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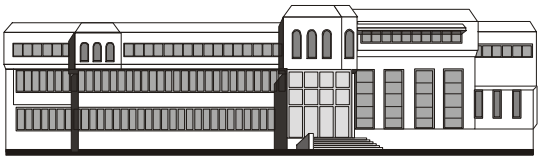

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The Tenth Conference of Agronomy Students

Čačak, Serbia

23 - 25 August, 2017

INTRODUCTION

Knowledge and skill of food production have a unique role in humans' lives. It is the foremost civilization skill. Nowadays, agriculture, as one of the oldest human activities, is a highly demanding production which merges knowledge from considerable number of fundamental and biotechnical sciences. This presents a challenge for the education of the students of agronomy and agricultural faculties.

The efforts to provide a student meeting point for discussing issues related to the acquired knowledge in the field of agriculture led to the First Conference of Agronomy Students which was held at and organized by the Faculty of Agronomy in Čačak, Serbia, in 1998. Today, we hope that the Tenth Conference of Agronomy Students will provide a forum for students-researchers in the field of biotechnical sciences contributing to the vibrant life of biotechnology research.

As the organizers of the Tenth Conference of Agronomy Students we encourage the students and their mentors to actively participate in this event by submitting projects, researches, experiences, and other theoretical and practical contributions as well as by transferring knowledge and expanding academic network.

This is the tenth Conference presenting 65 students' papers at three levels of study (bachelor, master, and doctoral) from XX universities and XX European countries. The submitted, presented, and printed papers are the part of scientific and research activities of the students at different levels of studies and of their mentors. The papers are printed as submitted by the authors.

We offer out thanks to all the participants of the Tenth Conference of Agronomy Students inviting them to participate in the next Conference as contributors or mentors.

Čačak, August, 2017

Sadržaj

Ten Conferences of Agronomy Students at the Faculty of Agronomy, Čačak: Participants from the Faculty of Agriculture, Osijek and their Current Status

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Abstract: This study provides an overview of collaboration between the Faculty of Agronomy, Čačak / Faculty of Agriculture, Lešak and the Faculty of Agriculture, J. J. Strossmayer University of Osijek. The collaboration includes, inter alia, the participation of our 12 students in four Conferences of Agronomy Students in Čačak from 2007 to 2013 and presentation of 16 papers. Five awarded papers were rearranged and published in *Acta Agriculturae Serbica*. Most of our participants in these Conferences are currently employed at the Faculty of Agriculture in Osijek, and their selection was based on high evaluation scores and experience gained in preparing and delivering lectures at the Conferences. All these young people gave their first public lectures in Čačak, and their bibliographies begin with papers published in the Conference Proceedings. We suppose that supervisors from the other participating institutions could write the same story. Sincere congratulations to the University of Kragujevac, Faculty of Agronomy - Čačak, Programme Committee and Organizing Committee on the successful organization of the ten conferences of agronomy students, with wishes for their continuation!

Key words: overview of collaboration, conferences of agronomy students, participants from Osijek

Chronology of collaboration

Scientific and educational collaboration between two faculties from Serbia i.e. Faculty of Agronomy, University of Kragujevac and Faculty of Agriculture, Lešak / Zubin Potok, University of Priština, on one side, and Faculty of Agriculture, J. J. Strossmayer University of Osijek (Croatia), on the other, has been initiated based on personal contacts between Desimir Knežević, Milan Biberdžić and Aleksandar Paunović from Serbia and Vlado Kovačević from Croatia, all of them as participants of annual conferences of agronomists of the Republic of Srpska held in Teslić (Bosnia and Herzegovina) in March from 2001 to 2006.

As a member of the Scientific Committee of Alps-Adria Scientific Workshops held under the patronage of the Hungarian Academy of Sciences, Vlado Kovačević invited the abovementioned colleagues from Serbia to take active participation in these workshops, which involved the publication of internationally peer-reviewed, accepted papers as special issues of the journal *Cereal Research Communications* indexed in the Science Citation (SCI) List. This initiative resulted in 23 papers written by teams of authors from Serbia, including colleagues from the two abovementioned faculties (CRC, 2006, 2007, 2008). These references contributed to the authors' promotion to higher scientific degrees and academic ranks.

On 26 - 29 June 2006, an international conference on "Improvement of Agricultural Production in Kosovo and Metohija" was held in Vrnjacka Banja, and organized by the Faculty of Agriculture, Priština - Lešak. As an invited speaker, Vlado Kovačević gave a presentation on "Improvement of acid soils by agromeliorative practices". The invitation came from Milan Biberdžić and Desimir Knežević. This conference paved the way for improvement and expansion of further cooperation.

The resulting improvement of collaboration was evidenced by the submission and acceptance of two bilateral projects between Croatia and Serbia under the patronage of the State Ministries of Science, as follows:

a) Bilateral Croatian-Serbian project (Faculty of Agriculture, Osijek and Faculty of Agriculture, Zubin Potok): Improvement of acid soils by liming and mineral fertilization (1 November 2008 - 31 October 2010; Project Leaders Vlado Kovačević and Miodrag Jelić).

b) Bilateral Croatian-Serbian project (Faculty of Agriculture, Osijek and Faculty of Agriculture, Čačak): Adaptation of management practices and cultivars of field crops to global climate changes (1 January 2010 - 31 December 2011; Project Leaders Vlado Kovačević and Aleksandar Paunović).

Grants associated with these projects covered the costs of reciprocal visits and participation in scientific events in Serbia and Croatia. Over the three-year period, the two projects resulted in a total of 26 scientific and review papers, 9 abstracts and participation in 10 international scientific events (Jelić, Kovačević and Paunović - final reports sent to state ministries, internal archive). Of the number, 11 papers and 4 abstracts were the result of mixed teams of authors from Serbia and Croatia.

Conferences of Agronomy Students

The expansion of collaboration was initiated by Aleksandar Paunović and his invitation for participation in the 5th Conference of Agronomy Students (CAS) with International Participation. Nada Parađiković and her students participated in the 5th Conference in August 2007. Our participation in the following three conferences (2009, 2011 and 2013) included more students and supervisors from Osijek (Table 1).

Table 1. List of papers of the Faculty of Agriculture, Osijek presented at Conferences of Agronomy Students

Author	Title of paper and supervisor's name (s.)
5th Conference: 30 August – 1 September 2007	
Iljkić D., Vinković T.	Hydroponic Growing and Biological Control of Pepper - s. Parađiković N.
Vinković T., Iljkić D.	Tomato Growing on Coconut Husks Substrate - s. Parađiković N.
6th Conference: 27 – 29 August 2009	
Jozić A.	Precipitation and temperature regime influences on maize yields in eastern Croatia - s. Rastija M.
Marijanović M.	Precipitation and temperature influences on wheat yields in eastern Croatia - s. Kovačević V.
Tkalec M., Vinković T.	Influence of Biostimulants on Growth and Development of Bell Pepper - s. Paradjiković N.
Iljkić D.	Precipitation and temperature influences on wheat yields in northwestern Croatia - s. Kovačević V.
Markulj A.	Precipitation and temperature regime influences on maize yields in northwestern Croatia - s. Kovačević V.
Vinković T., Tkalec M.	Application of different biostimulants in tomato growing - s. Paradjiković N.
7th Conference: 24 – 26 August 2011	
Jović J.	Precipitation and temperature regimes as factors of maize yield in Bosnian-Posavina County of Bosnia and Herzegovina - s. Rastija M.
Tomšić D.	Impacts of weather characteristics on yield of maize in Vukovar-Syrmium County, Croatia - s. Kovačević V.
Varga I.	Sugar beet production in Croatia from 2006 to 2010 - s. Antunović M.
8th Conference: 28 - 30 August 2013	
Dokić N.	Drought as a limiting factor of maize yields in Eastern Croatia and Vojvodina regions - s. Kovačević V.
Iljkić D.	Maize hybrids response on acid soils – s. Rastija M.
Stracenski S., Katić A.	Winter wheat management practice in PIK Vinkovci d.d. and weather characteristics in the period from 2010 to 2012 - s. Kovačević V.
Komljenović V.	Content of vitamin C in leaves and flowers of *Tropaeolum majus* L. - s. Paradjiković N.
Tkalec M.	In vitro propagation of Pelargonium - s. Paradjiković N.

Twelve students from the Faculty of Agriculture, Osijek participated in four conferences of agronomy students and presented 16 papers (Table 1). Five awarded papers were rearranged and selected for publication in *Acta Agriculturae*

Serbica (Paradjiković et al., 2007; Vinković et al., 2007; Markulj et al., 2010; Marijanović et al., 2010; Tkalec et al. 2010).

Current status of our Conference participants

Six students participating in Conferences of Agronomy Students are currently employed at the Faculty of Agriculture, Osijek; one student participant is currently holding a position at the Agricultural Institute, Osijek. Their selection was based on high evaluation scores and additional experience gained in preparing and delivering lectures at Conferences. All these young people gave their first public lectures in Čačak, and their bibliographies begin with papers published in the Proceedings of the Conference of Agronomy Students. Four participants have earned their PhD degrees, and have been involved in the education process as heads of some modules. We suppose that supervisors from Serbian and other international institutions whose students participated in the ten conferences of agronomy students could write the same story.

Based on these facts, sincere congratulations to the University of Kragujevac, Faculty of Agronomy - Čačak, Programme Committee and Organizing Committee on the successful organization of the ten conferences of agronomy students, with best wishes for a continuation of activities which started some twenty years ago! The current status of our students who participated in the Conferences is as follows:

Dario Iljkić: Graduated on 10 February 2009; employed at the Faculty of Agriculture, Osijek since 1 March 2009; earned a PhD degree on 6 February 2015; the process for appointment to the rank of Assistant Professor underway.

Tomislav Vinković: Graduated on 17 July 2006; employed at the Faculty of Agriculture, Osijek since 1 January 2017; earned a PhD degree on 11 November 2011; currently, Assistant Professor.

Marija Marijanović: Graduated on 12 July 2011; employed at the Agricultural Holding, Vinkovci as a Vegetable Crops Technologist.

Monika Tkalec: Graduated on 25 March 2011; employed at the Faculty of Agriculture, Osijek since 1 September 2011; earned a PhD degree on 6 February 2017.

Antonela Markulj: Graduated on 1 September 2011; employed at the Agricultural Institute, Osijek as a Research Assistant in the Department of Industrial Crops since 1 December 2011; currently a PhD student.

Jurica Jović: Graduated on 13 June 2013; employed at the Faculty of Agriculture, Osijek since 1 September 2013; currently a PhD student.

Ivana Varga: Graduated on 25 October 2010; employed at the Faculty of Agriculture, Osijek since 10 October 2010; earned a PhD degree on 11 November 2017; the process for appointment to the rank of Assistant Professor underway.

Stanislav Stracenski: Graduated on 10 July 2015; employed at the Agricultural Holding, Vinkovci as a Technologist.

Valentina Komljenović: Graduated on 30 September 2014; employed at the Faculty of Agriculture, Osijek.

Anita Jozić, Danijela Tomšić and Nikolina Dokić: Graduated, but their current status unknown.

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Characterisation of '*Candidatus* Phytoplasma prunorum' strains in Czech Republic

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Abstract: Molecular markers (*aceF*, *imp*, *pnp* and *secY)* are used for strain characterisation of '*Candidatus* Phytoplasma prunorum'. By sequencing of marker *aceF* should be possible to distinguish hypovirulent strains and its RFLP analysis enables monitoring of mixed infections of '*Ca*. P. prunorum' strains in samples. In this study 28 *Prunus* samples from experimental orchard in Lednice (Czech Republic) were used for strain characterisation of '*Ca*. P. prunorum'. As a result of RFLP analysis of *aceF* marker 5 samples were infected by mixed infections. Sequence analysis of *aceF*, *imp*, *pnp* and *secY* markers reveald new genotypes of '*Ca*. P. prunorum'. New genotypes and comparison of genotype composition of '*Ca*. P. prunorum' with other studies could indicate isolated evolution of '*Ca*. P. prunorum' in Lednice region. Finally, by sequencing of *aceF* marker it was not possible to distinguish candidate hypovirulent strains.

Key words: '*Candidatus* Phytoplasma prunorum', ESFY, Prunus, molecular markers

Introduction

One of the pathogens involved in premature death of stone fruit species is phytoplasma '*Candidatus* Phytoplasma prunorum', referred to as ESFY - European stone fruit yellows phytoplasma. It is present in sieve tubes or in parenchymal cells of phloem (Kůdela et al., 2002). Currently, there is no effective direct protection against phytoplasmas.

Typical ESFY symptoms on the genus *Prunus* are early leaf bud break prior blooming, yellowing or redness of leaves, their rolling and premature fallout. Affected apricot trees usually die within 2 years after appearance of first symptoms (Carraro and Osler, 2003).

Several '*Ca.* P. prunorum' strains were observed on the basis of the difference in symptomatic manifestation (Lorenz et al., 1994; Seemüller and Foster 1995; Kison and Seemüller, 2001; Ermacora et al., 2010). In the work of Ermacora et al. (2010) hypervirulent (strong symptoms) and hypovirulent (low or asymptomatic expression) strains were classified.

Despite the differences in symptomatic manifestation, routine phytoplasmal diagnostic techniques (sequencing and RFLP of the PCR product of rDNA) were not able to distinguish between '*Ca.* P. prunorum' strains on the molecular level (Kison and Seemüller, 2001; Seemüller and Schneider, 2004). Lately, several molecular markers to distinguish '*Ca.* P. prunorum' strains have been designed, but many have not shown variability sufficient for recognition hypo and hypervirulent strains. Among them are for example genes *tuf*, *fol*, *rpsC*, *tlyC* (Marcone et al., 2010), *pnp* and *secY* (Danet et al., 2011). Sequence analysis of *imp* gene of different '*Ca.* P. prunorum' isolates have shown relatively high variability, but it was not possible to distinguish hypo and hypervirulent strains (Danet et al., 2011, Marcone et al., 2010). Finally, the sequence analysis of the *aceF* gene showed sufficient variability (11 genotypes) and the possibility of distinguishing hypo and hypervirulent strains (Danet et al., 2011). More over, by RFLP analysis of the PCR product of *aceF* gene, mixed infections of various '*Ca.* P. prunorum' strains can be observed (Martini et al., 2010).

In this work, '*Ca*. P. prunorum' strains from Lednice, Czech Republic were characterized by 4 molecular markers (*aceF*, *imp*, *pnp*, *secY*). Marker *aceF* was analysed also by RFLP, which enables to reveal mixed infections of '*Ca*. P. prunorum'.

Material and methods

Plant material

Plant material under long term observation with typical symptoms or suspicion for '*Ca*. P. prunorum' infection (Table 1) was selected for the project. *Prunus armeniaca* L. (23 samples), *Prunus domestica* L. (3 samples) and *Prunus persica* (L.) Batsch (2 samples) species were analysed. All plants were located in the experimental orchard of Department of fruit growing, Faculty of Horticulture in Lednice, MEDNELU (CZ) of which 10 were kept under conditions of technical isolation.

DNA isolation

Total DNA was isolated from two-year-old shoots or from leaf stalks by modified protocol of Maixner et al. (1995). DNA was dissolved in 100 µl of TE buffer.

Table 1. Plant material

Species	Cultivar	Source	Origin	Symptoms
Prunus armeniaca	Saldcot	phloem	orchard Lednice	strong chlorotic leafroll, growth depression
	Polonais	phloem	orchard Lednice	strong leafroll, weak chlorosis, growth depression
	Antonio Erani	phloem	orchard Lednice	chlorosis, growth depression
	Hativ Colmer	phloem	orchard Lednice	growth depression
	Veselka*	phloem	orchard Lednice	weak chlorosis
	Reumberto	phloem	orchard Lednice	chlorosis
	Vestar	phloem	orchard Lednice	chlorotic leafroll
	Harglow	phloem	orchard Lednice	leafroll, growth depression
	NJA 35	phloem	orchard Lednice	weak chlorotic leafroll
	Olimp	phloem	orchard Lednice	chlorotic leafroll, growth depression
	Bai-Gon	phloem	orchard Lednice	leafroll, growth depression
	Hargrand (1)*	phloem	orchard Lednice	without symptoms
	Hargrand (2)	phloem	orchard Lednice	strong chlorosis, strong growth depression
	Hargrand (2/1)	leaves	technical isolator Lednice	chlorotic leafroll
	Hargrand (3)	leaves	Kobylí (technical isolator)	chlorotic leafroll
	Hargrand (4)	leaves	Kobylí (technical isolator)	chlorotic leafroll
	Hargrand (5)	leaves	Kobylí (technical isolator)	without symptoms
	Poyer (1)*	phloem	orchard Lednice	without symptoms
	Poyer (2)*	leaves	technical isolator Lednice	without symptoms
	Poyer (3)*	leaves	technical isolator Lednice	leafroll
	Poyer (4)*	leaves	technical isolator Lednice	leafroll
	Poyer (5)*	leaves	technical isolator Lednice	without symptoms
	Poyer (6)	leaves	technical isolator Lednice (spontaneous infection)	without symptoms
Pruns domestica	Carskaja	phloem	orchard Lednice	chlorosis
	M 49	phloem	orchard Lednice	chlorosis, growth depression
	Burbank	phloem	orchard Lednice	chlorosis
Prunus persica	13B	phloem	orchard Lednice	chlorotic leafroll
	GF 305	leaves	technical isolator Lednice (spontaneous infection)	chlorotic leafroll

* samples with candidate hypovirulent strains of '*Ca*. P. prunorum'

Detection of '*Ca*. P. prunorum'

Samples were analyzed for the presence of 16SrX phytoplasmas by nested PCR using P1/P7 (Schneider et al., 1995) and f01/r01 (Lorenz et al., 1995) primers. For specific detection of '*Ca*. P. prunorum' the real-time PCR protocol of Nikolic et al. (2010) with the TaqMan MGB ESFY probe was used.

Molecular markers

For the molecular characterization of '*Ca*. P. prunorum', molecular markers were used as in Danet et al. (2011). These markers are from the region of *aceF*, *pnp*, *secY* and *imp* genes.

The PCR product of *aceF* marker has size of 797 bp. In Danet et al. (2011) 11 distinct genotypes of '*Ca*. P. prunorum' were obtained. RFLP analysis of the

PCR product of this marker distinguished 6 different subgroups of '*Ca*. P. prunorum' (Martini et al., 2010).

The PCR product of markers *imp*, *pnp* and *secY* have a size of 673 bp, 549 and 664 bp respectively. In Danet et al. (2011) 14, 2 and 3, respectively distinct genotypes of '*Ca*. P. prunorum' were obtained.

Nested PCR

Nested PCR with two primer pairs was used for amplification of DNA region of each molecular marker. The 20 µl volume of one reaction consisted of 1 µl of DNA, 500nM of forward and reverse primer, 1.6mM Mg^{2+}, 0.125mM dNTP's, 1 U GoTaq G2 polymerase (Promega, WI, USA), 1x Buffer (Promega, WI, USA) and purified H_2O.

The temperature program of the PCR was the same for all molecular markers. First amplification: activation of the polymerase at 95 °C for 2 min and 20 cycles consisting of 95 °C for 30 s, 50 °C for 30 s, and 66 °C for 45 s and a final elongation at 66 °C for 5 min. For the second amplification 1 µl of the product from first amplification and nested primers were used in PCR with the temperature profile identical with the first amplification, however with higher number of cycles (35).

RFLP analysis of aceF marker

Three restriction endonucleases Bbsl, MluCI and HaeIII (New England BioLabs, MA, USA) were used for RFLP analysis of *aceF* marker PCR products. A 10 µl volume reaction consisted of 2 µl of PCR product, 3 U of respective endonuclease, 1X buffer and purified H_2O. Temperature program according to the manufacturer was used. RFLP products of Bbsl and HaeIII enzymes were separated by electrophoresis in 2% agarose gel. RFLP products of MluCI enzyme were separated by vertical electrophoresis in 5% PAGE. All products were visualized by intercalating dye GelRed (Biotium, Inc., CA, USA) in UV transiluminator.

Samples were divided into subclasses according to obtained RFLP patterns as in Martini et al. (2010).

Molecular markers sequence analysis

PCR products of each molecular marker were excised from the gel and purified by NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, D) and dissolved in 20 µl of elution buffer. Purified samples were sequenced by sanger method using the forward nested primer of respective marker in GATC Biotech (D) using ABI 3730xl.

Chromatograms were analyzed in the CLC program (CLC bio, DK), where obtained sequences were aligned with reference sequences from the NCBI

database. CLC program was also used in phylogenetic analyzes, where dendrograms of reference sequences together with newly discovered sequences were created by Nieghbour Joining.

Results and discussion

Detection of 'Ca. P. prunorum'

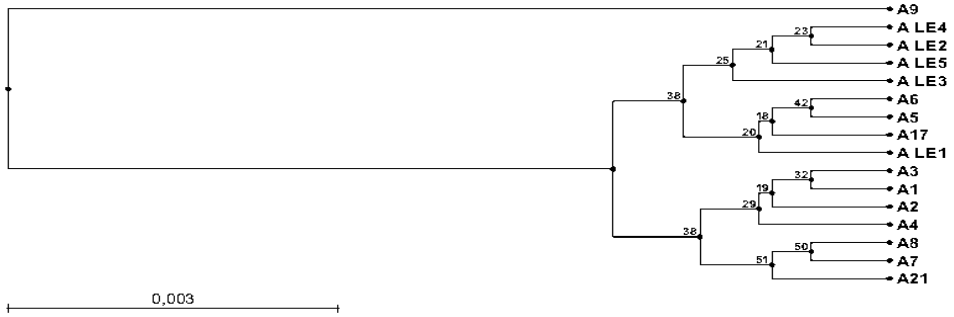
All tested samples were positive for the presence of phytoplasma from 16SrX and all of them were positive for 'Ca. P. prunorum' by nested PCR and real-time PCR, respectively.

Analysis of the molecular marker aceF

By RFLP analysis of the *aceF* marker 24 samples were divided into 3 subgroups (AceF-A, -C, -E) and 4 samples showed mixed infection (3 x AceF-B + E, AceF-A + C) (Table 2). The most representative subgroup was AceF-A (18 samples) and AceF-E (5 samples), which confirmed the results of Martini et al. (2010).

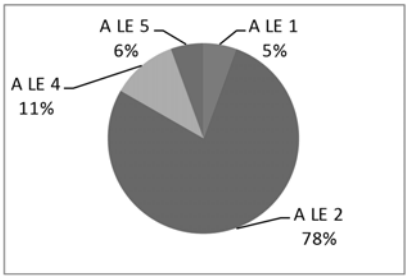
By sequencing of the aceF marker, complete sequences were obtained from 21 samples (Table 2). Alignment of obtained sequences (702 bp) with the reference sequences (Danet et al., 2011) showed that beside the sample Polonais, with '*Ca*. P. prunorum' genotypes A5 and A6, all other samples had unique sequences divided into 5 new genotypes (A LE1 - A LE5) (Table 2 and 3, Fig.1). The most common genotype among tested samples was the new genotype A LE2 (14 samples) and A LE4 (3 samples).

Figure 1. Dendrogram of aceF marker of phytoplasma 'Ca. P. prunorum'



Phylogenetic analysis by Neighbour Joining method, bootstrap value 100
Reference genotypes (Danet et al., 2011):A1 – A9; A17; A21
New genotypes (description in this work): A LE1 – A LE5

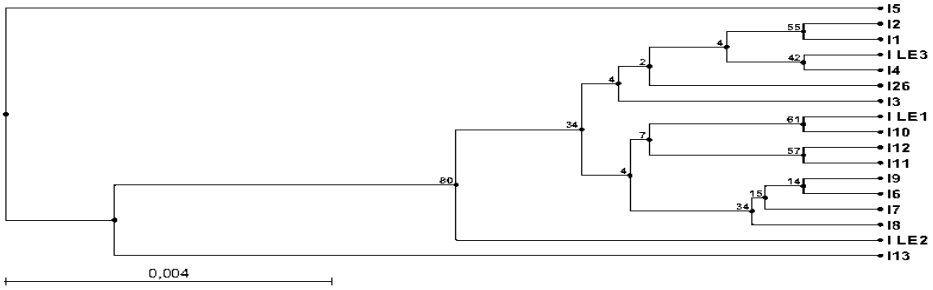
Figure 2. Genotypic composition of subgroup aceF-A of analysed samples



Analysis of the molecular marker imp

By sequencing of the imp marker, complete sequences were obtained from 18 samples (Table 2). Alignment of obtained sequences (607 bp) with the reference sequences (Danet et al., 2011) showed that besides known genotypes (I3, I4, I10), 4 samples had unique sequences (I LE1 - I LE3) (Tab. 2 and 3, Fig. 3). The highest proportion of tested samples were genotypes I4 (9 samples) and I10 (4 samples).

Figure 3. Dendrogram of imp marker of phytoplasma '*Ca*. P. prunorum'



Phylogenetic analysis by Neighbour Joining method, bootstrap value 100
Reference genotypes (Danet et al., 2011): I1 – I13; I14
New genotypes (description in this work): I LE1 – I LE3

Analysis of the molecular marker secY

By sequencing of the *secY* marker, complete sequences were obtained from 18 samples (Table 2). Alignment of obtained sequences (498 bp) with the reference sequences (Danet et al., 2011) showed that all samples had S2 genotype of '*Ca*. P. prunorum' (Table 2).

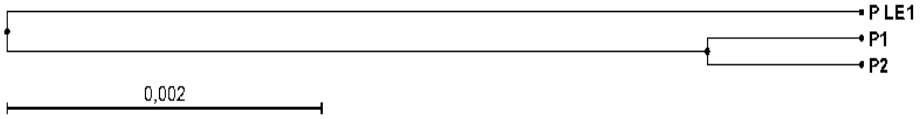
Analysis of the molecular marker pnp

By sequencing of the *pnp* marker, complete sequences were obtained from 20 samples (Table 2). Alignment of obtained sequences (464 bp) with the reference

sequences (Danet et al., 2011) showed that all samples, beside the sample Hargrand (4), had P2 genotype of 'Ca. P. prunorum' (Table 2, Fig.5).

Sequence of the sample Hargrand (4), P LE1, differed from reference genotypes P1 and P2 in 7, respectively, 6 SNPs (single nucletodie polymorhism) (Table 3, Fig. 4). This is a big difference considering that the difference between the genotype P1 and P2 is only 1 SNP. Inoculum of the Hargrand (4) was obtained from fruit orchard in Kobylí and compared to other samples, beside the marker *pnp,* it does not show significant differences in *aceF*, *imp* or *secY* markers.

Figure 4. Dendrogram of pnp marker of phytoplasma '*Ca*. P. prunorum'



Phylogenetic analysis by Neighbour Joining method, bootstrap value 100
Reference genotypes (Danet et al., 2011): P1, P2
New genotypes (description in this work): P LE1

From analysis of *aceF*, *imp*, *pnp* and *secY* markers of tested samples is evident the difference between '*Ca*. P. prunorum' genotype composition in the experimental orchard and results from work by Danet et al. (2011). The difference is proved by new genotypes in *aceF*, *imp* and *pnp* markers. In addition, new genotypes A LE2 - A LE5 differed from the reference genotypes A5 and A6 mainly in deletions of 688. and 695. nucleotide (Table 3), which is an interesting result when considering, that the reference genotype strains were obtained mainly from the western Europe. The A LE2 - A LE5 genotypes created a new, isolated branch in phylogenetic analysis (Fig. 1). Differences are also evident in *imp* marker genotype composition, where the most frequent genotypes of tested samples were I4 and I10 (13 of 18 samples), compared to I1 and I9 genotypes, which were the most frequent in Danet et al. (2011). For the *secY* and *pnp* markers, the most abundant genotypes were S2 (all samples) and P2 (19 of 20 samples), compared to work of Danet et al. (2011) were the genotypes S1 resp. P1 were the most abundant. These results may indicate isolated evolution of '*Ca*. P. prunorum' in Lednice in the Czech Republic.

RFLP and sequence results analyzes of *aceF* marker show clearly that RFLP analysis is not sufficient to fully distinguish '*Ca*. P. prunorum' strains, where, for example, the AceF-A subgroup was composed of four genotypes of '*Ca*. P. prunorum' (Figure 2). This confirmes the results of Martini et al. (2010). However, the advantage of RFLP analysis is the possibility of observation of mixed infections, which was confirmed in this work as well (Tab.2).

In Danet et al. (2011) analysis of the *aceF* marker was able to distinguish hypovirulent '*Ca*. P. prunorum' strains (genotype A6) from other strains. In this work, despite the candidate asymptomatic or weakly symptomatic individuals (Table 2 - labeled *), neither of them were genotype A6. In addition, all candidate hypovirulent strains differed from each other by the genotypic composition of all markers. However, natural recovery and possible persistence of asymptomatic expression even in plants propagated vegetatively is probably possible regardless on the phytoplasma strain (Osler et al., 2014; Osler et al., 2016).

Table 2. Results of analysis of '*Ca*. P. prunorum' molecular markers

Species	**Cultivar**	**Subgroups AceF-**[a]	**Genotypes *aceF***[b]	**Genotypes *imp***[b]	**Genotypes *secY***[b]	**Genotypes *pnp***[b]
Prunus armeniaca	Saldcot	A	A LE1	I LE1	-	-
	Polonais	A+C	A5+A6	I4	S2	-
	Antonio Erani	A	A LE2	I10	S2	P2
	Hativ Colmer	C	-	I4	S2	P2
	Veselka*	A	A LE4	I10	S2	P2
	Reumberto	C	-	I4	S2	P2
	Vestar	A	A LE2	I4	S2	P2
	Harglow	A	A LE2	I3	S2	P2
	NJA 35	A	A LE2	I4	-	P2
	Olimp	B+E	A LE4	-	-	P2
	Bai-Gon	B+E	-	-	-	P2
	Hargrand (1)*	A	A LE2	I10 + I LE 1	-	-
	Hargrand (2)	A	A LE2	I10	-	-
	Hargrand (2/1)	A	A LE2	I10	S2	P2
	Hargrand (3)	C	A LE3	I4	S2	P2
	Hargrand (4)	A	A LE2	I4	S2	P LE1
	Hargrand (5)	A	A LE2	I LE 3	S2	P2
	Poyer (1)*	A	A LE2	I4	-	-
	Poyer (2)*	A	A LE2	-	S2	P2
	Poyer (3)*	A	A LE2	I LE 2	S2	P2
	Poyer (4)*	A	A LE4	-	S2	-
	Poyer (5)*	A	A LE5	-	S2	-
	Poyer (6)	C	-	-	S2	P2
Pruns domestica	Carskaja	B+E	-	-	-	P2
	M 49	E	-	-	-	P2
	Burbank	A	A LE 2	-	-	P2
Prunus persica	13B	A	A LE 2	I4	S2	P2
	GF 305	C	-	-	S2	-

* samples with candidate hypovirulent strains of '*Ca*. P. prunorum'

[a] RFLP analysis classification by Martini et al. (2010)

[b] sequention analysis classification by Danet et al. (2011)

A LE1 – A LE5; I LE1 – I LE3; P LE1: new genotypes with different sequentions than reference sequentions in Danet et al. (2011)

Table 3. The differences between new and reference genotypes

New genotype	Closest related genotype[a]	Difference	Number of samples
A LE1	A6	deletion of 17. nt	1
A LE2		deletion of 695. and 688. nt	14
A LE4		deletion of 695. nt	3
A LE5		deletion of 695. nt, 3 SNP's	1
A LE3	A5	deletion of 695. and 688. nt	1
I LE1	I10	1 SNP	1
I LE2	I4	1 insertion, 2 deletions	1
I LE3	I4	1 insertion	1
P LE1	P1 resp. P2	6 SNP's resp. 7 SNP's	1

[a] according to Danet et al. (2011)

Acknowledgement

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Influence of biostimulants on growth and photosynthetic performance of young maize (*Z. mays* L.) plants exposed to chilling stress

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Abstract: The aim of the performed experiments was to study the effects of two biostimulants containing free amino acids and small peptides (Naturamin-WSP and Terra-Sorb Foliar) on the growth and photosynthetic performance of chilling-exposed young maize plants (*Z. mays* L., hybrid Kneza 307). The plants were grown hydroponically in a controlled environment at 25±1°C/20±1°C (day/night) temperature. At the appearance of third leaf stage, the maize plants were exposed to chilling (constant 10 °C) for 14 days. Seven days after the beginning of the chilling, the plants were sprayed by the biostimulants Naturamin-WSP (0.1%) and Terra-Sorb Foliar (1%) and left to grow for another 7 days. The plants were analyzed at the end of the experimental period. Plant growth parameters, leaf gas exchange, chlorophyll fluorescence and photosynthetic pigment content were determined. The results gave evidence that both applied biostimulants ameliorated to some extend the photosynthetic performance of chilling-exposed maize plants. The positive effect of Terra-Sorb Foliar was more clearly expressed.

Key words: *Zea mays* L., chilling, photosynthetic performance, biostimulants

Introduction

Maize (*Zea mays* L.) is a chilling – sensitive crop, especially in the early stages, during the transitional period from heterotrophic to autotrophic nutrition (Stamp, 1984). Young maize plants may be damaged when temperatures are within 5-15ºC range, causing chilling stress (Hola et al., 2007; Leipner, 2009). In the climate conditions of Bulgaria, early stages of maize plant development often go under suboptimal temperatures.

Chilling may induce different physiological disorders in young maize plants. It may provoke uncontrolled production of reactive oxygen species (ROS) damaging membrane integrity and enzyme activities (Farooq et al., 2008; Foyer et al., 2002; Aroca et al., 2003). The primary negative effects are further multiply on the cardinal physiological processes, such as water relations, mineral uptake (Aroca et al., 2003), photosynthesis (Foyer et al., 2002), etc. and lead to plant growth retardation (Leipner, 2009; Zaidi et al., 2010). Photosynthesis is very sensitive to chilling (Baker et al., 1994). It has been shown that chilling disturbs the overall photosynthetic process at different levels – pigments (Haldiman, 1998), photochemical processes (Kosova et al., 2005), CO2 assimilation (Bilska and Sowinski, 2010), etc.

Biostimulants are defined as agricultural products containing substances and/or microorganisms able to stimulate plant nutrient uptake and efficiency, increase plant stress tolerance as well as improve crop quality (du Jardin, 2012). Protein hydrolysates are one of the biostimulants sub-categories. They contain a mixture of small size peptides and free amino acids with plant or animal origin (Schaafsma, 2009). The application of protein hydrolysates has been used to improve plant tolerance to environmental stresses, such as temperature (Botta, 2013), drought (Petrozza et al., 2014), salinity (Ertani et al., 2013) and others. Surprisingly, there is a lack of information concerning the effects of protein hydrolysates on plants exposed to chilling. Therefore, we decided to study the effects of two biostimulants from this sub-category on the growth and photosynthetic performance of young maize plants under chilling stress.

Materials and methods

Growth Conditions and Experimental Design

The experiments were carried out in a climatic room of the Department of Plant Physiology and Biochemistry in the Agricultural University of Plovdiv, Bulgaria. Maize plants (hybrid Kneza 307) were grown hydroponically in ½ strength modified Hoagland nutrient solution, at controlled environment: photoperiod – 12 hours, PPFD (photosynthetic photon flux density) 200 μmol m-2 s-1, temperature – 25±1oC/ 20±1oC (day/night) and relative air humidity – 60±5%. Uniform maize plants at the appearance of the 3^{-red} leaf stage were used for the studies. The experimental design of the studies included 4 treatments, namely: (1) maize plants, grown at 25 ^{o}C, accepted as control; (2) maize plants, grown at 10 ^{o}C - chilling; (3) maize plants, grown at 10 ^{o}C and sprayed by 0.1% water solution of Naturamin-WSP and (4) maize plants, grown at 10 ^{o}C and sprayed by 1% water solution of Terra-Sorb Foliar. The products Naturamin-WSP and Terra-Sorb Foliar are biostimulants containing free amino acids and small peptides. The duration of the experiments was 14 days. The biostimulants

were applied 7 days after the beginning of chilling. Each treatment had 3 replications (pots) with 4 plants per pot. The experiment was performed twice.

Plant Growth Analysis

The plants were harvested at the end of the experimental period and fresh weight, plant height and root length were measured.

Leaf Gas Exchange Analysis

Leaf gas exchange (A – net photosynthetic rate, E – transpiration rate, g_s – stomatal conductance as well as c_i – internal CO_2 concentration) was measured by an open photosynthetic system LCpro+ (ADC, England) on the upper fully developed leaf, at PPFD of 450 μmol m^{-2} s^{-1}, after one hour adaptation.

Photosynthetic Pigments Content

Photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids) were extracted in 80% acetone, measured spectrophotometrically and calculated according to the formulae of Lichtenthaler (1987).

Chlorophyll Fluorescence Analysis

Chlorophyll fluorescence measurements were performed with a pulse modulation fluorometer (MINI-PAM, Heinz Walz, Germany) in the same leaves after dark and light adaptation. The measurements were done on both top-leaf zone and middle-lower part zone of the leaf lamina in no chilled and chilled plants. The maize plants were kept in the dark for 30 min before the start of the measurement. By switching on the measuring beam (0.02 – 0.20 μmol m^{-2} s^{-1}), the minimal level of fluorescence (F_0) was recorded. Immediately thereafter, a saturating light pulse of 5500 μmol m^{-2} s^{-1} with 0.8 s duration was sent out to record the maximal level of fluorescence in the dark-adapted state (F_m), from which the maximal quantum yield of PSII (F_v/F_m) was calculated (with $F_v = F_m – F_0$). After 30 min light adaptation at 450 μmol m^{-2} s^{-1} the steady-state level of photosynthesis was achieved and a saturating pulse with the same characteristics was applied. Fluorescence yield before triggering the saturation pulse (F); maximal (F_m') fluorescence, reached during the saturation pulse; as well as an apparent electron transport rate [ETR; ETR = Y*PAR*0.5*0.84 (Genty et al., 1989), where $Y=(F_m'-F)/F_m'$)] were determined. Both photochemical quenching (qP; $qP = (F_m' – F)/(F_m'– F_0)$ and non-photochemical quenching (qN; $qN = (F_m – F_m')/(F_m – F_0)$ were calculated according to Schreiber (2004).

Statistical Analysis

Statistical analysis was performed using one way ANOVA (for $P<0.05$). Based on ANOVA results, a Duncan test for mean comparison was performed,

for a 95% confidence level, to test for significant differences among treatments. In the figures and the tables, different letters (a, b and c) express significant differences at the $P < 0.05$.

Results

The applied 14-day-long chilling (10°C) significantly retarded the growth of maize plants (Figure 1). The chilled plants formed 70% less biomass, 41% shorter root length and 45% lower plant height as compared with the control plants. Some leaf yellowing in the middle of the lower part of the 3^{-red} leaf of chilled plants was observed. The application of both protein hydrolysates cased some positive effects on the plant growth parameters, but the detected differences were not significantly different from those of the untreated chilled plants.

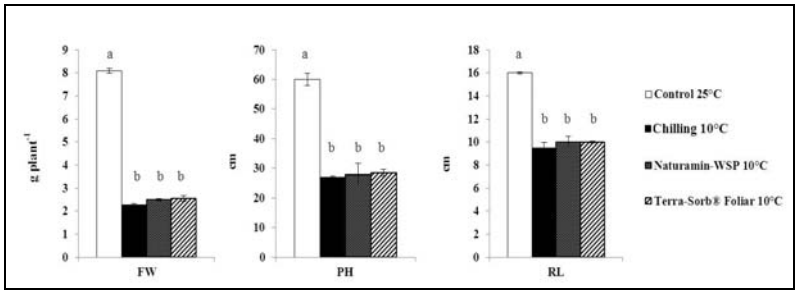

Figure 1. Influence of chilling and biostimulants on growth parameters of young maize plants. Different letters (a and b) following the mean values indicate significant differences at $P<0.05$.

The chilling treatment significantly decreased leaf gas exchange parameters in maize plants. The net photosynthetic rate (A) in chilled plants was diminished by 20%, transpiration rate (E) and stomatal conductance (g_s) by 36% and 50%, respectively (Table 1). Our results are in line with the observation of Kosova et al. (2005), who found that the chilling strongly diminished net photosynthetic rate. They also correspond with the data of Al-Shoaibi's (2008) reporting lower carboxylation efficiency in chilling-exposed maize plants. Leaf gas exchange parameters were less affected or unaffected in the chilled maize plants receiving biostimulant application. The A in plants-treated by Terra-Sorb Foliar was not significantly different from that in no chilled plants, while the A in plants-treated by Naturamin-WSP was inhibited by only 13%. The biostimulant application preserved water relations in the chilled plants as the measured values of E and g_s were significantly higher than those in the untreated chilled plants.

Table 1. Influence of chilling and biostimulants on leaf gas exchange in young maize plants from hybrids Kneza 307. A – net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); E – transpiration rate (mmol H_2O m^{-2} s^{-1}); g_s - stomatal conductance (mol m^{-2} s^{-1}).

Treatment	Leaf gas exchange					
	A		E		g_s	
Control 25°C	14.9[a]	(100)	0.96[a]	(100)	0.08[a]	(100)
Chilling 10°C	11.9[b]	(80)	0.61[c]	(64)	0.04[b]	(50)
Naturamin-WSP 10°C	12.9[b]	(87)	0.78[b]	(81)	0.05[b]	(63)
Terra-Sorb Foliar 10°C	14.6[a]	(98)	0.89[a]	(93)	0.05[b]	(63)

The data presented are an average. The values in brackets presented percentage (%) compared to the control 25°C. Different letters (a, b and c) following the mean values indicate significant differences at $P<0.05$.

The chilling cased negative impact on photosynthetic pigments in the maize plants (Figure 2). Chlorophylls and carotenoid contents in the chilled plants were diminished by 48 and 34%, respectively. The lower chlorophyll content in the leaves of chilled maize plants explained their visual chlorotic symptoms. Leipner et al. (2009) commented that the chlorotic symptoms on chilled maize plants are consequences of chlorophyll photooxidative damage. The stronger chilling effect on chlorophyll content than total carotenoids resulted in a change in their ratio. According to Haldiman (1998) the less affected total carotenoid content may be related to the essential role of these pigments as a protector of photosynthetic apparatus against oxidative damages. The application of both biostimulants ameliorated, but to different extent, the negative chilling impact on photosynthetic pigments. The positive effect of Terra-Sorb Foliar was better expressed than that of Naturamin-WSP. The decrease of both chlorophyll and carotenoid contents in Terra-Sorb Foliar-treated plants were smaller as compared with those in untreated chilled plants.

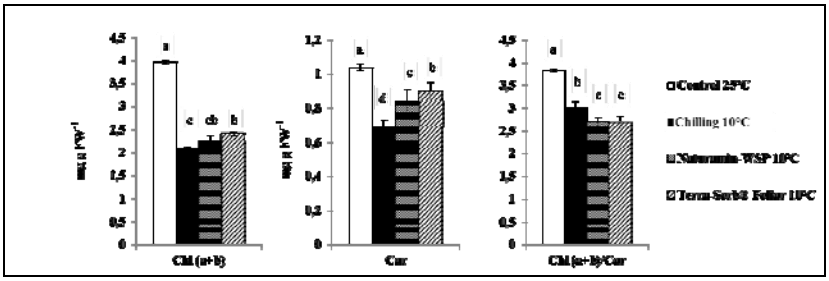

Figure 2. Influence of chilling and biostimulants on photosynthetic pigments content and ratios of young maize plants. Different letters (a, b, c and d) following the mean values indicate significant differences at $P<0.05$.

The chilling disturbed photochemical processes in the maize plants (Table 2). The maximal quantum yield of PSII (F_v/F_m) in dark-adapted the chilled plants was significantly decreased by 13%. The F_v/F_m value of the chilled plants was below the norm for healthy plants - 0.75-0.83 (Bolhar-Nordenkampf and Oquist, 1993), but in biostimulant-treated plants it was preserved. The lower F_v/F_m in the chilled maize plants is in line with the data of Bilska and Sowinski (2010) and Kosova et al. (2005), who explained this effect as the consequence of chilling-induced photoinhibition.

The chilling induced some negative changes in light-adapted leaves, too. The apparent electron transport rate (ETR) was retarded by 27%, the photochemical quenching (qP) was diminished by 9% and the non-photochemical quenching (qN) was increased more than twice. The apparent electron transport rate (ETR) is a calculated parameter, representing the linear electron transport rate of the overall *in vivo* photosynthetic process. The application of Naturamin-WSP and Terra-Sorb Foliar cased protective effect on ETR. The ETR values in the biostimulant-treated plants were lower than those in control plants, but the negative effect was less expressed, between 9 and 11%. Photochemical quenching (qP) indicates the proportion of open PSII reactive centers, while non-photochemical quenching (qN) – heat dissipation. The biostimulants application slightly increased the primary photochemistry (qP) and did not affect the heat dissipation (qN) in the chilled maize plants.

Table 2. Influence of chilling and biostimulants on selected chlorophyll fluorescence parameters in young maize plants from hybrids Kneza 307. F_v/F_m – maximal quantum yield of PSII; ETR – apparent electron transport rate; qP – photochemical quenching; qN – non-photochemical quenching.

Treatment	Chlorophyll fluorescence parameters			
	F_v/F_m	ETR	qP	qN
Control 25°C	0.77^{a} (100)	48.4^{a} (100)	0.499^{a} (100)	0.197^{c} (100)
Chilling 10°C	0.67^{b} (87)	35.5^{c} (73)	0.454^{b} (91)	0.431^{a} (219)
Naturamin-WSP 10°C	0.75^{a} (97)	42.9^{b} (89)	0.475^{b} (95)	0.409^{b} (208)
Terra-Sorb Foliar 10°C	0.75^{a} (97)	44.2^{b} (91)	0.589^{a} (118)	0.425^{a} (216)

The data presented are an average. The values in brackets presented percentage (%) compared to the control 25°C. Different letters (a, b and c) following the mean values indicate significant differences at $P<0.05$.

Discussion

The applied 14-day long chilling provoked leaf chlorosis and retarded growth of young maize plants, as might be expected. The observed negative effects on plant growth correspond to the results of other authors' researches, concerning

exposure of maize plants to chilling temperatures (Sowinski et al., 2005; Verheul et al., 1996). The results obtained are in line with our previous study with maize plants under 7-day long chilling treatment (Cholakova and Vassilev, 2015) and showed that the extended chilling duration had a stronger negative impact on maize plants. The growth inhibition of chilled maize plants was accompanied by different disturbances in the photosynthetic apparatus. Chilling provoked negative effects on photosynthetic pigments, leaf gas exchange and photochemical processes.

The application of both protein hydrolysates Naturamin-WSP and Terra-Sorb Foliar showed a small and insignificant positive effect on the growth of maize plants, but improved to some extent their photosynthetic performance. Our results confirm the potential of protein hydrolysates to ameliorate the physiological status of the stress-exposed plants. For example, they are in line with the data of Botta (2013) who found that the protein hydrolysates improved cold tolerance of lettuce plants. It has been also shown that protein hydrolysates ameliorated physiological status of salt-exposed maize plants (Ertani et al., 2013) as well as drought resistance of tomato plants (Petrozza et al., 2014).

We think the observed positive effects of the applied biostimulants have both direct and indirect nature. According to observations of Matsumiya and Kubo (2011) the amino acids and small peptides are easily absorbed by the leaves. Stiegler et al. (2013) confirmed this statement measuring enhanced nitrogen uptake in the plants after spraying with labeled nitrogen. The foliar application escaped the competition of microorganism having place in the soil. In addition, the leaf applied amino acid and peptides have shorter source – sink transportation. It was shown that the application of protein hydrolysates increased nitrogen assimilation and shoot biomass in hydroponically-grown maize (Schiavon et al., 2008) and secondary plant metabolism (Ertani et. al., 2011; Schiavion et al., 2010). The mentioned studies gave some evidence that leaf-applied amino acids and small peptides may get into metabolic pathways and support the recovering process in the stressed plants. Therefore, we may suggest that the applied biostimulants can stimulate recovering processes linked to photosynthetic pigments, primary photochemistry as well as carbon enzymes reactions.

In conclusion, the chilling retarded growth and induced negative changes in photosynthetic performance of young maize plants. The negative effects have been slightly ameliorated when the plants were sprayed by the biostimulants Naturamin-WSP and Terra-Sorb Foliar containing free amino acids and small peptides. Further studies are in progress to clarify the nature of the detected positive effects of the used biostimulants on photosynthetic performance of chilling–exposed maize plants.

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Weed flora and cereals corn vegetation in Posavotamnava region

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Abstract: Research of weed flora and cereals corn vegetation in the region of Posavotamnava was done during 2016 and 2017. Totally, 73 weed species were found, belonging to *Consolido-Polygonhum aviculare* association. The association is of therophyta character (54,79%) with large participation of perennial bulb weed 28,77%. In phytogeographical point of view, weed species of extensive geographical distribution dominate, (subeuroasian, Euroasian, cosmopolitan, circum-polar and subcircum-polar) with 82,19%, as well as the participation of submediterranean flora elements 8,22% being the consequence of submediterranean climate influence.

Key words: Posavotamnava, weed flora, weed vegetation, cereals corn

Introduction

Posavotamnava region presents an agricultural region. The total area is 595km^2, municipality of Vladimirci covers 338, and Koceljeva 256km^2. The northern and central parts of Posavotamnava have intensive crop farming production. The south and south-eastern parts of the region (hilly region) due to climate, edaphic and some other factors are focused on fruit production and livestock breeding, while crop farming production is rather extensive..

During constant efforts in acheiving high wheat yields, a lot of restrictive factors appear interfering established tasks and objectives. Among the above mentioned factors, weeds are the ones that appear, usually accompaning crops. Using modern agrotechnology (cultivation, protection, nutrition, assortment etc.) affected their adaptation and adjustment to the conditions that are predominant in agrophytocoenoses.

Harmful weed effect can be seen in decreasing plant growing yield, amount of water,and nutrients in soil, photosynthetic and other processes, reducing soil

temperature, bringing down the quality of plant growing, complicating land cultivation, as well as serve like focal points for spreading diseases and insect pests. Decreasing harmul effects in wheat and other cereals, being agrophytocoenoses of dense structure, can possibly be solved only by knowing floristic, phytocoenological and ecologicalweed ratio.

Examining weeds vegetation in cereals, especially in wheat, was the subject of many of the scientists ((Kojić, 1953, 1961, 1972, 1973, Kojić et al., 1972, 1976, 1982, Kovačević, 1953, 1956, 1973, 1976, Šinžar, 1965, 1967, Šinžar i Dejović, 1976, Gajić, 1955, 1956, Jelesijević et al., 1975, 1977, Slavnić, 1951, 1952, Milijić, 1980, Stepić, 1984, Milošević, 2008).

Research area

Posavotamnava region is slightly wavy land. The highest point is Konjski grob 389m situated on the south side, and the lowest is on the right bank of the river Sava, 75m.

Posavotamnava region is situated in the north-west part of Serbia. From the east, it is surrounded by Kolubara region, from the west by Mačva and Pocerina, and Srem from the north. The major part of the region has moderate-continental climate. North part is under a direct influence of Panonska nizija, so the elements of sub-humid and microthermal climate appear, as well as mediterraneanian from the south (Đukanović, 1981). The average air temperature is approx. $11,8^0$C, annual rainfall is approx. 800mm water precipitation per m^2.

Dominant type of soil is pseudogley which covers more than 75,00%. The other soil types are of less importance. In planting structure, about 70,00 % are field crops, while industrial plants, fruits, meadows, pastures and vegetables cover other areas.

Materials and methods

Weed flora and wheat vegetation were examined in the region of Posavotamnava during 2016/2017. Phytocoenological scannings were done by combined method of Swiss-French school (Braun and Blanquet, 1951). Phytocoenological examinations were done on twenty locations (tab. 1). The size of scanning was approx. $100m^2$, taking care that the locations were representative samples, representing a real status of yield weediness.

Life forms were processed according to Ujvarosi (1957), which represents the addition to Raunkier 's system.

The relation between weed species and ecological factors (land humidity F, pH value R, the content of nutritive materials especially nitrogen compounds N,

the content of organic materials H, soil aeration D, habitat lightening L, habitat temperature T, continentality K) is expressed by Landolt's (1977) ecological indexes.

Flora elements were done by Gajić (1980). Determination of weed species is done according to the works of well-known authors Josifović et al., Flora of Serbia (I-IX, 1970-1977). Javorka - Csapodi (1975), Čanak et al. (1978), Kojić (1981), Kovačević (1976), Todor (1968), Ujvarosi (1957), Stepić (1984), as well as by using some other literature.

Sintaxonomic status of weed vegetationof wheat in Posavotamnava region was determined according to conventional concept of Braun – Blanqu et and Tuxena,and by synthetic examination of phytocoenoses of Yugoslavia farming land (Kojić, 1975, 1982).

Results

According to the already published results during last two years, and on the basis of phytocoenological data analysis, weed vegetation of wheat can be presented in a sintaxonomic way, as it follows:

Class: *Stellarietea mediae* (No. - Bl. 1932) Tx., Lohm, Prague. 1950.

Order: *Centauretalia cyani* Tx. et Lohm. 1950

Bond: *Caucalion lappulae* Tx., 1950

Association: *Consolido - Polygonetum aviculare,* Kojić, 1973.

Association *Consolido – Polygonetum aviculare*, Kojić, 1973. is widely spread weed society in the territory of Serbia. Its presence is noticed in the region of Pomoravlje (Varvarin, Obrež, Jovac, Svetozarevo), surroundings of Belgrade, Smederevska Palanka, Kosovo (Kojić, 1975, 1982), south of Banat (Pančevo, Kovačica, Kovin, Vladimirovac, Vršac, Bela Crkva), south-east Srem (Šinžar, 1965, Šinžar i Dejović, 1976), Timočka Krajina (Milijić, 1980), north-west Serbia (Stepić, 1984), Mačva (Jelesijević et al. 1975, 1977).

Floristic content of this association, in the region of Posavotamnava contains 73 species (Tab. 1.). Characteristic species of the association are *Consolida regalis* and *Polygonum aviculare.* Both species give characteristic feature of the association, although *Consolida regalis*is in less phytocoenological screenings. The reasons for decreased exuberant and weediness of *Consolida regalis* are due to less agrotechnic level and long lasting using of herbicides in wheat and corn. Presence of characteristic species bonds of *Caucalion lappulae*is less than in other regions because of the same reasons as in association.

Higher sintaxonomic units of *Centauretalia cyani* and *Stellarietea mediae*are presented by the species: *Galium aparine, Avena fatua, Cirsium arvense, Papaver rhoeas, Vicia cracca,Veronica persica, Viola arvensis, Bilderdykia*

convolvulus, Myosotis arvensis. Characteristic composition of this association is made of 7 weed species (degree of continuity IV and V).

Among the tracers with significant weediness and degree of continuity, higher importance than others have: *Ambrosia artemisifolia, Agropyrum repens, Rubus caesius, Mentha longifolija* and *Poatrivialis. Ambrosia artemisifolia* has a significant place according to the results from 1984. (Stepić, 1984) although it was not determined as weed species. In the last 33 years, it spread so much and became a dominant weed species in the region of Posavotamnava, in all the farming crops (wheat, corn, soya etc.).

Biological spectrum of weeds (Tab. 2) clearly shows that this association is of therophyta character with significant participation of bulbs. Activity of therophyta is 54,79%, which is in relation to the same association in other regions of Serbia, significantly of lower value.

According to the data by Milijić (1980) the participation of therophyta in association *Consolido – Polygonetum aviculare* in the region of Timočka krajina is 71,43%, Kosovo 61,33% ((Kojići, Pejčinović, 1982), southeast Srem 69,84%, south Banat 71,40% (Šinžar, 1965), but according to the results by Stepić (1984), the difference between therophyta in this weed society 33 years ago was only 0,63%.The reasons for relatively low therophyta participitation and high bulb participation (28,77%), can be found in weed vegetation changes under the influence of using hormonal and other types of herbicides as well as distribution of association on soil, bad mechanical and pseudogley. High participation of bulbs 28,77% makes their effective control difficult, since they are very stable life forms. This resistant group weeds are: *Cirsium arvense, Vicia cracca, Agropyrum repens L., Rubus caesius and Mentha longifolija.*

Hemicryptophytes cover 16,44%, which is close to participation of this group of weeds in some other parts of Serbia, in association *Consolido - Polygonetum aviculare.* Obtained results regarding ecological (Tab. 3) indexes by Landolt (humidity F, pH value R, nitrogen supplying N, presence of organic material H, soil aeration D, lightening L, habitat thermofilicT, continentality K) are pretty realistic and maintain the characteristics of examined localities.

The basic phytogeografical characteristic of association *Consolido - Polygonetum avicular*eis a presence of flora elements of wide distribution (subeuroasian, Euroasian, cosmopolitan,, circumpolar, subcircumpolar, submiddle-European, middle European) 82,19%. The presence of submediterranean flora elements was also noticed (submediterranean, subpontic-submediterranean, pontic-submediterranean, subpontic-subcentral-submediterranean) covering 8,22%. The character due to the presence of mediterranean flora elements is given because of the following species *Legousia speculum veneris, Lathyrus aphaca, Bromus mollis, Lactucaserriola, Sambucus ebulus* (Tab.4).

Tab. 1 *Association Consolido – Polygonetum aviculare, Kojić*

Flora elemen	Life form	Plant species	Locality																			Cover value.	Degree of cont.	Ecological index							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			F	R	N	H	D	S	L
Characteristic association species *Consolido – Polygonetum aviculare Kojić*																															
Kosm.	T_4	*Polygonum avicular*	+	+	1.1	+	1.1		1.1	1.1	+	1.1	+	+	1.1	1.1	+	+		1.1	1.1		245	V	3_w	3	4	3	5	-	4
Subse.	T_2	*Consolida regalis*	+	+	+	+				+						+.1	+	+.1	1.1	+		1.1	1 17,5	III							
Characteristic bond species *Caucalion lappulae* Tx.																															
Subj.sib.	G_1	*Lathyrus tuberosus*			+	+		+	+							+		+	+	1.1	+		45	III	2_w	4	3	3	5	-	4
Kosm.	T_1	*Capsela bursa past*			1.1	+		+					+			+	+					+	40	II	2	3	4	3	4	-	4
Characteristic order species *Centauretalia cyani* Tx. et Lohm.																															
Evr.	T	*Galium aparine*	1.1	1.1	+	+		+	+	+		+	+	+	+	+	+	1.1	1.1		3.3	+	317,5	V	3	3	5	3	4	-	3
Subevr	T_2	*Avena fatua*		+.1	+		+	1.1	+			3.3	1.1	1.1	1.1	1.1	+	1.1	1.1		1.1		422,	IV	2	4	3	3	3	-	4
Subevr	G_3	*Cirsium arvense*		+	+	+	+	+			+.1	1.1	+		+	+		+		+		+	77,5	IV	3	3	4	3	4	+	3
Subevr	T_2	*Papaver rhoeas*	+	+	+							+			+	+		+	+	+		+	25	III	2	4	3	3	4	-	3
Evr.	G_1	*Vicia cracca*	+			+	+		+			+	+		+		+			+			22,5	III	3	3	3	3	4	-	4
Kosm.	G_3	*Convolvulus arvens*		+	+				+	+		+	+		+								17,5	II	2	4	3	3	4	-	4
Subm.	T_2	*Centaurea cyanus*								+					+	+	+						10	I	2	3	3	3	4	-	4
Subevr	T_2	*Vicia tetrasperma*						+						+						+			7,5	I	3_w	2	2	3	4	-	3
Se.	T_3	*Vicia sativa*																	+			+	5	I	3	4	3	3	4	-	3
Adv.	T_3	*Agrostemma githag*	+																				2,5	I	2	3	3	3	3	-	4

Evr.	T_2	*Matricaria chamom*	+																			2,5	I	3 3 3 3 4 - 4
Subevr	T_2	*Ranunculus arvensi*													+							2,5	I	2 4 3 3 4 - 3
Characteristic class types		***Stellarietea mediae*** **Tx., Lohm**																						
Adv.	T_1	*Veronica persica*			1.+	1.1	1.1	1.1	1.1			1.1	+	+		+	+	+	+	+	+	+	150	IV	3 4 4 3 4 - 4
Evr.	T_3	*Viola arvensis*	+	+	+	1.1	+	+	1.1	1.1	+		+	+.1	+	+					+		125	IV	3 3 3 3 3 - 3
Subevr	T_4	*Bilderdykia convolv*		+.1	+.1	+	+	+	+	+	+		1.1	+		+	2.1		1.1	1.1		+	1 72,	IV	2 3 3 3 4 - 4
Subevr	T_2	*Myosotis arvensis*	+	+	1.1	+						+	+	1.1	+				+				67,5	III	2 3 3 3 4 - 4
Adv.	T_4	*Erigeron canadensi*			+	+				+								+		+			12,5	II	2 3 3 3 4 - 4
Kosm.	T_1	*Stellaria media*	+			1.1			+										+				32,5	I	3 3 4 3 4 - 3
Subse.	T_2	*Anthemis arvensis*										+				+		+				+	10	I	2 2 4 3 4 - 4
Subevr	T_4	*Sonchus oleraceus*	+												+		+	+					10	I	3 4 4 3 4 - 4
Evr.	T_4	*Matricaria inodora*														+	+	+					7,5	I	
Pratilice																									
Adv.	T_4	*Ambrosia artemisifo*	1.1	2.2	2.2	1.1	2.2	2.2	2.2		4.4	1.1	1.1	1.1	1.1	2.2	1.1	2.2	1.1	3.1	1.1	3.3	1 525	V	2 3 4 2 2 + 4
Evr.	G_1	*Agropyrum repens*	1.1	2.2	1.1	1.1	1.1	2.2	+	1.1	+	2.2	1.1	2.3	1.1	1.1	2.3		1.1		1.1		8 92,5	V	3_w 3 4 2 3 + 4
Subj. sib.	G_3	*Rubus caesius*	+		+		+	+	+		1.1	+	+		+	1.1	+		+	+		+	80	IV	4_w 3 4 3 4 - 2
Kosm.	T_4	*Chenopodium albu*		+	+	+	+	+								+.1	+	+				+	45	III	2 3 4 3 4 - 4
Subse.	G_2	*Mentha longifolija*			+	+						+			+	+	+		+	+		+	22,5	III	4_w 4 4 3 5 - 3
Subevr	G_1	*Poa trivialis*				+				+				+		1.1		+		1.1			60	II	3_w 3 4 3 4 - 3
Subm.	T_3	*Legousia speculum*	+				+					+				+	1.1		+	+			40	II	2 4 3 3 4 - 4
Cirk.	H_5	*Artemisia vulgaris*	1.1	+						+					+				+	+			37,5	II	3_w 3 4 3 4 - 4

Subevr	G_3	*Roripa silvestris*		+	+			+							+		+			+			15	II	4_w 3 4 3 5 - 4
Subcir	T_4	*Polygonum lapathif*	+.1	+.1					+														52,5	I	3_w 3 4 3 3 - 5
Evr.	T_2	*Geranium disectum*			1.1		+																27,5	I	3 3 3 3 4 - 4
Cirk.	G_1	*Stachys palustris*								1.1	+												27,5	I	4_w 3 3 4 5 - 3
Adv.	T_4	*Xanthium strumariu*											+					1.1					27,5	I	3 3 5 3 2 +
Evr.	H_5	*Plantago major*	1.1																				25	I	3_w 3 4 3 5 + 4
Subse.	T_1	*Lamium purpureum*				+	+			+									+				10	I	3 4 4 3 4 - 4
Subevr	H_3	*Melandrium album*			+	+	+													+			10	I	
Evr.	T_4	*Polygonum persicar*					+	+	+												+		10	I	3 3 4 3 3 - 4
Subse.	T_4	*Ranunculus sardous*		+				+						+						+			10	I	4_w 3 3 3 5 + 4
Evr.	H	*Achillea millefolium*			+	+											+						7,5	I	2 3 3 3 4 - 4
Evr.	H_3	*Rumex crispus*				+						+					+						7,5	I	3_w 3 4 2 4 + 4
Subse.	H_3	*Rumex obtusifolius*					+			+										+			7,5	I	3 3 4 4 4 - 4
Kosm.	G_1	*Sorghum halepense*											+					+				+	7,5	I	1 2 3 3 3 -
Subcir	T_4	*Atriplex patula*		+												+							5	I	3 4 4 3 3 + 4
Kosm.	G_1	*Cynodon dactylon*												+		+							5	I	2 3 3 3 3 - 4
Subevr	H	*Dactylis glomerata*				+														+			5	I	3 3 4 3 4 - 3
Pont.-sub	T_2	*Lathyrus aphaca*													+					+			5	I	2 3 3 3 4 - 3
Cirk.	G_2	*Mentha arvensis*								+					+								5	I	3_w 3 4 4 5 - 4
Subevr	T_4	*Medicago lupulina*				+				+													5	I	2 4 3 3 4 - 3
Evr.	H_2	*Potentilla reptans*				+											+						5	I	3_w 3 4 3 5 - 4
Subpont. subm.	G	*Sambucus ebulus*							+											+			5	I	3 4 4 3 4 - 3

Evr.	G_3	*Sonchus arvensis*			+														+				5	I	3_w 3 4 4 4 + 3
Subm.	T_2	*Bromus mollis*				+																	2,5	I	3_w 3 4 3 4 - 4
Subevr	T_2	*Bromus secalinus*																	+				2,5	I	3_w 2 3 3 3 - 3
Subevr	H	*Campanela rapunculo*	+																				2,5	I	
Evr.	G_1	*Calystegia sepium*							+														2,5	I	4_w 4 4 3 5 - 3
Subevr	G_2	*Conium maculatum*																		+			2,5	I	3_w 3 4 3 3 - 4
Evr.	T_4	*Chenopodium polys*		+																			2,5	I	3 3 4 3 4 - 4
Subevr	T_4	*Euphorbia heliosco*																	+				2,5	I	3 3 4 3 4 - 4
Evr.	G_1	*Filipendula hexape*						+															2,5	I	2_w 3 2 3 5 - 4
Evr.	H_2	*Glehoma hederace*						+															2,5	I	3 3 3 3 4 - 3
Subevr	G_1	*Lathyrus pratensis*					+																2,5	I	3 3 3 3 4 - 3
Subpont. subca.-sub	T_4	*Lactuca serriola*			+																		2,5	I	2 3 3 2 3 - 4
Subse.	G_3	*Linaria vulgaris*								+													2,5	I	2 3 3 3 4 - 4
Subse.	G	*Ornithogalum umbe*					+																2,5	I	3_w 4 3 3 4 - 4
Kosm.	T_1	*Poa annuua*	+																				2,5	I	3 3 4 3 4 - 4
Kosm.	T_4	*Portulaca oleracea*				+																	2,5	I	3_w 3 4 3 4 - 4
Subevr	H	*Silene inflata*														+							2,5	I	
Subevr	P-H	*Solanum dulcamara*																		+			2,5	I	3_w 3 4 3 5 - 3

1.Mehovine, 2.Lojanice , 3.Matijevac, 4.Draginje, 5.Mali Bošnjak, 6.Koceljeva, 7.Družetić , 8.Koceljeva 2, 9.Brdarica, 10.Krnule, 11.Jalovik, 12.Jalovik 2, 13.Krnić, 14. Jazovnik , 15. Suvo selo, 16. Debrc, 17. Zvezd, 18.Trbušac, 19.Skupljen, 20.Kujavica.

Tab. 2. Biological spectrum Ass. *Consolido – Polygonetum aviculare*

Life form	**Number of plants**	**%**
T	39	54,79
G	22	28,77
H	12	16,44
Total	**73**	**100**

Tab. 3. Average agroecological index values of ass. *Consolido – Polygonetum aviculare*

F	**R**	**N**	**H**	**D**	**S**	**L**	**T**	**K**
186:68 =2,74	216:68=3,18	241:68=3,54	204:68=3,00	268:68=3,94	+ -	250:68=3,68	257:68=3,78	208:68=3,06
2,74	**3,18**	**3,54**	**3,00**	**3,94**		**3,68**	**3,78**	**3,06**

Tab. 4. Review of flora elements and their activity in Ass. *Consolido – Polygonetumaviculare*

Flora elements	Number of plants	%
Subevr.	20	27,40
Evr.	17	23,29
Kosm.	9	12,33
Subse.	8	10,96
Adv.	5	6,85
Subm.	3	4,11
Cirk.	3	4,11
Subcirk.	2	2,73
Subj.-sib.	2	2,73
Subpont – subm.	1	1,37
Se.	1	1,37
Subpont-subca.subm.	1	1,37
Pont. – subm.	1	1,37
Total	73	100

The knowledge of flora weed content in wheat crop, by using modern agrotechnic, correct soil cultivation and using combination of herbicides for the most important field crops, can lead to the acceptable and tolerant level of harm effects of weeds. In our agroecological conditions, it is not possible and rational to think about total weed destroying having in mind very serious far-reaching consequences.

Conclusion

Posavotamnava region, according to floristic researches in 2016/17 has 73 weed species. Biological spectrum of weed flora shows a significant participation oftherophyta (54,79%) followed by considerable presence of perennial weeds, bulbs (28,77%) and hemicryptophytes (16,44%).

In sintaxonomic point of view, weed vegetation of wheat in Posavotamnava region belongs to association *Consolido - Polygonetum aviculare,* bond *Caucalion lappulae,* order *Centauretalia cyani* and class *Stellarietea mediae*.

Concerning ecological conditions, weed flora of Posavotamnava region is adapted to moderate humidity (F), slightly acidic to neutral soil (R), slightly provided with nitrogen (N) and organic materials (H), relatively well soil aeration (D), adaptable for half shadow conditions (L), moderate warm habitats (T) and subcontinental climate (K).

In the region of Posavotamnava, flora elements dominate (Euroasian, subeuroasian, cosmopolitan, circumpolar, submediterranean and middle European) with 82,19%. Participation of submediterranean flora elements is 8, 22%.

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Pomological and phenological characteristic of selected varieties of Asian pears in the climate of the Czech Republic

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Abstract: Experiment with 23 varieties of Asian pears and a European pear variety ´Conference´ as the control was planted in 2012. In 2015 selected traits were evaluated: average fruit weight , average fruit yield, peel firmness, soluble solids content, titratable acidity, and date of the beginning of flowering. A total of 11 varieties had higher average fruit weight than the control variety (reaching an average of 215 g), with the highest weight obtained in ´Jin Hua´ variety (357 g) and the lowest weight in ´Niiseiki´ variety (90 g). All the varieties had lower soluble solids content than the control variety (16.53 %), the second highest content was found in ´Hosui´ variety (16.00 %) and the lowest in ´Ju Li´ variety (11.72 %). The earliest start of flowering was recorded in the varieties ´Jin Hua´, ´Wu Xiang´, ´Ya Li´, ´Ping Guo Li´ and ´Mut Chen´, which started flowering about five days earlier than the control variety, while the latest flowering variety was ´Talgarskaja krasavica´ which started to flower about four days after the control variety. Higher average fruit yield than the control variety (5 kg/tree) was observed in 14 varieties, of which ten varieties had at least two times higher yield than the control variety. Only three varieties had peel firmness values higher than the control variety (3.55 kg/cm^2) and the highest values were obtained in ´Wu Xiang´ variety (4.07 kg/cm^2), while the lowest values were measured in ´Hood´ variety (1.47 kg/cm^2). The percentage of titratable acidity was higher than that of the control variety (0.166 %) in 14 varieties with ´Kieffer´ variety having the highest acidity (0.405 %) and the variety ´Pungsu´ the lowest (0.084 %).

Key words: *Pyrus*, nashi, fruit quality, contained substances, introduction

Introduction

Pears are a significant fruit species in the temperate climate zone, rich both in vitamins and in minerals and especially in fiber. As concerns the total amount harvested crop, pears are the third most common fruit species in the Czech Republic (BUCHTOVÁ, 2014). The cultivation areas for European pears are mainly found in Europe, Northern America, Southern America, Southern Africa and Oceania. Among the most common European pear varieties there are e.g. ´Conference´, ´Abate Fetel´, ´Bosc ´, ´Blanquilla´, ´Williams´ a ´Red Williams´, ´Clapp's Favourite´, ´Anjou´ a ´Red Anjou´ (DECKERS and SCHOOFS, 2002). Most of the cultivation areas for Asian pears are situated in China, where the European pears represent less than 10 % of the production. The most common varieties are ´Suli´, ´Yali´, ´Cuiguan´ a ´Hosui´ (YUANWEN, 2011).

When it comes to the origin of cultural pear varieties there are no records of natural occurrence of pear species in the southern hemisphere, there are only records of their natural occurrence in Europe, Asia and northern Africa (HEDRICK, 1991). In Europe the history of growing pears goes as far back in history as antiquity (JANICK, 2002). The first mention of pears in Europe is found in Homer's Odyssey, where Homer calls pears a „gift of Gods“. The largest expansion of pears in Europe appeared at the time of the highest flourish of the Roman Empire (HEDRICK, 1991). In China, pears have been grown for over 3 000 years and for more than 2 000 years they have been grown in compact growing areas (NEČAS, 2010). Commercial cultivation of pears has been going on in China for hundreds of years (BEUTEL, 1990). One of the most significant milestones in the history of pear cultivation happened in the 9. century in France, when Charlemange was describing pear cultivation among other things in his Capitulaire de villis. Another important milestone was a publication of *Historia Plantarum* by Valerius Cordus in Germany in the first half of 16th century, a book in which Cordus described 50 varieties of pears (HEDRICK, 1991).

In Asia, pear cultivation appeared as early as the prehistoric period, when a group of pear varieties derived mainly from *Pyrus pyrifolia* and *P. ussuriensis* was cultivated, while the European group of pears was bred mainly from *P. communis*. Yet, both pear groups were formed through crossbreeding of many different kinds of pears (Jackson, 2003). Also, for both pear groups more suitable rootstocks are the ones bred from *P. communis* than rootstocks bred from *P. pyrifolia* and *P. ussuriensis* because of their tendency to suffer from certain physiological disorders (JANICK and MOORE, 1996). Both pear groups also differ in their content of substances: according to USDA 100 g of Asian pears contain an average of 88.25 g of water, 7.05 g sugar, 3.6 g fiber and 3.8 mg vitamin C, while the average for European pears is 83.96 g of water,

9.75 g sugar, 3.1 g fiber and 4.3 mg vitamin C. The difference in content of some substances is very significant, e.g. the content of calcium in European pears is twice the amount of the Asian pears and the content of iron in 100 g of European pears is 0.18 mg whereas in the Asian pears it is less than 0.01 mg (USDA, 2015). Both groups can be distinguished from each other by the shape and color of the fruit: in European pears the prevailing shape is pear shape while in Asian pears it is rather spherical. Asian pears have more coarse texture of the flesh and its firmness tells not so much about the ripeness of the fruit as it does in European pears (JANICK and MOORE, 1996).

Material and methodology

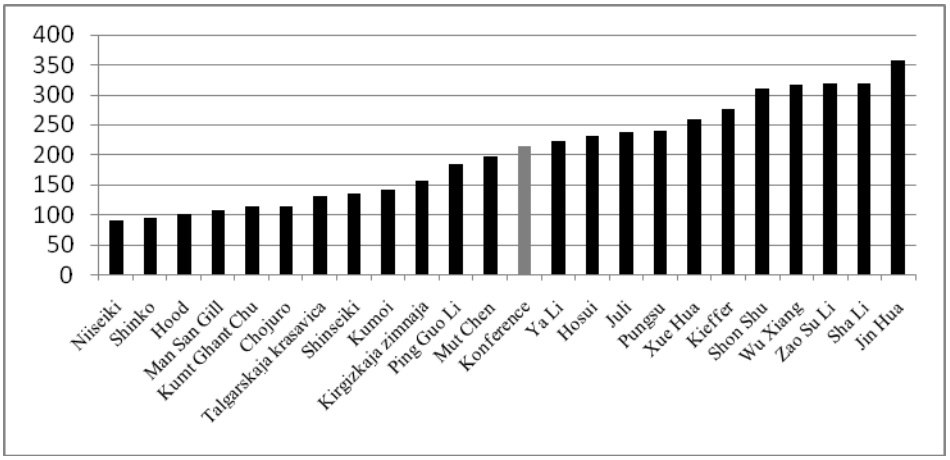
A total of 25 varieties on the pear seedling rootstock (H-TE-1) were used for the experiment, for each variety five plants were planted. The Asian varieties used in the experiment were partly obtained directly from China and partly collected from EU countries and the US. The experiment comprizes a control variety ´Conference´ and tested varieties ´Pung Su´, ´Shinseiki´, ´Man San Gill´, ´Ju Li´, ´Shinko´, ´Ping Guo Li´, ´Xue Hua´, ´Hosui´, ´Zao Su Li´, ´Chojuro´, ´Shon Shu´, ´Mut Chen´, ´Jin Hua´, ´Ya Li´, ´Wu Xiang´, ´Sha Li´, ´Kumt Ghan Chu´, ´Kumoi´, ´Niiseiki´, ´Talgarskaja krasavica´, ´Kirgizkaja zimnaja´, ´Kieffer´, ´Hood´.

In 2015 in the phase of ripeness 15 fruits of each variety was selected randomly and the following measurements were made: peel firmness using a stationary penetrometer Digital fruit firmness tester 53205, soluble solids content using a stationary refractometer AR 4D ABBE by Krüss and fruigt weight using a digital laboratory scale. Titratable acidity was always evaluated using three pieces of fruit, for each variety a fruit with the highest, an average and the lowest soluble solids contant was selected for which the titratable acidity was determined by titration with NaOH. The yield was evaluated visually in five trees for each variety. The beginning of flowering was determined as the date when 10 % of flowers were opened.

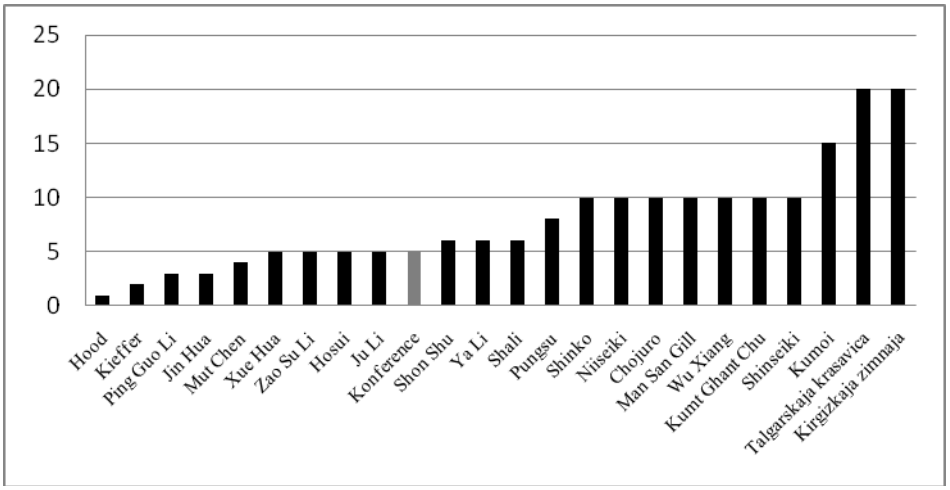
Results and discussion

Higher average fruit weight than in the control variety (215 g) was observed in 11 varieties with the highest weight measured in ´Jin Hua´ variety (357 g), followed by ´Sha Li´ (320 g), ´Zao Su Li´ (319 g), ´Wu Xiang´ (317 g) and ´Shon Shu´ (310 g), and the lowest weight measured in ´Niiseiki´ variety (90 g). In four varieties the fruit weight was below half of that of the control variety. Kappel et al., (1995), stated that an optimum fruit weight for a consumer is 150–250 g. YinSheng et al., (2002), measured the fruit weight for ´Niiseiki´ variety

from 110 g to 230 g, whereas in this experiment the average weight for this variety was 90 g, which could be a result of overproduction of the fruits.



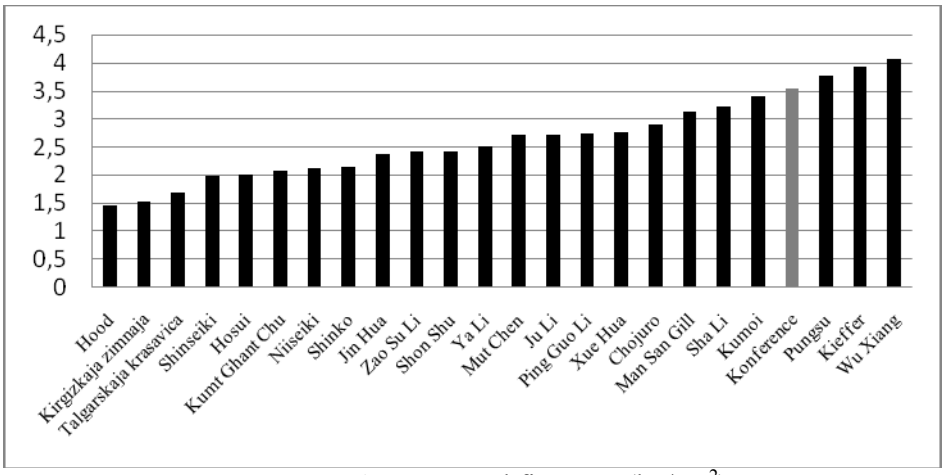
Graph 1. – Average fruit weight (g)



Graph 2. – Average fruit yield (kg/tree)

Higher average fruit yield than the control variety (5 kg/tree) was observed in 14 varieties with ten varieties having minimally twice the yield of the control variety. The highest yield was obtained in ´Talgarskaja krasavica´ and ´Kirgizkaja zimnaja´ varieties (20 kg/tree) and the variety with the lowest yield was ´Hood´ (1 kg/tree). Maas (2008) stated 11.4 kg as the average yield per tree in ´Conference´ variety of the same age grown in the sametraining system as in

presented study. The difference in the measured data may be caused by a combination of high temperatures and the lack of precipitation in the year when the data were recorded.

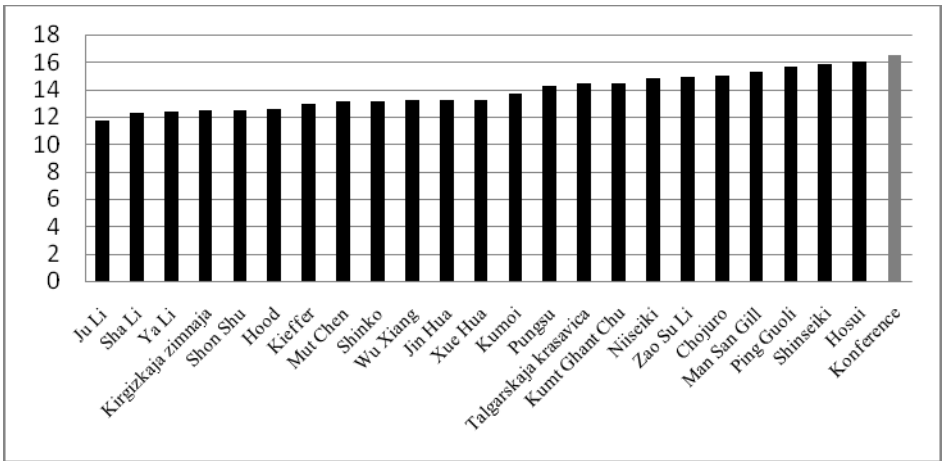


Graph 3. – Average peel firmness (kg/cm^2)

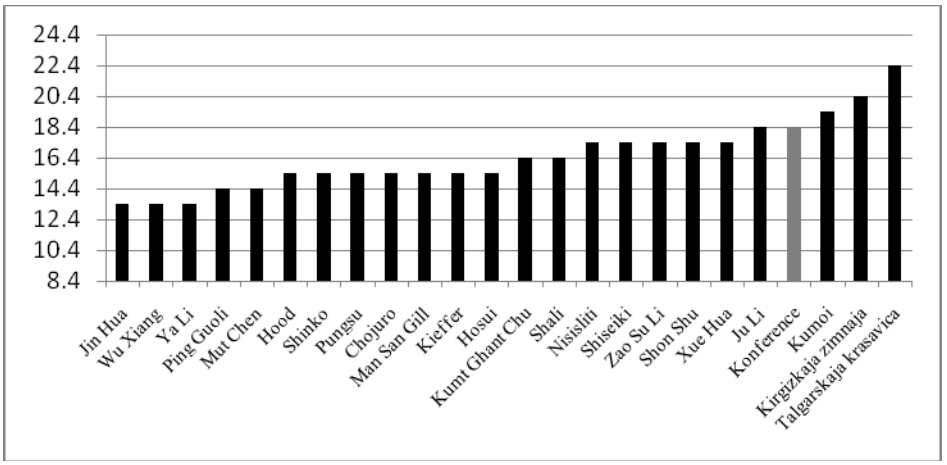
Higher firmness of the peel than in the control variety (3.55 kg/cm^2) was observed in only three varieties. The highest values were recorded in ´Wu Xiang´ variety (4.07 kg/cm^2), followed by ´Kieffer´ variety (3.92 kg/cm^2) and ´Pungsu´ variety (3.77 kg/cm^2). The variety with the lowest value was ´Hood´ (1.47 kg/cm^2). In a similar study by Jurick et al. (2015), the values of peel firmness reached from 1.13 to 2.88 kg/cm^2, with the varieties evaluated in Jurick's and present studies such as ´Hosui´ reaching 2.02 and 1.72 kg/cm^2, respectively. The differences for these varieties may result from a different determination of the harvest maturity. Kappel et al., (1995), states a peel firmness in pears as 1.69 to 3.43 kg/cm^2, which corresponds well with the results of this study.

The soluble solids content was lower in all tested varieties than that of the control variety (16.53 %), with the second highest content found in ´Hosui´ variety (16.00 %) and the lowest content found in ´Ju Li´ variety (11.72 %). According to Jurick et al., (2015), the ´Hosui´ variety had only 12.9 % of the soluble solids. The difference may be caused by different conditions in the year of Jurick's (2013) and presented (2015) studies, which is confirmed by Jurick's data from 2013, where he states the dry matter content for ´Hosui´ as 15.9 %. Hudina et al. (2000), stated that the European pears contained less sugar than the Asian pears, which is in conflict with the results of this study. YinSheng et al.,

(2002), stated the dry matter content in Asian pears being from 11.5 to 15.7 %, which corresponds with the results obtained in this experiment.

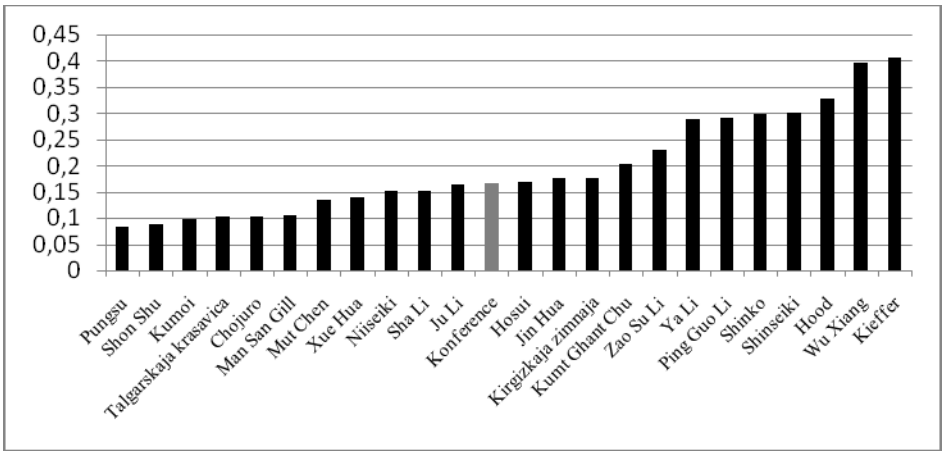


Graph 4 .– Average soluble solids content (%)



Graph 5. – The beginning of flowering

The earliest beginning of flowering was observed in ´Jin Hua´, ´Wu Xiang´, ´Ya Li´, ´Ping Guo Li´ a ´Mut Chen´ varieties, which started flowering as much as five days earlier than the control variety. The latest flowering variety was ´Talgarskaja krasavica´, which started to flower four day after the control variety.



Graph 6.– Average titratable acidity (%)

The titratable acidity was higher in 14 varieties than in the control variety (0.166 %). The varieties with the highest titratable acidity were ´Kieffer´ (0.405 %) and ´Wu Xiang´ (0.397 %) and the lowest titratable acidity was recorded in ´Pungsu´ variety (0.084 %). Jurick et al. (2015), stated the titratable acidity in nine Asian pear varieties from 0.07 to 0.21 % with the varieties evaluated in Jurick's and presented studies, such as ´Hosui´, reaching 0.17 % and 0.16 %, respectively. Although, YinSheng et al. (2002), mentioned similar values for soluble solids content in Asian pears, however the values for the titratable acidity were slightly different to those determined in this experiment, namely from 0.19 % to 0.39 %.

Conclusions

The results show that varieties of Asian pears have great variability in content of substances, fruit weight and date of flowering. In order to determine whether the selected varieties are suitable for cultivation in the Czech Republic, long term observations should be made, which can clarify frost resistance and other properties that could have negative effect on the cultivation. Data from this one year observation shows, that some varieties are more than suitable for the conditions of the Czech Republic.

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Could strongylid nematodes affect the processability of goat milk to cheese?

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Abstract: We evaluated the effect of strongylid nematodes on qualitative parameters of goat milk. In total, 331/334 (99.1 %) samples were positive for strongylids. *Haemonchus contortus*, genera *Trichostrongylus* and *Oesophagostomum columbianum* were the most prevalent strongylids recovered from incubated infective larvae. Prevalence of strongylids observed in our study was high but intensity of infection was mild; faecal egg output differs significantly between individual animals. Effect of strongylids on protein content, fat content and lactose was tested using generalized linear mixed models. Only protein content was affected by number of strongylids at determined intensity of infection. Fat content and lactose were affected seasonally. Results of our study may indicate that mild intensity of infection observed in our study does not significantly affect qualitative parameters of goat milk.

Key words: goat, gastrointestinal tract, disease, milk, nematode

Introduction

Agriculture focused on goat farming is important economic activity in European countries (Kantzoura et al., 2012). Products from goats milk are source of quality proteins. Protein content in milk is one of the main parameters that determine the cost of goat milk (Raynal-Ljutovac et al., 2008). Protein content affects cheese yield, processability and quality of milk (Amenu and Deeth 2007). Casein, the one of two major categories of milk proteins, forms

the structural matrix of cheese which retains moisture and fat (Lucey and Kelly, 1994).

Gastrointestinal nematodes represent a major constraint in goat production. Economic loses due to nematode parasitism can be as high as 20 % (Kantzoura et al. 2012). All grazing goats are infected with strongylid nematodes, low intensity of infection usually have a little impact on animal health. With increasing number of strongylids, signs, such as weight loss, diarrea and anemia may occur. The most important strongylid nematode in many European countries (and around the world) is *Haemonchus contortus* (Zajac, 2006). Presence of this abomasal nematode is attributed to decrease in protein level. Development of adult *H. contortus* may result in increased permeability of the abomasal epithelial lining, which allows leakage of protein into the abomasal lumen (Elsheikha, 2011). Subsequently, protein is directed from productive processes to rebuild damaged tissue (Coop and Holmes, 1996).

The aim of the study was to evaluate the effect of strongylids on qualitative parameters of goat milk. Our study was based on hypothesis that protein content, fat content and lactose content are affected by strongylids.

Material and methods

The study was carried out at a goat farm in the Czech Republic, which is specialized in the production of own organic products. During our study, more than 200 dairy goats (the White Shorthaired goat, a Czech national breed) were present on the farm. Milking was carried out twice a day. Goats were pastured throughout the year, and had no access to the pasture during the winter season (December - March). Lactation on the monitored farm took place from May to October. The farm produced an average daily milk yield of 328 kg (2.03 kg per goat), with an average protein and fat content of 2.87 % and 4.14 % respectively.

Table 1. The evaluation of milk component during lactation

	milk protein				**milk fat**			
month	min. %	max. %	median	SD	min. %	max. %	median	SD
V.	2.23	3.68	2.87	0.35	0.91	6.24	3.58	1.19
VI.	2.54	3.55	2.68	0.27	1.13	7.58	3.36	1.88
VII.	2.37	3.67	2.71	0.31	2.78	8.28	4.48	1.43
VIII.	2.46	4	2.78	0.32	2.39	8.89	4.61	1.66
IX.	2.51	4.47	2.97	0.37	2.0	6.43	3.42	1.12
X.	2.56	4.78	2.88	0.42	2.52	7.66	3.74	1.33

Table 1 shows the evaluation of milk components during lactation. Data regarding milk quality were recorded monthly and taken from alternate morning and evening milking time by the Association of Sheep and Goat Breeders of the Czech Republic.

Sampling procedure and parasitological methods

For the purpose of our study, thirty dairy goats were selected. Faecal samples were collected individually from each animal every month for one year. Faeces were obtained directly from the rectum and stored in labelled plastic bags at 4 °C until examination. For quantifying faecal parasite output, samples were investigated using a modification of McMaster method (Coles, 2003), with analytical sensitivity 50 eggs per gram of faeces. Saturated sodium chloride and glucose with a density of 1.28 $g.cm^{-3}$ was used as a flotation solution. The prevalence was expressed as a percentage of positively tested samples according to Bush et al. (1997). Because morphological identification of the most strongylid eggs to the genus level is virtually impossible, we chose to merge these eggs with the strongylid-type nematode (strongylids) group. To obtain infective larvae, for detailed strongylid identification, faeces were incubated for seven days at 27 °C. Obtained infective nematode larvae were identified to the genus or species level according to Van Wyk and Mayhew (2013). The results of the tests above determined the prevalence, intensity of infection and nematode species parasitizing in goats on a specific farm.

Statistical analysis

The effect of gastrointestinal nematodes on the qualitative parameters of milk was tested by generalized linear mixed models (GLMM). Separate tests for fat content, protein content, and lactose content were used. The statistical significance level was established as $\alpha = 0.05$. Each minimal adequate model was checked using standard statistical diagnostics in the end. All tests were computed using R statistical software, version 3.1.2 (R Development Core Team 2014).

Results and discussion

Goats are commonly infected by many species of endoparasites, which can affect qualitative parameters of milk and ultimately breeding economy. There are not many studies that have monitored effect of endoparasites on the qualitative parameters of goat milk. Moreover, results in recently published studies differ significantly. Intensity of strongylids infection differs depending on country and goat breed. During our study, 331/334 (99.1 %) samples were

positive for strongylids. The intensity of strongylid infection differs significantly between individual animals (Table 2).

Table 2. The evaluation of strongylids eggs recovered from faecal samples during whole study; EPG – eggs per gram, SD – standard deviation, minimal – min. and maximal – max. intensity of infection

	strongylids			
month	min. EPG	max. EPG	median	SD
I.	50	9 900	550	2 063
II.	100	2 700	800	1 073
III.	50	5 100	1 800	1 922
IV.	50	5 900	1 550	1 465
V.	200	6 250	2 175	1 569
VI.	50	4 000	1 600	1 129
VII.	200	5 000	1 400	1 112
VIII.	0	2 450	1 050	627
IX.	50	3 000	625	608
X.	50	2 050	400	463
XI.	0	1 950	400	446
XII.	100	2 650	650	695

Maximal eggs per gram (EPG) levels observed in our study (9,900) were lower than those observed in study of Alberti et al. (2014) and Holm et al. (2014); 10,452 EPG and 14,340 EPG respectively. By comparison, a study of Voight et al. (2016) observed an average EPG 620, and Etter et al. (2000) observed EPG 200 - 250.

The difference in EPG may be influenced by immunity of each individual goat and by breeding management. Local microenvironment on the pasture (temperature, humidity, density and length of the vegetation) affects species composition of parasites present on the pasture as well as their life cycle. These microenvironmental conditions on the pasture are crucial for development and survival of free living stages of the most gastrointestinal nematodes (O'Connor et al., 2006).

When evaluating effect of endoparasitic infections on milk quality, there is important to consider, apart from intensity of infection, parasite species composition. Some endoparasitic species may affect the milk quality more than the others.

Haemonchus contortus, *Trichostrongylus* spp. and *Oesophagostomum columbianum* were the most prevalent species in our study. When comparing the nematode species composition in goat herds with studies of Voight et al. (2016) and Stadalienė et al. (2015), it is evident that *Trichostrongylus* spp., *O. columbianum* and *H. contortus* predominates.

Etter et al. (2000) described decrease in protein and fat content in milk induced by *T. colubriformis*. Rinaldi et al. (2007) confirmed negatively impact of gastrointestinal nematodes on qualitative parameters of milk. In our study, protein content in milk was affected by presence and number of strongylids (ICC = 0.651), fat content (ICC = 0.177) and lactose content (ICC = 0.330) were affected stronger by month (Table 3). Alberti et al. (2014) confirmed that the level of infection affects milk quality. Results of their study indicate significant decrease in protein content caused by high number of gastrointestinal nematodes.

The low protein content in milk may have economic impact on farmers due to reduced cheese yield. Casein, the one of two major milk proteins, plays important role in processing of milk into cheese (Selvaggi et al., 2014).

Table 3. Maximal adequate models for surveyed protein content, fat content and lactose, minimal adequate model are in bold type

	d.f.	**F-value p**	**p-value**
Protein content			
month	5	**6.76**	**<.0001**
Strongylids	1	**5.26**	**0.0180**
month:Strongylids	5	0.7785	0.5677
Fat content			
month	5	**3.03**	**0.0062**
Strongylids	1	0.1472	0.7021
month:Strongylids	5	1.49	0.1732
Lactose			
month	5	**5.664**	**0.0001**
Strongylids	1	1.189	0.2783
month:Strongylids	5	1.350	0.2502

In conclusion, the prevalence of strongylids was high in our study but intensity of infection was mild. Variability in EPG was high between individual animals. From qualitative parameters of milk, only protein content was affected by number of strongylids at detected infection level. Due to recently increasing number of goats farmed in the Czech Republic and higher demand for goat products, it is necessary to solve the control of strongylids, because parasitosis is among one of the most important factors limiting goats breeding.

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Effect of genotype and environment on stem height in cultivars of wheat (*Triticum aestivum* L.)

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Abstract: Plant height is component significantly associated to yield potential. Height of wheat plant is controlled genetically under influence of environmental factors as well interaction of genotype/environment. The aim of this work was study of variability and components of variance for plant height in four wheat cultivars grown under different dose of mineral nutrition (control N_0=0, N_1=20, N_2=40 and N_3=80kg ha^{-1}). The experiment conducted in randomized block design in four replications on experimental field. The height of stem of 80 plants (20 plants per replication) were used for measurement at the full stage of maturity. The average values of stem height were significantly different among the wheat genotypes and variant of nutrition. The G-3617 had the highest stem height on all variant of nutrition, while the G-3625 had the lowest height of stem. The highest value of stem height had in average for all genotypes were found on N3 variant of nutrition. Phenotypic analysis of variance indicated that environmental factor had a larger impact of the total phenotypic variability for height of wheat plant stem (82.31%) than influence of genetic factors on expressing of analysed height of stem of wheat plant.

Key words: wheat, genotype, stem height, variability, phenotypic variance

Introduction

Plant height is an important trait related to other components of economic and biological yield. Height of stem plant of wheat is genetically controlled and variate under influence of environmental factors and interaction of genotype and environment (Zečević et al., 2008). Numerous major dwarfing genes -named as reduce heigh (*Rht*) in wheat were determined that have influence to plant height (Pestsova and Röder, 2002). The varieties possessing *Rht-B1b*, *Rht-D1b* had a

significantly shorter stem height than the varieties without *Rht* genes (Liatukas and Ruzgas, 2011). The introduction of dwarfing genes – reductors of height (*Rht*) of plant contributed to increasing of production of wheat at reduced costs (Fick and Qualset, 1973; Peng et al., 2003). The source of dwarfing gene were cultivar Norin 10, Saitama 27 which used in crosses in wheat breeding. Mostly prevail semi-dwarf wheat genotypes in the world due to intensive use genes *Rht-D1* and *Rht-B1* which shorten plant height in breeding program (Worland et al., 2001; Knopf et al., 2008). *Rht-D1* and *Rht-B1* genes are associated with susceptibility to *Fusarium* head blight (Miedaner and Voss, 2008) and lower competitivenes with weeds (Bertholdsson, 2005). Wheat stem height has very significant relationship to lodging stability. Harvest index increases at semi-dwarf wheat cultivars (Knezevic et al., 2008; Knezevic et al., 2015), spike length and productive tillering (Branković et al., 2015). Polygenes with quantitative influence to stem height of wheat identified on all 21 chromosomes (Korzun et al., 1998; Liu et al., 2006; Quarrie et al., 2006). In earlier genetic study of produced progeny of majority crosses of wheat were found that additive gene effects play the major components of variation, but in some crosses epistasis was the primary source of genetic variation of plant height (Wade et al., 2002; Zečević et al., 2005; McCartney et al., 2005). This indicate that different genes control height of plant at different phase of plant ontogeny and that interallelic interaction as well genes and environmental factor interaction have influence on variation of plant height (Ellis et al., 2004; Wang et al., 2010). However, environmental factors have influence to expression of stem height. The application of pesticides can influence to decrease stem (Božinović et al., 1998), while nitrogen nutrition influence to photosyntesis and production of organic matter and increasing of grain yield (Abid et al., 2016; Knežević et al., 2016).

The study of variability and components of phenotypic variance for plant height, give information to breeders about genetic and environmental influence on stem height heritability and can contribute to efficient selection of parent in breeding program.

The aim of this work was study of variability and components of variance for stem plant height in four wheat cultivars grown under different amount of nitrogen nutrition.

Materials and methods

The four winter wheat selected lines (G-3052, G-33625, G-3004 and G-3617) were tested in experiment was performed in randomized block design on $5m^2$ plots and 4 repetitions under different rate of mineral nutrition (control N_0=0, N_1=20, N_2=40 and N_3=80kg ha^{-1}). The stem height of wheat in full stage of maturity of 80 plants (20 plants per replication) were used for analysis.

Measuring stem height conducted as the shortest distance between root and base of ear. The analysis of variance was calculated according to randomize complete block design with two factors: A (genotype), B (N-dose), using ANOVA (MSTAT-C program, 1989). The significant differences among the means were grouped according to least significant difference (LSD). The analysis of variance was performed according to a random block system with two factors, allowing the calculation of the components of variance (σ^2_g-genetic, σ^2_{gl}-interaction; σ^2_E-environment; σ^2_f-phenotypic), Falconer (1981).

Climatic conditions during growing seasons

The average values of temperature and precipitation in the year of experiment in comparison with ten years long period were different (table 1). Average temperatures were similar during (8.3^{o}C) the year of experiment were not significantly different compared to average temperature of long-term period (8.5 ^{o}C).

Total amount of precipitation in year of investigation (533.7mm) was significantly higher than average sum of precipitation during ten years long period (417.8mm). The distribution and precipitation based on the average monthly values for the period of performance of the experiment for a number of months (October to February) was similar to the average amount for a ten-years period. In the year of experimental research in the period (January-June) of the phase of tillering to full maturity average rainfall was higher and more favorable for plant developmental phase, in comparison with the values of the average monthly rainfall during a period of ten years (table 1).

Table 1. Monthly mean temperatures and monthly and cumulative precipitation

Tem& Precpt	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Xm	Total
^{0}C	2005/06	11.5	5.6	3.3	-1.7	1.5	5.5	12.7	16.4	19.7	8.3	74.4
1990/2000		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.5	76.7
mm	2005/06	49.0	54.8	47.1	27.9	38.1	116	86.3	29.6	84.8	59.3	533.7
1990/2000		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	417.8

Results and discussion

The variation in stem height from 60.9cm to 87.6cm was found in analysis of wheat plants in four genotypes on each variant of nutrition (table 2).

In average for all variant of nutrition of wheat genotypes the stem height were different. The highest average stem height had G-3617 (79.0cm) and the lowest G-3625 (70.7cm) in average for all variant of nutrition.

The smallest stem height (60.9cm) was found on control variant for genotype G-3625. The same genotype had the smallest values of stem height on all dose of applied nitrogen in comparison to other studied genotypes, while G-3617 had the highest values of stem height (70.5cm) on control variant as well on each dose of applied nitrogen in comparison to other studied genotypes (table 2).

The variability of stem height of wheat depends on investigated genotypes and variant of applied nitrogen nutrition. There were very significant differences among the analyzed genotypes and average values of stem height. Significant differences between dose of nitrogen indicated response divergent genotypes (table 2).

The stem height expressed on control variant (without nutrition) was the lowest at all analysed genotypes in average (65.28cm) while on remain variant of applied dose of nitrogen average stem height value were successfully higher which followed the increasing of dose of nutrition. The highest stem height were found for on N_3 variant of nutrition with 80kg ha^{-1} N, in average for all genotypes (83.58cm) table 2.

This indicates that the effect of nitrogen on the investigated characteristic depends on applied N doses. Also, all wheat genotypes in experimental year condition and in average expressed higher values of stem height in dependence of increasing rate of N application. The values of analysed genotypes differ between between nutrition variant, which indicates that the weather conditions in the vegetation period were favourable and enabled more efficient nitrogen exploitation from soil, as well that the N_3 (80kg ha^{-1}) had the most positive effect.

Table 2. Average values of height of stem of winter wheat genotypes

Genotypes	N_0	N_1	N_2	N_3	Mean
G-3052	63.7i	70.6g	76.3ef	82.7bc	73.3d
G-3625	60.9j	66.8h	74.9f	80.2d	70.7c
G-3004	66.0h	70.8g	76.9e	83.8b	74.4b
G-3617	70.5j	76.2hi	81.9ef	87.6cd	79.0c
Average value	65.28	71.1	77.9	83.58	74.35

The sensitivity of stem height under environmental variation noticed (Gorjanović and Kraljevic-Balalic, 2004; Zečević et al., 2008).

The analysis of variance reveals significant differences in stem height among genotypes and N application rates in year of experiment (Tab. 2 and 3). Analysis of variance showed highly significant differences among genotypes (G) for stem height. Differences between investigated N dose (D) and analyzed interactions (G x D) were also high significant for those stem height of wheat genotypes.

The analysis of phenotypic variance established significant differences in the average value of stem height in the genotypes and dose of nutrition (Table 3).

The highest percentage of the whole phenotypic variability, was assigned to nitrogen dose (82.21%) and lower impact of genetic factor (15.79%) interaction of genotype/dose of nitrogen (0.27%).

Table 3. Components of phenotypic variance for plant height in wheat

Source of variance	Degree of freedom (DF)	Mean square (MS)	F-test	LSD		Components of variance	
				0.05	0.01	σ^2	%
Repetitions (R)	3	3.379	2.579^{ns}	-	-	-	-
Genotypes (G)	3	194.122	148.024**	1.288	2.364	12.00	15.79
N-Doses (D)	3	1001.130	763.396**	1.288	2.364	62.438	82.21
Interaction(GxD)	9	2.125	1.620^{ns}	1.832	2.631	0.204	0.27
Error	45	1.311	-	-	-	1.311	1.73
Total	63		-	-	-	75.953	100.00

In the earlier investigation of stem height heritability were established different contribution of genetic and environmental factors as well their interaction. So, Zečević et al. (2008) reported that the highest effect on plant height expression had genetic factors (66.16%), but lower impact belonged to environmental factors (26.75%), and only 3.31% belonged to genetic/environment interaction. Similarly, the highest impact of genetic factor presented in their previous study (Dimitrijevic et al., 2001; Zečević et al., 2004;) while significant impact of interaction genotype/environment were reported in study Petrović et al (2000).

Conclusions

On the base of obtained results, the varying of stem height of wheat genotypes were influenced by mineral N nutrition. Among the applied dose of mineral fertilizers, the variants of application of N_3 (80 kg ha^{-1}) led to the highest values of stem height of winter wheat plant in comparison with other variants of mineral nutrition. The highest value of stem height in genotypes G-3617 (79.0cm) was found. Nitrogen application had significant effect on stem height. By analysis of variance, it was established that stem height significantly depended to genotypes and increasing dose of N.

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Inheritance of resistance to *Plum pox virus* in apricot progeny from crossing of ´Harlayne´ x ´Betinka´

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Abstract: Inheritance of resistance to *Plum pox virus* (PPV, Sharka) is studied in 201 apricot hybrids resulting from a controlled pollination between the immune cultivar ´Harlayne´ and the resistant cultivar ´Betinka´. Each hybrid was inoculated at the base, with a bud from a plum tree infected with PPV (D and Rec strains), by a chip-budding method. Five symptom observations per year were made on the leaves of the hybrids during four growth periods. Leaves of hybrids were used to confirm the presence or absence of the virus by an enzyme-linked immunosorbent assay (ELISA) method. On the basis of these observations the hybrids were assigned to one of six groups according to evaluation of their response to PPV infection: immune, resistant, medium resistant, tolerant, susceptible, and very susceptible. About 75 % of the F1 hybrids were resistant and 15 % were tolerant. This is probably caused by two dominant loci with epistatic interaction of resistance to PPV.

Key words: PPV, sharka, stone fruit, symptoms, virus disease.

Introduction

The *Plum pox virus* (PPV), also called Sharka, belongs to the most harmful group of plant viruses, to the family of *Potyviridae*, genus *Potyvirus*. This group includes over 400 virus species, which is half of all known plant viruses (Polák *et al*. 2010). Severity of Sharka disease depends on the virus strain and on the susceptibility of the host variety. All European varieties are susceptible to the virus, yet there are differences in symptom manifestation, such as the period between infection and symptom apperance, symptom intensity and the way of

virus transmission (Bellanger, Audergon, 2006). The virus is transmitted by vegetative propagation, non-persistently by aphids and also mechanically – by plant sap, but it is not transmitted by pollen or seeds (Polák *et al.* 2010). Extent and fluctuation of the infection frequency depends on the pressure of the pathogen from the environment. The highest infection pressure was observed in years, when the growing season started early due to high temperatures. Susceptibility of plum trees to PPV infection also depended very much on the age of the tree. The highest infection rate was found in three years old trees (Navrátil, 2006). Short time protection measures against the virus include removing of infected trees and growing certified plant material free of PPV. Chemical protection against insect vectors of the virus is not efficient due to non-persistent transmission of PPV. That is why the only efficient solution is breeding and cultivation of new resistant varieties (Salava, Polák, 2014). Searching for new sources of PPV resistance and breeding new resistant genotypes of the genus *Prunus* are the two most important objectives of the breeding programs in Europe (Rubio *et al*. 2003). These programs are using different donors of resistance and tolerance. Former studies dealing with resistance against PPV in apricots presented different hypotheses which suggested that the resistance is controlled either by one (Dicenta *et al.* 2000), two (Moustafa *et al.* 2001) or three genes (Guillet-Bellanger and Audergon 2001, Salava *et al*. 2005, Pilařová, Krška, 2009).

Materials and methods

Evaluated progeny with the working title H998 which was produced by crossing a highly resistant to immune variety ´Harlayne´, bred in Canada, with a resistant variety ´Betinka´, originating from crossing of ´Vestar´ x ´SEO´ varieties carried out in 1984 at the Faculty of Horticulture in Lednice.

The hybrid progeny was cultivated in insect proof conditions. Each genotype was planted in three repetitions. Inoculation of PPV-D and PPV-Rec. strains was done by infected buds using the chip-budding method. Each year intensity of symptoms was evaluated five times during the vegetation period with the use of a modified four-point scale by Polák and Salava (2010, 2008) (Tab. 1):

Based on the existence and extent of the symptoms on leaves and subsequent verification of the presence of PPV in leaves by ELISA test, the evaluated progeny was divided into groups according to their resistance to Sharka using a modified methodology of Polák and Salava (2010) (Tab. 2).

To determine the segregation ratio, a summary of data collected from the classification of the hybrids in years 2013-2016 was used. Chi-square analysis of the F1 PPV-inoculated progeny was performed to find the best fit for segregation of the resistance trait (Fisher, 1970). Calculated segregation ratios were compared with the expected segregation ratios according to Mendel's laws

at the level of significance of $\alpha = 0.95$. For this analysis plants from Resistant and Medium resistant categories (Tab. 3) were linked together and considered as resistant (Tab. 5) and categories Susceptible and Very susceptible (Tab. 3) were linked together as well and considered as susceptible (Tab. 5).

Table 1. Scale for evaluation of intensity of the PPV symptoms on leaves

Point	Intensity of symptoms
0	**No symptoms**
1	**Symptoms of weak intensity** (Usually appears only on one or few leaves as lighter pale zones around the veins or as lackluster weak flecks and rings) – very weak diffuse patches of approx. 1–5 leaves.
2	**Symptoms of moderate intensity** Easily visible spots or rings on two or more leaves, covering approx. 50 % of the total leaf area.
3	**Symptoms of strong intensity** Light spots covering most of the leaf blade, very strong diffusion rings and spots on most leaves, leading to leaf deformation.

Table 2. Evaluation criteria of resistance to and susceptibility to *Plum pox virus*

	Classification	Intensity of symptoms on leaves, ELISA test, RT-PCR test
0	Immune	No symptoms on leaves, negative results of ELISA tests, negative results of RT- PCR tests and with negative results of verification on re-inoculated GF 305.
1	Resistant *	No symptoms on leaves, negative results of ELISA tests, positive and negative results of RT - PCR tests and with positive results of verification on re-inoculated GF 305.
2	Medium* resistant	Very mild or no symptoms on leaves (max. intensity 1) - overall average of symptoms below 0.5 and majority of negative ELISA test results or with the max. of 50% positive results.
3	Tolerant	Intensity of symptoms 1 and 2 - overall average of symptoms 0.6 – 1.4 or with positive ELISA test results - less negative tests, majority of positive tests of plants in a genotype.
4	Susceptible	Intensity of symptoms on leaves 1 to 3 - overall average of symptoms 1.5 – 2 or with positive ELISA test results - majority of positive tests over negative tests of plants in a genotype.
5	Very susceptible	Intensity of symptoms on leaves 2 to 3 - overall average of symptoms above 2 or with positive ELISA test results - majority of positive tests over negative tests of plants in a genotype.

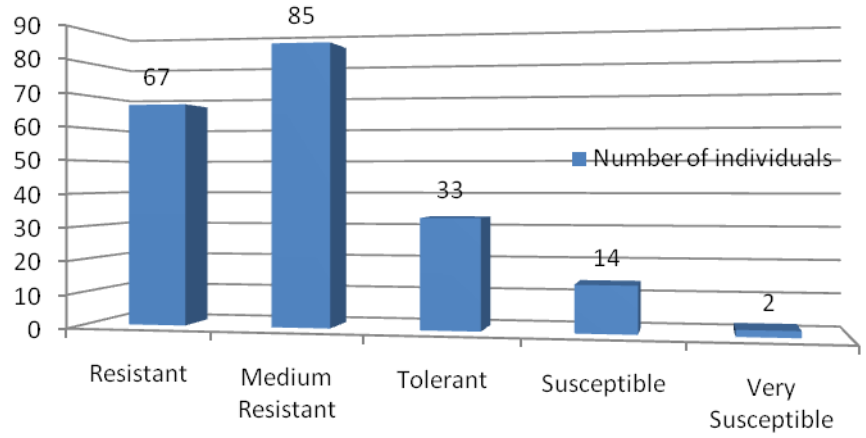
* Resistant (with an phenomenon of weakening of the symptoms and the presence of the virus – "silencing")

Results and Discussion

Based of four years (2013-2016) of observing visual symptoms and PPV detection by ELISA test the individual hybrids were classified into one of six groups (Tab. 2; Tab. 5) (Graph 1)

Table 3. Number of ´Harlayne´ x ´Betinka´progeny genotypes belonging to a specific group according to their resistance to PPV (a summary for the observation period 2013–2016).

H998 hybrids	Resistant	Medium resistant	Tolerant	Susceptible	Very susceptible	Total
Number of individuals	67	85	33	14	2	201



Graph 1. Results of the testing of hybrids in the 2013–2016

Table 4. Chi-square analysis of the evaluated progeny (´Harlayne´ x ´Betinka´)

Case	**Observed vs. Expected frequencies** (statistics 2013–2016) chi-square. = 1.536136 df = 2 p = 0.463909			
	observ.	**expect.**	**P - O**	**(P-O)^2/O**
R	152.000	150.750	1.250	0.010
T	33.000	37.690	-4.690	0.584
S	16.000	12.560	3.440	0.942
Sum	201.000	201.000	0.000	1.536

R = Resistant; T = Tolerant; S = Susceptible

Table 5. Segregation of resistance and susceptibility in apricot progeny from cross of an immune and a medium resistant variety (´Harlayne´ x ´Betinka´) after inoculation by PPV.

Progeny	Number of individuals				Expected ratio	χ^2	Probability
	R	**T**	**S**	**Total**			
´Harlayne´ x ´Betinka´	152	33	16	201	12:3:1	1.536	0.464

R = Resistant; T = Tolerant; S = Susceptible

The observed segregation ratio for the progeny of ´Harlayne´ x ´Betinka´(χ^2 = 1.536 ; P = 46.4 %) was not significantly different from the predicted 12:3:1 segregation ratio (Table 4;5). In data collected between 2013-2015 (3 years of observation) the evaluation of this progeny resulted in segregation ratio $\chi 2$ = 2.551 (P = 27.9%), which was not significantly different from the predicted 12:3:1 segregation ratio as well (Krška *et al.* 2017 *in* press). However, the results presented in this study from 4 years of observation are more accurate than the results from 3 years of observation of the same progeny.

Dicenta *et al.,* 2000, evaluated 291 seedlings from 20 different intentional crosses. As a source of resistance varieties ´Goldrich´, ´SEO´, ´Avilare´, and ´Lito´ were used. They found that the observed segregation ratios were close to the theoretical ratios which correspond to segregation of a monogenically inherited trait, where the resistance trait is dominant and the resistant parent is heterozygous for this trait. Apart from this, in Spain, Moustafa *et al*. (2001) were evaluating crossing of North American cultivars resistant to PPV (´Goldrich´, ´Harcot´,´Stark Early Orange´) and native cultivars susceptible to the virus. Seedlings from these crosses resulted in a segregation of 3:1 susceptible/resistant to the PPV. This segregation corresponded with the hypothesis of two independent dominant loci. The donors of resistance used would be heterozygous for both loci. Only those seedlings heterozygous for both loci, as the parental donors, would be resistant (Moustafa *et al*., 2001). Based on the study of progeny of ´Bergeron´ x ´Stark Early Orange´ French breeders suggest that the resistance to PPV of the cultivar ´Stark Early Orange´ is dominant and controlled by at least three genes (Guillet-Bellanger and Audergon, 2001). The ratios of resistant to susceptible plants in the progeny derived from the four apricot crosses ´Harlayne´ x ´Vestar´; ´Harlayne´ x ´Strepet´; ´Strepet´ x ´Harlayne´; Orangered x ´Harlayne´ are compatible with the hypothesis of three dominant genes being responsible for PPV resistance, with ´Harlayne´ being heterozygous for all three genes (Pilařová, Krška, 2009). Chi-square analysis of each progeny (three cross between cultivar ´Stark Early Orange´ (´SEO´) and selections LE-3241, LE-3246 (both ´Vestar´ × ´SEO´) and selection LE-3218) was performed to determine, whether their segregation ratio would

differ from the expected ratio. PPV resistance segregated in three apricot progenies in a 1:7 (resistant:susceptible) ratio, indicating that resistance was controlled by three independent dominant complementary genes. All the three dominant genes were needed for the resistance to be expressed and a lack of any one of the dominant alleles would result in susceptibility. This knowledge would help in effective planning of apricot breeding programs with this subjective (Salava *et al*. 2005). Soriano *et al.* (2008) found that several loci may be involved in the control of resistance; a two gene model would correspond with a double recessive epistasis in which the dominant alleles of both genes are necessary to provide resistance.

Conclusion

Findings of this study suggest that PPV resistance in apricot is controlled by two independent dominant genes with epistatic interaction, where the resistance is probably a dominant trait and the resistant parents (´Harlayne´ and ´Betinka´) are probably heterozygous for both loci. Tolerant plants are heterozygous for the hypostatic (masked) locus. Susceptible plants are homozygous recessive for both loci. The possible use of the ´Harlayne´ cultivar in apricot breeding programs to obtain resistant cultivars will be further discussed.

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Trypsin inhibitors content in four different cereal/ pseudocereal and soybean grains grown under conventional and organic conditions

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Abstract: Grains, especially maize, wheat and soybeans, are the most common types of crops that are growing in Serbia, while, recently, buckwheat has become an increasingly significant culture, due to the many benefits to human health. Also, an increasing interest for organic form of growing of crops among the consumers has been recorded. In addition to rich, the favorable chemical composition from the nutritional point of view, all types of grains contain anti nutritionally important components such as trypsin inhibitors. The aim of this work was to make a a comparative analysis of the amount of trypsin inhibitors, expressed via the trypsin inhibitors activity (TIA), that are presented in seeds of maize, wheat spelt, buckwheat and soybean produced in an organic and a conventional system during two seasons- 2015 and 2016. Organic grown maize seed from 2015 had a smaller amount of trypsin inhibitors (0.765 TIA / mg) in comparison to the same variety of organic and conventional seed from 2016. Organic grown spelt seed (2015) had the lowest amount of inhibitors (0.3493 TIA / mg), while the organic Soybean varieties from 2016 had the greatest amount of trypsin inhibitors (1335.7 TIA / mg) in comparison to all the other seeds types.

Key words: maize, spelt, soybean, buckwheat, trypsin inhibitors activity

Introduction

According to annual production (8-10 million tons) of grains Serbia is one of the leading countries in Europe. Wheat, maize and soybean are mostly

cultivated. Maize is traditionally the most cultivated crop in our area, which in production volumes significantly exceed wheat. According to a global annual consumption of maize (600-700kg per capita) Serbia is one of the leading countries of the world (Radosavljevic et al., 2002). Recently, due to their beneffits for human health, production of organic cultivated and alternative cereal (spelt) and pseudocereal (buckwheat) has started to increase. Growing of common buckwheat (*Fagopyrum esculentum* Moench) makes more than 90% of world production whereas tartar or bitter buckwheat (*Fagopyrum tataricum* Gaertn) is far less cultivated (Krkoškova et al., 2005). Common features of all cereal grains are: a) high content of nitrogen-free extractable substances (60-80%), mainly starch, b) low proteins content (7-15%) with a deficit of the essential amino acids lysine and tryptophan, c) low oil content (1.5-7%), which are mainly located in cells, d) relatively low crude fiber content (2-6%), e) low calcium and high magnesium, potassium and phosphorus content, f) sufficient content of microelements, g) high content of vitamin B and satisfying vitamin E content, h) very low contents of vitamins C and D and i) high digestibility of cereal grain (Žilić et al., 2007). In addition to nutrients, in soybean grain (as with all other legume) high content of some antinutritive bioactive components can be found, esspecially group of protein molecules commonly named trypsin inhibitors (TI). Two most important TI types are Kunitz and Bowman-Birk TI (Barać et al., 2015). The plant synthesizes them primarily as part of their defense system. In human body they block the action of proteolytic enzymes which can lead to increased pancreas secretion and then its hypertrophy. There are two opposing opinions about TI - while nutritionists considered them as undesirable components of food (by influencing digestion of certain proteins), pharmacologists emphasize their anti-cancer effects (Kennedy, 1998; Clemente et al., 2013).

The aim of this study was to examine the content of trypsin inhibitors-expressed as a trypsin inhibitors activity (TIA) in maize, wheat, spelt, soybean and buckwheat samples produced during 2015 and 2016, in two different production systems: conventional and organic .

Material and methods

Trypsin inhibitors content was investigated and determined in ten selected samples of different cereals, pseudocereals and soybean (Table 1).

Samples extraction procedure

Dried and milled samples of soybean and buckwheat (0.1g) and maize and spelt (1g) were extracted with 25cm^3 of distilled water during 30 minutes at room temperature. All samples were intensively shaken during extraction. After

that samples were centrifuged and supernatants were separated from remained solid residues 5 cm^3 of supernatants were mixed with 5 cm^3 of TRIS-HCl-$CaCl_2$ buffer solutions which was previously prepared. The mixture was thoroughly shaken 2 minutes by Vortex aparatus. Final solutions are prepared by mixing of 0.1 cm^3 of buckwheat and soybean samples i.e. 1 cm^3 of maize and spelt samples, with distilled water until final volume of samples (5 cm^3) was set (Pešić et al., 2017).

Table 1. List of samples with growing conditions and locations

Sample label	Growing plant	Plant Hybrid	Growing conditions/year	Locations
OK-15	Maize	Rumenka	Organic / 2015	Maize Research Institute "Zemun Polje"
OK-16	Maize	Rumenka	Organic / 2016	Maize Research Institute "Zemun Polje"
KK-16	Maize	Rumenka	Conventional / 2016	Maize Research Institute "Zemun Polje"
OSo-16	Soybean	Kaća	Organic / 2016	Institute of Field and Vegetable Crops, Novi Sad
KSo-16	Soybean	Kaća	Conventional / 2016	Institute of Field and Vegetable Crops, Novi Sad
OS-15	Spelt	Nirvana	Organic / 2015	Maize Research Institute "Zemun Polje"
OS-16	Spelt	Nirvana	Organic / 2016	Maize Research Institute "Zemun Polje"
KS-16	Spelt	Nirvana	Conventional / 2016	Experimental Field in Nova Varoš
OH-16	Buckwheat	Novosadska	Organic / 2016	Experimental Field in Nova Varoš
KH-16	Buckwheat	Novosadska	Conventional / 2016	Experimental Field in Nova Varoš

Determination of TIA

Blank: 2 cm^3 of BAPNA solution was mixed with 1 cm^3 of distiled water and 0.5 cm^3 of trypsin solution.

Samples: 1 cm^3 of final solutions of samples extracts were transferred to a test tube. After that 2 cm^3 of BAPNA solution and 0.5 cm^3 of trypsin solution were added in tubes. They were covered with metal caps and placed in water

bath at 37°C. After 10 minutes enzymatic reaction was stopped with adding of 0.5 cm^3 of 30% acetic acid. Finally, samples were intensively shaken and imediately absorption of yellowish solution was read at 410nm on spectrophotometer. TIA was calculated according to following equation:

$$\mathbf{TIA = (A_b - A_s)*100/m_{dry\ weight(mg)}}$$

where A_b and A_s are apsorbances of blank and samples, respectively and $m_{dry\ weight}$ is mass of dry samples expressed in mg.

Results and discussion

Biosynthesis of protease inhibitors is one of the most important plant defense mechanism against pathogens, predators and infections. Inhibitors content in plant material can be signifficant especially for those plants that belong to Graminaceae, Leguminoseae and Solanaceae families (Nakahata et al., 2011). The nutritional value of the raw soybeans cannot be adequately exploited, due to the presence of several anti-nutritional factors. Among them, the presence of trypsin inhibitors is one of the most important either because of effects that elicit or due to its significant total amount (6% of the total protein) (Radosavljević et al., 2002). This type of inhibitors can be divided in several families and subfamilies but two most important families (represent more than 80% of inhibitors activity in plants) are Kunitz trypsin inhibitors (KI) and Bowman-Birck trypsin inhibitors (BBI). KI inhibits trypsin enzime activity and reduces food intake by diminishing their digestion and absorption. Also, it causes the induction of enzymes of the pancreas, hyper secretion and the fast stimulation of pancreas growth, hypertrophy and hyperplasia (Mikić et al., 2009).

According to obtained results (Figure 1) seed of organic grown soybean possessed the highest content of TIA (1335.7 TIA / mg) among the all investigated samples. There was significant difference in TIA content between soybean cultivated under organic and conventional conditions- 1335.7 and 220.18 TIA/mg, respectively. A larger amount of TIA (428.57 TIA / mg), was detected in seeds of organic grown buckwheat (OH-16) compared with a conventional (357.14 TIA / mg). Organic maize seeds from 2015 had the least amount of trypsin inhibitors (0.765 TIA / mg) in comparison with seeds produced in 2016 - conventionally manufactured seed (1.504 TIA / mg) and organic (1.293 TIA / mg). Organic- 2015 spelt seed possessed the lowest content of TIA (0.3493 TIA / mg) compared to all other seeds. Similar contents of trypsin inhibitors of conventional (1.3825 TIA / mg) and organic (1.3715 TIA / mg) spelt from 2016 can be observed. It can be concluded that quantity of this type of enzimes inhibitors can be significantly different among the plant species. Also, year and growing conditions as well as geographical position can affected on divergent TIA content in seed of one plant species. Obtained results are in

agreement with results of other authors (Vollmann et al., 2003; Srebrić et al., 2010; Perić et al., 2009; Kostekli and Karakaya, 2017).

According to the results of the examination of the thermo labile antinutritional factors, Žilić et al. (2007) indicating that the urease and TI activity was higher in soybean (32.33 mg / g) than in the case of field pea grain (2.81 mg / g). Thermal treatment of grains causes degradation and partial (soybean) or total (pea) deactivation of inhibitors.

With the help of program of hybrids selection two different types of soybean without TI were produced in Maize Research Institute from Zemun Polje. Lana and Laura sort possessed TI content of 15.01 mg / g i.e. 15.35 mg / g respectively, which is about 50% reduced as compared to the genotypes with standard grain type (Perić et al., 2009).

TIA was affected significantly by genotype, fertilization treatment and environment (geographical location). Aplication of nitrogen fertilization increases proteins content in seed which affects on reduction of TIA for 15%. Also, TIA was reduced by nitrogen/sulphur application (Vollmann et al., 2003). Perić et al. (2009) pointed that genotypes containing the Kunitz trypsin inhibitor protein (KTI) exhibit a higher TI than genotypes lacking this protein but in both groups of genotypes TI was similarly affected by nitrogen application.

Huisman and Tolman (2001) defined the upper limit of TIA levels in the diet at ~0.5 mg / g. European Feed Manufacturers' Federation (FEFAC) recommends maximum TIA levels of 1.0 mg / kg per 10% CP content of the sample (mg / kg dry matter).

During trypsin and chymotrypsin investigation (CIA) in the extracts of wheat, rye mix, mixed cereals and, whole wheat flours and breads, Kostekli and Karakaya (2017) are determined that no TIA in the aqueous protein extracts obtained from whole wheat flour, dough, and bread. Previously, it was found that wheat had weak TIA and bran fraction of wheat had no TIA. Values for TIA were 5.13 TIA/mg protein, 15.85 TIA/mg protein, mixed cereals 26.92 ti/mg protein in wheat, rye mix and mixed cereals, respectively. Obtained results are significantly higer than our results obtained for spelt seed in both growing conditions and years (0.3493 - 1.3825 TIA/mg).

No TIA in whole wheat flour can be due to its bran content and possible interactions between protease inhibitors and complex polysaccharides found in bran. Trypsin activity in mixed cereals flour was significantly decreased during fermentation (3.6 times after fermentation) and baking (8.6 times after baking), which points to the fact that trypsin inhibitors found in mixed cereals lost their activities during fermentation and baking.

There is no quantitative data available on the trypsin inhibitors activity in maize kernels. In twelve assayed maize kernel samples, Brugger et al. (2015) are

proved that in-vitro assessment of TIA revealed an average activity of 1.27 ± 0.33 mg/g dry matter, ranging from 0.56 to 1.87 mg/g dry matter.

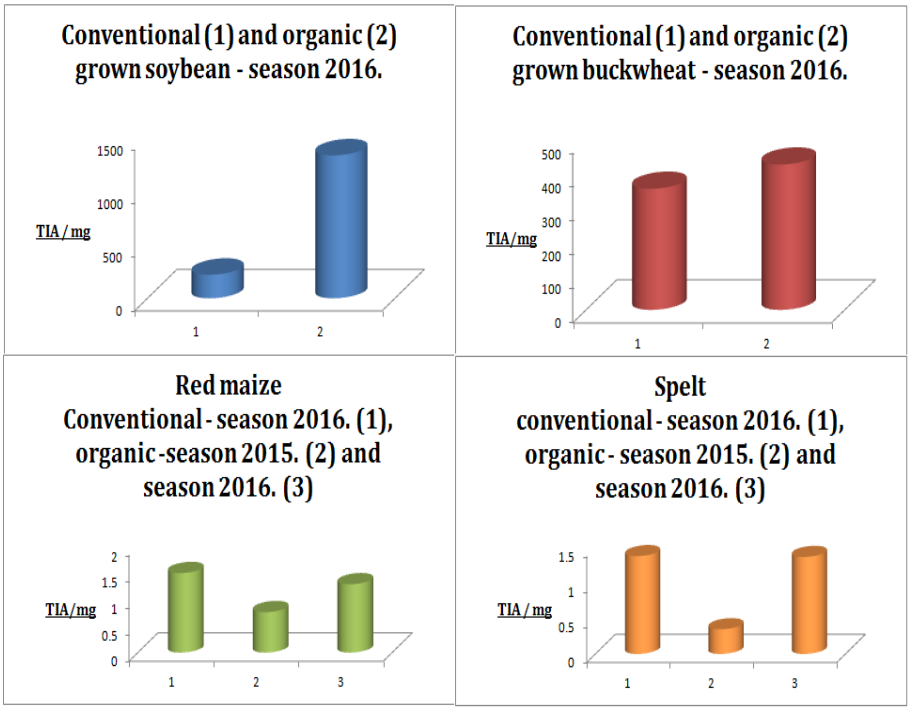

Figure 1. Trypsin inhibitors content in the samples expressed as a trypsin inhibitors activity (*TIA / mg*)

Wang et al. (2013) have shown that buckwheat trypsin inhibitors possess antimicrobial activity towards fungi, Gram-positive and Gram-negative bacteria and antitumor activity against various kinds of cancer cells. Protease inhibitors from buckwheat seeds are divided into two groups: anionic (suppressed growth and development of pathogenic fungi) and cationic (effectively inhibited some bacterial proteinases)-according to their physico-chemical properties (Dunaevsky et al., 1998).

Wang et al. (2013) examined trypsin-inhibitory activity (TIA), total protein content, and in vitro protein digestibility in buckwheat seed during germination process (1-7 days). Cu^{2+}, Zn^{2+}, Al^{3+} had a passive impact on eliminating trypsin inhibitory activity. During the first day of germination, TIA in four samples (19–22 U/g) decreased rapidly. The addition of 1000 mg/L Al^{3+} solution, TIA decreased significantly, with minimum values 4.90 U/g on day 3. Solution of Cu^{2+} (60 mg/L) decreased TIA from day 2 to day 5, while addition of zinc-

solution with concentration of 500 mg/L elicited the minimum activity of 5.43 U/g on day 5. The reduction of TIA showed a significant correlation with the increase of in vitro protein digestibility during germination.

Conclusion

Nutritional quality of grains of field crops depends on, both, content of many nutrients and antinutrients. Among antinutrients trypsin inhibitors play especially important role as one of the most spread type of protease inhibitors. According to obtained results it can be concluded that the amount of trypsin inhibitors vary not only in the different types of seeds but also between the seeds of the same species grown in different years and production conditions. For this reason it is necessary to continue research on the impact of growing conditions on the content of trypsin inhibitors in cereals/pseudocereals and soybean.

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Interspecific transplantation of brown trout and grayling germ cells into rainbow trout recipients

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Abstract: The peri-Adriatic river system holds a rich in variety of endemic freshwater fish species. Among them, salmonids such as the Adriatic lineage of grayling *Thymallus thymallus*, the Adriatic haplotype of brown trout *Salmo trutta*, *Salmo dentex*, softmouth trout (*Salmo obtusirostris*), marble trout *Salmo marmoratus* are considered morphologically and/or phylogenetically distinct endemic populations or species. The aim of this study was to develop the surrogate production technique for the grayling and the brown trout by inter-specific transplantation into rainbow trout recipients. In males of both species, spermatogonia type A (SGA) were the only germ cells present in the tissue. On the other hand, in the female germ-lineage we detected oogonia (OOG) as well as perinucleolar follicles (PNF). Sixty days after transplantation, fluorescently labelled cells could be detected within the recipient gonads. This indicated that both SGA and OOG from both donor species could migrate within the abdominal cavity of the rainbow trout recipients and colonize their gonads. Incorporation rates varied between 23% - 30%. Transplantation method demonstrated in this study could be used for the conservation and revitalization of genetic resources of endangered and endemic species or populations and thus give rise to new or improved management strategies for such species.

Key words: *Salmo trutta*, *Thymallus thymallus*, spermatogonia, oogonia

Introduction

The peri-Adriatic river system holds a rich in variety of endemic freshwater fish species (Sušnik et al., 2001). Among them, salmonids such as the Adriatic lineage of the grayling *Thymallus thymallus*, the Adriatic haplotype of the brown trout *Salmo trutta*, *Salmo dentex*, softmouth trout (*Salmo obtusirostris*), marble trout *Salmo marmoratus* are considered morphologically and/or phylogenetically

distinct endemic populations or species (Berrebi et al., 2000; Snoj et al., 2010; Sušnik et al., 2001). The majority of these species or populations are endangered in the Balkans. The main cause of this is the intense stocking of non-indigenous lineages and introgressions between them (Horváth et al., 2012). Negative effects of introgressions may be outbreeding depression and replacement of possibly locally adapted populations by allochthonous ones. Therefore, effective methods are needed for the conservation of endemic and endangered genetic resources of these species.

Cryopreservation of germplasm (including gametes, germ cells and embryos) is one of the most widely recognized methods which has a great power in species conservation. Namely, through the cryopreservation procedure, cells can be kept viable for an indefinite amount of time. The main problem in cryopreservation of fish gametes is the lack of successful and effective methodology for fish eggs and embryos, even though cryopreservation of spermatozoa has been developed for many fish species (Asturiano et al., 2017). A method that offers the possibility to overcome this barrier is the transplantation of germ cells (primordial germ cells, spermatogonia and oogonia) into suitable recipients (Okutsu et al., 2007; Yoshizaki et al., 2013). As indicated by Okutsu et al. (2007) for the first time, germ cells transplanted into the body cavity of recipient larvae have the ability to migrate within the recipient body cavity, colonize the genital ridge, proliferate and produce physiologically normal and viable gametes which will form the next generation. This way, germ cells can be used to revitalize endangered and extremely threatened populations of the peri-Adriatic salmonids.

The aim of this study was to develop the surrogate production technique for the grayling and the brown trout by inter-specific transplantation into rainbow trout recipients. Additionally, we have optimized the cryopreservation protocol for the germ cells of brown trout. Overall, the transplantation method demonstrated in this study could be used for the conservation and revitalization of genetic resources of endangered and endemic species or populations and thus give rise to new or improved management strategies for such species.

Material and methods

Fish sampling

Germ cells were isolated from immature brown trout *Salmo trutta* males (TL: 270.3 ± 16.8 mm) and females (TL: 255.5 ± 19.5 mm) as well as grayling *Thymallus thymallus* males (TL: 191.0 ± 39.2 mm) and females (TL: 197.3 ± 29.7 mm) sampled from the Bled fish farm (Slovenia). Fish were euthanized by an overdose of 2-phenoxyethanol and dissected for gonadal sampling at the fish farm. Dissected gonads were kept in the Leibovitz L-15 media supplemented

with 10% FBS on ice during transportation to the laboratory (maximum 1 hour). All gonads were cleaned from large blood vessels and adjacent connective tissue and were weighted before further manipulations.

Histological analysis

Pieces of both testicular and ovarian tissue of both species were sampled for histological analysis in order to distinguish and analyze cells present within the tissue. Samples were fixed in 8% neutral buffered formalin and stored at 4 °C until processing. Tissue pieces were processed in a series of ethanol and xylene and embedded into paraffin blocks. Each block was cut into 3-µm thick sections which were stained with the standard hematoxylin/eosin staining procedure. Sections were analyzed under a Nikon Eclipse 600 microscope and photographed using a QImaging Micro Publisher 3.0 digital camera.

Tissue dissociation

Tissue pieces were placed in an Eppendorf tube filled with dissociating solution (L-15 media + 10% FBS + 2 mg/ml collagenase [Sigma-Aldrich] + 10 µg/ml DNase [Panreac AppliChem]), cut into small pieces and digested for 60 min on a shaking plate at 23 °C. After incubation, cell suspensions were filtered through 50 µm filters and centrifuged at 200 ×g for 10 min at 10 °C. Pellets were resuspended in L-15 supplemented with 10% FBS and prepared for viability analysis. Among the female germ-lineage cells only oogonia had a diameter small enough to pass through the 50 µm mesh-size filters, therefore, only oogonia were analyzed by trypan blue live/dead staining and subsequently transplanted into recipients.

Germ cell transplantation

The transplantation procedure was performed by the MINJ-1 microINJECTORTM system (Tritech Research). Needles for micromanipulations were obtained by pulling glass capillaries (Narishige GD-1) by a Narishige PN-31 needle puller. Tips of the needles were gently broken by curved tweezers thus obtaining a sharp edge.

Prior to transplantation, isolated germ cells were labelled by a fluorescent membrane linker dye PKH-26 (Sigma-Aldrich). Labelling of cells (germ cells of both species) for transplantation was performed following the manifacturer's instructions. In short, isolated cells were washed twice in PBS, stained with 3 µl of dye / 1 million of cells for 5 min, and afterwards washed three times with L-15 + 10% FBS.

Three to five days post hatch (dph) recipient diploid (2n) rainbow trout larvae were anesthetized in 3 ppm 2-phenoxyethanol and transferred into a petri dish coated with 2% agar. Approximately 15000 germ cells were prepared and

injected into the abdominal cavity of each recipient larvae as firstly described by Okutsu et al. (2007). After transplantation, recipient larvae were kept at 10 °C overnight and were transported to the hatchery the following day where they were reared until further work.

Detection of donor-derived cells in recipient larvae

To confirm the colonization of the donor-derived germ cells into the gonads of rainbow trout recipients, 60-day post transplantation fry were sacrificed in order to localize the transplanted PKH-26-labelled cells. Fry were euthanized in an overdose of 2-phenoxyethanol and dissected by removing the head and tail and placed dorsoventrally under an epifluorescent microscope (Nikon Eclipse 600). The digestive organs were removed from the opened fish thus exposing the gonads. Detection of one or more red-fluorescent cells within the gonadal tissue was evidence of successful colonization of the recipient gonads by the transplanted cells.

Results and discussion

Based on the histological analysis, all individuals used in this study were immature and were at the same stage of development. In males of both species, spermatogonia type A (SGA) were the only germ cells present in the tissue (Figure 1A). On the other hand, in the female germ-lineage we detected oogonia (OOG) as well as perinucleolar follicles (PNF) (Figure 1A). It is important to note that among the female germ-lineage only OOG were present in the cell suspensions because of the selection caused by filtration (PNF having dimeters > 50 μm).

Approximately 2×105 SGA were isolated from a brown trout testis (~ 15 mg), 4.5×105 OOG from a brown trout ovary (~ 55 mg), 1.5×105 SGA from a grayling testis (~ 15 mg) and 2×105 OOG from a grayling ovary (~ 50 mg). Both cell types of both species displayed viability rates of > 85%.

Recipient larvae were reared until 60 days post-transplantation and the average survival rate was 59.5 ± 7.6% (Table 1). After dissection, fluorescently labelled cells could be detected within the recipient gonads (Figure 2). Fluorescent signal was of similar intensity in all recipients without regard to cells or donors (Figure 2). This indicated that both SGA and OOG from both donor species could migrate within the abdominal cavity of the rainbow trout recipients and colonize their gonads. Incorporation rates varied between 23% - 30% (Table 1). Control individuals displayed no fluorescence after dissection.

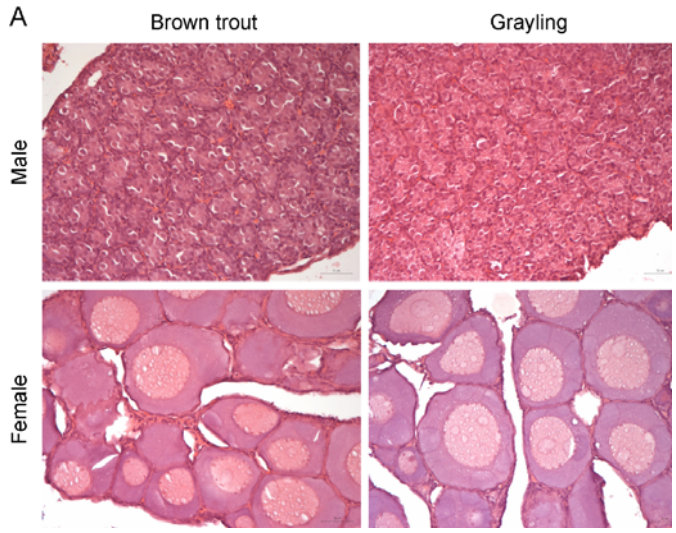

Figure 1. Histological sections of juvenile males used for the transplantation contained only spermatogonia type A (SGA; arrow) among the germ-line cells, while the juvenile females mostly contained perinucleolar follicels. Scale bars: 50 μm.

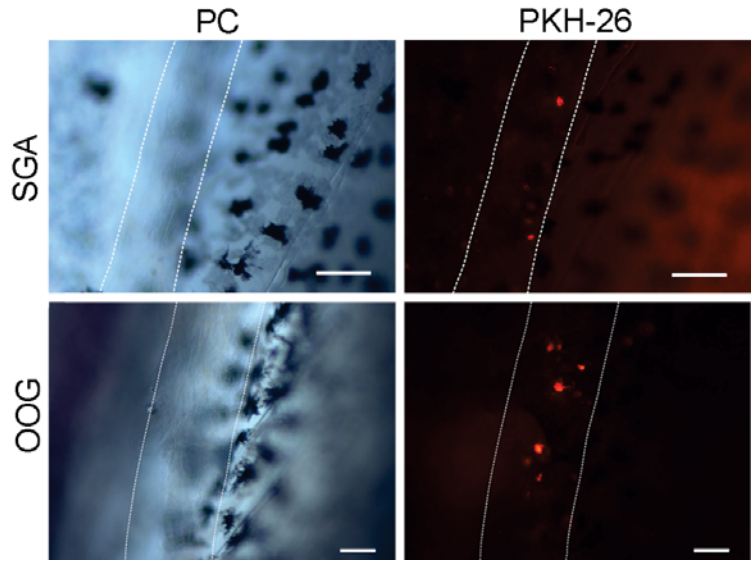

Figure 2. Intraperitoneal transplantation of brown trout and grayling spermatogonia (SGA) and oogonia (OOG) into rainbow trout recipients. Detection of the fluorescently labelled germ cells within the recipient gonads (delineated by white lines) signified successful incorporation of the donor-derived germ cells. Scale bars: 100 μm.

Table 1. Survival of rainbow trout recipients and germ cell colonization rates after inter-specific transplantation of brown trout (BT) and grayling (GR) spermatogonia (SGA) and oogonia (OOG).

Group	No injected	No survived	% survived	No positive	% positive
BT OOG	60	37	62%	10	27.03%
BT SGA	75	45	60%	12	26.67%
GR OOG	45	30	67%	7	23.33%
GR SGA	65	32	49%	9	28.12%
Control	100	79	79%	0	0%

This study is the first to demonstrate successful inter-specific transplantation of brown trout and grayling germ cells of both sexes into rainbow trout larvae as recipients. The only previous attempt of transplantation of brown trout germ cells was a successful intra-specific transplantation of spermatogonia resulting in 50% incorporation rates (Fernández-Díez et al., 2012). Intraspecific transplantation has its advantages since there are higher chances for successful end results and it can be used during conservation programs for some special populations of a well-known species. However, the development of an interspecies transplantation technique has an immense biotechnological value and potential since surrogate parents may be closely related species for which cultivation and breeding programs are more developed and standardized. This method also opens up the possibility of whole species or population rescue programs of highly threatened species. Inter-specific spermatogonial transplantation in salmonid fishes was firstly described by Okutsu et al. (2007) where the authors transplanted rainbow trout (*Oncorhynchus mykiss*) spermatogonia into wild-type masu salmon (*Oncorhynchus masou*) recipients. These two species belong to the same genus, but have been phylogenetically separated for at least 8 million years (McKay et al., 1996; Crete-Lafreniere et al., 2012). Here we demonstrate successful incorporation of germ cells (creation of germ cell chimeras) into the recipient that is phylogenetically much distant from the donor species and belongs to different genus. Molecular phylogeny places the genus *Thymallus* phylogenetically basal to other salmonids, with their most common ancestor dating 40-50 MY ago, while *Salmo* and *Oncorhynchus* are phylogenetically separated for more than 25 MY (Crete-Lafreniere et al., 2012; Shedko et al., 2013). We carried out transplantation of SGA and OOG, isolated from both donor species. While SGA have been more commonly used in transplantation, the isolation and transplantation of OOG has also been described in trout (Yoshizaki et al. 2010). Both SGA and OOG showed sexual plasticity after transplantation and differentiation into both male and female gametes. Our

results show that transplantation success was independent of the donor species or type of germ cells used; in all cases incorporation rates are equal (around 25%).

Sixty days after the intraperitoneal transplantation of germ cells into the abdominal cavity of rainbow trout larvae, fluorescently labelled germ cells were visualized inside the recipient gonads. This indicated that the transplanted germ cells, both SGA and OOG isolated from brown trout and grayling were able to migrate from the body cavity into the gonads and colonize them. Even though both germ cells and somatic cells were fluorescently labelled and transplanted into the recipients, Yoshizaki et al. (2010) demonstrated that only germ cells have the migratory and colonization potential, therefore suggesting that also in this study, only germ cells were the ones that colonized the recipient gonads.

Successful differentiation of donor-derived germ cells SGA and OOG from both species into functional sperm and eggs in the recipient gonads would shed a new light in the conservation of valuable Balkan trout genetic resources, for instance Adriatic grayling and marble trout, endemic to the Po and Soča river system in Italy and Slovenia. Both species are subject to different threats which may lead to their extinction. Current conservation efforts for the Adriatic grayling and marble trout consist of both *in situ* and *ex situ* strategies. Pure marble trout populations are maintained in isolated streams without the possibility of contact with allochthonous stocks. However, these are mostly small streams with low effective population sizes and with a constant danger of natural disasters, landslides, avalanches and floods (Crivelli et al, 2000). The main threat for the Adriatic grayling is hybridization with introduced grayling from Danubian drainage. Stocking with allochtonous grayling was banned for the Soča river, but grayling population is severely declining during last years (D. Jesenšek, personal communication and personal observation of authors). Sperm cryopreservation as an *ex situ* conservation measure is applied in the case of the Adriatic grayling as described by Horváth et al. (2012). Nevertheless, due to combination of threats and difficulties in rearing wild autochthonous salmonid fishes for the purpose of supplemental stocking, interspecific germ cell transplantation and offspring spawned from surrogate hatchery adapted parents could greatly improve the current management strategies. Furthermore, germ cell transplantation offers the possibility of producing genetically diversified seeds meaning that if donor fish originate from separate populations, a small number of surrogate parents may produce gametes which possess significant genetic diversity (Sato et al., 2014). Germ cell cryobanking and transplantation into hatchery adapted recipient species could play a significant role in revitalization of affected population.

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Sediment quality assessment with phytoindicators

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Abstract: Inappropriate and frequent discharge of untreated industrial effluents and sewage directly into the water reservoirs have led to contamination of water sources and sediments. Therefore, the possibilities of sediment use in agriculture have to be assessed and are intensively studied. Since contaminated sediment can pose a risk for cultivated plants, it is necessary to assess its quality, detect and characterize pollutants and their bioavailability to plants. This work aimed to assess the quality of sediment (in the form of interstitial water) from Nadela canal using comparative chemical and biological analysis. Chemical analysis (EPA 7000B) were used to determine the content of heavy metals, while biological effects were observed on germination and root and shoot length barley and cucumber and barley seedlings (ISTA Regulations book, 2011). Distilled water was the control variant. Chemical analysis detected high content of Ni^{2+} and Cu^{2+} in sediment, while the results of biotest indicate that germination was not a valid parameter of sediment contamination with abovementioned heavy metals, regardless of plant species. However, both of plants responded in morphological changes, inhibition of root and/or shoot of seedlings, to sediment quality. Thus, these parameters can be considered more reliable, and all species as good indicators of sediment contamination.

Key words: sediment, biotest, bioindicators, morphological and physiological parameters

Introduction

The problem of sediment contamination in agricultural regions is becoming more pronounced, because large amounts of sediment are dredged during regular seasonal maintenance of irrigation systems, and it need to be properly disposed. The possibility of sediment use in agriculture is increasingly studied, but since the contaminated sediment can presents risk to cultivated plants, it is necessary

to assess its quality, detect and characterize pollution and determine the bioavailability of certain pollutants on plants. Sediment quality can be assessed using physical and chemical methods, which comprise qualitative and quantitative aspect of pollution, but they are not sufficient to present the effects of pollutants on the living world. Therefore, to assess the quality of sediment and the detection of pollution it is necessary to include biological methods – bioindicators. Cultivated plants are of increasing importance as bioindicators. The results of assays with cultivated plants as bioindicators are direct indicator of opportunity for sediment disposal on agricultural. According to Ankley et al (1993), some plants are especially sensitive to elevated level of of certain pollutants, so they can be used as test organisms in verified protocols and risk assessment trials. Germination, root length and height / length of aboveground parts of seedlings are designated as the most striking parameters by numerous standard methods for assessment of phytotoxic effects of certain compounds. Also, Welbaum (citation from Mahmood, 2005) points out that tolerance of plants is evaluated through germination and growth of aboveground parts of seedling, because these are the crucial moments in plant development, and are the most dependent on environmental conditions. Liu et al. (2005) states that the sensitivity of plants to metals in water and sediment depends not only on the concentration and type of pollutant, but also on the stage stage of plant development (germination, germination, vegetative growth, etc.).

The aim of the study was assessment of sediment quality from the Nadela canal using test plants as bioindicators and assessment of potential of tested plants and parameters for detection of contamination of sediment, and also assessment of possibilities for sediment using on agricultural land.

Materials and methods

Sediment from Nadela canal was sampled according SRPS ISO 5667-12:2005, 2011 year and it was used in biotest in the form of interstitial water, obtained by decanting. The sediment quality classification was performed according to Dutch categorization.

As test plants, cucumber (*Cucumis sativus* L.) cultivar Tajfun (dicotyle plant) and barley (*Horedeum vulgare* L.) cultivar NS 525 (monocotyle plant) were used. Seeds of all plant species were collected during season 2016. The sediment quality from canal was assessed using physiological (germination - %) and morphological parameters (seedlings root and shoot length -cm).

In the study, a standardized method on filter paper was used, according to ISTA regulations (International rules for seed testing, 2011). Hundred seeds of cucumber and barley were placed in plastic boxes on pleated filter paper, previously moistened with 25 ml of interstitial water, and distilled water was

used as the control. Plastic boxes were put in thermostat in the dark and incubated at 25 ±2°C. In order to evaluate morphological parameters, after four days of incubation, ten plants from each repetition were separated and placed on previously moistened filter paper lanes (14 x 60cm), rolled in, put into PVC bags and returned to thermostat afterwards. Boxes with remained seeds and germinated plants were returned into thermostat also, and after seven (barley), or eight days (cucumber), germination was evaluated by counting germinated, atypical and non-germinated seeds compared to the total number of seeds. After the same period, seedlings root and shoot length from the rolls were measured.

The results were analyzed in statistical software SPSS 17, using t test (95% interval of confidence).

Results

Based on the Dutch classification of sediment, it can be stated that the sediment sample from the Nadele canal is moderately polluted by Ni^{2+} and Cu^{2+} (3 class).

Table 1. Physiological and morphological parameters of barley and cucumber in sediment from Nadela canal

		barley	cucumber
Germination (%)	interstitial water	90.0 ± 1.04 b	96.0 ± 4,62 a
	control	100 ± 0.00 a	93.0 ± 3,46 a
	t value	3.77**	0.79ns
Seedlings root length (cm)	interstitial water	11.60 ± 0.43 b	9.70 ± 0.90 a
	control	17.90 ± 0.72 a	8.90 ± 2.05 a
	t value	6.31**	1,85ns
Seedlings shoot length (cm)	interstitial water	14.20 ± 0.20 a	9.90 ± 1.16 b
	control	11.60 ± 1.50 a	13.30 ± 0.88 a
	t value	0.49ns	10.63**
Root/shoot	interstitial water	0.81	0.98
	control	1.54	0.67

Interstitial water from Nadela canal did not affect germination of barley (90%) nor cucumber (96%), and in all cases it was at the same level of significance with control (100%, 93%, respectively) (Tab 1). The length of the barley roots was under a strong influence of interstitial water quality and was significantly lower than the control (t=6,31**, p<0,01), while the shoot length

was at the same level of significance with control (t=0,49NS, p>0,05). Cucumber plants did not react to quality and sediment pollution by changes in root length, therefore all values are at the same level of significance with control (t=1,85NS, p>0.05). However, the growth of cucumber seedlings shoots in interstitial water was significantly inhibited (t=10,63** p<0,01) compered to the control.

It is evident that the germination, as physiological parameter, is not valid in sediment contamination detection with Ni^{2+} and Cu^{2+}, regardless on the plant species. Morphological parameters were more reliable indicators of changes in sediment quality and in the indication of the pollution with the specified heavy metals. The sensitivity of tested parameters and intensity of the changes depended on the plant species. These results are in accordance with the earlier citations of the Gvozdenac et al. (2012a, 2012b) and Prica et al. (2010).

Conclusions

- Germination was not a reliable indicator of sediment quality or pollution by heavy metals which are detected higher amounts, regardless of the plant species.
- Barley and cucumber are good indicators of increased content of Ni^{2+} and Cu^{2+} in the sediment and react by morphological changes.
- Intensity of change of morphological parameters is depending on the plant species.
- Sediment from Nadela canal could not be used or disposed on agricultural land, due to increased risk of phytotoxicity on barley and cucumber as a result of the presence of heavy metals detected by chemical analysis.

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Some Morphological Characteristics and Yield Parameter of Maize in Conditions of Artificial Western Corn Rootworm Eggs Infestation

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Abstract: Western corn rootworm (WCR) *Diabrotica virgifera* sp. *virgifera* Le Conte (Coleoptera, Chrysomelidae) is an oligophagous pest, native to America. WCR leaves serious consequences on maize plant and yield. A field experiment was carried out in Bečej, Vojvodina province (Serbia), during 2015 with Serbian cultivar NS-640. In experimental field selected and marked 96 plants and arranged in 48 pairs. Each pair represents two plants. The first is the artificially infested plant by agar egg suspension of WCR (D plant). The second represents control plant – in root zone injected distilled water (C plant). During an experiment, the field was inspected regularly. The following was recorded: number of caught specimens, plants height, plant diameters and mass of kernels. The first catch was on 9th July (9 specimens) and the last was on 10th September (1 specimens). The maximum measured height on D and C plants was 250 cm and 270 cm, respectively. The minimum measured diameter on D and C plant was 17.7 i.e. 15.2 mm respectively. The maximum measured mass of kernel of D and C plants was 247.57g and 195.28 g, respectively. Statistical analysis shows that there are no any significant differences between D and C plant.

Key words: WCR, artificial infestation, morphology, maize

Introduction

Western corn rootworm (WCR) *Diabrotica virgifera* sp. *virgifera* Le Conte (Coleoptera, Chrysomelidae) is native to America but today WCR is present worldwide in maize field (EPPO, 2015). It is an oligophagous pest, which leaves serious consequences on maize plant and yield (Hummel et al., 2008). WCR can feed on more than 20 plants from fam. Poaceae, but development and survival are less than on the maize which represents favorite host plant for this insect

species (Clark and Hibbard, 2004). The first introduction of WCR in Europe occurs near the Belgrade airport, Serbia in early `90 (Bača, 1993). Today this pest is present in almost every maize field around Europe (Hummel et al., 2008). The rate of WCR imago spread is up to 100 km per year (MacLeod et al., 2004; Baufeld, 2003). This pest is univoltine but leaves huge and serious damages on maize root and above – ground parts of plants (Bača, 1993, James et al., 2005; Ciobanu et al., 2009). WCR larval attack to the maize root living the most important damage in the field (Ivezić et al., 2001., Ciobanu et al., 2009; Wesseler and Fall, 2010). WCR larvae feeding on the nodal and lateral roots damaging the maize root system and it can lead to an impossibility for maize to uptake water and nutrients (Chiang, 1973; Kahler et al., 1985; Gavlovski et al., 1992; Riedell, 1997; Gray, 2009). Larval attack causing the plant lodging which can lead to the mechanical (inability to harvest maize during mechanical harvesting) and physiological yield loss (consequence of the inability of the injured roots to take up moisture and nutrients) (Hou et al., 1997; Tollefson, 2007; Dun et al., 2010). Plant lodging knows as goose necking is the main symptom that indicates the presence of WCR larvae in maize fields (Chiang, 1973; Wesseseler and Fall, 2010). Yield losses also result from the inability to harvest the maize kernel during harvesting because of the plant lodging (Spike and Tollefson, 1991). Environmental conditions, the type of the soil, moisture, and a number of WCR larvae in a soil are highly related to maize root damage (Ciobanu et all, 2009; Spike and Tollefson, 1989). Maize represents plant with strong, large root system (Gray et al, 1998). Decreased lodging and increased root system represent a measure of high tolerance of maize to WCR larvae attack (Riedell and Evenson, 1993).

Material and methods

The field experiment was carried out in Bečej, province of Vojvodina the Northern Serbia. It is performed from May 30th to September 10th 2015, with Serbian cultivar NS-640. The chosen field for experiment represents the field with low WCR natural infestation.

During the experiment 96 maize plants were selected, labeled and arranged into pairs. The plants are set up in two rows with space between labeled plants of 1 m. In each pair, one plant was artificially infested in root zone with 4 mL of WCR eggs 0.125% agar suspension (D plants). One mL of suspension contains 136 WCR eggs. The other plant from the pair was the control plant marked with C. In root zone of C plant was injected the same amount of distilled water (4 mL). After artificial infestation pheromone trap for WCR was set up in the middle of infested plant in an experimental field on 23rd June.

The field was inspected every week for five months. Each field inspection meant inspection of a sticky base in pheromone trap, replacement of sticky base and inspection on maize plants. The presence of WCR in the trap was recorded in every field inspection. Inspection of the plants included the measurement of the height and stem diameter. The measurements of stem diameter were made using a ± 0.05 mm precision Caliper, Pro-Max 67 IP Sylvac System.

Laboratory measurement was included measuring the mass of kernel using the technical balance (Kern EW 1500-2 M).

The differences between D and C plants, based on the height, stem diameter and mass of kernels, were analyzed using F-Test Two-Sample for Variances and T- test Two-Sample Assuming Equal Variances. Correlation between plant height, plant diameter and mass of kernel on D and C plants was determined by basic statistics (correlation matrix) at the importance level $p< 0.05$.

Results and discussion

The inspection of a sticky base in pheromone trap shows that the number of WCR specimens was fluctuating during vegetation period (Fig. 1). The first catch was in the beginning of vegetation on 9th July whit recorded 9 specimens. The last end the smallest catch was at the end vegetation on 10th September when was registered only one specimen. The biggest catch was in the middle of vegetation on 6th August with recorded 71 specimens on sticky base. The total number of caught WCR males recorded in pheromone trap during 2015 was 91specimens.

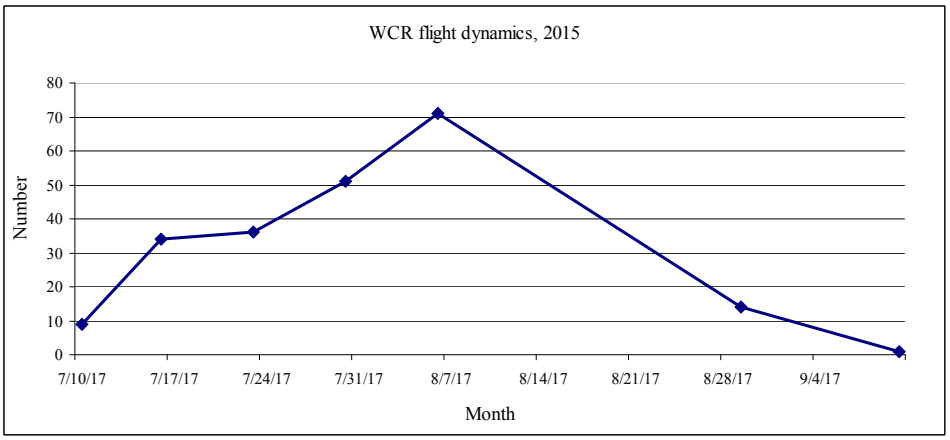

Figure 1. Population dynamics of WCR

Investigation (Bača et al., 1995) in Zemun polje, Serbia, shows that the largest number of WCR was recorded in the period of mass oviposition from 30th

July to 12th August. During research Sivicev et al. (2009) had the biggest catch in pheromone traps in the period of 25th July to 25th August in Serbia. These results are correlated with our results with the largest catch on 6th August (71 specimens). Results from 2014 and 2016 in Bečej (Tanasković et al., 2016; 2017) shows that the largest number of caught specimens was on 18th September (91 specimens) and on 30th September (181 specimens) respectively. On the odder hand (Tančić et al., 2006) in a period from 27th August to 10th September did not have registered catch in pheromone traps. Highest efficiency of pheromone traps during the experiment (Toth et al., 1996; Tollefson, 2007) was in the middle vegetation with the daily catch of 6 WCR. Our experiments and many other studies indicate the progressive and fluctuating catch of WCR adults depending on different vegetation periods.

The maximum measured height on D plants was 250 cm, and the minimum was 160 cm. On the other hand on C plants maximum and minimum measured height was 270 cm and 160 cm, respectively. A diameter of measured values on D plants was in the range of 17.7 – 40 mm and the measured stem diameter on C plant was in the range of 15.2 – 37.6 mm. The measured mass of kernel on D and C plants was in the range of 11.58 – 247.57g and 6.8 – 195.28 g, respectively (Figure 2).

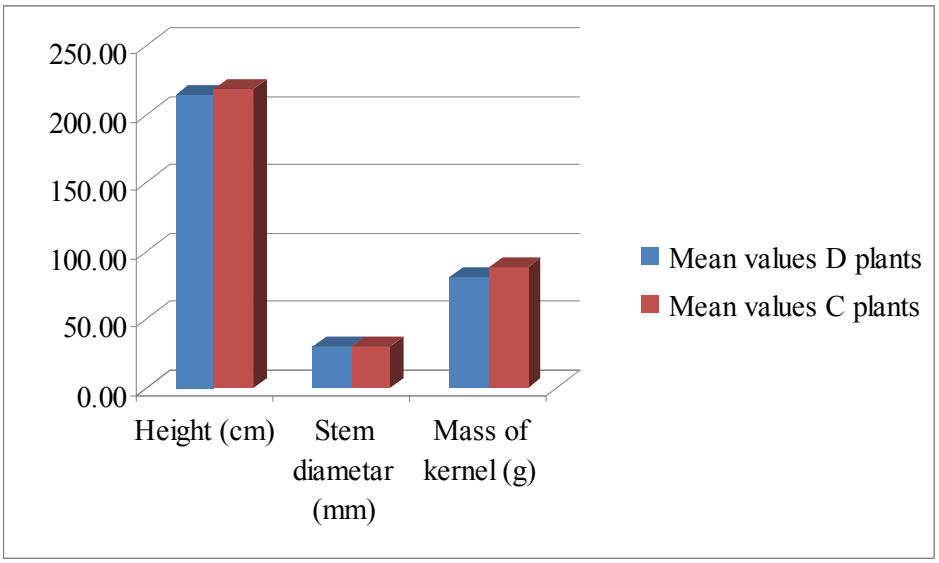

Figure 2. Maize morphology and yield parameter

The average height for D plants was 214.27 cm and for the C plant's were 218.85 cm. The average values of the plants diameter for D and C were 29.54

and 30.43 mm, respectively. The average mass of kernels for D plants was 80.76 g on the other hand for the C plants was 88.27 g.

Table 1. Differences between height, plant diameter, and mass of kernel of WCR infested plants and control plants

Observation	*Means values*		*t/Z**	*Sig.*
	D plants	C plants		
Height (cm)	214.27 ± 22.76	218.85 ± 20.99	1.0255	0.3077
Stem diametar (mm)	29.54 ± 6,41	30.43 ± 5.39	0.7816	0.4365
Mass of kernel (g)	80.76 ±76.5	88.27 ±64,37	0.7166	0.6045

Statistical analysis shows, using F-Test and T- test, that there are no any significant differences between D and C plant in their height, stem diameter and mass of kernels (Table 1).

Statistical analyses show that there is a correlation between height and mass of kernel on D and C plants ($p< 0.05$). The correlation coefficient on D and C plants was 0.66 and 0.75, respectively.

The harmfulness and increase of population of WCR in the field are caused by the cultivation of maize in monoculture (Sivcev et al., 2009). WCR larvae presence in maize monoculture can cause increase lodged plant from 3% to 15 % because of the strong root damage (Čamprag et al, 1998), and even up to 75% (Bača et al, 1998). In the research (Tanasković et al., 2016) WCR eggs infestation caused 95.7% damages on infested plant with different rate root damages.

Spike and Tollefson (1989) also point to that larvae presence in corn field leads to a decrease in yield. The same authors (1989) indicate that larvae make biggest root injuries and harmfulness increased by adult WCR feeding on the silk and kernels i.e. cobs. WCR shows tolerance to same control measures including cultural practices and pesticides control (Wright et al., 2000; Levine et al., 2002). The maize plants are the with high ability to recovering and his root tolerance is associated with a capability to grow new roots after larvae injury (Gray et al., 1998).

Conclusion

The obtained data indicate, in the condition of low natural plus artificial infestation by WCR eggs, that compare of the morphological characteristics (plant height and plant diameter) and mass of kernels as yield parameter, there not statistically significant differences between artificially infested and uninfested maize plants. Also, analyses show that there is a correlation between height and mass of kernel on D and C plants ($p< 0.05$).

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Appearance, harmfulness, and monitoring of *Xyleborus dispar* in western Serbia

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Abstract: *Xyleborus dispar* Fabricius, 1792. (Coleoptera: Scolytidae) is polyphagous European species widespread in the Europea. It represents a secondary pest or pest of weaknesses, and colonize plants with disrupted physiological functions. In the net system of Reporting and Forecasting Service of plant protection in Serbia the traps for this species deployed in localities Pirot, Leskovac, Šabac, Ruma, Jagodina and Užice since 2014. The appearance and harmfulness of *X. dispar* was registered near Čačak in 2016 (western Serbia) in pear orchards in the locality Miokovci (N 43^0 56'54" E 20^0 17'20,4", altitude 283m), orchard six years old, cv. Stark Delicious. The visual inspection identified activity on about 92% of the trees. Entry holes are registered up to 160 cm of height, on the tree and all primary branches. The average number of entry holes, on a random sample of 20 trees, at a height up to the level of the first branch was 27. This is the first report of a pest in the region of municipality Čačak. Appropriate monitoring of this species indicates that the appearance can be expected, depending on and temperature conditions, from the March.

Key words: *Xyleborus dispar,* pear, monitoring, harmfulness, Čačak

Introduction

In natural ecosystems and agroecosystems, plants are often exposed to various forms of stress (Taiz and Zeiger, 2006). Most often, the stress is caused by an external factor which enhances the effect of different deficits on the plants (Taiz and Zeiger, 2006).

Insects are the primary or secondary pests in plant production. Primary pests infested plant health authorities as well as their feeding and/or reproductive hosts. In contrast to them, the secondary pests infested in different ways the compromised systems (Mihajlović, 2015).

Changing the physiological status of the plant can be caused by different biotic and/or abiotic factors. The occurrence of late frosts in the spring or early autumn, drought periods, waterlogging caused by heavy rainfalls, soil pollution with heavy metals and hazardous substances are some of the factors that modify the physiological status of plants (Taiz and Zeiger, 2006). Also, flooding and poor soil drainage can provoke intolerant plants to become more susceptible to infestation by secondary pests (Ranger et al., 2016). Spring 2016 was characterized by good and abundant blossom, which was accompanied by expectations of higher yields. However, the high level of rainfall at the beginning of March unexpectedly flood wave caused of all Zapadna Morava tributaries (Figure 1).

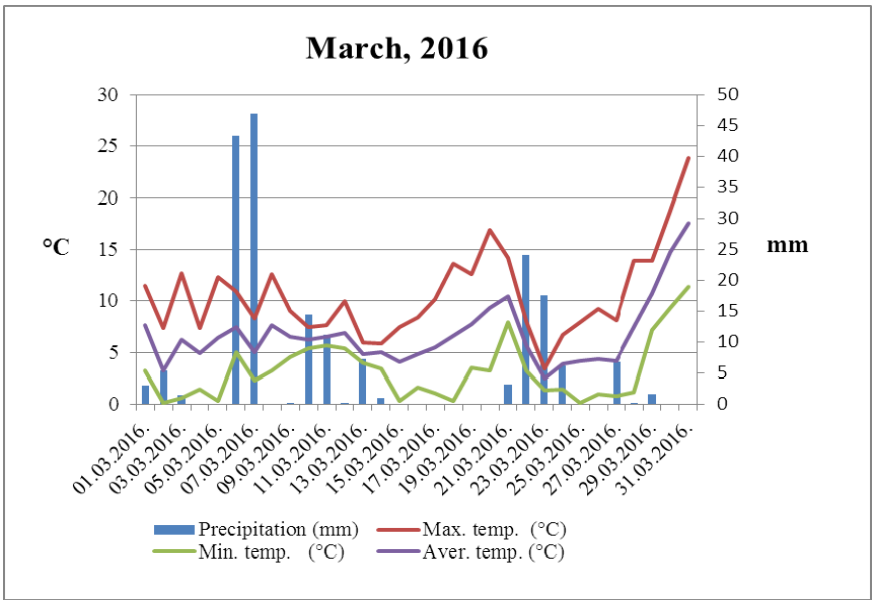

Figure 1. Air temperature and precipitation in Miokovci, March 2016 (https://www.fieldclimate.com/new/index_new.php)

During the full blossom in pear orchards on the right bank of the river Čemernica, registered the sudden signs of wilting on the trees. Decline increase in the orchard and described as the trees doused with hot water.

The aim of the research is to determine the cause of the sudden occurrence of pear trees wilting and/or decline, in an orchard in the vicinity of Čačak (locality of Miokovci). When the cause of decline was determined, biology and harmfulness of species were monitored. The aim of this was appropriate recommendations of necessary measures to the farmer.

Material and methods

The pear orchard is located in the village Miokovci, on the right bank of the river Čemernica. The geographical coordinates of the orchard are N $43^0$56'54" E $20^0$17'20.4", while the altitude is 283 m. The orchard is planted with 180 trees, out of which 177 trees are the variety Stark Delicious, two trees are the variety Santa Maria and one variety is Williams. All varieties were grafted on a quince rootstock MA. The plantation is in the sixth year of vegetation.

Field inspections were carried out every 30 days, starting from the first visit on 20th April 2016. During each field activity, symptomatic trees were visually inspected, and insects and plant parts were collected, which is all documented in photographs. Inspections were carried out on 20th April, 20th May, 21st June, 21st July and 21st August 2016.

Identification of the collected insect material was carried out according to Bright and Stark (1973) Determination Key, under the binoculars Leica M125 with LAS software system, at the Faculty of Agronomy in Čačak, Serbia.

On the same locality in vegetation 2017, on March 28th deployed red sticky bases as a trap. Below the bases installed attractant (alcohol) with the aim to increase the number of caught female specimens. In vegetation 2017 occurrence and abundance of insect monitored.

Results and discussion

During the field inspection on 20th April 2016, a uniformly modified habitus of pear trees in the orchard was registered. Changes were atypical and the trees looked like they were scalded (douched with hot water) (Picture 1).

Picture 1. The overall look of plants April, 20th 2016 (origin)

Picture 2. Entry holes and white frass trickles (origin)

Based on the general appearance, the symptom resembled the infection with bacterial fire blight caused by *Erwinia amylovora*, a disease well known to fruit producers. However, the symptoms differed from those of the bacterial infection in the absence of characteristic necrosis on the tips of the branches and on the flower buds. In a detailed inspection of trees, the symptoms of leaf and flower wilting were registered in the entire orchard. Irregularly scattered round holes – about 2 mm in a diameter – were recorded on the trees, in the trunk zone, and on the main branches. The openings on the surface of tree bark and branches were filled with a fine powder, similar to fine sawdust i.e. white frass trickles (Picture 2). The described changes were recorded on 165 trees, which account for 92% of the trees in the orchard.

Detailed counting of the number of openings on the trunk up to the height of the main branches (65-70 cm) indicates that it varied from 24 to 39 (Figure 2). The average number of holes at this height was 27. During the inspection, the presence of openings along the tree trunk at the height of 160 cm was also registered. The openings were present at all thick branches up to the mentioned height.

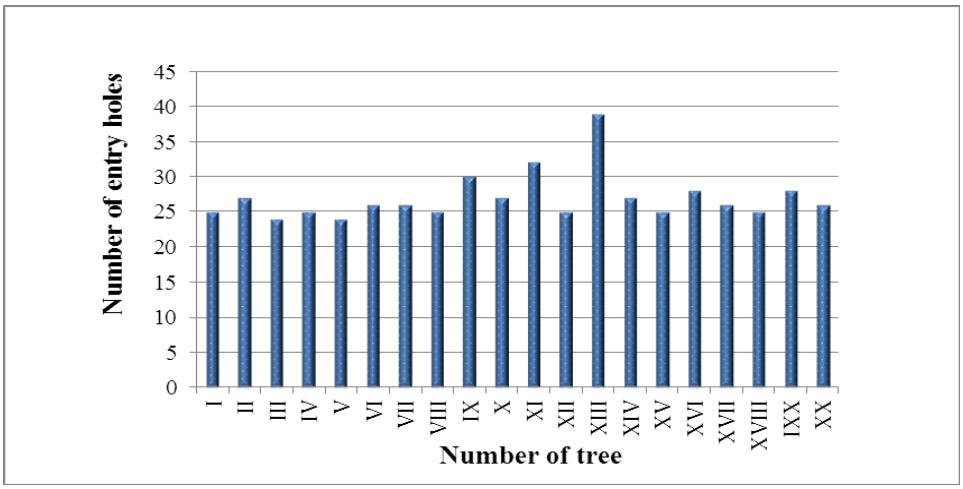

Figure 2. Number of entry holes in trees to a height up to 70 cm

The infested trees were divided into three categories based on the number of entry openings on the entire tree: trees with low infestation (up to 20 openings), trees with moderate infestation (35 openings) and trees with high infestation (more than 35 openings).

Round entry holes were discovered by incising the bark superficially at a boring position. They were set almost at the right angle to the central part of the trees. During the examination, the distal parts of the insect's body were found in

the holes, based on which it was concluded that the openings were the holes of a xylophagous species.

In the Laboratory for Entomology at the Faculty of Agriculture in Čačak, all the basic morphological parameters of the collected insects were observed with the binoculars. Based on these, the species was identified as *Xyleborus dispar*, an autochthonous insect of European entomofauna, better known under the trivial names as a pear blight beetle and European shot-hole borer (ambrosia beetle).

During the inspection on 20th May 2016, physiologically stronger trees with fewer insect holes continued further growth, fruit development, and foliation. This is a result of applying intensive care measures, such as fertilization and irrigation, which helped the plants to overcome the stress caused by cutting the vascular system by insect holes. The intensive boring of parental galleries by females ended. The disruption of the vascular system also ended as a result of intensive nutrition and irrigation of orchards and by establishing normal physiological processes of plants growth and development, although there were occurrences of registered leakage of sap from insect holes (Picture 3).

During the first inspection (20th May 2016), the emergence of the larvae was registered. The larvae feed on the mycelial form of fungi *Ambrosiella hartigii* (mycetophagous) (Picture 4) in parental galleries, which are colored in dirty white to gray color, resulting from the development of fungus mycelium on the walls.

Picture 3. Occurrence of sap from entry holes (origin)

Picture 4. The emergence of the first larvae (origin)

In the next field examination, carried out on 21st June 2016, the plants were generally more exuberant compared to the previous field inspections. However, trees that had the high level of infestation (> 35 entry openings) declined again and the fungus *Schizophillum commune* Fries (Picture 5) was reported to them.

When cutting the branches in transversal cuts, the system of canals with larvae and adults of a new generation was present. The walls of the canals in

which the insects completed their development were black. The change of color in the canals (Picture 6) is the result of oxidation of the fungus *A. hartigii* mycelia (Krstić, 1961; Langendorf, 1961; Mirić, 2004).

Picture 5. The appearance of *Schizophillum commune* (origin)

Picture 6. Black walls of galleries and new adults (origin)

During the fourth field inspection, on 21st July 2016, a satisfactory general appearance of the trees was registered. The fruits were small in number but of exceptional quality and could be classified as extra and/or first class.

The insect galleries were densely filled with adult insects, which were preparing for the winter imaginal diapause (Schvester, 1954).

The fifth and the final visual inspection prior to pear harvesting was on 21st August 2016. Compared to the previous field observation, no significant differences in the overall appearance of the plants were recorded. Bark cracking and its separation from the woody parts was progressing on the declined trees. Insect activity was reduced to a minimum and only the presence of adults was registered. Larvae and pupae were absent. The given results indicate that the pests development cycle was completed.

Based on the observations and the collected data it can be concluded that the cause of this sudden decline of pear trees in the village Miokvci is a pear blight beetle – *Xyleborus dispar* (Coleoptera, Scolytidae). This insect is a secondary pest, a pest that infests weak plants that have a physiological disorder caused by different biotic and/or abiotic factors. The infested pear orchard is located on the right waterfront of the river Čemernica, which had two immense floodings during the vegetation seasons of 2014 and 2016. The river Čemernica flooded the orchard in the period 13th-15th May 2014. During this period, about 100.4 mm per m^2 of rain precipitated, but without any adverse consequences to the trees in the orchard. A new incidence of flooding was registered on 7th March in 2016. However, the water retreated from the orchard very slowly – in about twenty days. Three weeks after the flood, at the end of March 2016, the first changes on plants were registered, and these changes were later observed and studied during the vegetation period.

Soaking of the roots and removal of the top soil layer in this period had the negative impact on the general physiological condition of the pear trees. Under such conditions, according to a number of authors, the plant responds by changing the chemical composition of specific volatile substances, so-called primary attractants and emits terpenic oil, such as alpha-pipen, delta-caren, limonene and camphene (Mihajlović, 2015). In the spring, with increases in day and night temperatures, the females begin with flights and selection of a suitable host. With the system of senses (olfactory receptors) the female registers trees that emit that specific volatiles.

Three weeks later the changes were registered on plants that are monitored in vegetation 2016.

The infested pear orchard is located between two apple orchards. All three plantations were flooded during both years. However, in apple orchards, the presence of a pear blight beetle was not recorded. This probably depended on grafting or how vigorous were the rootstocks on which the pears and apples were grafted. Pears were grafted on a quince rootstock MA, which is slightly stunted and has a shallow root system. This also results in high sensitivity to deficit or surplus of water in the root zone. Based on the obtained results, recommendation for the owner of the orchard is to carry out obligatory eradication of infested pear trees and incineration (burning) on the plant material by the end of February 2017.

The owner of Plantation was eradicated tree on 6th December 2016. However, the collected plant material remained stacked nearby. Farmer not complying with recommendations for mandatory incineration of collected plant material, but presence of this infested woody mass significantly affected the abundance of *Xyleborus dispar* at the same locality during 2017.

On 28th March 2017 started monitoring i.e. occurrence and abundance with red sticky traps and number of caught specimens shown in Figure 3.

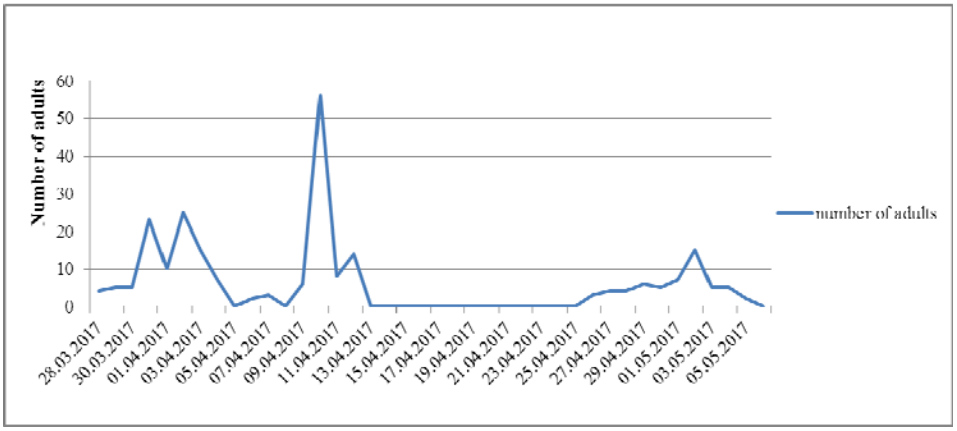

Figure 3. Number of caught *X. dispar* in 2017

From the Figure 3 can be seen that the insect activity registered during March. The number of caught specimens highly influenced by daily temperature and precipitation.

In the system of Reporting and Forecasting Service in plant protection of Serbia the traps for *X. dispar* deployed in localities Pirot, Leskovac, Šabac, Ruma, Jagodina and Užice since 2014. The practice is to set traps on 1st April. The results of our study showed that the activity of *X. dispar* females is a consequence of accumulated active temperature. This finding is completely harmonized with the research in European countries during last decade. The recent research indicates that adult flights begin when the maximum temperature reaches 14 ^{0}C (Speranza et al., 2009). In central Italy, in the region of North Lazio, an appearance of a pear blight beetle was investigated in a three-year period. It was found that the flights of this pest are in direct correlation with the amount of precipitation and temperature. During the rainy season, and lower temperatures, there are interruptions of the pest flights, but the flights are reactivated by improving weather conditions. Research from Lithuania in 2015 shows that the first flight of *X. dispar* in an apple orchard was registered at temperatures above 10 ^{0}C. In Lithuanian conditions, it is the beginning of April (Salmane et al., 2015). In Romania, the research indicates that the first flight was on 28th of March in 2003, at the temperature of 13.2 ^{0}C (Biociort and Marinescu, 2011).

Monitoring the insects and the changes on the pear trees indicate some conclusions, i.e. that:

- the cause of the sudden decline of pear trees in the village Miokovci is *X. dispar;*
- the average number of entry holes on the infested trees at the height up to 160 cm from the soil surface was 61;
- traps for monitoring *X. dispar* should be deployed from the beginning of March, depending on the temperature conditions.

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Development of Indian Meal Moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) on Three Maize Hybrids from Serbia

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Abstract: The objective of this study was to investigate the development of Indian meal moth, *Plodia interpunctella*, on three different maize hybrids from Serbia, on two kernels states (whole and broken). Development was assessed by following the life history parameters of this moth: mean developmental duration (MDD), adult emergence and longevity, and fecundity. The results of our study indicate that development of *P. interpunctella* is poorly influenced by the maize hybrid, compared to the state of kernel. Broken kernels are more suitable for *P. interpunctella* development, where the highest number of emerged adults and shorter MDD were recorded. Our study warrants further investigation of maize hybrids and their susceptibility to *P. interpunctella*. It also points out the importance of saving the kernel integrity and proper aeration of stored maize kernels.

Key words: *Plodia interpunctella*, life history, corn, kernels state, Serbia

Introduction

Plodia interpunctella (Hübner), the Indian meal moth, is a worldwide insect pest of processed cereals and cereal products (Mbata, 1990), dried fruits and vegetables (Perez-Mendoza and Aguilera-Pena., 2004), nuts (Johnson et., 1992) and confectionary products (Na and Ryoo, 2000). The larvae of this moth spin a silken web inside and on the food surface, and feed within the web. Since the webbing contains larval frass and exuviae it gives an upleasant odor and look to

the infested commodity. This can cause direct product loss and indirect economic costs through quality losses and consumer complaints (Phillips et al., 2000).

Maize is one of the most important exporting crop in Serbia (Anonymous, 2016). Presence and life activity of *P. interpunctella* on stored maize kernels can lead to deterioration of maize quality (Phillips et al., 2000) and also to the poor seed germination (Sallam, 1999). *P. interpunctella* larvae feeds on the kernels of the superficial layers of the stored kernels and reduce both quantity and quality of stored maize (Sedlacek et al., 1995).

The purpose of this study was to obtain additional data on how type of kernel and kernel state influence some of the life history parameters of *P. interpunctella*. The development of *P. interpunctella* was assessed by following life history parameters of this moth: mean developmental duration (MDD), adult emergence, longevity and fecundity.

Material and methods

Test insects

Plodia interpunctella culture used in this research originates from population reared for several years in chamber set, at 28 ± 1 °C and relative humidity 60 ± 10%, in transparent plastic containers for mass rearing (1.2 L in volume), on the standard laboratory diet for this moth (Silhacek and Miller, 1972). After eclosion of *P. interpunctella* adults, 50 pairs of one-day-old adult male and female *in copuli* were isolated from containers for mass rearing. Then, *P. interpunctella* adult pairs were placed into 2 L glass jars where females laid eggs. One-day-old eggs were used for the assays.

Maize

As a nutrient medium for development of *P. interpunctella*, three maize hybrids, developed in Institute of Field and Vegetable Crops, Novi Sad, Serbia, were used: NS 6130 (FAO 600, dent kernel type), an experimental NS hybrid (FAO 600, flint kernel type) and NS 1090 (FAO 170-190, semi-flint kernel type). Kernels were not treated with insecticides after the harvest. In order to eliminate the possible presence of any pest, kernels were exposed to deep freezing (-80 °C) before setting up the experiment. Maize kernels were weighted before and after the experiment had been conducted, and the percentage of quantitative losses in maize mass as a result of *P. interpunctella* larval feeding was calculated.

Experimental procedure

Experiment was set up as randomized 3 × 2 × 4 block design type. Three maize hybrids were used, in two different mechanical states (whole and broken

kernels). Each maize hybrid and kernel state was repeated four times, with a total of 24 replications. Each of 24 glass jars (0.25 L in volume) contained 100 g of mentioned kernel type and state, in which 100 one-day-old *P. interpunctella* eggs were added. Jars were sealed with swab of cotton paper coated with cotton cloth, for proper aeration. The jars were kept at chamber set, at 28 ± 1°C, r.h. 60 ± 10% and 14:10 (L:D) photoperiod.

After the emergence of the adults began, assays were checked once in every 12 h and the number of the emerged adults and their gender were recorded. Newly emerged unmated adults from the same assay were immediately paired. Each pair *in copuli* was isolated in a separate test tube in order to obtain data on longevity of adults and mean fecundity. The number of eggs laid per female was recorded daily until the females died. The mean development duration (MDD) was calculated for each *P. interpunctella* adult. MDD was defined as the average time (in days), from the start of the experiment to each adult emergence. Based on the percentage of adult emergence and the mean values of MDD, the susceptibility index (Dobie, 1974) was calculated, according to the Howe's method (1971). Susceptibility indices were rated according to Mensah (1986), who classified indices in five categories, from resistant to highly susceptible.

Data analysis

Data were statistically analyzed using the software package IBM SPSS Statistics 21. The results were presented as mean ± standard error (SE). Differences in number of emerged adults, mean developmental duration, adult longevity, fecundity, susceptibility indices and quantitative losses to maize among three tested maize hybrids and two tested kernels states were analyzed using one-way ANOVA and post hoc Bonferroni test. Comparisons between two mean values were analyzed by Independent-sample t test, while for comparing the mean values with literature data, one-sample t test was used. Pearson's test was used to establish correlation among the number of emerged adults and quantitative losses to maize. The level of confidence ($P < 0.05$) was used in all analyses.

Results and discussion

Adult emergence and mean developmental duration

The mean number of emerged adults and mean developmental duration (MDD) are presented in Table 1. Higher mean number of emerged adults was recorded on broken kernels (from 47.00 to 59.00) than on the whole ones (from 0.75 to 3.25). There were no statistically significant differences in the number of emerged adults among three maize hybrids in the state of whole ($P = 0.181$) and broken ($P = 0.128$) kernels. However, the number of emerged adults between

two states of kernels of the same hybrid was statistically different in all three tested hybrids (dent: $P < 0.0005$; semi flint: $P = 0.001$; flint: $P = 0.026$).

Table 1. Mean number of emerged adults, mean developmental duration and mean susceptibility index (±SE) of *Plodia interpunctella* on three maize hybrids from Serbia

Maize hybrid	*Kernel state*	*Mean number of emerged adult ± SE*	*Mean developmental duration ± SE*	*Mean susceptibility index ± SE* *
NS 6130 (dent)	whole	3.25 ± 0.85^{a1}	38.7 ± 3.003^{a1}	2.68 ± 1.01^{a1} (MR)
	broken	57.00 ± 2.48^{b2}	35.55 ± 0.29^{b1}	11.37 ± 0.19^{b2} (VS)
NS 1090 (semi flint)	whole	0.75 ± 0.75^{a1}	43.3^{a1}	0.635 ± 0.635^{a1} (R)
	broken	59.00 ± 2.61^{b2}	42.02 ± 1.01^{c1}	9.82 ± 0.29^{b2} (S)
Experimental hybrid (flint)	whole	2.50 ± 1.04^{a1}	48.2 ± 2.12^{a1}	1.73 ± 0.66^{a1} (R)
	broken	47.00 ± 5.87^{b2}	36.5 ± 1.42^{b2}	10.58 ± 0.27^{b2} (VS)

Vertical mean values of number of emerged adults, MDD and susceptibility indices having different letter (a, b, c, for compared different states of kernel) or different number (1, 2, for compared different types of kernel) in superscript are statistically different by Independent-samples t test and One-sample t test at $P < 0.05$.

* R - resistant; MR - medium resistant; S - susceptible; VS - very susceptible (Mensah, 1986).

On all three tested maize hybrids, MDD was shorter on broken (from 35.55 to 42.02 days) than on whole (from 38.7 to 48.2 days) kernels. The shortest MDD was observed in assays with broken dent kernels (35.5 days), while the longest MDD have had moths reared on whole flint kernels (48.2 days). There were no significant differences among MDD of *P. interpunctella* reared on whole kernels, of any of the three tested hybrids ($P = 0.150$). On broken kernels, MDD on semi flint hybrid was significantly longer than on two other hybrids ($P < 0.05$). Within the same hybrid, MDD was significantly longer on broken kernels only on hybrid in flint kernels ($P = 0.005$).

The results on adult emergence and MDD are in accordance to available literature data. All published data show that *P. interpunctella* have higher survival rate and develops significantly faster on broken maize kernels than on whole maize kernels (LeCato, 1976; Mbata, 1990; Kaliyan *et al*., 2005; Predojević *et al.*, 2017). For example, mean survival oh whole maize kernels varied from 28.4 to 35.3 days (Abdel-Rahman *et al*., 1968), i.e. from 37.2 to 42.4 day (Predojević *et al*., 2017) and 34.5 days (Arbogast, 2007), while on broken kernels, 25.9-38 days (Mbata, 1990), 28.5 days (Imura and Sinha, 1986) and 34-38 days (Predojević *et al*., 2017). In work of Predojević *et al.* (2017) the same maize hybrids were tested and *P. interpunctella* adult emergence was much higher on broken than on whole kernels also, but adult emergence in our experiment was significantly higher (almost twice) on broken kernels. The MDD

was almost the same in both studies. This difference was caused by the fact that in their study the surface of the substrate was disturbed every five days because of collecting the larvae for head capsule width measurements. Higher disturbance of maize kernels lead to the lower number of survived larvae and hence, the lower adult emergence. Effects of substratum disturbance was also confirmed by Predojević *et al*. (2016), who reported that disturbance of corn flour negatively influenced the adult emergence and mean developmental duration of *P. interpunctella*.

Susceptibility of maize hybrids to infestation by Plodia interpunctella

Susceptibility indices for the development of *P. interpunctella* on three maize hybrids from Serbia are given in Table 1. Based on our results, broken kernels are much more susceptible (indices values from 9.82 to 11.37) to infestation by *P. interpunctella* than whole kernels (indices values from 0.635 to 2.68). Whole kernels of tested semi flint and flint hybrids were resistant to infestation, while whole kernels of tested dent hybrid were medium resistant (Table 1). On the other hand, broken kernels of tested semi flint hybrid were susceptible to infestation by *P. interpunctella*, while broken kernels of tested dent and flint hybrids were very susceptible (Table 1). Susceptibility of maize kernel was only influenced by the state of kernels ($P < 0.05$) (Table 1). Many studies pointed out that the integrity of maize kernels is crucial for protection of stored maize (Mbata, 1990; Kaliyan *et al*., 2005; Predojević *et al*., 2017). They all reported that whole kernels are much less susceptible to attack by *P. interpunctella* larvae. Predojević *et al.* (2017) tested the susceptibility of same three maize hybrids to infestation by *P. interpunctella*, but with the factor of disturbance the surface of the maize kernels included in experimental procedure. These results are in accordance with our findings. However, susceptibility indices rates given in their study were more conservative almost in all cases by one rate, due to additional effects of disturbing the surface of the maize kernels. Our study once again confirms that whole kernels are more resistant to infestation by *P. interpunctella* than the broken ones, but also emphasis the significance of disturbance of the surface of the stored maize kernels. More frequent disturbance of stored kernels layers lead to the higher resistance of maize kernels to infestation by *P. interpunctella*.

Adult longevity and fecundity

The mean adult longevity and fecundity of *P. interpunctella* reared on broken kernels of three maize hybrids from Serbia are presented in Table 2. Data are presented only for broken kernels, because the number of emerged adults on whole kernels was very low and the emergence period was prolonged so we were not able to obtain sufficient data for statistical analysis. The highest mean adult longevity

was recorded on dent kernels (5.04 ± 0.13 days), while the lowest on semi flint kernels (4.8 ± 0.13 days). Females reared on dent kernels lived longer than on the other hybrids (4.88 ± 0.2 days), while among males, those reared on flint kernels (5.26 ± 0.12 days). Based on our results, males lived longer on all tested maize hybrids, but there were no significant differences in longevity between and inside the groups of males and females reared on tested hybrids ($P > 0.05$).

Mean fecundity was the highest for the moths reared on flint kernels (114.97 ± 8.22), while the lowest for those reared on dent kernels (104.51 ± 4.9). There were no significant differences in fecundity among different kernel types (i.e. maize hybrids) in the state of broken kernels ($P = 0.547$). In our study, adult longevity and fecundity were not influenced by the maize hybrid, i.e. the kernel type. Only a few studies published data on fecundity of *P. interpunctella* reared on maize. Abdel-Rahman *et al.* (1968) first reported that fecundity varied from 267 to 344, depending on variety of maize. Allotey and Goswami (1990) published that an average fecundity of moth on broken maize kernels from Nigeria was 174.2, while Arbogast (2007), in his study with farm-stored maize in South Carolina, USA, reported an average fecundity of 208.1. Mainly, fecundity is not highly influenced by the maize hybrid. Predojević *et al.* (2017) compared the fecundity data of *P. interpunctella* reared on the same maize hybrid as ours, but on whole, broken and ground kernels, with include disturbance of the maize substratum surface during the larval stage. They found that maize type does not influence the average fecundity on broken kernels, which varied from 109 to 113. These results show that neither the type of kernel nor the disturbance influence the fecundity of *P. interpunctella*. Mbata (1990) tested thirteen maize varieties from Nigeria and found that some hybrid varieties were more attractive for female moth to lay their eggs on to, because these kernels possess some attractants for *P. interpunctella*.

Table 2. The mean adult longevity and fecundity (±SE) of *Plodia interpunctella* on broken kernels of three maize hybrids from Serbia

Maize hybrid	*Adult longevity ± SE*			*Fecundity*		
	Females	*Males*	*Total*	*Mated females*	*Range*	*Mean ± SE*
NS 6130 (dent)	4.88 ±0.2	5.21 ± 0.17	5.04 ± 0.13	57	7-192	104.51 ± 4.9
NS 1090 (semi flint)	4.68 ± 0.16	4.92 ± 0.2	4.8 ± 0.13	66	4-242	108.74 ± 6.00
Experimental hybrid (flint)	4.59 ± 0.25	5.26 ± 0.12	4.92 ± 0.08	38	22-205	114.97 ± 8.22

Quantitative losses to maize due to food consumption of larvae

Mean percentage of quantitative losses in maize mass which arise from the food consumption by the *P. interpunctella* larvae are presented in Figure 1. Quantitative losses were significantly higher on broken kernels than on the whole ones in all tested hybrids ($P < 0.05$). The losses in maize mass among the hybrids were not significantly different on broken kernels ($P = 0.23$), while on whole kernels, the losses of flint type were significantly lower than on two other hybrids ($P < 0.05$). Pearson's test of correlation found significant strong positive correlation between the number of emerged adults and losses in maize mass ($R = 0.861$, $P < 0.005$). Our result point out that the maize hybrid does not influence the quantitative losses, but the state of kernels does.

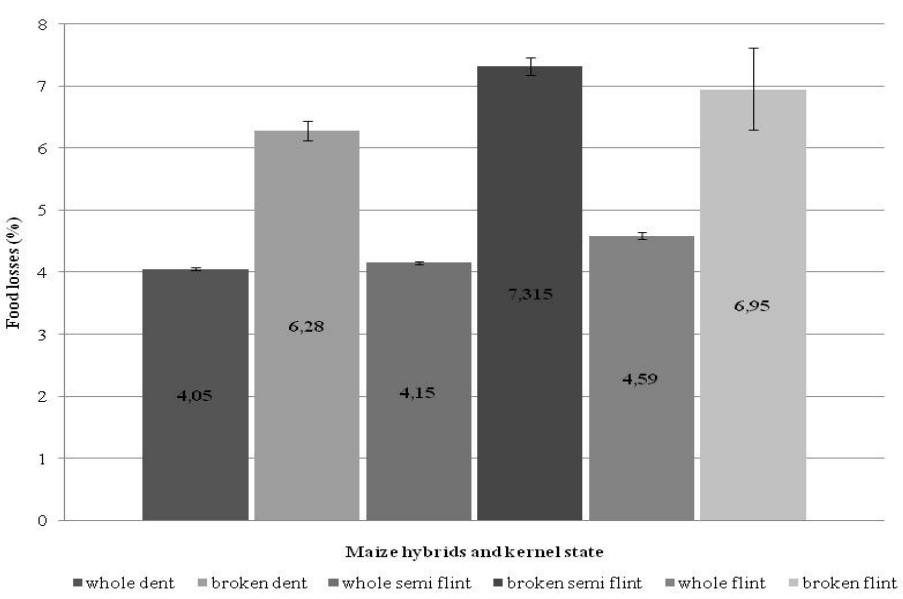

Figure 1. Mean percentage losses in maize mass caused by *Plodia interpunctella* larval feeding

Conclusion

Based on the results of our study, we can conclude that development of *Plodia interpunctella* is poorly influenced by the maize hybrid, i.e. kernel type. On the other hand, kernels state is a crucial parameter from which the success of *P. interpunctella* development is depended on, with broken kernels being much more suitable food for its development. Also, disturbance of kernels during infestation and development of early stages of

this moth leads to its lower survival and longer developmental period. Our results point out two major conclusions. First, the integrity of kernels should be preserved throughout the storage period and second, the disturbance of the kernels during the storage i.e. proper aeration of upper layers of stored maize should be used as additional method for reducing the number of *P. interpunctella*.

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Morphological and physiological response of a green-leaved and a purple leaved cultivar of sweet basil (*Ocimum basilicum* L.) to biosolids amendments

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Abstract: In order to assess the fertilization potential of biosolids for *Ocimum basilicum* L. crop, a series of morphological and physiological parameters were investigated in a pot experiment. Two basil cultivars, green-leaved Aromat de Buzau and purple-leaved Violet de Buzau were grown on different substrates, eroded soil 100%, biosolids 100% and a mixture of eroded soil 85% + biosolids 15%. Results showed that biosolids amendments to the eroded soil led to significantly increases on the fresh mass (+ 273%), plant height (+ 82%) and lateral stems (+ 127%) compared to the eroded soil in the case of green-leaved cultivar, while fresh mass and lateral stems significantly increased with + 120 % and + 28% respectively, in the case of purplea-leaved cultivar. Chlorophyll content and photosintetic activity of both basil cultivars were higher in biosolids 100% treatment compared to all the other treatments. Overall, eroded soil negatively impacted the investigated morpho-phisiologycall parameters of basil plants, while biosolids amendments significantly improved the growth and development of plants probably due to the large amounts of nutrients and organic matter.

Key words: basil, biosolids, chlorophyll content, eroded soil, photosynthesis

Introduction

Biosolids are sanitized sewage sludge that results from the waste water treatment plants (Burducea et al., 2016). Because larege amounts of sewage sludge are produced every year, approx. 10 milions t in European Union (EU)

the Sewage Sludge Directive (86/278/EEC) encourage the use of biosolids in agriculture as it contains high amounts of macronutrients and organic matter.

According to EUROSTAT (2017) at the EU level, there are countries that use the greatest percentage of sludge in agriculture. In the year 2012 Spain, Germany and United Kingdom were the largest sludge producers in EU, with 2577.2, 1844.4 and 1078.4 thousands t dry matter (d.m.) of which 74.5%, 29.3% and 78.2% were used in agriculture. On the oher hand, Hungary, Greece and Romania, despite the fact that they produced considerably amounts of sludge, its utilization in agriculture was limited (157.7, 118.6, 48.4, thousands t d.m. out of which only 9.5%, 11.8% and 4.1% were used in agriculture). The lack of awareness about the its benefits as a fertilizer, fear of contamination with heavy metals, the costs of sludge transport at agricultural site and costs for the environmental quality monitorization, are some reasons why the agricultural use of sludge is limited in some countries.

Most scientific papers shows positive effects of sludge on crop yield, due to the macro nutrients and organic matter that are present in the sludge (Cornfield et al., 1976; Vaca et al., 2011; Özyazıcı, 2013; Chrysargyris and Tzortzakis, 2015) and also on soil quality, by improving the physico-chemical properties suc as soil bulk density, aeration and even stabilization of eroded soils (Holz et al., 2000; Ros et al., 2003; Gu et al., 2013; Mihalache et al., 2014). Negative reports are related to the increase in heavy metals in plants and soils, pathogens and esthetic alteration of environment through smell (Singh and Agrawal, 2007; Mazen et al., 2010; Vaitkute et al., 2010; Collivignarelli et al., 2015). Regarding the physiology of plants cultivated on sludge amended soils, there are relatively few reports in the scientific literature (Singh and Agrawal, 2010).

Ocimum basilicum L. is a widely used medicinal and aromatic plant, well known for the volatile oils and phenolic compounds synthesized. Research indicate that basil can be grown on municipal sludge (biosolids) amended substrates (Zheljazkov and Warman, 2004), showing an increase in biomass while producing an essential oil free of contaminants. Since increasing agricultural land areas are affected by erosion (Darie and Ionita, 2013), the current paper aimed to quantify the morpho-physiological performance of basil plants cultivated on a highly eroded soil amended with municipal biosolids.

Material and methods

Experimental conditions

The experiment consisted of growing *Ocimum basilicum* L. plants in laboratory controlled conditions in various substrates. The basil cultivars used were the green-leaved Aromat de Buzau and the purple-leaved Violet de Buzau.

The seeds were obtained from the Agricultural Research and Development Station at Buzau, Romania.

For the growth of basil plants, 1 L plastic pots were used. The treatments were (v/v): eroded soil 100 %, eroded soil 85% + biosolids 15 %, biosolids 100 % and control (commercial substrate). The biosolids were obtained from the water treatment plant from Raducaneni commune, Iasi County, Romania. The concentration of heavy metals did not exceed the maximum admissible values for agricultural use. The soil was collected from an agricultural land situated at 46°20'29.0"N 27°41'44.6"E in Vaslui County, Romania and was represented by a loamy chernozem highly eroded by agricultural practices and environmental conditions. The physico-chemical characteristics were assessed at the County Office for Soil and Agrochemical Studies – Iasi (Table 1).

Each pot was seeded with 10 basil seeds. The plants were thinned at 2 individuals per pot after 1 week. Each treatment consisted of 10 pots. The pots were kept at constant temperature regimes, between 22 °C (night) and 25 °C (day). Artificial light was supplied by 4800K fluorescent tubes for 14 h photo period. The atmospheric humidity was relatively constant, around 50%. Plants were irrigated twice a week with distilled water.

Table 1. Physico-chemical properties of the eroded soil and sludge used in experiment.

Sample	pH	EC ($\mu S\ cm^{-1}$)	N (%)	P_2O_5 (% d.m.)	K_2O (% d.m.)	OM (%)
Eroded soil	8.05	221.4	0.068	0.09405	0.11689	1.24
Biosolids	6.75	4210	5.11	2.8	0.68	67.5
Comercial soil	6.5	509.1	0.41	0.4405	1.1626	41

(pH – potential of hydrogen, EC – electrical conductivity, OM – organic matter)

Morphological parameters

For each treatment, 8 individuals were assesed for stem height, number of lateral stems and fresh mass.

Physiological measurements

The photosynthetic activity was measured with an ADC Bioscientific LCi apparatus. Measurements were assessed during the light regime on 6 plants per each treatment and 3 leaves per plant.

Assimilatory pigments content was measured with a non-destructive portable chlorophyll content meter (SPAD 502) that measures optical absorbance, the readings being expressed as SPAD units on 3 leaves (flower, middle and upper regions of the plants) from 6 individuals each per treatment.

Statistical analyses

The statistical analyses conducted were represented by analyses of variance among treatments and the Tukey post hoc test at $p<0.05$, the results being expressed as means and standard errors.

Results and discussion

Regardless of the cultivar, basil plants recorded the lowest values for all the morphological parameters when were cultivated on the eroded soil. In the case of the green-leaved cultivar, biosolids amendments (15%) led to significantly increases on the fresh mass (+ 273%), plant height (+ 82%) and lateral stems (+127%) compared to the plants cultivated on eroded soil (Table 2). Also, plants cultivated on biosolids alone had significantly higher values for the investigated morphological parameters compared to plants cultivated on eroded or control soils, values that are comparable to those at biosolids 15% treatment.

Table 2. Growth parameters of green-leaved basil Aromat de Buzau cultivated on eroded soil amended with biosolids.

Treatment	Fresh mass (g)	Height (cm)	Stems (no.)
Control	31.21^{b}±0.7	40.13^{a}±1.73	17.25^{a}±0.37
Erroded soil	17.22^{a}±0.69	38^{a}±1.51	12.75^{a}±0.53
Biosolids 15%	64.35^{d}±0.7	69.19^{b}±3.42	29^{b}±2.1
Biosolids 100%	60.43^{c}±0.78	68.63^{b}±3.27	26.38^{b}±3.85

(values are means of 8 measurements ± standard errors, different superscript letters represent significant differences at $p < 0.05$)

Results for fresh mass and stems number of purple-leaved basil were the highest at biosolids 15 % treatment (+ 120 % and + 28 % respectively, compared to eroded soil) (Table 3). Regarding the plant height, all treatments including the control one led to significantly increases compared to eroded soil variant. Biosolids contain large amounts of nutrients and organic matter that are ready available for plants. Futhermore, biosolids may have also improved the physico-chemical properties of the eroded soil leading to better plant growth and development.

Similar results were obtained for other species, under biosolids cultivatio, with significant positive influences on similar parameters (Costa et al., 2010; Özyazıcı, 2013) but also for basil (Kashani et al., 2013). Some species may be sensitive to the toxic effects of sludge amendments, with reductions in root lengths and other morphological traits (Vaitkutė et al., 2010; Oleszczuk et al., 2012), however such effects were not recorded for basil grown on the analyzed substrates. The low performance of basil plants cultivated on eroded soil can be

explained by the low concentration of available nutrients and low organic matter content that affect the nutrients uptake by plant roots. The source and rate of fertilization with biosolids influences the availability of some essential nutrients such as magnesium (Chowaniak and Gondek, 2009).

Table 3. Growth parameters of purple-leaved basil Violet de Buzau cultivated on eroded soil amended with biosolids.

Treatment	Fresh mass (g)	Height (cm)	Stems (no.)
Control	$21.51^{b}\pm0.78$	$40.75^{b}\pm1.28$	$4.75^{a}\pm0.37$
Erroded soil	$14.74^{a}\pm0.75$	$31.25^{a}\pm0.53$	$4.5^{a}\pm0.63$
Biosolids 15%	$32.48^{c}\pm0.78$	$40.25^{b}\pm0.82$	$13.5^{b}\pm1.55$
Biosolids 100%	$31.44^{c}\pm0.82$	$38^{b}\pm1.54$	$12.75^{b}\pm1.73$

(values are means of 8 measurements ± standard errors, different superscript letters represent significant differences at $p < 0.05$)

Significanty higher photosyntetic activity was induced by biosolids amendments compared to the eroded soil only in the case of the purple-leaved basil (Table 4). Regarding the chlorophyll content expressed as SPAD units, biosolid 100% and biosolids amendments 15% led to significantly increases compared to plants cultivated on eroded and control soils for the bowth basil cultivars.

The elevated chlorophyll content of basil leaves cultivated on biosolis may be influenced by the higher nitrogen levels in substrate. Although some species may display reduction of chlorophyll contents under sludge amendment, this may be due to metal toxicity (Singh and Agrawal, 2007).

Table 4. Photosynthesis and chlorophyll content of green-leaved and purple-leaved basil cultivated on eroded soil amended with biosolids.

	Aromat de Buzau		Violet de Buzau	
Treatment	Photosynthsis (μmol CO_2 m^{-2} s^{-1})	Chlorophyll (SPAD units)	Photosynthsis (μmol CO_2 m^{-2} s^{-1})	Chlorophyll (SPAD units)
Control	$0.4^{ab}\pm0.03$	$25.99^{a}\pm0.65$	$0.31^{a}\pm0.03$	$27.49^{a}\pm0.64$
Erroded soil	$0.57^{ab}\pm0.05$	$25.04^{a}\pm0.54$	$0.36^{a}\pm0.03$	$27.52^{a}\pm0.87$
Biosolids 15%	$0.56^{ab}\pm0.07$	$29.73^{b}\pm0.87$	$0.61^{b}\pm0.05$	$31.86^{b}\pm1.01$
Biosolids 100%	$0.65^{bc}\pm0.05$	$34.31^{c}\pm0.57$	$0.67^{b}\pm0.04$	$35.72^{c}\pm1$

(values are means of 27 and 18 measurements for photosynthesis and chlorophyll content respectvely ± standard errors, different superscript letters represent significant differences at $p < 0.05$)

A positive correlation between nitrogen levels and chlorophyll amounts of leaves has been shown for several species (Mazen et al., 2010; Zhang et al.,

2013) and occurs due to the fact that nitrogen is included in the structure of chlorophyll molecules (Martinez and Ramos, 2015).

Other research regarding the usefulness of biosolids as fertilizers are also reported in Romanian literature. Biosolids were used especially for the high amounts of macronutrients and organic matter which improves soil quality and increase crop yield. For instance, in a two year study, 1994-1996, yield of maize (*Zea mays*) increased by 199.9% when it was cultivated on a soil amended with 10% sludge compost (Stan, 1996). Also, Ailincai et al., (2011), conducted a five year-crop rotation, winter rape-wheat-maize-sunflower-wheat obtaining increased yield of winter rape by 187% using 40 t ha^{-1} sewage sludge. Thus, the biosolids from Romanian waste water treatment plants can be used, with very good performances, for fertilization as well as soil conditioner, especially for the cultivation of higher crops but also for some medicinal plants such as basil, that are grown for the production of the essential oils.

Conclusions

The tested biosolids, can be used as amendments to eroded soil for basil plants cultivation with positive, fertilizing, effects on plant growth and development. The sludge exerts little toxicity on plant and the investigated physiological parameters were not affected. Further testing may be conducted to optimize biosolids dosage in order to improve eroded soil properties for basil crop fertilization.

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Toxic effect of caraway essential oil on adults of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae)

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Abstract: Yellow Mealworm Beetle *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae) is a stored-products pest from the family Tenebrionidae. It is widelyspread all over our country, particularly in mills. The damage is not reflected that much in destroying, but in the contamination by the waste products of insects which make the product unusable. The conventional methods of stored-products pests control implies the usage of insecticides. The constant pesticides application has led to reduced efficiency and resistance development. That caused the need for alternative ways of protection. Taking it into consideration, within this experiment the toxic activity of essential oil of caraway has been tested. The tested concentrations were: 150, 200, 250, 300 and 350 µl/ml. Four replications for all five tested concentrations were set up, including the controls. Ten insects were put in every Petri dish. The mortality was recorded after 24, 48 and 72 hours. After 24h caraway essential oil had the highest contact toxicity at the concentration level of 350 µl/ml, with the mortality rate of 20%. The mentioned concentration led to the mortality rate of over 60% after 48 hours.

Key words: *Tenebrio molitor*, insecticidal effect, caraway essential oil, contact toxicity

Introduction

In recent years essential oils have drawn attention due to their insecticidal effect as potential agents in pest control. As by-products of plant metabolism they are regarded as evaporable secondary metabolites of plants which are the mixture of mono and sesquiterpenes. The biological activity of essential oils depends on their chemical composition, the part of the plant they have been extracted from, phenological state of the plant, environmental conditions and the

extraction methods (Ukeh & Umoetok, 2011). Most essential oils are active in low concentrations (0,1 g/kg of food), which makes their broader application possible (Šućur, 2015) taking into consideration the fact that most of them are not toxic to mammals, birds and fish (Motazedian *et al.*, 2012).

The literature data say that citronella and eucalyptus oil (Batish *et al.*, 2008) are repellents to mosquitoes, cinnamon oil to aphids and mites, eugenol from basil or clove to various insects (Glare, 2015).

Caraway (*Carum* carvi) is an annual or biannual herbaceous plant with axial root system from *Apiaceae* family. The name of the genus is derived from the Greek *kar* or *kara*, which means „head“, due to the appearance of its inflorescence (Šarović, 2012). The main components of essential oils are considered responsible for its biological activity, which in the case of caraway are monocyclic monoterpenes carvone and limonene (Ebadollahi, 2013).

Yellow mealworm *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae) is a stored-products pest widely spread in our country, especially in mills. It is usually found in long-stored flour, factories of animal feed, on cereals, on a variety of packaging, mostly in dark, neglected places (Čamprag *et al.*, 2001). The damage is not reflected that much in destroying, but in the contamination by the waste products of insects which make the product unusable. It causes serious damage to packaging which the larvae bite through with their strong jaws as well as to the wooden parts in which they pupate.

Considering the damaging effects of stored-products pests the study of biological effect of caraway essential oil on the adults of *T. molitor* was carried out.

The need for the investigation primarily comes from the necessity to reduce the use of conventional insecticides and to give preference to the secondary biomolecules, the effect of which has not yet been fully studied. Since there are no data about the toxic effects of caraway on the yellow mealworm, the biological activity of this significant plant, whose essential oil has been extracted, is being studied.

The aim of the paper was to examine, by means of biological tests, the contact toxicity of caraway essential oil applied in different concentrations on the adults of species *T. molitor*.

The objective was to evaluate the insecticidal potential of the essential oil of caraway, obtained in the distillation by water vapor, as well as the possibility of its application as a means of biological struggle in the yellow mealworm control.

Material and methods

For the experiment the adults of *T. molitor* were used, grown in a thermostat on the nourishing substrate, under the controlled environmental conditions (temperature 24°C, humidity 60%), in the Laboratory for entomology at the

Faculty of Agriculture in Novi Sad. The caraway essential oil was extracted from the fruit (fructus) by hydrodestilation (HD) with n-hexane as an organic solvent/recipient, collected on a private farm at Mošorin (N45^{0}17'28.113 E20^{0}11'43.80936). The extraction of the essential oil was carried out in the Biochemistry Lab at the Faculty of Agriculture in Novi Sad and its composition was analyzed chromatographically at the Institute for Public Health in Belgrade, Laboratory for chromatography.

The chromatographic analysis of essential oil was carried out by recording of the mass spectrums of the detected components by gas chromatography with mass spectrometry (GC-MS). After confirming the components through retention times and Kovats indices, their quantification was done by gas chromatography with flame ionization detector (GC-FID).

The experiment was set in Petri dishes. There were 4 replications for each of 5 concentrations and 4 replications for control. The studied concentrations were: 150, 200, 250, 300 and 350 µl/ml. In each Petri dish there was some filter paper onto which 50 µl of a specific concentration of essential oil was placed, while in controls the same volume of n-hexane was spread. Then a disc made from water and wheat flour was placed in every Petri dish so as to exclude the stress factor as a possible mortality cause. Finally, 10 adult insects of yellow mealworm were put in each Petri dish, which were closed and sealed with parafilm. During the experiment the insects were kept in a thermostat, under the controlled environmental conditions of 24°C and 60% humidity.

The mortality rate, due to the effect of studied essential oil, was observed after 24, 48 and 72 hours.

To establish the statistical significance of the essential oil concentration (c), replication (r) and time (t) effect as the independent variables on the mortality rate as the dependent variable multifactorial and monofactorial analysis of variance (ANOVA) was applied by using the software package Statistica 13 (StatSoft, University licence).

Results and discussion

Contact toxicity of caraway essential oil

During the examination of the contact toxicity of caraway essential oil after 24-hour exposition of insects, the essential oil of caraway in the concentration of 350 µl/ml manifested the highest insecticidal effect (20%) on yellow mealworm, whereas at the concentrations of 150 to 300 µl/ml the insecticidal effect was 2.5%. After 48-hour exposition of insects the mortality rate was from 12.5 to 65% depending on the applied concentration, while after 72 hours the mortality rate at the highest concentration (350 µl/ml) of essential oil of caraway was 82.5% (Table 1), which can be regarded to be high efficiency for bioinsecticides.

Table 1. Insecticidal effect of caraway essential oil on adults of *T, molitor*

After 24h	Repetition				
Concentration (µl/ml)	I	II	III	IV	Mortality (%)
350	2	2	1	3	20
300	0	1	0	0	2.5
250	0	0	1	0	2.5
200	0	0	1	0	2.5
150	0	0	0	1	2.5
Control	0	0	0	0	0
After 48h	Repetition				
Concentration (µl/ml)	I	II	III	IV	Mortality (%)
350	4	5	7	10	65
300	4	5	3	7	47.5
250	4	3	3	2	30
200	1	3	2	2	20
150	0	1	1	3	12.5
Control	0	0	0	0	0
After 72h	Repetition				
Concentration (µl/ml)	I	II	III	IV	Mortality (%)
350	8	5	10	10	82.5
300	6	7	4	10	67.5
250	4	4	7	4	47.5
200	1	4	5	5	37.5
150	0	4	1	3	20
Control	0	0	0	0	0

There are various literature sources which speak in favour of the toxic effect of caraway essential oil on insects. Ebadollahi (2013) said that the caraway essential oil showed the contact toxicity on adult insects of maize weevil (*Sitophilus zeamais*), red flour beetle (*Tribolium castaneum*) and German cockroach (*Blatella germanica*). Kim *et al.* (2013) wrote about the insecticidal effect on the maize weevil and red flour beetle, while Ebadollahi and Mahboubi (2011) deal with the toxicity of the studied oil in case of lesser grain borer (*Rhyzopertha dominica*) and rice weevil (*Sitophilus oryzae*).

Statistical data analysis

The highest mortality rate of adults of *T. molitor* (82.5%) was registered by the application of caraway essential oil at the concentration level of 350 µl/ml, after 72 hours.

The multifactorial analysis of variance did not show the statistical significance between mortality rate as the dependent variable and combined effect of essential oil concentration (c), repetition (r) and time (t) as the independent variables. It also showed no statistical significance in case of combined effect of concentration and repetition (pcxr=0.931155 for p<0.05) (Picture 1), nor of combined effect of replication and time (prxt=0.981979 for p<0.05) (Picture 2). However, it showed the statistical significance between mortality rate as the dependent variable and combined effect of concentration and time as the independent variables (pcxt=0.001134 for p<0.05) (Picture 3).

	Univariate Tests of Significance for mortality (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	470,2222	1	470,2222	78,73488	0,000000
concentration (µl/ml)	237,6111	5	47,5222	7,95721	0,000016
replication	19,1111	3	6,3704	1,06667	0,372062
concentration (µl/ml)*replication	44,3889	15	2,9593	0,49550	0,931155
Error	286,6667	48	5,9722		

Picture 1. Effect of concentration and repetition on the mortality rate

	Univariate Tests of Significance for mortality (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	470,2222	1	470,2222	72,65236	0,000000
replication	19,1111	3	6,3704	0,98426	0,406316
time (h)	173,4444	2	86,7222	13,39914	0,000015
replication*time (h)	6,8889	6	1,1481	0,17740	0,981979
Error	388,3333	60	6,4722		

Picture 2. Effect of replication and time on the mortality rate

	Univariate Tests of Significance for mortality (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	470,2222	1	470,2222	238,4225	0,000000
time (h)	173,4444	2	86,7222	43,9718	0,000000
concentration (µl/ml)	237,6111	5	47,5222	24,0958	0,000000
time (h)*concentration (µl/ml)	70,2222	10	7,0222	3,5606	0,001134
Error	106,5000	54	1,9722		

Picture 3. Effect of concentration and time on the mortality rate

The monofactorial analysis of variance showed the statistical significance in the effect of essential oil concentration as an independent variable on the mortality rate as dependent variable (p=0.000002 for p<0.05), while Fisher's

LSD test showed the statistical significance between control and lower or higher concentrations (Picture 4).

Univariate Tests of Significance for mortality (Spreadsheet1)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	470,2222	1	470,2222	88,62827	0,000000
concentration (µl/ml)	237,6111	5	47,5222	8,95707	0,000002
Error	350,1667	66	5,3056		

LSD test; variable mortality (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = 5,3056, df = 66,000

Cell No.	concentration (µl/ml)	mortality Mean	1	2	3	4
1	0	0,000000		****		
2	150	1,166667	****	****		
3	200	2,000000	****			
4	250	2,666667	****		****	
5	300	3,916667			****	****
6	350	5,583333				****

Picture 4. Effect of concentration on the mortality rate with LSD test

The effect of repetition as an independent variable on the mortality rate as dependent variable showed no statistical significance ($p=0.519436$ for $p<0.05$).

The statistical significance was noted for the effect of time as an independent variable on the mortality rate as dependent variable ($p=0.000006$ for $p<0.05$), while Fisher's LSD test showed the statistical significance between the mortality rate observed after 24h and after 48h or 72h (Picture 5).

Univariate Tests of Significance for mortality (Spreadsheet1)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	470,2222	1	470,2222	78,30732	0,000000
time (h)	173,4444	2	86,7222	14,44208	0,000006
Error	414,3333	69	6,0048		

LSD test; variable mortality (Spreadsheet1)
Homogenous Groups, alpha = ,05000
Error: Between MS = 6,0048, df = 69,000

Cell No.	time (h)	mortality Mean	1	2
1	24	0,500000		****
2	48	2,916667	****	
3	72	4,250000	****	

Picture 5. Effect of time on the mortality rate with LSD test

Chromatographic analysis of caraway essential oil

From the obtained results it can be concluded that the main constituents of the caraway essential oil are carvone with 68.22% and limonene with 21.80% in content. The similar results were obtained by Ebadollahi (2013) who identified carvone with 37.9% and limonene with 26.5% as the main components of caraway essential oil. Beside carvone and limonene there were 25 constituents which in total make less than 10% of the studied essential oil. Considering the fact that the main components affect the biological activity of essential oil it can be concluded that the contact toxicity, dealt with in this study, is affected by monocyclic monoterpenes carvone and limonene.

Conclusion

Based on the study of the contact toxicity of essential oil of caraway, applied in the concentrations of 150, 200, 250, 300 and 350 µl/ml, it can be concluded as follows:

- After 24h, out of 5 studied caraway essential oil concentrations, the strongest toxic effect was manifested by the highest concentration of 350 µl/ml, with the mortality rate of 20%. The given concentration caused mortality of over 60% after 48 hours.
- The highest mortality rate of adults of *T. molitor* (82.5%) was registered by the application of caraway essential oil at the concentration level of 350 µl/ml, after 72 hours.
- Chromatographic analysis showed that the main constituents of the caraway essential oil are carvone with 68.22% and limonene with 21.80% in content.
- The multifactorial analysis of variance showed the statistical significance between mortality rate as the dependent variable and combined effect of concentration and time as the independent variables.
- The monofactorial analysis of variance showed the statistical significance in the effect of essential oil concentration as an independent variable on the mortality rate as dependent variable, while Fisher's LSD test showed the statistical significance between control and lower or higher concentrations.
- The statistical significance was noted for the effect of time as an independent variable on the mortality rate as dependent variable, while Fisher's LSD test showed the statistical significance between the mortality rate observed after 24h and after 48h or 72h.

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Postharvest diseases on stored ginger /*Gingiber officinale* Roscoe / and banana /*Musa sp./*

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Abstract: Post-harvest diseases can cause losses of banana fruits and ginger rhizomes both in terms of quality and quantity. Infected fruits have no market value. Many of post-harvest pathogens produce secondary metabolites as mycotoxins which pose serious threats to human health. The present article reported results of the preliminary study of the post-harvest pathogens of storage banana fruits and ginger rhizomes. Koch's postulates were applied to determine the etiology of the observed damages. In result of biological analysis of storage banana fruits and ginger rhizomes from markets in the Plovdiv were obtained 91 isolates. Eleven of them from banana and thirteen from ginger rhizomes showed positive pathogenicity reaction. Based on initial cultural and morphological characteristics two of isolates from ginger were determined as *Fusarium sp.* and these from banana belong to the *Colletotrichum musae*, *Fusarium sp., Botryosphaeria sp., Penicillium sp* and *Aspergillus niger.*

Key words: banana, ginger rhizomes, postharvest diseases, storage

Introduction

Fresh fruits are a major source of essential vitamins and minerals, such as vitamin A, vitamin C, and potassium, needed for human well-being.

The rhizome of ginger (*Zingiber officinale* Roscoe) and banana (*Musa paradisiaca* L.) are imported fruits for Bulgaria. Ginger is one of the most important spices in the world, and it is used as raw material in the food industry, pharmaceuticals, and cosmetics. Ginger descent from India from where it was introduced to Africa and Caribbean, however, no definite information on the primary center of domestication of ginger is available (Prabhakaran, 2013). It is now cultivated throughout the humid tropics and is a most widely used spice

worldwide. India, China, Indonesia, Nigeria, the Philippines, and Thailand are currently the main producers (Meadows, 1998; Plotto, 2002).

Banana fruit is one of the most important commercial fruit crops which grown all over the world in the tropical and subtropical areas. Major banana producing countries are India, China, Philippines, Brazil, Ecuador, Indonesia, Costa Rica, Mexico, Thailand and Colombia (Nath et al., 2015).

Losses due to postharvest disease may occur at any time, from harvest to consumption. When estimating postharvest disease losses, it is important to consider reductions in fruit quantity and fruit quality, as some diseases may not render produce unsaleable yet still reduce product value (Coates and Johnson, 1997). The temperature is the main factor determining the postharvest life of fruits and markedly affect their rates of respiration and general metabolism (Wills et al., 1998). Optimum storage conditions for mature ginger rhizomes can be stored at 12 to 14 °C with 85 to 90% relative humidity (RH) for 60 to 90 days. Storage at 13 °C with 65% RH leads to extensive dehydration and a wilted appearance (Akamine 1962, Paull et al. 1988). Superficial mould growth can occur if condensation collects on rhizomes, especially on the broken ends. Decay can be caused by rough harvesting and handling practices which result in injury to the skin and flesh of the rhizomes. Postharvest losses from diseases are caused by various microorganisms (Kaushal et al., 2017). According to Moreira et al., (2013) several pathogens as *Fusarium sp*. and *F. oxysporum, Lasiodiplodia theobromae* were able to cause rot of ginger rhizomes.

Banana fruits storage life decreased as temperature increased over the 15-35 °C (Marriott, 1980). According to Akter et al. (2013) the maximum anthracnose (*Colletotrichum musae*) level (44.58%) was observed in the treatement of 10°C in the var. Amritasagar, but cooling at 10°C least disease observed in fruits of all varieties. Important postharvest diseases of banana fruits are anthracnose (*Colletotrichum musae*)*,* crown rot (complex fungi, including *Fusarium spp., Verticillium spp., Acremonium sp. Colletotrichum musae*), black end (various pathogens including *Nigrospora sphaerica, Colletotrichum musae, Fusarium spp.*), Ceratocystys fruit rot caused by *Thielaviopsis paradoxa* (Coates and Johnson, 1997).

The most frequent pathogens on storage banana fruits are Fusarium semitectum, *Lasiodiplodia theobromae, Alternaria alternata* (Abd-Alla et al., 2014).

According to Antonissen et al. (2014) mycotoxins are secondary metabolic processes of fungi. They can be classified as field or storage fungi, such as the *Fusarium* species, produssed mycotoxins on the corps in the field, whereas storage fungi such as the *Aspergillus* and *Penicillium* species, produssed mycotoxins on the corps after harvesting.

For these reasons, the purpose of our study is to determine the etiology of the injured banana fruits and ginger rhizome.

This study aimed to define the post-harvest etiology of stored banana fruits and ginger rhizomes. To our knowledge, this is the first report of pathogens associated with injury of banana fruits and ginger rhizomes in our country.

Material and methods

Sampling of ginger rhizomes and banana fruits

A total of ten fruit markets and hypermarkets in Plovdiv town were surveyed for postharvest disease from December 2016 to May 2017. From each market, three banana and gingers rhizomes were considered for sample collection and taken for disease diagnosis in the laboratory.

Koch's postulates were applied to determine the etiology of injured banana fruits and ginger rhizomes.

Isolation

The visual damaged part from banana fruits and ginger rhizomes was cut into small bits, sterilized with ethanol and washed with sterilized distilled water and then transferred aseptically on potato dextrose agar (PDA) medium in Petri dishes. The Petri dishes were incubated at room temperature 24˚C for a development of fungus growth. The plates were observed daily, the initial growth observed was picked up aseptically, and it was transferred to PDA slants.

Pathogenicity test

The isolated pathogens have been subjected to pathogenicity test. Mature and semi ripen visual healthy banana fruits were collected from fruit market of Plovdiv and brought to the laboratory. The fruits were then surface sterilized by ethanol (70%), followed by washing with sterilized water and air dried and then separately inoculated with each of the isolates by Pin-Pricking method (Nath et al., 2015). Six fruits were separately inoculated with each of the isolates with three replicates for each isolate. For control banana fruit were pin-pricking with a sterile needle. The inoculated fruits, as well as control, were placed in sterilized, loosely tied polyethylene bags. A piece of sterilized wet absorbent cotton has been put inside each bag, and the bag was kept at 24˚C for symptoms development. Inoculated fruits were observed regularly. The test was reported on positive when there were necrotic spots and mycelial growth on inoculated places.

The first pathogenicity test for ginger assessed the ability of the isolates to colonize the cut surface of rhizomes. Healthy rhizomes were washed in tap water and sanitized by immersion in ethanol and cut with a flame-sterilized knife into slices about 3 mm thick. A mycelial disc of 9 mm diameter was taken from 10-day

old culture and then was placed on each slice of ginger rhizome. The control slices were no inoculated. The pathogenicity test was in a completely randomized design with three replications. The samples were incubated at 24ºC in a humid chamber on a previously sterilized Petri dish with a layer of filter paper moistened with sterile distilled water. The test was reported on positive if there was mycelial growth and rot on ginger slices (Moreira et. al 2013).

Cultural and morphological characterization of isolates

Cultural characteristics (color and density colonies, fruiting bodies, sclerotium-like bodies) were determined on PDA. Morphological characteristics (spore shape, color, size), were determine using microscopic method

Results and discussion

Symptoms that have been observed on banana fruits were rusty spots slightly sunken, small rugged dots with diameter approximately 1÷2 mm, brown spots with a black dot in the middle to blackish sunken spots, crown rot, and tip end rot. There were obtained 50 isolates from damaged fruits. Eleven of them showed a positive pathogenicity reaction (Table 1). No pathogen was isolated from any of the controls. On the basis of the preliminary cultural (colony and density, pigmentations, fruiting bodies) and morphological (spore shape, color) characteristics different fungi from different damaged\rotten parts of banana were discovered such as *Fusarium sp.* isolated of red spots on pericarp, *Colletotrichum musae* isolated of brown spot\pulp rot, *Botryosphaeria sp*. isolated of crown rot, blue molt *Penicillium sp* and *Aspergillus niger* isolated of tip end rot.

Table 1. Isolates from storage banana fruits and pathogenicity test

Isolates	**Crown rot**	**Brown spots\ pulp rot**	**Tip end rot**	**Red spots on pericarp**
Number of isolates	7	21	4	18
Pathogenicity test	2	3	2	4
Positive	1	2	2	3
Negative	1	1	2	1

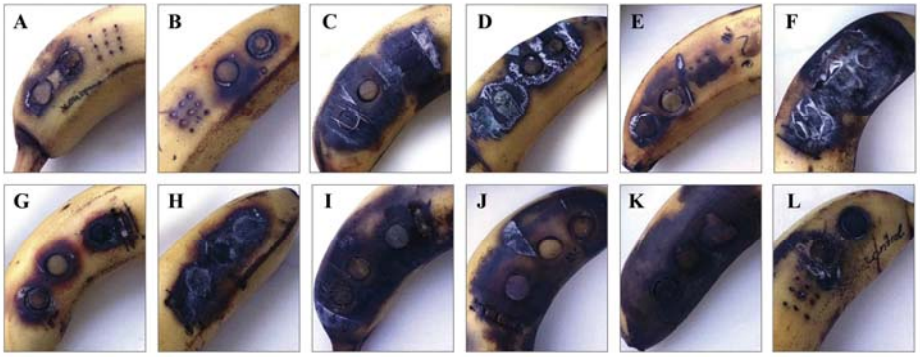

Figure 1. Pathogenicity test of banana. A. , L. *Control*, B. E *during the identification process*, C. *Fusarium sp.,* D. *Penicillium sp. ,* F. *Aspergillus niger,* G. *Botryosphaeria sp,.* H. *Botryosphaeria sp.,* I. *Fusaruim sp.,* J. *Fusarium sp.,* K. *Colletotrichum musae*

A pink-purple color of the skin, dry rot with mycelial formation, slightly concave spots, blue-green places of breaking rhizomes were the most common damages that were observed of injure ginger rhizomes. Several rhizomes were also examined without visible damage. There were obtained forty-one isolates. Thirteen of them showed the positive result (the fungus colony growth on the ginger slices). The others did not develop on the slice (Tabl.2). No pathogen was isolated from control ginger slice (fig.2). Based on initial cultural (color and density colonies) and morphological (spore shape, color) characteristics two of pathogen isolates were determined as *Fusarium sp.* The others pathogen isolates are during the identification process.

Table 2. Isolates from storage ginger rhizomes and pathogenicity test

Isolates	**Fungal rot**	**Place of breaking rhizome**	**Skin colour of pink-purple**	**Visual healthy**
Number of isolates	13	10	15	3
Pathogenicity test	5	3	4	1
Positive	4	3	4	0
Negative	1	0	0	1

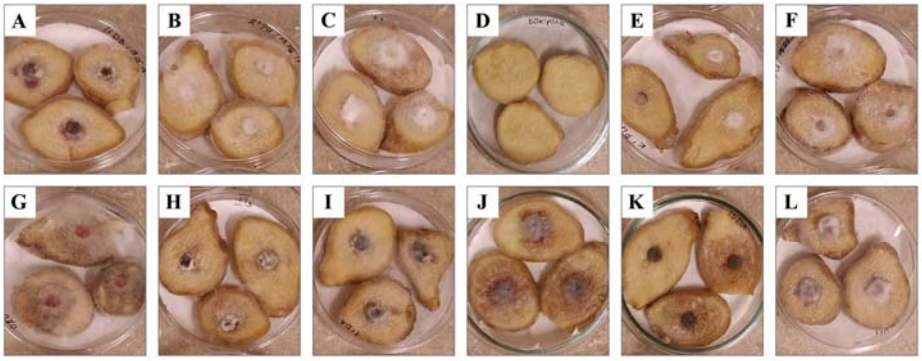

Figure 2. Pathogenicity test for colonization of cut ginger. A.,B., E, H., G.„I., J., K. *during the identification process*, C. *Fusarium sp.,* F. *Fusarium sp.,* D. *Control*

Monitoring of the damage on the fruits, vegetables and spice imported to into our country is important for clarifying the pathogenic microflora, which enters in our territory. Many of the post-harvest pathogens produce mycotoxins as secondary metabolites, which cause various diseases, including many allergies of consumers. This raises the question of improving storage conditions for reducing the incidence and development of diseases.

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Effect of mineral fertilizers on yield and quality of potato tubers

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Abstract: Research on the effect of different rates and methods of application of mineral NPK fertilizers on the yield and quality of potato variety 'Carrera' was conducted in 2015 on a luvisol of the Radočelo Mountain massif, at the Village of Bzovik. Treatments included an unfertilized control, NPK 16:16:16 (1500 kg/ha) applied in-furrow at planting, NPK 16:16:16 (1200kg/ha) applied in-furrow at planting, and NPK 16:16:16 applied at 700 kg/ha during seedbed preparation and 500kg/ha in-furrow at planting. Mineral fertilizers led to a very significant increase in tuber yield compared to the control, giving the highest total yield under 1500 kg/ha NPK treatment. NPK fertilizer application methods had no significant effect on potato yield at the same fertilizer rate applied, but a somewhat higher yield was obtained in the treatment involving pre-plant and in-furrow applications compared to the in-furrow applications of the total fertilizer rate at planting. Results on the nutritional value of potato tubers showed that the levels of tested nutrients were higher in the skin than in the flesh. The concentrations of tested nutrients in potato tubers were highest at the highest NPK fertilizer rate, whereas the lowest levels of all nutrients, except calcium and iron, were determined in tubers at NPK rates of 700kg/ha applied pre-plant and 500kg/ha applied at planting.

Key words: potato, NPK fertilizers, yield, tuber quality

Introduction

In Serbia, potatoes are grown on different soils, under different agroenvironmental conditions, on plots of land having non-uniform nutrient levels; therefore, no general recommendation can be made about the use of fertilizers. Mineral fertilizers are known for being effective in significantly increasing tuber yields of all potato varieties, with high importance given to their application methods. As emphasized (Bugarčić, 2015; Broćić and Stefanović, 2012; Dugalić et al., 2000, 2004), complex NPK fertilizers should be applied during primary tillage or seedbed preparation, or the recommended amount should be split into two applications, one pre-plant and the other as an in-furrow starter fertilizer.

Under the agroenvironmental conditions of Mt. Radočelo, Bošković-Rakočević et al. (2005) studied a number of potato cultivars and determined that the split application of fertilizer with half applied pre-plant and half applied in-furrow as a starter was more effective than plow-down applications with half plowed down in the fall and the other half plowed down pre-plant in the spring. Moreover, different rates of mineral fertilizers ($N_{160}P_{120}K_{120}$ and $N_{200}P_{150}K_{150}$) showed no yield patterns. Similar conclusions were drawn by Dugalić et al. (2004), whereas Noor Muhammad et al. (2015) found that the rate of NPK 175:110:90 was optimal for economic tuber yields. In similar studies, Akinpelu et al. (2011) applied four NPK rates (0, 200, 400 and 600 kg/ha) for potatoes and recommended 400 kg/ha as the most favorable rate, whereas Mona et al. (2012) suggested NPK fertilization at 120:80:100 kg/ha.

Excessive amounts of fertilizers can adversely affect not only yield but also tuber quality. High rates exceeding 1500 kg/ha can negatively affect the productivity and processing characteristics of potato tubers (Bugarčić, 2000). The amount of nitrogen used is negatively correlated with dry matter and starch levels (Hajšlova et al., 2005). Furthermore, nitrogen over-fertilization can cause an increase in nitrogen content in tubers relative to potassium content, thus increasing the susceptibility of tubers to after-cooking darkening, as well as a decline in their nutritional value (Rengel and Damon, 2008). In addition, Fe deficiency is another reason underlying after-cooking darkening as an adverse side-effect of heat treatment (Gvozden, 2016). Although not present in large quantities in potato tubers, Ca and Mg play an important role in controlling physiological disorders in tubers and increasing their disease resistance (Brown et al., 2012).

Based on the abovementioned statements, the objective of this study was to evaluate the effect of different rates and methods of application of mineral fertilizers on tuber yield and quality of potato cv. 'Carrera' grown in an upland luvisol in Western Serbia.

Material and methods

The effect of different rates of mineral fertilizers on the yield and quality of 'Carrera' potatoes was examined in 2015. The experiment was laid out in a randomized block design with three replications at the village of Bzovik, Municipality of Kraljevo (Latitude 43^0 25' 33" N, Longitude 20^0 25' 53" E; 1107 m altitude), on a luvisol of the Radočelo Mountain massif.

Fertilization treatments included: T1 – (unfertilized) control; T2 - NPK 16:16:16 applied at 1500 kg/ha in-furrow at planting; T3 - NPK 16:16:16 applied at 1200 kg/ha in-furrow at planting; T4 - NPK 16:16:16 applied at 700 kg/ha during seedbed preparation, and at 500 kg/ha in-furrow at planting. 'Carrera' potatoes were planted on 30 April 2015 at a spacing of 70 x 25 cm, and harvested on 28 September 2015.

Before trial establishment, soil samples were collected from a depth of 30 cm for the following analyses: soil pH was measured at a 1:2.5 ratio of soil to distilled water and 1M KCl; humus content was determined by oxidation with $KMnO_4$ solution (according to Kotzman); total nitrogen was estimated by Kjeldahl analysis (Gerhardt Vapodest); available phosphorus and potassium were evaluated by extraction with 0.1M NH_4-lactate and 0.4M CH_3COOH, according to Egner-Riehm (P was analyzed spectrophotometrically by the phospho-vanadate colorimetric method and K was determined by flame photometry); available Al was measured by Sokolov's method; available Ca and Mg were assessed by extraction with 1M NH_4CH_3COO. Available Cu, Fe, Zn and Mn were determined by extraction with 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA solution, pH=7.3 (Lindsay and Norvell, 1978), and analyzed using atomic absorption spectroscopy.

After harvesting, potato tubers were sampled for separate analyses of major macro and micronutrients in the skin and the flesh. Nitrogen content was determined by elemental analysis using a Vario EL III Elemental Analyzer; the levels of K, Ca, Mg, Fe, Mn, Cu and Zn were assessed by digestion in concentrated HNO_3 and 30% H_2O_2, with K readings made by a flame photometer and measurements of the other elements taken by an ICP.

Results on potato yield were subjected to the mathematical and statistical method of an analysis of variance (ANOVA), and the significance of differences was determined by the LSD test at 0.01 and 0.05 significance levels.

Results and discussion

The results of agrochemical testing (Table 1) showed an acid reaction of the soil, indicating the necessity for soil amendment practices such as liming and humification in further potato production as the coefficient of nutrient use efficiency in such soils is lower than in less acidic soils richer in organic

matter (Bošković-Rakočević and Bokan, 2003). Regardless of the strongly acid reaction of this soil, mobile aluminum content (3.38 mg/100 g soil) is within the limits tolerable to plants (Jakovljević et al., 1991), which is of high importance given the particularly deleterious effect of excess mobile aluminum in the arable layer as evidenced by the decrease in root penetration depth and, hence, reduction in the uptake of nutrients and water from the soil (Foy, 1974).

The levels of soil micronutrients were within the optimal range. Among micronutrients, only available Zn (1.52 mg/kg) was moderately supplied (Ankerman, 1977). The contents of DTPA-Fe (55.6 mg/kg) and DTPA-Mn (61.5 mg/kg) were high, whereas the concentration of available Cu (0.64 mg/kg) was low (Ankerman, 1977).

Table 1. Agrochemical characteristics of the soil

Depth (cm)	pH/ H_2O	pH/ KCl	Humus (%)	N (%)	Al	P_2O_5	K_2O	Ca	Mg	Cu	Fe	Mn	Zn
					mg/100 g					mg/kg			
0-30	4.75	3.80	3.1	0.21	3.38	23.3	47.3	128.5	13.3	0.64	55.6	61.5	1.52

Results on the effect of different rates and methods of application of NPK fertilizer on potato tuber yield revealed a significant fertilization-induced increase in tuber yield in all treatments compared to the control (Tab. 2).

Table 2. Yield of potato cultivar 'Carrera'

Treatments	Yield (kg/ha)	Index (%)
T1	11200[c]	100
T2	60 653[a]	541.5
T3	51 699[b]	461.6
T4	52 330[b]	467.2

**The same letters in columns indicate non-significant differences among means at $P \leq 0.05$ by LSD test*

As compared to the unfertilized control, the lowest increase in yield (361.6%) was obtained by the in-furrow application of the whole amount of NPK fertilizer (1200 kg/ha) at planting, and the highest (441.5%) after in-furrow treatment with NPK 16:16:16 at a rate of 1500 kg/ha at planting.

The effectiveness of NPK fertilizer in significantly increasing tuber yield in all potato varieties under different soil and agroenvironmental conditions was confirmed by a number of authors both in Serbia and worldwide (Stoiljković and Šušić, 1975; Dugalić et al., 2000, 2004; Noor Muhammad et

al., 2015). However, few data are available on the effect of fertilizer application methods on potato yield under different soil and agroenvironmental conditions. In the present experiment, the total NPK fertilizer rate (1200 kg/ha) applied in-furrow at planting gave a somewhat lower tuber yield (51,699 kg/ha) compared to the same amount of fertilizer split into two applications i.e. 700 kg/ha during seedbed preparation and 500 kg/ha applied in-furrow at planting (52,330 kg/ha). The difference in tuber yield between the two treatments was not significant (Tab. 2). Results indicated that a somewhat higher tuber yield of potato cv. 'Carrera' was obtained after the split application of NPK fertilizer, consistently with the findings of Dugalić et al. (2004). Splitting the same NPK rate into two applications i.e. pre-plant and starter planting applications, although non-significantly increasing tuber yield compared to the single in-furrow application of the total fertilizer rate, can be recommended as a better treatment, considering the practical experience that high NPK fertilizer rates applied in-furrow at planting can damage potato sprouts in some soils, apart from unfavorably affecting the productive and processing traits of potatoes (Bugarčić, 2000). Similarly, in a study by Bošković-Rakočević et al. (2005), under the same agroenvironmental conditions of Mt. Radočelo, greater effectiveness in all cultivars was achieved by applying half of the fertilizer rate pre-plant and the other half in-furrow as a starter.

The increase in NPK 16:16:16 rate to 1500 kg/ha applied as an in-furrow starter treatment was highly significantly effective in increasing tuber yield compared to the control, as well as to treatment T3 (in-furrow application of the total NPK fertilizer rate of 1200 kg/ha NPK) and treatment T4 (700 kg/ha applied pre-plant and 500 kg/ha applied in-furrow).

The results of the analysis of the nutritional value of potato tubers show that the levels of all nutrients were higher in the skin than in the flesh (Tab. 3). The nitrogen content of the flesh was slightly higher than the average values of 1.56% (Bártova et al., 2013) and 1.49-1.80% (Rostami et al., 2015), whereas the level of K was somewhat lower than the range of 2.6-3.6% (Trehan and Sharma, 2002). These findings were particularly evidenced in treatments with 1500 kg/ha NPK fertilizer, in support of the reports by Rengel and Damon (2008) who found that excessive nitrogen application can increase nitrogen content in tubers relative to potassium. Cu, Fe, Zn and Mn levels in the potato flesh in all treatments were within the average range determined by Kabata-Pendias (2011).

Table 3. Nutritional value of potato tubers

Treatments		N	K	Ca	Mg	Cu	Fe	Mn	Zn
		%		mg/kg					
Skin	T2	2.61	4.02	1710	2022	9.04	98.78	87.43	36.88
	T3	2.48	3.70	1352	1889	9.32	117.55	71.58	23.38
	T4	2.42	3.72	1252	1684	6.50	158.07	50.85	25.37
Flesh	T2	2.16	2.34	559	1530	5.48	28.56	17.03	20.09
	T3	2.00	2.29	412	1489	5.43	21.82	16.20	16.98
	T4	1.89	2.16	539	1147	3.26	23.85	10.26	16.00

As indicated by the analysis of the effect of different rates and methods of application of NPK fertilizer on tuber quality, the highest levels of all tested nutrients were obtained at the highest rate (1500 kg/ha), as opposed to the lowest levels of all nutrients, except Ca and Fe, under NPK treatment at 700 kg/ha applied pre-plant and 500 kg/ha applied at planting, with all values being within the optimal range.

Based on the comparative analysis of the obtained yields and nutritional value of 'Carrera' potato tubers grown under the agroenvironmental conditions of Mt. Radočelo, the rate of 1200 kg/ha NPK 16:16:16 split into two applications i.e. 700 kg/ha applied pre-plant and 500 kg/ha applied at planting can be recommended as the most favorable and most cost-effective application rate, given the 367.2% increase in yield compared to the control, with optimal levels of nutrients obtained.

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Cornelian cherry selections (*Cornus mas* L.) in Novi Sad - morphological fruit properties and production of planting material

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Abstract: Until recently, Cornelian cherries were mostly growing in the natural population. Systematic collection, breeding programs, propagation and cultivation in orchards have been recently established in the Serbia. The main and the only one collection of Cornelian cherry in region were established 2011 year at the nursery of Faculty of Agriculture in Novi Sad. The collection orchard includes, among the some registered varieties, twenty promising selections from natural population in Serbia. The paper present some fruit properties of five the best Cornelian cherry selections from Novi Sad (CPC16, APRANI, BACKA, R1 and PPC1) during 2015 followed by the fruit length, fruit width, fruit weight, flesh to stone ratio and yield per tree in 5th vegetation (18.46 to 27.84 mm; 15.48 to 19.50 mm; 3.62 to 6.82 g; 79.44 to 87.00%, 243.55 to 1378.95 g, respectively). Due to a great importance of Cornelian cherry fruits as safe food and a wide interest in growing, the best selections were grafted onto generative rootstocks of Cornelian cherry by bud grafting. The best grafting success has been achieved in the APRANI (84.53%) and BACKA (77.64%) while the weakest was at CPC16 (59.25%). Depending on the selection, investigated quality parameters did not show great variability.

Key words: Cornelian cherry, selection, fruit properties, grafting

Introduction

The Cornelian cherry is a semi domesticated plant suitable for the organic production which can be used as both food and medicine. Fruits can be consumed fresh or in the form of various processed products (Bijelić, 2009;

Bijelić et al., 2011a). Also, the Cornelian cherry may have large applications in medicine and cosmetics (Celik et al., 2006), or as ingredient of herbal preparations used in the treatment of diabetes (Jia et al., 2003; Jayaprakasam et al., 2005). In addition to the above, the Cornelian cherry is suitable for hedges, anti-erosion protection and as greenery in urban areas, as it tolerates high levels of air pollution. The Cornelian cherry wood is strong and resilient and it can be used in carpentry and wood turning industry (Chatfield, 2006). It is also an important honey plant because it blooms very early (Bijelić, 2011). It is highly tolerant to diseases and pests, so it is a rare plant species that can be grown without application of chemicals. This feature is quite important in the light of the current campaign for more intensive production of safe food. In recent years increasing attention has been paid by consumers to less known fruits such as the Cornelian cherry (Ercisli et al., 2008; Bijelić, 2011), which have unusual flavors, qualities and balance chemical composition and many of which are rich with antioxidants and anthocyanins (Yilmaz et al., 2009a; Popović et al., 2012; Gunduz et al., 2013). Serbia has a rich gene pool of Cornelian cherry genotypes adapted to different local conditions in different regions of the country (Bijelić, 2011). Most trees are open pollinated seedlings of wild genotypes which vary widely in terms of productivity and fruit characteristics. Seed propagation and long-term human selection has also given rise to a great diversity of trees (Yilmaz et al., 2009a; Bijelić, 2011). Despite its wide usage in Serbia and its region, there are no established standard cultivars of the Cornelian cherry, and there are not many of them in the world either, in comparison with other commercial fruits. In these regions, 99% of Cornelian cherry crop is harvested from open pollinated seedlings of wild genotypes (Bijelić, 2011), just like in Iran (Hassanpour et al., 2012), Turkey (Ercisli 2004; Yilmaz et al., 2009b), Ukraine (Klimenko, 1990; 2004) and other regions. Considering that the needs of the processing industry and market for Cornelian cherries are much greater than the quantity of fruits that can be collected in nature, it is necessary and desirable to grow large-fruit selections and cultivars of the Cornelian cherry in plantations, and the basic prerequisite for starting a profitable Cornelian cherry plantation is the production of quality planting material of selected large-fruit genotypes. It is the first step in creating conditions for the transition from fruit gathering to plantation production of this fruit species. Currently in our region, Cornelian cherry plantations is increasing in line with an increase in the production of planting material, since there is a great interest of producers.

The first and the only Cornelian cherry collection orchards planting in the region represents a wealth of biological and economic potential, as well as a rich source of material for further breeding and selection. It is carried out monitoring of all stages of growth and development in order to develop and improve technology for this new fruit variety introduced to the cultivated area.

The aim of this paper is to present some results from breeding programe of Cornelian cherry in Novi Sad, reviews some morphological fruit properties and show success and quality production of the best Cornelian cherry selections suitable for recomendation for orchards.

Material and methods

The study was conducted in a nursery at the experimental field of the Faculty of Agriculture in Novi Sad. The nursery is close to Novi Sad, where the soil type is chernozem with very favourable physical and chemical properties.

This paper reviews morphometric characteristics, during the 2015 year, of the five selections (CPC16, APRANI, BACKA, R1 and PPC1) that were found to be superior in comparison with the other tested genotypes in collection orchard (Bijelić et al., 2011b; 2011c). Fruits of the selected trees were picked at the stage of full maturity. Samples that contained 30 fruits per selection, in three replications, were then subjected to morphometric measurements of the above fruit characteristics. Measurements were done by means of precision analytical scales and digital micrometer calipers. Flesh to stone ratio was interpreted as a share of the mesocarp compared with fruit weight, expressed in percentage.

In order to spread the best selections of cultivated area, founded the technology of planting material by grafting (Bijelić et al., 2016). The paper presents success of production of quality plant material during the 2015 year of the best Cornelian cherry selection for growing in orchards.

The results obtained were statistically analyzed using StatSoft (2017) by analysis of variance (ANOVA). Significance of mean differences among the characteristics was tested by Duncan's multiple range test for the significance level of 0.01%. The results are presented in tables.

Results and discussion

Consumers worldwide are increasingly showing interest in natural high quality fruits, what exactly is a Cornelian chery. On the basis of pomological characterization for five the best Cornelian cherry selections that can be recommended for the growing (Table 1), clearly stands out PPC1 from the fruits of maximum mass (6.82 g) and flesh ratio (87.00%), followed by R1 (4.92 g, 84.55%, respectively). Directions of Cornelian cherry selection should primarily focus on the greater fruit weight, which has the strongest direct correlation with the mesocarp mass (Bijelić et al., 2007).

Tab. 1. Some morphometric fruit properties of the best Cornelian cherry selections, Novi Sad

Selections	*Fruit length (mm)*	*Fruit width (mm)*	*Fruit weigth (g)*	*Flesh/stone ratio (%)*	*Yield per tree (g) in 5th year*
APRANI	21,58 c	15,48 d	3,62 c	79,44 d	262,10 c
R1	25,52 b	18,29 b	4,92 b	84,55 bc	1.378,95 a
CPC16	18,46 d	17,35 c	3,73 c	84,72 ab	243,55 c
PPC1	27,84 a	19,50 a	6,82 a	87,00 a	1.303,10 a
BACKA	22,25 c	16,85 c	4,50 bc	81,76 c	853,70 b

Values in the same column for each trait with different case letters are significantly different at $p < 0.01$.

Featured selections are very valuable genetic material for future breeding program, because can compete with the cultivars (Klimenko, 2004), as well as selections of Cornelian cherry (Yalcinkaya, 1999). In addition to the fact that in Serbia there are genotypes noteworthy breeders by the results of previous studies on higher flesh ratio and a significant fruit weight (Korać, 1996). Cornelian cherry population in Slovakia have flesh ratio of 77-86% (Brindza, 2005), while authors in Turkey (Pirlak et al., 2003) found genotypes with higher flesh ratio. Cornelian cherry varieties grown in Ukraine are very large fruit and flesh ratio, 85 - 92% (Klimenko, 2004).

Thanks to the successful production of quality plant material (Bijelić et al., 2016), every year in Serbia and the around increase the orchards with the grafted Cornelian cherry selections. Shown below (Table 2) is the success of young trees production during 2015.

Tab. 2. Success of grafting the best Cornelian cherry selection and some quality parameters of young plants

Selections	*Success of grafting (%)*	*Height of plants (mm)*	*Diameter of plants (mm)*	*The average number of root*	*The average length of root (mm)*
APRANI	84,53 a	756,50 b	13,92 a	21,00 a	144,55 a
R1	63,56 bc	911,22 a	12,10 ab	12,00 d	101,50 d
CPC16	59,25 c	746,75 b	11,67 ab	14,00 c	118,10 c
PPC1	69,87 b	763,20 b	10,15 c	15,00 bc	122,11 bc
BACKA	77,64 a	893,75 a	11,05 b	17,00 bc	138,20 ab

Values in the same column for each trait with different case letters are significantly different at $p < 0.01$

The greatest grafting success was achieved with APRANI in the period I (84.53%) and with BACKA (77.64%), while the poorest success was with

CPC16 (59.25%). PPC1 and R1 had similar bud grafted success (69.87% and 63.56%, respectively). Concerning the other examined parameters of young tree quality, the greatest height was observed with R1 (911.22 mm) and BACKA (893.75 mm). Other genotypes formed shorter young trees of uniform height. Young trees produced during this research were shorter than the ones from the research of Ekaterina (2008), who reported that the Cornelian cherry young trees were 120–150 cm in height in the first vegetation, with 4 to 5 side branches, and that they could be planted at a permanent location. The largest diameter of a young tree was formed with APRANI (13.92 mm) followed by R1 (12.10 mm) and CPC16 (11.67 mm). That is in agreement with the results of Klimenko (1990) who reported diameters from 1.3 to 1.5 cm. The largest number of roots formed was observed with APRANI (21.00) while the smallest number of roots was formed with R1 (12.00). Klimenko (1990) received a significantly smaller number of roots (from 10.6 to 14.4). The longest root system was formed with APRANI and BACKA.

The results of this study indicate that the best Cornelian cherry selections from Novi Sad can be recommended for orchards growing and established production technologies of planting material suitable for mass production and the introduction of this fruit of the concept of organic cultivation. These results, combined with our other studies are significant for recommending optimal cultivation technology of grafted Cornelian cherry selections in orchards.

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External changes in eggs depending of the storage conditions and hybrid lines

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Abstract: The purpose of this research was examination of the external changes that occur in the quality characteristics of eggs for consumption, during storage. As a material for research were used eggs from two hybrid lines ISA Brown and DeKalb. A total 112 eggs from category L (63 -73 g) were used for the investigation. Half of the eggs (from both hybrid lines) are kept at room temperature (13 to 22°C, avarage 16°C) and half in the refrigerator (0-4°C). Weight loss, air cell size and other physical characteristic were recorded at 0, 10, 21 and 40 days of storage. The egg weight loss in eggs stored at 4 °C significantly ($p<0.01$) lower than the eggs stored at 16 °C. Gradually, as time passes by, the mean values of air cell size, starting from 10^{th} day, 21^{th}, until 40^{th} day of eggs storage from both hybrid lines increase, which indicates drastic changes regarding the values.

Key words: eggs, hybrid lines, storage, changes, quality,

Introduction

The poultry industry, which is mainly focused on egg production, shows constant development in the last twenty years, and the private individual farms which increase their activity in this sector are the greatest contributors.

The egg and broilers production in small family agricultural economies has long tradition and representation in the Republic of Macedonia and it is also a part of the rural heritage. In egg production part, the main goal is keeping the current situation of total self-sufficiency on the domestic market with domestic production through keeping and advancing of the domestic consumption.

According to data from the Statistical Office from 2015, the total number of laying hens is 1.352.564 and the average egg production by laying hen is 150. In total production, 109.977 is from individual producers and 93.406 is a part of egg production in the business sector.

Global production and egg consumption, as well as their effects on human's health is result of their easy availability and accessibility, high-quality proteins, vitamins and minerals, which are necessary for healthy and quality life Zaheer, (2000). Eggs are available for everyone through the year, easy for cooking and also tasty and useful. Eggs are perfect food for balanced and healthy diet.

In the developed countries and in the developing countries, continuous enlargement of production and consumption of eggs is determined, as well as significant improvement of quality properties of eggs.

Beside the way of farming, the genetic base of laying hens, feeding, the laying hens' age, ambient temperature and lightening regime also affect the egg quality (Škrbić et al., 2004 and Daghir, 2004). Many researches confirm the assumption that the egg's quality immediately after laying is the best (Pavlovski et al, 2000).

Their freshness in the following 2-3 weeks after hatching could be preserved by proper collection and storage of eggs in suitable conditions (Jin et al., 2011).

In fact, that is immediate result of interaction of two variables: the initials egg characteristics and storage conditions (Vasilev and Kitanovski, 2005). The obvious changes of the egg are expressed primary in weight loss, decreasing of volume mass, increasing of air chamber, with clearly expressed smell, colour change, and sometimes the histological structure, too.

Material and methods

Source of material

Eggs from two hybrid lines: (Isa Brown which lay brown eggs) and De Kalb which lay white eggs), grown in modern private poultry farm, were taken as research material in this researches. According to quality, they belong to A "fresh eggs" class intended for consumption.

Eggs from L category with weight 63 - 73 g or in total 112 eggs from both hybrid lines were taken from each hybrid line for foreseen research.

Half of the eggs (from both hybrid lines) are stored at room temperature (13 - 22°C), and half of them in refrigerator (0 - 4°C).

Examinations of physical characteristic of eggs for consumption from both hybrid lines are conducted in Meat and Meat Products Control and Quality Laboratory at the Faculty of Biotechnical Sciences - Bitola.

For the necessary examinations, seven eggs from each hybrid line were taken immediately after laying, then on 10^{th} day, 21^{st} and 40^{th} day.

In the last group eggs from both lines, after 40 days, on each 10 days the mass change of eggs and the height of air chamber were followed individually. Egg mass was measured every day with digital beam scale.

The height of air cell size was determined with candling. After each measurement, mean value was calculated. Egg length and width was measured with calliper in order to calculate egg shape index.

Statistical analysis

The data obtained was analyzed by using the statistical program (Ms.Excel), so as to compare the treatments and to made analysis of variance.

Results and discussion

From the results presented in (Table 1), it could be determined that the mean values of egg mass on laying day of white and brown eggs stored at room temperature (16°C) are: 58±0.93; 59.14±1.02.

Table 1. Changes in egg mass

Storage condition	Hybrid lines	Parameter	Day of storage			
			0	**10**	**21**	**40**
(16°C)	DeKalb	x	58.00±0.93	57.71±0.52	55.28±1.13	52.14±0.96
		min	55.00	56.00	53.00	52.00
		max	62.00	60.00	59.00	58.00
		Sd	2.44	1.38	2.98	2.54
		Cv	4.22	2.35	5.39	4.70
	ISA Brown	x	59.14±1.02	57.71±0.75	54.42±0.68	51.42±1.18
		min	55.00	55.00	53.00	46.00
		max	62.00	61.00	57.00	56.00
		Sd	2.67	1.97	1.81	3.15
		Cv	4.51	3.42	3.33	6.13
(4°C)	DeKalb	x	/	58.85±0.74	56.71±0.89	53.43±1.16
		min	/	57.00	54.00	49.18
		max	/	62.00	61.00	57.34
		Sd	/	1.95	2.36	3.06
		Cv	/	3.31	4.02	5.74
	ISA Brown	x	/	58.85±0.51	56.28±1.11	53.16±1.06
		min	/	57.00	54.00	50.93
		max	/	61.00	62.00	58.54
		Sd	/	1.34	2.92	2.80
		Cv	/	2.28	5.02	5.28

On the 10th day of storage, a decrease in the average mass from 57.71±0.52 of white eggs and 57.71±0.75 of brown eggs has been noticed. On 21st day, bigger

decrease in the weight of eggs from both hybrid lines, such as: 55.28±1.13; 54.42±0.68. On the 40th day of storage of the egg at room temperature, white eggs mass is 52.14±0.96, and brown eggs mass is 51.42±1.18. The obtained results for eggs stored in refrigerator (4°), starting from the 10th, until the 40th day also show decrease in the weight of the egg mass.

The difference in the decrease of values from the 10th, until the 40th day of egg storage in refrigerator is 5.42 of white eggs and 5.69 of brown eggs.

Our results are similar to the results of (Akyurek and Okur, 2009) and Chonkas et al., (2014).

The dynamics of changes in eggs height during different days of storage (at room temperature and in refrigerator) are expressed through average mean values shown in (Table 2).

The difference in decrease of height at average room temperature (16˚C) of white eggs is 1.57, and the decrease of height of brown eggs is 2.57.

Table 2.Dynamics of changes in eggs height

Storage condition	**Hybrid lines**	**Parameter**	**Day of storage**			
			0	**10**	**21**	**40**
(16°C)	DeKalb	x	56.85±0.70	56.85±0.88	55.71±0.18	55.28±0.47
		min	54.00	54.00	55.00	53.00
		max	59.00	60.00	56.00	57.00
		Sd	1.86	2.34	0.48	1.25
		Cv	3.27	4.11	0.87	2.26
	ISA Brown	x	56.14±0.67	56.28±0.52	54±0.38	53.57±0.57
		min	54.00	55.00	52.00	51.00
		max	59.00	59.00	55.00	55.00
		Sd	1.77	1.38	1	1.51
		Cv	3.15	2.45	1.85	2.82
(4°C)	DeKalb	x	/	56.42±0.57	56.28±0.89	55.14±1.28
		min	/	55.00	53.00	50.00
		max	/	58.00	60.00	59.00
		Sd	/	1.51	2.36	3.38
		Cv	/	2.67	4.12	6.14
	ISA Brown	x	/	54.85±0.40	54±0.81	54±0.48
		min	/	54.00	52.00	53.00
		max	/	57.00	58.00	56.00
		Sd	/	1.06	2.16	1.29
		Cv	/	1.94	3.92	2.39

The obtained results for eggs stored in refrigerator (4˚C), starting from the 10th, until the 40th day also show decrease in height.

A statistically significant difference at level ($p<0.05$) has been determined for both hybrid lines.

From the given data, it could be ascertained that the change becomes as a result of the mass of the egg white and egg yolk on one side, and as a result of evaporation of water from the egg shell during eggs storage. Also, similar results were obtained by (Scott and Silversides, 2001) and Hagan, (2013).

The temperature and the time of storage affect the outer quality of the egg – as time of storage is increasing, along with the temperature, the egg width is changing, (Table 3).

Table 3. Dynamics of changes in eggs width

Storage condition	**Hybrid lines**	**Parameter**	**Day of storage**			
			0	**10**	**21**	**40**
(16°C)	DeKalb	x	42±0.38	43.42±0.29	43.70±0.17	42.85±0.26
		min	40.00	42.00	43.00	42.00
		max	43.00	44.00	43.00	44.00
		Sd	1	0.78	0	0.69
		Cv	2.38	1.81	0	1.61
	ISA Brown	x	43.14±0.41	45.57±1.61	43.42±0.20	42.57±0.20
		min	42.00	43.00	42.00	42.00
		max	45.00	55.00	43.00	43.00
		Sd	1.06	4.23	0.53	0.53
		Cv	2.47	9.29	1.25	1.25
(4°C)	DeKalb	x	/	44±0.38	43±0.31	42.42±0.43
		min	/	43.00	42.00	41.00
		max	/	54.00	44.00	44.00
		Sd	/	1.00	0.81	1.13
		Cv	/	2.27	1.89	2.67
	ISA Brown	x	/	44.28±0.28	43.57±0.53	42.57±0.29
		min	/	43.00	42.00	42.00
		max	/	45.00	46.00	44.00
		Sd	/	0.75	1.39	0.78
		Cv	/	1.70	3.20	1.84

On the initial, 10^{th}, 21^{th} and 40^{th} day the width of white eggs is: 42±0.38, 43.42±0.29, 43.70±0.17, 42.85±0.26.

On the same days and under the same ambient conditions, brown eggs have the following values: 43.14±0.41, 45.57±1.61, 43.42±0.20, 42.57±0.20.

As a result of inner changes which occur in the eggs, there are changes in width and egg shape index. The increase of egg width occurs as a result of analogous decrease of height.

The obtained results for eggs stored in refrigerator (4˚C), the mean values on the 10^{th} day are 44±0.38 of white eggs and 44.28±0.28 of brown eggs.

A decrease in width from 43±0.31 of white eggs and 43.57±0.53 of brown eggs is noticed on the 21^{th} day.

The difference in decrease of values from the 10th day until the 40th day of eggs storage in refrigerator is 1.58 of white eggs and 1.71 of brown eggs.

From the results in (Figure 1), it could be stated that on the initial day, at room temperature, the air cell size has smallest value 5.8±0.38 of white eggs and 6.4±0.51 of brown eggs.

Gradually, as time passes by, the mean values of air cell size, starting from 10th day, 21th, until 40th day of eggs storage from both hybrid lines increase, which indicates drastic changes regarding the values.

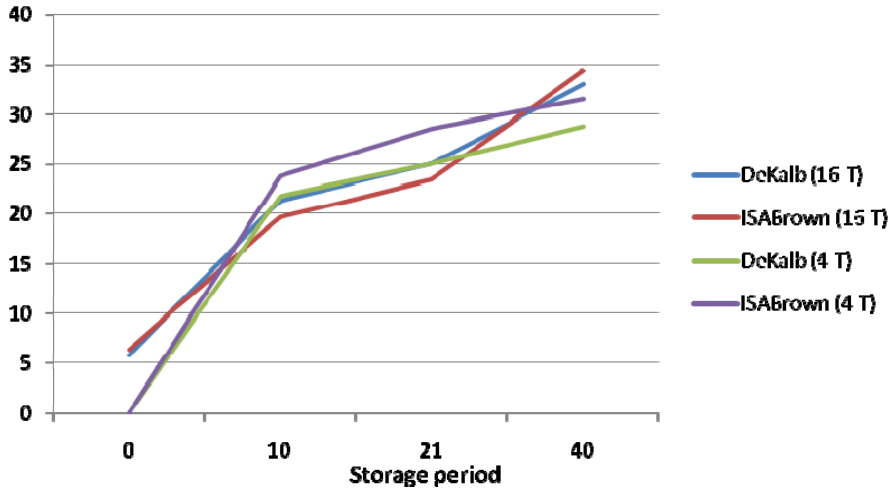

Figure 1. Dynamics of changes of air cell size during storage period

In comparison with other author for air cell size in the same or similar conditions, our results are similar to the results of Chonkas et al.,(2014); (Vasilev and Kitanovski, 2005), Kralik et al., (2014).

Conclusion

From the study, it was observed that egg weight, height and width, air cell size indices decreased with increase in storage period. The regression relations given for the external evaluation parameters can be used for estimating optimum period for loss in egg qualities and for planning storage facilities. It can be concluded that the quality of an egg is affected by the method and length of storage and also a hybrid line.

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Laryngeal Hemiplegia in Horses - Clinical Signs and Diagnostic Approach

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Abstract: Eighteen horses with breathing and wheezing problems were examined by endoscopy of the upper respiratory tract, where a different degree of left-sided laryngeal paresis was diagnosed. I most of cases (12 horses) there was established a second degree of paresis. In four cases was determined a third degree of paresis and two horses were diagnosed with fourth degree hemiplegia. In ten patients endoscopic examination established additional pathology, including dorsal displacement of the soft palate, entrapment of the epiglottis, rostral displacement of the palatopharyngeal arch and blood in the trachea. Clinical parameters (body temperature, heart rate, respiratory movements, color of mucous membranes) were in physiological norms.

Key words: horse, laryngeal hemiplegia, endoscopy

Introduction

Laryngeal hemiplegia is a common and a long time is considered as the most important equine upper airway disease of horses. It usually affects the left side of the larynx and occurs most commonly in larger-breed horses (>160 cm in height) (Cahill and Goulden, 1987). The pathology is described as a failure of abduction of structuraly normal arytenoid cartilage because of decreased or completely absent motility of the main abductor of the cartilage *m. cricoarytenoideus dorsalis*. In most of the cases no cause for this condition is found, thus it has been termed idiopathic laryngeal hemiplegia (ILH). Different theories explain the neuropathy as result of mechanical compression or strech of the left recurrent laryngeal nerve, bacterial or viral infection, vitamin deficiencies (Cahill and Goulden, 1987), perineural injections, neoplasia, trauma of the neck and paralaryngeal abscessation (Rose et al., 1981; Hillidge, 1986).

An assumption for genetic predisposition has been proposed by the same authors. The most significant clinical signs are expressed in exercise intolerance and inspiratory noise with different amplitude and sound like roar and whistle (Dixon et al., 2003). Currently, endoscopy is the gold standard for assessing laryngeal dysfunction in the horse so this article focuses on it.

Material and methods

This study was conducted within three years and is expressed in endoscopic examination of the nasal cavity, larynx and trachea of 18 horses with respiratory problems, accompanied by manifestations of shortness of breath during exercise, wheezing and worsening physical condition. Clinical investigation included determining the body temperature, the color of the conjunctiva, heart rate and respiratory activity. The gender distribution was as follows: 6 mares, 8 stallions (incl. 1 foal) and 4 geldings. The horses were representatives of the following breeds: Pure Arabian (3), Thoroughbred (3), Trotter (7), Warmblood (2) and Draft horse (3). The age of patients varied from 6 months to 14 years. Evaluation of laryngeal hemiplegia was carried out at rest using a 4 grading system (Hackett et al. 1991). I Grade - Synchronous and full abduction of the arytenoid cartilages; II Grade - Asynchronous movement (hesitation, flutter, abduction weakness, etc.) of the left arytenoid cartilage during any phase of respiration. Full abduction of the left arytenoid cartilages (when referenced to the right) is observed either by swallowing, nasal occlusion or the use of respiratory stimulants; III Grade - Asynchronous movement (hesitation, flutter, abduction weakness, etc.) of the left arytenoid cartilage during any phase of respiration. Full abduction of the left arytenoid cartilages (when referenced to the right) cannot be induced either by swallowing, nasal occlusion or the use of respiratory stimulants; IV Grade - Midline or paramedian position of the left arytenoid cartilage and no substantial movement of the left arytenoid cartilage can be induced by swallowing, nasal occlusion or the use of respiratory stimulants. The endoscopy was performed after a slight sedation with Xylazine (0.3-0.5 mg/kg, I.V.). A slap test in the saddle area was used to stimulate the movement of the cartilage.

Results and discussion

Clinical examination of all horses from this study showed no abnormal physiological parameters. In all horses involved in this survey was diagnosed a presence of a left laryngeal hemiplegia (Fig. 1, 2). In 8 patients it was the only pathology, while in the remaining 10 cases were identified and another abnormality in respiratory organs. In 3 horses, there was evidence of blood or

traces of it in the trachea, and in 3 horses a dorsal displacement of the soft palate (DDSP) was observed (Fig. 3). In 2 patients, transient entrapment of the epiglottis was detected (Fig. 4), and in two cases a rostral displacement of the palatopharyngeal arch (RDPA) was diagnosed (Fig. 5, 6). A second degree of paresis was documented in 12 horses, and in 4 of the cases there was a 3 degree of paresis. In 2 animals the endoscopic examination showed complete paralysis of left arytenoid cartilage. During endoscopic examination was revealed that the find is dynamic and changes over time. Such a finding was also expressed by Dixon et al., (2003). In our opinion, the use of a low-grade sedation results in a change in the motor activity of the arytenoid cartilages and thus influences the real assessment of their mobility. Such a conclusion is expressed by Valdes-Valquez et al., (1993). In this order for such kind of study is more appropriate to be performed without sedation, but this is sometimes risky and unfeasible. This research has shown that often laryngeal paralysis is accompanied by other abnormalities, which undoubtedly contribute to the more severe manifestation of this neuropathy. According to us the use of the 4-Grading system in practice is justified because of the easy differentiation of the various degrees of paresis / paralysis of the larynx, which correlates with the statement of others (Archer et al., 1991; Ducharme et al., 1991).

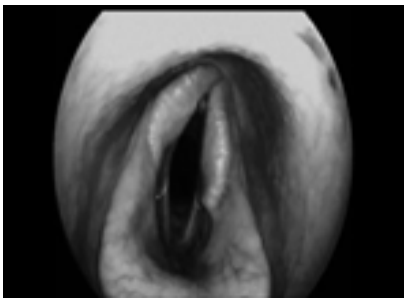

Fig. 1. Laryngeal hemiplegia IV Grade.

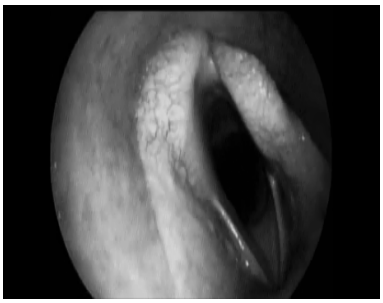

Fig. 2. Laryngeal hemiplegia IV Grade.

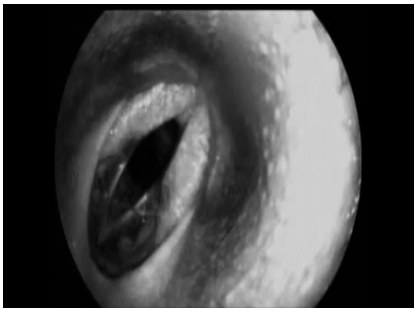

Fig. 3. Laryngeal hemiplegia II Grade and DDSP.

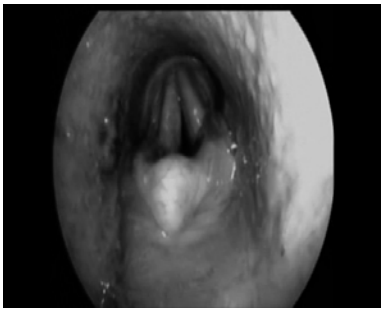

Fig. 4. Laryngeal hemiplegia II Grade and epiglottis entrapment.

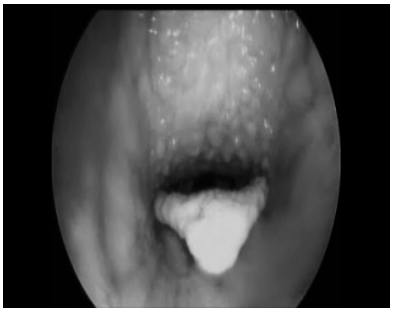

Fig. 5. Laryngeal hemiplegia and RDPA.

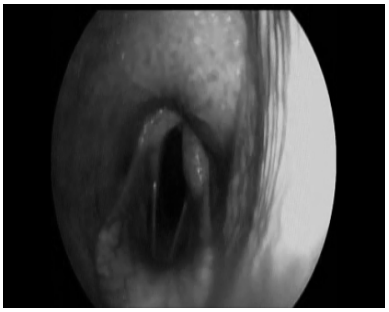

Fig. 6. Laryngeal hemiplegia IV Grade and RDPA.

Conclusion

Laryngeal hemiplegia in horses is a common neuropathy. Often, it is accompanied by other pathologies, resulting in significant breathing difficulties and decreased horse performance. Resting horses do not usually exhibit deviations in clinical parameters (body temperature, heart rate, respiratory frequency). Laryngoscopy is critical in diagnosing laryngeal paresis and determining its severity. It should be done if possible without prior sedation of the patient. The four-grade system is objective and easy to apply for practical purposes.

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Spectrophotometric determination of total flavonoids in grape skins using different standards

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Abstract: Grape skin is recognized as very important source for the recovery of a large number of biologically active compounds, such as polyphenols, most of which are flavonoids. The objective of this study was to estimate the total flavonoid content of seven white and red grape skin varieties using two different standards: quercetin (QE) and catechin (CE). The significant differences (p<0,05) between the content of the total flavonoids for each analysed samples expressed as mg QEg^{-1} DM and mg of CEg^{-1} DM were obtained. An approximately 3.6 to 4.3 fold differences in total flavonoid content were found. It is recommended that the flavonoid content should be estimated by a flavonoid standard which is predominant flavonoid in grape skins.

Key words: polyphenols, total flavonoid, quercetin, catechin, grape skin

Introduction

Grapes are very widespread crop in the world and are generally used in the production of wine. However, grapes (*Vitis vinifera*) are known not only as a source for the production of wine, but also as a significant source of polyphenolic compounds (Mitić et al., 2012), which offer health benefits (Yang et al., 2009). Polyphenols are important constituents of the grape, and consider as one of the major factors in the quality of the grapes and wine (Ferreira et al., 2016; Rodriguez Montealegre et al., 2006). Phenolic compounds found in grapes are associated with different impacts on human health, such as antimicrobial, anti-inflammatory, anti-aging activity, prevention of cardiovascular disease (Ferreira et al., 2016) and anticancerogenic activity (Yang et al., 2009). By-products obtained after wine production, such as the residue after pressing for white wines or vinification for red wines (skin and seed), contain substantial

amounts of polyphenol components (Spigno et al., 2007), and can be used as food supplements (Rockenbach et al., 2011). Parts of the grapes, which are generally rich in polyphenols are the skins and seeds (Katalinić et al., 2010; Guerrero et al., 2009; Rodriguez Montealegre et al., 2006). Distribution of polyphenols in the grape berry is different, therefore, it has been found about 60-70 % of total extractable polyphenols in the seed, around 28-35% in the skins and only 10% in the pulp (Pantelić et al., 2016). Generally, all the polyphenols which are found in grapes can be divided into two groups: non-flavonoids (hidroxybenzoic and hidroxycinnamic acids and stilbenes) and flavonoids (flavan-3-ols, flavonols and anthocyanins) (Anter et al., 2014; Mitić et al., 2012; Yang et al., 2009). Grapes contain mainly flavonoids (Rockenbach et al., 2011). The most abundant flavonoids in most of grape skins are flavonols, yellow pigments that contribute to the colour of white grape and wine (Rodriguez Montealegre et al., 2006) and anthocyanins, which are responsible for red, purple and blue pigments in coloured grapes (Ferreira et al., 2016; Rodriguez Montealegre et al., 2006). Flavan-3-ols are a large family of polyphenols and mainly include catechin, epicatechin, gallocatechin, epigallocatechin and their polymers (Guerrero et al., 2009) and predominantly are located in the grape seed (Di Lecce et al., 2014; Jayaprakasha et al., 2001; Pantelić et al., 2016; Santos-Buelga et al.,1995) but could be present in a significant amount in grape skins depending on grape varieties (Di Lecce et al., 2014; Katalinić et al. 2010).

Generally, the content of the polyphenols is determined by several factors such as the type of grapevine and is influenced by viticultural and environmental factors (Katalinić et al., 2010). However, the dominant flavonoid in most of grape skins are quercetin (Pantelić et al. 2016; Rodriguez Montealegre et al., 2006), although catechin could be present in a similar or higher amount in some grape varieties (Di Lecce et al., 2014; Katalinić eta al. 2010).

One of the abundant methods for determination of total flavonoids in grape skins is colorimetric assay with aluminium chloride. For quantification of the results different flavonoid standards are used in literature, mainly catehin (Ivanova et al., 2011) and quercetin (Costa Braga et al., 2016; Medina-Meza & Barbosa-Canovas., 2015; Putnik et al., 2016). Knowing that each substance has different extinction coefficient and due to it show different relation between concentration and absorbance, we could ask whether the quantification of total flavonoids by different standards can be comparable. In other words, whether the content of total flavonoids expressed as catehin equivalents is significantly different of that expressed as quercetin equivalents? So, the aim of this study was to compare the results obtained for determination of total flavonoid content in grape skins by spectrophotometric assay using quercetin and catehin as flavonoid standards.

Material and methods

Chemicals

Standards of catechin and quercetin were supplied by Sigma-Aldrich Chemie GMbH (Munich,Germany). Standard solutions and dilutions were prepared using water purification system (Androna SIA, Latvija). Other chemicals and solvents were of analytical grade.

Grape samples

The seven different grape varieties, four red varieties: „Hamburg", „Prokupac", „Merlot", „Cabernet Sauvignon" and three white varieties: „ Smederevka", „Riesling Italien" and „Tamjanika" were obtained from vinery located in Aleksandrovac, center of the Župa district of Serbia in 2016 year. All grapes were pressed for winemaking process and grape pomace samples were immediately dried in an drying oven (Thermo Scientific Haraeus, MA, USA) at 60^{o}C for 72h (final water content about 15%). Then, the skins were manually separated from the seeds and were ground in a small laboratory coffee grinder. The skins with particle size <0,6mm were used for analysis. The material was maintained at -20 ^{o}C in vacuum-packed plastic containers until further analysis.

Preparation of grape skin extracts

Grape skins were prepared according to the slightly modified method described in Pantelić et al., (2016). The skins, approximately 2g, were extracted with 20 ml methanol containing 0,1% HCl and stirred for 1h on a mechanical shaker. After shaking at room temperature, the extracts were placed in the dark, in the fridge at 5 ^{o}C for 22h. Thereafter, extracts were filtered through Whatman No.42 filter paper and then pure supernatant was collected. Extractions were repeated three times and all three supernatants were combined and evaporated to dryness by rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) under reduced pressure at 40 ^{o}C. After evaporation, the residues were dissolved in 15ml milliQ water. These solutions were used for further testing.

Determination of total flavonoids

The total flavonoid content of the grape extract was determined using the slightly modified colorimetric method proposed by Ribeiro et al., 2015. Briefly, 125µl an appropriate dilution of the grape extracts was mixed with 625µl of milliQ water and subsequently with 37,5µl 5% sodium nitrite solution. An aliquot of 75µl 10% aluminium chloride was added 6min later and after 5min, 250µl of 1M sodium hydroxide was added. MilliQ water was added to bring the final volume of the mixture to 1250µl. The absorbance of the mixture was immediately measured at 510nm wavelength using an Shimadzu UV-Vis

spectrophotometer (UV-1800, Shimadzu USA Manufacturing Inc, UR, USA). The absorbance was measured against the blank (prepared in the same way with methanol instead of sample).

The flavonoid content was determined by a catechin and quercetin standard curves and expressed as the mean values of three replications (miligrams of catechin equivalvents (CE) and quercetin equivalents (QE) per 1g of dry matter).

Statistical analysis

Statistical analysis was performed using Statistica software ver 6.0 (Statsoft Co., Tulsa, OK, USA). Differences between means were estimated using *T*-test, at level of significance $p < 0,05$. The data were reported as the mean ± SD.

Results and discussion

The results obtained for the total flavonoid content of grape skins using two different flavonoid standards are presented in Figure 1. The total flavonoid content in seven grape skin varieties varied from 1.11±0.044 to 4.26±0.061 mg CEg^{-1} DM and 4.00±0.219 to 18.19±0.441 mg QEg^{-1} DM. The grape skin of variety Cabernet Sauvignon had the highest content for both total flavonoid content as mg CEg^{-1} DM and as for mg QEg^{-1} DM, whereas the skin of variety Riesling Italien had the lowest values.

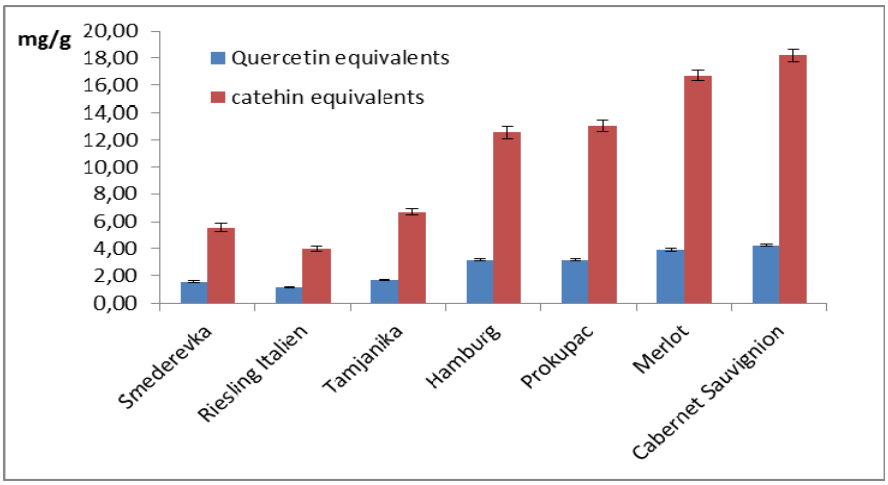

Figure 1. Total flavonoid content of grape skins estimated by different flavonoid standards (mean ± SD, $n = 3$).

It could be noticed that significant differences ($p < 0,05$) existed between the content of the total flavonoids for each analysed samples expressed as mg QEg^{-1}

DM and mg of CEg^{-1} DM. An approximately 3.6 to 4.3 fold differences in total flavonoid content were found between the values estimated as quercetin equivalents compared to those estimated as catehin equivalents. These differences are due to different standard curves obtained for quercetin and catehin. Coloured complex of catehin showed lower UV-absorbing capacity compared to that of quercetin at 510nm.

Significant differences among the results for total flavonoids in grape skins could be found in the literature. For example, analysing the four Macedonian red and white grape skins, Ivanova et al. (2011) obtained the total flavonoid content from 3.12±0.12 to 10.2±0.04 mg CEg^{-1} DM, whereas Putnik et al. (2016) registered significantly higher amount for Merlot red grape skin (18.03±1.29 to 54.03±0.85 mg QEg^{-1} DM depending on extraction conditions) or Costa Braga et al. (2016) for Pinot Noir red grape skin (22.43±2.89 mg QEg^{-1} DM). These differences could be, beside variety, environmental and extraction conditions, also due to standard applied for quantification.

Most of varieties characterised by predominant presence of flavonols in the skin among which quercetin is predominant (Pantelić et al., 2016; Rodriguez Montealegre et al., 2006). For these varieties, the use of quercetin as a standard for quantification of total flavonoids is recommended. But, for varieties in which the similar amount of quercetin and catehin (as dominant flavonols and flavanols) is found (De Lecce et al., 2010), the use of mixed standard of catehin and quercetin should be considered for total flavonoids quantification. Similar, the determination of total flavonoid content by catehin standard for those varieties (Katalinić et al. 2010) in which catehin is predominant flavonoids could be justified.

Conclusions

Concerning that flavonoid composition of grape skins was variety dependent, the choice of adequate standard for quantification of total flavonoids should be based on predominant flavonoid present in the selected skins. In this way, the great variability of the total flavonoid content among different studies could be avoided and the more precise results will be obtained.

Acknowledgements

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Phytochemical Screening of Lupine Flour after Different Methods of Grain Soaking

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Abstract: Lupine (*Lupinus albus*) seeds are a rich source of protein for animal and human nutrition. The nutritional quality of lupine seeds can be improved through removal or elimination of some antinutritional compounds by soaking. The effect of two different methods of lupine grain soaking on the presence of: alkaloids, tannins, flavonoids, terpenoids, sterols, saponins, coumarins and glycosides in lupine flour were investigated. Lupine seeds were soaking, three days, in 70% ethanol and distilled water. Soaking process led to decrease in presence of: alkaloids, tannins, terpenoids, sterols and saponins in lupine flours. The changes in the presence of glycosides were not registered, while flavonoids and coumarins were not detected in any sample of lupine flours. Extraction of these biological active components was more intense in 70% ethanol then in distilled water. Soaking of lupine seeds in 70% ethanol and distilled water led to decreases of antinutritional factors in lupine flour. Soaked lupine seeds is a potential source as nutrient supplement and functional agent for foods systems.

Key words: lupine flour, grain soaking, phytochemical screening, soaking solutions

Introduction

The sweet lupine seed (*Lupinus albus*) is a rich source of protein, lipids and dietary fiber for animal and human nutrition in different parts of the world, not only for their nutritional value, but also their adaptability to marginal soils and climates. Lupine is a legume valued primarily for its high protein content similar

to that of soybean, and for its important dietary lipids, fiber, minerals and vitamins (Martinez-Villaluenga et al. 2006; Dueńas et al. 2009). In contrast to soybean, lupine can be grown in more temperate to cool climates and it can be considered as the strongest potential competitor of soybean.

Seeds of white lupine have a protein content ranging from 33% to 47%, according to genotype and location, with full range of essential amino acids. Lupine proteins contain a low amount of sulphur-containing amino acids, and contrary to other legumes, a high amount of lysine (Dervas et al. 1999). Oil content varies from 6% to 13% according to genotype, with a high concentration of polyunsaturated fatty acid (Huyghe, 1997).

In our country (Serbia), the lupines still has not widely accepted in human nutrition. However, white lupine, which has been consumed as a food in a narrow area for a long time, was accepted for human consumption by the Australian government in 1987 and by the United Kingdom government in 1996 (Cox, 1998; Swam, 2000).

Lupine flour can be used in production of different fermented products. It can be added to pasta, crisps, bread and emulsified meat products to increase nutritional value and aroma and to modify texture. Moreover, protein isolate can be produced from lupine seeds (Erbaş et al. 2005).

However, the limitation of use of lupines is due to their high content of bitter alkaloids and other antinutrients. The antinutritional factors being structurally divided into two categories: proteins (such as trypsin inhibitors and lectins) and others such as tannins and phytic acid (Martin-Cabrejas et al. 2009). The nutritional quality of legume seeds can be improved through removal or elimination of such antinutritional compounds by soaking, dehulling, germination or cooking (El-Baltegy, 1996; Ä lvarez- Ä lvarez et al. 2005; Nalle et al. 2010; El-Sayed et al. 2010; Mohammed et al. 2017).

Soaking has been documented to be an effective treatment to remove antinutrional factors, which can be eliminated with the discarded soaking solution. Some studies have shown that soaking significantly reduced phytate, protease inhibitors activity and tannins content (El-Adawy et al. 2000; Vijayakumari et al. 2007; Mohammed et al. 2017).

The main objective of this study was to determine the effect of soaking of lupine seeds in 70% ethanol and in distilled water on the presence of: alkaloids, tannins, flavonoids, terpenoids, sterols, saponins, coumarins and glycosides in lupine flours.

Material and methods

Plant materials

The sweet lupine seeds (*Lupinus albus*) were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

Soaking methods

Lupine seeds were soaking, three days, in 70% ethanol and distilled water at room temperature; lupine: soaking solution ratio of 1:10 (w/v). Soaked seeds were separated from the soaking solution, dried (12 hours at 50°C) and then were ground to a coarse powder by a Micro-Mill grinder (Fisher, Germany). The lupine powders were extracted and prepared for phytochemicals screening.

Extraction procedure

Lupine flours were extracted with 70% ethanol at ratio of 1:10 (w/v) using mechanical stirrer (Tehtnica EV-102) at room temperature. The samples were filtered with Whatman No. 1 filter paper. Extracts were evaporated on vacuum evaporator (Heidolph laborata 4000 efficiend) to a volume of 5 ml, at 50°C. Extracts were tested for presence of phytochemicals.

For checking the presence of phytochemicals in soaking solutions, the solutions were evaporated on vacuum evaporator to a volume of 5 ml (70% ethanol at 40°C, and water at 50°C).

Phytochemical screening

Extracts of lupine flours and soaking solutions were tested for the presence of numerous phytochemical constituents following the methods described by Harborne (1998).

Alkaloid test: 1 ml lupine flours extracts and soaking solutions were added with 5 ml of 1% HCl and heated on steam bath at 60ºC for 15 minutes. After filtration, 1 ml filtrates were separated into 3 tubes. 1 ml of Mayer's reagent, Dragendorff's reagent, and Wagner's reagent were added to each tube. The yellow, orange and turbid brown color precipitate indicated the presence of alkaloid.

Phenol and tannin test: 1 ml of the extracts was added with 1 ml of 3% $FeCl_3$. Formation of greenish-black indicated the presence of hydrolyzed tannin including phenol or blue color specified the presence of condensed tannin.

Flavonoid test: 2 ml of the extracts were dissolved in 3 ml of 50% ethanol. Very small piece of magnesium strips were put in the tube and boiled for 30 minutes, and then 2 drops of concentrated HCl was added. Formation of pink color indicated the presence of flavonoids.

Terpenoid test: 5 ml of the extracts were mixed with 2 ml chloroform and then carefully added with 3 ml concentrated H_2SO_4. A reddish brown coloration between upper and lower layer specified the presence of terpenoids.

Sterol test: 2 ml of extracts were added with 2 ml of concentrated H_2SO_4. Red precipitation revealed the presence of steroidal ring.

Saponin test: 2 ml of the extracts were mixed with 5 ml of distilled water and shaken strongly for 5 min. Persistence of foams for 30 minutes revealed the presence of saponins.

Coumarin test: Qualitative filter papers were soaked in 10% NaOH and hang upon the tubes containing 5 ml of the extracts and evaporated for 30 minutes in water bath. The soaked filter papers were observed after placing under UV light for 10 min. Fluorescence spot specified the presence of coumarins.

Glycoside test: 1 ml of extracts was dissolved in 1 ml distilled water and a few drops of 10% NaOH. Formation of yellow color indicated the presence of glycosides.

Results and discussion

The qualitative phytochemical screening of lupine flours and soaking solutions was performed on 8 groups of compounds (Table 1). It was found that both methods of grain soaking cause reduction antinutrients of analyzed samples. But, extraction of these biological active components from lupine seeds was more intense in 70% ethanol then in distilled water.

Table 1. Phytochemical screening of lupine grain extracts and soaking solutions

Bioactive component		*Lupine flour*			*Soaking solutions*	
		No soaked grain	Soaked grain			
			H_2O	70% C_2H_5OH	H_2O	70% C_2H_5OH
alkaloids	Mr	++++	++	+	++	+++
	Dr	++++	++	+	+++	+++
	Wr	++++	++	+	+++	+++
tannins		+++	-	-	+++	+++
flavonoids		-	-	-	-	-
terpenoids		+++	++	+	-	+
sterols		++++	++	+	+++	+++
saponins		++	++	+	+	++
coumarins		-	-	-	-	-
glycosides		+	+	+	-	-

Mr - Mayer's reagent; Dr - Dragendorff's reagent; Wr - Wagner's reagent

Results indicated the presence of alkaloids is greater in flour made from the grain soaked in water than in ethanol. This is confirmed by all three different tests: with Mayer's reagent, Dragendorff's reagent and Wagner's reagent (Table 1.). All three tests show reduced presence of alkaloids in lupine flour, but not complete absence. The presence of alkaloids proves to be non-toxic at low

concentrations. Since most alkaloids of lupine are water-soluble, the alkaloid level of lupine (0.5–4%) can be decreased to 0.04% by soaking in running water or brine (Erbaş et al. 2005). Reducing the presence of alkaloids in the lupine grain is very important, considering that lupines species can not be consumed directly because they contain quinolizidine alkaloids, mainly sparteine and lupanine, giving a bitter taste in white lupine and causing respiration problems and liver damage (Erbaş et al. 2005).

Tannins, which are present in the hull, can be effectively reduced by removal of hulls (Longstaff and McNab, 1991). But, our results show that, soaking process in distilled water and 70% ethanol leads to a complete absence of tannin from lupine flour (Table 1.). Namely, a similar presence of tannins is registered lupine flour prepared from no soaked grain and in soaking solutions (Table 1.). Extraction of tannins from the lupine grain hull is favorable since the tannin reduction can improve the nutritional quality of the seeds, as it increases the bioavailability of minerals (Jiménez-Martínez et al., 2003).

Glycosides are some of undesirable compounds found in lupines (Allen 1998). Although traditionally they have been known as antinutritional factors, these compounds may also have beneficial effects, such as antioxidant effects and prevention of cancer (Rochof and Panozzo 2007). Our results show that, applied soaking methods of lupine grain generally does not lead to a reduction in the presence of the glycosides in the flours (Table 1.). But, our results also show that, some type of glycosides such saponins, are extracted in 70% ethanol.

Saponins are glycosides present in many plant with a bitter taste. Their antinutritional effects are mainly reflected in depressed feed intake that causes growth inhibition to animals and reduced animal reproduction (Francis et al. 2002). Their adverse effect may be related to an increase of the permeability of the small intestinal mucosa cells, which leads to an inhibition of active nutritient transport (Johnson et al. 1986). Then, saponins are bipolar compounds possessing characteristics of emulsifier and hemolytic (membrane) activity which might lead to damage of the red blood cells membrane (Šošević 2012).

A similar degree of extraction of terpenoids, strerols as well as saponins are registered in analyzed samples (Table 1.). The plant-derived terpenoids are considered to be the most potent anticancer and anti-inflammatory compounds known (Sultana and Saify, 2013). Plant sterols have health effect because they may lower blood cholesterol levels, in the diet (Vieno et al. 2000). Therefore their presence is desirable in flour. The applied methods of soaking can not completely remove these compounds.

Our results show that, flavonoids and coumarins are not present in the tested samples (Table 1.).

In summary, the sweet lupine seed (*Lupinus albus*) is a rich source of biologically active components. Applied soaking process of lupine seeds led to

decrease in presence of: alkaloids, tannins, terpenoids, sterols and saponins in lupine flours. But, generally does not lead to a reduction in the presence of the glycosides in the flours. Flavonoids and coumarins are not present in the tested samples. So, applied soaking process led to decrease in presence of some antinutrents from lupine seeds, and its flour can be incorporated into a wide range of foods.

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Effect of grafting on dynamics of ripening and yield of tomato *(Lycopersicon esculentum* Mill.)

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Abstract: Tomato (*Lycopersicon esculentum*, Mill.) is one of the most important vegetable in the world. Also, tomato is mostly bred in growing substrate in greenhouses. However, greenhouses are used for the longest time possible which resulted with soil-born diseases. Grafting is a possible chemical free solution that shows some changes in plants thanks to the vigorous rootstock. Tests were conducted in a greenhouse on tomato cv. Buran F1 and cv. Berberana F1, where were two varieties of seedlings, grafted and non grafted, grown in the substrate with EC = 1.7 dS/m. Dynamics of ripening and yield were observed on the first four branches of tomatoes. The obtained results show that grafting induces faster ripening at one of two cultivars for 1 – 2 days. Unlikely, in our research, non-grafted variants had higher yield for 0.3 – 0.4 kg per plant in comparation with grafted variants.

Key words: tomato, grafting, ripening, yield

Introduction

Tomato (*Lycopersicon esculentum*, Mill.) is one of the most important vegetable in the world. Also, it is the second most commonly consumed vegetable in the Europe. Fresh tomato and tomato products are good sources of vitamins, carotenoids, and phenolic compounds, which can be beneficial for the prevention of oxidative stress and degenerative disorders.

Greenhouse horticulture has been developed to protect crops from unfavorable environmental conditions. The result of the management of microclimate conditions throughout the year, according to the biological requirements of cultivated plants, is a significant increase in yield per plant (Kastori et al., 2013).

Environmental stresses present the most limiting conditions for horticultural productivity and plant exploitation worldwide. Important environmental stress factors include: water, temperature, nutrition, light, oxygen availability, metal-ion concentrations, and pathogens (Colla et al., 2010; Savvas et al., 2010). Soilless culture avoids problems associated with decreasing fertility of natural soils, due to disease limitations and the increase in salinity (Verdonck, 1975). A specific method of modifying plants to resist environmental stresses comprises grafting commercial cultivars onto selected vigorous rootstocks (Lee and Oda, 2010). Grafting is nowadays regarded as a rapid alternative to the relatively slow methodology of breeding for increased environmental-stress tolerance of fruit vegetables (Flores et al., 2010).

The advantages of grafting, a popular and valuable technique used for years in the production of vegetables, are unquestionable, one of the most important being protection against soil-borne diseases and nematodes (Louws et al., 2010). Another important advantage of grafting is protection against abiotic stresses such as high/low temperatures, salinity (Colla et al., 2010), drought or excessive water soil content (Schwarz et al., 2010) and elevated concentrations of heavymetal and organic pollutants in soils (Savvas et al., 2010). Furthermore, grafting is very popular among farmers because it results in fruit yield increases and enhanced overall plant vigour. The grafted plant takes up water and nutrients from the soil more efficiently, and retains its vitality for longer periods during the growing season (Aloni et al., 2010; Lee et al., 2010).

Several conflicting findings have been published on changes in fruit quality caused by grafting, and on whether these effects are advantageous or deleterious (Davis et al., 2008; Alexopoulos et al., 2007; Flores et al., 2010; Rouphael et al., 2010; Bekhradi et al., 2011; Kyriacou and Soteriou, 2012). The differences in reported results may be attributable in part to differences among production environments and methods (e.g., soilless vs. insoil culture, irrigation, and fertilization), types of rootstock/scion combinations, and harvest dates.

Therefore, the aim of the present paper is to examine the effect of grafting on dynamics of ripening and yield of tomato. Based on obtained results will determine whether grafting and to what extent has an impact on maturation and yield of tomato.

Material and methods

Research was conducted in the greenhouse on tomatoes A1- cv. Buran F1 (Enza Zeden, Netherlands) and A2- cv. Berberana F1 (Enza Zeden, Netherlands) variants on which were monitored fruits. Seedlings were in two variants of nongrafted (B1) and grafted (B2) cv. Buran F1 and cv. Berberana F1. Seedlings were grafted on Maxifort and bred at level of salinity EC = 1.7 ds m^{-1}. The

plants were planted in pots 26 cm in diameter charged with 12.5 l of soil media "Klasmann Deilman TS 3". The experiment was set under greenhouse controlled conditions at the surface of 100 m^2, the object of Faculty of Agriculture in Banja Luka. The experiment was divided according to a randomized block design (Randomized block system) with three repetitions in each block.

Fruits were carefully picked in full physiological ripeness from the first four bearing branches (C1, C2, C3, C4). During the process of cultivation were applied common agricultural measures (nutrition, protection, irrigation), as well as specific measures of care for the tomatoes (nipping laterals, removal of lower leaves, decapitation after the fourth bearing branch).

The changes in fruit skin color and the time of ripening were monitored until harvest.

Colorimetric analysis

Changes in the color of epidermis were observed at intervals of four days, with the device Chroma meter CR-400/410 from the moment of the first change of color to full maturity, taken into consideration on how to examine always the same point on the fruit. Color cards of changes in ripening were obtained by entering the colorimetric values of a *, b * and L * in Lab tool of Corel Draw x8. The values of a *, b * and L * were converted into the nearest value of the standard colors.

Yield per plant

Yield per plant was calculated after obtaining exact number of fruit set per brach at first four branches of tomato cultivars and mesuring the waight of every fruit. After that, calculation resulted the yield per plant up to the fourth brach in kg per plant.

Results and discussion

Under standard growth conditions, grafting did not have any effect on fruit yield no significant differences were found among the different graft combinations evaluated in the three experiments, a result which corroborates previous ones obtained with different shoot tomato genotypes (Estan et al., 2005; Martinez- Rodriguez et al., 2008).

Number of ripening days

Color development in tomatoes is temperature sensitive with better plastid conversion occurring above 12°C and below 30°C (Thai et al., 1990). Tijskens and Evelo (1994) demonstrated that b* suffered big changes if tomatoes were ripened at high temperatures (over 30°C) and yellowing took place due to the

inhibition of lycopene synthesis and the accumulation of yellow/orange carotenoids. This may indicate that under other than normal ripening conditions, changes in the b* values may compensate or exaggerate a* magnitudes, depending on their mathematical relationship, leading to misleading results. This indicates that under abnormal ripening conditions, b* and L* changes may be important and the ripening index to be used should be carefully selected.

Table 1. Number of ripening days

Variation	Buran F1 (A1)		Berberana F1 (A2)		$\bar{x}$	*F*
	B1	B2	B1	B2		
C1	18	19	17	15	17.25	
C2	15	15	17	14	15.25	
C3	12	13	9	9	10.75	22.659**
C4	11	14	12	11	12.00	
$\bar{x}$	14	15.25	13.75	12.25	13.8125	
	A		B		AxB	
$LSD_{0.05}$	2.7843		1.1028		2.8811	
$LSD_{0.01}$	5.1109		1.6707		5.0209	

The average duration to the ripening of the fruits on the branches was 13.8 days (Tab. 1). It should be noted that the ripening of the first branch, irrespective of variations, was significantly longer in relation to each subsequent branch. Therefore, the fruits from the first branches were ripening after 17.25 days, whereas the third branch took 10.75 days (Tab. 1). Testing of the significance level showed that there was a highly significant difference in the time of fruit ripening, depending on their position on the plant or branch. Also, a significant difference was found between the cultivars, while grafting had no significant impact on the ripening process.

Colorimetric analysis

Color changes during tomato ripening were the result of changes in the values of L*, a* and b*, although the more important ones were along the a* axis, related to chlorophyll degradation and lycopene synthesis. Accurate color identification among the six ripening stages based on USDA (1975) visual classification should include the three parameters.

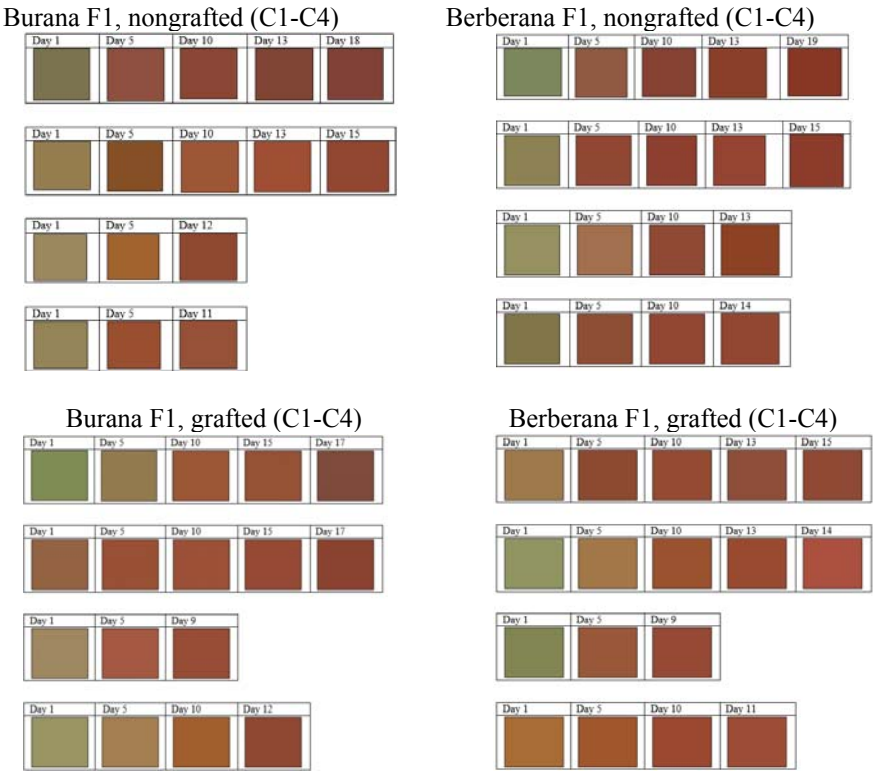

Figure 1. The color change after days of ripening

Number of fruits per brunch

Table 2. shows that the average number of fruit per branch was 4.75. The highest average number of fruit per branch was on non-grafted variant of Berberana F1 with 5.5 fruits per branch, and the lowest was on a grafted variant of the same cultivar. There were no differences in average numbers of fruit per branch between variants of Burana F1. Testing levels of significance did not show a significant difference in the number of fruits per branch in relation to the position of fruit on the plant.

Our results confirmed the results of Mutai (2012) who showned that irrespective of disease condition, grafting on any of the rootstock did not significantly affect the number of fruits produced in comparison to nografted plants. Ibrahim et al. (2001) also found that the total number of fruits per truss in nongrafted plants was statistically different from the total for grafted plants.

Table 2. Number of fruits per brunch

Variation	Buran F1 (A1)		Berberana F1 (A2)		$\bar{x}$	*F*
	B1	B2	B1	B2		
C1	6	5	6	4	5.25	
C2	5	4	5	4	4.5	
C3	4	5	6	4	4.75	1.5^{ns}
C4	4	5	5	4	4.5	
$\bar{x}$	4.75	4.75	5.5	4	4.75	
	A		B		AxB	
$LSD_{0.05}$	0.6495		0.7898		0.9812	
$LSD_{0.01}$	1.1923		1.1964		1.5515	

Mean mass per fruit

In our experiment, the average weight of a fruit was 116.87 g, whereas no significant difference was found in the average weight of the fruit. Grafted Berberana F1 had the highest average fruit weight in the value of 130.98 g (Tab. 3.). Unlike Berberana F1, grafted Buran F1 had the lowest value of an average fruit mass of 100.99 g. The first branch of all variants had the highest value of the average fruit weight of 135.12 g, while the average fruit weight on the other branches was similar- generally about 110 g.

Table 3. Mean mass per fruit (g)

Variation	Buran F1 (A1)		Berberana F1 (A2)		$\bar{x}$	*F*
	B1	B2	B1	B2		
C1	86.97	71.37	126.84	127.31	103.122	
C2	143.9	117.22	124.39	155.69	135.3	
C3	135.91	107.25	95.67	105.09	110.98	2.399^{ns}
C4	116.48	108.12	111.92	135.81	118.083	
$\bar{x}$	120.815	100.99	114.705	130.975	116.87	
	A		B		AxB	
$LSD_{0.05}$	44.9808		10.327		45.3021	
$LSD_{0.01}$	82.5685		15.6445		81.4067	

Mutai (2012) has shown that non-inoculated control plants had heavier fruits than grafts, averaging 69.8 g and 36.1 g, respectively, which confirmed our study. Grafting did not have signicifant influence on mean mass per fruit.

Yield per plant

In our research, average yield per plant was 2.11 kg. This shows that grafting has no significant influence on yield per plant (Tab. 4). According to Maršić

and Osvald (2004), total fruit yield of non-grafted plants was significantly higher in comparison to that of the plants grafted onto both rootstock cultivars. Similarly, Romano and Paratore (2000) stated that vegetable grafting does not improve the yield when the selection of the rootstock is not suitable. The yield advantage of grafted plants has been shown to be clear when they are grown on infested soil (Vuruskan and Yanmaz, 1990; Augustin et al., 2002; Besri, 2002).

Table 4. Yield per plant (kg)

Variation	Buran F1 (A1)		Berberana F1 (A2)		$\bar{x}$	*F*
	B1	B2	B1	B2		
$\bar{x}$	2.13	1.81	2.44	2.05	2.1095	86.962^{ns}
	A		B			
$LSD_{0.05}$	0.4702		0.3325			
$LSD_{0.01}$	2.3557		1.6657			

Conclusion

Our results lead to the conclusion that there was no significant differences between grafted and non-grafted variants, regardless on tested cultivars.

Also, there was a slight difference between the cultivars, where cv. Berberana F1 showed better results in tested parameters, ripening was for one to two days shorter, mean mass per fruit was higher, as well as yield per plant.

Testing of the significance level showed that there was a highly significant difference in the time of fruit ripening, depending on their position on the plant or branch.

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Influence of seed age and moisture on the germination of banjaluka's wheat varieties

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Abstract: Water stress and seed age are important factors that affect agricultural production, leading to a significant reduction in yield. The availability of water and its movement in the seed are very important for the process of germination. Therefore, in the laboratory of the Faculty of Agriculturewe set up experiments in which we examined the germination of three domestic varieties of wheat (Bosanka, Nova Bosanka and Jelena) in conditions of the lack of water caused by different concentrations of mannitol. To test the viability of seed, we used seed produced in 2012 and 2015. The research included: germination energy, total germination and vigor classification. Based on the results, it was found that the osmotic effect of different concentrations of mannitol has an impact on the growth of seedlings in the early stages of development in all tested wheat varieties, while the older seed in most parameters yielded worse results than younger seed.

Key words: seed, drought, variety, germination, wheat

Introduction

Conditions in field, such as high temperature, extremely wet conditions or heavy soils, can lead to a slowdown of germination, feeble seedlings and in some cases, loss of seed viability. In such circumstances, the crop, the yield and quality can be significantly reduced (*Mathur S B, Kongsdal*, 2003). The stressful conditions from the surrounding environment are limiting factors for the agricultural production worldwide (*Roy et al.*, 2011). Drought, increased salt concentration, as well as other abiotic factors, have a negative impact on the quality (*Wang and Frei*, 2011) and quantity (*Dolferus et al.*, 2011) of all produced cereal crops, including wheat. Drought is one of the most important

abiotic stress factors for plants and represents a complex form of stress, which affects various metabolic processes (physiological, biochemical and molecular) of the whole plant, in almost all plant organs and plant cells (*Yordanov et al.*, 2000).

At the stage of germination of winter wheat often comes to a deficit of water in the soil. Drought may cause the decrease in photosynthetic rate, and the reduction of biomass and yield (*Bala et al.*, 2006; *Hnilička et al.*, 2007).

Seed age significantly affects the seed quality - germination energy and total germination (*Miladinovet al.*, 2014). Due to the unstable and often unplanned production in our country, area and yield keep changing each year. Sometimes for that reason, seed produced in particular year can't be used for sowing in the next year, but it can be used 2-3 years later (*Mrdja et al.*, 2010). Seed vigor is variable in the most agricultural cultures (*Saxen et al.*, 1987; *Rapčan et al.*, 2006), and depends on a number of factors, including seed age.

The aim of our research is to identify the local winter wheat genotypes that tolerate drought stress at the stage of germination of seeds and determine the influence of seed age of the specified varieties on the quality of the seeds in the early stage of wheat growth.

Material and methods

In the laboratory of the Faculty of Agriculture we carried out the research in which we examined the germination of domestic wheat cultivars. The research included three factors:

Factor A - variety (Research was performed on three varieties of wheat that are created at the Agricultural Institute of the Republic of Srpska in Banja Luka. Varieties are Bosanka, Nova Bosanka and Jelena).

Factor B - concentration of mannitol (0 Mpa, -0.3 MPa and -0.6 MPa).

Factor C - the age of wheat (wheat seed produced 2012 and wheat seed produced in 2015).

The seeds were sterilized in 96% alcohol and then 100 equal and healthy seeds were taken and placed in Petri dishes, for each variety and treatment (*Džamić et al., 1999*). Dishes were equipped with a filter paper saturated with the solutionof a particular concentration (of the mannitol), with the water potential close to zero - control, -0.3, -0.6 MPa (*Braccini et al., 1996*).

Seeds were placed in an oven (Binder), 7 days at a temperature of 20 ° C, until the first true leaf appeared. For each variety, concentration of mannitol and seed age, experiment was set up in 4 repetitions. During experiment we observed:

- germination energy (*Brasil, 1992*), 4th day of experiment were counted all germinated seeds by that period (%);

- percentage of germination, or the total number of germinated seeds compared to a total number of seeds from a single experiment type (%);
- vigor test (*Nakagawa, 1999*) or strong seeds percentage (%).

The results of the research were processed by the variation-statistical analysis, assessment of the significant difference by LSD test and were shown in tables.

Results and discussion

The quality of seed is a complex trait that is primarily determined by its germination. Determination of germination by standard germination method (*ISTA, 2003*) is implemented in ideal conditions, that is why results of this test are only valid for optimum conditions in the field (*TeKrony, 1995; Siddique and Wright, 2004; Bukvic et al., 2009*), but standard germination often exceeds the germination in the field conditions (*Hamman et al., 2002*). By increasing of the storage period, the germination of the most field crops significantly reduces (*Saxena et al., 1987 .; Andrić, 2004*). Mannitol is used to cause water stress (*Chen et al., 2010*), as osmoticum in the germination test and it's a penetration-resistant solution, which doesn't damage the embryo (*Almansouri et al., 2001*) that causes the inhibition of seed germination by affecting the water uptake.

Germination energy of wheat seeds

Table 1 shows the influence of seed age and mannitol on the vigor of wheat.

The average germination energy of all varieties, regardless of seed age and the use of mannitol, was 69.6%. Germination energy was the highest by variety Bosanka (72.8%), and by variety Jelena was the lowest (63.2%). Identified differences were statistically significant, as well as differences between the varieties of Nova Bosanka and Jelena.The differences between varieties Bosanka and Nova Bosanka had no statistical significance. Under controled conditions, the effect of drought on the germination energy of five banjaluka's wheat varieties was examined by *Jovović et al.* (2015). In this study, variety Bosanka stand out, which is in accordance with our results.

The highest percentage of wheat seeds germination was found at the water potential of 0 MPa (81.1%), while the lowest germination energy (59.8%) was found at a water potential of -0.6 MPa. Identified differences were statistically very significant. Statistically highly significant differences in germination energy were found in water potential conditions of 0 MPa and -0.3 MPa, as well as between -0.3 MPa and -0.6 MPa. The obtained results are in accordance with the results of *Jovović et al.* (2015), from experiments with wheat seeds in which they found that increasing water potential leads to a significant reduction in germination of seeds.

Table 1. The influence of seed age and mannitol on germination of wheat (%)

Variety	*Mannitol*	*Year*		*Average for varieties*
		2012	2015	
Bosanka	0	92.0	88.0	90.0
	-0,3	81.5	38.5	60.0
	-0,6	77.5	59.2	68.3
	Average	83.7	61.9	72.8
Nova Bosanka	0	79.7	97.0	88.3
	-0,3	63.0	75.0	69.0
	-0,6	52.0	68.5	60.3
	Average	64.9	80.2	72.5
Jelena	0	74.0	56.0	65.0
	-0,3	66.5	80.5	74.5
	-0,6	49.5	52.0	50.2
	Average	63.7	62.8	63.2
Average for the mannitol	0	81.9	80.3	81.1
	-0,3	71.0	64.7	67.8
	-0,6	59.7	59.9	59.8
Average per year		70.9	68.3	69.6

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0,05	3.863	3.863	3.154	6.690	5.462	5.462	9.461
0,01	5.151	5.151	4.204	8.919	7.281	7.281	12.614

Seed produced in 2012 had the germination energy of 70.9%, while the seed produced in 2015 had germination energy of 68.3%. Depending on the seeds age the differences in the germination energy didn't have statistical significance. Most authors found that during a long period of storage, the quality of seed decreases (*Avdic et al*, 2011; *Heeger*, 1956; *Jevđović et al.*, 1999; *Jevđović and Radanovic*, 2000; *Jevđović*, 2001), while *Beljo et al.* (2012) in experiments with tobacco found that physiological maturity significantly affects the % of germination, because older seed had a higher percentage of germination, compared with younger seed. Physiological maturity affected the germination energy of wheat seed, so the seed produced in 2012 had better germination energy compared to seed produced in 2015, that is why our results are similar to the results obtained by *Beljo et al.* (2012) in experiments with tobacco seed.

Results of testing germination energy of different varieties, whose seeds germinated on soil with different concentrations of mannitol, showed that the maximum values for all varieties, were in the control (in the water potential of 0 MPa), except for the Jelena variety, where the maximum value in the water potential was of -0.3 MPa. By increasing water stress, in all varieties, the value

of germination energy decreased and it had statistical significance. The exception was the variety Bosanka, which had better germination energy in water potential of -0.6 MPa, compared to a water potential of -0.3 MPa. By changing the water potential to a more negative, there is a reduction of germination energy, proven also by *Pratap & Sharma* (2010) vigna seeds testing, where the germination energy and percentage of germination were inhibited by increasing the osmotic potential.

It was established that variety Bosanka had the highest germination of seed produced in 2012 and in water potential of 0 MPa, while the lowest germination was at seed produced in 2015 and in water potential of -0.3 MPa.By the variety Nova Bosanka, the highestgermination energy had seed produced in 2015 and in water potentialof 0 MPa, while the lowestgermination energy had seed produced in 2012 and in water potential of -0.6 MPa. By the variety Jelena,the highest germination energy had seed produced in 2015 and in water potential of -0.3 MPa, while the lowest germination energy had seed produced in 2012 in water potential of -0.6 MPa.

Wheat seed germination in total

Table 2 shows the influence of the seed age and mannitol on germination of some varieties of wheat created at the Agricultural Institute of Republic of Srpska.

Average percent of total germination rate of all examined varieties, regardless of seed age and water stress, was 80.2 %. Careful analysis showed that variety Bosanka had the largest number of germinating seeds 90.3%, whereas variety Jelena had the lowest number of germinating seeds 73.8%. Furthermore, it was found that differentiation between two varieties Bosanka and Nova Bosanka was statistically highly significant, but differentiation between varieties Nova Bosanka and Jelena was significant. Average results of total germination, previously mentioned, were a lot better compared to the experiment conducted by *Velijević et al*. (2016) whose parameters of total germination ranged from 75% to 78%, in the experiment which used certain varieties of clover and Italian ryegrass as samples, whereas the experiment carried out by *Poštić et al.* (2005) on a pepper showed total germination of 80%. *Lomović et at.* (1995) examined a wheat seed germination and found that variability of total germination exists depending on an examined variety.

At a water potential of 0 MPa the highest percentage of germinating wheat seeds was 90.3%, whereas the lowest percentage was 70.5% at a water potential of –0.6 MPa. Determined differentiation was statistically highly significant. Control parameters for all varieties indicated that the percentage of total germination was above the minimum of 88%, which represents a legal requirement for a high quality germination of seed, and moreover, is in line with

findings by *Mladenovski et al.* (2000) whose experiment was carried out on Macedonian wheat varieties in controlled conditions at water potential of 0 MPa.

Table 2. The influence of the seed age and mannitol on total wheat seed germination (%)

Variety	*Mannitol*	*Year*		*Average for variety*
		2012	2015	
Bosanka	0	94.0	96.0	95.0
	-0,3	87.0	93.0	90.0
	-0,6	81.3	90.8	86.0
	Average	87.4	93.3	90.3
Nova Bosanka	0	80.8	99.0	89.9
	-0,3	73.0	78.5	75.7
	-0,6	70.0	66.0	68.0
	Average	74.6	81.2	77.9
Jelena	0	74.0	98.0	86.0
	-0,3	71.5	81.5	76.5
	-0,6	59.3	58.5	58.9
	Average	68.3	79.3	73.8
Average for the mannitol	0	82.9	97.7	90.3
	-0,3	77.2	84.3	80.7
	-0,6	70.2	71.7	70,5
Average for the year		76.8	84.6	80.2

LSD	*A*	*B*	*C*	*AxB*	*AxC*	*BxC*	*AxBxC*
0,05	4.028	4.028	3.289	6.978	5.698	5.698	9.868
0,01	5.371	5.371	4.386	9.304	7.595	7.595	13.156

Seed produced in 2012 had a value of total germination rate 76.8 %, whereas a value of total germination rate of seed produced in 2015 was 84.6%. Changes in total germination rate influenced by seed age were statistically significant. Higher seed age was followed by a lower seed germination rate, which is in line with results obtained by *Jevđović* (2001) where valerian seed age was examined. In all examined varietes the highest germination rate was marked down at water potential 0 MPa. The increase of water stress is followed by a decrease in germination rate within every type examined, which is consistent with findings by *Madžo* (2013) whose experiment showed the influence of water stress on germination and root length. This experiment used different concentrations of water solution (5% and 10%). The experiment showed that water solutions caused inhibition of germination and root length.

This experiment showed that germination rate of older seed was low, whereas germination rate of younger seed was higher. Variety Bosanka had the highest

germination rate (96.0%) within seed produced in 2015 at water potential of 0 MPa, whereas seed produced in 2012 at water potential of –0.6 MPa had the lowest germination rate (81.3%). The variety Nova Bosanka showed certain differentiation. Seed produced in 2015, at water potential of 0 MPa had the highest germination rate (99.0%), whereas the lowest germination rate (66.0%) was marked down within seed produced in 2015. At water potential of –0.6 MPa variety Jelena had the highest germination rate (98.0%), within seed produced in 2015 at water potential of 0 MPa, whereas seed produced in 2015 at water potental of –0.6 MPa had the lowest germination rate (58.5%). Given results are in line with the experiment carried out *by Jovović et al.* (2015) where varieties Bosanka, Nova Bosanka and Jelena were analysed.

Vigor test for wheat seed

Table 3 shows the wheat seed vigor test results which represents the number of seed able to withstand different stress factors.

Table 3. The influence of seed age and mannitol on vigor test (%)

Variety	*Mannitol*	*Year*		*Average for varieties*
		2012	2015	
Bosanka	0	37.0	34.8	35.9
	-0,3	33.0	37.3	35.1
	-0,6	34.3	32.9	33.6
	Average	34.8	37.9	34.9
Nova Bosanka	0	34.1	68.0	52.5
	-0,3	34.0	52.5	43.3
	-0,6	29.3	44.5	36.9
	Average	33.4	55.0	44.2
Jelena	0	30.0	75.0	52.5
	-0,3	31.3	51.5	41.4
	-0,6	25.0	43.5	34.3
	Average	28.8	56.7	42.7
Average for the mannitol	0	34.7	59.2	46.9
	-0,3	32.8	47.1	39.9
	-0,6	29.5	40.3	34.9
Average for the year		32.3	48.9	40.6

LSD	*A*	*B*	*C*	*AxB*	*AxC*	*BxC*	*AxBxC*
0,05	3.631	3.631	2.964	6.288	5.134	5.134	8.894
0,01	4.840	4.840	3.953	8.383	6.846	6.846	11.858

Results showed 40.6% vigor, which is exquisite given the opinion of *Sarić-Krsmanović* (2013) who says germination depended on the development of

vigor. The highest vigor (44.2%) was shown by Nova Bosanka variety, whereas variety Bosanka had the lowest vigor (34.9%). Determined differentiation was statistically highly significant, whereas vigor test differentiation had no statistical significance. *Đekić et al.* (2013) defines vigor test as an indicator of phytic acid presence, which is significant for wheat seed health. Furthermore, it has been proved that phytic acid increases a vigor of seedlings and inhibits a development of alfatoxins in a wheat seed.

At water potential of 0 MPa the highest vigor noted was 46.9%, whereas the lowest vigor value was 34.9% at water potential of –0.6 MPa. Determined differentiation was statistically highly significant. Statistically highly significant differentiation in vigor test was evident at water potential range between 0 MPa and –0.3 MPa, as well as between –0.3 MPa and –0.6 MPa. Vigor seed and sprout length, followed by the root and coleoptile length, were the most sensitive to drought stress (*Dhandas et al.* , 2004).

For seed produced in 2012. vigor was 32.3%, whereas for seed produced in 2015. vigor was 48.9%. Determined differentiation was statistically highly significant.

Mannitol concentration stress vigor test obtained following results: the highest values were shown by all varietes at water potential of 0 MPa, whereas the increase of water potential led to decrease of vigor levels. These results are consistent with findings by *Vujaković et al.* (2011) where winter vetch seed was examined and it was determined that decrease in germination rate was influenced by adverse conditions applied during the vigor test. Every examined wheat variety with younger seed had higher vigor compared to older seed, whereby differentiation was statistically highly significant.

Conclusion

Results obtained by this experiment showed that osmotic effect combined with different concentrations of mannitol has the influence on a seedling growth at early development stages of every wheat variety tested. Close examination of adverse conditions, different osmotic potentials, demonstrated that every tested variety showed a reduction in germination rate at water potential of –0.3 MPa, which can be an indicator of sensitivity to stressful conditions. Seedling growth was less reduced at water potential of –0.3 MPa, whereas at –0.6 MPa reduction was greater. Based on results, it can be concluded that threshold limit value of water potential, influenced by mannitol, to which all varieties can have acceptable seedling growth, is –0,3 MPa (lower stress level).

When it comes to seed age, the experiment indicated that older seed (2012) exposed to different mannitol concentrations has given weak results, as opposed to younger seeds (2015).

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Economic viability of raising apiary

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Summary: The aim of this work is to determine the economic feasibility of investment in the production of honey. Honey production is important in beekeeping. Besides honey, other products are pollen, propolis and wax. There are a number of possibilities and ways of honey production, and the two basics ways are mobile and stationed beekeeping. In our example the stationary production model is shown. For the evaluation of the economic feasibility the dynamic methods of evaluation instruments (net present value, internal rate of return and payback period) and economic indicators (profitability and cost-effectiveness) are used. The largest part of the investments goes for the equipment which has to be purchased in the first year. The total investments are 26 152,-KM. The investment is financed from the loan of 20 000, - KM, and the rest is financed from the own source. Total annual revenues go up to 47 000,-KM. Most of the costs consist of labor work, food and packaging. All economic parameters show economic feasibility of investment in the production of honey. In the end the most important thing in order to become a beekeeper, amateur of specialized worker is to know theoretical and practical knowledge as well as developing a selfless love for these winged insects.

Key words: bees, honey, economic justification, stationed production model, investing

Introduction

Beekeeping is an agricultural production activity that can be enjoyed by people of different professions, age and gender (Pavlić, 2014). In beekeeping we distinguish these two types; migratory beekeeping (in which the beekeeper moves the hive near grazing depending on which season), stationed beekeeping (in which the beekeeper places the hive mainly in one place) and, as a new branch, eco beekeeping. Beekeepers are specific types of livestock producers because they do not depend on the land, often they do not even have a land or if they do it is very little. The benefits of keeping bees are direct and indirect. Direct benefits are honey, pollen (flower and powder), propolis, beewax, bee venom, royal jelly, queen bee, bees, and the indirect benefits is the role of bees in the pollination of crops for human and animal consumption. Number of hives in an apiary mainly depends on the capacity of bee pastures in the region of 6-8 km in diameter as well as the saturation of that region of the number of hives (Umeljić, 2010). Most often this number varies from 50 to 80 in one bag apiary. Each hive needs to have its clearly highlighted number, which corresponds to the number in beekeeping diary in which are entered all operations and observations for this particular bee society (Kulinčević, 2006). The aim of this study is to determine the economic viability of raising the apiary with the model of 100 beehives, and on that basis calculate the parameters that reflect the economic feasibility of certain investments.

Materials and methods

For this work literature from various authors was used, authors who not only deal with the description but also with application of beekeeping and its production. Methods used in this paper are: collecting data from available literature and websites, data analysis, analysis types and cost structures of honey production, assembly calculation, and calculation of economic indicators of production of honey. For economic calculations and tables, we used the data collected through interviews with beekeepers who are involved in the production of honey. Based on the analyzed data and economic calculations different models of production of honey, and the economic feasibility of production were compared, applying the calculation of Excel.

For the assessment of economic feasibility, we used dynamic estimation methods: net present value (cumulative net cash flow at the end of the project), internal rate of return (based on excel financial functions -Irr) and discounted payback period:

In addition to the dynamic methods of evaluation of investment, we have used and financial indicators: cost-effectiveness and profitability (Ivanković and Vaško, 2013).

Production of economy $= \frac{(total\ revenue)}{(total\ expenses)}$ Profitability $= \frac{Profit}{(uk.expenses)} *100$

Results and discussions

At the very beginning beekeeping requires all equipment, from the hive to the accessories and for that initial investments are needed in order to start a business.

Table 1. *Investment costs of raising apiary*

Investment cost(2018)	Unit of measure	Quantity	Price (KM)	Value
Beehives	Piece	100	70	7 000
A swarm of bees	Piece	100	60	6 000
Queen bee	Piece	100	18	1 800
Smoke machine	Piece	1	22	22
Suit	Piece	2	40	80
Electric whirligig	Piece	2	30	60
Knife for tilting Honeycomb	Piece	2	6	12
Beekeeping knife	Piece	2	4,5	9
Brush bees-fastener	Piece	2	3,5	7
Queen excluder	Piece	100	0,6	60
Means of transport	Piece	100	4,7	470
Total for 2018				**25 520**
Year 2019				
Honey extractor	Piece	1	365	365
Tub for titling the cap	Piece	1	7	7
Bucket for pouring honey	Piece	1	180	180
Bucket for pouring honey	Piece	1	80	80
Total for 2019 year				**632**
The total investment cost of raising apiary				**26 152**

Table 1 shows the investment cost for the equipment of the apiary with 100 hives. Economic lifetime of the investment is 10 years (0 + 9), the investment begins on 2018 year, and a period of effectuation of the investment is to 2027. The investment is made through the period of two years, in the first year is reserved for collecting the equipment that is needed, while in the second year investments are made to those assets that are necessary for extracting honey which will bring first income.

Total number of investment is 26 152,-KM, out of that number 25 520 ,-KM goes to the first investment, and 632 to the second year. Out of the total investments 20,000 - KM is invested from the loan, while the rest is self-financing.

Table 2. *Realized revenues*

Type of income	Unit of measure	Quantity	Price (KM)	Value
Honey	Kg	3 000	12	36 000
Propolis	Kg	20	150	3 000
Pollen	Kg	20	30	600
Wax	Kg	100	14	1 400
A swarm of bees	Piece	100	60	6 000
Total				**47 000**

Since the first year goal is to strengthen communities so that they can successfully overwinter, we cannot expect the yield of honey and other products of bee-keeping, and therefore we do not have income in the first year of the economic lifetime of the project. If there are no unforeseen circumstances, the assumption is that we will in the coming years the project achieve the same yields of honey, propolis, pollen, wax and income from the sale of swarms of bees. Total projected revenues per year amount is 47 000, - KM.

Table 3. Costs that occur in all years

Type of expenditure	Unit of measure	Quantity	Price	Value
Cake	Kg	300	3	900
Sugar	Kg	2 000	15	3 000
Protective devices	L	100	10	1 000
The coast of the transport vehicle	L	1 000	1.8	1 800
Salary costs	Month	12	800	9 600
Total				16 300

Table 3. presents the expenditures that occur in each year of effectuation of the project. The most significant cost is the salary of an employee. The following table shows the cost of packaging used for packaging and storage of bee products. Since the first year is without the profit, the costs of packaging arise only from the second year. Since the yield is constant, ie. does not change from year to year, we have the same costs for the next nine years. The cost of packaging represent a significant item in total costs, and amounts per year 2 800, - KM.

Tabela 4. The costs of packaging

Packaging	Unit of measure	Quantity	Price	Value
For honey (750ml)	Piece	4000	0,60	2 400
For propolis (20ml)	Piece	1000	0,20	200
Pollen (20ml)	Piece	1000	0,20	200
The total cost of packaging				2 800

The project of raising apiary is largely financed by bank loan which provides loan at an interest rate of 8% and payback period of 5 years. For this reason, we have and interest costs from 2017-2022, which declines year after year.

Table 5. Interest expense

Year	2018	2019	2020	2021	2022
Total	1 478	1 196	892	562	204

Depreciation is an expense, but unlike other costs, it does not constitute an outflow of funds. For this reason, we consider it separately from other costs (expenses). In the analysis depreciation of bee means and means of transport are calculates, and the results in the first year are 1276, - KM, while the remaining 9 years are 1 308, - KM.

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For the assessment of the cost-effectiveness of the project, dynamic estimation methods (discounted payback period, internal rate of return, net present value) and financial indicators (cost-effectiveness and cost-effectiveness) were used.

Table 6. Elements for the calculation of investment and economic indicators

Year	2018	2019	2020	2021	2022	2023	2024	2025	2026
Revenue	47 000	47 000	47 000	47 000	47 000	47 000	47 000	47 000	47 000
The residual value									13 108
Total receipts	47 000	47 000	47 000	47 000	47 000	47 000	47 000	47 000	60 108
Operating expenses without depreciation	17 778	20 296	19 992	19 662	19 304	19 100	19 100	19 100	19 100
Amortization	1 276	1 308	1 308	1 308	1 308	1 308	1 308	1 308	1 308
Total expenditures	19 054	21 604	21 300	20 970	20 612	20 408	20 408	20 408	20 408
Gross profit	-19 054	25 396	25 700	26 030	26 388	26 592	26 592	26 592	26 592
Income tax	-	2 539	2 570	2 603	2 639	2 659	2 659	2659	2 659
Net profit	-	22 857	23 130	23 427	23 750	23 933	23 933	23 933	23 933
Net cash flow	-43 298	23 532	24 438	24 735	25 057	25 241	25 241	25 241	25 241
The discounter factor (8%)	1,000	0,926	0,857	0,794	0,735	0,681	0,630	0,583	0,540
Discount net expected cash flow	-43 298	21 791	20 943	19 640	18 417	17 189	15 902	14 715	13 630
Cumulative net cash flow	-43 298	-21 507	-564	19 076	37 493	54 682	70 584	85 299	98 929

Internal rate of return is 55.43%, net present value at the end of the economic life is 117 448, - KM, Discounted payback period is two years, months and 11 days. Since the internal rate of return is greater than the interest rate, the net present value is positive and the payback period is shorter than the economic lifetime of the project concluded that it is reasonable and profitable investment in this project.

Honey production does not require high investment costs and achieves the high product yields, and consequently forms the high coefficients of profitability and cost-effectiveness. Except for the first year when there was no income, and from that we can conclude based on the given odds that the project shows the feasibility of investments.

In our project, efficiency and profitability ratios are presented for the period in which revenues are realized. The goal of any business entity that economy

coefficient is above one, and that total revenues will be higher than the total expenditures. Economy grows from year to year as a result of reduction in total expenditure, or a reduction of interest expense. In 2023 the age achieved the maximum that is constant to the end of the project.

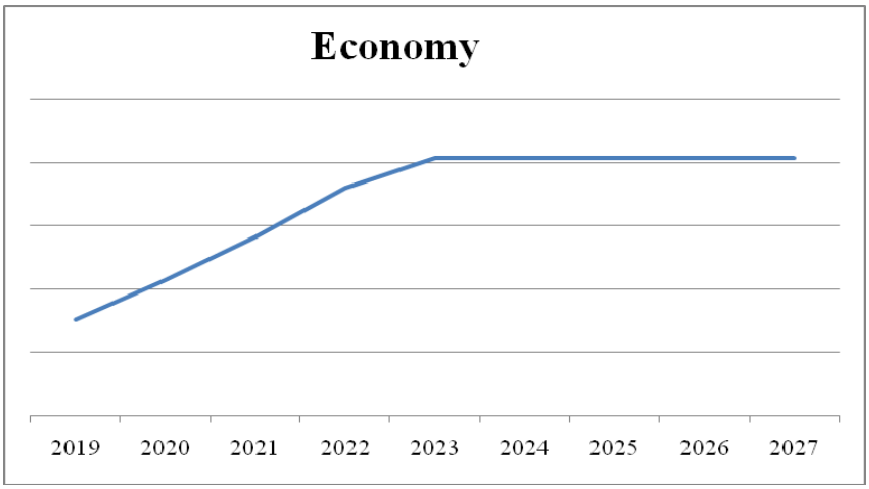

Chart 1. The coefficient of economy

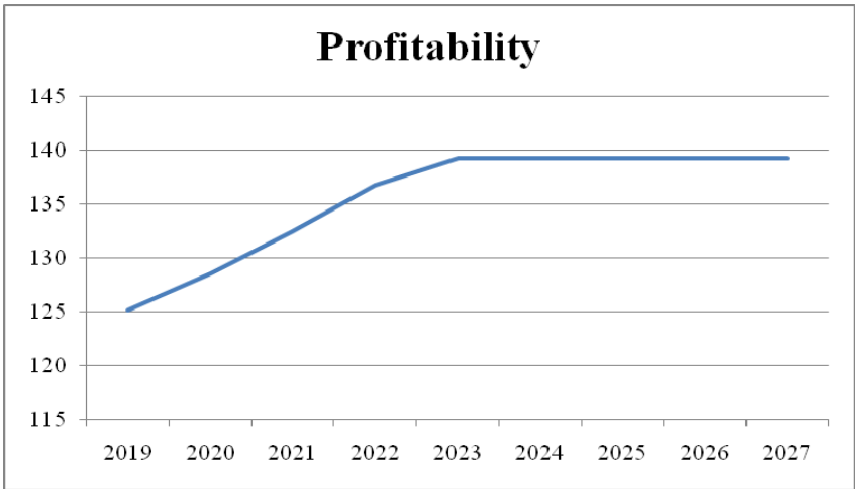

Chart 2. The coefficient of profitability

The goal of profitability is to show how many units achieved to get one unit of money invested. In this project profitability exceeding 100%, which means

that the profit is higher than the total cost, and the largest in the last 4 years effectuation of the project, when it amounted to 139%, which means that one invested KM, realizing a net profit of 1.39 KM.

And cost-effectiveness and profitability show that it is economically profitable to invest in a model stationed breeding bees.

Conclusion

Beekeeping is an agricultural production activity that with enough knowledge of theoretical and practical knowledge can achieve a high yield of honey, propolis, pollen and wax per hive. The main goal is to get large quantities of honey, with the least cost of production. Total investments amount is 26 152, - KM. Payback period is approximately two years. The high rate of income and economic indicators, as well as cost-effectiveness and profitability show that beekeeping branch of agriculture can ensure the existence of all people who work with it. Stationary production model is recommended for farmers with and without experience in this sector.

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Effect of Organic and Organo-Mineral Fertilizers on Grapevine Leaf Chlorophyll Content

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Abstract: This research was carried out to determine the effects of different organic and organo-mineral fertilizers on grapevine leaf chlorophyll content of Okuzgozu grape varieties during growing season. As organic fertilization treatment; O1: Lifebac NP, O2: Bactoguard and O3: Humanica, and Organi-Mineral Fertilization treatment; OM1: Bactolife Super Organo Power, OM2: Bactolife Quality Organo, OM3: Bactolife High Organo 5-5-0, OM4: Bactolife High and C: untreated control plant were applied. To determine the chlorophyll content of leaves during growing season in the 5th and 6th nodes of the shoot were measured by using a SPAD device. There were significantly differences in terms of the amount of chlorophyll in the leaves of Okuzgozu grape variety considering the experiment tretaments. At the end of the growing season (10.07.2017), the highest chlorophyll concentration was determined in vines treated with organic Humanica treatment (36.7). The least chlorophyll content was determined in leaves of untreated grapevines for all of the fertilizer treatments

Key words: Grapevine, Okuzgozu, Organik Fertilizer, Chlorophyll, SPAD

Introduction

Turkey is situated between latitudes 36-42° north and longitudes 26-45° east, a favourable area for viticulture that has a long history in the cradle of civilization. Turkey is, thus, one of the top producers of grape and the total vineyard area is 468.792 ha and the grape production is approximately 4 million tonnes. Over 77 million ton of grapes are produced in the worldwide using more than 7.1 million ha. Turkey ranks fifth in terms of growing area after Spain, France, China, and Italy. And ranks sixth in production after China, Italy, USA, Spain and France (Soylemezoglu et al., 2016).

A large peninsula in Turkey. Anatolia is surrounded by the Mediterranean Black and Aegean seas. This peninsula is connected to the Asian continent in the east and also to Caucasia in the north-eastern corner. which is believed to be the primary origin of cultivated grapes. Anatolia includes the area of origin of *Vitis vinifera* ssp. sylvestris (wild grape). which can now be found all over the country especially on river banks. shores of lakes and in forests. Anatolia is also called Asia Minor. In Turkey. grapes have been mainly grown as table grapes (52%) for raisins (38%) and for fruit juice and wine (10%) with around 80 standard cultivars grafted onto mainly six standard rootstocks in nine viticultural regions (Soylemezoglu et al., 2015). Turkey has about 7% of the world's area of vineyards. and produces 6.4% of the world's grape production. In addition. productivity in Turkey has improved by about 40% in the last 15 years from 6654 kg ha^{-1} in 1998 to 9249 kg ha^{-1} in 2012 (Anonymous, 2014).

Turkey and eastern neighbour countries known as Asia minor are the motherland of grapevines. Grapes is cultivated since 8th century BC in this area. A neo Hitite rock-cut relief in Divriz in South central Turkey shows that grapevine was well known fruit in ancient Hitite civilization. The rock relief depicts the late 8th century BC (ca 730- 710BC). Tabalian(Tuwanuva) King Warpalawas and storm or fertility-God Tarhunzas is accompained with grapes. wheat and hieroglphic Luwian inscription. The relief express the thanksgiving of Warpalawas to the God Tarhunzas. Turkey is located in the northern hemisphere between the 36^{o} - 42^{o} northern parallel and it is one of the mediterrranean countries. Because of favorable ecological conditions grapevines can be grown all over the country. For this reason it possesses over 1200 grape varieties. About 37 % of fresh grapes grown in Turkey goes into juice and other local consumptions such as kofter, sucuk, pekmez and 3 % into wine. while 27% is sold as table grape and 33% is dried as seedless (raisin) and seeded grapes (Uzun and Bayır, 2008).

The aim of this study was to determine the effects of different organic and organo-mineral fertilizers on grapevine leaf chlorophyll content of Okuzgozu grape varieties during the vine gowing season.

Material and methods

In this study Okuzgozu grape variety was used as a material. The most valuable red wines of Anatolia are made from this grape. It enchants always the experts with their dark cherry-purple color, aromatic properties and plump structure. The amount of Alcohol is 12.5-13.5% and amount of acid is 5.5-7 gr / l. It fits to rest it for long years. The cluster is conical, large (400-500 g) and full grain.

The study was carried out in 2015 in a fully automated greenhouse belonging to the Department of Horticulture of the Faculty of Agriculture of the University of Dicle. The plants are growth in a black plastic bag at a ratio of 1: 1: 1 (Farmyard manure: Sand: Soil).

This study O1: Lifebac NP, O2: Bactoguard and O3: Humanica fertilizers were used as organic fertilizer treatments. And OM1: Bactolife Super Organo Power, OM2:Bactolife Quality Organo, OM3: Bactolife High Organo 5-5-0, OM4: Bactolife High Organo 5-5-5 fertilizers were used as organo-mineral treatments (Anonymous. 2017). C: Control plants was untreated fertilizer.

Lifeback-NP is a microbial fertilizer containing natural Bacillus subtilis and Bacillus megaterium isolates. It proliferates rapidly by covering plant roots/leaves and generates nutrient-intake promoting secretions without feeding from plants. It enables plants to fix free nitrogen in the atmosphere through their leaves and roots.

Bactoguard. derived from plants. is a liquid organic fertilizer containing organic acids. amino acids. antioxidant enzymes and hormones in natural form. It provides effective protection against cold. hail. water. and salt stresses. It is contained Organic Matter: 35%; Nitrogen (N): 2.5%; Organic Carbon: 24%.

Humanica is an organic matter containing and soil conditioning fertilizer based on Leonardite which has a dark color and soft texture. Soils factors require reconditioning if their structures suffer disruption along with efficiency and productivity losses due to certain factors. It is contained Organic Matter: 40%. Total Acid (Humic+Fulvic): 50%. Maximum Humidity: 35%.

Table 1. Content of Organo-mineral fertilizers.

Organomineral Fertilizers	*Bactolife Super Organo Power*	*Bactolife Quality Organo*	*Bactolife High Organo 5-5-0*	*Bactolife High Organo 5-5-5*
Organic Material	%20	%20	%20	%20
Total Nitrojen	%10	%4	%5	%5
Organic Nitrojen	%2	%1	%1	%1
Urea Nitrojen	%8	%3	%4	%4
Water soluble Boron	%0.2	%0.2	%0.2	%0.2
Water soluble Copper	%0.2	%0.2	%0.2	%0.2
Water soluble Iron	%0.2	%0.2	%0.2	%0.2
Water soluble manganese	%0.2	%0.2	%0.2	%0.2
Water soluble Zinc	%0.2	%0.2	%0.2	%0.2
Chlorine rate	%0.12	%0.1	%0.1	%0.1
pH range	3.5-5.5	3.5-5.5	1.5-3.5	1.5-3.5

Results and discussion

SPAD readings in vine growing seasons revealed that organic and organo mineral fertilizer treatments significantly affected the leaf chlorophyll content of the Okuzgozu grape variety (Table 2 and 3). The beginning of the growing season (22.05.2017) the highest chlorophyll concentration was determined in vines treated with organic Bactoguard treatment (31.2). The least chlorophyll content was determined in leaves of untreated vines (27.2).

Date of 26.05.2017, the highest chlorophyll concentration was determined in vines treated with organic Bactoguard treatment (31.7). Date of 05.06.2017, the highest chlorophyll concentration was determined in vines treated with organic Humanica treatment (31.9). Date of 12.06.2017, the highest chlorophyll concentration was determined in vines treated with organo-mineral Bactolife Super Organo Power treatment (33.3). Date of 19.06.2017, the highest chlorophyll concentration was determined in vines treated with organo-mineral Bactolife Super Organo Power treatment (34.6). Date of 26.06.2017, the highest chlorophyll concentration was determined in vines treated with organo-mineral Bactolife High Organo 5-5-5 treatment (36.0). Date of 03.07.2017, the highest chlorophyll concentration was determined in vines treated with organic Bactoguard treatment (37.1). Date of 10.07.2017, the highest chlorophyll concentration was determined in vines treated with organic Humanica treatment (36.7). All of the fertilizer treatment the least chlorophyll content was determined in leaves of untreated vines (Table 2 and 3).

Table 2. Leaf chlorophyll content (SPAD) during the growing season of Okuzgozu grape variety (first period)

Fertilizer*	*22.05.2017*	*26.05.2017*	*5.06.2017*	*12.06.2017*
O1	29.3	29.8	29.8	30.8
O2	31.2	31.7	31.0	31.0
O3	30.2	29.9	31.9	31.6
OM1	31.1	31.5	30.8	33.3
OM2	31.1	30.8	30.6	29.5
OM3	30.8	30.8	29.3	30.1
OM4	28.8	29.2	29.5	29.0
C	27.2	28.3	28.4	28.5

* Organic Fertilizer; O1: Lifebac NP, O2: Bactoguard and O3: Humanica Organi-Mineral Fertilize; OM1: Bactolife Super Organo Power, OM2: Bactolife Quality Organo, OM3: Bactolife High Organo 5-5-0, OM4: Bactolife High C: untreated control plant

Table 3. Leaf chlorophyll content (SPAD) during the growing season of Okuzgozu grape variety (second period)

Fertilizer[*]	*19.06.2017*	*26.06.2017*	*03.07.2017*	*10.07.2017*
O1	32.9	34.1	35.4	36.3
O2	33.7	31.9	37.1	35.0
O3	34.3	34.7	36.1	36.7
OM1	34.6	35.7	36.8	35.7
OM2	32.6	33.7	34.6	36.2
OM3	32.8	32.4	34.2	35.0
OM4	32.8	36.0	36.2	34.7
C	29.4	29.9	30.2	33.3

* Organic Fertilizer; O1: Lifebac NP, O2: Bactoguard and O3: Humanica Organi-Mineral Fertilize; OM1: Bactolife Super Organo Power, OM2: Bactolife Quality Organo, OM3: Bactolife High Organo 5-5-0, OM4: Bactolife High C: untreated control plant

Conclusion

Leaf chlorophyll content is directly related to plant stress and aging. Therefore the amount of leaf chlorophyll in the vine plant is the manager of environmental and plant nutrient changes. It affects the yield of grapevine, quality of grapes and wine quality. Changes in the amount of chlorophyll in the vine leaves are detected using the chlorophyll-measuring instrument (SPAD). As a result of the study we determined that organic and organo-mineral treatments were effected the leaf chlorophyll content of Okuzgozu grape variety.

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Vitrification of zebrafish *Danio rerio* testicular tissue

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Abstract: Vitrification is a novel cryopreservation technique which prevents the negative effects of ice formation, thus offering a potent alternative to the conventional slow-rate freezing. Therefore, the main aim of this study was to develop a needle immersed vitrification (NIV) method in order to cryopreserve the whole zebrafish testes. Testes were pinned on an acupuncture needle, passaged through equilibration (ES) and vitrification solutions (VS) containing different combinations and concentrations of methanol (MeOH), propylene glycol (PG) and dimethyl sulfoxide (Me_2SO)and subsequently plunged into liquid nitrogen. 3 ES and 3 VS (totaling to 9 test groups) were used in order to find optimal concentrations of ES and VS which will result in viable cells after warming. Warming was done by putting samples in different warming solutions (L15, sucrose and FBS in different percentage) for varying periods of time. Fresh and cryopreserved tissue was dissociated and the obtained single cell suspension was stained by trypan blue to determine cell viability. The final viability was determined as the number of viable cells recovered from the cryopreserved tissue compared to the number of viable cells obtained from the fresh tissue. Contrary to ES, VS had a significant effect on spermatogonia viability after warming ($p<0.05$). Vitrification and equilibration solutions containing equal cryoprotectant concentrations (ES: 1.5 M MeOH + 1.5 M PG; VS: 3M DMSO and 3M PG) yielded the highest spermatogonia survival rate of 48.5%. Since this two solutions gave highest survival rate, they were used in the subsequent experiment with 5 different zebrafish strains. Viability of all 5 strains was higher than 50%. This shows that NIV of whole zebrafish testes is efficient method for storing zebrafish genetic resources, especially since after transplantation they can mature into both male and female gametes.

Key words: Vitrification, testicular tissue, spermatogonial stem cells

Introduction

Cryopreservation plays a significant function in the tissue banking which is necessary for storing genetic material not only of endangered species, but also of transgenic lines which are nowadays created thanks to the rapid development of science and different technologies. Even though cryopreservation offers many advantages, the slow-rate freezing generates several conditions that may lead to cell damage (intracellular and extracellular ice formation, cell dehydration, cryoprotectant toxicity…) Recently, vitrification as a technique of fast cooling rate procedure which significantly prevents the negative effects of ice formation has been applied in the cryopreservation of fish gametes.

Vitrification enables hydrated living cells to be cooled to cryogenic temperatures in the absence of ice by leading to a glass-like solidification (Tavukcuoglu et al., 2012). This is achieved by using ultra-fast cooling and warming rates, together with using high cryoprotectant concentrations (Lujic et al., 2017). Vitrification simplifies and frequently improves cryopreservation because it eliminates mechanical injury from ice, eliminates the need to find optimal cooling and warming rates (Fahy and Wowk, 2015).

Besides mice and *Drosophila*, zebrafish (*Danio rerio*) is one of the most common model organisms with many mutant lines. Research with *D. rerio* has yielded advances in the fields of developmental biology, oncology, toxicology (Hill et al., 2005; Bugel et al., 2015), reproductive studies, genetics, stem cell research, regenerative medicine, evolutionary theory etc. Even though the protocols for cryopreservation of zebrafish sperm have been developed in a past years, protocols for cryopreservation of fish eggs and embryos are still not developed, mostly due to their complex structure (Carmichael et al., 2009; Yang et al., 2015). The aim of this study was to develop and optimize the vitrification procedure for zebrafish testes. Additionally, repeatability of the method is demonstrated in five different zebrafish strains (wild AB type [ABwt], casper, leopard [ABleo], vasa [vasa::egfp] transgenic line and Wilms tumor [wt1:gfp] transgenic line).

Material and methods

Animals and sampling

All idividuals used in this study were housed in a Zebtec recirculating system (Tecniplast Zebtec, Tecniplast, Buguggiate, Italy) at the Department of Aquaculture, Szent István University, Hungary. Fish were maintained at 25±0.5 °C in a 14-h light/10-h dark cycle. Fish were euthanized by overdosing with2-phenoxyethanol. To avoid contamination after excision, testes were put in 70%

EtOH for 1-3 seconds and then in 96 well microplate filled with Leibovitz L-15 media kept on ice.

Experimental design

Firstly, optimal vitrification procedure for wild AB type [AB_{wt}] zebrafish testis was developed by testing nine different test groups with three vitrification and three equilibration solutions containing different concentrations and combinations of cryoprotectants (Table 1). These test groups were chosen according to Marques et al. (2015) with minor modifications. Extender for all test groups consisted of L-15 media supplemented by 10% FBS, 25 mM HEPES and 0.5 M trehalose. After determination of the optimal vitrificaiton procedure, the repeatablity of the optimal method was tested on five different zebrafish strains (wild AB type [AB_{wt}], casper, leopard [AB_{leo}], vasa [*vasa::egfp*] transgenic line and Wilms tumor [*wt1:gfp*] transgenic line).

Table 1. Test groups for the vitrification of zebrafish testes with three equilibration (E1-E3) and three vitrification solutions (V1- V3) containing different combinations and concentrations of methanol (MeOH), propylene glycol (PG) and dimethyl sulfoxide (Me2SO).

	Equilibration solution (E)			Vitrification solution (V)		
	E1	E2	E2	V1	V2	V3
MeOH (M)	1.5	1.5	-	1.5	1.5	-
PG (M)	1.5	-	1.5	4.5	-	3
Me_2SO (M)	-	1.5	1.5	-	5.5	3

Tissue vitrification

Three testis were pinned on a sterile acupuncture needle which was kept in an Eppendorf tube (2ml) filled with Leibovitz L-15 medium until vitrification. In order to prepare the tissue for vitrification, needles with pinned testes were transferred into equilibration solution for 5 minutes, afterwards they were transferred into vitrification solution for 30 sec, during this time cryoprotectants have impregnated tissue. Subsequently needles were removed from vitrification solution, dried a bit by sterile paper towel and plunged into liquid nitrogen placed in a Styrofoam box. Needles were kept in liquid nitrogen in a closed Styrofoam box for 5 - 10 minutes, transferred into precooled cryotubes, placed onto a metal cane and then stored into cryobank until further work.

Warming procedure

Each needle was warmed separately. Cryotubes were detached from the metal cane, plunged into liquid nitrogen and needles were released and still kept in a Styrofoam box. Each needle was incubated in 3 serial warming solutions

(WS) at 24 °C) for various period of time. Firstly, needles were transferred into WS1 (L-15 + 10% FBS + 0.1 M trehalose) for 1 minute, then into WS2 (L-15 + 10% FBS + 0.1 M trehalose) for 3 minutes and finally into WS3 (L-15 + 10% FBS) for 5 minutes. Afterwards testes were released from needles and placed individually in a separate well (96-well plate) filled with L-15 supplemented with 10% FBS.

Tissue digestion

Warmed tissue was added into digestion solution containing 2 mg/ml collagenase type I, 1.5 mg/ml trypsin and 20 µg/ml DNase I and cut into smaller pieces. Cut tissue was incubated on a shaking plate for 90 minutes at 24 ˚C. Digestion process was stopped by adding 400 µl of L-15 and 100 µl of FBS (10% FBS v/v). Obtained solutions were filtered through 50 µm filters and centrifuged at 200 ×g for 10 minutes at 24 ˚C. Supernatant was carefully removed and the pellet was resuspended in 20 µl of L15 supplemented with 10% FBS. Cell suspension was kept on ice until further use.

Viability evaluation and cell counting

Equal volume of cell suspension and 0.4 % trypan blue were mixed, incubated for 3 minutes and viability of each sample was checked in a Bürker-Türk type hemocytometer under the microscope. Number of live cells (unstained by trypan blue) was calculated in 15 fields per each sample.

Statistical analysis

All percentage data were logit transformed prior to statistical analysis. Two-way ANOVA was used to test the effect of different equilibration and vitrification solutions on cell viability. All statistical analysis were performed in Statistica v12 software (Statsoft Inc., USA).

Results and discussion

Viability of freshly digested testicular cells exceeded 99%. The vitrification procedure caused a decrease in the number of live cells compared to the control. Protocol using E1 and V3 yielded in highest viability (48%), however clear statistical differences could not be delineated. Two-way ANOVA displayed that vitrification solutions had a significant effect on viability ($F_{(2,18)}$=3.56, p<0.05) while equilibration solutions did not have a significant effect $F_{(2,18}$=0.24 p>0.05) (Figure 1). Since vitrification solutions containing equal concentrations of cryoprotectants (V3) generally yielded the highest viability, this indicates that the 1:1 cryoprotectant ratio is more optimal and that high concentrations of 4.5 and 5.5 M could be toxic for cells.

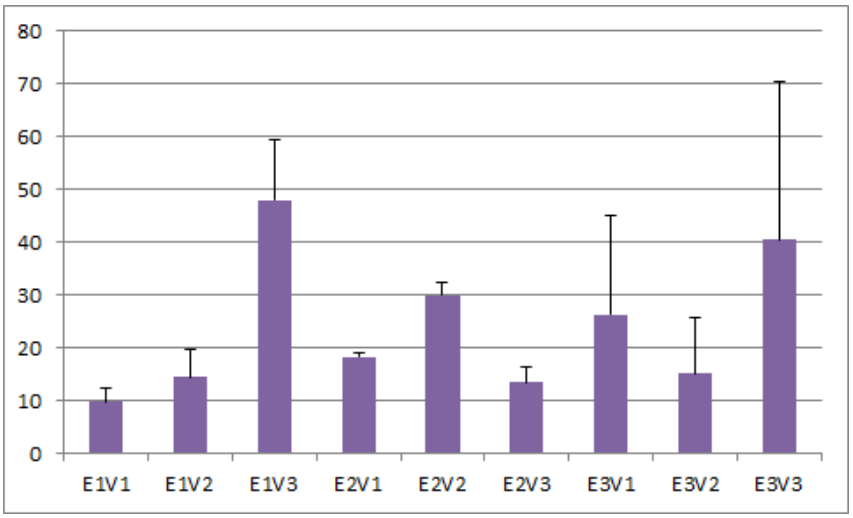

Figure 1: Effect of different combinations equilibration solutions (E) and vitrification solutions (V) on zebrafish testicular cells viability. Data are displayed as mean ± standard deviation.

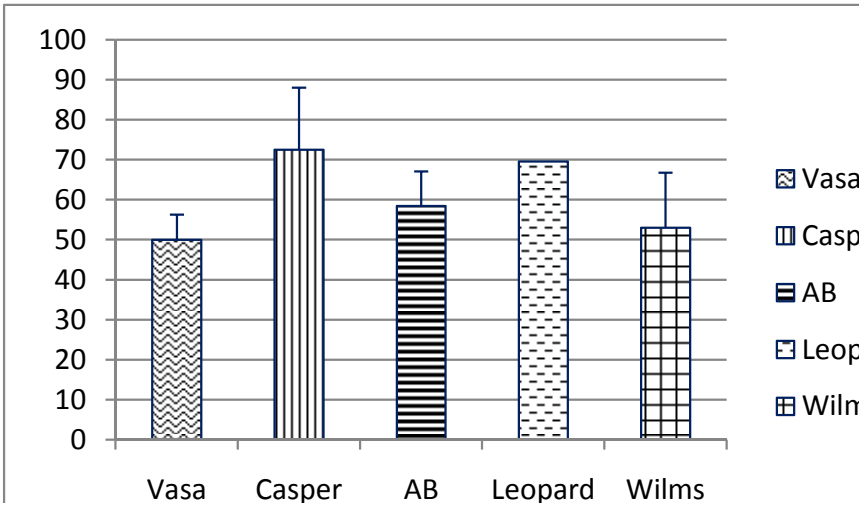

Figure 2: Percentage of viable isolated zebrsfish (different stains) testicular cells after vitrification with equilibration solution (E1) and vitrification solution (V3) Data are displayed as mean ± standard deviation.

After determination of the optimal vitrification protocol which led to highest viability, repeatability of this protocol was verified by cryopreserving testes of 5 different zebrafish stains - wild AB type [ABwt], casper, leopard [ABleo], vasa

[vasa::egfp] transgenic line and Wilms tumor [wt1:gfp] transgenic line. Viability was the highest for Casper stain of zebrafish (72,49%), while for the others are between 50% and 70% (Figure 2).

In present study we have demonstrated for the first time successful vitrification of zebrafish testicular tissue. Different concentrations and combinations of cryoprotectants in equilibration and vitrification solutions had a different effect on viability of zebrafish spermatogonia. Furthermore, the application of the optimal vitrification protocol was repeatable as demonstrated on different zebrafish lines. As previously mentioned, cryopreservation plays a significant role in the tissue banking which is necessary for storing valuable biological material The main advantage of NIV method is the direct exposure of testes to liquid nitrogen with minimal volumes of cryoprotectants being attached to them, therefore maximizing the cooling rate.

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Properties of Kenger Plants' Seeds *(Gundelia tournefortii* L.) grown naturally in Karacadag Region of Turkey

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Abstract: Kenger is naturally grown in Lebanon, Syria, Palestine, Israel, Jordan, Iraq, Iran, Azerbaijan, Armenia and Turkey, which have mostly semi-arid climates. In Turkey: Eastern Anatolia, Southeastern Anatolia, Mediterranean and Aegean regions, high places, often seen in the rocky regions of the mountains. The mineral composition of wild Kenger plants grown in Turkey with chemical composition and myrtle flower bud (*Gundelia tourneforti L.)* is rich in crude oil, crude protein and fiber. In the Eastern Anatolia Region in Turkey, it is effective for the treatment of vitiligo disease in folk medicine. Kenger 's fresh seeds are used as pickles and at the same time an effective diuretic. Fried seeds are used as kenger cups. In this study, germination of seeds collected in Karacadağ Region in petri dishes, transfer of seeds in greenhouse kenger seeds and nitrogen-protein values of these seeds were analyzed.

Keywords: kenger, *Gundelia tournefortii L*., seed, farming

Introduction

The kenger plant, which is known as the herald of spring in the Southeastern Anatolia Region and emerged from the first months of spring, is a plant with barbed and sultry till about 40-50 cm in height. It is a perennial and herbaceous plant and considered as a vegetable. Despite the fact that the plant is in a thorny structure, the first output is off-season. In the middle of thorns, a branch called "neri" emerges. Kenger *(Gundelia tournefortii),* Asteraceae (Asteraceae) family. Other names are known hook plant, hook, enger, henger, kalağan, kengi grass, kepre, kingar, sweet kenger, kanak, kaluğa (Ertuğ, 2000). Diyarbakir Karacadag near kenger found in abundance on the people of the region collected and sold in markets or by the side of the road, kenger gum is obtained and is presented to the market as Malatya Darende region. In this study, the general characteristics of

kenger to make available for the cultivation of seeds germination and cultures to determine the methods on how to resolve issues with. Kenger from the seed cultivation and cultivation techniques for the development of the study are reviewed. And at the same time, kenger was to determine the nutritional content of food and seed by determining the properties of consumption.

Materials and Methods

1. Materials

In the experiment the kenger seeds were used as the material collected from the region of Karacadag

2. Methods

The experiment was carried out in the unheated polycarbonate sera and laboratory belonging to the Garden Department of the Faculty of Agriculture of the University of Dicle in the spring semester. In research applications reviewed under the following headings;

2.1. Seed germination: In this experiment seeds + 4 ° C refrigerator in the laboratory under conditions were made. The seeds were separated from the thorns and outer shells and placed in petri dishes. The study consisted of 4 replicates and a total of 200 seeds were used, 50 each. Plastic and three-compartment Petri dishes were used with 10 seeds in each Petri fairies and a total of 20 were used. The number of seeds leaving during the experiment was observed by observation every day morning and evening.

2.2. Transferring germinated seeds to the greenhouse petri: In the previous experiment in a Petri dish filled with peat and germinated seeds in the greenhouse violes added to the experiment was established. In this study, a total of 70 germinated seeds were transferred to the violes. The number of seeds leaving during the experiment was observed and observed every day morning and evening.

2.3. Transplanting of seeds extracted from thorns and outer shells in violes in greenhouse conditions: In this application, a test was made by planting 200 seeds, peat stuffed with violets, with spines and outer shells removed. The number of seeds leaving during the experiment was observed and observed every day morning and evening.

2.4.Nitrogen and Protein Analysis in Kenger Seed: This application was made by applying the Kjeldahl method at the Gap International Agricultural Research and Training Center. For analysis the seeds were removed from the outer shells and dried. Dried seeds made into flour at the mill and prepared for analysis. For analysis to be performed on two samples, 1 g of each sample is taken and started to be applied. Nitrogen and protein analysis is usually done

when nitrogen and nutrients in food and feed are converted into ammonium sulfate as a result of burning with concentrated sulfuric acid. The resulting ammonium sulphate is converted to ammonia with a concentrated sodium hydroxide solution**.** The resulting ammonia is held in boric acid solution and the resulting ammonium borate is titrated with 0.1 N HCl solution. The total amount of nitrogen in the sample is found from the amount of spent HCL. This value was multiplied by the factor determined for each product and the amount of protein was found (KOKSAL et al., 2011) , (KUTLU, 2008).

% Nitrogen = [0.014 x N x (V1-V2)] x 100 / m

V1 = volumetric mL of HCl acid solution consumed in titration

V2 = volume mL of HCI acid solution consumed in titration in the witness assay

N = Concentration of the hydrochloric acid solution

M = weight of received sample (g)

% Protein =% Nitrogen x FACTOR

2.5. Determination of General Characteristics of Kenger Seed: With this application, it has been tried to determine the general characteristics of kenger seed. The general structure of the Kenger seed was determined by two different applications, crustacean seeds and thorns and outer shell-free seeds.

2.5.1. Features of Shell Kenger Seed: This application was made using a total of 400 kenger seeds. Kenger seed weight, height, diameter, number of thorns, length of thorns, weight per thousand seeds and height / diameter index were determined by this application.

a.) Seed weight for seeds of kenger 400 grams 100 units of the application to be weighed on a precision balance sensitive to 0.01 in 4 repetitions, the average weights were determined.

b.) For the seed diameter, 400 seeds of Kenger seeds were divided into two groups of 200 seeds and their diameters were measured with calipers. The values found in the groups were divided into 200 seeds and their average diameters were found. By combining two groups with average diameters, we were able to find the average diameter of 2 split and 1 kenger seeds with the group number.

c.) For the seed size, 400 seeds of Kenger seed were divided into two groups of 200 seeds and their lengths were measured with calipers. The values found in the groups were collected and divided into 200 seeds and the average length was found. Two groups with an average tumbler group were collected to provide the average number of 2 split and 1 Kenger seeds with the group number.

d.) For the seed length / diameter index, 400 seeds of Kenger seeds were divided into two groups of 200 seeds and the values measured by calipers with the sizes and diameters of the samples were divided. The values in the groups were collected and divided into 200 seeds, and the average height / diameter

value was found. Two groups with average height / diameter values were collected to find the average height / diameter index of 2 divided and 1 Kenger seeds with group number.

e.) The seed weight thousand of seed shellfish for the application to be 4 repetitions of 400 seeds 100 units of 0.01 grams kenger weighed on a precision balance sensitive to the average weights were determined.

f.) For the number of seed thorns, in practice two groups of 400 Kenger seeds 200 were separated and the number of thorns of the samples was written as the observation result. The values found in the groups were collected and divided into 200 seeds, and the average number of thorns was found. The two groups with the average number of thorns were grouped together to find the average number of thorns of 2 divided and 1 Kenger seeds with the group number.

g.) For seed thorn height, 400 seeds of Kenger seeds were divided into two groups of 200 seeds and their thorns were measured by calipers. The values found in the groups were collected and divided into 200 seeds and mean thorns were found. The two groups with mean thorns were combined to obtain the average thorn height of 2 divided and 1 Kenger seeds with the group number.

2.5.2. Freed from the thorns of the seed shell and Kenger Properties: This application was made using a total of 400 kenger seeds. The weight, height, diameter, grain weight and height / diameter index of the Kenger seeds free of crust and thorns were determined by this application.

a.) For the weight (g) of the Kenger seed which is free of crust and thorns, in practice 400 clams and thorns were weighed on a precision weighing scale of 0.01 gauge per 100 gauge Kenger seeds, and their average weights were determined. These values are summed and divided by the total number of seeds, 400. The result was to find the average weight of Kenger seed, which is free from 1 shell and thorn.

b.) For the diameter (mm) of the Kenger seed, which is free of crust and thorns, in practice, 400 pieces of shells and spikes of Kenger were removed from the two groups of 200 seeds and their diameters were measured with calipers. The values found in the groups were divided into 200 seeds and their average diameters were found. We collected two groups with average diameters to find out the average diameter of Kenger seeds divided into 2 groups and 1 shell and thorns.

c.) For the size (mm) of Kenger seeds which are free of shells and thorns, 400 pieces of shells and spikes of Kenger were removed from the spout and two groups of 200 seeds were separated and their size was measured with calipers. The values found in the groups were collected and divided into 200 seeds and the average length was found. We collected two groups with an average

tumbling number, and we found that the average number of Kenger seeds divided into 2 groups and 1 shell and thorn were found.

d.) For the height / diameter index of the Kenger seed which is free of shells and thorns, two groups of 200 Kenger seeds, separated from 400 shells and thorns, were separated and the values measured with calipers were divided by the size and diameter of the samples. The values in the groups were collected and divided into 200 seeds, and the average height / diameter value was found. Two groups with average height / diameter values were collected to find the average height / diameter index of Kenger seeds divided into 2 groups and 1 group of shells and thorns.

e.) For the weight of 1000 grain of Kenger seeds which are free of crust and thorns, in average 400 weights of Kenger seeds purified from 400 crusts and thorns were weighed and weighed on 4 sensitive weighers sensitive to 0.01 grams.

These values are summed and divided by the total number of seeds, 400. The result is multiplied by 1000 to find the weight of an average thousand grains of Kenger seed purified from the crust and thorns.

Results and discussion

1. Results On The Properties Of Seed Shellfish Kenger

When the characteristics of Kenger seed were examined, it was determined that the seed weight value was 0.21 gr. When the width and width of the seed were examined, it was determined that the width of the seed was 7.04 mm and the value of the seed was 12.15 mm. Seed index (height / width) was 1.74 (Table 1).

The average number of thorns in the Kenger plant was 7 and the mean thorn height was 5.01 (Table 2).

Table 1. Properties of seed shellfish Kenger

Seed Weight (g)	*Seed Diameter (mm)*	*Seed Length (mm)*	*Length / Diameter Index*	*Thousand grain Weight (g)*
0.21	7.04	12.15	1.74	208.65

Table 2. Features of seed shellfish bumps Kenger

Number of thorns	*Thorn length*
7	5.01

2. Freed from the thorns on the properties of the shell and the seeds of Kenger Results

When the characteristics of the Kenger seed, which is free of crust and thorns, are examined, it is determined that the seed weight value is 0.09 gr. When the average diameter and size of the seeds were examined, it was determined that the diameter value was 5.45 mm and the height value was 10.20 mm. Seed height / diameter index was determined as 1.93 (Table 3).

Table 3. Properties of Kenger seeds free of crust and thorns.

Seed Weight (g)	*Seed Diameter (mm)*	*Seed Length (mm)*	*Length / Diameter Index*	*Thousand grain Weight (g)*
0.09	*5.45*	*10.20*	*1.93*	*94.7*

3. Findings Related to Nitrogen and Protein Content of Kenger Seed

Determination of the nitrogen ratio was carried out on 2 specimens taken from the seeds. The values were calculated from the % nitrogen formula and the average percentage of nitrogen and% protein were found. The value of the factor used for protein detection in Kenger seed was multiplied by the general factor value of 6.25 (Table 4).

% Nitrogen detection

% Nitrogen=[0,014 x N x (V1-V2)] x 100/m

1.For example;
V1 = 49.86 ml
V2 = 0.26 ml
N = 0.1 ml
m = 1 gr
% Nitrogen= 6.94

2.For example;
V1 = 48.49 ml
V2 = 0.26 ml
N = 0.1 ml
m = 1 gr
% Nitrogen= 6.75

Nitrogen the average value of % 6.85 was found.

% Protein detection

1. For example; % Protein =% Nitrogen x FACTOR %Protein: 43.375
2. For example; % Protein =% Nitrogen x FACTOR %Protein: 42.187

The average protein value was 42.78%.

Table 4. Nitrogen and Protein Content of Kenger Seed

Nitrogen	***Protein***
%6.85	*%42.78*

4. Germination of seeds:

The trial was established as 4 replicates and consists of a total of 200 seeds. Each recipe was applied in the form of 10 seeds in 50 seeds and petri dishes.

The petri dishes contained in these experiments were numbered and were checked daily and the number of germination was written. 1. total of 27 germination with 27 turf root formation in total, total 40 germination with 30 turf root and 10 turf grass leaf formation in 2, 3. total 24 germination with 20 turf root and 4 cotyledon leaf formation in repeat, 24 turf root in 4th repeat A total of 24 germination occurred

The total number of germination was 115, and the germination rate of carcass seeds at +4 C was 57.5% in 4 repeated test refrigerated conditions.

5. ***Transfer of germinated seeds in petri to the greenhouse:***

Approximately 70 seeds germinated at +4 C under refrigerator conditions were planted in unheated polycarbonate greenhouse at 28 C and 75-80% moisture conditions, and daily outputs were observed. As a result of this observation, a total of 13 plant developments were observed.

Conclusion

Kenger seeds in the region need a long time to germinate in natural growing areas. In order to develop the method of cultivating the kenger plant, the propagation material must first be identified. Seeds are an important generative method that can be used for multiplication. Although there is not much study on the seeds of Kenger plant, it can be said that the germination rate is very low and requires a long period of time due to the presence of seed dormancy and natural conditions. In order to break this dormancy shown by Kenger seed, it was found out that thick shell was removed at +4 degrees and germination was achieved at 57.5% at suitable humidity conditions. It has been observed that increasing or decreasing the degree of temperature can change the rate of grassing, as well as removing the outer shell of the seed and increasing the rate of germination and germination at low temperature grade dormancy.

At the end of the study; Germinated seedless seeds have been found to be more abundant than ungerminated seedless seeds.

There have been no studies on the nutrient content of Kenger plant seed before in Turkey. Therefore, the nitrogen content and the protein content of the samples were found to be 6.85% and 42.78%, respectively. In addition to medically evaluating the nutrient content of the seed, the use of Kenger seed as a feed for poultry (chicken) is even more important because of its high protein content. With the nutrient content, it is possible to spread the breeding by providing faster germination in a short time. In the region, the farmer does not

make any efforts to grow the crop while reducing the annual yield. The farmer lack of knowledge about farming these days, it is noteworthy that kenger areas is reduced. The farmer lack of knowledge about farming these days, it is noteworthy that kenger areas is reduced.

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The effects of wastewater use on yield of cotton and soil pollution

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Abstract: The aim of this study is to determine the effects of treated domestic wastewater on cotton yield and soil pollution. The study was carried out in the experimental years of 2011 and 2012. The domestic wastewater was provided from the reservoir treated by anaerobic + stabilization. After treated wastewater, suspended solids (28-60 mg l^{-1}), biological oxygen demand (29-30 mg l^{-1}), and chemical oxygen demand (71-112 mg l^{-1}) decreased significantly compared to those in the untreated wastewater. There was no heavy metal pollution in the water used. The cotton-seed yield (31.8 g $plant^{-1}$) in the tanks where no commercial fertilizers were applied was considerably higher compared to the yield (17.2 g $plant^{-1}$) in the fertilized tanks where the recommended amount of nitrogen fertilizer was used. There were no significant differences between the values of soil pH. soil electrical conductivity (EC) after the experiment increased from 0.8-1.0 dS m^{-1} to 0.9-1.8 dS m^{-1}. The heavy metal pollution did not occur in the soil and plants because there were no heavy metals both in the treated waste water. It could be concluded that treated domestic waste water could be used for growing crops in a controlled manner, which would not be used directly as human nutrients, such as cotton.

Key words: treated wastewater, irrigation, soil pollution, crop yield, cotton

Introduction

Considering increasing population, industrilization processes and climate change in the world, fresh water amount and quality decrease year by year. On the other hand, , irrigation is the most important input for cotton growing since it consume much more amount of irrigation water compared to the other crops (Kanber et al., 1991; Cetin and Bilgel, 2002). The irrigation water requirement for the cotton plant is, thus, considerably higher.

Consequently, use of wastewater resources for irrigated agriculture is one of the solutions for this purpose. Use of wastewater in cotton irrigation might be considered. One of the advantages of use of wastewater for cotton crop is that cotton is not directly placed in the food chain.

In developed countries where environmental standards are applied, much of the wastewater is treated prior to use for irrigation of fodder, fiber, and seed crops and, to a limited extent, for the irrigation of orchards, vineyards, and other crops. Wastewater and its nutrient content can be used extensively for irrigation and other ecosystem services. However, wastewater reuse also exacts negative externality effects on humans and ecological systems, which need to be identified and assessed.

Most crops give higher than potential yields with wastewater irrigation, reduce the need for chemical fertilizers, resulting in net cost savings to farmers. If the total nitrogen delivered to the crop via wastewater irrigation exceeds the recommended nitrogen dose for optimal yields, it may stimulate vegetative growth, but delay ripening and maturity, and in extreme circumstances, cause yield losses.

The impact of wastewater irrigation on soil may depend on a number of factors such as soil properties, plant characteristics and sources of wastewater. The impact of wastewater from industrial, commercial, domestic, and dairy farm sources are likely to differ widely.

Impact from wastewater on agricultural soil, is mainly due to the presence of high nutrient contents (Nitrogen and Phosphorus), high total dissolved solids and other constituents such as heavy metals, which are added to the soil over time. Wastewater can also contain salts that may accumulate in the root zone with possible harmful impacts on soil health and crop yields. The leaching of these salts below the root zone may cause soil and groundwater pollution (Bond, 1999).

This study aims to evaluate the effects of treated domestic wastewater on the soil pollution risks and cotton plant yield.

Material and methods

Experimental Site

This research was conducted over a 2-year period at the Research Station of Agricultural Faculty, Dicle University, in Diyarbakır, Turkey, during the 2011 and 2012 growing season.

The location of the site is 37° 54' N, 40° 14' E at an elevation of 660 m above the sea level. The soil texture at this site is mostly clay (approximately % 65). The bulk density ranged from 1.19 to 1.27 g cm^{-3} in the soil profile. The infiltration rate was 8 mm h^{-1}. There was no specific risk in terms of water table

or soil salinity. The organic matter content, pH and lime content of the soil were 1.67%, 7.9 and 8.5% respectively. The climate of the area is typical terrestrial climatological characteristics. The average annual rainfall is 487 mm and it occurs mainly during the months of winter and spring season. The average annual (growing season) and daily maximum evaporation from Class A pan are 976 and 8.4 mm, respectively (Üzen, 2014).

Experimental Tanks

The metal tanks (containers) of 1,00 m height and 0,60 m diameter were used for the experiment. Soils were collected from the fields, dried and sifted through a sieve with an aperture of 6.35 mm, and pressed into tanks in layers of 5 cm considering the bulk density of soil in the fields. Bottom layer of the tanks were filled with 5 cm of sand-gravel mixture for drainage.

Experimental Treatments and Agronomic Applications

The different treatments were applied for each experimental year. Thus, it was aimed to expose, the distinctive effects of wastewater on providing nutrients for soil and crops. The split-plots design were used in completely randomized design with 4 replications (Table 1).

Table 1. The experimental treatments

2011		*2012*	
Main treatments	*Sub treatments*	*Main treatments*	*Sub treatments*
F_1: Recommended fertilizer (N, P) **F_2:** No fertilizer	**I_0:** Freshwater (100% of Class A Pan evaporation, Ep) **I_1:** Wastewater (100% of Ep) **I_2:** Wastewater (120% of Ep) **I_3**: Wastewater (80% of Ep) **I_4**: Diluted wastewater (50% wastewater + 50% freshwater) (100% of Ep)	**F_1**: Recommended fertilizer (N, P) **F_2:** 50% of the recommended fertilizer (N, P) **F_3**: No fertilizer	**I_1:** Freshwater (100% of, Ep) **I_2:** Wastewater (100% of Ep) **I_3:** Diluted wastewater (50% wastewater + 50% freshwater) (100% of Ep)

Fertilization and other cultural practices

The treatments were the recommended amount of fertilizer for the farmers, 50% of the recommended fertilizer and no fertilizer for the main plots (tanks).

The sub-plots were different levels of treated wastewater and fresh water. Cotton seeds were sown at 20th May 2011 and 10th May 2012. After emergency of plants, young plants were decreased considering row distance of 20 cm. totally three plants were grown in each experimental tank.

Appropriate fertilization for nitrogen phosphorus for the study area were recommended as 130 kg N ha^{-1} and 80 kg P_2O_5 ha^{-1} for the recommended fertilizer under the farmer conditions (Özer and Dağdeviren, 1986; Karademir et al., 2005).

The amount of irrigation water calculated according to the experimental treatments were applied to the tanks by means of a volumetric cap. Irrigation scheduling was based on Class A Pan evaporation (Kanber and Güngör, 1986) and irrigation interval applied as 10 days. The volume of irrigation water was calculated based on the cumulative pan evaporation coefficient, K_{pc}=1.00, during identified irrigation interval, surface area of the tank and based on soil depth (0.90 m) (Cetin and Bilgel, 2002).

Water, Plant and Soil Analyses

At the end of the study, the analysis were made to determine the content of macro, micro and some heavy metals in the soil due to fertilization and wastewater usage. Soil samples for the analysis were taken from two different depths (0-30 cm and 30-60 cm) of the tanks.

Wastewater samples were collected to analyze before each irrigation cycle.

Bulk density, field capacity, wilting point, pH and electrical conductivity, lime, organic matter, cation exchange capacity (CEC), exchangeable sodium percentage (ESP) for the soils and water analyses (pH, EC, cations and anions) were determined according to the principles given in Tüzüner (1990). Soil pH was determined through a pH meter (McNeal, 1982). EC was determined through a conductivity meter. Total N was using Kjeldahl procedure (Bremner and Mulvaney, 1982). Concentrations of soluble Ca and Mg were found out using EDTA titration method, while Na and K measured applying aflame photometer (Richards, 1954). Phosphorus was determined using OLSEN extraction (0.5 M $NaHCO_3$) (Olsen and Sommers, 1982) procedure.

Statistical Analysis and Evaluation

Split-plots with randomized design using four replications were realized to evaluate the effects of the treatments on yield and yield components. Yield of cotton data were evaluated for a single plant because there are only 3 plants in the experiment and a tank such as container was used. All data were analyzed using a JUMP. Variance analyses were made for each experimental year. Statistical evaluation of data was conducted under the principles given by Yurtsever (2011).

Results and discussion

Cotton-seed yield

According to the experiment results there were no significantly interactions between applications of commercial fertilizers different amount of the treated wastewater. The cotton-seed yields were, thus, ranged from 14.1 to 29.5 g plant^{-1} and from 24.8 to 30.3 g plant^{-1} depending on experimental treatments in 2011 and 2012, respectively. The highest cotton-seed yield (29.5 g plant^{-1}) was obtained from the treatment of no commercial fertilizer and the amount of irrigation water that the wastewater was applied based on 100 % of Class A Pan (F_2I_1) in 2011. Considering the data and the results, there were considerably differences between the main treatments (applications of commercial fertilizer) in 2011 (Table 2). Consequently, the wastewater increased considerably the cotton-seed yield without commercial fertilizer. However, there were no significantly differences between the treatments in 2012 (Table 2). These data have showed that use of wastewater increased the cotton-seed yield compared to the treatment of no fertilizer The yield obtained from the application of commercial fertilizer (F_1) was, therefore, lower 45.9% compared to those from the application of no commercial fertilizer (F_2) (Table 2). Similarly, Dawson and Hilton (2011) stated that the plant could provide nutrients from the wastewater. Tsadilas and Vakalis (2003) and Gündüz (2013) also were determined to use the same level of wastewater increased the yield of cotton compared with clean water. Several researchers have also mentioned that the irrigation with wastewater increases the cotton-seed yield. Alikashi et al (2012) reported that the cotton yield, number of bolls, leaf area index (LAI) and plant height were significantly higher when the crop irrigated with treated municipal wastewater rather than with freshwater. The irrigation with wastewater increased in cotton yield and its components in comparison to the plants irrigated with freshwater as well (Silva et al., 2009).

According to yield data in the year 2012 as well in 2011, there were no statistically significant difference between the applications. It means that a significant difference did not occur in yield between the wastewater source and commercial fertilizer applications. The applications of untreated wastewater and without commercial fertilizers in 2012 resulted in less than yield than those in 2011. The reasons for this could be some problems on weak emergency of the plants at the beginning of the growing season and growing the plants in the same tanks using same fixed treatments in the second experimental year. In other words, the yield of crops watered with wastewater each year could reduce due to cumulative effects of wastewater in the soils in the tanks. As similar to these findings, Camp et al. (1985) reported in that the excessive nitrogen application decreased yield because it could wash the nitrogen remaining after the plant

reaches maximum degree. Same researcher have emphasized that in some cases the application too much level of nitrogen in the plant from any source can reduce marketable yields, delay the date of maturity and reduce fruit size.

Table 2. Effect of wastewater irrigation on cotton-seed yield.

2011					
Main treatments (Level of fertilizer)	Cotton-seed yield (g plant^{-1})	Proportional decrease in yield (%)	Sub treatments (Irrigation water)	Cotton-seed yield (g plant^{-1})	Proportional decrease in yield (%)
F_2	31.8	45.9	I_1	23.1	3.2
			I_0	22.4	13.5
			I_2	20.0	15.3
F_1	17.2	-	I_4	19.6	27.2
			I_3	16.8	-
2012					
F_1	27.6	1.03	I_2	28.9 (a)	8.0
F_2	27.3	6.10	I_3	26.6 (b)	12.3
F_3	25.9		I_1	25.3 (b)	-

There were differences in terms of lint yields between the experimental years. The main reason for that could be the difference in sowing date, which was 20th in 2011 because of the climatic conditions. Many researchers reported that a delay in sowing can reduce yield (Karademir and Sakar, 1999; Edmisten, 2008). The cotton-seed yield (31.8 g plant^{-1}) in the tanks where no commercial fertilizers were applied was considerably higher compared to the yield (17.2 g plant^{-1}) in the fertilized tanks where a common nitrogenous fertilizer was utilized.

Effects of wastewater on soil pollution

According to the some soil analysis results, the values of pH in the samples of soils after the study were 7.8 – 8.2 while those were 7.8 - 7.9 before the study. It could be stated that the use of wastewater increased the values of pH of soil. Concerning the EC in the soil, EC were 0.97 - 1.79 dS m^{-1} at the end of the experiment compared to the values of EC (0.80-1.00 dS m^{-1}) in the soil before the experiment. Organic matter (OM) content of the soil was relatively low, ranging between 0.27% and 0.65%. For all experiment treatments, the amount of OM is higher in the upper layers of soil and decreases with depth. Uyanöz (2000) reported that in the lands irrigated with wastewater for many years, OM is adequate or high in 0-30 cm soil layer.

The amount of phosphorus in the soil samples varied between 4,62 and 13,16 ppm at the end of experiment . The highest content of available P (13,16 ppm)

in the soil after the experiment was observed in the treatment F_2I_2 (50% of the recommended fertilizer and wastewater (100% of Ep) in the soil depth of 0-30 cm. The lowest content of available P (4,62 ppm) in the soil after the experiment was observed in the treatment F_1I_2 and F_1I_3. According to the literatures (Olsen et al., 1954; Follet and Lissay 1970), phosphorus levels between 6.1 - 12.2 ppm are adequate levels in the soil.

It could be concluded that wastewater caused the increase in P content. Organic carbon content values in the soil were observed close to each other and varied between 0.16% and 0.38%. The values of the other some micro elements were Fe: 4.23 - 5.54 ppm; Cu: 0.49 - 1.19 ppm; Zn: 0.29 - 1.59 ppm; Mn: 3.99 - 6.89 ppm. Considering these data, it could be stated that all these values were not higher compared to threshold values in the soil. Boron was not observed also excessive value (0.40-0.80 ppm). However, the content of Pb values (5.3-6.9 ppm) in the soil were insignificantly higher compared to the critical value (5 ppm). The content of Cd were below the threshold value given by the Regulation on Water Pollution Control in Turkey. It could be stated that there were no any heavy metal pollution and risks using domestic wastewater. However, the risk of contamination by trace metals must be considered when wastewater is applied and understanding of the behavior of metals in the soil is essential for assessing environmental risks of applying wastewater in agro□ecosystems (Khai et al., 2008).

Conclusions

According to the experimental results on cotton-irrigated by the treated wastewater, the treated wastewater increased considerably the cotton-seed yield compared to fresh water and without using commercial fertilizers. It could be concluded that there were significantly effects of wastewater on supplying nitrogen for the crop. In addition, there were no any pollution and any accumulation in the crop leaves and soil in terms of heavy metal and other harmful elements for two years. Because the wastewater used for this experiment was domestic wastewater not included heavy metals.

As a result, domestic and treated wastewater could be used for cotton irrigation. Because cotton is not directly in a food chain for human. In addition, treated wastewater might be a potential and economical resource to the water resources and the expansion of irrigated agriculture. However, the quality of each wastewater could differ due to the source of wastewater, population movements, seasons and climate conditions. Thus, each wastewater might be used for irrigation purposes taking into account some precautions on quality of wastewater and risk analysis for the special uses. In addition, the wastewater and its effects must be monitor for long term use.

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The impact of walnuts, almonds, and hazelnuts as a nutrient medium on fecundity of *Plodia interpunctella*

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Abstract: Indian meal moth (*Plodia interpunctella* Hübner, Lepidoptera, Pyralidae) is one of the most important insect pests of storage facilities worldwide. The larvae feed on different stored products, cereals, dried fruits, dried vegetables, herbs, chocolate etc. The most important damages that this moth causes to the infested food are visual i.e. quality losses, while losses in weight are negligible. Although *P. interpunctella* is one of the most important pests of dried fruits in Serbian facilities, there is still very little available data about this pest's ecology, biology and population dynamics of local populations. The aim of this study was to determine the influence of different nutrient medium (broken kernels of walnuts, hazelnuts, and almonds) on the fecundity of *P. interpunctella*. According to obtained data, it can be concluded:

- The highest mean number of eggs (468) was recorded on hazelnuts, while lower on the almond and walnut (438 and 405, respectively).
- The lowest mean number of eggs (3 eggs) was recorded on hazelnuts. The lowest number on the walnuts and almonds was slightly higher (16 and 22, respectively).
- The shortest period until *P. interpuctella* male and female copulation (time spent on courtship), was registered on the walnuts (221.0 ± 302.0 s), while on the hazelnuts this period was up to five times longer (1193.0 ± 1456.1 s).
- The fecundity is not statistically significantly influenced by the nutrient medium on which larvae were reared.

Key words: *Plodia interpunctella*, walnut, hazelnut, almond, eggs, fecundity

Introduction

Indian meal moth - IMM (*Plodia interpunctella*, 1813 Hübner) (Lepidoptera, Pyralidae) is one of the economically significant insect pests of storage facilities

worldwide (Fasulo and Knox, 2008). This moth has been the object of numerous studies conducted by many researchers globally (Tzanakakis, 1959; Mbata, 1985; Aguilera-Pena and Perez -Mendoza, 2004). Biology and ecology of *P. interpunctella* is well-studied, but in Republic of Serbia (RS) there is very little available data about this pest's ecology, biology and population dynamics of local populations (Vukasović et al., 1972; Kljajić et al., 2002; Almasi, 2008; Almasi and Poslončec, 2010; Vukajlović at al., 2013). *P. interpunctella* is one of the most important secondary storage insect pests (Rees, 2004). According to the Almaši and Poslončec (2012), this moth is one of the most important insect pests of dried fruits in the RS. The most important damages in the food infested by this moth are visual changes i.e. quality losses, while losses in weight are negligible (Almaši, 2008; Miljković, 2017). The larvae are polyphagous and they feed on wide range of stored products, such as cereals, dried fruits and vegetables, herbs, chocolate etc. The amount, type and nutritive quality of available food highly influence the fecundity of *P. interpunctella,* besides environmental conditions (relative humidity, light and dark period, temperature) in which population of *P. interpunctella* develops. Also, female size and it's physiological condition are important factors that affect the fecundity (Mbata, 1985). Depending on all of the mentioned factors, data on *P. interpunctella* fecundity differs from study to study (Allotey and Goswami, 1990; Deseo, 1976; Mbata, 1985; Vukajlović, 2012). Fecundity has been defined as the maximum physiological ability to produce functional gametes by an individual (Pešić, 2011). In our experiment, fecundity was considered as the total number of eggs, fertilized or unfertilized, laid after the mating.

The aim of this study was to determine the influence of different nutrient medium (broken kernels of walnuts, hazelnuts, and almonds) on the fecundity of *P. interpunctella*.

Material and methods

The experiment was conducted in the Laboratory of General and Applied Entomology, Institute of Biology and Ecology, Faculty of Sciences, University of Kragujevac. Nutrient medium, on which experimental *P. interpunctella* populations were reared, were broken kernels of walnuts (*Junglans regia* L.), hazelnuts (*Corylus avellana* L.) and almonds (*Amygdalus communis* (L.) Spach). Walnuts were purchased from a country household, while hazelnuts and almonds were purchased in health food store "Biomax".

The experiment was set in three series based on the nutrient medium that was used (walnut, hazelnut, almond), with 12 jars in each treatment. Each marked with its own serial number (1-12). Depending on the type of nutrient medium jars is characterized by the following designations: O - walnut, L - hazelnut, B -

almond. Each jar within the treatment contained 100 mL of the nutrient medium (walnut, hazelnut or almond) and 50 one-day old *P. interpunctella* eggs. After that, jars were placed in a thermostat chamber at a temperature of 28 ± 1 ^{0}C, humidity 60 ± 10%, light regime of 12 h light: 12 h darkness. Fourteen days after setting up the experiment, we started measuring the width of larval head capsule by using micrometer in ocular of microscope. This was performed until the last pupation.

During examination of jars in search for larvae to measure, each founded pupa was isolated in separate test tube. Each test tube was labeled appropriately and contained information on date of pupation, the designation for the repetition from which pupa were isolated and unique serial number for each pupa. After that, test tubes were checked on every eight hours until eclosion of imago. Data on date of eclosion and gender were recorded for each imago. In order to get fecundity data, male and female that ecloded on the same day and on the same nutrient medium were immediately paired in a separate test tube. Time spent on courtship was measured for each pair of *P. interpunctella* adults by using the stopwatch (TPMC). Every pair of adults was then isolated in the separate empty test tube were female laid eggs.

Analysis of fecundity was performed by using two parameters, the period of time spent on courtship and the total number of laid eggs.

Data were statistically analyzed using IBM Statistics 19 software package. The observed biological parameters were compared using the Duncan test ($P < 0.05$)

Results and discussion

Obtained results in female fecundity indicate that the shortest TPMC of 9s registered on walnut, pair O2L2 – O5L3 (Figure 1, Table 2). A number of laid eggs of this pair is 352. The longest recorded TMPC is 1381 s, pair O10L3 – O4L1. Fecundity of this pair is 280 eggs.

On hazelnut, the shortest TPMC is 27s, pair L5L3 – L5L5 (Figure 1, Table 2), and fecundity 42 eggs. The longest time of 3845 s is recorded for pair L4L1 – L4L3 and registered fecundity is 394 eggs.

On almond, the shortest TPMC is 22 s and 323 laid eggs recorded on pair B8L3 – B2L1 (Figure 1, Table 2). In the other hand, the longest TPMC of 2215 s and 193 laid eggs registered on pair B4L3 – B3L1.

On broken walnut as nutritive medium recorded the largest fecundity of 405 eggs in O5L4 – O7L1 pair (Figure 2, Table 2). The smallest number of laid eggs (16) listed of female in pair O9L2 – O9L1. On this medium average number of laid eggs were 231.5 ±93.1 (Table 1).

Table 2. Fecundity and TPMC of adults reared on walnut hazelnut and almond as a nutritive medium

	Walnut											Haselnut												Almond											
Pair	Copulatin	Number of laid eggs (from 1st after mating)									Fecundity	Copulatin	Number of laid eggs (from 1st after mating)										Fecundity	Copulatin	s (from 1st after mating)										Fecundity
	(s)	1.	2.	3.	4.	5.	6.	7.	8.	9.		(s)	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.		(s)	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	
I	328	0	89	20	34	45	28	5			221	161	0	5	119	27	7	0						158	2215	4	84	83	22								193
II	46	0	23	81	49	0	2				155	7441	17	154	57	14	0	14						256	200	20	77	0	2	0	0	8	14	4	16		141
III	487	0	0	32	0	6	9	0	0	0	47	3845	0	234	80	47	33	0	0					394	101	73	0	0									73
IV	47	96	111	12	0						219	1002	108	118	43	11	11							291	151	59	123	53	1								236
V	383	69	132	42	16	8	6				273	2871	45	63	85	0	63	0						256	153	32	96	52	14	0	7						149
VI	59	0	25	103	25	0	0	3	0	0	156	1264	0	5	0	57	5	22	11	0	4			104	642	0	126	47	0	18	0						191
VII	70	0	0	167	23	0	0				190	262	92	46	17	7	0							162	109	98	197	0	15								310
VIII	163	5	146	0	0						151	77	0	0	3	0	0	0	0					3	77	102	179	45	21								347
IX	14	84	30	0	21	14	0	0			149	658	7	207	176									390	43	93	166	0	0	50	0						309
X	681	119	22	2	2	31					176	555	62	85	50	24	0							221	49	42	123	78	22	0							265
XI	119	63	214	5	56	11	0	9			358	166	121	2	242	1								366	2645	112	89	66	0	8	1						276
XII	66	72	15	11	136	26	3				263	2622	3	73	97	27	4							204	1608	148	93	4	11								256
XIII	34	186	35	13	12	2					213	59	0	21	179	0	0	10	0					210	1463	193	88	42	9	7							339
XIV	66	193	53	15	8	3					272	210	100	177	58	0	3							338	49	259	0	86	8	11	3						367
XV	37	10	19	0	7	18	0	3			57	1710	115	168	5	27	0	0						315	249	241	59	21	0	22							343
XVI	130	4	0	0	7	4	0	1			16	1710	74	201	18	25	0	1						319	46	5	161	20	21	19							226
XVII	283	178	63	0	39	17	0	1			298	27	0	0	0	0	0	0	0	24	18			42	87	4	198	1	0	13	20						236
XVIII	42	188	100	0	47	6	0	0			341	54	0	0	14	0	13	1	0	8	0	0	56	92	37	51	30	113	1								195
XIX	108	159	24	36	25	0					244	2960	0	0	0	14	49	158	8	0				229	356	140	20	80	41	9							290
XX	1232	190	51	23	13	0					277	50	51	39	51	9	3							153	70	101	0	83	11	2							197
XXI	195	95	77	0	37	7					216	148	157	33	10	0	0							200	83	153	99	62	5	4	2	7					332
XXII	59	156	68	0	14	0	0	0	0		238	575	124	0	135	21	38	3						321	112	7	96	116	0								219
XXIII	83	10	0	0	25	17	54	2	2	0	110	35	89	190	2	0	53	0	0					334	58	20	17	3	7	44	19	8					118
XXIV	36	88	205	29	71	0	12	0			405	44	8	221	0	0								229	134	170	97	120	46	4	1						438
XXV	65	97	144	35	36	2					314	907	14	0	81	0	15	0	3					113	22	34	131	97	40	21	0						323
XXVI	594	85	172	0	72	3	4	0			336	2515	67	55	38	28	0	0						188	99	113	62	11	0	0	9						195
XXVII	65	12	126	12	22	0	0				172	1211	88	29	66	19	0	0						202	71	1	49	13	8	0							71
XXVIII	101	0	195	60	1	42	12				310	131	0	138	111	70	3	1						323	54	19	36	0	2	35	3	0					95
XXIX	78	0	128	41	35	27	0				231	1135	0	122	26	12	19	0	0	0	0			160	180	20	175	100	38	0							333
XXX	1381	0	258	22	0	0					280	283	74	148	80	43	0	0						345	230	22	186	89	11	4							312
XXXI	69	134	23	136	26	5	7	0			331	3690	0	56	4	24	38	4	9					135	63	0	103	80	10	0	1						194
XXXII	223	68	55	34	27	11	0				195	945	167	119	101	64	17							468	1932	0	0	10	12								22
XXXIII	278	0	3	6	8	7	14	5	4		47	931	72	78	75	8	2	4	0	2	0			241	125	102	84	95	25	0							306
XXXIV	84	134	0	176	0	5	16				331	118	109	65	99	32	0	0						305	123	40	74	36	5								155
XXXV	9	215	39	73	22	3					352	1109	0	161	83	34	0	0						278	1079	134	105	0	20	1							260
XXXVI	101	172	3	105	30	0					310														120	182	17	54	25	0							278
XXXVII	427	0	121	54	30	1	0				206														28	139	33	33	17	0							222
XXXVIII	131	0	156	83	0	23	0				262														423	0	116	103	58	23	5	7					312
XXXIX	453	54	84	101	0	8					247														467	88	129	60	12	0	0						289
XL	78	1	87	114	55	0					257																									

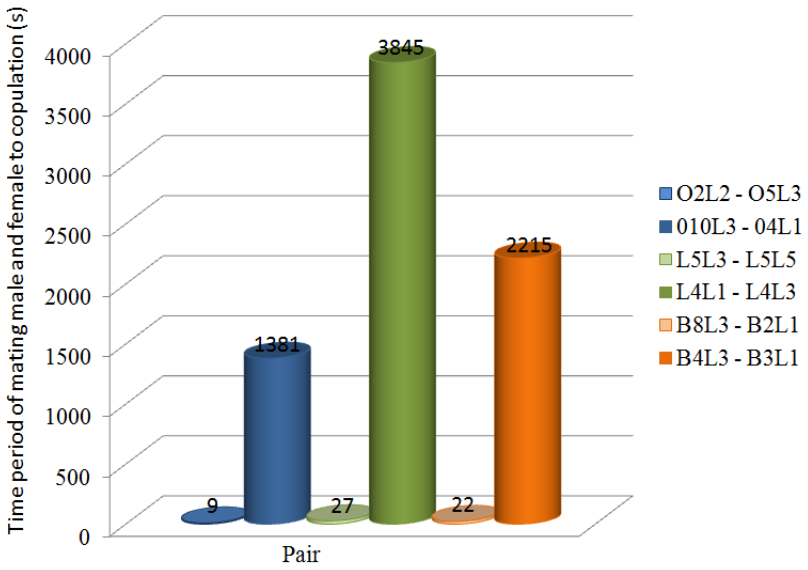
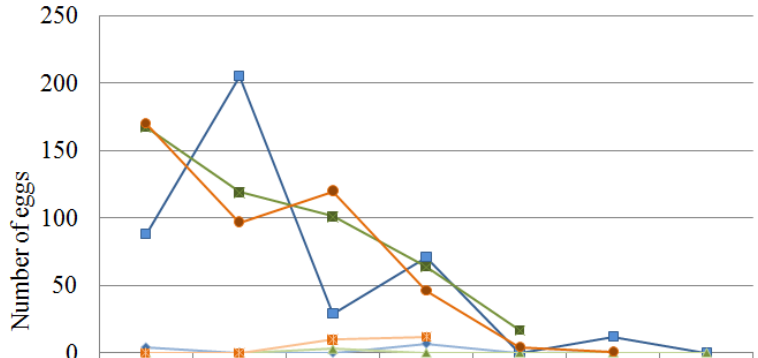


Figure 1. Minimum and maximum value of TPCM of adults reared on walnut, hazelnut, and almond as nutrient medium



	1. day	2. day	3. day	4. day	5. day	6. day	7. day
O5L4 - O7L1	4	0	0	7	0	1	
O9L2 - O9L1	88	205	29	71	0	12	0
L6L1 - L8L3	0	0	3	0	0	0	0
L4L2 - L4L5	167	119	101	64	17		
B5L2 - B11L2	0	0	10	12			
B6L2 - B1L4	170	97	120	46	4	1	

Figure 2. Minimum and maximum fecundity value of females reared on walnut, hazelnut, and almond as nutrient medium

The largest number of laid eggs in the experiment recorded as the largest number on hazelnut pair L6L1 – L8L3 and reaching 468 eggs (Figure 2, Table 2). Also, the smallest fecundity recorded on this media is 3 eggs on L4L2 – L4L5 pair. An average number of laid eggs were 239.6 ±107.2 (Table 1).

On almond the largest fecundity (438 eggs) recorded on pair B5L2 – B11L2, and female of B6L2 – B1L4 pair laid only 22 eggs (Figure 2, Table 2). An average number of laid eggs on almond were 242.1 ±92.1 (Table 1).

The shortest TPMC (time period of mating male and female to copulation) enrolled for adults reared on walnut, then on almond and hazelnut (Table 1). On moths reared on hazelnut TMPC was and five times longer than on walnut. Differences in a time period between pairs reared on different media are statistically very important (F= 13.11*, p<0.01 – Table 1). However, differences in a number of laid eggs are not statistically significant depending on nutritive medium i.e. different kernels (F=0,13nz, p>0.05).

Table 1. Comparative analysis of fecundity between fruits

Days	*Nutrient medium*			
	Walnut	Hazelnut	Almond	F ratio
TPMC	221.0±302.0 b	1193.0±1456.1a	350,1±581,0 b	13,11**
Number of eggs	231.5±93,1 a	239.6±107.20 a	242,1±92,1 a	0,13nz

TPMC - Time period of mating male and female to copulation

Results in research of Johnson at all. (1992) registered the largest fecundity value on females reared on the nutritive medium of ground walnuts, pistachios, and almonds, from those obtained in this study. On ground walnuts, at temperature 28.3 ^{0}C registered average fecundity of 291.3±22.08 eggs, and on 31.7 ^{0}C listed 262.9±15.95 eggs. On ground pistachios at 28.3 ^{0}C average fecundity was 317.2±7.56, and at 31.7 ^{0}C recorded 301.1±3.78 eggs. In the same temperature conditions average fecundity on ground almond is similar i.e. 308.5±6.49, and 311.3±4.63 eggs, respectively to temperatures. Obtained results indicate that fecundity is inversely proportional to the temperature increase, i.e. a temperature increase on the substrate of ground walnut and pistachio caused fecundity reduction.

Much lower fecundity values recorded in research of Allotey and Goswami (1990). Average fecundity value on wheat was 96.8 and on broken corn kernel 174.2 eggs. Average fecundity of 170 laid eggs registered in females reared on dried fruits (Simmons and Nelson, 1975). Aguilera-Peña and Perez-Mendoza (2004) quote 212±38 eggs as average fecundity in the female feed on garlic.

Vukajlović (2012) quoted that average fecundity on wheat germ 137.41, oatmeal 97.36, corn flour (cornstarch) 56.41, whole wheat flour 69.33, and on

standard laboratory medium 146.24 eggs. This data indicates that fecundity influenced by the type of nutritive medium. Females whose larvae reared on nutritive medium with higher protein and lipid amounts released in average the biggest fecundity (Vukajlović, 2012).

Obtained results in this research indicate that:

- TPMC (time period of mating male and female to copulation) is shortest for adults reared on walnut (221.0±302.0s). This time for moths reared on hazelnut is up to five times longer (1193.0±1456.1).
- Difference of female's fecundity statistically not significant depending on nutritive medium on which specimens reared (231.5±93.1 eggs on walnut, 239.6±107.20 on hazelnut, and 242.1±92.1 on almond).
- The biggest fecundity value recorded on hazelnut (468 eggs), but slightly lower value listed on almond and walnut (438 and 405 eggs, respectively).
- The minimum fecundity value listed on hazelnut (3 eggs), and on walnut and almond recorded slightly higher values (16 and 22 eggs, respectively).

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Medieval arable farming and technological mentalities Eastern Slavs on Ukrainian lands

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Abstract: The articleconducts a compilativeanalysisof agricultural farming at the Middle Ages in Ukraine.It has been considered the different plowing tools wereused there undercereals growing within the ethnic Ukrainian territory at different landscapes and soil-climatic zones.The seasonal plowing operations, different technological practices, organic fertilizers inputs and farming systems have been also reviewed in the paper.It is emphasized that the tillage practices implementation relied upon: socio-economic needs, crops'positioning in rotations, soil-climate conditions, existing technical base, agricultural knowledge, historical and cultural features, etc.

Key words:agrarian history,Ukraine, Middle Ages, agriculturalpractices, soiltillage, arable farming system, organic fertilizers.

Introduction

Thesustainableland use and cutting-edge agricultural development in Ukraine requiresadetailedstudyingandanalysisofitshistoricalbackgroundinthepast.The purpose of that paper is to exploremedieval arable farming and technological mentalitiesof EasternSlavsonUkrainianlands. To succeed the assignedscientific and practical tasks it was provided a comprehensive analysis of existing factual materialsrelated to the land using in the past, the then technological systems of soil cultivation onthe ethnic Ukrainian lands during the Middle Ages.

The manuscript represents the generalized reviewed analysis of fundamental writings onmedievalagriculture and technological mentalitiesof Eastern Slavson Ukrainian landsthat had been appeared at the end of the XX - at the beginning of the XXI centuries.

Material and methods

The systemic-historical analysis and synthesis methodology was used in our research.

Results and discussion

Ukrainians, in the Middle Ages, had already perfect, as of that era,arablefarmingtools, having indicated a fairly high level of grain-growing culture in general.Written information, ethnographic materials of the XIII-XVII centuries testify that a variety of arable land was used at Ukrainian farms for technologically consistent stages for growing cultivated cereals.

A wooden plow (рало) still had been used, because it in some regions of Ukraine performed a special soil-working function: occasionally as an instrument of plowing, the ridges loosening - as additional soil tillage operation. There were changes in the design of the wooden plow –were appeared the additional rudders-teeth (from two to five and even more), the rider moves into a triangular or quadrangular body, a wooden plowwas used with and/or without a cultivator point.

At the ethnic territory of Ukraine (first of all in the Middle Podneprovian and Polesye as the ancestral homelandof arable farming) and other Slavic nations was approved other arable implement – the plough, which emerged in the late first millennium AD in several grain-growing areas of Europe, including in the Middle Podnestrovian and was improved over many centuries(Krasnov, 1987).

Theplough was used to turn up the soil layer before crops sowing. The soil-landscape local features of different ethnographic regions generated regionalplough models: Carpathian-mountain model, Polissyaplough, Volynplough, etc.

The Ukrainian traditional plough consisted of two main parts - the ploughand the draft fore carriage. The main part of theplough, which connects all the other parts in a one constructive system, is the beam.To the constructive parts of plow are: lifterand plough-tails (chepigi). Working parts of a woodenplough: the gouter, the soleon which the plough-share and the benchwere planted. Subsequently, from the iron was made the gouter, the plough-share andlater on - the bench.

In chronicles, theploughdetermined the unit of the familytaxation. The taxation was calculated by the number of oxen in the household. Eight oxen composed a «plough» - an artificial unit of taxation(Bilodid, 1975).

Sokha had a split workpiece (rassosh), which was connected in the upper part with twoteam poles, in which a horse harnessed.The main difference is thesokhafrom theploughwas that the sokha did not turn the layer of soil, but only shoved it to the side.Sokha, as an instrument of the work of one family, also served as a unit of taxation in Rus(Busel, 2009).Depth of soil tillage bysokhawas - up to 12 cm. In comparison with the plough, it was necessary for the field processing less traction effort of the horse, and greater physical tension and skill of the peasant.In the Forest-Steppe zone of Ukrainewas useda sokha-stag beetle with a horizontal arrangement of openers.

Among the complex of tillage implements a prominent place was belonged to the instrument of the second soil preparation stage: drapaks, harrows of various designs and etc.The first rupture devices (harrows-breeches)had the simplest construction, were made mainly the branch-type – the tops of coniferous trees with chopped branches or branches with sharp spits – hawthorn, blackthorn and etc. In Rus, the farmers brought the field to the technological completeness by bar brushes, most often wicker type, which later acquired a variety of forms, were noted by different ways of fixing the teeth.

Everywhere there were four-staged seasonal plowing: spring ploughing under the spring crops, bare fallow plowing, summer ploughing, stubble remains cultivating, finally fall-plowing for winter crops sowing.The ratio of the mentioned stages in different regions was dictated by socio-economic needs, the predominance of winter or spring crops. Technical ploughing operations corresponded to natural, soil, historical and cultural features, the existing technical base, agricultural knowledge.

The technological system of the soil cultivating was multiplied, with the involvement of the plough andthe wooden plow, the plough and the sokha, and even less commonly - all three tools or the wooden plow and the sokha. The complicated technological soil preparation to crops' sowing in Ukraine (XIII-XVcenturies) having involved two main tools used inthe fallow farming, mostly under winter crops.Each instrument was assigned to a specific operation of the soil cultivation system:a plough was used for pioneering soiltillage, the wooden plowdestroyed soil ridges ("cross-over plowing"), after fallow the soil treatment was fulfilled along the ridges ("lengthen plowing"). Sometimes arable lands were lengthened for the second time by a plough, and cultivated by a sokha.

To maintain and restore a soil fertility were used various arable systems: extensive - slash-and-burn system of agriculture, fallowfarming,two-field crop rotation; intensive farming (usually was based on classical tree-field crop rotationwhilein Padilla was happened four-fields in rotation). Somewhere from

the of tree-field crop rotationremained only idea of fallow wedge, because sometimes the field was divided into four or five or even six and eight plots. Fallowfarming was arisen as a result of the arable tools improvement as by the presence of winter and spring cereals in rotation (Pavlyuk,1986).

Slash-and-burn system of agriculturewas especially widespread in the Forest and Forest-Steppe zones and was caused by the presence of large forest areas and numerous human settlements. The selected trees and bushes of the forest in autumn or winter were cut down and left then on the ground forfurtherburning action in the spring.The glades ("apiary") were loosened and sowed (without ploughing) by millet or flax – at the first year and byrye, oats, etc. - in subsequent years. After the soil depletion, the field was abandoned and farming was occurred in other forest areas. The cycle of operation of the site was as follows: from 1-3 to 5-7 years on the cleared area it was used for crops cultivating, then - used for haying or pasturing (up to 10-12 years). If the lend wasn't used for fallowing 40- 60 years – it was restored by forest planting (Andrianov,1988).

The two field fallowrotationon Ukrainian lands was implemented mainly in the Southern Steppe areas and was used after the several years of cerealscultivating: millet, rye, wheat, etc.; after the depletion of the soil productivity it wasabandoned for 10 to 25 years for the «rest» - for natural soil recoverin, after which the land was ploughed again (Ponomarev,1993).

Two-field fallow crop rotation was also widespread in the Steppe zone of the ethnic territory of Ukrainians.After the fallow were predominantly grown cerealswithin a two-rotation system. For example, one year was sown a winter rye, in the second year - the field was resting.There was a peculiar order of crops, when half of the field was sowed not for one year, but for two or three years in a row (depending on the soil fertility), then it lie fallow the same amount of time.

During the Kyivan Rus three-field rotation system of agriculture was the most widespread.All arable lands were divided into three parts: one part was given under winter crops, the second - spring crops, the third - fallow.Lands that were used under fallow could be used as a pasture for livestock. Next year, that plot was used for wintercrops (rye, wheat), after that – for spring cereals.The plot with the spring crops, after harvesting went to fallow farming. Thisfields design was made possibleto grow different crops simultaneously, as well as improved the soil fertility (Lozko,2004).

As a result of the cultural and economic development of arable land on the ethnic territory of Ukraine, along with the advanced technological soil operationsdevelopment had been started use the organic fertilizers. Variety methods of soil nutrition supply had been developed inKyivan Rus, which can be classified on the base of two main features: the general soil fertilization and

the soil fertilization for certain crops (Pavlyuk, 2001). During theKyivan Rusperiod was formed an integral system of using organic fertilizers: ash soil fertilizing after the wood burning, regularmanure application under a tree-field crop rotation system, green manure sowing and its burning by plowing. The highest rates of fertilizers were used for plots with the cereals growing – the most profitable crops in that time.In Polissya. That plot was called «pomirko», at the Left Bank – «pidmetom» or «konoplyanik».

The harvesting tools included: thesickle (widely represented in the cultural layers of Kyivan Rus), the scythe (distributed in the XIII-XIV centuries) and theflail (widely used for threshing grain crops). The flail consists of a handle up to 190 cm in length and a working part - a beater, made of an oak bat with a length of 110 cm. Both parts were connected bytawingtugin different ways, that allow a freely rotation of working area on the handle.The cereal sheaves were spread on the threshing-floor together in two rows, after that they beign taken with two hands were wiped with stickonears (Ponomarev, 1993).Other methods of threshing were also used:a roller with involving animals or only animals, who stepped on the outspread of sheaves and dehusked of ears.

Each of the technological procedures was accompanied by series of mandatory ceremonial actions of and ritual activities.

Conclusions

In the Middle Ages, Ukrainian farmers usedmanifold agricultural tools for crop tillage: the wooden plow, the plough, thesokha.Among of the tillage implements a prominent place was belonged to the instrument of the second stage of afield preparation - drapaks, harrows of various designs and etc. The farm implements included following harvest tools: the sickle (widely represented by the cultural layers of Kyivan Rus), the scythe (distributed in the XIII-XIV centuries) and the flail (widely used since the time of Kyivan Rus for threshing grain crops). Everywhere there were four-stages seasonal plowing: spring ploughing under the spring crops, plowing black pairs, summer ploughing and cultivating stubble under winter crops, finally – under-winter ploughing.

The technological system of soil cultivating was re-usable, with high amount of different tools which were pointed to the rational exploitation of the field. To maintain a soil fertility and restore its nutritional potential, were used various farming systems: extensive - slash-and-burn system of agriculture, fallow and two-field rotation; intensive farming systems – agronomic efficiency distinguished classical tree-field crop rotation, and partly four-field crop rotation (in Padilla). Sometimes the field was divided into four, five, six or eight plots.

As a result of the cultural and economic development of arable lands on the ethnic territory of Ukraine, along with: the soil technological preparations by different tools;changes in plots designation;time adjusting; crop sequence – becamepossibleusing organic fertilizers: ash application during the wood burning, manure application into the tree-field crop rotational system, green manure (grasses) application.Each of the technological procedures was accompanied by a series of mandatory ceremonial actions and ritual activities.

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Stripe effect on different spring barley varieties

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Abstract: Spring barley takes up 90 percent of a barley planted acreage in Ukraine. Barley is used for food, feed and technical objectives. It's considered that barley is a valuable fodder-grain crop. Unfortunately, barley undergo such diseases as: common bunt, loose smut, stripe rust, brown rust, black rust, root rot, spot blotch, stripe of barley. The most negative effect on grain quality and yield has a stripe of barley. Our findings demonstrate, that barley stripe influenced mainly leaf blade, stem and head. The deases reduced seeds germination by 13-58% and by 4.5% crop yield was lost. Some barley varieties were better adopted to the affection of stripe, because they were spread it through aerial dissemination and/or locally concentrated it in tissues. Our results demonstrated, that Vakula, Hetman, Phoenix varieties significantly reduced intensity of the pathogen mycelium spreading by 12.5-22.6% at a stem elongation stage and by 14-37.6 % at a dough development stage. After all, the studied varieties of spring barley, were classified as unstable.

Key words: barley, stripe, diseases, Drechslera graminea.

Introduction

Barley is the most widespread crop in world agriculture. In global structure of crop acreage, barley takes fourth place after wheat, rice and corn. In Ukraine, it's running second to winter wheat and occupies 2.86 million hectares among of

60 million hectares of a total land area, 42 million hectares of agricultural lands. According to State Statistics preliminary data – 9.4 million ton of barley was harvested and 4.7 million ton exported abroad in 2016. Therefore, Ukraine takes a third position in the global rating rank between countries-exporters of barley. The main importers of Ukrainian barley are: Saudi Arabia, Libya, China, EU, Algeria, Israel, Tunisia, Jordan, Morocco, the UAE and Turkey (State Stitastics, 2016).

Spring barley is typically planted in April and harvested in August. It's the most popular crop for spring reseeding of damaged cereals after cold and dry winter. Winter barley is a vernalization-sensitihitive crop (the plant needs to be influenced to low temperature before it can flower), whereas facultative and spring barley varieties are unneedful of it. Winter barley is usually sown in the fall for exposure to low temperatures during the winter and then it development is completed during the following spring and summer. Spring barley does not require to be influenced to low winter temperatures and can be sown in spring. Winter varieties of barley are usually matured somewhat earlier than spring ones (Anderson, 2013).

Barley is used as a main animal and fish fodder component. It is essential in malting and beer brewing production. 1 kg of barley grains contains 1.2 fodder units, 100 g of digestible protein, more than 12% of protein, about 77% of carbohydrates, almost 2% of fat and 3% of mineral elements. Barley protein is nutritional in amino acids, whereas lysine and tryptophan are dominated ones. Application a barley bran in an animal diet has positively effect on their mass increasing and resistance to unfavorable natural conditions. Barley may be grown for green feed and hay. Barley straw is used as a roughage in animal husbandry: 1 kg of this product is equivalent to 36 fodder units. Barley is an important food crop. Glassy grain large-kerneled barley contains 9-11% of protein, 82-85% of starch and flour. It is widely used as an additive in the bakery. The grain quality may be influenced by diseases. The most harmful disease of barley is a striped spot - *Drechslera graminea*, that under epiphytotic conditions reduces barley yield by 4.5 times and has tremendous negative effect on grain deteriorating (Maslak, 2012).

The purpose of this research was studying of the spring barley varieties resistance to the striped spot. To achieve this goal, we expertized: the distribution of striped spot on spring barley at the university arable lands; stability of different spring barley varieties to the striped blotch - *Drechslera graminea.* (Peresypkin, 1989).

Material and methods

The research was conducted by the Plant Growing Department of the National University of Life and Environmental Sciences of Ukraine over a

period of 4 yrs. on a Typical Chernozem in the Forest-Steppe zone of Ukraine in the township of Mytnytsya, in the Kyiv region (lat. 50°07′43″N, long. 30°14′35″E). The average annual temperature is 7.6°C with a mean temperature during the vegetative period of 12.4°C. The local climate can be defined as temperate with annual precipitation about 562 mm (273 mm of it falls during the vegetation period). The soil was classified as a Haplic Chernozem according to the FAO Soil Classification (Meadow Chernozem in Ukrainian Soil Classification), a Black Chernozem according to the Canadian system of soil classification (Soil Classification Working Group 1998) or a Mollisol according to the USDA Soil Classification. According to Ukrainian soil texture classification, the soil was classified as medium loam with 42.8% physical clay (Σ<0.01%), and 31.1% of clay (Σ<0.001%). The bulk density was in the range favorable for plant growth: 1.12-1.21 g cm^{-3}. The soil pH was in the range of 7.0-7.5, soil organic matter was 4.4%, cation exchange capacity - 32.5 meq 100 g^{-1}. The amount of available N, P, K was 7.3, 8.2, 17.1 mg 100 g^{-1}, respectively.

The experiment was initiated in 2014 as a split-plot design. Each plot was 2 m wide by 5 m long (10 m^2). Replication of experiment – 4 times. A 2 year study based on minimum tillage (shredding and disking stalks after harvest, followed by spring cultivators to a depth of 20-22 cm and field cultivation, then bedding). Row spacing was 0.15 m. Seed grains was sowed in recommend for this region time. Seed grains were placed to a depth of 4-6 cm. The mineral fertilizers were not used. Other husbandry operations such as weeding, pest and disease control were not applied.

Spring barley explorations were timed to following stages: 1) tillering (10 - 15% of plants appeared as plumule sheath of side shoots); 2) stem elongation (50% of plants come into ripe ear); 3) dough development. Plants were examined on a disease affection and intensity. Plat damages grading by stripe were evaluated by Heshele scale (Heshele, 1971): 0 - no symptoms; 1 - flying spot or takes up 5% of the leaf stem; 2 - spot coating takes up 10% of the leaf stem; 3 - spot coating covers 25% of the leaf stem; 4 - spot coating covers 40% of the leaf stem; 5 - spot coating covers 65% of the leaf stem; 6 - spot coating covers over 65% of the leaf stem. Spring barley varieties were: Aeneas, Vacula, Hetman, Phoenix, Sontsedar, Sebastian, Corona. Means and standard errors were calculated for each measured parameter. Least significant differences (LSD) between measured parameters were evaluated at 0.05 significance level (Dospehov, 1985).

Results and discussion

Our findings demonstrate, that the resistance of some spring barley varieties to the stripe wasn't found at a stage of tillering.The first diseas symptoms were reaveled at the stem elongation stage. The stripe distribution

at that time was 30% in 2014 and 29.5% in 2015. The development of the disease covered 7.22 and 6.76% of plants respectively (Table 1). The stripe distribution and development had been increased during the dough stage. Thus, the spreading of the disease was 56% in 2014 and 55% in 2015. The development of the disease was 27% and 20.15% in above mentioned years respectively.

Table 1. The stripe distribution and development at the different spring barley growth stages.

Years	Stage of tillering		Stem elongation stage		Dough development stage	
	Distribution	Development	Distribution	Development	Distribution	Development
2014	-	-	30.0	7.22	56.0	27.0
2015	-	-	29.5	6.76	55.0	20.15

Table 2. A different spring barley varieties resistance to the leaf stripe affection.

Varieties	Stem elongation stage		Dough development stage	
	Distribution,%	Development,%	Distribution,%	Development,%
Aeneas (st.)	13.8	2.9	39.3	8.7
Vakula	1.25	0.65	14.25	3
Hetman	1.3	0.7	25.3	4.8
Sontsedar	23.8	6.2	51.8	11.6
Phoenix	1.25	0.65	14.25	2.8
Sebastian	23.8	6.4	51.8	12.3
Crown	12.5	1.6	29.3	4.7
LSD_{05}^{*}	2.1	0.4	3.1	1.2

LSD_{05}^{*} is the least significant difference at 0.05 deviation.

A spring barley resistance to stripe was differentiated depending on crop's varieties. Despite this, all tested varieties were subjected to disease and demonstrated a high susceptibility to leaf spot. However, such varieties as Vakula, Hetman and Phoenix, in the stem elongation stage, were less infected by leaf stripe than others. The distribution of a disease was found at 1.25% of plants in Vakula and Phoenix varieties, 1.3% - in Hetman one (Table 2). Sontsedar and Sebastian varieties were infected of disease at 23.8% level, while its development was 6.2 % and 6.4 % respectivelly. The higher intensity of a disease was found at the dough plant growing stage. The number of infected plants reached: 14.25% in the Phoenix and 51.8% in the Sebastian varieties. The intensity of the disease was respectively between 2,8-12,3%.

Conclusions

1. The first evidences of spring barley stripe were revealed at a stem elongation stage. Leaf stripe was distributed on 30% of plants in 2014 and on 29.5% - in 2015. The intensity of the disease was 7.22% and 6.76% respectively. At the dough development stage the disease was distributed on 56% of plants in 2014 and on 55% - in 2015. The intensity of the disease was 27% and 20.15% respectively.

2. Vacula, Hetman and Phoenix were lower damaged by striped spot than other varieties at a stem elongation stage. The plant lesions reached 1.25% for Vakula and Phoenix and 1.3% for Hetman. The stripe of barley was distributed on 23.8% of Sontsedar and Sebastian varieties and developed on 6.2% crops. The greatest development (14.25%, 51.8% on Phoenix and Sebastian spring barley varieties respectively) and intensity (2.8-12.3% on all varieties) of disease were found at the dough development stage.

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The effect of frozen storage time on some parameters of sugar beet quality

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Abstract: The basic indicators of the sugar beet quality are amount of sucrose and non-sugar compounds. Sugar beet, since its arrival to sugar plant, should be processed in short time. However, for the purposes of extensive research, scientists can't analyze all parameters of quality during that short period of time. Thus, the effect of storage time and storage temperature on chemical properties of sugar beet should be examined. In this paper it was found that there were no significant changes in amount of sucrose and alpha-amino nitrogen during the 41 months storage at -18 °C. Based on amount of alpha-amino nitrogen it was clear that sugar beet from season 2012 was more suitable for processing than beet from season 2013. Sugar beet from both seasons (2012, 2013) belonged to the beet varieties with high contents of sucrose.

Key words: sugar beet, sucrose, alpha-amino nitrogen

Introduction

Sugar is produced in over 100 countries worldwide; 78% of the world's sugar supply is derived from sugar cane and 22% from sugar beet, mainly cultivated in industrialized sountries. The worldwide sugar production reached 160 million tons and total world sugar trade is projected to increase by 19,9% between 2010 and 2020 (Rezbova, 2013). Although sugar cane is still the world's no.1 crop for sugar production, its use for this purpose has been stagnating. On the other side, sugar beet shows both, qualitative and quantitative growth potential.

Sugar beet (*Beta vulgaris saccharifera*) is the only industrial plant, cultivated in temperate climates, which can be used for sugar production. Although sugar gives value to the sugar beet crop, the byproducts of sugar production, such as pulp, molasses, beet particulate and carbonation lime, give an added value. Molasses is one of the main raw material in processing and fermentative

industries, particularly for the production of baker's yeast. Due to good nutritive composition, sugar beet molasses is used as a supplement in feed production. The biggest world sugar beet producer is Russia with more than a million hectares of sugar beet yields and the main European producer in 2015 was France.

Since EU has deficit of 3-4 million tons of sugar per year, development of sugar beet varieties with maximum root and sucrose potential has priority. The sugar production in Serbia reached 400 000 t.

For profitable sugar production, sugar beet root must have certain chemical, morphological, biochemical and microbial properties. Quality of sugar beet depends on the content of sucrose and content of non-sugar compounds, primarly alpha-amino nitrogen, potassium and sodium. The sugar content in sugar beet can vary from 12% to 20% and according to European norms the sugar beet is marketable if it contains 14% sugar or more. Ideal efficancy of 130 kg sugar per ton of standard sugar beet processed at a sugar plant can be achieved only if sugar beet has a sugar content of 16%.

The sugar extraction rate depends on content of sucrose, potassium, sodium and alpha-amino nitrogen in sugar beet (Sklenar et al, 2000). Excessive fertilization and unfavorable agroecological conditions result in high amount of nitrogen compunds accumulated in sugar beet. High concentration of alpha-amino nitrogen decrease the yield of crystalization of sucrose. Thus, all nitrogen compounds present in sugar beet, except proteins (which can be precipitated during the process of purification) are marked as unwanted nitrogen.

Sugar beet should be quickly processed and according to this, experimental results about contents of sucrose and alpha-amino nitrogen in frozen sugar beet during different storage time at -18°C is impotrant for scientists, particularly for multiyear researches.

Material and methods

The alpha-amino nitrogen was determined using L-glutamine as standard for preparing series of calibration solutions in concentration range from 1,44 to 14,4 $mmolL^{-1}$. Other chemicals were:$Cu(NO_3)_2$, $Pb(CH_3COO)_2 \cdot 3H_2O$, PbO, CH_3COOH and CH_3COONa.

The copper reagent was prepared as follows: 250 g $CH_3COONa \cdot 3H_2O$ and 10g $Cu(NO_3)_2 \cdot 3H_2O$ were dissolved in a 1000 mL flask, filtrated and adjusted pH to 6 with concentrated acetic acid.

The stock solution of the base lead acetate was prepared as follows: 300g $Pb(CH_3COO)_2 \cdot 3H_2O$ was mixed with 100 g PbO. Then, a 1000 mL of distiled water without $CO_2$was added to this mixture. After standing for 7 days the solution changed its color to white or reddish-white, then filtered.

The diluted solution of the base lead-acetate was prepared by mixing 25 mL of a stock solution of the base lead-acetate with 975 mL of water.

The sucrose content was determined by a polarimeter (POL-1, Optic, Italy), and the alpha-amino nitrogen content by spectrophotometry (Photolab 6100 VIS, Germany). Samples were prepared by the method of cold digestion (Milić et al., 1992). 26.00 g of pulp sugar beet was mixed with 177 mL of diluted basic lead acetate. The mixture was transferred to a blender where the mixing time 3 minutes at 6000 r / min. After stirring the content was filtered and the resulting filtrate was used for the determination of sucrose and alpha-amino nitrogen. The concentration of sucrose in the filtrate determined from Biots Law:

$$\alpha^t_\lambda=[\alpha]^t_\lambda \cdot c \cdot l \qquad (1)$$

where: α^t_λ-degree read at polarimeter, °, $[\alpha]^t_\lambda$-a specific rotation of sucrose, which is +66.54 °mL$g^{-1}$$dm^{-1}$ at 20ºC and at a wavelength of 589 nm., c-sucrose concentration, gmL^{-1}, l-tube length, dm was used to calculate the content of sucrose in sugar beet. According to the above method, it is assumed that 26 g of pulp sugar beet containing 23 mL of juice, on the basis of which the weight of sucrose (g)/ the amount of L-glutamine (mmol) from the pulp are present in 200 mL of a solution (177 mL of basic lead acetate and 23 mL of sugar beet juice).

It was mixed 5 mL of a solution of L-glutamine of particular concentration, 5 mL of acetate buffer pH 6 and 2 mL of copper reagent. The intensity of the blue color of the complex formed amino acid and Cu^{2+} ions were measured at 600 nm, with distilled water as a reference value. The alpha-amino nitrogen in sugar beet was determined from calibration curve of L-glutamine based on the linear dependence:

$$A = f(c) \qquad (2)$$

where: A - the absorbance _red at spectrophotometer, c- concentration of L-glutamine, 1-mmol L^{-1}

Results and discussion

In this paper it was determined the content of sucrose and alpha-amino nitrogen in sugar beet from 2013 which was stored 41 months at -18 ° C. The results are compared with the results, which were obtained after 5 and 17 months of storaged the same sample of sugar beet at the same temperature. Also, it was compared contents of sucrose and alpha-amino nitrogen of beet from 2012 and 2013.

The sucrose content after 5 months of storage was 15.62 wt.% ± 0.41, after 17 months of 16.58 wt.% ± 0.67 and after 41 months of storage 15,62 wt.% ± 0.86 (Figure 1). Based on the value standard deviation measurements can be assumed that content of sucrose does not change during storage and that the differences were caused by experimental error. The sucrose content in the sugar beet in 2012 determined after 5 months of storage at -18 ° C was 16.48 wt.% ± 0.98. The difference in sucrose content of the beet from the 2013 and 2012 are in the range of experimental error, which can not express the assumption that sugar beet has a higher content of sucrose.

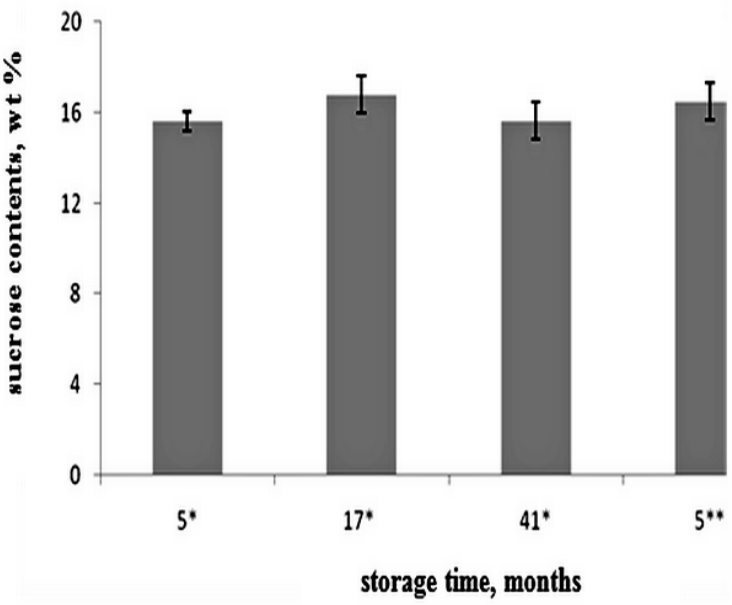

Figure 1. The sucrose content in function of storage time of sugar beet:
* from 2013 and ** from 2012

Jamaz (Jamaz, 2015) found that in 2012, despite the unfavourable_climate conditions, the average content of sucrose in sugar beet was the largest (15.1%) for a three-year period (from 2011 to 2013), while the sucrose content was depended by varieties of sugar beet and decreased in the following order: varieties *Belinda,Gina* and *Espirt,Chiara*. Due to lack of information about varieties of analyzed sugar beet, and to the high content of sucrose it can be assumed that sugar beet from 2012 and 2013 belonged to varieties with high sucrose content.

For precise determination of the alpha-amino nitrogen in the sample it was constructed calibration curve of the standard L-glutamine (Figure 2).

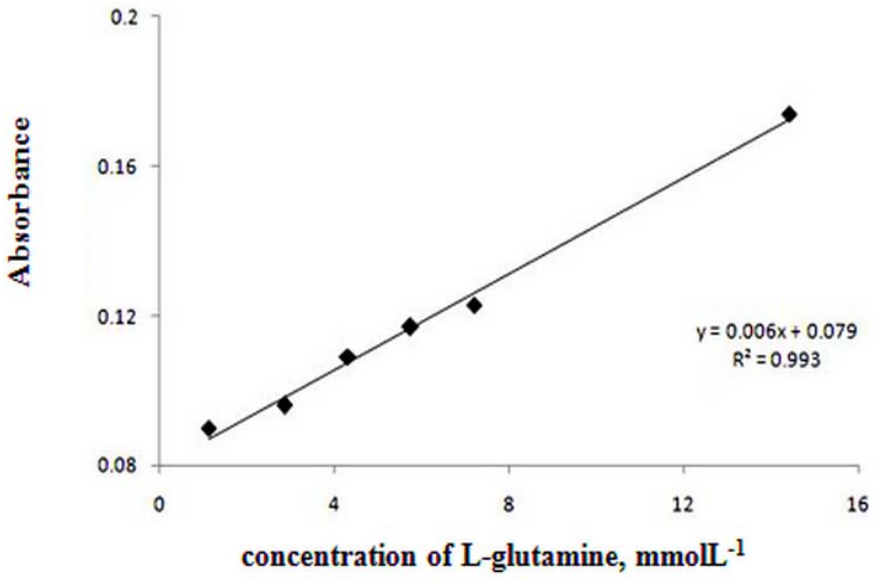

Figure 2. The calibration curve of L-glutamine

The content of alpha-amino nitrogen in the sugar beet was 4.21 mmolL-1 ± 0.39 after 5 months of storage at -18 ° C, 3.64 mmolL-1 ± 0.37 after 17 months of storage and 3.64 mmolL- 1 ± 0.42 after 41 months of storage at -18 ° C (Figure 3).

Based on the values of standard deviation can be assumed that there was no significant changes in amount of alpha-amino nitrogen storaged at -18 ° C. Sugar beet in 2012 after 5 months of storage had a significantly lower amount of alpha-amino nitrogen 1.66 mmolL-1 ± 0.31. It can be assumed that the sugar beet from 2012 was more suitable for processing because usually lower content of alpha-amino nitrogen because better utilization during the crystallization of sucrose.

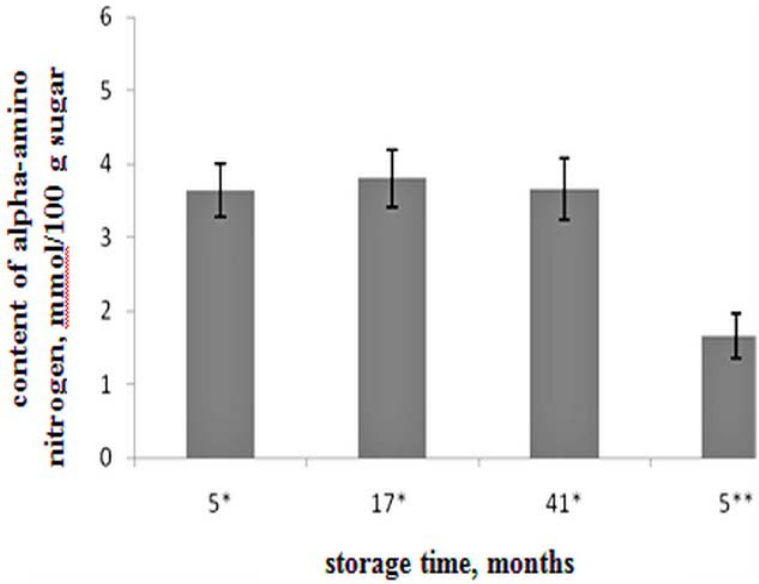

Figure 3. The content of alpha-amino nitrogen in function of storage time of sugar beet: * from 2013 and ** from 2012

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The effect of light spectra on cucumber plant grown under salinity stress

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Abstract: Light is very important factor necessary for plant growth and development as well as for the concentration of organic compounds contained therein. The influence of the quality composition of light is also very important for the chemical composition of the plant cell. In presented study we investigated the effect of an additional illumination with red, blue or a combination of red: blue light in ratio 7:1 or 4:1. We found that the blue light and a combination of red and blue light in a 7: 1 ratio increased the most the rate of net photosynthesis in both control plants and those under salinity stress. The blue light was most effective in chlorophyll and soluble proteins synthesis, and the red light was most effective for sugar accumulation in cucumber plants (cv. Regina).

Key words: cucumber, light quality, salinity stress, stress markers

Introduction

Light is one of the most important environmental factors necessary for plant growth and development as well as for the concentration of organic compounds contained therein (Kopsell and Kopsell, 2008). There are many studies on the influence of light intensity on the chemical composition of plants (Keller and Hrazdina, 1998), but controversial results are often reported for the influence of the quality composition of light. For example, red light stimulates the synthesis of anthocyanins in fruit of cranberries (Zhou and Singh, 2002) while the same effect in tomatoes is achieved by blue light (Giliberto et al., 2005), and in lettuce the additional illumination with blue and UV-A light increases the content of anthocyanins and carotenoids (Li and Kubota, 2009).

Attempts have been made to establish a relationship between the ratio of red and blue lights and the regulation of plant growth and morphogenesis. According to some authors (Nhut et al., 2003), plant growth was better at 10%

blue LEDs. A number of studies have shown that LEDs also affect the plastid pigment content. According to some authors, blue light stimulated chlorophyll synthesis (Kim et al, 2004), while red light reduced chlorophyll content (Tanaka et al., 1998; Li et al., 2010), and stimulated the accumulation of beta-carotene (Fan et al., 2013).

The quality of light also affects the content of sucrose, starch, soluble sugars, and the activity of plant photosynthesis. An increase in sugars and starch content was observed in cotton grown under red light (Li et al., 2010), while the combination of blue and red light was effective in the vine (Heo et al., 2006).

The regulation of carbohydrate metabolism through the quality of light is well documented (Kowallik, 1982). There is a hypothesis that red light can inhibit the translocation of photosynthetic products, thereby increasing starch accumulation (Soebo et al., 1995). Goins et al. (1997) found a higher rate of net photosynthesis and increased stomatal conduction in wheat leaves when combining red and blue light. Similar results are found in chrysanthemum (Kim et al., 2004).

Agricultural crops are often subject to water scarcity and/or salinization, which negatively affects their growth and development as well as yields. Understanding plant responses to these or other stress factors are important in helping the productivity of stressed plants. Photosynthesis and cell growth are one of the first processes that affect saline stress or water deficiency (Munns et al., 2006; Chaves, 1991). These effects may be associated with lowering available CO_2 due to stomatal or mesophilic constraints (Flexas et al., 2004, 2007), or due to changes in photosynthetic metabolism (Lawlor and Cornic, 2002), or due to the occurrence of secondary oxidative stress (Chaves and Oliveira, 2004).

Studies on salt stress in plants are many and various, but studies on the influence of light composition on plants subjected to salt stress are scarce. This fact motivated our study of the impact of additional illumination with red, blue and combined red with blue light, on cucumbers plants (cv. Regina) subjected to salt stress in the hydroponics.

Material and methods

Plant material, growth conditions and light treatments

The cucumber seeds (cv. Regina) were germinated on perlite moistened with water for one week, after which they were placed in containers and grown in Knope nutrient solution supplemented with micronutrient elements. Plants were grown under controlled conditions (relative air humidity 65%, temperature 25/18 °C day/night, light duration 14 hours, light intensity (PAR) 250 μmol m^{-2} s^{-1}). Plants were divided into four groups with different additional illumination. The

light sources used were luminescent white light lamps and additional LED lamps for red, blue or a combination of 7: 1 red: blue and 4: 1 red: blue light. Upon the appearance of a second true leaf, one part of the plants from each light group was treated with 50 mM NaCl. This concentration of NaCl causes moderate salinity stress in cucumber plants (Harizanova, 2017). Under these conditions, the plants were grown for 10 days and the nutrient solution was changed every two days. At the end of the experimental period, the plants of all variants were analyzed for photosynthetic activity and some biochemical parameters. The variants are presented in Table 1, and each variant consisted of three replicates of four plants per repeat.

Table 1. Variants used in this study

Type of additional light illumination (light groups)	Variants
I. Red:blue LED in ratio 7:1 (R:B 7:1)	1. Control
	2. Salinity 50 mM NaCl
II. Red:blue LED in ratio 4:1 (R:B 4:1)	3. Control
	4. Salinity 50 mM NaCl
III. Red LED (R)	5. Control
	6. Salinity 50 mM NaCl
IV. Blue LED (B)	7. Control
	8. Salinity 50 mM NaCl

Photosynthetic and biochemical measurements

The parameters of leaf gas exchange (A – photosynthesis, E- transpiration, gs – stomatal conductance) were determined by a portable photosynthetic system LCpro$^+$ (ADC, England). The photosynthetic pigments were extracted with cooled 85% acetone, measured spectrophotometrically using Pharo 300 (Merck, Darmstadt, Germany) and calculated according to Lichtenthaler (1983). The content of reducing sugars was determined by the Hagedorn -Jensen method with modifications of Popov (Koleva-Valkova et al., 2016). The amount of soluble proteins was determined by Bradford (1976). The free proline content was determined spectrophotometrically by the method of Bates (1973).

Statistical analysis

All measurements were evaluated for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the $p<0.05$ level.

Results and discussion

Based on the analysis of photosynthetic activity, it can be said that the quality of light significantly changed the rate of net photosynthesis (Table 2). The highest photosynthetic activity was determined in plants from the light group I (combined light R: B 7: 1, variant 1), followed by the plants from light group IV (B light, variant 7). The lowest photosynthetic activity was measured in plants from variant 6 (grown with additional R light). The higher photosynthetic values in the plants from variants 7 and 8 (B light) correlated with the higher values obtained for plastid pigments in the same variants (Table 2). The transpiration rate and stomatal conductance followed the same trend as with the photosynthesis. Photosynthetic activity decreased in the control plants (variants 1, 3, 5 and 7) depending on the light quality. It was observed that the plants from the light group I (R:B 7: 1), followed by those from light group IV (B) were best represented, followed by the plants from light group II (R:B 4: 1) and finally from light group III (R). For plants under salinity stress, the influence of the type of illumination followed the same trend (Table 2). With regard to plastid pigments, the blue light increased the concentration of chlorophyll a, b and carotenoids more in the control and slightly less in salinized plants (Table 2, variants 7 and 8). In the plants which were grown with an additional illumination of R:B 4: 1 light, higher values of the plastid pigments in variant 4 were observed. The lowest values of plastid pigments were recorded in plants grown with an additional R:B light 7: 1 (variant 2) illumination. The present results demonstrated that the blue light plays an important role in the synthesis of chlorophyll, which was in accordance with the results of other authors (Li et al., 2013; Li and Kubota, 2009).

As an important stress marker, the content of free proline was measured (Table 3). The highest levels were recorded in the leaf of variant 5 (control plants were grown under an additional red light). In the plants from I and IV light groups, the content of proline was higher in the leaves of the salinized plants. In contrast, higher values of free proline were recorded in the leaves of control plants (variants 3 and 5) of light groups II and III compared to those of the salinized plants of the same groups.

The soluble protein content changed significantly under the influence of the type of light or the salinity stress (Table 3). Higher values were reported in the leaves of control plants from all light groups except light group IV. A similar tendency was found in the roots but with two exceptions – light groups II and IV, the salinized plants from variants 4 and 8 accumulated more soluble proteins in the roots compared to those of the control plants of the same groups. Our results differ from those of other authors which stated that the concentration of soluble proteins did not change under the different light spectra (Li et al., 2013). A possible explanation may be the difference in the experimental patterns, the different combinations of light, and the presence of salt stress in our experiment.

Table 2. Leaf gas exchange parameters and concentration of plastid pigments in young cucumber plants grown under different additional light illumination and exposed to salinity stress. Photosynthetic rate - A (μmol CO_2 m^{-2} s^{-1}), transpiration rate - E (mmol H_2O m^{-2} s^{-1}) and stomatal conductance - gs (mol m^{-2} s^{-1}).

	Leaf gas exchange			*Plastid pigments mg/g FW*		
Variants	*A*	*E*	*gs*	*Chl a*	*Chl b*	*Caroten*
1	17,06a	2,30a	0,34a	1,19b	0,29c	0,45a
2	15,94ab	2,38a	0,36a	0,99c	0,27c	0,35b
3	12,54c	1,84b	0,23b	1,14b	0,32b	0,38b
4	13,55c	1,66b	0,19c	1,48a	0,42a	0,50a
5	10,52c	1,10b	0,11c	1,18b	0,33ab	0,42a
6	9,68d	1,32b	0,14c	1,18b	0,35a	0,42a
7	16,53a	2,00a	0,30a	1,30a	0,38a	0,46a
8	15,19b	2,18a	0,33a	1,15b	0,32b	0,42a

Values followed by the same letter within the column do not significantly differ (by the LSD test, p=0.05).

Table 3. Free proline, soluble protein and reducing surars concentration in leaves and roots of young cucumber plants grown under different additional light illumination and exposed to salinity stress.

	Free proline μg g^{-1}FW		Soluble protein mg g^{-1} FW		Reducing sugars %	
Variants	leaves	roots	leaves	roots	leaves	roots
1	29,49c	18,04b	18,43b	9,33a	0,922b	0,345c
2	41,23b	16,69c	14,86c	9,03a	0,403d	0,776a
3	36,27b	22,41a	19,7a	8,2b	0,533c	0,286c
4	32,95b	18,64b	18,9b	8,73b	1,171a	0,536b
5	77,23a	21,96a	19,4a	9,01a	1,752a	0,204c
6	29,19c	18,34b	17,98	8,62b	0,668c	0,252c
7	23,61c	17,89b	19,8a	8,74b	0,536c	0,348c
8	24,97c	23,61a	20,1a	9,75a	0,732c	0,497b

Values followed by the same letter within the column do not significantly differ (by the LSD test, p=0.05).

With regard to the sugar content, it was found that the different light combinations stimulated the synthesis of sugars mainly in the leaves (Table 4). The exception was variant 2 where in the roots of the salinized plants was measured higher content of reducing sugars under the influence of an additional R: B 7: 1 light. The highest concentration of sugars was recorded in the leaves of

control plants under the influence of red light (variant 7), followed by variant 4 (R: B 4: 1), variant 1 (R: B 7: 1) and variant 7 (B light). In the roots, the sugar content did not follow the same trend depending on the light, but higher values were observed for all salinized plants than in the control plants of the same light group. This may be due to the fact that the stress factor collides first with the roots, and there was a need to accumulate more metabolites used in cellular breathing to provide the cells with energy to cope with the stress. On the other hand, in the leaves, the sugar content was much higher than in the roots and was strongly influenced by the type of the light. This may be due to the fact that they are synthesized in the leaves in photosynthesis, and secondly, that some of them act as osmolytes and protect the cells in salinity stress. It is obvious that the light quality regulated carbohydrate metabolism, and the red light increased the content the most. This observation was in accordance with the results of other authors (Li et al., 2013; Lin et al., 2013; Chung et al., 2010).

Conclusion

In conclusion, it can be said that the light composition influenced the photosynthetic activity and the biochemical content of the plants subjected to salt stress. Under the influence of blue light and a combination of R: B light in a 7: 1 ratio, the rate of net photosynthesis was higher in both control and salinized plants compared to plants exposed to red light or a combination of R: B light in a 4: 1 ratio. The blue light was most effective in chlorophyll and soluble proteins synthesis, and the red light was most effective for sugar accumulation in cucumber plants (cv. Regina).

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Soil research in the areal of the town Lyubimets for growing vineyards

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Abstract: Based on several soil profiles from the Lyubimets region in determining important soil indicators for vineyards, such as soil pH, particle size composition, the presence of carbonates, organic matter, etc. there are made conclusions about the suitability of soils for the establishment of vineyards.

Key words: soil, organic matter, pH, vineyards

Introduction

The soil is one of the very important factors influencing its chemical and physical properties on growth, vine productivity and grape and wine quality. The development of the root system and the depth it reaches depend on its mechanical composition and its overall physical, water, air, and thermal characteristics.

Each of the three components - soil, subsoil and material scale, has a different but strong influence on the formation of the vegetative and generative organs of the vine and the technological characteristics of the grapes.

Therefore, when zoning and when defining the micro-regions and their areas, strict requirements of the vine varieties for the soil conditions are strictly observed. It is no coincidence that in the countries with highly developed viticulture, the area where the grapes were grown is also marked for the well-established wine brands.

The aim of the present work is to study some soil indicators in the region of Lyubimets and to combine the climatic conditions to make recommendations for growing vineyards.

Material and methods

The subject of the study is several soil profiles located in two localities of Lyubimets.

The preparation and the analysis of the samples were conducted at the laboratory of the Department of Agrochemistry and Pedology at the Agricultural University – Plovdiv. The following soil indicators were analyzed:

- *Particle size composition of photosedimentografy on FRITISCH*
- *Organic matter by Tiurin*
- *pH (H_2O) value - potentiometric*
- *Total carbonates by Shaibler*
- *Exchange Ca^{2+}+Mg^{2+} - complexometric*
- *Mobile potassium - in 2n HCL*
- *Mobile forms of phosphorus – according to Egner - Reem*
- *Nitrate and ammonium nitrogen with 1% KCL*

Results and discussion

Climatically, the territory of the town of Lyubimets is related to the Continental - Mediterranean climate area.

The average annual rainfall in the area is 595 to 775mm. The seasonal maximum is in winter, with the months with the most rainfall being November and December. An important minimum is recorded during the summer and mainly during August.

The average annual air temperature is in the range of 10.0 to 12.5 °C. Winter is mild, with average temperatures in the coldest month - January about 1.0 to -1. 5 °C. Absolute minimum temperatures fall very rarely below -10.0 ÷ -15.0°C. The summer looks like hot, with average absolute maximums of 34.0 - 36.0° C

The absolute temperature maximum measured in the area is 39.2^{o}C for August. The average value for the same month is 34.8 °C and ranges from 32.7 to 36.9 °C. From 1 it can be seen that temperatures above 35 °C can be expected during June-September. The recording of such temperatures can cause damage to the vine if combined with low soil moisture conditions, mainly due to August.

The soil cover in the region of Lyubimets is represented by two soil differences:

The first - According to the FAO-Unesco classification, they refer to the Chromic Cambisols. Their soil profile consists of Humus Accumulative and Metamorphic Horizons. In different parts of the terrain, the soil is characterized by a heavy to medium sand-clayey Particle size composition. The amount of clay ranges from 17.3 to 48.5%.

The averaged values for all profiles are shown in Table 1.

Table 1a. Physico – chemical characteristics of Chromic Cambisols

Depth cm	*Total $CaCO_3$ %*	*pH H_2O*	*Organic matter, %*	*Particle size composition (≤ 0,01 mm), %*
0-20	0.0	5.92	2.67	36.40
20-40	0.0	6.20	1.81	43.55
40-60	0.0	6.52	0.98	45.60
60-80	4.62	7.25	0.63	35.94
80-100	7.10	7.00	0.33	32.05

The total carbonate content of the total area studied was 1.81% at the upper limit of 12.6%. Table 1 show the average values of calcium carbonate by depth of the soil profile.

Carbonates are washed at a depth of 60-80 cm, where the average value is 4.62%, at an upper limit of 12.6%.

The presence of common carbonates is found mainly in the lower parts of the terrain and in the deep soil layers. This is not a limiting factor for the development of the vine in the studied terrain.

Soil response (in H_2O), average for the site is 6.52 and ranges from 5.00 to 8.00. The soil reaction is defined as moderately acidic to neutral. It is believed that the vine develops normally at pH values of 5 to 7. In this sense, the established mean pH values are included within the indicated range.

The average humus content for the entire site and for the whole depth of the studied soil is 1.39% and is estimated to be low, ranging from 0.33% to 2.9%. The distribution of humus in depth is presented in Table1.

There is a clear tendency to reduce humus in depth, which is a natural process. The content of organic matter in the site is assessed as favorable for the development of the vine.

The second - The Vertisols occupy the most depressive forms of the Lyubimets terrain. The soils tested are characterized by a heavy Particle size composition. They contain from 58.7 to 65.8% clay (Table 2a), making them less suitable for growing vineyards. It is known that vines can have excellent growth, development, great productivity and high grape quality when the soil clay content does not exceed 50%. Apart from their unfavorable part size composition, these soils are also characterized by unfavorable general physical properties, especially in the layer below 60 cm. The bulk density is from 1.40 to 1.45 g / cm3, and the total porosity is below 50%. The amount of air in the lower layers of the soil is less than 10%, which in many wet years can lead to asphyxiation of permanent crops such as vineyards, peaches, cherries, plums, etc. The leachers are deeply decarbonated. Carbonates are below 1.00 m. The reaction is slightly acid to neutral. The soils are very well stocked with humus,

total and digestible nitrogen. They are well-stocked in phosphorus and especially potassium. The exchange cations (Ca^{2+} and Mg^{2+}) are sufficient for the normal course of chlorophyll photosynthesis. The data are presented in Table 2a,b.

Table 2a. Physico – chemical characteristics of Vertisols

Depth cm	*Total $CaCO_3$ %*	*pH H_2O*	*Ca^{2+} meq/100g*	*Mg^{2+} meq/100g*	*Particle size composition (≤ 0,01 mm), %*
0-20	0.0	6.38	13.88	2.95	60.30
20-40	0.0	6.60	14.98	3.38	61.30
40-60	0.0	6.75	16.55	4.10	63.98
60-80	0.0	6.95	17.73	4.53	65.80
80-100	0.0	7.23	19.40	5.45	58.70

Table 2b. Physico – chemical characteristics of Vertisols

Depth cm	Organic matter, %	Total nitrogen %	NO_3^{2-} NH_4^+ mg/kg	P_2O_5 mg/100g	K_2O mg/100g
0-20	3.33	0.24	77.93	23.68	40.87
20-40	2.38	0.15	58.50	16.53	35.00
40-60	1.23	0.08	25.55	8.45	29.37
60-80	0.73	0.00	9.43	4.95	26.50
80-100	0.35	0.00	6.48	3.80	25.35

Conclusion

Based on the study of the soils in the region of Lyubimets, Svilengrad, as well as the climatic peculiarities of this region, we came to the following conclusions:

1. Climatically, the area of Lyubimets meets the requirements for growing vineyards.
2. Regarding the mechanical composition and physico-chemical properties, cinnamon forest soils distributed in the area under consideration meet the requirements for growing vineyards, while the reeds are better suited for fruit growing - apples and pears.
3. Due to the low content of humus, total nitrogen and phosphorus, it is recommended fertilization with well-aged manure, nitrogen and phosphorous fertilizers with norms that are consistent with soil availability.

In conclusion we can say that the cinnamon forest soils in the Lyubimets region are suitable for growing vineyards by applying the necessary agro-technology, fertilization and plant protection measures.

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Evaluation of insecticides for control of box tree moth – *Cydalima perspectalis* (Walker, 1859)

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Abstract: *Cydalima perspectalis* or the box tree moth is a pest of Buxus trees. It originates from South East Asia and recently has been introduced in Europe, and in 2014 in Bosnia and Herzegovina. It has quickly spread over the country and started to make devastating damage to this ornamental plant. Buxus ornamentals are usually grown in private gardens or in public parks. Its damaging potential requires implementation of control measures at regular basis. In this study two insecticides from two groups, neonicotenoides and piretroides, were tested. Caterpillars of the box tree moths were collected from naturally infested trees during April and May and treated with insecticides in laboratory assay. The treatments included evaluation of inhalation, digestion and contact effects of insecticides on the larvae. Treatments included 100% recommended dose, five times lower and control without insecticides. Inhalation treatments did not affected the larvae. In the digestion assay piretroid did not caused mortality, while for neonicotenoide mortality was 20% and 8% for full and five times lower dose, respectively. In the contact assay higher mortality was produced with neonicotenoide insecticide that has reached 100% with full dose. This results can help in choosing the most effective insecticide and its way of application when we understand way of action of pesticide.

Key words: Buxus, piretroids, neonicotinoides

Introduction

The box tree moth (*Cydalima perspectalis* Walker) belong to the family Crambidae (Lepidoptera). The origin of the species is East Asia. In Europe, it was first reported from south-western Germany in 2007 (Krüger, 2008), and it

was probably introduced with containered *Buxus* seedlings imported from Asia. Since then, the moth has spread to several other European countries. For example, it was found in Bosnia and Herzegovina for the first time in 2014 (Ostojić *et al.*, 2015).

The pest have several synonyms: *Diaphania perspectalis* Walk., *Glyphodes perspectalis* Walk., *Palpita perspectalis* Walk., *Phakellura perspectalis* Walk. (Fora *et al.*, 2016). It is reported that the pest has 2-3 generation per year in Central Europe (Strachinis *et al.*, 2015), and overwinters in the caterpillar stage (Koren, Črne, 2012).

The host plants for *C. perspectalis* are species from genus *Buxus*. The damage in Bosnia and Herzegovina, was observed only on *Buxus sempervirens* L. Larvae of the moth are significant defoliators of box trees. After they eat all leaves, they feed on bark of the branches. The clearest evidence of *C. perspectalis* damage is the presence of a high number of were not alive leaves and the presence of webbing, spun by the larvae in box tree canopy.

In Europe, in this moment, there are no reported natural enemies of *C. perspectalis*. Even birds don't attack pest caterpillars because they have a toxic alkaloids content from their host-plant (Leuthardt *et al*., 2013). Only reported effective biocontrol agent is *Bacillus thuringiensis*. However, this product is not available for garden owners in Bosnia and Herzegovina. Therefore, through this study effect of insecticides with different MoA (Mode of Action) on larvae of the box tree moth was evaluated.

Material and methods

Caterpillars collecting

Third instar and older caterpillars were collected in several public and private gardens and parks in the urban environment of Banja Luka, Republic of Srpska, Bosnia and Herzegovina. Larvae of the box tree moths were collected from naturally infested trees in early morning hours during April and May 2017. Gathered caterpillars were placed in empty jar with perforated cover cover, with the box tree leafs offered as a food source.

Application of insecticides

Two groups of insecticides – neonicotinoides and piretorides were used in this assay.

First treatment was with cypermethrin 200g/l (Cipkord 20 EC, 1,5ml/10l of water). This insecticide is with contact and digestion effects, from piretroides group of insecticides. Second treatment was with acetamiprid 200g/kg (Volley 20 SP; 2,5g/10l of water). Acetamiprid is active substance from neonicotinoides group of insecticides. It has contact and digestive effect on pests.

The treatments included evaluation of inhalation, digestion and contact effects of insecticides on the larvae. Treatments included 100% recommended dose, five times lower (20%) dose and control (without insecticides). For inhalation and contact treatments five caterpillars were placed in plastic Petri dish 5.5 cm diameter, with five replicates, and for digestion treatment one larva was placed in each Petri dish, with 15 replicates.

In inhalation treatments, in each Petri dish 350µl of water solution of tested insecticides was added on filter paper and than one larva was placed inside the dish. The Petri dish was sealed with parafilm, to prevent evaporation. For contact treatmans caterpillars were submerged with tweezers in water solution of insecticides for approximately two seconds and placed in a Petri dish. In digestion assay individual leaves were also submerged in water solution of insecticides. Individual leaf was offered to one larva. For examination of digestion effects of cypermethrin, average weight of fresh leaves was 0,0089g and after dipping them in water solution of product it was approximately 0,0231g. Average weight of fresh leaves for testing of digestion effects of acetamiprid was 0,0184g, and average weight of same leaves after submersion was 0,0286g. Larvae moratlity was checked after one, two, three and 72 hours (treatments with cypermethrin), and one and 24 hours (treatments with acetamiprid).

For each treatment there was control group, which included the same number of Petri dishes like each treatment, but the larvae or leaves were not treated with insecticides. They were fed with leaves of box trees whole time.

Results and discussion

Treatment with cypermethrin

At both concentrations for inhalation treatmans, there were no mortality at all.

In experiments with contact effects, there were different results. On full dose one hour after beginning of assay, all caterpillars moved very slowly; after two hours they look all were not alive, meaning that they did not react on touching them. After three hours from treatment one larva in one replicate was alive, and it was moving only with touching head, but after 72 hours four larvae within all replicates were alive, meaning that mortality was 86%. In control treatment all larvae were alive and readily fed on offered food. In treatment with 20% dose, after one hour all larvae were alive, but they were moving very slowly. Two hours after teratment, 11 larvae were were not alive, while three hours after treatment three more larvae were were not alive (14 were not alive in total), but after 72 hours from start 10 (40%) were alive, nine were were not alive (36%), and six (24%) were missing because of cannibalism.

In trial with digestion effect, at 100% dose, after one hour less than half caterpillars were eating treated leaves and they were moving slowly. Two hours after start of the trial, caterpillars from nine Petri dishes were were not alive (in five of them, caterpillars were eating, while in other four they were not eating treated leaves. It was assumed that cause of mortality was contact and not digestive effect). After three hours, four caterpillars were were not alive, one is moving by itself, while other 10 were moving only with touch. However, after 72 hours, all caterpillars were alive. At 20% of recommended dose, after first and second hour about 70% of larvae fed, but they were all alive. After three hours, one caterpillar looked paralysed, but after 72h all were alive. Results from this part of assay are shown it Table 1.

Table 1. Rate of mortality of caterpillars in the assay with cypermethrin

	1h			2h			3h			72h		
D1	Inh	Dig	Con	Inh	Dig	Con	Inh	Dig	Con	Inh	Dig	Con
	-	-	-	-	9	25	-	4	24	-	-	21
	1h			2h			3h			72h		
D2	Inh	Dig	Con	Inh	Dig	Con	Inh	Dig	Con	Inh	Dig	Con
	-	-	-	-	-	11	-	1	14	-	-	9*

*six caterpillars were missing because of cannibalism

Even in the control group it was noted appearance of cannibalism, because two caterpillars were missing.

Treatment with acetamiprid

As well as in the first case, inhalation treatmans didn't have any results. One larva was missing due to cannibalism.

In the assay with contact effects, for full dose after only an hour from the beginning of the assay 24 larvae were not alive, and after 24 hours there were no alive caterpillars. On lower dose, after one hour, eight caterpillars were not alive, and after 24 hours 14 caterpillars were not alive, in total 22 or 88%.

Table 2. Rate of mortality of caterpillars in the application of acetamiprid

	1h			24h		
D1	Inh	Dig	Con	Inh	Dig	Con
	-	-	24	-*	5	25
	1h			24h		
D2	Inh	Dig	Con	Inh	Dig	Con
	-	-	8	-	2	22

*one caterpillar is missing

In the experiments with digestive effects, for full dose, after one hour, there were no mortality (three caterpillars fed), and after 24 hours five (33.3%) caterpillars were not alive (of the remaining ten, seven fed). In part of assay with lower dose of acetamiprid, after one hour all caterpillars were alive, but after 24 hours only two (13.3%) of them were not alive (from remaining number of larvae, four ate whole leaf, seven of them ate only a part of leaf, and two didn't eat at all). Results from this part of assay are shown it Table 2.

Discussion

In this study piretroid and neonicotinoide insecticides showed moratlity effect on the different stages of the larvae of the box tree moth, but this was highly dependent on type of treatment and dose. As expected, inhalation treatments did not affected the larvae in all concentrations and types of insecticides.

In the digestion assay piretroid did not caused mortality, while for neonicotenoide moratlity was 20% and 8% for full and five times lower dose, respectively. In the contact assay higher mortality was produced with neonicotenoide insecticide that has reached 100% with full dose.

Fora *et al.* (2016) discovered that active substance deltamethrin 250g/kg (Decis 25 WG) have the maximum larvae control effect 7 days after application, thiamethoxam 25% (Actara 25 WG) 14 days after application, lambda-cyhalothrin 50g/l (Karate Zeon) 21 days after treatments, thiacloprid 480g/l (Calypso 480 SC) and imidacloprid 75g/l + deltamethrin 10g/l (Confidor Energy) 21 days after treatments. They applied the insecticides with a back-pack sprayer.

Recovery of caterpillars treated with the piretroid can be explained with the fact that this group of insecticides have so-called "knock-down" effect. It may be defined as the state of intoxication and partial paralysis, which usually precedes to death, but at most of the cases it presented a temporary paralysis followed by complete locomotion recovery.

Results of this study can help in choosing the most effective insecticide and its way of application when we understand way of action of pesticide. Because the mode of action of those active substances are different, it can be recommended to combine them to increase the efficacy against this pest.

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Dynamics of amylase activity in dogs with experimental acute pancreatitis

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Abstract: Acute pancreatitis is a severe inflammation of the gland related to damage of acini cells and activation of inflammatory cells. The purpose of this study was to establish plasma amylase profiles during the time course of experimental acute pancreatitis and to compare variations of different amylase isoforms in dogs. For that purpose, acute pancreatitis was surgically induced in 6 dogs by ligation of both pancreatic ducts. Total amylase, pancreatic amylase and extra-pancreatic amylase concentrations were determined before (hours -72 and 0) and after (from 3rd to 96th hour) surgery. Total amylase/pancreatic amylase and pancreatic amylase/extra-pancreatic amylase ratios were determined as well. Concentrations of total amylase and amylase fractions tended to gradually increase from the 6th to the 48th hour after surgery. After that all isoforms begin to slowly decrease, and on the 96th hour, the extra-pancreatic amylase levels are near the baseline values. These results confirm the occurrence of an inflammatory reaction, characterized by an increase in the total and pancreatic amylase accompanied by a change in ratios between different amylase isoforms, during acute pancreatitis induced in dogs by ligation of excretory ducts.

Key words: amylase, pancreatic amylase, pancreatitis, dog

Introduction

Amylase is an enzyme that is synthesized in the pancreas and hydrolyses alpha-bonds in starch. The determination of the amylase value is recommended for the first time by Elman in 1929, and for almost 100 years is considered a universal laboratory test for the diagnosis of AP (Elman et al. 1929).

The main advantages of determining amylase values over other biochemical tests in patients with suspected AP are its easy technical performance, low cost

and relatively high sensitivity - 85 to 95% (Yadav et al., 2002). The main disadvantage is the relatively low specificity, especially in patients with hyperlipidemia, as well as in patients studied late in the course of the disease (Yadav et al., 2002). The total amylase values are basically determined by the isoenzymes salivary (S) amylase and pancreatic (P) amylase.

Salivary amylase is synthesized and secreted by the parotid glands. It has the same function but different electrophoretic motility from pancreatic amylase. In order to increase the specificity of the amylase measurement it is better to be measured amylase isoforms than the total amylase. P-isoamylase is the most reliable indicator that the increase in total amylase level is derived from the pancreas (Agarwal, 1990; Bunch, 2003; Watson, 2004; Mix and Jones, 2006). The specificity and sensitivity of p-amylase for diagnosis is higher than the total amylase, but this test cannot yet be applied universally (Bunch, 2003).

P-isoamylase levels may also increase in the absence of AP. Causes of this may be diseases of the bile ducts, small intestine perforation, intestinal obstruction or ischemia (Simpson and Lamb, 1995; Bunch, 2003; Ruaux, 2003; Steiner, 2003; Watson, 2004)

The purpose of this study was to establish plasma amylase profiles during the time course of experimental acute pancreatitis and to compare variations of different amylase isoforms in dogs.

Material and methods

Experimental animals and protocol design

Six experimental mongrel dogs from both sexes, 4-5 years old, weighing 13.5-18.0 kg, provided by the municipality of Stara Zagora, were used in the present study and the same dogs served as auto-controls on hours -72 and 0. Prior to the experiment, the animals were vaccinated with vaccine Nobivac® DHPPiLR (Intervet International B.V) and treated orally against internal parasites with Caniverm® (Bioveta, A. S. Czech Republic, ~ 1 tablet 10 kg B.W.) and against external parasites with Bolfo® Powder (Bayer, Germany). They were fed according to their age and had free access to tap water. The experimental procedure was approved by the Ethical Committee of the Faculty of Veterinary Medicine in Stara Zagora.

Experimental dogs were premedicated with a subcutaneous injection of atropine sulphate (Vetprom Ltd, Bulgaria, 0.02 mg/kg) and 10 minutes later, by an intramuscular injection of acepromazine hydrochloride (Combistress®, Kela – Belgium, 0.2 mg/kg). Twenty minutes after, thiopental sodium (Thiopental VUAB®, Slovakopharma, Slovakia) at 10 mg/kg was intravenously injected for the anaesthesia induction and the anaesthesia was maintained using 1.5-2 V% halothane (Narcotan ®, Spofa, The Czech Republic) and oxygen flow 2-2.5

L/min, with a Bain breathing circuit. Fluid maintenance was performed with 0.9% saline (Troyapharm, Bulgaria) at a rate of 5-10 mL/kg/h. The experimental pancreatitis was induced by ligation of both pancreatic ducts. The surgical approach was created by cranial median laparotomy. The initial part of the duodenum was brought outside the abdominal cavity and isolated from inflow of new gastric and duodenal content by intestinal clamps. Duodenotomy was performed by linear incision of the side, opposite to the pancreas. Both ducts were cannulated through the minor and the major duodenal papillae. Around the excretory ducts, ligatures were placed around the excretory ducts openings by two-layer (purse-string and cross-stitch) sutures of the absorbable suture material polygalactin (Vicryl®, Ethicom Inc, USP 4-0). The duodenal wound was closed by Schmiden's suture and continuous Lambert polyglyconate sutures (Maxon, Davis-geck, USP 2-0). The abdominal cavity was closed routinely. Dogs have been fasted for 2 days after surgery but 24 hours after surgery, dogs have had free access to the drinking water. On the 5th day after surgery, all dogs were euthanized with thiopental and potassium chloride.

The experimental procedure was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Stara Zagora.

Determination of plasma amylase profiles

Blood samples (10 mL) were collected from the puncture of the *v. cephalica antebrachii* into sterile heparinised tubes prior to the operation (hours -72and 0) and 3, 6, 24, 48, 72 and 96 hours after the surgery. Heparinised blood was centrifuged (600g, 10 minutes, at 4°C) within 30 min after collection. Plasma was immediately separated and analyses were performed.

Total amylase values (U/L) were determined colorimetrically with an automatic biochemical analyzer (Mind ray BS-120, China) and commercial kit (Biolabo Diagnostics SA, France).

Pancreatic amylase (U/L) was determined with an automatic analyzer (Reflotron Plus, Germany) and commercial kit of Roche Diagnostics Ltd, GB.

Extra-pancreatic amylase values (U/L) were determined as a difference between total amylase and pancreatic amylase levels.

Statistical analysis

Data were statistically processed by ANOVA (one-way analysis of variance) (Statistica for Windows, Stat Soft Inc., USA 1993). All results are presented as mean and standard error of the mean (mean ± SEM). The statistical significance of parameters, according to time, was determined in the LSD test at $p < 0.05$.

Results

In the course of the experiment, we found a marked increase in the total amylase values (Table 1 and Figure 1). Output values varied between 436 and 1012 U/L, at an average of 654 ± 86 U/L 72 hours before the study and 816 ± 57 U/L at 0 hour. At the 3rd hour after surgery, the level of total amylase started to rise, and values from 24th to 72nd hours were significantly higher ($p < 0.01$) than measured at 0 hour. The maximum value established at 48th hour (14911 ± 3167 U/L) was approximately twenty times higher than those in the preoperative period.

Table 1. Plasma levels of Total Amylase (TA), Pancreatic Amylase (PA), Extra Pancreatic Amylase (EPA) (U/L) and relationship between them in dogs with experimentally induced acute pancreatitis via pancreatic channel ligatures. The results are presented as mean ± SEM; n=6; [a]$P<0.05$; [b]$P<0.01$

Time	PA	EPA	TA	TA/PA	PA/EPA
-72h	272,5 ±30,1	381,2 ±79,5	653,7 ±85,8	2,45±0,26	0,92±0,27
0 h	307,2 ±26	508,7 ±60,4	815,8 ±57,3	2,76±0,34	0,67±0,12
3 h	413,5 ±50,8	640 ±109,3	1053,5 ±120,1	2,74±0,37	0,78±0,21
6 h	985,5 ±162	420,5 ±94,3	1406 ±160,7	1,55±0,19[b]	3,74±1,755
24 h	5480,3 ±472[a]	3480,2 ±708,4[b]	8960,5 ±612,6[b]	1,68±0,15 [a]	3,36±2,03
48 h	10496 ±2968,4[b]	4415,2 ±1266,3[b]	14911,2 ±3167,2[b]	1,53±0,21[b]	3,23±0,89
72 h	6288,5 ±1362,5[b]	1635,2 ±514,5	7923,7 ±1826,8[b]	1,24±0,04[b]	4,72±0,71 [a]
96 h	3339,7 ±851,8	801,7 ±220,6	4141,8 ±1060,1	1,23±0,03[b]	4,86±0,65 [a]

Similar dynamics were observed in changes in pancreatic amylase levels (Table 1 and Figure 1). Output values were 273 ± 30 U/L at -72nd hour and 307 ± 26 U/L at 0 hour. Statistically significant increase ($p < 0.05$) was recorded at the 24th hour, after which the increase was continued ($p<0.01$) at 48th and 72nd hours. The highest average (10496 ± 2968 U/L) was reported again at 48th hour after the start of the experiment - almost thirty-five times more than the average value of PA recorded at 0 hour.

The baseline values of extra pancreatic amylase (Table 1 and Figure 1) were 381 ± 79 U/L at -72nd hour and 509 ± 60 U/L at 0 hour. A statistically significant

increase ($p<0.01$) was found at 24th and 48th hours. Here, the maximum values (4415 ± 1266 U/L) were recorded again on the 48th hour since the beginning of the experiment, but immediately after that the levels started to decrease and on the 96th hour they approach the initial - 802 ± 221 U/L. By the EPA, the rising of the initial levels during the experiment was less than 10 times.

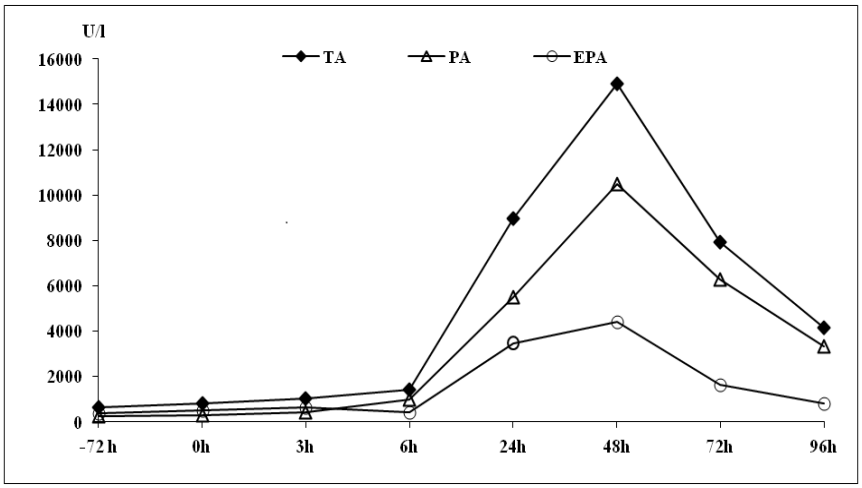

Figure 1. Plasma levels of Total Amylase (TA), Pancreatic Amylase (PA), and Extra Pancreatic Amylase (EPA) (U/L), according the time, in dogs with experimentally induced acute pancreatitis via pancreatic channel ligatures.

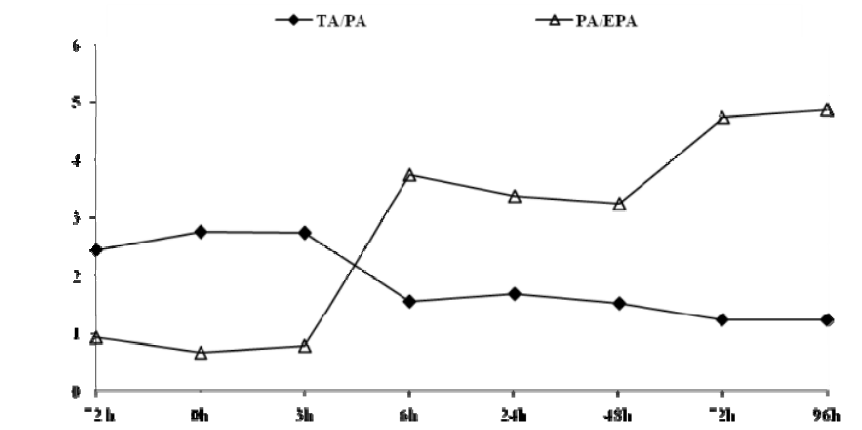

Figure 2. Total amylase/pancreatic amylase ratio (TA/PA) and pancreatic amylase/extra pancreatic amylase ratio (PA/EPA), according the time, in dogs with experimentally induced acute pancreatitis via pancreatic channel ligatures.

Prior to the start of the trial, the ratio of total amylase and pancreatic amylase was 2.45 ± 0.26 and 2.76 ± 0.34 in favor of total amylase, respectively at -72nd and 0 hours. From the 6th hour to the end of the experimental staging, we observed a steady, statistically significant decrease in this ratio, with the lowest being average value on the 96th hour - 1.23 ± 0.03 (Table 1 and Figure 2).

In the pancreatic/extra pancreatic amylase ratio we observed a reverse trend. The values here started from 0.92 ± 0.27 at -72nd hour and 0.67 ± 0.12 at 0 hour, to reach a maximum of 4.86 ± 0.65 at 96th hour. The increase was noted throughout the experiment, but a statistically significant deviation ($p < 0.05$) from the baseline was noted at 72nd and 96th hours (Table 1 and Figure 2).

Discussion

Under the term acute pancreatitis are understood various, acutely occurring morphological changes in the pancreas consolidated only on the basis of a single mark - the severity of their occurrence and development. The course of experimental pancreatitis (induced by ligation of the pancreatic ducts) resembles that of spontaneous, which is caused by obstruction due to cholelithiasis, intestinal parasites, foreign bodies, inflammation or duodenal hyperplasia. The clinical manifestation is highly dependent on changes in the gland and the involvement of other organs and systems in the inflammatory process. Diagnosis is most often based on elevated levels of pancreatic enzymes in the blood (Ruaux, 2003; Steiner, 2003; Mix and Jones, 2006). Their determination is mandatory in patients with suspected acute pancreatitis, with an unclear origin of abdominal complaints and those with unspecified pain localization.

Increased levels of amylase and lipase, at least twice the upper limit of normal, place the laboratory diagnosis of acute pancreatitis. Amylase level is more susceptible if the study is done soon after the onset of symptoms (Estourgie et al., 93, Smotkin, 2002). The values begin to rise between the 2nd and the 12th hour, with a peak around the 48th hour. Because of their short half-life, however, they can be normalized until the fifth day after the elevation (Smotkin, 2002). The results obtained by us fully support this claim, as at the 96th hour from the start of the experiment the mean values of total amylase and amylase isoforms were times lower than the maximum.

Similar statements have been made by other authors (Estourgie et al., 93), which note that serum amylase reaches its maximum at 48th hour and after 7 days restores its initial level. In our experiment, the maximum individual amylase value (28 326 U/L) was recorded exactly at 48th hour post surgical intervention. At the same time, the same individual had a pancreatic amylase value of 24 600 U/L. This means that the ratio of TA/PA at this time is 1.15 and the PA/EPA ratio is 6.54, at mean baseline values of 0.67 at 0 hour. These values

unambiguously show that amylase isoforms can be determined to increase specificity of amylase measurement.

A similar assertion is also expressed by other authors (Agarwal, 1990; Watson, 2004, Mix and Jones, 2006). In their view, the use of P-isoamylase is most appropriate for confirming that the increase in the total amylase level arises from the pancreas, although P-isoamylase levels may increase in the absence of acute pancreatitis (Bunch, 2003, Ruaux, 2003; 2003; Watson, 2004). Causes of this may be diseases of the bile ducts, small intestine perforation, intestinal obstruction or ischemia.

On the other hand, many conditions other than AP are associated with an increase in amylase levels: abdominal inflammatory processes, mumps, macro-amylasemia, kidney failure, and others (Mansfield and Jones, 2001), which is a prerequisite for determining the different isoforms and the correlation between them.

The parallel changes in the values of total amylase and p-amylase accepted by many scientists (Mia, 1978; Brobst, 1970; Attix, 1981; Strombeck, 1981; Edwards, 1990) as an essential criterion for the diagnosis of acute pancreatitis, were also found in our experiment.

Conclusion

Our results give us reason to believe that the level of amylase is a good diagnostic test for both diagnosis and follow-up of acute pancreatitis. However, when diagnosing elevated levels of total amylase, differentiation should always be made with hyperamylasemia of other origins and the values of this laboratory indicator should be interpreted along with the values of pancreatic amylase.

Determination of the ratio between total amylase and different amylase isoforms gives a clearer picture of the origin of amylase, and would be useful for the diagnosis of pancreatic diseases.

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Effects of chlorantraniliprole on antioxidative defense system in European Corn Borer (*Ostrinia nubilalis*)

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Abstract: The European Corn Borer (*Ostrinia nubilalis)* is one of the most damaging corn insect pests and it has adetrimental influence on corn production. This work examines the effects of insecticide chlorantraniliprole on the antioxidative defense system of *Ostrinia nubilalis* larvae from Rimski Šancevi (near Novi Sad, Serbia). The experiment setup consisted of a completely randomized block design with 4 replicates. Two experimental groups were formed: control group (C) and T (chlorantraniliprole – 0,1ml ha^{-1}). Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application, after which the homogenates of whole larvae were made. Ultimately, determination of the lipid peroxides (LPO), reduced glutathione (GSH) and oxidased glutathione (GSSG) concentration was performed. By comparing experimental groups, it was found that chlorantraniliprole significantly increases concentration of lipid peroxides in the treated groups compared to the control, while the concentration of oxidized and reduced glutathione did not change significantly. Increased lipid peroxidation suggests that chlorantraniliprole significantly affects the damage of the cell membrane and increase oxidative stress in larvae of *Ostrinia nubilalis* and thus reduce the abundance of this pest.

Key words: chlorantraniliprole, *Ostrinia nubilalis*, oxidative stress, glutathione, lipid peroxides

Introduction

Genus *Ostrinia* has over 20 species. Four of those are marked as pest insects species, which cause damage in maize production. *Ostrinia nubilalis* is polyphagous species, the most important species of this genus, lives in over 200 different plant species. *Ostrinia nubialis* has about six generations, depending on

conditions, while in our area usually it has two or three generations. The female lays about 15-45 eggs clustered on the underside of the leaf. After hatching the larvae feeds the surface part of the leaf. A few days later, after the initial feeding, the larvae bore the stem or cob of the corn. At the end of July, larvae converts into pupas, and after that into butterflies that give generation of caterpillars that will survive the winter. They are very resistant to low temperatures, and a large number of them survive the winter. Also, a very important characteristic of this pest is exerted sexual dimorphism, where females are larger, brighter in color with a wingspan of up to 35 mm, while males are darker with a range of up to 30 mm. Thus, the direct damage caused by *Ostrinia* is the reduction in yield per cob of corn from 3 to 11% (Popović et al., 2015). In addition to reducing the impact on productivity, these pests lead to breaking plants, declining of cob ahead of time. Larval damages constitutes "open windows" for the development of fungal infections and mycosis (Kojić, 2009). In our country, the suppression of *Ostrinia* registered the following insecticides: Coragen 20-SC, Avaunt 15 EC, Ampligo 150 ZC and Talstar 10 EC. In this experiment we investigate effects of chlorantraniliprole (trade name: Ampligo®, Syngenta, Serbia) on larval antioxidative defense system (concentration of lipid peroxides, oxidated and reduced glutathione). Ampligo is an anthranilic diamide insecticide that binds with ryanodine receptors, resulting in the unregulated release of calcium from insect muscle cells and leading to the cessation of feeding, lethargy, muscle, paralysis, and death (Cao et al., 2017). The aim of this study is to prove whether chlorantraniliprole has significant influence on the increase in the concentration of lipid peroxides in the treated group compared to the control, and whether it is an altered concentration of the oxidized and reduced glutathione.

Materials and Methods

Location description

The experiment was carried out in Rimski Šančevi, near Novi Sad, Serbia (45°19'47.72"N, 19°51'1.95"E, altitude 78 m a.s.l.) during 2016. The experiment setup consisted of a completely randomized block design with 4 replicates and was based according to EPPO guidelines (nr. PP 1/13(3)). Each plot consisted of 4 rows of maize, separated from other plots with one untreated row on each side. The length of each plot was 10 m, with a spacing of 2 m between blocks. The control plot wasn't sprinkled nor treated with insecticides and fungicides, whereas treated plot was sprinkled with chlorantraniliprole (Apmligo®, Sungenta, Serbia, formulation: suspension concentrate (SC)) in a concentration of 0,1ml ha^{-1}. The insecticide application was performed during the peak flight of the European Corn Borer, using a backpack sprayer unit with

high clearance attachment with 4 nozzle boom (model 315-HCB-4) from Bellspray Inc dba R&D Sprayers. The working height of the sprayer is manually adjustable (0,6 – 4,2 m) and the spray volume is 400 l ha^{-1} at a pressure of 200 kPa with an operation speed of 4-6 Km h^{-1}. Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application. Larvae were euthanized immediately in liquid nitrogen for further analyses.

Preparation of homogenates and measuring protein concentration in samples

Values of antioxidative defense components were determined from homogenates of whole larvae. Nakon eutanazije, larvae (100 mg of fresh larval mass) were homogenized using a homogenizer (IKA-Werke) in 2mL of sucrose buffer (0.25M sucrose, 0.05Mtris-HCl, 1mM ethylenediaminetetraacetic acid; pH 7.4) on ice (3 cycles of 10 s at 2000 rpm). Homogenates were then sonicated for 3 cycles 15 s (using a Bandelin sonoplus HD2070 instrument) and centrifuged on an ultracentrifuge (Beckman L7–55) at 105 000 g and 4 8C. The supernatant was extracted and frozen at –24 ºC until further experiments.

Determination of antioxidative defense activity

The determination of lipid peroxides (LPO) concentration was based on the reaction of lipid peroxidation products (malondialdehydes) with TBA (thiobarbituric acid reactive substances—TBARS) (Ohkawa et al., 1979) and was expressed in nmol/mL extract. The concentration of reduced glutathione (GSH) was determined based on GSH oxidation by 5.5-dithio-bis-6.2-nitrobenzoic acid (Beutler, 1975) and was expressed in μmol/mL extract. The concentration of oxidized glutathione (GSSG) was determined enzymatically by glutathione reductase (Beutler, 1975) after inhibition of GSH oxidation by 4-vinylpiridine and was expressed in nmol/mL extract. Concentration of LPO was determined by spectrophotometric (UV-VIS Spectrophotometer Shimadzu UV 1800). Reduced and oxidized glutathione concentrations were determined by ELISA microplate reader (Microplate reader, RT-6100; Rayto, China).

Statistical analysis

Results were processed using the SAS Ver 9.1.3 statistical package. The prerequisite for analysis of variance was the normality of distribution within a group achieved through logarithmic transformations of the traits (Sokal and Rohlf, 1981). Differences in the average values between control and treated group were assessed by one-way ANOVA.

Results and Discusion

Oxidative stress is defined as a disruption of the prooxidant antioxidant balance in favor of the former, leading to potential damage (Sial and Brunner, 2012). In this experiment, we followed the concentration of lipid peroxide, glutathione, as part of the antioxidant protection, as well as the concentration of glutathione in its reduced (GSH) and oxidased form (GSSG). Glutathione is a tripeptide which is found in cells in millimolar concentrations were as thiol in the reduced state, and, in smaller amounts, as a disulfide in the oxidized form. Investigation of the effects of chlorantraniliprole to the components of the oxidative protection in larvae of *Ostrinia nubilalis* it was found that under the influence of the insecticide of this significantly increased the concentration of lipid peroxides ($F= 133,28$; $p < 0.001$***) (Figure 1.), whereas the concentration of reduced glutathione (Figure 2.) ($F= 3,09$; $p>0,05$ ns – not significant) and oxidased glutathione (Figure 3.) doesn't change significantly ($F=1,67$; $p>0,05$ ns – not significant). Rodrigues et. al. (2015), investigating effect of chlorantraniliprole on Chironomid larva, sugesting that larvae were not under oxidative stress, since short exposures to chlorantraniprole did not affect LPO levels, despite the significant inhibition of glutathione S transferase and catalase. It seems that detoxification of reactive intermediates and ROS is still achieved due to glutathione consumption, since total amount of glutathione (GSH) were significantly decreased in organisms exposed to chlorantraniliprole. Moreover, it was observed that CEA was disturbed due to increased activity of the electron transport system (ETS), suggesting extra energy expenditure in larvae.

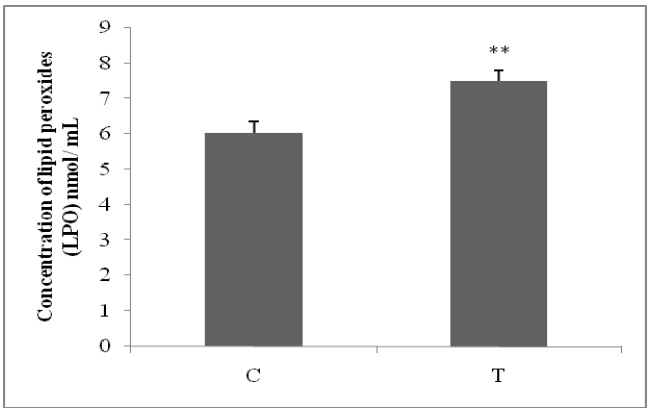

Figure 1. Concentration of lipid peroxides (LPO) in *Ostrinia nubilalis* larvae in control (C) and group treated with chlorantraniliprole (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance ($F=133,28$; $p<0,001$***).

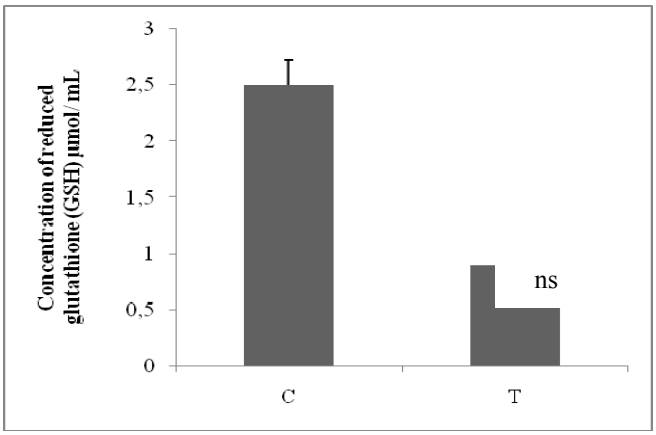

Figure 2. Concentration of reduced glutathione (GSH) in *Ostrinia nubilalis* larvae in control (C) and group treated with chlorantraniprole (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=1,67; ns – not significant).

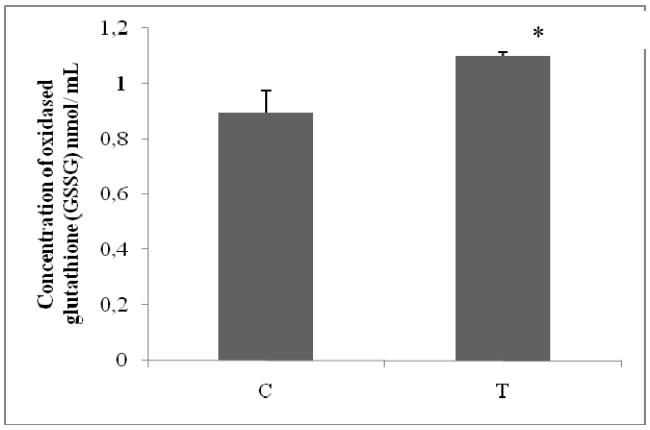

Figure 3. Concentration of oxidased glutathione (GSSG) in *Ostrinia nubilalis* larvae in control (C) and group treated with chlorantraniliprole (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=3,09; ns – not significant).

Conclusion

Chlorantraniliprole doesn't change significantly concentrations of oxidased and reduced glutathione but significantly increase the level of lipid peroxides. So, Chlorantraniliprole indirectly increase the oxidative stress in larvae of

Ostrinia nubilialis and that makes him a very powerful insecticide weapon against pest insects

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Effects of Indoxacarb on antioxidative defense system in European Corn Borer (*Ostrinia nubilalis*)

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Abstract: The European Corn Borer (*Ostrinia nubilalis)* is one of the most damaging corn insect pest and it has detrimental influence on corn production. This work examines the effects of insecticide indoxacarb on the antioxidative defense system of *Ostrinia nubilalis* larvae from Rimski Šancevi (near Novi Sad, Serbia). The experiment setup consisted of a completely randomized block design with 4 replicates. Two experimental groups were formed: control group (C) and T (indoxacarb – 0,25 ml ha^{-1}). Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application, after which the homogenates of whole larvae were made. Ultimately, determination of the lipid peroxides (LPO), reduced glutathione (GSH) and oxidased glutathione (GSSG) concentration was performed. By comparing the experimental group it was found that indoxcarb significant influence on the increase in the concentration of lipid peroxide and oxidized glutathione whereas the concentration of reduced glutathione decreases significantly. The results suggest that significant changes in the components of the antioxidant protection system indicator successfully reduced performance of these insects by applying insecticides indoxcarb ant its effectiveness in combating these corn pest.

Key words: indoxcarb, *Ostrinia nubilalis*, oxidative stress, glutathione, lipid peroxides

Introduction

Indoxacarb is a proinsecticide. It acts by inhibiting sodium ion entry into the nerve cells, resulting in paralysis and in death of target insects (Wing et al., 1998). Indoxacarb is an oxadiazine that makes treated insects stop feeding and go into mild covulsions or permanent paralysis (Wing et al., 2000). It is effective

against several lepidopteran pests, like *Ostrinia nubilalis*, also known as European maize borer. *Ostrinia nubilalis* is one of the most widely studied agricultural pests in the world. *O. nubilalis* is a very important corn pest in our country. It belongs to a group of polyphagous insects, but its main host plants are corn and peppers. Outbreaks of pests, abundance of population and the number of generations depend on weather conditions, corn hybrid and of agrotechnical measures. In our conditions, *O. nubilalis* has two generations, or if the weather conditions are favorable, it can have three generations (Kojić, 2009). Corn borer reduces the yields of corn from 2 to 25% (Popović et al., 2015). The aim of the experiment is to demonstrate the toxicity of indoxcarb, in other words – the effectiveness of insecticides in controlling pests. Presence of Indoxcarb leads to an increase of prooxidant substances which disturb the homeostasis of the redox status in cells, damage the nucleic acids, proteins, and cell membranes. Thus, due to the presence of toxic substances there is an increase of oxidative stress of treated organism *O.nubilalis*. In response to oxidative stress a level of components of antioxidative protection system rises. By tracking the enzymes, which play a role in antioxidant protection, in this experiment we investigated the mechanism of acting indoxcarb on corn pest Ostrinia nubilalis.

Materials and Methods

Location description

The experiment was carried out in Rimski Šančevi, near Novi Sad, Serbia (45°19'47.72"N, 19°51'1.95"E, altitude 78 m a.s.l.) during 2016. The experiment setup consisted of a completely randomized block design with 4 replicates and was based according to EPPO guidelines (nr. PP 1/13(3)). Each plot consisted of 4 rows of maize, separated from other plots with one untreated row on each side. The length of each plot was 10 m, with a spacing of 2 m between the blocks. The control plot was not sprinkled nor treated with insecticides and fungicides, whereas treated plot was sprinkled with indoxicarb (Avaunt®, DuPont, Serbia, formulation: emulsifiable concentrate (EC)) in a concentration of 0,25 ml ha^{-1}. The insecticide application was performed during the peak flight of the European Corn Borer, using a backpack sprayer unit with high clearance attachment with 4 nozzle boom (model 315-HCB-4) from Bellspray Inc dba R&D Sprayers. The working height of the sprayer is manually adjustable (0,6 – 4,2 m) and the spray volume is 400 l ha^{-1} at a pressure of 200 kPa with an operation speed of 4-6 Km h^{-1}. Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application. Larvae were euthanized immediately in liquid nitrogen for further analyses.

Preparation of homogenates and measuring protein concentration in samples

Values of antioxidative defense components were determined from homogenates of whole larvae. After being euthanized, the larvae (100 mg of fresh larval mass) were homogenized using a homogenizer (IKA-Werke) in 2mL of sucrose buffer (0.25M sucrose, 0.05Mtris-HCl, 1mM ethylenediaminetetraacetic acid; pH 7.4) on ice (3 cycles of 10 s at 2000 rpm). Homogenates were then sonicated for 3 cycles 15 s (using a Bandelin sonoplus HD2070 instrument) and centrifuged on an ultracentrifuge (Beckman L7–55) at 105 000 g and 4 ºC. The supernatant was extracted and frozen at –24 ºC until further experiments.

Determination of antioxidative defense activity

The determination of lipid peroxides (LPO) concentration was based on the reaction of lipid peroxidation products (malondialdehydes) with TBA (thiobarbituric acid reactive substances—TBARS) (Ohkawa et al., 1979) and was expressed in nmol/mL extract. The concentration of reduced glutathione (GSH) was determined based on GSH oxidation by 5.5-dithio-bis-6.2-nitrobenzoic acid (Beutler, 1975) and was expressed in μmol/mL extract. The concentration of oxidized glutathione (GSSG) was determined enzymatically by glutathione reductase (Beutler, 1975) after inhibition of GSH oxidation by 4-vinylpiridine and was expressed in nmol/mL extract. Concentration of LPO was determined by spectrophotometric (UV-VIS Spectrophotometer Shimadzu UV 1800). Reduced and oxidized glutathione concentrations were determined by ELISA microplate reader (Microplate reader, RT-6100; Rayto, China).

Statistical analysis

Results were processed using the SAS Ver 9.1.3 statistical package. The prerequisite for analysis of variance was the normality of distribution within a group achieved through logarithmic transformations of the traits (Sokal and Rohlf, 1981). Differences in the average values between control group and treated group were assessed by one-way ANOVA.

Results and Discussion

The system includes a non-enzymatic antioxidant protection, and enzymatic components. Glutathione, as one of the non-enzymatic components has an important role in maintaining the redox balance, glutathione protects cells from hydrogen peroxide and organic hydroperoxide, an organic radical, peroxy radical, and hydroxyl peroxide. There are two of these forms: a reduced (GSH) and oxidized (GSSG). Glutathione reductase catalyses the reduction reaction of oxidized glutathione into reduced glutathione, with the participation of NADPH

as a reducing cofactor. Oxidized glutathione goes back into its reduced form of glutathione reductase operation with the help of NADPH as an electron donor. In this manner, rounding of the cycle prevents wear and glutathione ascorbate, and the flow of electrons going from the NADPH to hydrogen peroxide. Investigation of the effects on the components of oxidizing indoxcarb protection code Ostrinia nubilalis larvae were established under that influence of the insecticide. Indoxcarb significantly increases the concentration of lipid peroxide (Figure 1.) (F=8.97; p<0,01**) and concentration of oxidized glutathione (Figure 2.) (F=9,73; p<0.01**). Concentration of reduced glutathione (Figure 3.) in treated group decrease significantly in contrast to control group (F=6,20; p<0,05*). Tulasigiriyappa et al. (2012) experimentally proven impact indoxicarb to changes in the antioxidant protection system in the albino mice. Results of the present study on antioxidants, oxidative stress byproducts and oxidative stress enzymes of the liver in mice revealed that the mice treated with 6 and 12 mg indoxacarb showed no significant change in the level of antioxidant, oxidative stress byproducts and oxidative stress enzymes. Whereas, treatment with 18 and 24 mg indoxacarb caused significant decrease in the level of GSH and ascorbic acid and significant increase in the level of TBARS and glutathione stransferase enzymes when compared with that of the control mice.

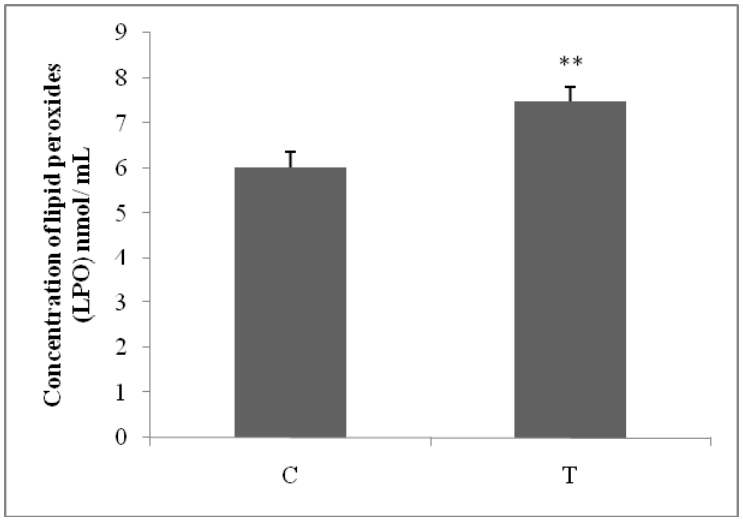

Figure 1. Concentration of lipid peroxides (LPO) in *Ostrinia nubilalis* larvae in control group (C) and group treated with indoxcarb (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=8,97; p<0,01**)

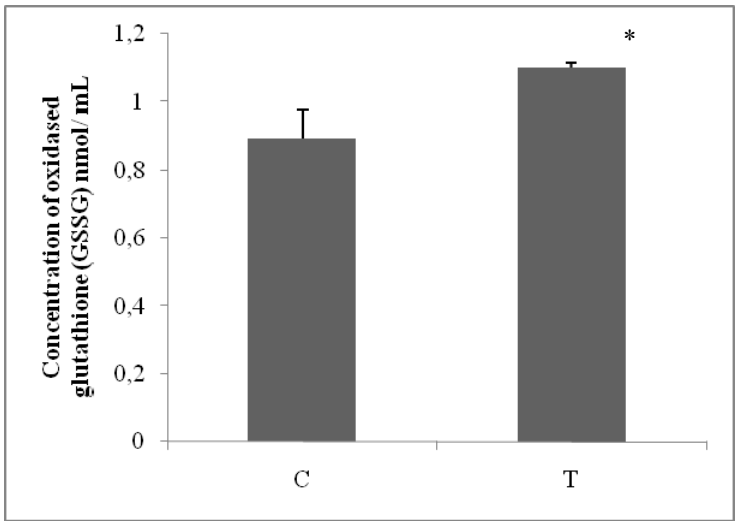

Figure 2. Concentration of oxidazed glutathione (GSSG) in *Ostrinia nubilalis* larvae in control gropup (C) and group treated with indoxcarb (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=9,73; p<0,01**)

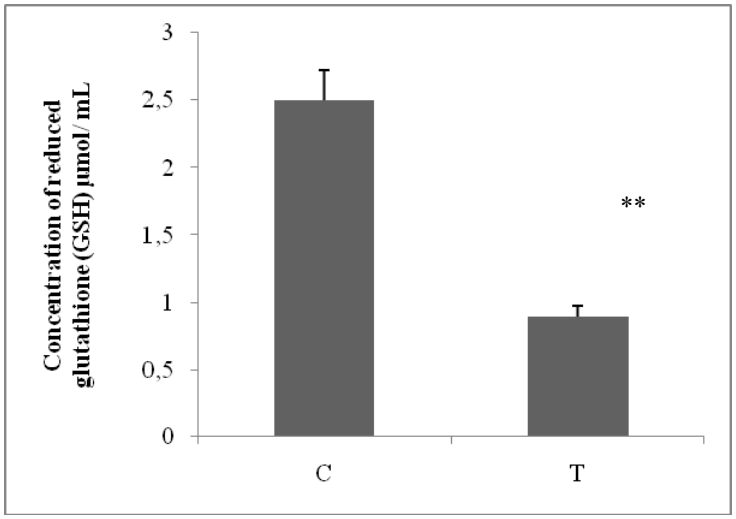

Figure 3. Concentration of reduced glutathione (GSH) in *Ostrinia nubilalis* larvae in control group (C) and group treated with indoxcarb (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=6,20; p<0,05*)

Conclusion

Indoxcar significantly increased levels of lipid peroxidation and oxidative stress, which is reflected in the increase of concentration of lipid peroxide and oxidized glutathione whereas the concentration of reduced glutathione decreased significantly. A significant oxidative stress indicates indoxcarb important role in protecting corn from invasion of the pest.

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Implementation of Innovations in Agribusiness Companies and their Impact on Marketing Efficiency

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Abstract: This paper analyzes thetendency to develop innovative competences in relation to business and especially marketing efficiency of agricultural businesses. Through the paper the terms of innovation, marketing and marketing efficiency are defined. The paper also lists the different classifications of innovation and the detailed process of innovation adoption process with essential factors in the implementation of innovations. Paper focuses on the impact of innovation on agribusiness enterprises with an emphasis on enterprise performance and sustainability and challenges in agricultural businesses. Part of the paper was the study on 75 randomly selected representatives of agribusiness companies from Republic of Macedonia where their innovation competences related to the marketing function were examined. Paper showed that implementation of innovative competencies favors business performance and competitiveness.

Key words: innovations, marketing efficiency, business performance

Introduction

Inovations are considered as a key factor in growth and the development of the agrobusines and represent the main driving force of the economy in many countries. So, given the high competition in business, agribusiness enterprise is forced to invest in developing and introducing innovations if that company wants to be successful. Today, agriculture in the world is not perceived as an attractive type of business, but on the other hand, demand for food is increasing day by day. Accordingly, the agro food sector should strive for new opportunities through the development and application of technological innovations.

In Republic of Macedonia there are major problems in the agro-industry, but despite these problems there is still place for affectin the agro-food sector. Macedonian producers of food products have good natural conditions for developing agro-industrial production, as well as experience and tradition. By producing quality products this sector can achieve significantly effective and competitive production from the existing Agrifood Industry, and the production of quality products and developing agribusiness can only come through development and learning. On the other hand, rural society is torn between tradition and innovation, and for fear of investment and lack of motivation the innovation incentives does not apply to the extent that is possible.

Research objectives

The purpose of this paper is to empirically analyze the orientation towards the development of innovation competence in terms of business and in particular the marketing efficiency of agribusiness companies. The aim of the research is to show how enterprises in the field of agribusiness can by focusing on developing innovative skills to influence their own business and marketing efficiency. Moreover, the aim of this paper is through theoretical and practical knowledge to show how the implementation of innovation affects the business and competitiveness of agribusiness companies.

Hypotheses of the research

H1 innovative agribusiness companies develop greater environmental and social performance.

H2 propensity to innovation enables the achievement of business goals and marketing in agribusiness enterprises.

H3 innovative agribusiness enterprises tend to encourage investments in the development and education.

Material and methods

Based on the research literature on innovation and innovation in marketing, marketing efficiency, marketing of SMEs has been maid selection of enterprises that represent a series of new particles for the proposed variables. The empirical research was conducted using the method of questionnaire investigation. The survey was conducted on a random sample that comprises of enterprises specializing in agro-food sector of the country. The research involved 75 randomly selected representatives of agribusiness enterprises. In order to collect data about the underlying ideas, opinions of respondent's innovation competencies and marketing efficiency of agribusiness enterprises it has been used a questionnaire consisting of 32 questions. The level of agreement with the

given claims has been evaluated on Likert scale of 1 to 5 levels where 1 means - it does not matter, 2 - somewhat important 3 - Important (average), 4 - important, 5 - extremely important.

For statistical analysis of the collected data is used the program package Statistics 7.1. For the purposes of analysis and data processing were used methods of descriptive statistics which calculated an arithmetic average and standard deviation for each particle (matter) so it can be determined the homogeneity / heterogeneity of respondents. Reliability and internal consistency of certain scales measured with Cronbach alpha coefficient that determined whether their measure was true. In correlation analysis it has been verified the degree of linear dependence between variables, and then was made a simple regression analysis that determined the statistical significance of the impact of certain independent variables on the marketing efficiency of agribusiness enterprises.

Results and Discussion

The first part of the survey clearly shows that in the sample are significantly more prevalent the male representatives with 51 representative (68%), compared to 24 women (32%). The highest percentage of respondents are at the age of 40-49 years (32%) and the least are older than 50 years (16%). In the educational structure prevalent is high vocational education with 38 respondents (51%), followed by higher vocational education (Bachelor) in 28 respondents (37%) and Masters - 9 respondents (12%). Most of them are employed in 67 agribusiness enterprises with fewer than 10 employees (90%), in whicj prevale family agribusinesses in the sample were 47 (63%) (Table 1).

At the outset of the data processing it has been needed to determine the reliability of the variable or the measuring scales, and all this in order to determine their reliability. Reliability of measurement scales (variable) is measured using the coefficient Cronbach's Alpha. Cronbach's Alpha coefficient is a measure of the internal consistency of the set of claims, and may take a value between 0 and 1. The closer the value is to 1, the more reliable the measuring scale is. While it is desirable that the values of the coefficient are greater than 0,7 (Nunnally, Brenstein, 1994),when the values are about 0.6 it can be considered acceptable (Robinson et. All. 1991).

It has been established the reliability of the dependent variable - marketing efficiency, and three independent variables - methods, tools and standards of production; new products and new markets; research, development and education that are make up the structure of marketing innovation. This means that among the aforementioned variables exists connection so it can be said that they do measure the same phenomenon, and that it is realistic to expect

measuring the impact of independent variables on the dependent. In other variables that were not shown as reliable (Cronbach's Alpha <0,60) it is not possible to establish their mutual influence, ie independent and uncertain variables have no effect on the dependent (secure) variable and it is not possible to be involved in further research (Table 2).

Table 1. Description of the sample (n = 75)

Demographic characteristisc	***Frequency***	***%***
Gender		
Male	51	68
Female	24	32
Age		
< 30	21	26
30 – 39	18	24
40 – 49	24	32
> 50	12	16
Education		
High vocation education	38	51
Magister (Bachelor) Profeission	9	12
Higer vocation education	28	37
Size of the company		
< 10	67	90
10 – 49	7	9
50 – 249	1	1
Type of the company		
Family agribusiness	47	63
Trade	21	28
Trade Company	6	9

Source: Reasrch of the author

Because of the low reliability of further processing it had been eliminated two uncertain variables. These are IT technologies and presence on the Internet (alpha = 0,47) certification, labeling and design (alpha = 0, 574). They because of their values for Cronbach's Alpha coefficient are uncertain and can not be included in further analyzes.

By applying descriptive statistics were calculated average values and measures of variability (standard deviation) for all tested variables, ie their individual particles. Most favorable opinion of the respondents is in evaluating improvements to create new consumers where it is determined average of 3, 97 (Table 3).

Table 2. Coefficient Cronbach's Alpha

Variables	***Cronbach's Alpha***
Methods, tools and production standards	0,71
New products and new markets	0,74
IT technologies and online presence	0,47
Certification, labeling and design	0,49
Research, development and education	0,61
Marketing efficiency	0,90

Source : Reasrch of the author

Table 3. Values of arithmetic average and standard deviation for all particles of a certain variables

Particles	***Arithmetic average (M)***	***Standard Deviation (SD)***
New methods and tools	3,52	0,68
Innovative environmentally friendly technologies and processes	3,79	0,70
Standard for management of the relationships with customers	3,45	0,89
Improving the production and / or distribution	3,53	0,66
New product / services in the market	3,51	0,91
Practice for encouraging the entry of new markets	3,31	0,73
Investing in the development of research development projects	3,57	0,64
Educating employees	3,44	0,92
Credibility of the company	3,59	0,64
Image of enterprise	3,89	0,67
Differentiation	3,57	0,64
Creation of new customers	3,66	0,69
Satisfaction and consumer confidence	3,97	0,74
Positioning	3,66	0,80
New product	3,63	0,88

Source: Research of the author

The values of the smallest standard deviation (0.64) suggest greatest homogeneity, with the highest agreement of respondents with respect to that conclusion. Worst rated are the new products and / or services. From the standard deviation (0.92) is more visible disagreement or heterogeneity among respondents on the issue of investment in the development of research and development projects. To determine the importance of the variables or the connection between the examined variables it has been made a correlation analysis (Table 4). The table checks the level of dependence between three variables models, ie the level of dependence between two features. For the

purpose of this research is used Pearsons coefficient of linear correlation (r) and it has been used a two-way testing (English. 2- tailed)for the significance of the correlation. The value of the Pearsos coefficient from 0.6 to 0.8 refers to the existence of a strong secondary correlation. Values of 0.9 to 1 represent a strong correlation, while 1 represents the complete correlation. Values between 0 and 0.5 represent weak correlation. Values between 0 and 0.5 represent weak correlation. The results of correlation analysis showed that statistically significant correlates of marketing efficiency of agribusiness enterprises represent the following variables :methods, tools and standards of production (r = 0,536; p <0,01) research, development and education (r = 0,588; p <0 01) where it has been determined a strong correlation. Although with its existence with a weak level of correlation not less important is the correlate of the variable - new products and new markets (r = 0,432, p <0,01).

Table 4. Correlation of changing marketing innovation and their perception of the impact of marketing effectiveness on agorbiznis enterprises

	Perceived impact of marketing efficiency in angrobiznis enterprises (dependent variable)
Methods, tools and production standards	r = 0,536 p = 0,000 (statistically significant correlation, p<0,01)
New products and new markets	r = 0,432 p = 0,008 (statistically significant correlation)
Research, development and education	r = 0,588 p = 0,004 (statistically significant correlation)

Source: Research of the author

Moreover, it can be determined that the methods, tools and standards of production and research, development and education represents those factors in this research that are in the stronges conection with the marketing efficiency of agribusiness enterprises. Not so much as earlier mentioned two factors, but still relatively important factor is the factor new products and new markets.

To determine which variables have a significant impact on the marketing performance of agribusiness enterprises it has been useed a simple regression (Table 5). It has been tested the individual influence by independent variables (methods, tools and standards of production, new products and new markets, research, development and education) of the marketing efficiency on the argobisines enterprises, which is considered as a dependent variable.

Table 5. Model simple linear regresion (dependent variable: marketing efficiency of agribusiness enterprises)

	β	t	CE	Sig.	R^2
Methods, tools and standards of manufacture	0,536	28,847	0,21	0,0001	0,287
New products and new markets	0,432	29,496	0,22	0,0001	0,187
Research, development and training	0,588	37,730	0,15	0,0001	0,346

$p < 0,05$; *Source: Research of the author*

The results derived from the model of simple linear regression showed that three independent variables are significant impact on the marketing efficiency of agribusiness enterprises. The most significant impact is expressed through the variations in the investments in the research, development and training of employees ($\beta = 0, 588$, $t = 37,730$, $p < 0,05$) and variable methods, tools and standard production ($\beta = 0,536$, $t = 28,847$, $p < 0.05$). The investigation of the dependence of one phenomena from three independent phenomena (variables) that is relatively independent influence on the independed on the dependent variable. Also,it has been determined the existence of linear dependence between variables.

A smaller impact on the marketing performance of agribusiness enterprises variable, has the variable new products and new markets ($\beta = 0,432$, $t = 29,496$, $p < 0,05$)

Conclusion

The application of new ideas, knowledge or practice in the particular context in order to create positive change is called innovation.The innovation had always been necessary for competitiveness and economic growth and success of the companies in the field of agribusiness. There are different classifications of innovation, and between different types of innovation in agribusiness are distinguished the following: organizational, technological and social. But it is not enough to only develop the innovations in the company feor the eficience performance of the company they should be adopted and implemented. Given that innovation are bringing something new there is some resistance and fear because the adoption period often takes considerable time while innovation is developed, available in the market and the time when the same will be used. As the small and medium agribusinesses are facing greater competition they have to as soon as possible to meet the challenges of the market and react to new innovations.

A key reason for the innovation and application of innovation in agribusiness is the desire to increase their business performance and market competitiveness acomplishing certain business and marketing purposes. The research in this paper shows how agribusiness enterprises through innovative practice can affect their own business and marketing efficiency.

As for the variables that were proved as unreliable with Cronbach alfa coefficient - IT technology and application of the Internet (alpha = 0,47) and certifications, labeling and design (alpha = 0,574) they can not be taken into further processing because they were under Cronbach alfa <0,60 and it is not acceptable and they can not be considered in further processing.

In changing variables - methods, tools and production standards it is observed that the results of correlation analysis are in average strong corelation high (r = 0,536; p <0,01), and that the outcome of ednostvano Linari correlation shows a relatively strong impact on marketing efficiency agribusiness enterprises (ß = 0,536, t = 28,847, p <0,05), therefore it is possible to accept the hypothesis that says:

H1 innovative agribusiness companies develop greater environmental and social performance.

That means that the agribusiness enterprises apply methods and tools because of innovative environmentally friendly technologies and processes, both in production and administration, and use standards for the management of customer relations that take into account social and environmental aspects.

When it comes to variable - New products and new markets, we can talk about the importance of this correlate to the marketing efficiency of agribusiness enterprises (r = 0,432, p <0,01), as well as their impact on the marketing efficiency of agribusiness enterprises (ß = 0,432, t = 29,496, p <0,05) and that alowes to be accepted the hypothesis:

H2 inclination towards innovation allows achieving of business and marketing purposes in agribusiness enterprises.

Since it is likely that agribusiness companies are working to improve the production and / or distribution primarily through placing new products / services to market in a way to encourage innovative new forms of creating new segments and / or penetration of new markets.

The research shows that the third independet variable - Research, development and education was defined (medium) strong correlation (r = 0,588; p <0,01) indicating a good correlation with the dependent variable. But the simple linear regression shows the greatest significance of this variable (ß = 0,588, t = 37,730, p <0,05), so it is possible to accept the third hypothesis:

H3 innovative agribusiness enterprises are aimed at encouraging investment in the development and education.

Because agribusiness enterprises surveyed stick to investing in development research development projects and education of employees.

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Improving plant

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Abstract: The improvement of plants through selection and crossbreeding is a basic view of the development of modern agriculture. Improving plant happened again with the earliest domestication. Developments in the last century in the field of cross-plant combined with those in agriculture, plant physiology, molecular biology and genetics have contributed to the technical achievements that led to unprecedented advances in yield in all major plants. Scientists estimate that about half of the benefits the yield of the crop are a result of genetic improvement. The selection of plants is an important part of successful farming in the United States. The research of plants and plant provided collecting genetic material that is used to create improved species for agricultural and horticultural use. The importance of this work was recognized at the end of the 19th century.

Key words: plants, genetics, improvement, agriculture, research.

Introduction

The yield of the agricultural cultural plants depends on genotype and from environmental factors. The maximum yield of a genotype is limited genetic potential of variety and can be realized only under ideal conditions the external environment. Because these conditions are not right, the variety exercised greater or lesser percentage of svojotgenetski potential yield. To be a larger percentage, it needs to know the impact of environmental conditions on certain components of yield. Agricultural biotechnology, consisting of scientific techniques used for creating or transforming plants now used to obtain desirable properties of plants.

The plants are selected according to desired properties such as size, increased yield and resistance to pests. Significant achievements in perfecting the plant

during the 20th century are the result of the rediscovery of fundamental principles in genetics, as explained Gregor Mendel, and the understanding of inherited traits. The selection of plants is an important part of successful farming in the United States. The research of plants and plant provided collecting genetic material that is used to create improved species for agricultural and horticultural use. The importance of this work was recognized at the end of the 19th century. In 1898 the Ministry of Agriculture in the United States founded the Office for imported seeds and presenting plant in order to promote agricultural research. Since then, dedicated breeders and collectors wandering the world in search of new plants for the United States. These research often traveled in underdeveloped countries suffer from diseases, consequences animals, extreme weather conditions and by their desire to hunt plants.

Today, the National geoplasmatic system of plants in the Ministry of Agriculture of the United States still conducts targeted research plant. The ministry also keeps seeds and other plant parts of the world collections, old and new species, and herbaceous species. The collection includes 450,000 units kept in more than 20 specialized locations throughout the United States.

DNA, chromosomes and genes

To understand how to improve plant must learn basic genetics. Genetic processes taking place inside cells. Plant cells have three main constituent parts: a cell wall, nucleus and cytoplasm. Cytoplasm containing various organelles with specific important functions in plants. The core contains various organelles with specific important functions in plants. The core contains chromosomes that control the inharitance. Chromosomes are made of DNA, organized in units called genes which determine all the metabolic activities of the cell. The Hromosome contains from hundreds to thousands of genes. DNA and genes are composed of units which are called nucleotides. The string of these nucleotides dictates the sequence of amino acids during photosynthesis. [3]

Mitosis and meiosis

Genetic material is passed through two important processes: mitosis and meiosis. Mitosis is a dividing cell into two new cells that are identical to the original cell, a key process for growth and development. Meiosis is another type of cell division that reduces genetic content in half. Meiosis is associated with sexual reproduction takes place in male and female reproductive organs.[3]

Mendels genetics

His observations led to the formation of inharitance laws, which today still apply in cross plant. The laws of heredity based on predictive behavior of chromosomes and the transmission of genes from parents to offspring. They are

based on three factors: (1) genetic traits are controlled by two alleles, which can take one of two forms of homologous htomozomi; (2) when there are two different alleles, one determines the phenotype responsible for that quality; (3) during the meiosis of the chromosomes from one parent accidentally seprenese each gamete. Based on these three principles can predict the result of cross-pollination. Mendel worked on the cross which is selfed plant.

Because it is homozygous all gametes of green pea-parents wear G-allele and those of yellow peas parent carry g- allele. Their offspring are called F1-generation, when giving the first fruit of crossbreeding. In F1-generation all be heterozygous, which means they contain both forms of the allele. Because G-form of the allele is dominant, all plants of F1-generation will have a green meshumki.Isto so, if only to take account of this trait during the formation of gametes, all plants will produce two types of gametes G or g. Thus, if two individual, the F1-generation are cross would have three possible genotypes in their offspring: GG, Gg and gg. The relationship between these genotypes would be 1: 2: 1, GG, Gg and gg, subsequently, giving respect to phenotypes 3: 1 green: yellow. [4]

Selection of plants

The fact of 'similar plants "was theoretically guide the selection. Some modern plants, such as maize, very little resemble their wild predecessors because thousands of years long selection of people. As agriculture developed so farmers chose seeds from superior plants and used for further sowing. This approach resulted in the creation of "earthy varieties" or "diversity." The main goal of any program to create plants to produce superior varieties than permanent. [1]

Qualitative relations

Qualitative properties of cereals and fodder include selection on the characteristics of fermentation of starch and digestive of fodder. These features include the following:

- Forage crops like alfalfa, corn and perennial grasses are selected to increase the foliage for increasing the value of the food because the leaves are better than trunks.
- The barley was selected as sweet corn with enhanced features that affect the quality of the beer.
- Wheat was selected as grains with improved quality for bread production, such as the content of gluten and starch properties.
- Cotton was selected for Increasing quality of hair and color.[5]

Selective strategies

Once you choose the purpose of the selection program for creating plants it is necessary to determine the selection strategy. Specific strategies will depend on the method of reproduction, pollination, and the species themselves. Basic steps for most of the selection programs are: (1) collecting genetic material (2) selection of plants and the selection program: (3) hybridization of plants and re-selection of superior offspring, and (4) evaluating new material . Different aspects of the program are conducted in various areas due to the influence of certain factors, such as rainfall, temperature and soil fertility on the expression of form.[5]

Banks for seeds

US began to keep plants and seeds with interesting features in the 90s of the 19th century stations around the country. In 1958 in Fort Collins, Colorado, was built National Laboratory for storing seeds, a collection of collections. Today this institution named National Center for storage of genetic resources include germoplazma of plants, animals, microorganisms and insects. Examples of zazhuvan material include modern and older varieties of plants, wildflowers and plants. This facility also contains a large collection of important horticultural and landscape plants are grown, maintained and explore the related institutions around the country. Seeds stored in a cool, have a lifespan of 20 to 50 years, but still need to test for some time and re-seeding. The partner institutions across the country specialize in certain types of plants. The institutions include:

- Aberdeen, Idaho. National Collection of small grains is located at this location. Plants include: barley, oats, rice, rye, wheat and other cereals.
- Urbana, Illinois. This facility keeps the national collection of corn and soybeans.
- Griffin, Georgia. This location is kept a variety of plants, such as sorghum, egg (black) patlodzhan, peanuts, peppers, sweet potatoes and millet.
- Riverside, California. Keep collections US agrumski fruits vkluchuvakji oranges, grapefruit and lemons. Also keep germoplazma of dates. [2]

Table 1 presents the area we cover genetically modified plants that are grown in the US

Corn	Soybeans	Cotton	Sugar beet	Papaya	Canola
50%	97%	96%	95%	40%	90%

Collecting genetically different materials

Collecting genetic material is the first step of the selection program and consists of a collection of seeds from a wide range of resources, often in order to maximize the diversity of a certain trait, such as resistance to disease. The greatest genetic diversity of a species commonly found in the center of origin. Researchers at plants around the world for sources of features that can be used in the selection programs.

These germoplazmatichni collections of seeds from different locations. Breeders use germoplazma-kits for the identification of plants with desired traits and try to combine them into new varieties. Breeders can start with the selection process and existing varieties.[4]

Assessing the new material from plants

The end products of the selection programs grow in experimental products in different environments. If the experimental product show favorable and useful properties compared to other varieties can be released as a commercial variety and is sold to manufacturers. Creating a new breed is a long process, an average of five years, but can last up to 12 years after the first crossing.[4]

Pedigree- selection

Pedigree-selection method is often used in the hybridization of selfed plants.
Stages of this method are:

- Seed from the initial intersection of two selected plants are sown. Lumberjack plants showing superiority select.
- The seeds from these selected plants are collected and sown on new lines. Each line is made from seeds of a plant. Unwanted seeds are removed.
- The additional selection of plants within the ranks is happening϶ with additional sowing of selected seeds up to six times the F6-generation.
- In F6- generation plant families from among uniformed and become seeds of the selected families are collected and evaluated in experimental trials.

These experimental trials will determine whether the selected lines are superior to comparable permanent varieties, and whether they have the desired properties.[3]

Hybridization

The process of hybridization used sodzavanje tugjooplodni varieties of plants. The creation of hybrid maize comprises the following steps:

1. Creating natural hybrids of self-fertile plants of maize bred over several generations to obtain uniform variety.

2. A true hybrid maize was created by crossing two plants obtained with different genetic traits; a plant used as the male parent and another as female parent. This first obtained hybrid has typical properties that are superior to those of both parents. The superior ability of spunk called heterosis or hybrid.[3]

Genetically modified plants: friends or not?

The development of genetic engineering is probably one of the most important achievements in the improvement of plants in recent years. Also, it is one of the most controversial topics. Some see genetic engineering as a powerful tool for improving the plant, while others considered it a threat to the environment and human health.

The possible benefits of genetic engineering include the possibility of introducing new traits in plants, such as:

1.The biological resistance to pests and diseases eliminates the need for applying insecticides that have a negative impact on the environment.
2.Tolerance of herbacidi reduced amount herbicides that are applied to some plants (eg. plant "Round-up-ready" tolerant that non-selective herbicide glyphosate has limited durability and showed little impact on the environment). Using this technology also promoted conservation tillage practices because Round-up can be used several times after eruption of the plant.
3.The adjustment of the stress on the environment (for example, tomatoes have a long life on the shelves) .
4.Desirable nutritional characteristics (eg golden rice has a high concentration of betacarotene in wheat).

However, the creation of genetically modified organic plants also raised some concerns, such as:

1.The overall safety of GMO genetic engineering.
2.The impact on human health because we know the long-term result of eating food derived from GMO plants.
3.The ability of GMO plants to cross with fivite relatives or the possibility that they themselves become difficult to control.
4.Unintended effects on organisms not targeted (eg, a possible negative effect on beneficial insects of some proteins introduced into plants).
5.Creating insects and weeds that are resistant to Bt or glyphosate, consequently, it is the result of excessive use of this technology.
6.Ethical and religious issues related to genetic engineering and ownership of genes introduced into GM plants. [4]

Conclusions

Based on the above, one can draw the following conclusions:

- To understand how to improve plant must learn basic genetics. Genetic processes taking place inside cells.
- Genetic material is passed through two important processes: mitosis and meiosis.
- People practiced methods of plant selection since we first tamed plants with selection of the desired type of wild plant populations. After a short time people have noticed the inherited variation in plants.
- The collection of genetic material is the first step of the selection program and consists of a collection of seeds from a wide range of resources, often in order to maximize the diversity of a certain trait, such as resistance to disease.
- The Ministry of Agriculture of the United States still conducts targeted research plant. The ministry also keeps seeds and other plant parts of the world collections, old and new species, and herbaceous species. The collection includes 450,000 units kept in more than 20 specialized locations throughout the United States.
- US began to keep plants and seeds with interesting features in the 90s of the 19th century stations around the country. Examples of saved material include modern and older varieties of plants, wild plants and species of plants and seeds stored in a cool, have a lifespan of 20 to 50 years.

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Influence of rooting hormones on propagation of lavender (*Lavandula angustifolia* L.)

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Abstract*: Lavandula angustifolia* L, most significient lavender, was used in this research. The aim of this study was to investigate the effects of using two different hormones from IBA group (Clonex® gel and Rhizopon® for green cuttings), which were applied on green steam cuttings. The study period conducted from May,2015 to Avgust,2016. There were 90 root cuttings which were divided into 3 groups of 30 plants in each with 3 repeats (Clonex, Rhizopone and Control plants). The morphological characteristics (plant height, number of leaves, buds, plant diametar, number of lateral branches and flowers, and at the end fresh and the dry weight) were analyzed. We did seven measures to follow morphological characteristics. The results were proved better rooting to Clonex® treatment, better growth and early blooming which is rare in first year of production. From the results it could be concluded a reasonable application of the using rooting hormones as biostimulators in the propagation of *Lavandula angustifolia* L.

Key words: Lavender,propagation, hormones, cuttings, Clonex

Introduction

Lavender is a genus of family *Lamiaceae.* A perennial herb, shrubs and subshrubs mainly native plant to warm temperatures Mediteranian region. Although it can be grow up to 1 700m height above to sea level. Lavender is usually grow because of essential oil from blooming fragrant flowers. It is used in perfumery, industry, and for pharmaceutical preparations (El-Keltawi, Croteau, 1987). It has a great therapeutic value as antifungal, anti-inflammatory,

carminative, antispasmodic and in skin care. Nowdays, it is highly prized in aromatherapy and is emerging as one of the cash crops of Kashmir valley (Chisty et al., 2006).

Lavender could be grow by the seeds, but that it is slow and the plants exhibit too much variation in growth rate and oil composition to be commercially used and dependence of many factors (Andrade et al.,1999). Some hybride sorts have steril seeds (Šilješ et al., 1992). Since the discovery of natural plant rooting hormones (Thimann and Went, 1934), for propagation it has been intuitive to apply these substances to the basal end of cuttings to produce new roots. Propagation of moderate and difficult-to-root species with rooting hormones may enhance rooting percentages. Exogenously applied hormones also facilitate rooting where cultural practices or environmental conditions are not ideal. Examples include uneven misting, suboptimal propagation temperatures and, in some cases, reduced light levels during winter.

Nowdays, the based of almost every hormone, which is use like biostimulators is auxin. Auxin is a plant hormone that aids in the initiation of adventitious roots. Indole acetic acid (IAA) is the naturally occurring auxin found in plants. IAA is involved in nearly every aspect of plant growth and development. Some of the processes regulated by IAA include formation of embryo in development, induction of cell division, stem and cleoptile elongation, apical dominance, induction of rooting, vascular tissue differentiation, fruit development, and tropic movements such as bending toward light. Synthetic forms of auxin are available commercially in the form of Indolebutyric acid (IBA) and napthaleneacetic acid (NAA). Commercial preference given to these synthetic compounds and less to IAA is illustrated by the large number of rooting products available containing one or both of them. Generally speaking, auxinbased rooting products are applied at concentrations of 500-1,500 ppm for herbaceous and softwood cuttings. In addition, rates between 1,000 and 3,000 ppm may be used for woodier tissue, but the maximum recommended concentrations are not more than 5,000 and 10,000 ppm for semi-hardwood and hardwood cuttings, respectively (Cerveny and Gibson,2005). Basal methods used are the Quick Dip and Basal Long Soak Methods, using rooting solutions, and the Dry Dip Method, using rooting hormone powders (Kroin, 2012).

Propagation of lavender from cuttings is easy and more likely to be successful than growing the plants from seeds. Propagation with cuttings is faster and it makes use some inhibitors of grow possibile. The aim of this study was to investigate the influence of using rooting hormones in a propagation of *Lavandula angustifolia* L.

Material and methods

This study was conducted during the years of 2015(May) and 2016(Avgust) on the family farm in the greenhouse, owener Marko Kovačević in Mišin Han, municipality of Banja Luka. The steam cuttings are taken from the two and three years old parent plants, which were grow also in the greenhouse.

The Aplication of Clonex® and Rhizopon® hormones

The cuttings are shorten to the lenght of 6-7cm and the lower leaves are removed. It is used bevel cut for the cuttings. The cutting was done with disinfected scissors. It have been used 90 of cuttings and it disposed over 30 in each group, so there were three groups. One of its groups was the Clonex® hormone treatment group and another was the Rhizopone® hormone treatment group of plants. The third group was the Control plants group. Both hormones are from the IBA group of hormones. The Clonex® hormone in gel-form contains 4-indool-3-ylbutyrlc acid 3g/l, and Rhizopon® in powder-form with 0,5% IBA active ingrediant. After the application of hormones, the cuttings were put in the containers with perlite substrate (Gramoflor Special Mixture-peat moss). Than, they were watered and left at the shady and cooler place. After alomost 2 weeks are noticed the plants treated by Clonex® hormone were started to callus, and after four weeks got the real small roots, but at the rest of treated and untreated plants have just started to callus.

The plants transplanting and measuring

After mounth and a half, the plants were trasplanted into pots of 13cm in growing substrate (Domoflor Mix 4-mixture of black and white moss peat, 50-60% organic matter, 20-25% dry matter, fine structure 0-10mm, pH=5,5-6,2). The pots with young plants were put at the desk in the greenhouse, divided into groups of 30 with 3 repeats. At the same time, there were done the first measure, which showed intesive growth of plants treated by Clonex® and better root growth. They stayed at this place until Avgust of 2016 when they moved outside. During that period were get 7 measures (5 inside and 2 outside) and followed the temperature conditions in the greenhouse twice a day (in the morning, at the afternoon). The plants were watered by need. In the Septembar of 2016, the plants were prepeared for measuring the dry and fresh weight.

There were picked up 9 plants by chance in each group. Washed and dried with the paper towel the fresh plants were measured at the analytic scale with two decimales, each part particulary (root, above ground).

Weighead above-grounds parts and roots are packed in separate paper bags, properly labeled and placed in a tray drying chamber up to 70^{o} C. Drying the

plants have lasted to a constant weight. After the plants put out and measured again.

The mass of fresh and dry plants was expressed in grams (g). The obtained data were statistically analyzed using the analyse of variance and the differences between hormones used F-test in a computer program VVSTAT (Vukadinović, 1994).

Results and discussion

In order to determine the influence of rooting hormones on growth and development of lavender (*Lavandula angustifolia* L.) through plants morphological indicators and the fresh and dry weight of roots and above-ground parts, the following results were obtained. During the experiment, measurements were made of morphological indicators of plant growth and development. Table 1. shows the average value of the indicator.

Table 1. Morphological indicators of growth and development lavender *Lavandula angustifolia* L. under the influence of rooting hormones

Treatment variant (A)	Plant height (cm)	Number of leaves	Number of lateral branches	Lenght of lateral branche	Plant diameter (cm)
Control (A1)	15.05	59.69	8.32	6.67	20.48
Treatment with Clonex® (A2)	16.32	158.70	15.19	9.36	24.35
Treatment with Rhizopon® (A3)	15.98	174.32	15.45	7.59	21.92
Average	15.79	130.89	12.99	7.88	22.25
Analysis of variance - F	5.73^{*}	309.69^{**}	359.86^{**}	98.79^{**}	52.63^{**}
LSD	Plant height (cm)	Number of leaves	Number of lateral branches	Lenght of lateral branche	Plant diameter (cm)
0.05	0.9530	0.6301	0.7371	0.4760	0.9319
0.01	ns	0.9053	1.1167	0.7211	1.4118

ns = *not significant*

Plant height was under significant ($p \leq 0.05$) influence of rooting hormones treatment. It is recorded significant differences between tretament with Clonex® (A2) and control plants (A1), but there is no significant differences between

treatment with Rhizopon® (A3) and control plants (A1) as well as between plants treated with different rooting hormones (A2 and A3). The highest average height of lavender was recorded on treated plants with Clonex® 16.32 cm and the lowest hight was recorded on control plants with 15.05 cm.

Number of leaves, number of lateral branches, lenght of lateral branches and plant diameter were under a very significant ($p \leq 0.01$) influence of rooting hormones treatment.

The highest average number of leaves (174.32) was recorded in A3 variant (treatment with Rhizopon®) and the lowest of 59,69 belong to the control plants (A1). It is recorded significant differences between control (A1) and treatment plants (A2 and A3) as well as between plants treated with different rooting hormones (A2 and A3).

The highest average number of lateral branches belong to treatment with Rhizopon® (A3) 15.45, while the lowest was in the control group of plants (A1 8.32). It is also recorded significant differences between control (A1) and treatment plants (A2 and A3) but there is no differences between treated plants (A1 and A3).

The highest lenght of lateral branches 9.36 cm belong to variant A2 (treatment with Clonex®), while the lowest belong to the control group of plants (A1 6.67 cm). This time were recorded differences between tretament with Clonex® (A2) and control plants (A1) as well as between plants treated with different rooting hormones (A2 and A3), but there is no significant differences between treatment with Rhizopon® (A3) and control plants (A1).

The highest plant diameter 24.35 cm was recorded in A2 variant (treatment with Clonex®) and the lowest of 20.48 cm was recorded in A1 variant (control plants). It is also recorded significant differences between treatment with Clonex® (A2) and control plants (A1) as well as between plants treated with different rooting hormones (A2 and A3), but there is no significant differences between treatment with Rhizopon® (A3) and control plants (A1) (Table 1.).

After analyzing the morphological indicators of growth and development of lavender (*Lavandula angustifolia* L.) measurement of fresh and dry weight of plants were carried out and the obtained results are shown in Table 2.

Fresh weight of above-ground part was under a very significant ($p \leq 0.01$) influence of the rooting hormones. The highest recorded average value 92.65 g belong to the treatment with Clonex® (A2). The lowest recorded value was 78.01 g and belong to the control variant (A1).

Dry weight of above-ground parts, fresh weight of root and dry weight of root were under significant ($p \leq 0.05$) influence of rooting hormones treatment.

The highest value of the dry weight of above-ground parts belong to the variant A2 (treatment with Clonex®) 33.10 g and the lowest value belong to variant A1 (control) 26.64 g.

Table 2. Fresh weight (FW) and dry weight (DW) of root and above-ground part of lavender *Lavandula angustifolia* L. under the influence of of rooting hormones

Treatment variant (A)	Above ground part FW (g)	Above ground part DW (g)	Root FW (g)	Root DW (g)
Control (A1)	78.01	26.64	35.93	7.22
Treatment with Clonex® (A2)	92.65	33.10	48.72	11.07
Treatment with Rhizopon® (A3)	78.55	26.28	37.84	7.28
Average	83.07	28.67	40.72	8.52
Analysis of variance - F	22.51**	5.46*	6.47*	10.39*
LSD	Above ground part FW (g)	Above ground part DW (g)	Root FW (g)	Root DW (g)
0.05	6.0528	5.6843	9.3899	2.3654
0.01	9.1695	ns	ns	ns

ns = *not significant*

The highest fresh root weight was recorded in variant A2 (treatment with Clonex®) 48.72 g and the lowest recorded value was in control group (A1 35.93 g).

Also,the highest dry root weight was recorded in variant A2 (treatment with Clonex®) 11.07 g and the lowest recorded value was in control group (A1 7.22 g).

In all parameters were recorded significant differences between treatment with Clonex® (A2) and control plants (A1) as well as between plants treated with different rooting hormones (A2 and A3), but there is no significant differences between treatment with Rhizopon® (A3) and control plants (A1) (Table 3).

Root promoting compounds such as rooting hormones are used to increase the percentage of cuttings which form roots, reduce the time to root initiation, increase number of roots produced per cutting and to increase uniformity of rooting (Parađiković et al., 2013). Indole-3-butryc acid and 1-naphthalenacetic acid are commonly used in commercial propagation because of their consistency in promoting adventitious root formation on cuttings (Boyer and Graves, 2009). In the study of Ayanoğlu and Özkan (2000), three different treatments, 100 ppm, 200 ppm IBA application and no IBA application (control) were used on *Salvia officinalis* L. cuttings and on the 15th and 30th days their rooting ability was investigated. The 100 ppm IBA treatment had the highest rooting ratio (78.75%), number of roots and the longest roots. The rooting capabilities of the cuttings of

Karabaş lavender which were gathered from two different locations, were investigated by Ayanoğlu et al. (2000). The cuttings were treated by 1000, 2000 and 4000 ppm IBA doses and the cuttings with no IBA treatment were used as a control. The hormone doses positively affected the rooting of cuttings gathered from both places, and the rooting ratios, the length of roots and the number of roots per cutting increased with the hormone doses. The highest rooting ratio (70%) was obtained from the cuttings treated with 4000 ppm IBA dose. Vârban et al. (2016) investigate influence of perlit and peat with two products to stimulate rooting cuttings of *Salvia officinalis* L.: Clonex gel which contains 4-indol-3-butyric acid in the amount of 3g/liter and Radi-Stim no. 2 powder. From the results obtained it was observed that rapid rooting of sage cuttings is carried out using as substrates perlite + gel (Clonex), peat + gel (Clonex) and perlite + Radi-Stim no. 2.

Conclusion

During this research, it is confirms significant application of rooting hormones on a propagation of lavender. The average values of all investigated parameters (plant height, number of leaves, plant diametar, number of lateral branches, length of lateral branches, and at the end fresh and the dry weight) were statistical more significant at plants treated with hormones than the average values of control plants. Also, the average values of the fresh and the dry mass were statistical significant at the use of treatment with Clonex® hormone in comparation of treatment with Rhizopon® and Control plants.

Now, we can say that the existing of hormones as biostimulators in propagation of lavender (*Lavandula angustifolia* L.) is useful way of growth and propagate this sort of plants. It is faster and more efficiency way of production.

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Chemical features and quality assessment of the natural mineral waters in the Vrnjačka Banja area, Serbia

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Abstract: Water is one of the most precious natural resources. The aim of the study was a preliminary examination on mineral waters quality (S1–S5) in the Vrnjačka Banja area, Serbia, through the standard physico-chemical parameters: temperature, pH value, conductivity, turbidity, content of chloride as well as the total organic matter. All samples of water were collected and analysed in the period April-May 2017. The obtained results were compared to the National and World Health Organization (WHO) water quality standards. The results for temperature, pH and conductivity were within the values defined by Regulation on the quality of mineral water, except for the conductivity in samples S2 and S5 that were slightly above prescribed (2980 and 3460 μS cm^{-1}, respectively). Increased turbidity was observed in the sample S4 (5.24 NTU). The concentrations of total of organic matter in all analyzed samples were around 45 mg L^{-1} which indicates that the found values were 9 times greater than allowed and it can be result of a number of natural factors or the geographical location of the source itself.

Key words: mineral waters, water quality, chemical characteristics, Vrnjačka Banja

Introduction

Water quality is of a vital importance for mankind given the direct connection between water and human survival (Rajic et al., 2012). It is an important substance to all life both living and non-living and also is regarded as a universal solvent capable of dissolving nearly all solutes. Water quality is known to perform essential roles in human health (Boe-Hansen, 2001). Water is

a resource that has many uses, including but not limited to: recreation, transportation hydroelectric power-making, domestic, industrial, commercial uses (Bartram and Balance, 2006). In light of the current global development, water protection and management are positioning themselves as top priorities for preservation of the modern civilization. Despite the enormous amounts of water available in nature, its usability has been significantly decreased by pollution (Jiang, 2006). Human body is mostly made out of water (Turgut et al., 2005). Mineral spring waters represent a significant natural source of minerals necessary in the human organism (Baba et al., 2008). Water qualifies as "healing" or mineral if it contains more than one gram of dissolved matter per liter. Mineral waters which spring from greater depths, often have an increased temperature and therefore qualify as thermo-mineral. According to the density of occurrences and the diversity of physical and chemical features of the mineral waters, the territory of Serbia makes one of the richest areas of the European continent, but only a small quantity of these mineral waters is being bottled (Petrović et al., 2010). The global geological conditions dictate the speed of water exchange, but they do not correspond completely with the quality of the ground water. Therefore, the knowledge of the physical, chemical and biological parameters of water is very important for determining its type and quality (Kostić et al., 2016).

The purpose of this research was the preliminary quality assessment of mineral waters from the Vrnjačka Banja area, Serbia, through analysis of the following chemical and physical features: temperature, pH value, conductivity, turbidity, content of chloride and the total organic matter (TOM). Results were compared to the National and World Health Organization (WHO) water quality standards.

Material and methods

Study area

The Raška district is one of eight administrative districts of Šumadija and Western Serbia. It expands to the south-western part of the country. The administrative center of the Raška district is Kraljevo, which is about 25 kilometers away from Vrnjačka Banja (43° 37'/ N,20°54'/E). The geographical position of the locations is shown in Figure 1. The following samples are collected in the Vrnjačka Banja area: thermo-mineral source Snežnik (S1), Jezero (S2), thermo-mineral source Topla voda (S3) thermo-mineral source Borjak (S4), as well as thermo-mineral source Beli izvor (S5). All samples of water were collected and analysed in the period April-May 2017, in dark glass bottles, which had previously been rinsed with distilled water and sterilized with 70% alcohol.

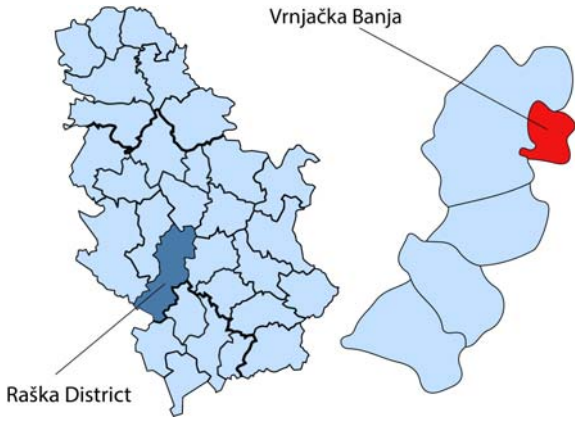

Figure 1. Geographical position of Vrnjačka Banja area

Determination of physicochemical properties

The temperature of the samples was measured with a thermometer and expressed in °C (Yu et al., 2009). The pH value and conductivity were measured using a pH meter and conductometer (sensION+ MM 374 GLP 2 channel Benchtop Meter) (APHA, 2012). Turbidity was measured by nephelometry using Handheld Turbiditimeter (Turb 430 IR) (APHA, 2012). The chloride content was determined by volumetric titration using standard solution of silver-nitrate (0.1 mol L^{-1}) with potassium-chromate (K_2CrO_4) as an indicator (Mohr's method) (Waters-Doughty, 1924). The total content of organic matter was determined using Kubel-Tiemann method (titration with a potassium-permanganate in acid solution) (Feliks and Škunca-Milovanović, 1990).

Results and discussion

The results of analyzed physicochemical parameters in water samples are shown in Table 1.

The temperature of analysed water samples was in the range 17.0–36.0 °C. The highest temperature value was recorded for the sample S3, and water from that source belongs to moderately warm waters. It is important to mention that mineral water from that spring is used to enhance digestion and help treat stomach and gallbladder illnesses. It improves epithelialization of the gastrointestinal and the urogenital mucosa. This mineral water is applied orally (through drinking), through inhalation, as an enema and as a vaginal spray in treatment of the above mentioned illnesses. It is also utilized in treatment of degenerative and inflammatory diseases of skeletal system as a mineral bath.

The pH value of the analysed water samples was in the range 6.42–6.88, which is within the value range defined by the recommendations of UNESCO/WHO/UNEP

(Chapman and Kimstach, 1996). Due to the influence of the pH value on the chemical properties of water, determining it is very important (Saritpongteeraka and Chaiprapat, 2008). The obtained results showed that the studied water samples were moderately acidic as expected. The lowest pH value (6.42) was measured in sample S1 (Snežnik) while the highest pH value (6.88) was recorded in S4 (Borjak).

Conductivity is the electrical property of water, and depends on the ions present in it - their concentration, mobility and charge, as well as the temperature on which it is measured. According to the legislation of the Republic of Serbia and the World Health Organization (WHO) the maximum allowed conductivity in natural mineral water is 2500 μS cm^{-1} (Official Gazzete, 2008; WHO, 2008). As presented in Table 1, samples S3 and S5 had higher values than allowed, 2980 and 3460 μS cm^{-1} respectively, which indicates that those samples contained an increased concentration of dissolved salts.

Turbidity of water is caused by suspended inorganic and dispersed organic substances, and is the result of the optical activity of substances dissolved in it. In most tested water samples, measured turbidity was < 5.00 NTU, except in the sample S4 (5.24 NTU) that had a slightly higher value than allowed. This may be linked to several factors such as the geology of the surrounding terrain, the presence of organic and inorganic materials, and the sudden inflow of surface water in the rainy season.

Table 1. Physicochemical parameters of investigated water samples

Sample	**S1**	**S2**	**S3**	**S4**	**S5**	MAC*
Temperature (^0C)	17	27	36	19	22	-
pH	6.42	6.49	6.48	6.88	6.55	6.5-9.5
Conductivity ($\mu S\ cm^{-1}$)	1397	2980	2150	1215	3460	2500
Turbidity (NTU)	2.77	1.44	0.89	5.24	4.24	5
Cl^- ($mg\ L^{-1}$)	21.62	35.09	34.74	20.56	46.79	250
Total organic matter ($mg\ L^{-1}$)	48.40	45.60	46.80	47.60	44.00	5

*maximum allowed concentration (MAC) in water for human use (Official Gazzet, 2008)

In all tested samples the presence of chloride was in the range from 20.6 to 46.8 mg L^{-1} (Table 1). The greatest amount of chloride ions was found in sample S3, while sample S4 showed the lowest values of this parameter. There is no evidence that the increased concentration of chloride can affect human health. However, chloride may have an effect on taste and colour of water (WHO, 2008).

In water containing organic substances of human, animal, plant or industrial origin, a certain amount of potassium-permanganate is spent for their oxidation (depending on the amount of organic matter in water) (Chapman and Kimstach, 1996). According to the law of the Republic of Serbia, the maximum allowed concentration of total of organic matter in natural mineral waters is 5.0 mg L^{-1}. All the analyzed samples had similar values of around 45 mg L^{-1} which indicates that the found values were 9 times greater than allowed. The organic substances present in the water do not have to be pollutants, but may naturally be present in a sample because of the field geology.

Conclusion

In this research we obtained the results of physicochemical parameters for 5 different water samples from the Vrnjačka Banja area. All the samples turned out to be thermo-mineral. The analyzed parameters were within the following ranges: temperature 17–36°C, pH 6.42–6.88, conductivity 1215–3460 μS cm^{-1}, turbidity 0.89–5.24 NTU and chlorides 20.56–49.79 mg L^{-1}. Obtained values were then compared to the referent maximum values of the analyzed natural mineral water parameters stated in the Regulations on water quality. The results showed that pH value was within the prescribed range in all samples, but the conductivity values were slightly above the prescribed in samples S2 and S5. Turbidity was higher than the prescribed maximum value in sample S4. All the tested samples displayed significant deviations from the maximum prescribed values for total organic matter, which can either be a result of a number of natural factors or the geographical location of the source itself.

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Study of selected physicochemical parameters in real water samples of the Zlatibor area, Serbia

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Abstract: The quality of water is of vital importance to mankind since the water is directly related to human survival. This study was conducted to assess the quality of water (S1–S4) from the Zlatibor area, Serbia, through the standard physico-chemical parameters: temperature, pH value, conductivity, total hardness and content of ammonia. The results were compared to the National and World Health Organization water quality standards. The results for temperature, pH and conductivity were within the values defined by the Regulation on the quality of drinking water. Sample S3 shown the highest value of conductivity (985 μS cm^{-1}), which was on the limit of permitted value. The values of total hardness for investigated samples were in the range from 179–370 mg L^{-1}. Samples S1 and S2 belong to the category of *soft waters* (179 and 180 mg L^{-1}, respectively), S4 belongs to the category of *medium hard waters* (370 mg L^{-1}), while S3 belongs to the category of *hard waters* (460 mg L^{-1}). Sample S1 had slightly elevated concentrations of ammonia (0.146 mg L^{-1}), while in sample S3 this value was on the verge of permitted (0.1 mg L^{-1}).

Key words: drinking water, water quality, physicochemical properties, Zlatibor area

Introduction

Water is the basis of life (Samuel, 2013). It is the primary ingredient of every living being. The human body consist of 55-75% water and that percentage must be maintained to help the body function normally (Montain et al., 2006). Adult person needs to drink in average two liters of water per day to compensate natural daily loss of fluids. Water is essential for the body because it regulates body temperature, removes harmful substances from the body and transports

nutrients vital for the human body. The total amount of water in the body is in an extremely dynamic balance within the extracellular space of each cell. It constitutes about 92% of blood plasma, 80% of muscle tissue, 60% of red blood cells and over half of most other tissues such as intestines, stomach and kidneys (Turgut et al., 2005).

Many water resources in developing countries are unhealthy because they contain harmful physics, chemical and biological agents (WHO, 2008). Industrial development, urbanization and population growth have a negative impact on water quality (Su et al., 2011). The contamination of groundwater is due to excessive use of mineral and natural fertilizers, waste disposal, wastewater discharge, excessive use of pesticides and air pollution. Additionally, there are numerous diseases that are transmitted through polluted water (Boe-Hansen, 2001). According to World Health Organization (WHO), drinking water should be clear, colorless, odorless, tasteless, and free of pathogens or other toxic chemicals (WHO, 2008). Therefore, knowledge of the physical-chemical parameters of water is very important to characterize the type and quality of water (Kostić et al., 2016). These parameters are binding factors for the survival of organisms, first of all flora and fauna.

The aim of this research was to test water quality in the territory of Zlatibor District, Serbia, through the selected physical-chemical parameters: temperature, pH value, conductivity, total hardness and content of ammonia. Results were compared to National and World Health Organization water quality standards.

Material and methods

Study area

The Zlatibor District is one of eight administrative districts of Šumadija and Western Serbia. It is located in the western, mountainous part of Serbia. The district was named after the mountain region of Zlatibor (43°51'00" N; 019°51'00" E). The geographical position of the locations is shown in Figure 1. The samples are collected from municipalities of Požega: tap water from Gornja Dobrinja (S1), city's tap water (S2), well's water from Gornja Dobrinja (S3) as well as pool's water from Gornja Dobrinja (S4). All samples of water were collected and analysed in the period of April-May 2017, in dark plastic bottles, which were previously rinsed with distilled water and sterilized with 70% alcohol.

Determination of physicochemical properties

The temperature of the samples was measured by a thermometer and expressed in °C (Yu et al., 2009). pH value and conductivity were measured using a pH meter and conductometer (sensION+ MM 374 GLP 2 channel

Benchtop Meter) (APHA, 2012). Total water hardness was determined in complexometric way, by titration with standard solution of ethylenediaminetetraacetic acid (EDTA) of 0.01 mol L^{-1} concentration (Rajković, 2012). A Shimadzu (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements. The colorimetric method was used for determination of ammonia (Nessler's reagent, 425 nm) (APHA, 2012).

Figure 1. Locations of water samples

Results and discussion

The results of investigated samples of water are compiled in Table 1. The measured temperature of analyzed samples was in the range from 17 to 20 ºC. The recorded values are within the acceptable limits for the survival, metabolism and physiology of aquatic organisms. Water temperature is a highly important component of the aquatic ecosystem as it affects the living organisms and the physical and chemical characteristics of water.

All water samples had the pH between 6.5 and 8.5 which are values accepted by the World health organization (WHO) standards (Chapman and Kimstach, 1996). The results showed that the studied water samples were neutral to moderately alkaline. The lowest pH value was measured in sample of pool's water (S3, pH 7.32) while the highest pH value was recorded in a S1 (pH 8.12). These changes in pH from one sample to another are due to the CO_2 content of the water (Touridomon al., 2009).

Conductivity is an indicator of the degree of mineralization of water. It depends upon concentration and degree of dissociation and migration velocity of ions in the electric field. In the present investigation it varies from 354 μS cm^{-1} to 985 μS cm^{-1} which is within the recommended values according to the legislation of Republic of Serbia (Official Gazzete, 2008). The electrical conductivity is correlated with total dissolved solids (Rashenahalli et al, 2011).

Hardness is imparted to the water mainly by calcium and magnesium ions. Calcium is essential element for human beings (nearly 2 g per day) and plant growth. However, hard water is generally undesirable because it forms precipitate with soap, produces scales in boilers on heating and has high boiling point due making it unsuitable for cooking. In the present study, the mean total hardness ranged from 179–370 mg L^{-1} which were lower than maximum permissible limit of 500 mg L^{-1} (WHO, 2008). According to the received results of water hardness analysis (in mg $CaCO_3$ L^{-1}) it can be conclude that City supply system water in samples S1 and S2, belong to the category of *soft waters* (<200 mg L^{-1}). Water from S4 belong to the category of *medium hard waters* (200–400 mg L^{-1}). Water from spring S3 belong to the category of *hard waters* (400–600 mg L^{-1}).

Ammonia is a biologically active compound found in most waters as a normal biological degradation product of nitrogenous organic matter (protein). In water ammonia reacts to form ammonium (NH_4^+) and hydroxyl (OH^-) ions. When pH is above 7.2, some free NH_3 remains and this increases with increasing pH. It has been known that ammonia (NH_3) is toxic to fish and that its toxicity increases with increasing pH and temperature of the water (Medeiros et al., 2016). According to the legislation of the Republic of Serbia the maximum allowed presence of ammonia in drinking water is 0.1 mg L^{-1} (Official Gazzete, 2008). Sample S1 had slightly elevated concentrations of ammonia, while in sample S3 this value was on the verge of permitted. In the remaining two samples (S2, S3) content of ammonia were far below the maximum permissible value (Table 1).

Table 1. Physicochemical parameters of drinking water of Zlatibor area

Sample	*Temperature (^0C)*	*pH*	*Conductivity ($\mu S\ cm^{-1}$)*	*Total hardness ($mg\ CaCO_3\ L^{-1}$)*	*NH_3 ($mg\ L^{-1}$)*
S1	19.9	8.12	408	179	0.146
S2	18	7.76	354	180	0.003
S3	17.1	7.40	985	460	0.101
S4	19.2	7.32	708	370	0.049
MAC*	-	6.5-8.5	1000	500	0.1

*maximum allowed concentration (MAC) in water for human use (Official Gazzet, 2008)

Conclusion

In the analyzed samples only certain parameters were outside the range of permissible values for drinking water. All water samples had the pH between 6.5 and 8.5 and these results showed that the studied water were neutral to moderately alkaline. The largest differences between the samples are shown with conductometric assay and the degree of water hardness. Sample S3 has shown the highest value of conductivity (985 μS cm^{-1}), which is on the limit of permitted value. Additionally, sample S3 had the highest values of total hardness (460 mg L^{-1}) and belongs to the category of *hard waters*, while samples S1 and S2 have shown the lowest hardness value (~180 mg L^{-1}) and belong to the category of *soft waters*. The amount of ammonia in the sample S3 is on the verge of the maximum allowed (0.1 mg L^{-1}), while in the sample S1 observed value is slightly greater the maximum allowed for this parameter (0.146 mg L^{-1}). Further physico-chemical investigation would give a more complete picture of the quality of water from this district, which is the final aim of the started research.

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Milk production during first and second lactation from Holstein-Friesian breed

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Abstract: This study was carried out to investigate the effect od somatic cell count and a total number of bacteria on milk production in cow milk of Holstein – Friesian breed in first and second lactation in a duration of six months on the farm in the region Pelagonia, Republic of Macedonia. They will be investigated a total number of somatic cells, the total number of bacteria and their impact on milk production. Somatic cells are a natural ingredient in milk and their number can't be more than 400 000/ml. His number increase as result of infection of the mammary gland and high bacteria number in the milk. The average values of somatic cells in the milk of the first lactation was 219 491/ml and in the milk of the second lactation was 275 817 /ml. The total count of bacteria was 146 111/ml in first lactation and 218 194 /ml in second lactation period. Milk production averages over six months amounted in milk from cows in first lactation 24,69 kg milk, while from cows in the second lactation was lower, amounted to 22,83 kg milk. This study indicates that high SCC and a total number of bacteria negatively affects not only milk production but also milk composition and quality.

Key words: milk, somatic cells count, total count of the bacteria, milk production, Holstein – Friesian breed

Introduction

Milk quality is determined by chemical composition, physical characteristics and hygienic parameters. The main indicators of hygienic quality of milk are a total number of bacteria and somatic cell count (SSC). One of the most important factors which influence to increasing the number of somatic cells in the milk is an infection of mammary gland may be infected with entering to the

bacteria over the nipple's canal (galactogenic); over the demaged skin (lymphogenic) and over blood (hematogenic). Usually provocate Staphylococcus aureus, Streptococcus agalactia, Streptococcus (uberis, pyogenes, Dysgalacticae) and coliform bacteria. They can increase the number of somatic cells to the 1.000 000/ml. Increasing the bacteria number is appear, damage on the udder as result of that; somatic cells are increased. The most important environmental parameters are the status of udder infection, the age of cow, stage of lactation, the number of lactation, breed, housing, geographical area and season, herd size, stress, heavy physical activity, and milking. a farmer himself can control a great number of environmental factors using good managing practice and permanent education (Z.Cacic et al.,2003)

According to the regulations on the quality of raw milk in R. Macedonia limit of somatic cells count (SSC) is ≤400 000/ml milk and total count of bacteria (TCB) is ≤100 000 /ml milk.

If the number of somatic cells is over 400 000/ml the milk is mastitic, and the consequences are: low milk secretion, low milk production, changes in chemical contain and physical, bacteriological and technological characteristic of the milk.

Material and methods

In the course of preparing this labor was examined cow milk of Holstein – Friesian breed in first and second lactation. The examination was followed by a period of six months lactation. The cow was cultivated in control condition on farm with 860 cow capacity in Pelagonia region in R. Macedonia in the same condition of attendance and diet.

Total count of somatic cells was examined and continual twice in one month at the same samples of milk with Somacount (ISO 13366-2:2006).

Microbiological analysis of all samples exerted once of month during of six months. Total bacteria count (TBC) was determined with Bactocount.

Control of milk production from cows of Holstein – Friesian breed in first and second lactation was conducted using vector - gauge of milk. We follow the amount of milk for six months.

Results and discussion

From our research can be noticed that in the both lactation parameters of somatic cells count and total count of bacteria was larger at the start of lactation.

Average values of somatic cells in second lactation is higher, 275 817/ml, cv (%) is 66,7, and in first lactation 219 491 /ml and cv (%) is 66,7 .There is the increasing number of somatic cells in a fourth, fifth and sixth month.(Table.1 and figure.1)

Table 1. Parameters of somatic cells count (SSC) in I and II lactation

months	***n***	**I lactation**			**II lactation**		
		$\bar{x}$	*s*	*cv* (%)	$\bar{x}$	*s*	*cv* (%)
1	12	203.174,20	53.975,30	26,57	328.845,80	298.972,90	90,92
2	12	151.960,00	64.402,80	42,38	245.769,20	242.675,70	98,74
3	12	195.081,20	75.868,60	38,89	189.156,20	126.887,70	67,08
4	12	188.908,30	72.501,20	38,38	326.625,00	161.096,70	49,32
5	12	273.722,50	63.331,60	23,14	293.385,00	820.28,8	27,96
6	12	203.174,20	53.975,30	26,57	271.120,80	77.125,20	28,45
total values	**72**	**219.491,40**	**113.734,70**	**51,82**	**275.817,00**	**183.959,30**	**66,7**

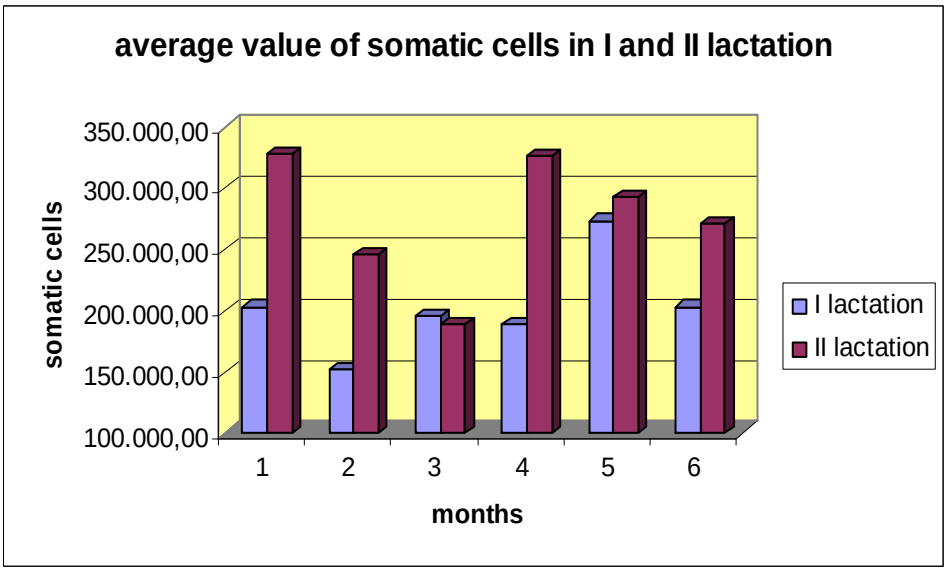

Figure 1. Average value of somatic cells count in I and II lactation

Breed of the second lactation have a higher value of a total number of bacteria 218 194/ml; cow in first lactation average value is 146 111/ml of expression in percentage 60.92%.(Table.2 and Figure.2)

Breed in first lactation total count of bacteria is 100 000 – 204 166/ml milk and coefficient variation is 22.46- 77.77%. Breed in the second lactation has average value of total bacteria count from 95.000/ml to 325 000/ml and coefficient of variation 29,09 – 76, 37%. Breed in the second lactation has higher value of total bacteria count in second, fourth, sixth-month compared to breed in the first lactation because of minimizing the resistance of this breed.

Table 2. Parameters of total count of bacteria (TCB) in I and II lactation

months	n	I lactation			II lactation		
		$\bar{x}$	s	cv (%)	$\bar{x}$	s	cv (%)
1	12	180.833,33	124.787,70	69,01	95.000,00	72.550,92	76,37
2	12	204.166,67	158.771,32	77,77	291.666,67	84.834,96	29,09
3	12	140.833,33	46.015,48	32,67	175.833,33	98.577,00	56,06
4	12	100.000,00	22.462,09	22,46	325.000,00	133.994,57	41,23
5	12	141.666,67	47.258,16	33,36	199.166,67	137.010,84	68,79
6	12	109.166,67	31.176,43	28,56	222.500,00	84.436,64	37,95
total values	**72**	**146.111,11**	**92.695,25**	**63,44**	**218.194,44**	**126.505,87**	**57,98**

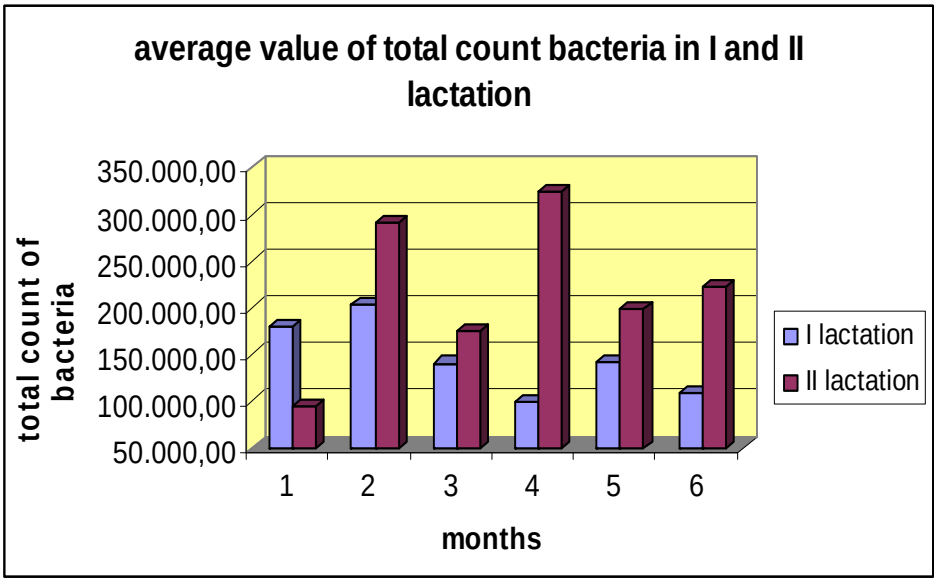

Figure 2. Average value of total count bacteria in I and II lactation

The higher number of bacteria in this month is a result of higher number of mastitic diseases e.t. infections of the mammary gland. During July the values of the total count of bacteria are higher and that is as result of higher outside temperature. The stress of higher temperature and moisture are stimulated bacteria number and infection in the summer period. There is a lot of variations in the total count of bacteria during six months depending on the conditions of animals cohabitation and subjective factor- man.

Table 3. Milk production in I and II lactation

months	n	I lactation			II lactation		
		$\bar{x}$	s	cv (%)	$\bar{x}$	s	cv (%)
1	12	27,9167	9,2585	33,16	26,6667	7,0367	26,39
2	12	27,4167	7,3418	26,78	26,0000	6,4244	24,71
3	12	27,2500	6,5661	24,10	25,4167	7,3788	29,03
4	12	25,0833	4,3788	17,46	22,5000	6,0076	26,70
5	12	21,9167	3,4761	15,86	20,0833	4,4407	22,11
6	12	18,5833	3,9648	21,34	16,3333	4,2923	26,28
total values	**72**	**24,6944**	**6,8805**	**27,86**	**22,8333**	**6,9099**	**30,26**

Milk production averages over six months amounted in milk from cows in first lactation 24,69 kg, while that of milk from cows in second lactation 22,83 kg milk. This study indicates that high SCC and total bacteria count negatively effects on milk production, reduce milk production. The same result from another study is that SCC and total bacteria count negatively effects on milk production, milk composition and quality (Cinar et al., 2015)

To accomplish a lower number somatic cells and bacteria Garsia, 2004 in his studies indicate of 2 key factors: cleaning and adequate hold and transport of the milk. In this context may remember conclusion to Yayaro et al, 2004 and Zrinka Cacic et al, 2003 that the somatic cells and the total count of bacteria in milk may be indicators of breed control, health udder of the animal and milk quality.

With the increase of somatic cells over 400 000/ml, the milk is mastitic tells Antunac. N.et all, 1997, and consequences are reduce milk secretion, changes in chemical contain, physical, bacteriological and technological characteristics of the milk.In this context may be remembered and research of (Majic, 1989).

Relationships between somatic cell count and variation in milk production at the cow level must be reviewed to provide average reference values suitable for the assessment of economic losses due to subclinical mastitis (Philipe Hortet and Henri Seegers, 1998).

Conclusion

1. Average values of the total count of somatic cells (SSC) for the first lactation was 219 491/ml, for the second lactation was 275 817/ml in six month lactation period, so we can make a conclusion that in the breed in second lactation we have higher values of somatic cells count.

2. Average values of the total count of bacteria from the breed in the first lactation is 146 111/ml. From the breed in the second lactation is 218 194/ml, so the higher values of the total count of bacteria in the second lactation as results of common disease of mastitis.

3. Milk production averages over six months amounted in milk from cows in first lactation 24,69 kg, while that of milk from cows in second lactation was lower and it amounted to 22,83 kg milk.

4. Therefore, it is suggested that monthly control of SSC, the total count of bacteria is the most efective methods to monitor and evaluate changes in the amount of milk and milk quality. We must pay attention for better control, diagnosis of mastitis diseases on time, respect the higienical standards linked with holding and keeping of breeds in farms.

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Physico-chemical and microbiological changes during manufacture of white brined cheese

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Abstract: The aim of research was to evaluate changes of physico-chemical characteristics, somatic cell count and microbiological changes during ripening of white brined cheese. During the process of ripening from one bathes was taken sample from curd and cheese samples at 1-st, 5-th and 10-th day. From each cheese two samples were taken, one from the middle and one from the cheese rind. The Escherichia coli count was reduced during the ripening process. It was found negative coefficients of correlation between content of NaCl, pH and Escherichia coli counts ($r=-0.86$ $p<0.001$; $r=-0.98$ $p<0,001$, respectively).

Key words: cheese, Escherichia coli, ripening

Introduction

Cheese is the generic name for a group of fermented milk-based food products, delivered in an extensive variety of flavors and forms all through the world (Fox, F.P., et al., 2000). The cheese itself is regarded as a nearly complete source of valuable nutrients, especially conjugated linoleic acid, calcium, phosphorus and high – quality protein (Fox, P. and McSweeney P, 2004; Ercan D., 2009; Waleed A. M., 2013). Cheese is a biochemically dynamic product and undergoes significant changes during ripening (Fox, F.P., et al., 2000), they may be grouped into primary (lipolysis, proteolysis and the metabolism of residual lactose and of lactate and citrate) or secondary (metabolism of fatty acids and of amino acids) biochemical changes (McSweeney P., 2004).

White brined cheeses are the most widely produced and consumed cheeses in Macedonia. Currently, white brined cheeses in Macedonia are produced by both traditional and industrial methods. Traditionally, white brined cheeses have usually been made from unpasteurized or medium heat-treated milk, without starter cultures and in small dairy plants and households using simple

equipment. Macedonian white brined cheese might be characterized as a soft (50 - 60% moisture), high fat cheese (25-30%), with protein content of 12–21% and high salty (3–5%) with a pH range of 4.20 – 5.05 (Mojsova S., 2013).

The microbiological quality of raw milk can be affected by several factors, such as milking, housing, farming system (organic, conventional), and the season of the year (Bogdanovicova K, et al., 2016). E. coli bacteria are considered an important hygiene indicator throughout the process of raw milk obtaining, storage, transport, and sale. E. coli is commonly found in the intestinal microflora of humans and warm-blooded animals, but it may become a pathogenic organism (Costa et al. 2009). In heat untreated raw materials of animal origin, such as raw milk, Escherichia coli occurs quite frequently (Badri et al. 2009).

It is evident that the rate of survival and/or growth of pathogenic bacteria in cheeses depends on the ecological conditions (Aw (water activity), pH, salt content, temperature of maturation) within the cheese and/or brine. Although storage in brine is thought to cause a decrease in the populations of undesirable contaminants, there is great concern that the brine can also serve as a reservoir of certain salt-tolerant pathogens (Bintsis T., and Papademas P., 2002). Generally, bacteria belonging to the family Enterobacteriaceae, such as E. coli, do not tolerate high salt levels (Fatimah A.B., and Anderson J.G., 2009)

Material and methods

Cheese manufacture and sampling

Sample analyses were conducted on traditionally produced cheese in laboratory conditions at Faculty of Biotechnical sciences - Bitola. Raw cow's milk was used to manufacture the cheese. Samples of milk, curd, cheese over dry salting process (1st day of ripening), and 5-th and 10-th days of brine ripening were analyzed. The curdling was performed with liquid commercial rennet for 45-50 min without heating the milk. Further on, the curd was cut in cubes of 2 cm^3, resting for 5 minutes and afterwards pressed in cheese mold for 24 hours. Than the curd was cut into four parts and salted on the surface with coarse-grained salt. Cheese blocks were placed in plastic cans filled with brine solution of 15 g NaCl/100g. During the ripening period of first 10 days the cheese was held at 15-17°C (Figure 1 White-brined cheese traditional technology).

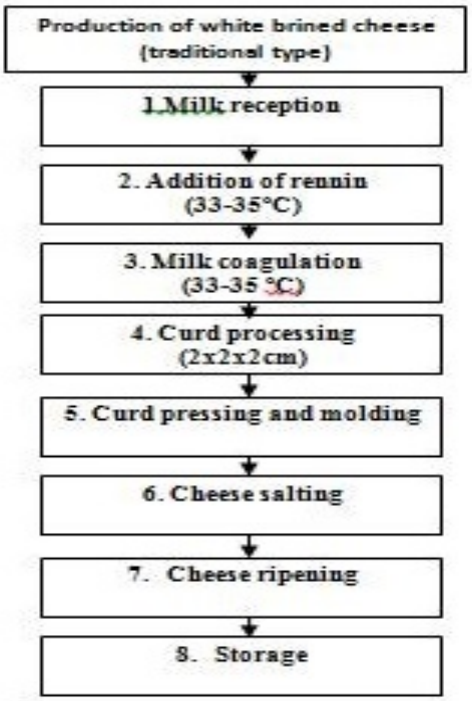

Figure 1. Technological scheme of traditional cheese (Popovski N. et al., 2015)

Microbiological and physicochemical analysis of the cheese

Physicochemical and microbiological analysis was examined on milk, curd and cheese samples on the 1st day, 5th day and 10th day of manufacture. Lactoscan MCC was used for physicochemical analysis of milk. The determination of fat content, pH and ^{0}SH, dry matter (%), water content (%), NaCl (%) was done on curd and cheese samples. The determination of the content of milk fat in cheese is determined by the Gerber method (Caric at al. 2002). The pH of milk and cheese samples was measured using a digital pH meter (model MP120FK Mettler Toledo, Greifensee, Switzerland). Titratable activity (TA) was determined by titration using Soxlet-Henkel method. Dry matter and water content was determined by MJ33 Mettler Toledo. The NaCl contents in curd and cheese during ripening were determined by the Mohr method. All analyses were performed in triplicate Milk sample examined by Beta star screening kit (Neogen, USA) which is used for rapid detection of the betalactam antibiotics such as penicillin, ampicillin, amoxicillin, cloxacillin, and cephapirin.

Samples for microbiological analysis were taken under sterile conditions, placed into plastic sterile dishes and kept under refrigeration until analysis. Microbiological analyses were preformed within 2 h after the sampling at Anima

Vet laboratory (Bitola). Aliquot of 10 mL of milk or 10 g of cheese was homogenized with 90 ml of buffered peptone solution. Decimal dilution was made with sterile physiological solution. The enumeration of E. coli was performed according to the method defined by MKC EN ISO 16649-2:2008, known as Horizontal method for the determination of β-glucuronidase-positive Escherichia coli, by the technique of counting colonies cultured at 44°C, using 5-bromo-4-chloro-3 indolyl-β-d-glucuronide.

Results and discussion

The quality of cow's milk used for the manufacture of white brined cheese is presented in Table 1. The average somatic cell and total bacterial count in the milk was $9{,}8*10^5$ cells/ml and $5{,}836*10^6$ cells/ml which is much higher than allowed regulations (Official Gazette no. 197/2016). This confirms the poor hygienic conditions at farm level. The chemical parameters in milk are in compliance with the requirements (Official Gazette no. 96/2011) also the results obtained in this study confirmed earlier findings of Mojsova S., et al., (2013).

Table 1. The quality of cow's milk used for manufacture of white brined cheeses

Chemical parameters of the milk		**Microbiological parameters of the milk (CFU/ml)**	
Fat (%)	3,80	Enterobacteriaceae	$0{,}033*10^6$
Solids non fat SNF (%)	8,30	Coliform bacteria	$5{,}1*10^6$
Density (%)	27,99	Staphylococcus	$0{,}42*10^6$
Lactose (%)	4,55	Eschericia coli	$0{,}0024*10^6$
Solids (%)	0.68	Total bacterial count	$5{,}836*10^6$
Protein (%)	3,04		
^{0}SH (%)			
Condictivity (%)	3,43		
Freezing point	-0,528		
Antibiotic residues	No positive detection		
SCC (cells/ml)	$0{,}98*10^6$		

The physico-chemical characteristics of white brined cheese during manufacturing and ripening are presented in Table 2. Acidity is one of the most important properties of cheese which influences the structure, rheological, sensory characteristics and overall quality of the cheese (Savic Z., 2015). During

the first 10th days of ripening period the average pH was 5,34. According to Levkov V., et al., (2014) decreasing of the pH was a result of lactic acid production by lactic acid bacteria (LAB). But in our study a gradual increase of pH value during cheese brine ripening (1, 5 and 10 days of ripening) was observed. This might be a result of yeast metabolic activity which uses lactic acid as a source of carbon, or a result of great amounts of alkaline compounds released during proteolytic activities (Volken de Souza et al., 2003). As a result of diverse metabolic activity titratable acidity gradually increased during cheese ripening (Dubrova Mateva et al., 2008), but lower value of titratable acidity was recorded on 5th and 10th day. According to Velevski S., (2015) lack of lactic acid leads to reduction of titratable acidity and adulteration of cheese during the ripening time.

The combined method of salting (dry salting and brining) was used. During cheese ripening, the content of NaCl gradually increased as a result of salt diffusion, which in turn depends on cheese size and format as well as on size and quantity of cheese fat globules. In addition, hydration of casein micelles might interfere with the salt diffusion in cheese (Guinee and Fox, 2004).

Table 2. Changes in the physicochemical parameters during manufacturing and ripening of white brined cheese

Parameters	**Cheese making**		**Ripening cheese (days)**		
	Milk	**Curd**	**1**	**5**	**10**
pH	6,67	5,30	5,29	5,29	5,44
Titratable acidity (^{0}SH)	7,2	72	80,8	42	42,4
Dry matter (%)	12,08	48,94	49,29	48,82	47,02
Water content (%)	/	51.06	50,71	51,18	52,98
Fat (%)	3,9	27	27	27	27
NaCl (%)	/	/	1,60	7,68	8,17

The traditional cheeses contain original microflora (Beresford et al., 2001) which evolved during ripening as a result of nutritive and environmental changes in cheese (Williams et al., 2002). The evolution of Escherichia coli counts during the manufacturing and ripening of white brined cheese are shown in Table 3 and

Figure 2, and the maximal average values of Escherichia coli were attained in the curd ($1,76*10^6$). The counts of Escherichia coli are reduced constantly during the ripening period. The negative coefficients of correlation between content of NaCl and Escherichia coli counts ($r=-0.86$ $p<0.001$) suggest the inhibitory effect of high salt content. The negative coefficient of correlation between pH and the investigated group of bacteria ($r=-0.98$ $p<0,001$) might indicate the possible adaptation of microorganisms to lower values of pH in cheese.

Table 3. Changes in counts of Escherichia coli (CFU/ml) during manufacturing and ripening of laboratory produced white brined cheese

Parameters	**Cheese making**		**Ripening cheese (days)**		
	Milk	**Curd**	**1**	**5**	**10**
Escherichia coli (CFU/ml)	$0,0024 *10^6$	$1,76 * 10^6$	$0,2 *10^6$	$0,11 * 10^6$	$0,011 * 10^6$

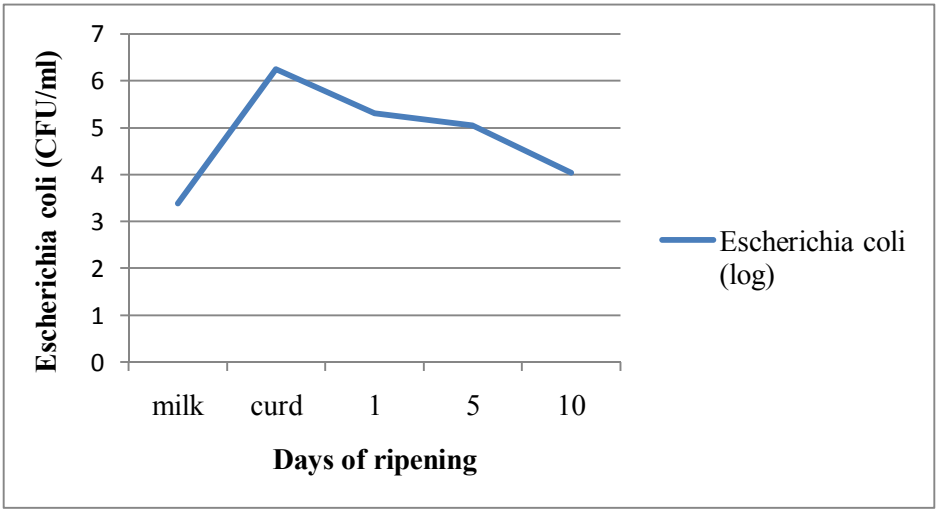

Figure 2. Changes in counts of Escherichia coli log (CFU/ml) during manufacturing and ripening of laboratory produced white brined cheese

Conclusion

The obtained results indicated a high number of all investigated groups of microorganisms in all stages of production, starting from the raw milk. The presence of high counts of Escherichia coli demonstrated the poor sanitary and

hygienic conditions at raw milk. Reduction of Escherichia coli content during the first 10 days of cheese ripening is influenced by salt content in cheese.

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Production technology and quality evaluation of fresh pork sausages

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Abstract: The aim of the research was to determine the quality and sensory characteristics of fresh sausage made from pork meat, fat and spices produced in the laboratory of the Faculty of Biotechnological Sciences, Bitola, Macedonia. For this purpose, was determined the chemical composition of the raw material (pork meat and adipose tissue) for the production of sausages. The average moisture, protein, fat, ash, content was 74.38%; 22.05%; 1.98%; 1.19% for pork meat and 36.03%; 7.27 %; 55.08%; 1.44% for adipose tissue. The obtained results of the sensory evaluation shown a high average grade 4.31 for sensory properties of sausages. The proposed technological procedure enables the production of sausages at home conditions.

Key words: sausages, pork, technology, sensory evaluation, quality,

Introduction

Meat preparations pose the greatest assortment of meat products from meat processing facilities. Generally, they are divided into several groups, of which the most common are sausages (durable, semi-durable and fresh); delicatessen products (durable and semi-durable) and cans.

In our country, meat processing facilities generally have observed this variety of production, although we have seen a growing import of meat products from neighboring countries or countries of the European Union.

It is known from the history that people have used the sausages as a food product. The ancient Romans made sausages from special recipes and spent during various religious ceremonies and holidays. In the Middle Ages, the people of Europe ate different kinds of sausages, (Kuzelov, 2013).

The sausages are products obtained by filling natural or artificial casings with a mixture of different types and quantities of minced meat, fat, skins, offal, remains of connective tissue and additional ingredients (Regulation on Quality of minced meat and meat preparations no. 63/2013). The appearance and other sensory characteristics are specific to the specific type of sausage.

Sausages are mainly produced for rapid consumption. If we want to keep the sausages for a longer time, they previously somehow must be preserved by smoking, drying, cooking, (Akesowan, 2013).

Fresh sausages are among the best known and most widely used sausage from the population, primarily because of their good quality (not used additives) have beautiful irreplaceable flavor, can be dried a few days and immediately consumed or little to volatilize and consume. They are known also, as homemade sausages. This type of sausage can be produced by meat shops and households.

In the sausage production, the recipe and nomenclature vary much. Sausages that come to market under the same name differ much as for its quality and taste, which depends primarily on the basic raw composition and use of spices and the skills of the technologist who produces them.

Material and methods

The technological process of production of homemade sausages is carried out in the Laboratory of quality and safety of meat and processed meat at the Faculty of Biotechnological Sciences. Produced are sausages from a standard traditional recipe using meat, fat and spices with the equipment available at the laboratory.

The main raw material which is used in the production of sausage is pork I category ham, weighing about 10 kg, cut into the basic parts. As fat is used bacon and spices such as leeks, red (Bukovska pepper), black pepper, muscat nut and cloves. Salt is added in an amount of 20g / kg. Additional raw materials have been used such as natural pork intestines previously soaked in hot water.

Chemical composition

Chemical composition (Moisture, ash, protein and fat) of the raw material (pork meat) and pork fat (adipose tissue) were determined according to the standard AOAC.

Sensory evaluation

The evaluation of sensory properties was carried out by students of the Faculty, by using the method of corrected stitch five-point system and method of ranking (Radovanovich Popov Rajlic, 2001/2001). Sensory evaluation included six properties (external appearance, the appearance of the intersection, consistency, aroma, color and taste). For each of the tested properties was fixed factor of importance. Evaluated properties are valued on a scale from 1-5.

Production technology of fresh pork sausage

The performing the whole technological procedure was performed on a satisfactory hygienic level, cutting on Inox tables, using a specialized set of butcher knives and glove. From the machines, it was used a machine for grinding the meat (wolf) and manual filling machine.

The technological production process includes multiple steps:

1) –Cutting (preparing meat of required pieces);
2) - Grinding (sieving) of the meat and the filling preparation;
3) –Preparation and sieving of leek;
4) – Stirring the filling and adding of spices and leek;
5) – Preparing the intestines;
6) - Filling the intestines;
7) Maturing of sausages (period of five days)

Cutting the meat - the task of cutting the swine ham is to perform separation of meat from bones, separation of blood vessels, fascia, tendons, cartilage. The cutting also involves separating of main parts (Figure 1). Once is performed the separation of the bones, the meat is cut into pieces of a suitable size ie They are prepared for grinding. For the production of these sausages, we have used ratio 80% pork meat (ham); 20% fat (bacon);

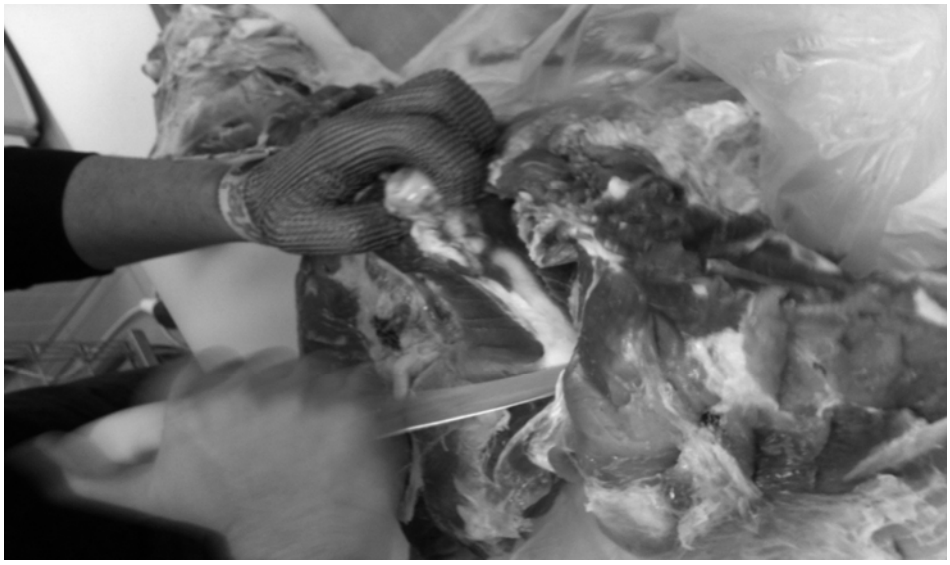

Figure 1. Cutting (preparing meat of required pieces)

The cut meat into pieces ***is grinded*** in grinding machine for meat (Figure 2).

Figure 2. Grinding (sieving) of the meat and the filling preparation

For grinding the meat is meant such a treatment process in which the muscles of the slaughtered animal ie the meat under the influence of special blades is mechanically separated from their physiological relationships and lead to a degree of magnitude that matches the type of final product. The beef is grinded into smaller pieces, unlike the pork.

The pieces of pork with bacon pieces are ground to bigger pieces ie plate with a larger diameter of 8 mm (is grinded once in a machine).

Preparation and cutting of leek - leek is added raw, just it is cut in the wolf machine where did the sieving of the meat. Cutting the leek is the size of 0.5 cm. Also if we want to, we can brown the leeks and such browned add to the meat batter. There is no difference in the taste of the final product while adding raw or browned leeks, the difference is that the browned leeks are safer to avoid spoiling of the product ie contamination with microbial the sausages and therefore it is recommended to sear it before adding into the filling. In sausages, we added about 1kg leek for 10 kg meat batter.

In further technological step charge, ***we mixed the filling along with leeks and spices were added.***

Spices that were added during the mixing of the required homogeneous filling for the production of sausages are:

-1 kg leek is added to 10 kg meat mass;

- 160-180gr salt is added to 10kg of meat mass;
- red pepper 50-80gr is added to 10 kg of meat mass;

- black pepper 40g is added to meat 10kg weight;
- muscat nut 5-10gr is added to the meat mass of 10kg;
- clove of 5 grams is added to the meat of 10kg weight

These are the basic spices that we added in the production of homemade sausages in the laboratory, but there is no limit for adding other spices because each sausage maker has his own technology in the production of the final product. Thus, the added raw materials ie the meat batter and the spices are stirred until achieving homogenization of raw materials.

Preparation of the intestines - for the production of sausages are used natural thin pig intestines with a diameter of 28-30-32mm. Before using the intestines if they are salted they need be unsalted. After mechanical removal of salt is carried rinsing with warm water of 10 to 20 minutes and then are placed in warm water and finally in cold (well wash out) and they are stored in a cool place and thus made the intestines can be used up to two to three days.

After preparation of the intestine, the filling is put into a manual filling machine, through which is done the filling of the sausage casing ie the intestines. As casings as already previously mentioned are used only thin pork intestines with diameter 28-30-32mm, (Figure 3 a, b).

Figure 3. Sausage filling in manual filling machine

During filling, the intestines should be well salted, because changes can appear they change their appearance because they collect water, a fat that reduces the viability and appearance of the sausages.

Once the intestines are filled, they are cut usually on 100-120gr and length 15-20cm piece.

Finished sausages are put to hang on metal sticks and it is important not to touch each other because the places where they touch each other appears brighter color that results from a lack of air and subsequently lower effects of smoke on that sites. At those places is also collected moisture, which leads to the appearance of mold and mucus.

Thus prepared sausages are left ***to ripen*** for about five days because these sausages are thermally unprocessed ie fresh sausages and their sustainability is 2-3 days, (Figure 4).

Figure 4. Ripening of pork sausages

During the period of ripening, we performed continuous control and occasional airing. Simultaneously we checked the hygiene of the room to get the quality and proper health product.

Results and discussion

Based on the previously exported technology of production of sausages can be determined that the used meat in sausage production is the meat of I category (ham) that meets the quality criteria and provides a tasty product. During the production of sausages are used spices produced in our country (leek, red pepper).

The results of chemical composition of the pork meat (I category) and fat are given in (Table 1).

Table 1 Chemical composition of pork meat and fat (adipose tissue)

Variable	Pork meat (I category)	Adipose tissue
Moisture (%)	74.38	36.03
Protein (%)	22.05	7.27
Fat(%)	1.98	55.08
Ash (%)	1.19	1.44

To produce quality charge and finished product quality, the content of the main components has a great influence on consistency, ripening of sausages and their nutritional value.

The average values for the water content was 74.38% ; fat content, 1.98%; protein amount 22.05%; and mineral 1.19%.

The average water content in the pork fat used as a material for sausage production was 36.03%; the content of fat in adipose tissue was 55.08%.

The average protein content was 7.27 % and the average content of minerals is 1.44%. Adipose tissue after muscle tissue as a significant component in the charge, it has a major impact on the quality of product. Of particular importance is its hardness and resistance to oxidative changes.

Our results are similar to results from the analysis of the chemical composition of pork in most literature data Okrouhlá et at., (2006); Butko et.al., (2007); Migdał et al., (2007).

Sensory quality of the sausages was investigated based on evaluations of appearance, consistency, color, flavorl and taste.

The results of evaluating the properties by the evaluators are shown in (Table 2).

Table 2. Sensory analysis of fresh sausages

Selected sensory properties	Scores
General acceptability	3.2±0.40
Consistency	4.0±0.28
Juiciness	4.7±0.51
Color	4.4±0.67
Flavor	4.3±0.69
Taste	4.5±1.03
Average value	4.31

After the completed production period of ripening (five days) we consumed the sausages and found excellent organoleptic properties (taste and odor primarily) and satisfactory quality of them with an average grade 4.31. The high

average grade of the sausages is the result of quality raw material and basic hygiene regulations with which the possibility of microbial contamination is minimized. Our results are similar to results of: Vuković et al.,(2009); Vasilev et al.,(2011).

Conclusion

Chemical and nutritional evaluation showed better compliance (or adherence) to food legislation and improved nutritional quality in relation to the guidelines and the recommendations for healthy food consumption.

The proposed technological procedure enables the production of sausages at home conditions.

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Trout farming generates oxidative stress in amphipod crustacean species *Gammarus balcanicus*

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Abstract: The freshwater amphipod *Gammarus balcanicus* is one of the most widespread crustacean species in Serbian streams. Gammarus shows high level of intolerance on nitrite and phosphate concentration in water habitat. Since trout farming can lead to increse of these compounds, we have chosen these species as bioindicator on the level of oxidative stress biomarkers. Samples were collected from two upstream localities (L1, L2) and two downstream localities (L3, L4). Total amount of Glutathione (GSH), glutathione S transferase (GST) and glutathione reductase (GR) activities have been measured. Concetration of GSH at the locality L3 is significantly lower when compared to L1, L2 and L4. Activity of GR was decreasing at localities L3 and L4 when compared to the L2, while the activity of GST at L4 was significantly lower in comparison to L1. Changes in antioxidative enzyme activity registered at the downstream localities come as a direct consequence of the increase in phosphates and nitrates in the localities down the trout farm, due to the increased concentration of fish feaces and the remains of fish food. Thus, antioxidative defense components and *Gammarus balcanicus* can be considered as excellent bioindicators of the river ecosystems' pollution as the result of fish farming.

Key words: *Gammarus*, oxidative stress, trout farm, pollution

Introduction

A man constantly affects the environment and usually the effect is negative in all aspects of its functioning. The anthropogenic effect is mirrored mostly in clearcutting of forests, construction of plants, storage of large quantities of waste of variouse consistency, water, air and ground pollution by large quantities of waste of various consistency, pollution of water, air and ground by large quantities

of organic and unorganic pollutants, which are a direct result of the industrial processing and of other human activities. The negative effect on the environmnent and different forms of agricultural production such as farming, animal husbandry and fish farming, are also present. One of the aforementioned factors, the fish farming, represents a significant and disconcerting problem due to organic pollution of the river course and intensive construction of the necessary facilities. The number of trout farms in Serbia doubled during the 10-year period from 1997 to 2007, and their annual production reached a level of approximately 2000 tons fish/yr, with a total production growth rate of up to 17.4% (Marković et al., 2007). Moreover, the experimental region of the present study (southwestern Serbia, in the vicinity of Novi Pazar) is experiencing continued population growth and the consequent increase of food requirements. All these factors call for intense and constant fish production, which endangers preservation of the environment and water quality, particularly during periods of low water levels when the pollutant concentration is increasing at a higher rate (Boaventura et al., 1997). Fishponds are usually constructed in the upper flow of the river ecosystems where the water is of the best quality. However, after the water vacates the fishpond, it takes with itself large quantities of uneaten fish food as well as fish feaces which alternate physical and chemical characteristics of the river water, first of all reducing the quantity of dissolved oxygen and in turn affect the life of the rivers. Decay of food and feaces releases large quantity of nitrates and phosphates which have stressogenic effect on the river zoobenthos. Gammarids as detritivorous species play an important role in the trophic food chain of the aquatic environment. They decompose organic matter and serve as prey for amphibians, insects, flatworms, other crustaceans such as crabs and crayfish, and fish (MacNeil et al., 2002). Gammarids have shown to be very sensitive to pollution, in particular from wastewaters (Schneider et al., 2015). They have been used in various studies for toxicity evaluation of river waters and effluents through in situ exposure or mesocosm study, by measuring toxicity endlocalities such as reproduction. Alterations in reproduction endlocalities such as molting cycle, fertility and fecundity may be caused by various pollutants in the effluents and can result in population dynamics impairment (Coulaud et al., 2015). Increased concentration of phosphates and nitrates on a biochemical level can lead to an increase in oxidative stress and can activate antioxidative system of protection which protects the cell from any impairment caused by the stress. Antioxidative system of protection is composed of enzymatic and nonenzymatic components and a change in their activity or concentration is a clear evidence about the induced stress in which case they can serve as excellent bioindicators of the environmental condition. To date, researchers have focused mainly on the examination of physicochemical parameters, biodiversity, and composition of macro zoobenthos communities; the structure of the river bed; and the effects of pollution on activity

of the antioxidative defense system. However, there are relatively few studies on the effects of fish farms and their organic pollution on the antioxidative defense system of crustaceans, particularly in groups such as *Gammaridae*. Aim of this paper is to contribute to better understanding of effects that fishponds have on the environment by analyzing the level of oxidative stress in *Gammarus balcanicus*.

Material and methods

Location description

Raška is a river in southwestern Serbia. The basin of Raška River lies on a limestone terrain, with shale, serpentine, and igneous rocks. In keeping with the specific geomorphology of the terrain, the river flows across a 6.8-m high waterfall at 1 point, which renders it suitable for electric exploitation and also makes it a good supplier for the trout farm located in the village of Pazarište on the outskirts of Novi Pazar. Four locations, 2 upstream (L1, L2) and 2 downstream (L3, L4) from the trout farm, along the channel of the Raška River (vicinity of Novi Pazar, Serbia) were chosen to determine the effects of increased antioxidative stress levels in *Gammarus balcanicus*.

Gathering of samples

After the crustacean were collected with tweezers, they were euthanized immediately in liquid nitrogen for further analyses. Sampling was performed in November 2012, and after measuring and identification, crustaceans were kept in a refrigerator at –24 ºC until the preparation of homogenates. Crustaceans were used to determine antioxidative defense system components. In the series of experiments organized for standardization of a method specific for the species *G. balcanicus*, there were 3 replications in all experimental groups. Physical and chemical water parameters Basic physical and chemical water parameters were measured directly in the field: temperature, dissolved oxygen concentration, pH, and conductivity were ascertained using a water field kit (PCE-PHD meter). Analyses of total phosphorus, orthophosphates, and ionized ammonia were performed in the laboratory according to American Public Health Association protocols (APHA, 1998).

Preparation of homogenates and measuring protein concentration in samples

Values of antioxidative defense components were determined from homogenates of individuals. Crustaceans (100 mg of fresh larval mass) were homogenized (using a homogenizer from IKA-Werke) in 2mL of sucrose buffer (0.25M sucrose, 0.05Mtris-HCl, 1mM ethylenediaminetetraacetic acid; pH 7.4) on ice (3 cycles of 10 s at 2000 rpm). Homogenates were then sonicated for 3

cycles 15 s (using a Bandelin sonoplus HD2070 instrument) and centrifuged on an ultracentrifuge (Beckman L7–55) at 105 000 g and 4 ºC. The supernatant was extracted and frozen at –24 ºC until further experiments.

Determination of protein concentration, antioxidative defense activity

Protein concentration was determined according to Bradford (1976) using bovine serum albumin as the standard. Glutathione reductase activity (GR) was determined according to Glatzle et al. (1974). In brief, changes in the amount of NADPH consumed by the reduction of standard amount of oxidized glutathione (GSSG) were measured spectrophotometrically at a wavelength of 340 nm. The activity was expressed as nanomoles NADPH per minute per milligram protein. Glutathione-S-transferase activity (GST) was determined according to the method by Habig et al. (1974). GST catalyses the conjugation of 1-chloro-2.4-dinitrobenzene (CDNB) with the SH groups of glutathione. The amount of derived CDNB–glutathione complex was measured spectrophotometrically at 340 nm and expressed in nanomoles GHS per minute per milligram protein. Determination of the total concentrations of glutathione was conducted according to the method described by Griffith (1980). Sample proteins were precipitated in the homogenates by sulphosalicylic acid. The method is based on the recycling procedure where the oxidation of GSH with DTNB (producing 5.5-dithiobis-nitrobenzoic acid) and its reduction by glutathione reductase with NADPH are conducted reciprocally. The rate of formation of 2-nitro-5-thiobenzoic acid was monitored spectrophotometrically at 412 nm, and the concentration of total glutathione was calculated in accordance to the standard and was expressed per grams of wet mass.

Statistical analysis

Results were processed using the SAS Ver 9.1.3 statistical package. The prerequisite for analysis of variance was the normality of distribution within a group achieved through logarithmic transformations of the traits (Sokal and Rohlf, 1981). Differences in the average values between different upstream and downstream locations were assessed by one-way ANOVA followed post hoc by Fisher's least significant difference test.

Results and discussion

Upon analyzing physical-chemical paramteres in the river, it was noticed that the amount of phosphates, nitrates and free ammonia was significantly increased downstream from the fishpond, while at the same time the concentration of dissolved oxygen is dropping (Table 1).

Table 1. Values of chemical parameters along the investigated locations at the Raška River measured in November 2012. DO - dissolved oxygen; PO4 - orthophosphates; NH4 - total ammonia; NO3- nitrates; All values of measured chemical parameters are shown in mg/L

location	Chemical parameters			
	NNH4	SPO4	NNO3	sDO
L1	00.032	1<0.01	57.50	810.4
L2	00.029	10.016	16.70	111.0
L3	00.314	10.016	17.90	17.0
L4	00.222	00.017	18.30	18.0

Concetration of different matters which come from the fishpond significantly influence the change of the activity of glutathione reductase (Figure 1.) with the organisms that live downstream from the fishpond in comparison to those that live upstream from the fishpond ($F=3,53$; $p<0,05$*). LSD test shows significant decrease of activity with the organisms from the second donwstream locality(L4) when compared to organisms from the second dupstream locality(L2). Glutathione reductase plays an important role in the antioxidative protection of the cell because it catalyzes regeneration of glutathione (GHS) from its oxidative form (GSSG). Change of GR activity in terms of its decrease can be interpreted as chronic action of stress on *G.balcanicus* in localities downstream from the fishpond. Actually, Ensibi et al. (2013) localityout that the activity of antioxidative enzimes can be reduced by chemical stress depending on the intesity and duration of the stress. Glutathione S transferase catalyzes the reactiong of conjugation, making the conjugation of electrophile matters easier, such as reactive forms of oxygen, which make it more hydrophilic for transport and excretion (Ensibi et al., 2013). By comparing organisms from the upstream to those from the downstream localities of the fishpond, it has been established that the activity of glutathione S transferase does not change significantly (Figure 2.). However, LSD test shows that the activity on the second donwstream locality(L4) is significantly lower than the activity of GST at the first upstream locality(L1). Similar results can be found with Barros and associates (2017) who tested the infulence of triclocarban (TCC) on the acitivity of GST with *Gammarus locusta*. In fact, it showed that by increaseing the amount of TCC, the activity of GST is falling which can be understood by the presence of a larger quanity of lipid peroxides that appear as a result of increased concentration of pollutant when oxidative defense is no longer efficient which concurs with our results. The amount of total glutathione (Figure 3.) is heavily dependant of the presence of matters which

come from the fishpond (F=3,94; p<0,05*), and the amount of glutathione is significantly lower at the first downstram locality(L3) when compared to the upstream localities. Reduction of concentration of total glutathione at L3 and its significant increase at L4 when compared to L3, is connected with the change in the dissolved oxygen in the water and the disruption of the reversible cycle of oxidation and the reduction of glutathone during the detoxification of organisms (Gismondi et al., 2012). These changes usually happen in normal circumstances as well on a yearly level and during different seasons when the level of production in ecosystems is changing together with the amount of availabel oxygen.

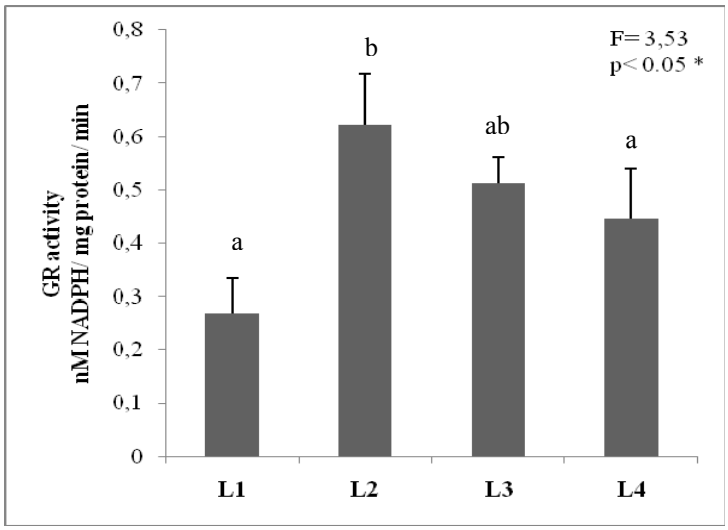

Figure 1. Glutathione reductase (GR) activity in *Gammarus balcanicus* at 4 locations, 2 upstream (L1, L2) and 2 downstream (L3, L4) from the trout farm on the Raška River. Bars represent the mean values ± standard error. Significance of the effects was tested by one-way analysis of variance and compared post hoc by Fisher's least significant different test (p<0,05*)

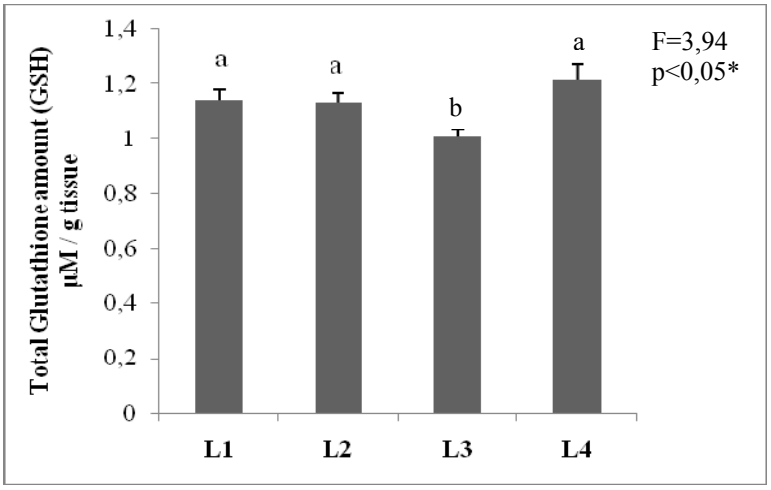

Figure 2. Total amount of Glutathione (GSH) in *Gammarus balcanicus* at 4 locations, 2 upstream (L1, L2) and 2 downstream (L3, L4) from the trout farm on the Raška River. Bars represent the mean values ± standard error. Significance of the effects was tested by one-way analysis of variance and compared post hoc by Fisher's least significant different test (p<0,05*)

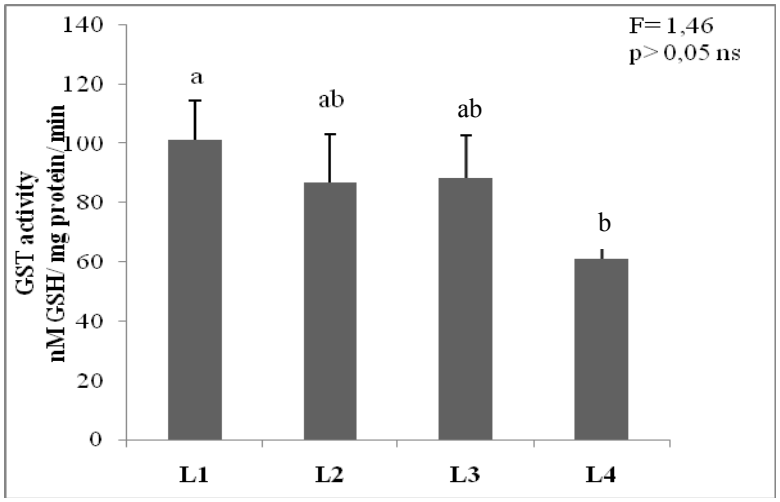

Figure 3. Glutathione S transferase (GST) activity in *Gammarus balcanicus* at 4 locations, 2 upstream (L1, L2) and 2 downstream (L3, L4) from the trout farm on the Raška River. Bars represent the mean values ± standard error. Significance of the effects was tested by one-way analysis of variance and compared post hoc by Fisher's least significant different test (p>0,05 ns – not significant).

Conclusions

The presence of fishponds affects the change of physical and chemical characteristics of water in Raska River. As a consequence of the changed environmental conditions, first of all reduced amount of oxygen and increased amount of phosphates, ammonia and nitrates, oxidative stress in organisms such as *G. balcanicus* that live on the benthos of the river bed can be seen. The results indicate that the species *G. balcanicus* can serve as an excellent bioindicator of freshwater pollution. Changes in the concentration of GSH and GST and GR activities of *G. balcanicus*, as a very sensitive aquatic macroinvertebrate, can be used as biochemical biomarkers of freshwater pollution.

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Testing the level of financial literacy of urban and rural population in Republic of Srpska

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Apstract: The aim of the study was to investigate knowledge on the most important financial terms related to the loan and some relations connected to it, of both urban and rural population in the Republic of Srpska. The subject of the research is to determinate the level of the financial literacy of the population as well as the comparison of knowledge and familiarity between urban and rural residents of our country. The work consists mainly of primary data collected by surveying respondents. The secondary data was collected from several different sources of finance and banking. The methods used for the processing of the above-mentioned data are: descriptive statistics and χ^2 test. The SPSS program was used to determinate the posibile existence of significant differences between the responses obtainted in the survey of both urban and rural residents. The results obtained by the analysis and processing of statistical data show an increased level of financial literacy of the populatin in the Republic of Srpska.

Key words: literacy, population, credit, urban, rural

Introduction

Testing the finacial literacy can be defined as testing knowledge and familiarity of our population on many different financial termes that, in our case, are realated to loans and credit relations. The loan is one of the terms we can often hear about nowadays, and because of that we wanted to check the level of knowledge of our population connected to the credit realitions with the

assumption that majority of our population is actually very well familiar with few financial termes considering the above-mentioned relations.

In the modern age, a loan represents an essential part of every country's economy but also to some people, it represents a big part of their lifes. Many of us, due to insufficient monthly income, are not able to afford certain things and in those type of situations we turn to banks or other financial institucions. People take different types of loans with different amount which of course, depends on their needs. By taking the loan they become deptors or borrowers and they need to pay a certian price that comes with the credit realtions. The amount of price they pay depends on the type of the loan, the repayment period and other important factors.

A loan, as one of the many funding sources also represents the most common external form of financing in agriculture that has a very high risk of borrowing money for banks and other finanacial institucions. It is necessary to find a way to decrease the risk which will enable better loan deals to agriculture along with the farmers who will be able to adapt to the new and easier credit conditions.

According to the research of various institutions in Croatia that took place back in 2015, using the OECD methodology, testing the financial literacy is actually extremely important for the country. The research vas conducted by Ipsos agency. The results were presented in 2016 by the Croatian National Bank and the Croatian Financial Services Supervisory Agency.

Material and Methods

In our scientific research we used two types of methods: the survey of urban and rural population in the Republic of Srpska and for processing data we collected from the survey, we used descriptive statistics methods and the $\chi 2$ test. The above-mentioned data was processed in the SPSS. The survey included 332 people, of whom 185 of them were from the urban and 147 from the rural areas of our country.

By reducing the gap between the number of urban and rural residents, we were able to get more accurate results on their knowledge of the particular financial terms which in our case are realated to a loan and lending conditions. That allowed us to have a better comparisson on the level of the financial literacy between urban and rural population.

The respondents were asked questions regarding their personal expiriences with loans, which allowed as to separate those who took and those who have never taken a loan at a certain financial institucion. Some of the respondents were interviewed "face to face" (73) while the rest of them (259) were questioned via the Internet. Those we questioned verbally were immediately informed regarding some of the financial terms they were not familiar with.

As for the age, the majority of urban respondents (69.5%) are young people, age 18 to 25. The rural population shares a similar case in this situation. Just a little more then a half of the rural population (51.52%) are young people, age 18 to 25. Although the population of aforementioned age was not our target group, we did sucessed in examing the knowledge of the young population who lives in Republic of Srpska, regarding the financial terms that are very common nowadays, which is certainly a great benefit for the final results of the reasearch. Of course, in our further presentation, we will not put the emphasis exclusively on the respones of the young people, age 18 to 25, but to all answers we collected from all age categories.

The χ^2 test is based on the χ^2 distribution which can be used for solving several problems. In our case, we used the well known testing the equality (or differences) of the propotions of three or more sets, which is also know as testing the homogenity of the observed phenomenon. (2006. Lovrić, M., Komić, J., Stević, S.)

$$\chi^2 = \sum_{i=1}^{n} \frac{(fi - fi*)^2}{fi*}$$

Results and discussion

As far as the experience of our respondents with loans is concerned, the situation is similar in both urban and rural areas of Republic of Srpska. The answers we received are expressed in percentages that can be seen in the table below.

Table 1. Previous experience of our respondents with the loan

Offered answer	Urban	Rural
Yes I have taken a loan	50.7 %	46.8 %
No I haven't taken a loan	49.3 %	53.2 %

Most of the urban residents who took the loan responded that they had a fairly good credit experience (38.5%) as well as the rural residents, though with a slightly larger share of 53.3%.

Some of the respondents who haven't taken the loan yet, have also been asked why they haven't decided to take a certain type of loan. Most of the urban population stated that they don't have the need for a loan. As far as the answers of our rural population go, reasons why they have not taken the loan are high interest rates and "other" which is one of the answers we offered in our survey.

Previously obtained answers can also be presented in a tabular form which is shown below:

Table 2. Reasons for not taking the loan

Offered answer	Urban	Rural
High interest rates	26.1 %	25.8 %
Lack of trust	18.8 %	18.3 %
Insufficient knowledge about the offer	2.9 %	7.5 %
Not having the need	36.3 %	22.5%
Other	15.9 %	25.8 %

When talking about financial institutions, the vast majority of the urban population states that banks are the financial institutions where they took the loan. Generally speaking, this relation is not that realistic, given the fact that although microcredit organizations offer loans at much higher interest rates, the population and the economy are more likely to decide on those types of loan, because conditions of the loan are not so much demanding. The relationship between bank and micro-credit organizations' loans is somewhat different in the case of rural population.

Table 3. Type of the financial insitution where respondents took the loan

Offered answer	Urban	Rural
Bank	86.4 %	66.67 %
Microcredit organisation	11.4 %	33.33 %
Leasing company	2.2 %	-

We have started to question the financial literacy of the population by asking our respondents how familar they think they are with financial terms related to credit relations. This way we have obtained their personal opinion about their own knowledge on this terminology.

In both cases we can link the obtained answers with the level of education of the population. Most of the highly educated both urban and rural people think that they are either familar or partially familiar, while all those who are not that familiar have either primary or secondary education. There is a strong correlation between the level of ones qualification and their financial literacy.

In order to examine the knowledge of our respondents about the loan and terms realted to it in more detail, we have also asked some questions regarding: interest rate, grace periods and some collaterals as loan security instruments. The interest rate as a price of the loan, represents a significant factor in credit relations (2011. Mikerević, J., D.) and huge majority of both urban and rural population in Republic of Srpska have at least heard about the concept of interest

rate and they know what it means. For most of them, the interest rate greatly influences their choice when choosing a loan.

Table 4. Familiarity of the respondents with financial terms

Offered answer	Urban	Rural
Totally familiar	6.3 %	-
Familiar	31.2 %	26.9 %
Partially familiar	51.6 %	59.7 %
Not familiar at all	10.9 %	13.4 %

There are several types of interest rates but those that are most important in our case are nominal and effective interest rate. Since the effective interest rate is always higher than the nominal (2011. Mikerević, J., D.) because it is actually the nominal interest rate increased by the loan approval expense, we were interested in whether our residents actually know the difference between these two terms.

Table 5. Opinion of the respondents about the difference between NIR and EIR

Offered answer	Urban	Rural
NIR > EIR	7.3 %	27.1 %
NIR < EIR	36.8 %	23.3 %
NIR = EIR	1.5 %	3 %
I don't know	54.4 %	46.6 %

There is a somewhat different situation regarding questions about the grace period which can be defined as a postponed financial obligation. This is actually a time period where debtors only pay the amount of the interest rate (2013. Vaško, Ž., Ivanković, M.). The majority of the population are very well familiar with the meaning of aforementioned financial term.

It is often questioned whether the grace period is in fact a financial relief for the debtor or simply an additional expense. Although it represents both, we were interested in whether our respondents would decide if grace period is a cost or a relief for the debtor.

Most of the urban and rural population believe that the grace period is a financial relief for the loan users, while the smallest part of the respondents decided that grace period is an additional cost. Of course, the collected data can also be shown via percentages in tabular form.

Testing the financial literacy was finalised by questions regarding the endorser as one of several types of collaterals. Collaterals represent instruments that are usually used by a bank or other financial institution to decrease a potential risk of losing their money. Both urban and rural population have heard

about the term "endorser" and they know what it means, and a small part of the respondents had an endorser or they have been one before. Most of them are familiar with the term "collateral".

Table 6. Opinion of the respondents about the true meaning of grace period

Offered answer	Urban	Rural
Financial relief	65.1 %	56.7 %
Additional expense	9 %	9.3 %
I don't know	25.9 %	33.6 %

Table 7. Is an endorser a collateral?

Offered answer	Urban	Rural
Yes	78.3 %	71 %
No	8.7 %	13 %
Maybe	5.8 %	9.4 %
I'm not sure	7.2 %	6.5 %

We have finalized the survey with issues related to agriculture as an economic activity. Agriculture is one of the high risk activities when it comes to investing and financing. At the same time, it is quite risky for banks and they often have an aversion on giving loans to the farmers.

In the Republic of Srpska the situation concerning agriculture and farmers is at a quite low level. With insufficient support of our country on the one side and demanding credit conditions on the other, our agricultural producers have been brought to a very difficult position.

More than a half of both urban and rural residents agree that credit conditions in our country are extremely demanding. They also agree with the fact that agriculture as an economic activity requires better and less demanding credit conditions.

The survey was finalised with the last question also regarding our agriculture. We wanted to know how many of our respondents are actually engaged in the agricultural production, and also how many of them work at their own farm.

Table 8. Testing the engagment in agricultural production

Offered answer	Urban	Rural
No and I don't plan to engage	58.3 %	21.8 %
No but I do plan to engage	18.7 %	21.8 %
Yes and I have my own farm	16.7 %	34.5 %
Yes but I don't have my own farm	6.3 %	21.8 %

We have already mentioned using the χ^2 test to show a possibile significant statistical difference between answers of both urban and rural residents of our

country. The following table shows some of the questions we asked our respondents and the value of the obtained coefficient.

Table 9. Testing the equality with the χ^2 test

Number	Name of the question	χ^2
1.	Previous expirience with loans	0.718
2.	The impact of interest rate	0.343
3.	Knowing the difference between NIR and EIR	0.042
4.	Understanding the concept of an endorser	0.817

Based of testing the equality of two sets, using the chi square test, we concluded that there was no statistically significant difference between the number of urban and rural population who have taken a loan, because the significance coefficient is above 0.05.

By testing the statistical significance, we concluded that the urban and rural population had the same answer on the question: Does the interest rate influence their choice when taking a loan? The majority of respondents agreed that the interest rate does affect their choice when taking a loan.

On the other hand, we found out there are significant differences between rural and urban population in understanding the concepts of nominal and effective interest rate.

In the end, we came to a conclusion that there is no statistically significant difference in understanding the term "endorser", and the majorty of both urban and rural population of our country are familiar with the fact that an endorser is actually one of many collaterals.

Conclusion

By processing the collected data we have previously shown, we concluded that the majority of both urban and rural population of the Republic of Srpska are in fact familar with terms that are loan related, and therefore we believe that the vast majority of respondents are actually financially literate. This allows us to justify our previous assumption and therefore we have accepted the hypothesis that the majority of people are very well familiar with some of the financial terms we already mentioned.

As for those people who are not that financially literate, we believe that it is necessary to find an appropriate way that will expand their awareness and improve their knowledge, especially when it comes to the younger generations of our country. This way, we want to increase the awareness of people on concepts related to credit relations because they will be better oriented in

situations when they actually need a loan but aren't so sure or don't know at all what some of the financial terms related to the loan mean.

The majority of our residents have partially good experiences with loans, which can also be seen as a confirmation of their financial literacy. Our goal is to keep this level of financial literacy in the future, which will influence next generations of our residents to have this or maybe even higher level of financial knowledge.

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The Presence of Fish in Population's Diet

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Abstract: Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium and iodine. Fish are sources of other important nutrients, including the long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA), which are associated with reduced heart disease risk. This paper is done on the basis of analysis the fish consumption among the population in city of Bitola (Macedonia), and the level of their awareness of its nutrition value, regardless of age and sex of the responders. The methodological approach in this paper is based on the descriptive method on the basis of the data that has been collected from the field.

Key words: fish, health

Introduction

Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium and iodine. Its protein, like that of meat is easily digestible and favourably complements dietary protein provided by cereals and legumes that are typically consumed in many developing countries.

Fish are an important source of protein worldwide. Globally, they comprise about 6 percent of dietary protein, but for 3 billion people, fish account for up to 20 percent of the average per-capita intake of animal protein (FAO, 2014). Fish are sources of other important nutrients, including the long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA), which are associated with reduced heart disease risk.

Because of the potential health benefits of fish, the Dietary Guidelines for Americans (DGA) recommend that people consume 8 ounces of seafood per

week, especially marine-derived "oily" fish such as salmon, mackerel, sardines, pompano, anchovies, swordfish, trout and tuna, to provide an average daily consumption of 250 mg of EPA/DHA per day (USDA & HHS, 2010). Other fish provide these fatty acids, but levels are low enough that very large amounts of fish would have to be consumed each day to meet the recommendation. Although another omega-3 fatty acid, alpha linolenic acid (ALA), can be converted into EPA and DHA, the conversion is fairly limited in humans.

The aim of this research was to determine the fish consumption among the population in city of Bitola (Macedonia), as well as, the level of their awareness of its nutrition value, regardless of age and sex of the responders.

Material and methods

This paper is done on the basis of analysis the fish consumption among the population in city of Bitola (Macedonia), as well as, the level of their awareness of its nutrition value, regardless of age and sex of the responders.

The methodological approach in this paper is based on the descriptive method on the basis of the data that has been collected from the field.

The data is collected from own research obtained from the field by using the method of examination. The examination is done by using empirical techniques of survey and personal interviews with a pre-structured questionnaire to group participants from Bitola (The questionnaire is provided in Appendix).

Results and discussion

Analyses for the fish consumption among the population in Bitola were made on a total of 92 participants, all of them from Bitola, from which, 47 were woman and 45 man. All of them were being above the age of 20. When they were asked if they are taking care of their health, 63 responded with "*yes*", 20 with "*no*" and 9 with "*I don't know*". 65 of the responders answered that they are "*healthy*", 24 of them that they "*have heart disease*" and 5 of them that they "*have allergy*", but neither to a food, nor fish. When they were asked if they take any supplements, 73 answered "*no*" and 18 "*yes*". Most used supplements were: *multivitamins* (4), *vitamin C* (8), *B complex* (6), Fe (2) and *Mg* (5).

The following results are from the answers of the section "SPECIFIC QUESTIONS" in the Questionnaire:

Question 1. *"Do you eat fish?"* 83 participants "*eat fish*" and 9 participants "*don't eat fish*" because they are vegetarians, don't like the taste, don't like to clean fish bones, or because they can't afford it.

Question 2. *"How often do you eat fish?"* 41 participants answered "*once per week*", 9 participants answered "*twice per week*", 3 participants answered "*more*

than twice per week", 23 participants answered "*once per month*", 11 participants answered "*rarely*" and 4 of them "*never"*.

Question 3. *"How do you like your fish prepared?*" 9 participants answered "*canned*", 19 participants answered "*baked*", 4 participants answered "*boiled*", 34 participants answered "*fried*", 14 of them answered both *"canned" and "fried*", 2 of them answered *both "baked" and fried*", 6 participants answered "*canned", "baked" and fried*" and 4 of them "*don't eat fish"*.

Question 4. *"Which type of food do you usually eat"* 22 participants answered that they prefer *"low-fat*" and 70 answered that they prefer "*regular*" food.

Question 5. "*Do you think that fish is low fat food?"* 71 participants said "*yes*" and 21 participants said "*no*".

Question 6. *"What kind of fat the fish have more?"* 16 participants answered "*saturated fat*" and 76 participants answered "*unsaturated fat*".

Question 7. *"Do you think that fish is excellent source of omega- 3 fatty acids?"* 85 participants answered "*yes*" and 7 participants answered "*no*".

Question 8. *"Do you think that omega-3 fatty acids are good for your health?"* 13 participants answered "*no*" and 79 participants "*yes*", 28 of them answered that they "*don't know why omega-3 fatty acids are good*", 30 participants answered that they are "*good for the heart and circulatory system*", 10 participants answered that they are good for "*general health and well-being*", 8 answered that they are "*good for the eyes*", 6 answered that they "*can help keep your brain healthy and reduce your risk of cognitive decline*" and 4 answered that they "*can help to keep your bones healthy*".

Question 9. *"Do you think that fish is excellent source of protein?"* 77 of the participants answered "*yes*" and 15 participants answered "*no*".

Question 10. *"Do you think that fish contains enough minerals and vitamins?"* 64 of the participants said "*yes*" and 28 participants answered "no".

Question 11. *"Which one do you think has more vitamins and minerals?" with possible answers a) freshwater fish and b) salt water fish,* 24 of the participants answered *" freshwater fish"* and 68 participants answered *"salt water fish"*.

Question 12. *"Do you think that fish is calorie rich food?* 62 of the responders answered "*no*" and 30 of the responders answered "*yes*".

Question 13. "*Eating fish may reduce the risk of stroke, heart disease, Alzheimer's disease…" there were two possible answers a) true and b) false.* 65 of the responders answered *"true*" and 27 of the responders answered *"false"*.

Question 14. *"Do you prefer saltwater fish or freshwater fish?"* 41 responders answered *"salt water fish",* 45 of them answered *"freshwater fish",* 2 of them answered that prefer *"both salt water fish* and *freshwater fish" and* 4 *answered "don't eat fish"*.

Question 15.*"Which species of freshwater fish do you often eat?"* 63 participants said "*brown trout*", 43 participants answered "*carp*", 3 participants "*sheatfish*", 5 participants "*common bleak*", 2 participants "*the northern pike*", 4 participants "*grass carp*", 2 participants "*European chub*", 1 participant "*bighead carp*", 4 of the participants said "*don't eat fish at all*" and 4 answered "*don't like freshwater fish*".

Question 16. "*Which species of saltwater fish do you often eat?"* 56 participants answered "*European hake*", 41 participants answered "*scomber*", 25 answered "*tuna*", 14 answered "*Atlantic salmon*", 10 answered "*European pilchard*", 2 answered "*Atlantic cod*", 4 said "don't eat fish at all" and 3 answered "don't like salt water fish".

Table 1. The use of fish per year

Fish species	**Average use of fish per year**						
	1 per day	**1 per week**	**2-3 per week**	**1 per month**	**2-3 per month**	**1 per year**	**Never**
Carp	/	5	2	27	5	35	18
Brown trout	/	8	2	43	8	13	18
European chub	/	/	/	/	1	5	86
Grass carp	/	/	/	4	/	15	73
Bighead carp	/	1	/	1	/	15	75
The northern pike	/	/	/	/	/	3	89
Sheatfish	/	/	/	5	/	24	63
Common bleak	/	/	3	3	3	24	59
Atlantic Salmon	/	3	/	18	2	20	49
Atlantic cod	/	/	/	2	/	3	87
Atlantic herring	/	/	/	1	/	6	85
European pilchard	/	24	5	15	8	5	35
Scomber	/	10	3	34	6	13	26
Tuna	/	26	2	31	10	3	20
European hake	/	17	4	38	14	7	12
Canned fish	/	39	5	24	8	2	14

Conclusion

Even in small quantities, fish can have a significant positive impact in improving the quality of dietary protein by complementing the essential amino acids that are often present in low quantities in vegetable-based diets.

But recent research shows that fish is much more than just an alternative source of animal protein. Fish oils in fatty fish are the richest source of a type of fat that is vital to normal brain development in unborn babies and infants. Without adequate amounts of these fatty acids, normal brain development does not take place.

Eating fish is an important source of omega-3 fatty acids. These essential nutrients keep our heart and brain healthy. Two omega-3 fatty acids found in fish are EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Our bodies don't produce omega-3 fatty acids so we must get them through the food we eat.

Omega-3 Fatty Acids:

- help maintain a healthy heart by lowering blood pressure and reducing the risk of sudden death, heart attack, abnormal heart rhythms, and strokes.
- aid healthy brain function and infant development of vision and nerves during pregnancy.
- may decrease the risk of depression, ADHD, Alzheimer's disease, dementia, and diabetes.
- may prevent inflammation and reduce the risk of arthritis.

Omega-3 fatty acids are found in every kind of fish, but are especially high in fatty fish. Some good choices are salmon, trout, sardines, herring, canned mackerel and canned tuna.

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Trends in fruit production in the federation of Bosnia and Herzegovina for the period 2006-2015

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Abstract: The subject of this research are the trends of four the most common kind of fruits in Federation of Bosnia and Herzegovina for the period from 2006 to 2015. Although positive trends in the number of birth trees and the total yield of all types of tested fruit trees have been recorded from year to year, the average yields have a negative trend, which is actually about the disproportionate growth of the number of birth trees and the total yield, ie the trend of the growing trees grows at a higher rate than the total yield. In this paper was noted a great fall in production of all types of examined fruits during the years of adverse weather conditions and natural disasters (drought, flood, frost, hail). That fact points out that in the Federation of BiH there are no yet fully adequate agrotechnical precautions, adequate techniques and technologies to prevent the negative impact of mentioned climatic conditions on agricultural production. Also, there is a lack of budget support by entity / cantonal authorities to the producers to mitigate the adverse effects of climate change.

Keywords: fruit production, trend, Federation of Bosnia and Herzegovina.

Introduction

Fruit growing as an important segment of agricultural production, after cattle breeding, has the most favorable conditions for exploitation in BiH. In the most important fruit regions of our country there is a moderate continental climate, with average annual temperatures of 11-12°C. The Federation of Bosnia and Herzegovina, one of two BiH entities, with its diverse emblem and the mellow climate has very significant natural potentials for fruit production. Despite the favorable climatic and soil conditions for the cultivation of a large number of fruit trees, both continental and mediterranean, FBiH is still dominated by

extensive orchards with low average yields. Manufacturing capacities are very modest, and permanent placement channels are still unmanaged. All this leads to the conclusion that the fruit production sector is still at a low level of development, despite positive tendencies in planted areas (number of trees) over the last decade (Federal Ministry of Agriculture, Water Management and Forestry, 2014). As an important segment of agricultural production in the Federation of Bosnia and Herzegovina fruit production has undergone extensive expansion in the post-war period, since intensive production began immediately after the war. Due to the optimum climate and soil conditions in the Federation of Bosnia and Herzegovina the most prominent is cultivation of apples and pears in the group of apple fruit as well as plum and cherry from the group of horticultural fruit. However, it is important to note that plums occupy more than 50% of the total fruit production structure in the Federation of BiH. All of the above was a motive for researching the trends in fruit production of the Federation of Bosnia and Herzegovina during the previous ten-year period (2006-2015), which could predict the events in the forthcoming period. Therefore, the purpose of this paper is to establish, research and analyze the trends of fruit production during a ten-year period in the Federation of BiH. The aim of the paper is to show the trends of selected major fruit production in the Federation of Bosnia and Herzegovina in the period 2006-2015, and according to them to predict production in 2017.

Material and method

In order to determine the trends of the researched phenomenon in the fruit production of the Federation of Bosnia and Herzegovina, it was necessary to collect secondary data related to the research period from 2006 to 2015. In this sense, as sources were served statistical yearbooks by the Federal Bureau of Statistics. In addition to this individual statistical bulletins at Canton level (cantons in figures) have been used, as well as thematic bulletins published by the same institution - the Federal Bureau of Statistics. The tendency method was used to determine tendencies of the observed phenomena. The trend is the tendency of one phenomenon observed over a certain period of time. When calculating the trend, time is taken as independent variable (ti), and the observed phenomenon as a dependent variable (Yi). Accordingly, the trend is expressed in the following way: Yi = f (ti). Depending on the actual trend of some phenomenon the trend may be straight (linear) and curve. In this paper was used a linear trend, where the straight trend line is determined by the following equations: Yi = a + b ti, where: Yi = the average estimated value of the observed phenomena over a given time period; A = average initial level of phenomena; B = average increase or decrease of phenomena in a given time unit and ti = time

point for which the calculated value (Yi) is. Determining the trend equation is calculated by computing the parameters (a) and (b), and then by presenting the trend equation graphically. (Horvat, Mijoč, 2012). The obtained trend also allows predicting the further development of the phenomenon, based on the known data, in the series. When the development of the phenomenon is represented in advance or reversed we use an extrapolation. Determination of unknown values of the examined phenomenon within a given series is called interpolation.

Results and discussion

This chapter presents the results of the research. Trends in the total number of trees, total production and average yields were made for apple, pear, plum and cherry. By the number of birth trees, but also by production, the predominant is the plum, then apple while the pear and cherry are less represented in the production. However, the trend of average cherry yield showed a positive trend, while in other fruit trees a negative trend of average yield. This actually tells us about the disproportionate growth of the number of birth trees and the total yield, ie that the trend of birth trees grows at a higher rate than the total yield of apples, plums and pears. The following table shows the number of birth trees, total and average yield of four selected types of fruit in the Federation of Bosnia and Herzegovina for the period 2006-2015.

The following graph shows the trends of the number of birthtrees of apple, plum, pear and cherry in the Federation of Bosnia and Herzegovina for the period 2006-2015.

On the basis of the chart 1 it can be observed positive trends of birth trees of all examinated types of fruits. In the observed period, the number of apple trees increased by 259.790. new trees or by 12.2% on an average yearly basis. However, there is a noticeable decrease in the number of apple trees in 2010, and this is due to drought and spring frosts that hit the area of Bosnia and Herzegovina that year. Then the number of apple trees is experiencing a jump in 2011 and continues to grow until 2016. The number of pear trees increased by 41.448. trees annually or by 3,76%, while the number of plum trees increased on average by 99.999. trees annually or by 1,84%. In the observed period the number of cherry tree increased by 15.308. annually or by 3.43%. If the stated tendency of increase continues in 2017 we can expect the following number of fruit trees in the Federation of BiH:

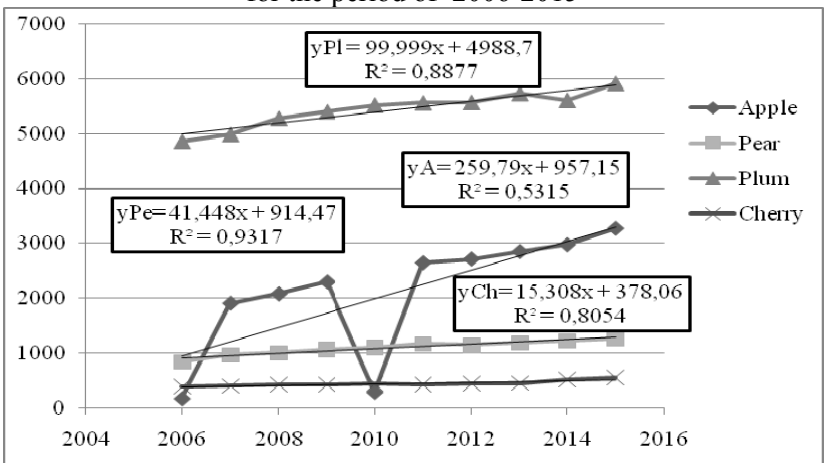
- apple 3.814.840
- pear 1.370.398
- plum 6.088.689
- cherry 5.787.342

Table 1. Number of birth trees, total production and average yield of selected fruit type in FBiH for 2006-2015.

Fruit	*Year*									
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Apple										
Number of trees, in 000	175	1915	2085	2305	292	2653	2722	2856	2980	3279
Total production, tons	20604	19702	21057	26492	26032	29000	15751	32652	16852	41929
Average yield, kg/tree	11,8	10,3	10,1	11,5	10,5	10,9	5,8	11,4	5,7	12,8
Pear										
Number of trees, in 000	847	967	1008	1069	1106	1175	1147	1196	1233	1262
Total production, tons	7875	7458	8280	9891	9053	9963	5847	11554	5181	11719
Average yield, kg/tree	9,3	7,7	8,2	9,3	8,2	8,5	5,1	9,7	4,2	9,3
Plum										
Number of trees, in 000	4860	4988	5275	5398	5516	5557	5563	5723	5601	5908
Total production, tons	37950	45277	35044	53434	56557	57232	35312	83342	26818	45685
Average yield, kg/tree	8,1	9,1	6,6	9,9	10,3	10,6	6,3	14,6	4,8	7,7
Cherry										
Number of trees, in 000	387	401	422	420	442	431	443	451	517	556
Total production, tons	3995	4567	4805	5314	5336	6191	4282	6116	4952	6440
Average yield, kg/tree	10,3	11,4	11,4	12,6	12,1	14,4	9,7	13,6	9,6	11,6

Source: Federal Bureau of Statistics (2006-2015).

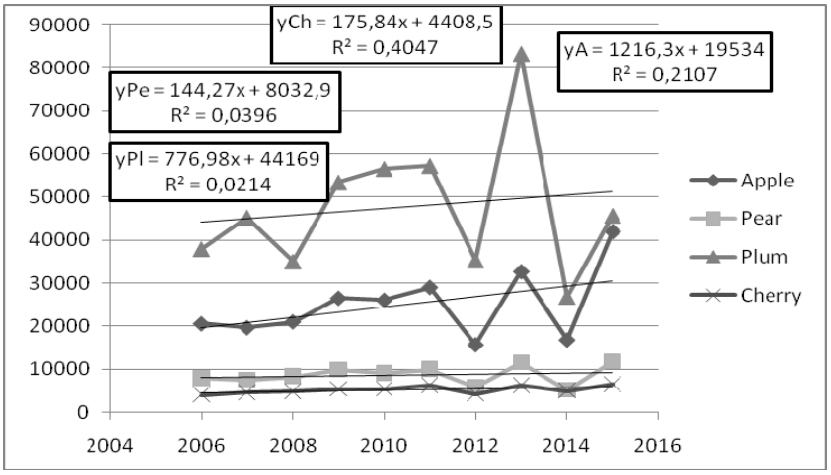
Graph 1. The trends of the number of apple and horticultural fruit trees in FbiH for the period of 2006-2015



Source: Federal Bureau of Statistics (2006-2015).

The following graph shows the trends of total production of selected fruit types in the Federation of BiH for a given period.

Graph 2. The trends of total apple and horticultural fruit yields in FBiH for 2006-2015



Source: Federal Bureau of Statistics (2006-2015).

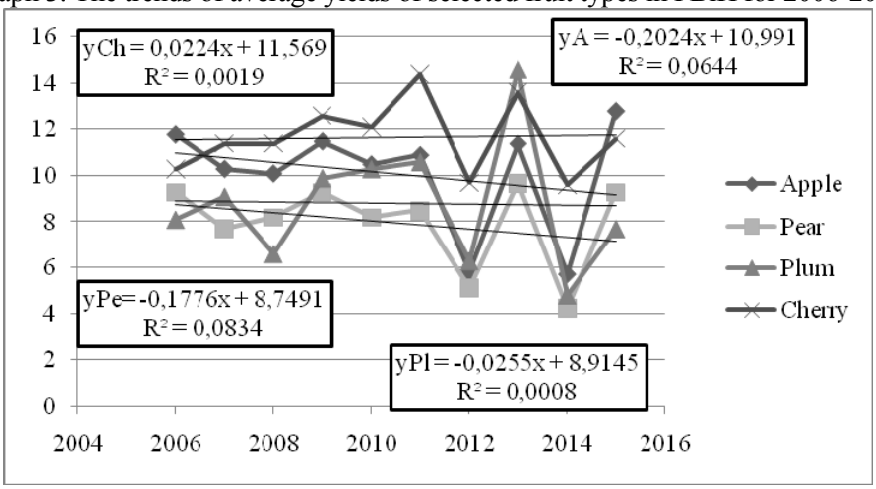
On the basis of chart 2, positive trends can be observed in all four types of fruit types surveyed in the Federation of BiH. In the observed period, total apple production increased annually by 1216.3tons, or by 4.86%. The total production of

pears increased annually at a lower rate than apple by 1.66% or 144.27t.The value of total plum production increased annually by 776.98t, or by 1.63%. Plum is the most cultivated crop in the FBiH area. What emerges as the main conclusion by observing the established trend of total plum production is significant variations from year to year. Considering that in 2008, 2010 and 2012 in Bosnia and Herzegovina in the summer months it was drought, it is obvious that the observed oscillations in the total yields achieved are the result of such unfavorable climatic conditions. Total cherry production increased annually by 175.84tons or by 3.38%. From the chart 2 we can also notice a large drop in production in all four examined fruit types in 2014, with plum being the biggest drop. The reason for this is that the May floods that occurred that year and caused enormous damages and losses in agricultural production. If the increase tendencies continue in 2017 we can expect the following total fruit production in the Federation of BiH, expressed in tons:

- apple: 32.913,3;
- pear: 9.619,8;
- plum: 52.715,7;
- cherry: 6.342,7.

The following chart shows trends of average yield of four selected fruit types in the Federation of BiH for the given period.

Graph 3. The trends of average yields of selected fruit types in FBiH for 2006-2015



Source: Federal Bureau of Statistics (2006-2015).

Based on Chart 3, we see that a positive trend of average yield was observed only in cherry, while other types of fruits observed a negative trend of average yield. In the observed period, the average yield of apples decreased annually by -0,20 kg per tree or by -2.01%. Average pear yields decreased annually more than

an average apple yields rate (-2.23% or -0.18 kg / tree). The average plum yield value was reduced at least annually, ie by -0.03 kg / tree or at -0.29%. Unlike those three fruit types, average cherry yield in the observed period increased annually to 0.02 kg per tree or 0.19%. If the noted movements of average yield continue to go in the same direction, in 2017 we can expect the following average fruit yield in FBiH, expressed in kg / tree:

- apple : 8.76;
- pear: 6.79;
- plum: 8.63;
- cherry:11.82.

Conclusions

In this paper, the aim was to present the trends of selected major fruit production in the Federation of Bosnia and Herzegovina in the period 2006-2015, and based on it to predict their production in 2017. Using the trend method, it was established that in the period 2006-2015 in all the examined fruit types the trend of the number of birth trees and total production has positive tendencies. The average yield trend has negative tendencies in apple, pear and plum, while in cherry has a positive trend of average yield in the period 2006-2015.The obtained results show that in selected fruit crops the yields (total and average) are still most dependent on climatic conditions. For now, there is no adequate agrotechnics in the FBiH, a strategy for the development of the agricultural sector has not been implemented, nor does the entity / cantonal government allocate resources to help the agricultural producers.

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Effects of Ampligo insecticid on oxidative stress in European Corn Borer (*Ostrinia nubilalis*)

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Abstract: The European Corn Borer (*Ostrinia nubilalis)* is one of the most damaging corn insect pests and it has a detrimental influence on corn production. This work examines the effects of insecticide Ampligo on the antioxidative defense system of *Ostrinia nubilalis* larvae. The experiment setup consisted of a completely randomized block design with 4 replicates. Two experimental groups were formed: control group (C) and T (chlorantraniliprole+Lambda Cyhalothrine - Ampligo®, Syngenta, Serbia) in a concentration of 0,20ml ha^{-1}. Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application, after which the homogenates of whole larvae were made. Ultimately, determination of the lipid peroxides (LPO), reduced glutathione (GSH) and oxidased glutathione (GSSG) concentration was performed. By comparing the experimental groups it was found that Ampligo significant influence on the increase in the concentration of the LPO in the treated group compared to the control group, as well as significant decrease in the concentration of GSH, whereas the concentration of GSSG is not significantly changed. This proves that Ampligo has significant influence on change in cell redox balance, and therefore a reduction in the number of larvae of this insect pest.

Key words: Ampiligo, *Ostrinia nubilalis*, oxidative stress, glutathione, lipid peroxides

Introduction

Normal functioning of the cell metabolism depends on the production of reactive oxygen species, ROS, and reactive nitrogen species, RNS, as well as the established equilibrium between prooxidant and antioxidant. Thus, oxidative stress may be defined as seriously disturbed balance between the production of

reactive oxygen and nitrogen species on the one hand, and of antioxidant protection on the other side (Halliwell and Gutteridge, 1999). Sies (1991) provides the definition of its oxidative stress, where the oxidative stress is described as disturbance between prooxidant-antioxidant balance in the direction of the first, leading to potential damage. This paper studies the effect of insecticides on Ampligo components of oxidative protection in larvae of *Ostrinia nubilalis*. This insect in temperate regions attacks a large number of plant species. *O.nubilalis* is especially damaging to maize is grown on 30% of all cultivable surfaces, as well as damages to about 7% (but can be up to 50%) (Popović et al., 2015). In the production conditions of corn borer it usually develops 1 to 3 generations. The main damage is caused by larvae bore in the leaf and stem of the ear of corn, leading to the attack of fungal diseases and mycotoxins (Kojić, 2009). One of the registered insecticide against O. nubilalis is Ampligo 150-ZC, based on active substance chlorantraniprole and lambda-cyhalothrin. Lambda-cyhalothrin is a contact and digestive synthetic pyrethroid insecticide of the large number of insect pests. Chlorantraniliprole is an anthranilic diamide insecticide that binds to ryanodine receptors, resulting in the unregulated release of calcium from insect muscle cells and leading to the cessation of feeding, lethargy, muscle paralysis, and death (Han et al., 2012; Cordova et al., 2006). It is highly active against many chewing pest insects, primarly by ingestion and by contact (Dinter et al., 2009; Bassi et al.,2007). Chlorantraniliprole has a wide spectrum, including Lepidoptera. The aim of this work is to present how insecticide Ampligo change antioxidative redox balance. This may indicate that ampligo can trigger cellular detoxification metabolism, for example that of glutathione, which has primordial role in maintaining, a major mechanism by which oxyradicals can cause oxidative damage.

Materials and Methods

Location description

The experiment was carried out in Rimski Šančevi, near Novi Sad, Serbia (45°19'47.72"N, 19°51'1.95"E, altitude 78 m a.s.l.), during 2016. The experiment setup consisted of a completely randomized block design with 4 replicates and was based according to EPPO guidelines (nr. PP 1/13(3)). Each plot consisted of 4 rows of maize, separated from other plots with one untreated row on each side. The length of each plot was 10 m, with a spacing of 2 m between blocks. The control plot wasn't sprinkled nor treated with insecticides and fungicides, whereas treated plot was sprinkled with Ampligo (Chlorantraniliprole + Lambda Cyhalothrine (Ampligo®, Syngenta, Serbia, formulation: suspension concentrate and capsule suspension combination (ZC)) in a concentration of 0,20ml ha^{-1}. The insecticide application was performed

during the peak flight of the European Corn Borer, using a backpack sprayer unit with high clearance attachment with 4 nozzle boom (model 315-HCB-4) from Bellspray Inc dba R&D Sprayers. The working height of the sprayer is manually adjustable (0,6 – 4,2 m) and the spray volume is 400 l ha^{-1} at a pressure of 200 kPa with an operation speed of 4-6 Km h^{-1}. Larvae from maize stems (hybrid NS 6030, FAO group 600) were collected following 20 days after the insecticide application. Larvae were euthanized immediately in liquid nitrogen for further analyses.

Preparation of homogenates and measuring protein concentration in samples

Values of antioxidative defense components were determined from homogenates of whole larvae. Upon being euthanized, larvae (100 mg of fresh larval mass) were homogenized using a homogenizer (IKA-Werke) in 2mL of sucrose buffer (0.25M sucrose, 0.05Mtris-HCl, 1mM ethylenediaminetetraacetic acid; pH 7.4) on ice (3 cycles of 10 s at 2000 rpm). Homogenates were then sonicated for 3 cycles 15 s (using a Bandelin sonoplus HD2070 instrument) and centrifuged on an ultracentrifuge (Beckman L7–55) at 105 000 g and 4 8C. The supernatant was extracted and frozen at –24 ºC until further experiments.

Determination of antioxidative defense activity

The determination of lipid peroxides (LPO) concentration was based on the reaction of lipid peroxidation products (malondialdehydes) with TBA (thiobarbituric acid reactive substances—TBARS) (Ohkawa et al., 1979) and was expressed in nmol/mL extract. The concentration of reduced glutathione (GSH) was determined based on GSH oxidation by 5.5-dithio-bis-6.2-nitrobenzoic acid (Beutler, 1975) and was expressed in µmol/mL extract. The concentration of oxidized glutathione (GSSG) was determined enzymatically by glutathione reductase (Beutler, 1975) after inhibition of GSH oxidation by 4-vinylpiridine and was expressed in nmol/mL extract. Concentration of LPO was determined by spectrophotometric (UV-VIS Spectrophotometer Shimadzu UV 1800). Reduced and oxidized glutathione concentrations were determined by ELISA microplate reader (Microplate reader, RT-6100; Rayto, China).

Statistical analysis

Results were processed using the SAS Ver 9.1.3 statistical package. The prerequisite for analysis of variance was the normality of distribution within a group achieved through logarithmic transformations of the traits (Sokal and Rohlf, 1981). Differences in the average values between different upstream and downstream locations were assessed by one-way ANOVA.

Results and Discussion

We have already mentioned that the reactive oxygen and nitrogen species presented in every organism, are part of the normal functioning of metabolism. But factors such as pesticides, heavy metals, etc. disturb the balance in the relationship of antioxidant and prooxidant. Investigation of the effect of the insecticide Ampligo on the components of oxidative protection in *Ostrinia nubilalis* larvae found that under the this influence the concentration of lipid peroxide (Figure 1.) significantly increases ($F = 16.54$; $p<0,01$**) which contributes to the loss of fluidity of the membrane and its selective permeability, whereas the concentration of reduced glutathione (Figure 2.) significantly reduced ($F= 5.02$; $p<0,01$*). Ampligo has no significant influence on the change of concentration of oxidized glutathione (Figure 3.) ($F=4.74$; $p>0.05$). Accordingly, glutathione, glutathione reductase and glutathione peroxidase (GSH-Px) are important indicators of oxidative stress. As a water-soluble tripeptide, glutathione is the most abundant intracellular thiol small molecule and a predominant defense against ROS and tissues. Glutathione reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation, promotes the regeneration of α-tocopherol, and serves as a substrate for GSH-related enzymes, e.g. glutathione peroxidase (GPx) and glutathione S-transferases (Habig, 1974). A very important role of glutathione reductase is that this enzyme catalyses the reduction reaction of oxidized glutathione into reduced glutathione because it is often used as a mesure of cellular toxicity (Pastore et al., 2003). Rodriges et al. (2015) investigated the effects of chlorantraniliprole on Chironomus riparius and prove that total glutathione levels were significantly reduced in all chlorantraniprole treatments. In contrast, LPO and AChE activity were not significantly altered with exposure to chlorantraniliprole.

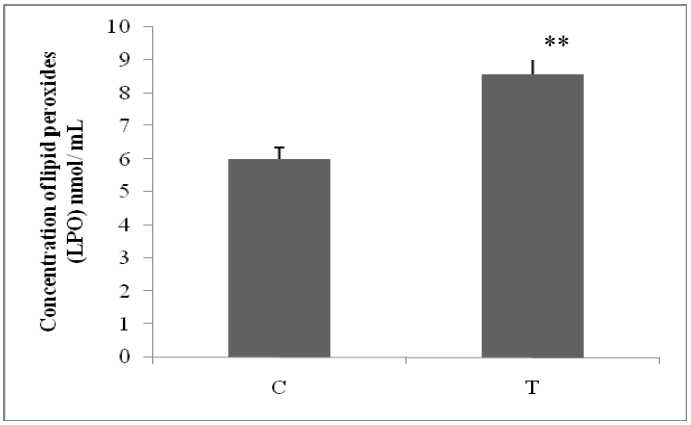

Figure 1. Concentration of lipide peroxides (LPO) in *Ostrinia nubilalis* larvae in control group (C) and group treated with Ampligo (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance ($F=16,54$; $p<0,01$**).

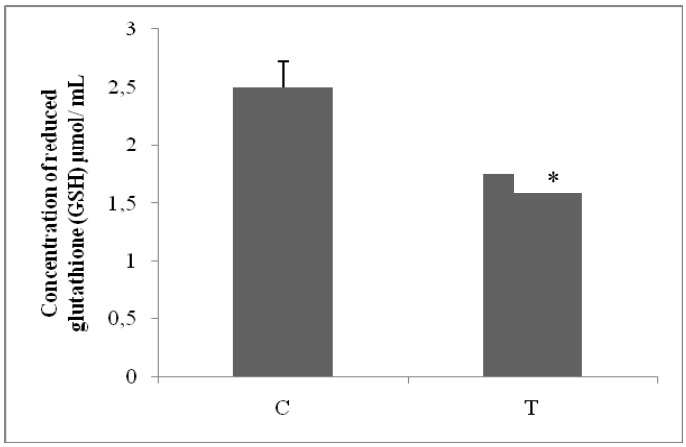

Figure 2. Concentration of reduced glutathione (GSH) in *Ostrinia nubilalis* larvae in control group (C) and group treated with Ampligo (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=5,02; p<0.05*).

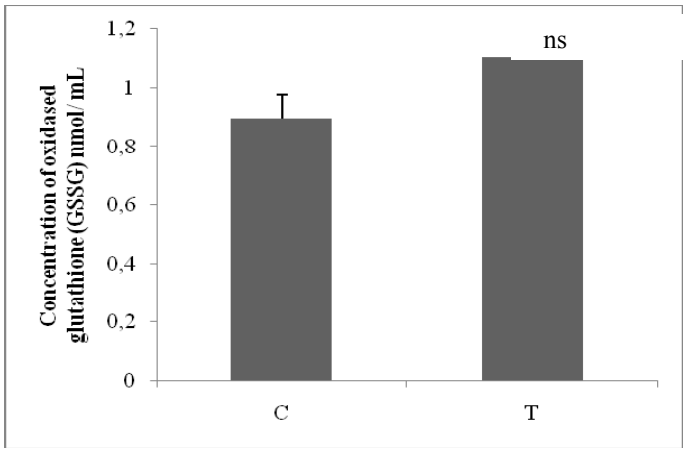

Figure 3. Concentration of oxidazed glutathione (GSSG) in *Ostrinia nubilalis* larvae in control group (C) and group treated with Ampligo (T). Bars represent the mean values ± standard error. Significance of the efects was tested by one-way analysis of variance (F=4,74; p>0.05 ns – not significant).

Conclusion

Ampligo changes significantly the concentration of lipid peroxides and reduced glutathione and so indirectly increases the oxidative stress in *Ostrinia nubilialis* larvae, and that makes it a very powerful insecticide weapon against this pest insects.

Acknowledgements

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Faunistic research of true bugs (Heteroptera) in light traps

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Abstract: True bugs (Heteroptera) are a diverse and complex group of insects. True bugs are regular inhabitants of many ecosystems, particularly agro ecosystems. Many species can be important for crop production, and in addition to affecting the yield can threaten its quality. In order to monitor the presence of insects in Sombor and Čelarevo light traps type RO Agrobečej were placed, using the mercury vapour lamp, 250W power. The traps were daily operated from dusk till dawn. Samples were pulled out from the traps each morning and examined in entomological laboratory at the Faculty of Agruculture, University of Novi Sad. Species identification was done according to morphological characteristics of examined species, in accordance with available keys for identification and web sites. The results revealed fauna of true bugs monitored for two months, from early May to the end of June 2016. The dominance index was calculated using Berger-Parker index and species were categorised according to Tischler's scale. The results are presented in tables and graphs, and the paper will indicate dominant and the most economically important heteropteran species.

Key words: true bugs, Heteroptera, light traps, Berger-Parker index

Introduction

True bugs (Heteroptera) are ecologically very diverse group of insects that inhabit almost all habitat types, both natural and those created by man. Despite their ubiquity, true bugs in Serbia are poorly trained group and there are only few professionals who deal with these insects (Kereši, 1999; Protić, 2011; Šeat, 2011; Konjević, 2015). However, true bugs have their place in nature, as well as specific roles in the ecosystem, regardless of whether .These insects generally prefer warm and dry habitats so grass habitats are extremely rich in species.

Most of the true bugs are herbivores dependent on the vegetation, so the kinds of plants, the structure of the vegetation cover and microclimatic conditions to the vegetation forms, define the communities bug, their abundance and even the presence of certain species (Henry, 2009; Fauvel, 1999). This paper indicates the most abundant and economically important species for agricultural production active during May and June.

Material and Methods

True bugs sampling was conducted from 1 May to 30 June 2016, by light traps. Traps type RO Agrobečej were used having mercury lamps with power of 250 W as light source. One trap was placed in Sombor and the other one in Celarevo (Figure 1). According to UTM map light trap was set up in the square labelled CR56 for Sombor and in Čelarevo labelled with the tag CR81. Trapped insects were collected each morning and classified according to their taxonomic belong. Operating time of the trap was approximately 1 hour before sunset to one hour after sunrise. Identification of sampled specimens was done according to morphological characters and available keys for identification: Protić (2011), www.macroid, Savage (1989) and similar.

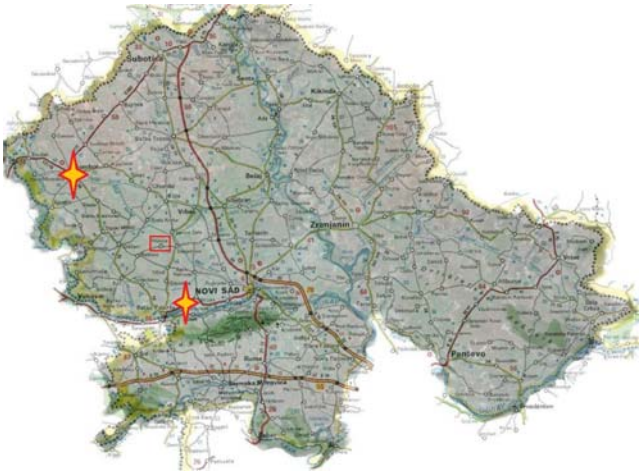

Figure 1. Map of the trapping points

During the processing of the collected material, the dominance was calculated by using Berger-Parker dominance index (*d*) as the expression of the

proportion of certain species in total catch, according to Southwood and Henderson (2000):

$$d = N_{max}/Nt,$$

whereas Nt is the total catch and N_{max} number of specimens of certain species. Species were categorised according to Tischler's scale for species dominance (Tischler, 1949): eudominant 10 % ≤ Di ≤ 100 %; dominant 5 % ≤ Di < 10 %; subdominant 2 % ≤ Di < 5 %; recedent 1 % ≤ Di < 2 % and subrecedent 0 % < Di < 1 %. In Table 1. dominance is shown in percentage (d*100%). In order to show similarity between observed locations Sörensen's similarity index was also calculated, as

I=2J/(a+b)*100% (in Kereši, 1999),

where J is a number of joint species for both localities, a – number of recorded specimens at locality A, and b- number of specimens recorded at locality B.

Results and discussion

Results of insect collecting by light trap at two localities revealed 998 heteropteran specimens in total. All collected Heteroptera, registered in the region of Bačka, during the sampling period from May to June 2016 belong to four families (Figure 2.) within the 42 species identified (Table 1). There is a huge dominance of family Miridae, representing 94% of the total sample. It was followed by families which were present in less than 5% in total sample: Lygaeidae (4%), Anthocoridae (1%) and Pentatomidae (1%).

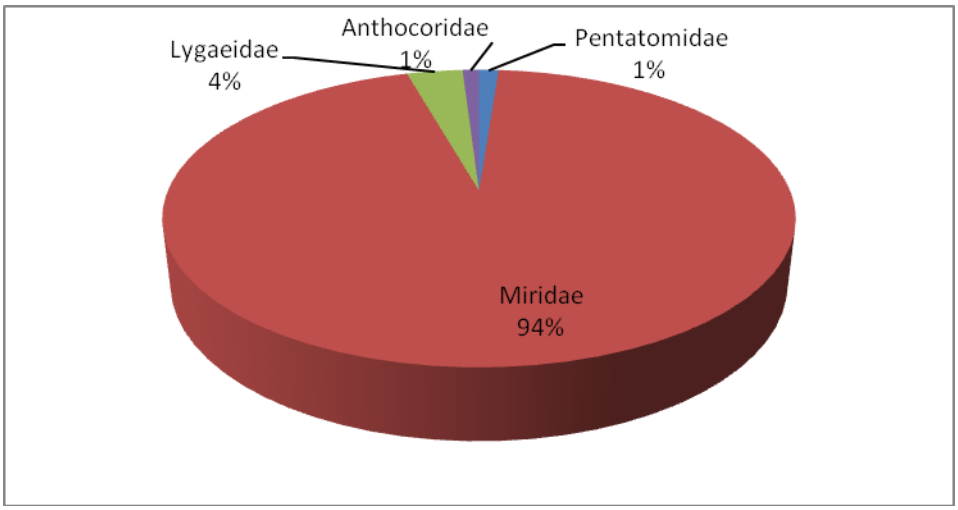

Figure 2. Composition of Heteropteran families in total sample

Table 1. List of Heteroptera species captured with light traps during May and June 2016 in Sombor and Čelarevo

Family	Species	Sombor	%	Čelarevo	%	∑	%
Pentatomidae	*Nezara viridula*	2	0.34	1	0.24	3	0.30
	Dolycoris baccarum	-	0.00	5	1.20	5	0.50
	Eysarcoris ventralis	-	0.00	1	0.24	1	0.10
	Palomena prasina	-	0.00	1	0.24	1	0.10
	Rhaphigaster nebulosa	1	0.17	-	0.00	1	0.10
	Acrosternum heegeri	-	0.00	1	0.24	1	0.10
Miridae	*Polymerus vulneratus*	4	0.69	3	0.72	7	0.70
	Lygus punctatus	-	0.00	2	0.48	2	0.20
	Polymerus unifasciatus	7	1.20	88	21.10	95	9.52
	Sthenarus rotermundi	2	0.34	13	3.12	15	1.50
	Acetropis carinata	2	0.34	-	0.00	2	0.20
	Adelphocoris lineolatus	188	32.36	77	18.47	265	26.55
	Orthotylus interpositus	1	0.17	-	0.00	1	0.10
	Teratcoris antennatus	3	0.52	11	2.64	14	1.40
	Phylus melanocephalus	-	0.00	1	0.24	1	0.10
	Stenodema calcarata	24	4.13	1	0.24	25	2.51
	Agnocoris reclairei	4	0.69	11	2.64	15	1.50
	Apolygus spinolae	4	0.69	11	2.64	15	1.50
	Phylus coryli	2	0.34	1	0.24	3	0.30
	Deraeocoris olivaceus	1	0.17	2	0.48	3	0.30
	Lygus rugulipennis	194	33.39	54	12.95	248	24.85
	Stenotus binotatus	18	3.10	4	0.96	22	2.20
	Orthorylus viridinervis	-	0.00	1	0.24	1	0.10
	Trigonotylus caelestialium	-	0.00	5	1.20	5	0.50
	Globiceps flavomaculatus	10	1.72	15	3.60	25	2.51
	Orthocephalus saltator	14	2.41	15	3.60	29	2.91
	Orthocephalus vittipennis	2	0.34	1	0.24	3	0.30
	Polymerus cognatus	1	0.17	1	0.24	2	0.20
	Closterotomus norwegicus	1	0.17	1	0.24	2	0.20
	Closterotomus trivialis	-	0.00	1	0.24	1	0.10
	Lygus gemellatus	27	4.65	2	0.48	29	2.91
	Polymerus palustris	14	2.41	37	8.87	51	5.11
	Brachycoleus decolor	7	1.20	2	0.48	9	0.90
	Deraeocoris ventralis	2	0.34	16	3.84	18	1.80
	Phytocoris ulmi	7	1.20	1	0.24	8	0.80
	Lygus pratensis	17	2.93	5	1.20	22	2.20
	Camptozygum aequale	1	0.17	-	0.00	1	0.10
	Alloeonotus egregius	-	0.00	2	0.48	2	0.20
	Adelphocoris seticornis	-	0.00	1	0.24	1	0.10
Anthocoridae	*Orius sp.*	-	0.00	10	2.40	10	1.00
Lygaeidae	*Metopoplax origani*	-	0.00	2	0.48	2	0.20
	Megalonotus chiragra	21	3.61	11	2.64	32	3.21
∑	42	581	58.22	417	41.78	998	

Among all collected specimens, according to Tischler's categories, there were two eudominant species: *A. lineolatus* with 265 specimens captured, representing 26,55% of the total sample, and *L. rugulipennis* with 248 sampled specimens, representing 24,85%. Both eudominant species of total sample belong to family Miridae, common family of true bugs with mostly polyphagous specimens (Schaefer and Panizzi, 2000). These species might be abundant and of economic importance in different agricultural crops. The rest of the total sample consisted of 2 dominant species: *Polymerus unifasciatus* and *Polymerus palustris*, followed by seven subdominant, six recedent and 25 subrecendent species.

During two months of sampling heteropteran species there were 29 species recorded at locality Sombor, beloging to only three families: Miridae, Lygaeidae and Pentatomidae, among which the dominant was family Miridae. Results of the sampling at named locality revealed also two eudominant species: *Lygus rugulipennis* and *Adelphocoris lineolatus,* representing 33,39% and 32,36% of the sample, respectively (Fig. 3). The most numerous species in Sombor was *L. rugulipennis,* which was the second abundant species in total sample. Both larva and adult of this herbivore are harmful, phytophagous and feed on green parts of different plant. *L. rugulipennis* is common species that often attains strikingly high density (Protić, 2011). The alfalfa true bug, *A. lineolatus* reached the highest abundance in total sample, and was the second most abundant in Sombor. It is an agricultural pest which might endanger the yield and quality of alfalfa and similar crops by causing stunted growth and deformation of flowers and leaves. The rest of the sample in Sombor consisted of seven subdominant species, four recedent and 16 subrecedent species (Table 1).

At the second observed location Čelarevo there were three eudominant species: *Polymerus unifasciatus*, *Adelphocoris lineolatus* and *Lygus rugulipennis* (Fig.4). They were followed by one dominant (*Polymerus palustris*), nine subdominant, three recedent and 22 subrecedent species, making it 38 species recorded at this locality in total (Table 1).

By looking at the whole period of sampling, during May there were 35 species recorded, while in June there were 26 species captured by light traps, indicating higher activity of certain species whose adults are more active during the spring. Among all true bugs species which are plant feeders and are possible pests there was only one exception from family Anthocoridae, specimens of genus *Orius sp.,* which are predatory species. These insect might be used in biological control of thrips and aphids having in mind their high efficiency (www.greenmethods.com). Although captured in only five specimens, species *Dolycoris baccarum* belonging to family Pentatomidae is highly poliphagous, common species, which can make damages to small grains, sunflower, tobacco, lucerne, clover, sugar beet, potato, corn and similar crops (Protić, 2011). Low

number of sampled Pentatomidae, the second largest family of true bugs (Henry, 2009) might indicate lower photophilic preferences of stink bugs in comparison to much more abundant mirids recorded in light traps.

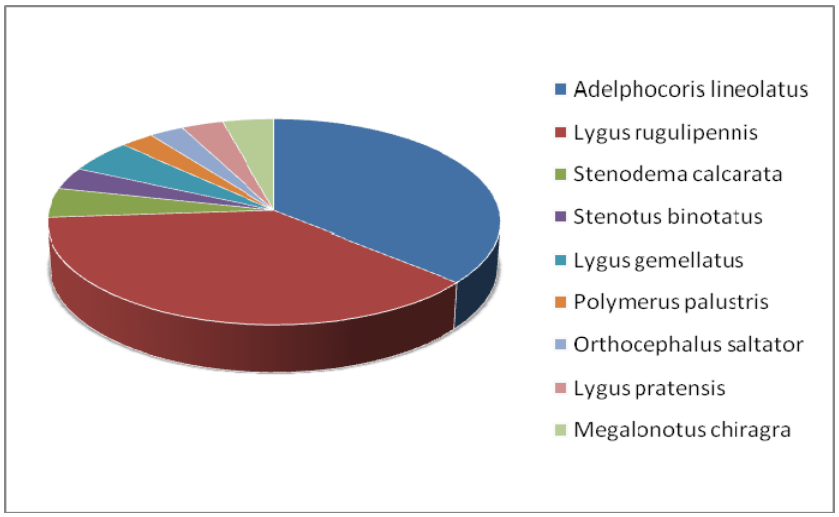

Figure 3. Composition of eudominant and subdominant species of true bugs in Sombor

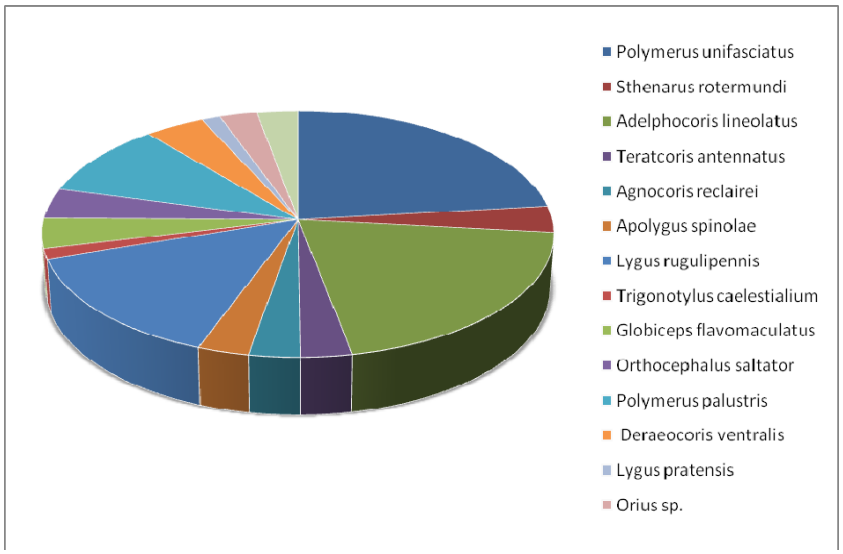

Figure 4. Composition of eudominant, dominant and subdominant species of true bugs in Čelarevo

There were 25 joint species that were captured at both observed localities. Therefore Sörensen's similarity index which was calculated was 0,746, indicating high interspecific association between captured heteropteran specimens from localities Sombor and Čelarevo.

Conclusions

During May and June 2016 there were in total 998 specimens of true bugs captured by the light traps set on two localities. Eudominant species, represented with more than 10% of the sample, at both observed localities were *Adelphocoris lineolatus* and *Lygus rugulipennis*, followed by two dominant species: *Polymerus unifasciatus* and *Polymerus palustris*. At locality Sombor there were two eudominant species: *Lygus rugulipennis* and *Adelphocoris lineolatus*. In Čelarevo the most abundant were three eudominat species: *Polymerus unifasciatus*, *Adelphocoris lineolatus* and *Lygus rugulipennis*. Sörensen's similarity index showed relatively high interspecific association in heteropterans of named localities.

In total sample of true bugs which were captured by light traps during spring and very early summer of 2016 the most important species with the economic importance for agriculture was the most abundant *A. lineolatus* which can seriously endanger alfalfa production. The second abundant species *L. rugulipennis* is highly polyphagous species with preferences to many agricultural crops, therefore it might be also of economic importance to agriculture. They both might achieve high abundance in certain crop and endanger yields.

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Eco-friendly control of *Xanthomonas euvesicatoria*

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Abstract: Plant pathogenic bacteria are a chronic threat to food production worldwide. With the intensification of agriculture, producers became heavily dependent on agrochemicals as a reliable method for crop protection. Most agrochemicals present on the world market are of synthetic origin with numerous side-effects on humans and the environment. Integrated management of bacterial plant diseases control include prevention of the spread of the bacteria, introduction of resistant varieties, cultivars or hybrids, application of copper or Bordeaux mixture and use of antibiotics. However, use of antibiotics in plant production is prohibited in many countries, including Serbia. Also, copper treatments are often insufficient for bacterial disease control, and there is an annual limit for quantity of this substance to be used in concept of organic production. Biological control strategies offer environmentally friendly alternative for plant disease control. This paper evaluates inhibitory effects of several alternative biocontrol substances (secondary metabolites of *Streptomyces hygroscopicus*, liquid culture of *Bacillus* spp., essential oils of wild oregano, clove and cumin) on significant pepper pathogen *Xanthomonas euvesicatoria.* The obtained results suggest that liquid culture of *Bacillus* spp., as well as essential oils of wild oregano and cumin show great potential for eco-friendly control of *X. euvesicatoria.*

Key words: *Xanthomonas euvesivcatoria,* essential oils*, Bacillus subtilis, Streptomyces hygrpscopicus*

Introduction

Pepper (*Capsicum annuum* L.) is one of the major vegetable crops grown in Serbia. However, the yield and quality of fruits are often reduced due to disease problems (Mijatovic et al., 1999; Obradovic et al., 1997). *Xanthomonas*

euvesicatoria (Jones et al., 2006) causes bacterial spot disease on pepper (*Capsicum annum* L.) in warm, humid areas worldwide. It induces lesions on the leaves, stems and fruits (Jones et al., 2000; Stall et al., 1994). Leaf infection results in blight, necrosis and early leaf fall. These cause a reduction in photosynthesis, while fruit infection results in direct economic losses (Jones et al., 1991; Obradovic et al., 2004; Stall et al., 1994). *X. euvesicatoria* is listed among A2 quarantine pests by EPPO. Bacterial spot of pepper caused by *X. euvesicatoria* is a very important disease of pepper in Serbia (Balaž, 1994; Obradovic et al., 1997; 1999; 2000; 2001a).

Control of bacterial spot disease is extremely difficult. Management mostly relies on bactericides as fixed coppers - copper hydroxide, copper oxychloride, copper sulphate (Conover and Gerhold, 1981). However, due to presence of copper-tolerant strains (Adaskaveg and Hine, 1985), under favorable conditions for the disease development chemical control alone is often insufficient. Although antibiotics are effective against bacterial diseases, the number of antibiotics used in agriculture is modest compared to applications in human and veterinary medicine. In Serbia, use of antibiotics for control of *bacterial diseases* on *plants is prohibited* (Obradović and Ivanović, 2007).

Difficulties in control of bacterial spot disease caused by *Xanthomonas* spp. with antibiotics and copper-based products are frequently reported. Also, in organic production, application of copper compounds is limited. Therefore, interest in eco-friendly plant disease control alternatives is increasing and researches in this area are intensified worldwide (Dianese et al., 2003; Flaherty et al., 2000; Ji et al., 2003; Louws et al., 2001; Moss et al., 1997).

Among eco-friendly alternative disease control measures, bacteria from *Streptomyces* and *Bacillus* genera, as well as different essential oils exhibit promising antimicrobial properties. The most significant characteristic of Streptomycetes is the ability to produce secondary metabolites such as antibiotics. *S. hygroscopicus* synthesize complex of antibiotics, which inhibit the growth of different bacterial disease agents (Bogatzevska et al., 1989).

Genus *Bacillus* is one of the most utilized bacterial genera in the biocontrol of phytopathogenic microorganisms. This genus comprehends a heterogeneous group of Gram-positive, aerobic or facultative anaerobic, endospore-forming bacteria. The endospores are termotolerant structures, resistant to dryness, ultraviolet radiation and organic solvents. These properties, associated with the ability of producing peptide antibiotics, contribute to the utilization of this genus in the biocontrol of plant diseases (Backman et al., 1997; Kloepper, 1997; Melo, 1998). Numerous studies suggest antimicrobial activities of plant compounds against many different types of microorganisms, including plant pathogens (Conner, 1993; Srinivasan et al., 2001; Özcan et al., 2001; Friedman et al., 2002; Erkmen et al., 2004; Biavati et al., 2004). In this study, effects of *S.*

hygroscopicus, *Bacillus* spp. and three essential oils against *X. euvesicatoria* originating from pepper leaves are investigated.

Material and methods

Bacterial isolates

Two bacterial isolates were used in the study (PL 1 and PL 1). The isolates were obtained from pepper leaves in 2016, and were identified as *X. euvesicatoria* based on pathogenic, morphological and biochemical-physiological properties. Identification of the isolates was also confirmed by PCR method using species-specific primers (Xeu 2.4 and Xeu 2.5). The isolates belong to Microbial culture collection of Laboratory for detection of pathogens, pests and weeds, Faculty of Agriculture, University of Novi Sad.

For the trial purposes, the isolates were grown on YDC medium for 48 hours at 26 °C. After incubation, bacterial suspension of each isolate adjusted to concentration of approximately 10^8 cfu/ml was prepared.

Preparation of biocontrol substances

The agent *S. hygroscopicus* was obtained from Microbial Culture Collection of the Faculty of Technology in Novi Sad. Secondary metabolites were produced by cultivation of the agent in liquid media containing glucose (15.0), soybean meal (10.0), $CaCO_3$, (3.0), NaCl, (3.0), $MgSO_4$, (0.5), $(NH_4)_2HPO_4$, (0.5), K_2HPO_4, (1.0). The pH of the medium was adjusted to 7.2 ± 0.1. *S. hygroscopicus* was grown in a 100 ml shake flask containing 30 ml of the culture medium. The fermentation medium was inoculated with 10% (v/v) of a 48 h old preculture and incubated at 26 ± 1°C for 72 h under conditions of spontaneous aeration on a rotary shaker at 150 rpm. After cultivation, the sample of the cultivation medium was centrifuged at 10,000 g for 10 min and the cell-free supernatant containing secondary metabolites was used for growth inhibition assay.

Liquid culture of *Bacillus* spp. is a commercially available biofertilitzer containing $6x10^{10}$spores of different strains of *B. subtilis, B. sicheniformis* and *B. megaterium* suspended in water with addition of sucrose and meat extract.

S. hygroscopicus and *Bacillus* spp. were applied without dilution and as a 10% dilution. A 10% suspensions of *S. hygroscopicus* and *Bacillus* spp. culture were obtained by suspending centrifuged supernatant or the commercial biofertilizer in sterile distilled water, respectively.

Essential oil of clove (*Syzygium aromaticum*) was extracted by hydro-distillation using an all glass Clevenger-type apparatus. Essential oils of wild oregano (*Origanum vulgare*) and cumin (*Cuminum cyminum*) were of commercial quality and destined for human use.

Testing of X. euvesicatoria growth inhibition by biocontrol substances in vitro

Meat peptone agar medium (MPA) was prepared and poured to test tubes (10 ml of medium to each tube). After autoclaving and cooling prior to gelling, 500 µl of bacterial suspension of each isolate (PL1 i PL2) was transferred to each test tube, homogenized, and poured into Petri dishes placed on a flat, horizontal surface. After solidification, sterile blank filter paper disc (Ø 6 mm) was placed in the center of each petri dish. On each disc, 3 µl of tested biocontrol substance was added. In control, 3 µl of sterile distilled water was applied to filter discs. Petri dishes that contained essential oil were sealed with laboratory parafilm to avoid eventual evaporation of the essential oils. The plates were incubated at 28 ^{o}C for three days.

Inhibitory effect of the treatment against *X. euvesicatoria* was determined by measuring the diameter of zones of inhibition (in mm) that formed around filter paper discs.

Data analysis

The obtained data were processed by factorial ANOVA, and singnificance of differences was calculated by Duncan's multiple range test using software Statistica 13.

Results and discussion

The study confirmed the broad potential of some bioactive compounds and their activity against phytopathogenic bacteria. Results of factorial analysis of variance (Table 1) showed that applied biocontrol substances were highly significant source of variation for *X. euvesicatoria* growth inhibition ($p \leq 0.01$).

Table1. Factorial ANOVA: effect of biocontrol substances on growth inhibition of *X. euvesicatoria*

Effect	Tests of Significance for diameter of inhibition zone (mm)				
	SS	Degr. of Freedom	MS	F	p
Biocontrol substances	17225.59	7	2460.80	2451.22	0.000000
Isolate	1.72	1	1.72	1.72	0.196450
Biocontrol substances*Isolate	9.68	7	1.38	1.38	0.236311
Error	48.19	48	1.00		

According to Duncan's multiple range test (Table 2), regardless of the *X. euvesicatoria* isolates, the most significant growth inhibition was obtained by oregano and clove essential oils, significantly higher compared to all other tested biocontrol substances. *Bacillus* spp. also exhibited significant inhibition effects on growth of *X. euvesicatoria*, however it was significantly lower compared oregano and clove oil (Figure 1). In the case of the isolate PL1, lower concentration of *Bacillus* spp. (10%) caused significantly smaller growth inhibition diameter compared to the pure culture liquid of *Bacillus* spp. No inhibition effect on *X. euvesicatoria* was recorded for secondary metabolites of *S. hygroscopicus* and cumin essential oil.

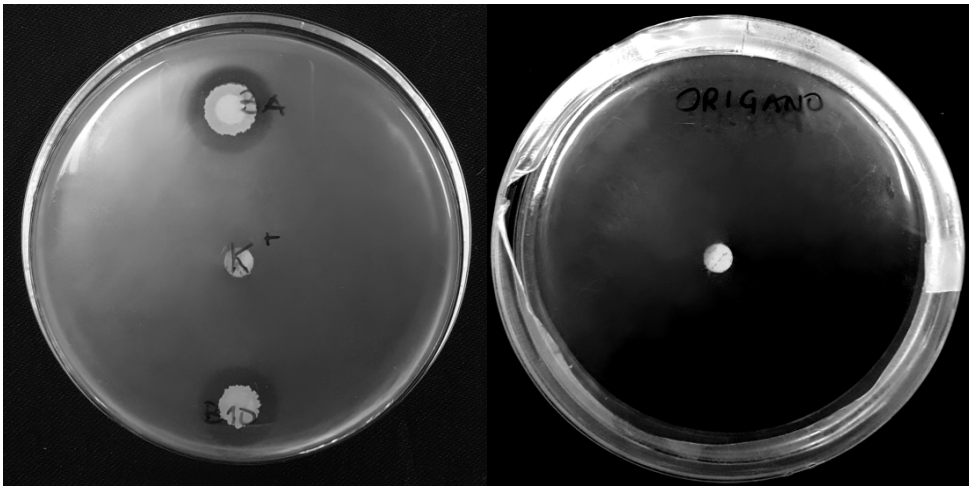

Figure 1. Inhibition effects on growth of *X. euvesicatoria* obtained by *Bacillus* spp. and control in center of Petri dish (left) and oregano essential oil (right)

Recent studies show that essential oils of oregano (*Origanum vulgare*) are among the most active compounds against different bacteria (Hammer et al., 1999; Smith-Palmer et al., 1998), which is in agreement with the results of our study. Also, Lucas et al. (2012) found that clove *(Syzygium aromaticum)* essential oil reduce the severity of tomato bacterial spot and induces an increase in activities of β-1,3-glucanase, chitinase, and peroxidase, which supports the results obtained in our trials. Nikolić et al. (2013) registered that 6 of 52 tested *Bacillus* sp. isolates inhibited the growth of plant pathogenic bacteria, primarily the species of *Xanthomonas* genus.

Our study confirmed high activity of *Bacillus* spp. against *X. euvesicatoria.* In trials conducted by Encheva-Malinova et al. (2014), three Streptomyces strains isolated from Antarctic soils were screened for *in vitro* antibacterial activity against bacterial species isolated from pepper plantations in Bulgaria

and Macedonia – *X. vesicatoria, X. gardneri* and *X. euvesicatoria. X. euvesicatoria* was registered as the most sensitive species to the antibacterial substances synthesized by the streptomycetes, which is contrary to the results obtained in our study which revealed no activity of *S. hygroscopicus* secondary metabolites against *X. euvesicatoria.* The differences in the antibacterial activity among the strains used in these studies might be due to the nature of the produced substances.

Table 2. Duncan's multiple range test: effect of biocontrol substances on diameter of growth inhibition zone of *X. euvesicatoria*

Biocontrol substances	Isolate	Diameter of inhibition zone (mm)
S. hygroscopicus metabolites	PL1	0.00[a]
	PL2	0.00[a]
S. hygroscopicus metabolites 10%	PL1	0.00[a]
	PL2	0.00[a]
Bacillus spp.	PL1	17.75[c]
	PL2	18.00[c]
Bacillus spp. 10%	PL1	14.38[b]
	PL2	16.75[c]
Clove	PL1	40.00[d]
	PL2	40.00[d]
Oregano	PL1	40.00[d]
	PL2	40.00[d]
Cumin	PL1	0.00[a]
	PL2	0.00[a]
Control	PL1	0.00[a]
	PL2	0.00[a]

*Values followed with the same letter are at the same level of significance

Conclusion

According to the results, it can be concluded that oregano and clove essential oils as well as *Bacillus* spp. exhibit strong activity against *X. euvesicatoria* and can be considered as promising eco-friendly bactericides. However, antibacterial activity of these agents should further be investigated in field trials, under production conditions, to confirm their activity in field.

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Sanitary State and Species of Poplar: Case Study Perm, Russia

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Abstract: The paper presents the analysis of species composition and the sanitary state of trees of the genus *Populus* L., found in the territory of the city of Perm. As a result, 1663 plants were detected, 12 species and hybridogenic taxa were identified. These are *P. alba, P. balsamifera, P.* × *berolinensis, P. deltoides, P. laurifolia, P.* × *moscowiensis, P. nigra, P.* × *sibirica, P.* × *sowietica pyramidalis, P. suaveolens, P. tremula, P. generosa*.

Key words: species composition, genus, poplar, category of sanitary state, types of damage.

Introduction

Relevance of the research topic

Functionally important and irreplaceable component of the urban environment are green plantations, perfoming sanitary hygienic and decorative-planning functions. For successful development of plants in adverse ecological conditions, it is necessary to conduct scientifically-grounded selection of the range of tree species (Albensky 1946; Tsarev, 1985; Theodoronsky, 2007). By virtue of high productivity, decorative and easy poplar breeding, they are widely used in planting, protective and recreational plantings (Redko, 1975; Tsarev, 1985; Plotnikova, 1994).

Poplar is a fast-growing tree species that effectively delays dust and soot, neutralizes harmful chemical compounds and actively absorbs carbon dioxide. It enriches the air with phytoncids, releasing volatile and non-volatile substances that slow the growth and development of pathogens. It features high gas

resistance, resistance to pests and diseases, does not require significant care (Pauley, 1949; Grozdinsky, 1975; Tokin, 1980; Aksenov, 2000).

The disadvantages of poplars are fragility, damage by rot and damage by pests (Redko, 1975; Aksenov, 2000). Adult trees is characterized by the presence of a crown with a large mass, a large crown in turn makes poplar dangerous, prone to falling trees (Abaimov, 2009). In urban conditions, a significant defect of poplars is brittle wood (Kolesnikov, 1974; Tsarev, 1985).

The purpose of the research was: to study the role and prospects of different species of the poplar genus in the greneery planting in the city of Perm.

Objectives: 1) to study the species composition of the genus poplar, and to determine the representation of different species in the greenery planting of the city of Perm; 2) to study the sanitary condition of species of the genus poplar.

Systematics of the genus Populus

Populus is a genus of deciduous fast-growing trees of the family *Salicaceae*, of the order of *Salicales*, subclass *Dilleniidae*, class *Magnoliopsida* (dicots), department *Magnoliophyta*. There are 110 species in the genus *Populus* (Tsarev, 1985; Isebrands and Richardson, 2014).

According to the classification of the International Poplar Commission, the genus is divided into 5 sections (Pauley, 1949; Redko, 1975; Zsuffa, 1975; Medvedeva, 2015):

1) *Turanga* Bge; 2) white poplars (*Leuce* Duby). Includes two subsections: a) real white poplars (*Albidae* Dode); b) aspen (*Trepidae* Dode); 3) black poplars (*Aigeiros* Duby); 4) balsam poplars (*Tacamahaca* Spach.); 5) swamp poplars (*Leicoides* Spach).

Characteristics of the studied species

SUBGENUS *Tacamahaca* Spach

Section 1 *Tacamahaca* Spach

1. Poplar Fragrant (*P. suaveolens* Fisch.) is distributed in Eastern Siberia, the Far East, Chukotka, Mongolia and northern China. In the upper part of the trunk, the crust is smooth, greenish-gray (Abaimov, 2009). A large tree with a dense ovoid crown. Young shoots are resinous, fragrant, cylindrical, shiny with white lenticels (Novikov, 1965). Buds are sharp, glutinous. Leaves on pubescent petioles, leathery, oval-lanceolate with short-pointed apex and broad-wedged base, crenate-serrate along margin. Male flowers are short with 15-30 stamens, female - large, multiflorous, pistil in flowers with a bifid stigma. Blooming time in May at the same time as the leaves bloom. Fruits are naked, ovoid (Aksenov, 2000; Abaimov, 2009). Frosty, not whimsical to soil conditions. Gives abundant root siblings. Used in single and group plantings in gardens and parks, casing roads and reservoirs (Aksenov, 2000).

2. Poplar Moscow (*P.* × *moskowiensis* R.I. Schrod.) is a hybrid between *P. suaveolens x P. laurifolia*. A tree with a weakly branched, ovoid crown and a light gray bark of the trunk. Shoots are cylindrical, the buds are long, gluey. It blossoms simultaneously with the opening of the leaves, in the fall, early leaves the leaves. Leaves are on short, slightly pubescent petioles, elongated, pointed, at the base cuneate or rounded, at the base of the leaf there are two glands. It grows quickly, frosty, decorative. Fruiting occurs at the age of 9 years (Albensky, 1946; Kolesnikov, 1974; Tsarev, 1985).

3. Laurel poplar (*P. laurifolia* Ledeb.) is distributed in the Western, Eastern, Southern Siberia, the Altai and the mountains of Central Asia. A large tree with a tent-shaped, unbranched crown. The trunk is thick, slightly flaky with a deeply cracked bark. The shoots are ribbed, due to the narrow longitudinal cork wing-like processes. The buds are large, resinous, covered with 2-4 scales. Fruit 2-3 fold egg-shaped capsule, seeds numerous with long hairs. Leaves are glabrous, large, gradually acuminate to the apex, ovate with a rounded base, wavy or glandular-dentate at the margin. Male earrings are up to 8 cm long, dense, anthers are purple. Pestle has a yellow-green stigma. The tree blossoms simultaneously with the opening of the leaves. Frost resistant, low demand for soil, medium–weather resistant. It is used in ordinary plantings. In gardening, female specimens should be avoided, giving abundant fluffy seeds (Albensky, 1946; Aksenov, 2000; Abaimov, 2009).

4. Balsam poplar (*P. balsamifera* Ledeb.) is naturally prevalent in North America. A large tree with a broad, spreading crown (Zasada and Phipps, 1990). The flowers are in catkins that appear early in spring before the leaves. It is characterized by rapid growth at a young age (up to 2 meters per year). The buds are ovate-conical, large, gluey. Young shoots are ribbed, later rounded. Leaves are ovate, dark green above, lighter, naked below. The petioles are round in cross section, long. Box 3-4 fold. Blossoming time is in April-May. Winter and frost resistant, not demanding to soils, light-loving it is recommended for forest reclamation plantations, field shelter plantations, forest crops, casing of river and road banks, single and group planting (Zasada and Phipps, 1990; Aksenov, 2000; Abaimov, 2009). Fruiting occurs at the age of 10 years (Albensky, 1946; Zasada and Phipps, 1990; Isebrands and Richardson, 2014).

5. Poplar Renewable (*P. generosa* Henry Gard) has ovate, glistening on top, green, from below gray-green leaves. On brachyblasts, ovate leaves up to 10 cm long with a sharp elongated apex, the base of the leaf is straight, rounded or broadly wedged. There are two glands at the base of the leaf. On the coppice leaves the base is cordate, less often straight. On the growing shoots the base is from the straight to the wedge-shaped. Petioles with a visible groove, oblate, up to 5-6 cm long (Tsarev, 1985).

Section 2 *Aigeiros* Duby

6. Eastern Cottonwood (*P. deltoides* Bartl. Ex. Marsh. Subsp. *monilifera* (Ait.) Eckenw) naturally grows in North America and southern Canada to the foothills of the Rocky Mountains. A tree with a straight trunk and a wide-oval crown (Cooper and Van Haverbeke, 1970). The elongated shoots in the crown are slightly ribbed, and the coppice is strongly ribbed. Leaves 3-8 cm long and 3-6 cm wide, densely skinned, dark green shiny, triangular with straight or wide wedge-shaped base and short-pointed apex, leaves at the base with two or three glands, along the edge blunt-dentate. Petioles with two glands in the place of transition into the leaf blade, without groove, oblate (Molganova and Ovesnov, 2016). Fruits have 3-4 folding boxes. Gas and smoke resistant, it is used in protective gardening, casing roads, territories of industrial enterprises in group and alley planting, in single planting, forest parks, river floodplains (Kolesnikov, 1974; Isebrands and Richardson, 2014).

7. Poplar Black (*P. nigra* Ledeb.) is distributed in Central and Southern Europe, Western Siberia, Altai, Central Asia. The tree is large with a wide-spread crown. The shoots are cylindrical, almost naked or with hardly noticeable ribbing. The buds are pointed, sticky, with a bent apex. Leaves are fragrant, dense, from above dark green, from below light, with pointed tip, along the edges small-toothed. Petioles in upper half distinctly laterally oblate, along upper margin without groove. Blooms in April-May before the leaves bloom, abundantly and annually. Thermophilic, able to endure a long flood, light and hygrophilous. It is well resumed by root offspring and cuttings, which is why it is recommended for anti-erosion planting, casing rivers and reservoirs (Albensky, 1946; Abaimov, 2009; Isebrands and Richardson, 2014).

SUBGENUS *Populus* Dode

Section 3 *Trepidae* Dode

8. Common Aspen (*P. tremula* Ledeb.) occurs everywhere, grows in Western Europe, China, Mongolia, North Korea. A tree of the first magnitude (up to 35 m in height) with a cylindrical trunk (up to 1 m in diameter) and a rounded crown. The bark of young trees is smooth, light green. The old cortex is black or dark gray, with deep cracks in the lower part of the trunk. Buds are large, oblong-ovate-pointed or conical, firm, glabrous or hairy, gluey. Flower buds larger than leafy, egg-shaped, laid in summer on brachyblasts. Blossoms abundantly and annually, before the leaves blossom (late April-early May), begins to bloom from 10-12 years. The duration of the flowering period is one week. Bracts scales densely pubescent with long hairs. The leaves are rounded with a blunt apex, at the base wide-wedged, along the edges with uneven, large blunt teeth. The petiole is almost equal in length to the leaf blade, bare, closer to the base of the leaf, oblate. On young shoots the leaves are triangular-ovate with a pointed apex. Fruit is a capsule with a lot of seeds. The seeds are small, black or yellowish-gray, with hairs, ripen

on average 35 days after flowering, are carried by the wind. Winter- and frost-resistant, well tolerates excessive moisture, medium-to-fertile soil fertility and moisture, poor resistance to rot, light-loving. Used to strengthen the slopes of ravines and river banks, gives abundant root shoots (Kolesnikov, 1974; Smilga, 1986; Abaimov, 2009; Isebrands and Richardson, 2014).

Section 4 *Populus*

9. Poplar White, or Silver (*P. alba* Ledeb.) is distributed in the European part of Russia, Siberia, Central Asia, Western Europe, China, Asia Minor. The tree is large with a wide-spread crown. It possesses a strongly developed root system. Buds are small, ovate, not sticky, at first pubescent, later glabrous. The leaves are dense on the pubescent petioles, on the ayxyblasts 3-5 lobed, from below are white, on the brachyblasts – round-ovoid. Bracts are whole-sided or marginally unequal. Blooms in April-May until the leaves bloom, fruits ripen in June. Demanding to soil moisture, medium-demanding to fertility, is able to tolerate salinity, winter hardy, light-loving. Pruning and shaping of the crown is poor, part of the branches wither, and the crown loses decorativeness, a duplicity is formed. It is recommended for single and group plantings, strengthening river banks and reservoirs. Due to the abundance of root offspring and a strong root system in urban gardening is not desirable (Albensky, 1946; Kolesnikov, 1974; Abaimov, 2009; Isebrands and Richardson, 2014).

10. Poplar Soviet Pyramidal (*P. × sowjetica pyramidalis* Jabl.) is a hybrid between P. *alba x P.bolleana* received by Soviet scientist A.S. Yablokov. Distributed in the European part everywhere, not north of the forest zone. A large tree with a narrow pyramidal crown. In the lower part of the trunk the cortex is slightly cracked, in the upper part it is more smooth. The leaves can be unbroken, along the rim, notched-toothed and 3-5 lobed. On top dark green, white pubescent beneath the leaf. Drought-and gas-proof, demanding for fertility and soil moisture. It is used in single and group plantings, alleys (Plotnikova, 1994).

Intersectional hybrids

11. Poplar Siberian (*P. × sibirica* G. Kryl. Et Grig. Ex A. Skvorts.) is a tree up to 25 m high, a hybrid between *P. balsamifera x P. nigra*. Leafy plates broadly ovate with a rounded base, strongly protruded apex, from above dark, from below pale green, along edges dull-crenate-serrate, with well-marked glands. Pincushion on top with a groove, at the transition to the base of the sheet without iron. Shoots, leaves and earrings are often bare or rarely pubescent. The capsule is almost sessile, bivalve (Skvortsov, 2007).

12. Poplar Berlin (*P. × berolinensis* Dippel.) is a hybrid between *P. laurifolia x P. nigra*. A tree with a wide-pierced crown. The bark of the trunk at the top is light gray, smooth, beneath it is dark gray, fissured. Young shoots are ribbed, later rounded, with sparse lenticels. Leaves on the pubescent petioled, flattened at the base of the leaf, with a narrow groove. The leaf plate is oblong-ovoid in shape,

unequally serrate on the edge, with pointed top, two glands at the base of the leaf. The buds are gluey, greenish. It grows quickly, undemanding to the soil, and wintering. Well tolerates the shaping and pruning of the crown. The tree is suitable for street and boulevard landings, along the banks of reservoirs. Fruiting occurs at the age of 8 years (Novikov, 1965; Kolesnikov, 1974; Aksenov, 2000; Ovesnov et al., 2007).

Material and methods

In July 2016, a survey of 30 streets in 4 administrative districts of the city of Perm (58°01′ N, 56°16′ E, 87 m. a. s. l.) with a total length of 20.7 km. The work was carried out by the route method, according to the methodology of the tree inventory. Each tree was assigned a sequence number, and it was determined: 1) species identity; 2) types of damage; 3) the category of sanitary state.

Species attribution was determined from a number of sources (Rychin, 1950; Novikov, 1965; Ovesnov et al., 2007). Assessment of the sanitary state was carried out in accordance with the scale of "Sanitary rules in the forests of the Russian Federation" for a set of visual signs: defects and damage to the trunk, the presence and proportion of shrunken branches, the density of the crown, the presence of fruit bodies and kappas. With the subsequent distribution of the number of trees of each species into 6 categories of viability. Healthy, weakened, severely weakened, shrinking, fresh dead wood, old dead wood (Alekseev, 1989; Sanitary rules…, 1992; Mozolevskaya, 1998). The degree of weakening (condition) as a whole or of each tree species was defined as the weighted average of the estimates of different categories of the state (Guidance on the design…, 2007).

Results and discussion

Species composition of the genus Populus and the representation in the planting of greenery

As a result, 1663 plants were examined, 12 species and hybridogenic taxa were identified (Table 1): 2 subgenus, 4 sections and 2 intersectional hybrids.

Of the 1663 plants studied, 41.5% (Table 1) are – *P.* ×*berolinensis*. In second place in the prevalence was *P.* × *moscowiensis* - 20.1%. 8.8% of the poplar is a *P.* × *sowietica pyramidalis*. *P. suaveolens*, and *P. alba* is much less common, accounting for 7.8%. The share of other trees is not significant and does not exceed 4% for each species: that is, trembling (4.0%), *P.* × *sibirica* (3.7%), *P. balsamifera* (2.4%), *P. laurifolia* (2.4%), *P. deltoides* (0.8%), *P. nigra* (0.6%), and *P. generosa* (0.1%).

Table 1. The proportion of species from the total number of plants

Species name		Proportion of species (%)
English	Scientific	
Subgenus *Tacamahaca* Spach		
Section 1 Balsam poplar (*Tacamahaca* Spach)		
Fragnant poplar	*P. suaveolens* Fisch.	7.8
Poplar Moscow	*P.* × *moscowiensis* R.I. Schrod.	20.1
Poplar Laurel	*P. laurifolia* Ledeb.	2.4
Balsam poplar	*P. balsamifera* L	2.4
Poplar Renewable	*P. generosa* Henry Gard.	0.1
Section 2 Black poplar (*Aigeiros* Duby)		
Eastern Cottonwood	*P. deltoides* Bartl. ex Marsh. subsp. *monilifera* (Ait) Eckenw.	0.8
Black poplar	*P. nigra* L.	0.6
Subgenus *Populus*		
Section 3 Aspen (*Trepidae* Dode)		
Common Aspen	*P. tremula* L.	4.0
Section 4 White poplar (*Populus*)		
White poplar	*P. alba* L.	7.8
Poplar Soviet Pyramidal	*P.* × *sowietica pyramidalis* Jabl.	8.8
Intersectional hybrids		
Poplar Berlin	*P.* × *berolinensis* Dippel.	41.5
Poplar Siberian	*P.* × *sibirica* G. Kryl. et Grig. ex A. Skvorts.	3.7

Types of damage

As a result of the research, it was found that all kinds of injuries are of different nature. In accordance with the affected plant organs, the types of damage were divided into the following: mechanical damage and bark detachment; dryness; the shriveled skeletal branches; frost cracks; hollow; pathological shape of the trunk (bending, fusion, slope, curvature, asymmetry, 2 trunks and more); fruit bodies of mushrooms; caps (outgrowths). Figure 1 shows the percentage of damage types in the species studied.

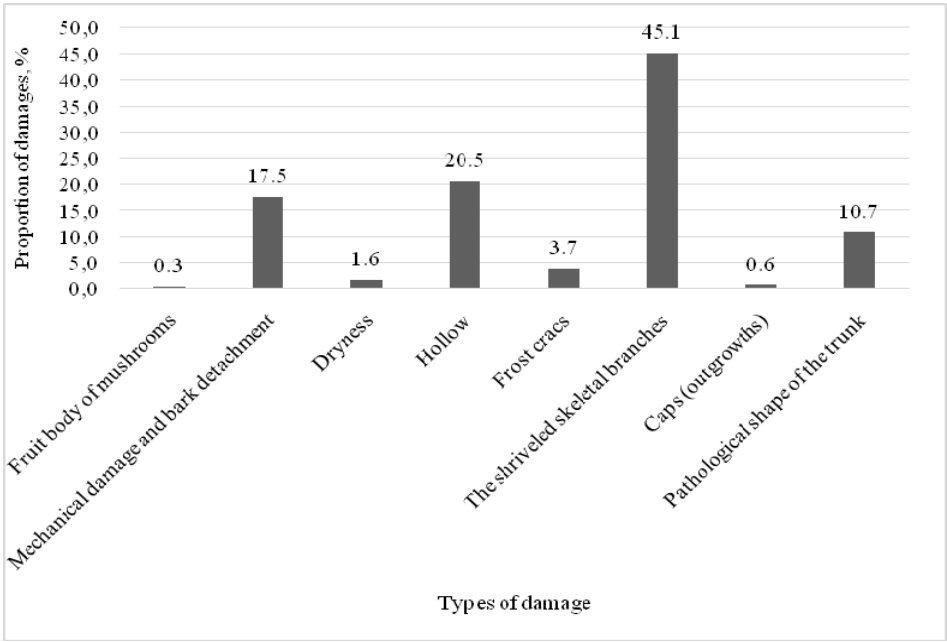

Figure 1. Correlation of damage types in the species of trees under study

From the data in Figure 1, the dominant types of damage in the studied plants are: the shriveled skeletal branches (45.1%), hollows (20.5%), mechanical damage and bark detachment (17.5%), pathological form of the trunk (10.7%). The least common types of damage are: frost cracks (3.7%), dryness (1.6%), caps (0.6%) and fruit bodies (0.3%).

One of the external signs of damage to rot is the presence of fruiting bodies, hollows, mechanical damage to the trunk. Rotting reduces the mechanical strength of the trunk, the resistance of trees to the wind, disrupt transport and metabolic processes in the tree. Fruit bodies were found in 11 (0.3%) of the examined trees: Poplar Tinder *Oxyporus populinus*; Oyster mushroom *Pleurotus ostreatus*.

Assessment of sanitary condition

The greatest number of plants constitute the 2 category of the sanitary state (SS) (weakened) – 53% (845 plants) of the total number. To severely weakened (3 category SS) – 33% (589 pieces). Without signs of weakening (1 category SS) 10% of trees (150 pcs.). To 4 categories SS (shrinking) is 3% (69 pcs.). Fresh dead wood (5 category SS) constitute the 1% (10 pcs.) of trees, dead past years (6th category SS) is absent. The average weighted category of the SS of the poplar genus is 2.3 (weakened). Average category of SS by species as a whole, and separately the trees undergone topping is presented in Table. 2

Table 2. The distribution of the number of trees by the category of sanitary state (CSS)

Species	Proportion of species (%)					Average CSS	Trees subjected to topping	
	Category of sanitary state						Share of total plants (%)	Average CSS
	1	2	3	4	5			
P. × berolinensis	5	47	42	5	1	2.5	44	3.1
P. × moscowiensis	13	57	25	5	0	2.2	9	3.1
P. × sowietica pyramidalis	8	60	24	8	0	2.4	-	-
P. alba	9	74	15	2	0	2.1	-	-
P. suaveolens	6	43	45	4	2	2.5	41	3.2
P. tremula	55	45	0	0	0	1.4	0	-
P. × sibirica	0	18	82	0	0	2.8	81	3.0
P. balsamifera	17	51	32	0	0	2.1	12	3.0
P. laurifolia	0	20	80	0	0	2.8	55	3.0
P. deltoides	0	100	0	0	0	2.0	-	-
P. nigra	0	22	56	11	11	3.1	-	-
P. generosa	0	100	0	0	0	2.0	-	-

From the data in Table 2 in the best SS there is a species: that is a *P. tremula* average CSS of 1.4. Without signs of weakening constitute the 55% (36 pcs.), 45% (30 pcs.) belong to the 2 categories from the investigated plants. The worst SS is observed in the species *P. nigra*, average CSS – 3.1. It has more than half of the plants 56% (5 pieces) of the 3rd category, 27% in 2 categories (2 pcs.), 11% (1 pc.) of plants of the 4th category, of the investigated plants, 11% (1 pc.) is 5th category. The average CSS - 2.8 (severely weakened) is characteristic of the species *P. laurifolia* (the third category is 80%, the second category is 20%), and the *P. × sibirica* (18% - the 2nd category of the SS, and 82% the third category of the SS). *P. generosa* and *P. deltoides* belonged to the second CSS (100%). Category of sanitary state – 2.1 is characteristic of the species: *P. balsamifera* and *P. alba.* The 5th category is less common and is noted in species: *P. ×berolinensis* (1%), *P. suaveolens* (2%), *P. nigra* (11%). As a result of topping or rejuvenating trimming of trees, even with exact observance of the rules of poplar keeping in the city, occurs the deterioration of the sanitary state. Among the species: *P. × berolinensis*, *P. × moscowiensis, P. suaveolens*, *P. × sibirica*, *P. balsamifera* and *P. laurifolia* were found plants that underwent topping, the average category of sanitary state is 3.1. Pruning – direct human intervention in the life of plants, growth processes, branching and molding. Pruning leads to a decrease in the total biological productivity, reduces the total area of the leaves, disturbs the growth processes,

changes the natural ratio between the mass of underground and aboveground organs (Albensky, 1946; Kolesnikov, 1974).

Conclusion

As a result of the works, 1663 trees were surveyed in the territory of the city of Perm, 12 species and hybridogenic taxa, 2 subgenus and 4 sections were found. The most common species are: *P.* × *moscowiensis* and *P.* × *berolinensis*, the least: that is *P. nigra* and *P. generosa*.

Among the types of damage, the largest share is made up of: shrunken skeletal branches (45.1%), hollows (20.5%), mechanical damage to the trunk and cortical detachment (17.5%), pathological form of the trunk (10.7%). The least common types of damage are: frost cracks (3.7%), dryness (1.6%), caps (0.6%) and fruit bodies (0.3%).

The greatest number of plants constitute the 2 category of the sanitary state (SS) (weakened) – 53% of the total number. To severely weakened – 33%. Without signs of weakening 10% of trees. To 4 categories SS belong 3% of trees. Fresh dead wood constitute the 1% of trees, dead past years is absent. The average weighted category of the SS of the poplar genus is 2.3 (weakened). Good indicators of the sanitary state in the conditions of Perm are characteristic for species: *P. tremula* (Average CSS – 1.4), *P. deltoides* (Average CSS – 2.0) and *P. generosa* (Average CSS – 2.0).

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Soil Contamination by Heavy Metals

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Abstract: This article deals with the problem of soil contamination with heavy metals based on microprobe analysis of the magnetic phase of the soil. The studies carried out reflect the different structural contents of components of technogenic origin in the soil. These components, which are called magnetic sphere, which prevail in the composition of magnetic particles, are a factor in the production of the metallurgical industry. Heavy metals are a chemically and mineralogically complex form of production waste. Falling into the soil, heavy metals are mixed with the soil solution, thereby releasing to the bottom various mobile forms of zinc, copper, nickel and other metals, and metalloids. The content of such elements in the soil is not a favorable factor for agricultural production, as well as human life's.

Key words: Magnetic particles, spherules, microprobe analysis, urbo-sod-podzolic soils, microsphere.

Introduction

The role of soil in human life is great. A man gets almost everything necessary to maintain his existence from the soil. Soil is the most important and irreplaceable source of food resources. It is the main means of agricultural production and forestry. In soil there is a significant part of metabolic processes between animate and inanimate nature.

Soil pollution is a serious environmental problem affecting human health and the ecosystem. Industrialization and urbanization lead to an increase in heavy metal contamination of urban soils. The soil cover of urbanized areas is a complex natural-anthropogenic system. The products of technogenesis fall on the earth's surface, accumulate in the upper horizons of soils, change their chemical composition and are again included in the natural and technogenic migration

cycles. Technogenic halos in the soils record the intensity of pollution during the last 20-50 years. Abnormal zones of metal concentration in soils are indicators of anthropogenic pollution and pose a danger to the environment. (Yazikov, 2001).

The magnetic properties and the ecological and geochemical status of urban soils directly depend on the amount and composition of their magnetic phase (Vasiliev, 2014).

Objective: to study the morphology and elemental chemical composition of the particles of the magnetic phase of urbo-sod-podzolic soils in the middle Kurya microdistrict and urbanozems on ancient alluvial deposits on the right bank of the river Kama.

Objectives of work:

1. Search and processing of theoretical information on the morphology of the magnetic phase of soils.
2. Separation of the magnetic phase from the soils.
3. Microprobe analysis of the magnetic phase of soils and interpretation of its results.

Magnetic spherules in natural-technogenic sediments represent one of the most common groups of components of technogenic origin. They are found in practically all areas under technogenic pressure. Magnetic spherules are easily distinguished in the laboratory in the process of magnetic separation (Menshikova, 2015).

Sources of origin of magnetic spheres in natural and technogenic sediments in the valleys of the Urals are mainly metallurgical and coking plants, as well as some municipal facilities. They are associated with metal processing processes and accumulate in the waste of the metallurgical industry, are common components in the ash of thermal stations using solid fuels, are formed during welding and other technogenic processes using high temperatures (Menshikova, 2015).

Viewing the magnetic fraction of sod-podzolic soils under a microscope shows that it consists of aggregates formed by fragments of minerals, among which there are many particles of regular spherical shape. Demagnetization, ultrasonic treatment, alternating magnetic field and multiple separation lead to disintegration of aggregates and promotes an increase in the concentration of the spheres in the studied preparations (Glebova, 1986).

The magnetic fraction, isolated from sod-podzolic soils, contains not only spherules, but also products of their destruction. They have cracks, holes that expose the inner cavities. Large spherules consist of separate fragments of more or less regular shape. When separating some of the spheres with a bright metallic sheen float up. When crushed under a microscope, these particles scatter into fragments with a wall thickness of one tenth or less of the diameter. The inner side of the shell is spongy, especially in spheres floating in the water during their separation from soil samples. On the surface and inside large disintegrated hollow spheres sometimes smaller ones are located (Babanin et al, 1995).

The inhomogeneity of the structure of spherical particles is clearly visible on the sections of the magnetic fraction. In the natural state and during grinding, surface and internal structures can be easily observed, sinks and caverns are opened. Most balls do not have a monolithic surface - it often consists of blocks, facets, furrows, needle crystals, plates or dendrites can be observed on the surface and inside (Babanin В.Ф, 1995).

According to morphological features, particles from sod-podzolic soils can be divided into groups (Glebova, 1986):

1 - spherules 1-2 mkm in diameter with a smooth surface (most often found on larger particles in the form of "kidneys");

2 - particles 2-3.5 mkm in diameter, covered with "amorphous" tubercles, between which a smooth surface is visible (they can occur as "kidneys" on large diameter spheres);

3 - spherules 3-8 mkm in size, the entire surface is covered with scales, cubic crystals; on these particles, sometimes particles of the first two groups are attached;

4 - particles more than 8 mkm (up to 40-1000 microns), the surface is covered with scales, tiles, weakly bent particles, often destroyed, holes in the spherules of this group open the hollow part.

Gennadiev found that forest soils are enriched more by spherules than soils of treeless forests. This is due to increased capture of atmospheric dust by forest phytocenosis. Thus, in the case of sod-podzolic soils of the Moscow Region (Solnechnogorsk district), the content of spherical magnetic particles (5-13 g / m2) in soils not covered by forest was shown to be 3-3.5 times less than in analogous soils, Covered with forest (26-35 g / m2).

Also, the research conducted by Gennadiev (2004) in the city of Elektrougli (Moscow region) demonstrated the heterogeneity of the distribution of magnetic particles from the source of emissions. It was established that bog-podzolic soils at a distance of about 500 m from industrial enterprises and railroad contain more than 70 g / m2 of spherical magnetic particles, and at a distance of 1.5 km only 55 g / m2. Due to the easy granulometric composition of the studied soils, the spherules penetrate to great depth. The concentration of the magnetic phase at a depth of 0-20 cm is 0.2 g / kg.

Menshikova's detailed study (2015) of surface of the spheres from the sediments of the river valleys of the Urals under the electron microscope allowed her to trace the progressive change in them under the influence of agents of the external environment. One of the main processes is partial dissolution, as well as oxidation. Signs of dissolution of the spheres are caverns, depressions, microcracks, pores, etc. The results of the microprobe analysis showed significant differences in the chemical composition of the investigated spheres. Among them, according to the chemical and mineral composition of Е.А Меньшиковой (2015) distinguishes

the following groups: glandular (magnetite); Silicate-ferruginous (altered magnetite) and silicate.

The glandular spherules are distinguished by the most regular spherical shape, strong semimetallic luster, black color, smooth surface and small dimensions (usually less than 100 microns). They have the strongest ferromagnetic properties. In their chemical composition, the proportion of iron oxides exceeds 80% (Osovetsky, 2006).

Thus, the magnetic spheres of soils are diverse in genesis, elemental chemical composition and morphology. Their quantity, composition and properties in the soil are indicators of man-made pollution. The study of magnetic spheres is an actual research direction in soil science.

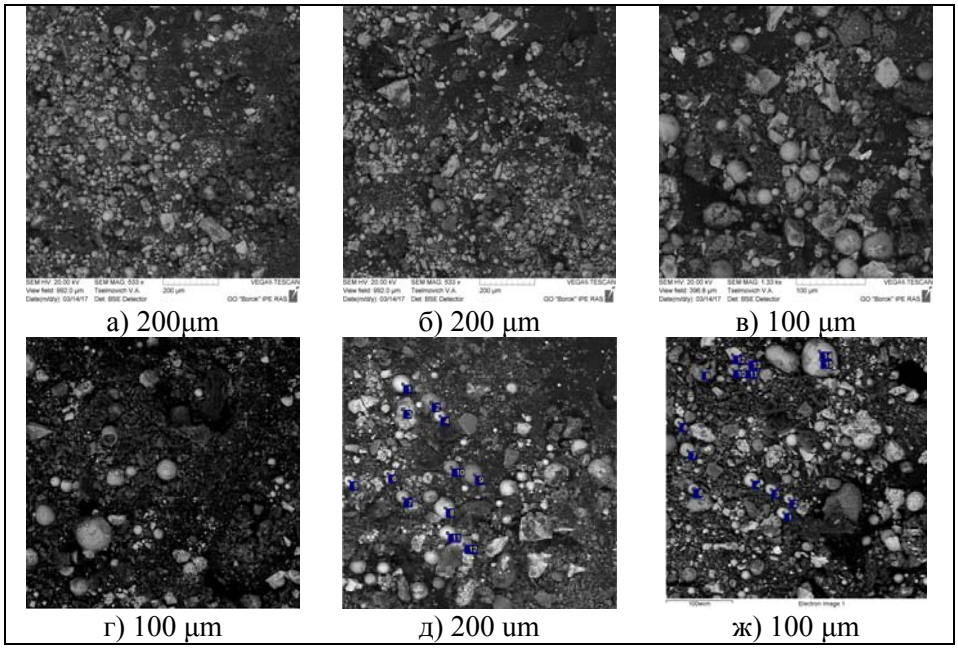
Results and discussion

Study of sandy urbo-sod-podzolic soils under forest in the middle Kurya district of Perm. The magnetic susceptibility of fine earth samples of sandy loamy urbo-sod-podzolic soils varies from 41 to 44 x 10-8 m3 / kg-1.

From the soil samples of the strongly decomposed forest litter with the help of a ferrite permanent magnet, the magnetic phase was extracted by the method of dry separation. The content of the magnetic phase was 0.3% of the mass of the initial sample of the layer. The magnetic susceptibility of the magnetic phase extracted from the soil is about 25,000 x 10-8 m3 / kg-1.

The phase composition of the magnetic particles was studied in the geophysical observatory Borok of the Institute of Earth Physics of the Russian Academy of Sciences using a scanning electron microscope Teskan VEGA II. The elemental chemical composition of magnetic particles is determined using an energy-dispersive spectrometer complete with a microprobe "Teskan VEGA II". The method of electron-probe microanalysis (EZMA) makes it possible to achieve locality of the analysis of ~ 1.5 - 0.3 mkm in depth and 0.8 - 0.3 mkm wide (Tselmovich).

On the picture 1 presents panoramic or general micrographs of samples of magnetic particles. Black background on microphotographs is an electrically conductive carbon scotch on which samples of magnetic phase particles are applied for analysis. In micrographs, the particles in reflected electrons are inhomogeneous in color and composition: gray particles are aluminosilicates with a small fraction of iron, light gray particles contain aluminosilicate particles with the inclusion of a higher proportion of metals with a high atomic mass (iron and heavy metals). The lightest (silvery) particles contain an increased amount (70% or more of the mass) of iron and heavy metals. Figure 1 shows that the silver phase is dominated by silvery spherical particles (spherules), polyhedral particles and irregularly shaped particles.

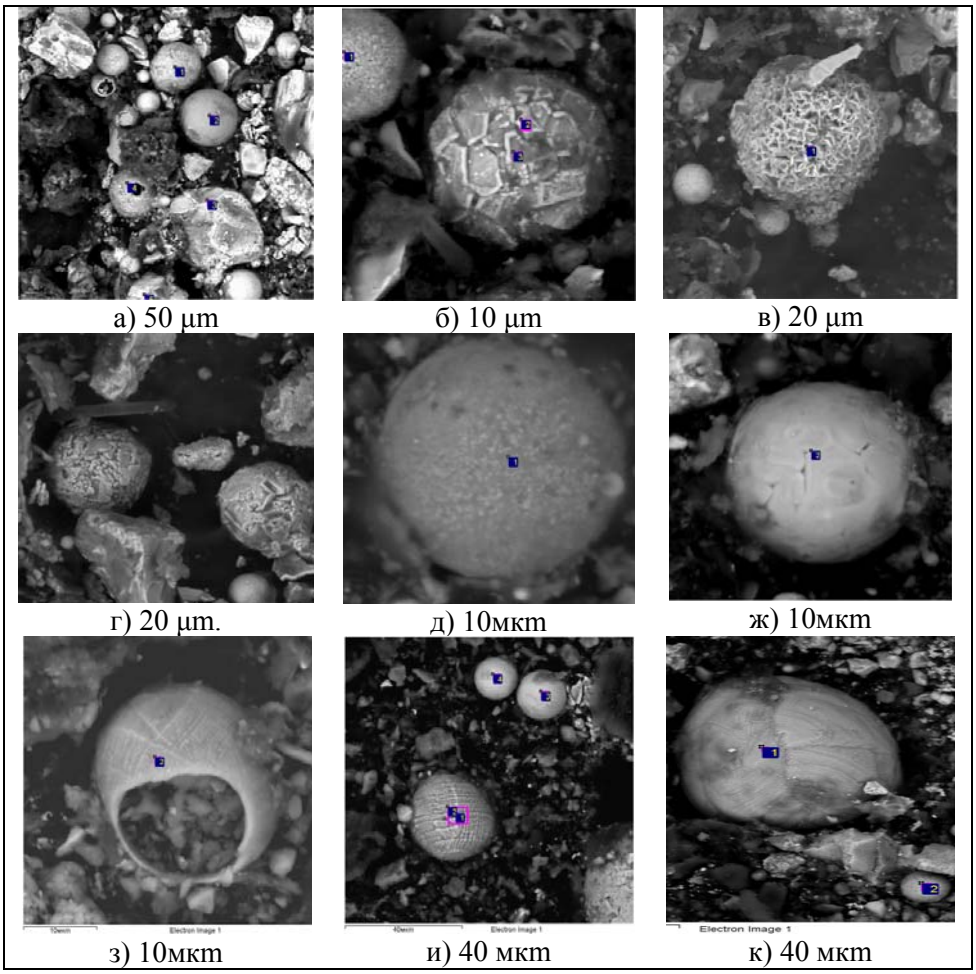


а) 200μm б) 200 μm в) 100 μm

г) 100 μm д) 200 um ж) 100 μm

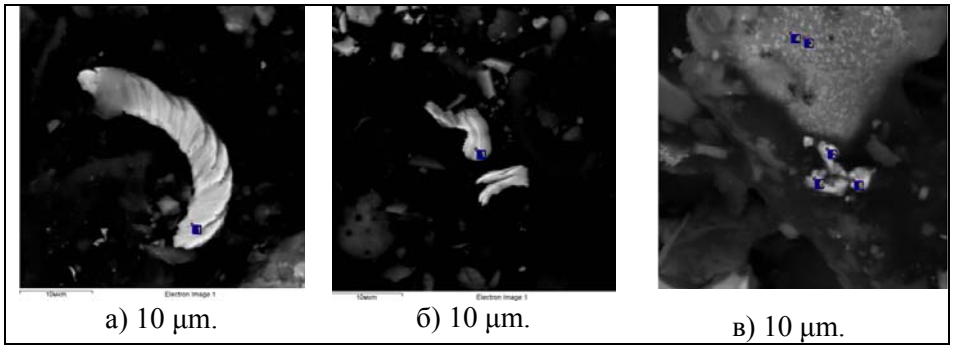
Picture 1. Electron microprobe snapshot of the magnetic phase (MF) of soils in the right-bank part of Perm, 2017.
1-15 – number of points for spectroscopic analysis in particles MF.

On the picture 2 shows pictures of spherical magnetic particles. The texture of spherical particles is diverse and is represented by several forms: smooth, rough, furrowed, scaly, spongy. The roughened surface of the spheres (a-1, a-4, s, u-1, 2, k-1) is more often encountered, but there are also particles with a smooth (a-2, u-4, 3, -2) (B-1, d), granular (a-4, d), spongy (in), scaly or segmental (b), honeycomb (g) surface. The dimensions of the spheres range from 20 to 50 mkm. Some spheres have open cavities (a-2, x, z), which allows them to be transported by air streams to considerable distances from emission sources to the atmosphere through the pipes of industrial and power enterprises. The technogenic source of the spheres on the right-bank territory of Perm can be the emissions of the metal-working shops of PJSC Motovilikhinskiye Zavody and the steelmaking plant of OOO Kamastal. The enterprises are located in relation to the sampling site in the Kurya microdistrict at a distance of 2-3 km from the leeward side and on the high left bank of the river. Kama. Annually, the steelmaking plant of OOO Kamastal produces about 400 thousand tons of alloyed steel, which is naturally accompanied by emissions of pollutants into the atmosphere.



Picture 2. Electron microprobe snapshot of the magnetic phase (MF) of spherical shape: a) spherical smooth (2), rough (1), granular (3); B) spherical furrowed (1), polygonal, scaly (2, 3); C) spherical spongy; D) spherical furrowed e) spherical granular; G) spherical segmental honeycomb, with facets; H) spherical with an open cavity; E) spherical smooth (4, 3), rough (1, 2); I) spherical roughened (1), smooth (2). 1-5 - numbers of spectroscopic analysis points in the MF particles of the forest urbo-sod-podzolic soil of the Medium Kur'ya microdistrict of Perm, 2017.

On the Picture 3 shows an electron-microprobe snapshot of a spiral-shaped particle from the magnetic phase of the forest urbo-sod-podzolic soil of the middle Kurya microdistrict in Perm. Their size is up to 20 microns (picture 4).



а) 10 μm. б) 10 μm. в) 10 μm.

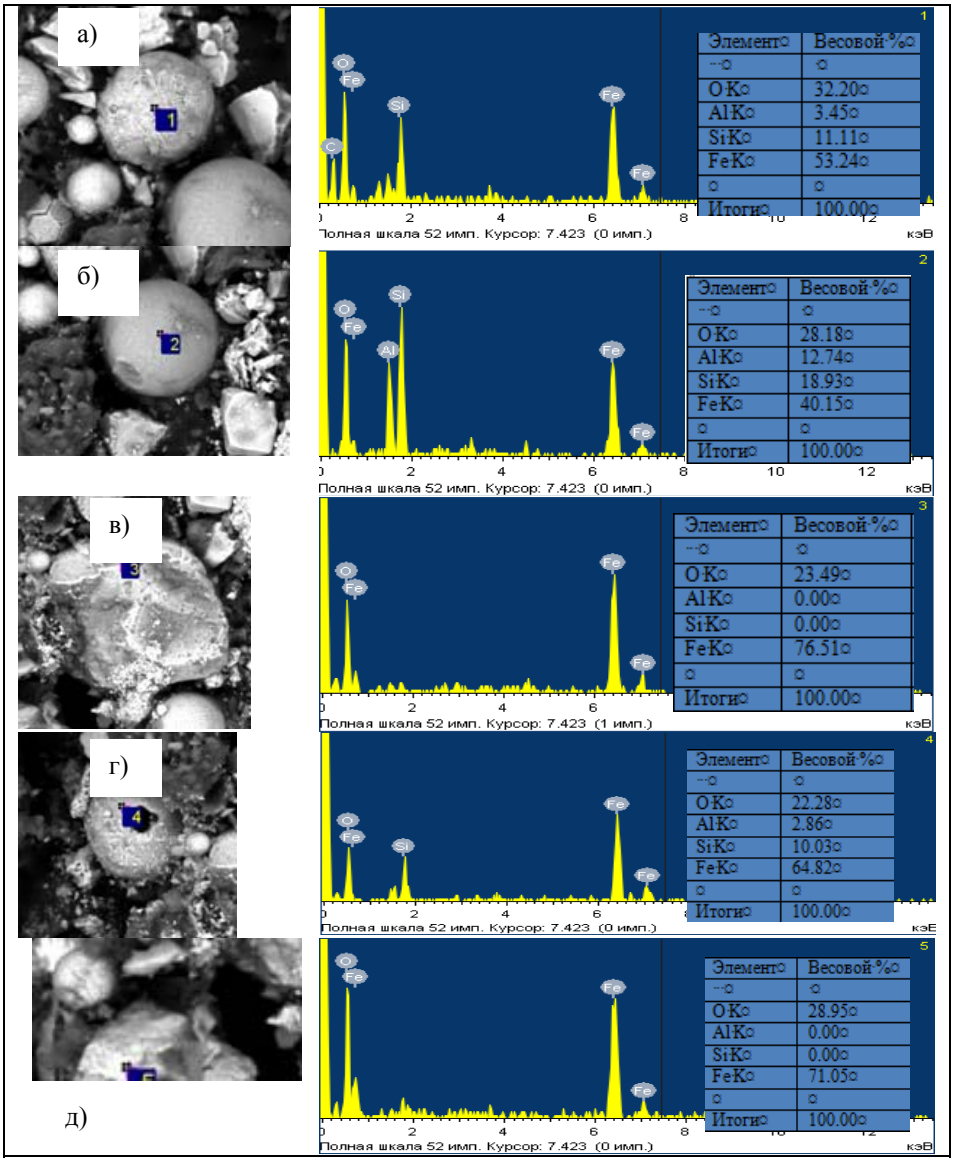
Picture 3. Electron microprobe snapshot of the magnetic phase: a) spiral shape; B) irregular shape; C) irregular shape; 1-6 - numbers of spectroscopic analysis points in the MF particles of the forest urbo-sod-podzolic soil of the Medium Kur'ya microdistrict of Perm, 2017.

Table 1 - Chemical composition of magnetic particles in the sample of the magnetic phase No. 1 of the forest urbo-sod-podzolic soil of the medium-sized Kurya microdistrict of Perm (%), 2017.

Range	O	Fe	Al	Si	Result
1-1(а)	32,20	53,24	3,45	11,11	100
1-2(б)	28,18	40,15	12,74	18,93	100
1-3(в)	23,49	76,51	0,00	0,00	100
1-4(г)	22,28	64,82	2,86	10,03	100
1-5(д)	28,95	71,05	0,00	0,00	100
Medium	27,02	61,15	3,81	8,01	100
Standard deviation	4,09	14,59	5,24	8,08	
Maximum	32,20	76,51	12,74	18,93	
Minimum	22,28	40,15	0,00	0,00	

On the picture 4 shows the spheres and polyhedral magnetic particles of maghemite (a, b, r,) and magnetite (c, d). These particles have predominantly iron with impurities Al, Si, O. Some particles (c, d) have a purely ferruginous composition, without Al and Si impurities. Enriched with oxygen. The chemical composition of magnetite (Mt) (c) is represented by: Fe - 76.51% and O - 23.49% by weight. Fe ions on the spectrum have three peaks. The chemical

composition of magnetite (e) is represented by: Fe -71.25% and O-28.95% (Table 1).



Picture 4. Electron microprobe snapshot of particles and energy-dispersion spectra of points (1-5) of microprobe analysis of a sample of the magnetic phase No. 1 of forest urbo-sod-podzolic soil of the middle Kurya microdistrict of Perm, 2017.

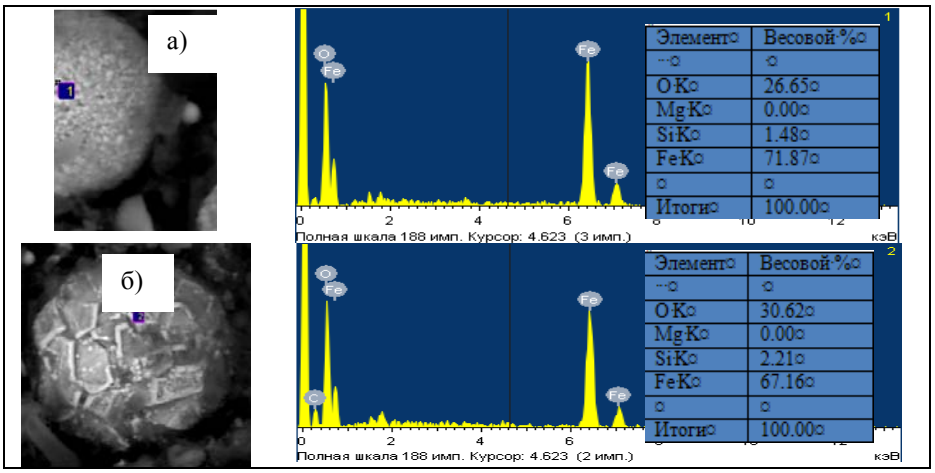
Magnetic particles in the sample of the magnetic phase № 1 have on the average the following elemental composition: Fe - 61.15%; O - 27,02%; Al, 3.81%; Si - 8. 01%.

The magnetic particles on the picture 5 are represented by maghemite (b) and magnetite (a). They have mainly a ferruginous composition with impurities of Mg, Si.

The average content of elements in the sample: Fe - 67.58%; O - 28.19%, Mg - 1.96%; Si - 2.26%. The chemical composition of slightly oxidized magnetite (e): Fe -71.87% and O-26.65% (Table 2).

Table 2 - Chemical composition of magnetic particles of the sample of the magnetic phase № 2 of the forest urn-sod-podzolic soil of the middle Kurya microdistrict of Perm (%), 2017.

Range	O	Mg	Si	Fe	Result
2-1(а)	26.65	0.00	1.48	71.87	100.00
2-2(б)	30.62	0.00	2.21	67.16	100.00
2-3	27.31	5.87	3.10	63.72	100.00
Medium	28.19	1.96	2.26	67.58	100.00
Standard deviation	2.13	3.39	0.81	4.09	
Maximum	30.62	5.87	3.10	71.87	
Minimum	26.65	0.00	1.48	63.72	



Picture 5. Electron microprobe snapshot of particles and energy-dispersion spectra of points (1-2) microprobe analysis of the magnetic phase of sample No. 2 of forest urbo-sod-podzolic soil of the middle Kurya microdistrict of Perm, 2017.

Table 3 - Chemical composition of magnetic particles in a sample of the magnetic phase of forest urbo-sod-podzolic soil of the medium-sized Kurya microdistrict of Perm (%).

Energy dispersive spectrum	O	Na	Fe	Result
3-1(а)	19.50	0.00	80.50	100.00
3-2	27.54	1.67	70.79	100.00
3-3(б)	26.01	4.51	69.47	100.00
3-4(в)	21.99	0.00	78.01	100.00
Medium	23.76	1.55	74.69	100.00
Standard deviation	3.68	2.13	5.39	
Maximum	27.54	4.51	80.50	
Minimum	19.50	0.00	69.47	

The average content of elements in sample No. 3: Fe - 74.69%; O - 23.76%; Na -1.55%.

Conclusion

Magnetic particles in the zone of emissions of metallurgical enterprises are a chemically and mineralogically complex form of production waste, which is the carrier and source of heavy metals in soils in the areas of operation of smelters. In the composition of magnetic particles that pollute the soil, spherules predominate. The matrix of the spheres consists mainly of iron oxides (magnetite, hematite) mixed with iron silicates (olivine, pyroxenes). The rims on the surface of the spheres consist of magnetite and hematite. The mineralogical and chemical composition of soil spheres depends on the composition of the ore, waste and temperature conditions at the time of their formation, as well as on the activity of soil solutions during the weathering of the spheres.

The magnetic particles of the strongly decomposed litter of forest urbo-sod-podzolic soils of the middle Kurya microdistrict of Perm, in general, have the form of hollow microspherules with a varied texture of their surface. According to the mineralogical composition, these are predominantly iron oxides: magnetite and maghemite with impurities of aluminosilicate particles. Their origin is high-temperature and is associated, most likely, with the emissions of the steelmaking plant of OOO Kamastal, Perm. The particles of the magnetic phase are carriers of heavy metals: Cr, Ni, Mn, Zn, and Ti and Mg are typical elements of the impurities of emissions from metallurgical enterprises.

The hollow form of the spheres and their location in the layer of forest litter testifies to anthropogenic aerial pollution of the components of the environment of Perm in the zone of action of metallurgical enterprises. Hollow micro- and

nanospheres from the atmospheric air can enter the upper respiratory tract of city residents, which poses a potential threat to their health.

Weathering of magnetic particles when they are involved in the process of soil formation can be accompanied by the release into the soil solutions of mobile forms of zinc, copper, nickel and other metals and metalloids and lead to contamination of the city's hydrosphere.

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Long-Term Lending of Ukrainian Agrarian Operators

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Abstract: long-term bank crediting of agricultural enterprises has been analyzed for the period of 21 years. We found out what has driven the volumes of long-term bank crediting of agricultural enterprises of Ukraine and proposed the ways of development.

Key words: long-term lending, loans, agricultural enterprises

Introduction

Enhanced impact of long-term bank crediting in agricultural sphere is the issue, which requires comprehensive scientific research. The latter opens the opportunities for in-depth analytical studies on efficient crediting tools for investment projects of agricultural enterprises. It is noteworthy to mention that long-term bank loans as the source of investment resources can duly streamline the activity of agri enterprises and boost their economic growth.

Material and methods

Agricultural sector of Ukrainian economy is one of the most important fields of business activity in this country. Sustainability of rural area depends on this sector as well as food safety of the population. As compared to others, this sphere of economy of Ukraine requires actual, comprehensive support of the state and the banks.

Ukraine, as producer and exporter of agricultural commodities, should provide Ukrainian agri enterprises with necessary support and assist in bettering conditions of production and improvement of competitiveness on domestic and foreign markets. Long-term bank loans to agri enterprises can ensure its

development, provided that credit resources are properly allocated. Development of long-term bank crediting of agricultural enterprises had the following trends:

It was upward in the period of 1996 - 2008, 2010 years, and in 2009, 2015, 2016 crediting volume considerably dropped, in 2011- 2013, 2017 - it went up, though the share of long-term volumes varied, depending on political and economic situation in Ukraine and globally (Table 1, Fig.1).

Table 1. Crediting volumes in Agriculture, mln UAH.

Year	Total	Including long-term loans by types of currency		
		UAN	in foreign currency	share, %
1996	169	30	-	17,8
1997	268	25	-	9,3
1998	329	31	62	28,3
1999	390	43	119	41,5
2000	745	58	98	20,9
2001	1676	111	139	14,9
2002	2593	338	239	22,2
2003	4561	1540	525	45,3
2004	5218	1768	668	46,7
2005	8192	3090	105	50,5
2006	11878	5026	1867	58,0
2007	16508	7136	3077	61,9
2008	28812	12296	5665	62,3
2009	26026	10821	4960	60,6
2010	26545	13211	4703	67,4
2011	34143	14822	5985	60,9
2012	36488	13375	6603	54,8
2013	39158	14253	5097	49,4
2014	55335	19864	10518	54,9
2015	54577	20053	13593	61,6
2016	48425	18215	8151	54,4
2017 April	57514	32133	6873	67,8

Upward trend of agricultural crediting volumes was stipulated by state support and preferential credits. Along with that, decrease of share in long-term loans was associated with crises issues, political instability and finance and economic situation in the country and testified to the lack of confidence of the bank institutions to funding recipients in agricultural sector, caused by: risks in the branch, level of financial solvency and instability of agricultural sector, driven by seasonality, cyclicality, depending on natural weather conditions and difficulty in forecasting the yields and price formation of domestic and foreign agri markets.

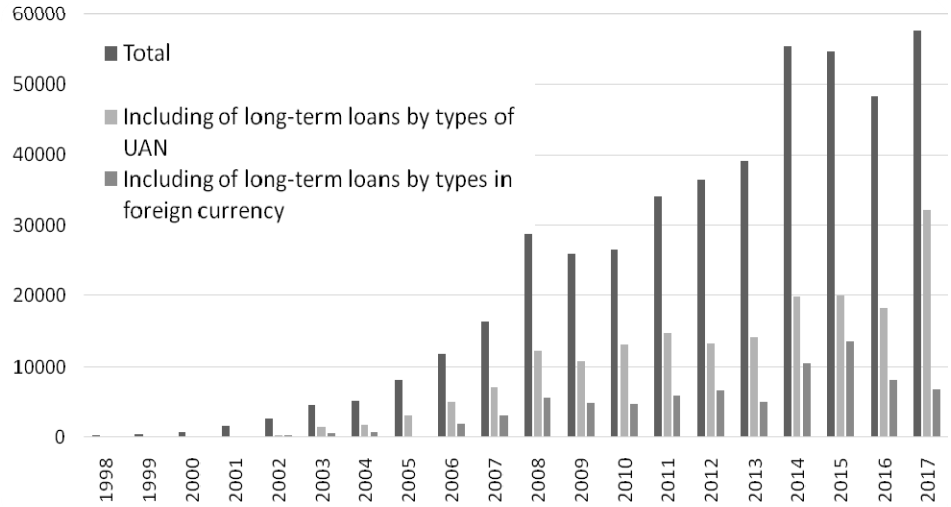

Fig.1. Crediting volumes of agricultural enterprises (mln UAH)

Development of agricultural enterprises requires long-term bank crediting. Investments are necessary to expand business and modernize fixed assets (equipment and facilities). Banks provide long-term loans to the borrowers on condition of availability of liquid assets, which might be used as a pledge. Farmland may serve as the main pledge for agrarian enterprises. Private farmland in Ukraine is of low cost. Besides, it is under ban for trade.

For agri enterprises to be able to develop financially, it is necessary to tackle the issue of liquid assets to be pledged, so that banks will be able to make long-term investments in main assets of agri enterprises, having abated credit risks. Nevertheless, development of agri sector of Ukraine is not possible without support of the state, which envisages preferential subsidy, budgetary appropriation, donations, actual state programs for support of the rural area, preferential taxation. One of the afore-mentioned ways is capital investments, the sources of which are based on: the costs from state budget, state funds, investments, long-term commercial and bank loans. Special state agrarian bank will be efficient creditor.

Results and discussion

Based on the analysis, we can identify the drivers of long-term crediting of agricultural enterprises. The external drivers are: agrarian policy (development and reforming of the following agri laws by Verkhovna Rada of Ukraine: mortgage, pledge, lands, state guarantee fund, agri market development fund, proper support of domestic producers, etc.); state budget (state income and expenses related to

agriculture); state financial support of agriculture (provided in the budget: expenditures, donations, compensations, subsidies, benefits); commodity, stock market (development of commodity market of Ukraine with engagement of agricultural enterprises: as an opportunity for profitable sales of agricultural commodities; development of fund market in terms of agricultural enterprises : opportunities to obtain additional financial resources by issuing mortgage bonds as an alternative for long-term loans); transparent controlled pricing for agricultural (regulation and identification of price range for agricultural commodities), land market (development of farmland market),guarantees and pledge (state, property collateral guarantors, etc.), (reliability of collateral, other opportunity for settlement with creditors as for paying off the debt in case of unforeseen circumstances).

Internal factors include: business solvency of the borrower (ability to comply with obligations in full scope and in due terms; rating of the borrower, which is identified depending on the state of the enterprise, its reputation, the risks related to payoff, project plan, collateral (pledge, etc.), liquidity of the pledged assets and asset value in future period), which is determined by: 1. Business reputation. 2. Credit history. 3. Financial soundness. 4. Quality of project plan. 5. Loan collateral.

It is necessary to consider crediting of agri investments projects as allocation of resources for investments focused on expanded reproduction of capital due to the principal of banking crediting and support of the state.

Conclusions

Tackled issues of long-term crediting shall streamline the following: stabilization of financial and economic situation in the country, using mortgage banking institutions, including the state ones, which will be of mutual benefit; long-term credit resources of the banks; adequate analysis of credit score of agricultural enterprises (including financial soundness , seasonality); establishing high-quality legal base; network of credit history bureau, optimization of the system of property registration (to be improved: land cadastre and state registration of ownership rights, etc., state guarantee (as redistribution of risks); state financial support; increase of irrevocable grants to agricultural enterprises of the country, counter and preferential crediting provided by the state, efficient regulation of NBU credit market, development of the insurance.

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Efficiency of new liquid fertilizers chap liquid in immortelle plantation production system (*Helichrysum italicum* (Roth) G. Don fil.)

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Abstract: Demand for safe products of plant origin, including medicinal substances produced according to the principles of organic production, is becoming increasingly important each day. As the requirements for organic raw material of immortelle also considerably increase, the aim of these study was to investigate the efficacy of new liquid organic fertilizer Chap Liquid on crop yields and other parameters of immortelle growth in plantation. In the experiments, besides liquid organic fertilizer Chap liquid, both organic-mineral fertilizer Sapro elixir variant, and unfertilized control variant were included. Experiments were carried out in a commercial plantation in the vicinity of Podgorica. In all variants of organic fertilizer application, Chap fluid had a significantly greater effect on increasing the average number of shoots (39.4 and 42.2), their height (41 and 45 cm) and average yield per plant (276.7 and 366.7 g) compared to the control variant (31.5, 36 cm and 183.3 g). Differences in the studied parameters between liquid organic fertilizer Chap liquid and conventional fertilizer Sapro elixir did not have statistical significance. Results have shown that the new fluid organic fertilizer Chap Liquid can successfully be used in the organic production of immortelle.

Key words: immortelle, plantation, liquid fertilizers

Introduction

In recent decades, interest in medicinal, aromatic and spicy herbs has been increasing worldwide and it is reflected in increased use of medicines of plant origin, growing demand of plant material by the industry, and the growth of areas under plantations. It is estimated that more than 3,000 species are present

in international trade (Schippmann, 1997). As a result of the increased ecological awareness, the growth of living standards, and the desire of people to return to nature, the demand for products of plant origin produced according to the principles of organic production significantly increases. Such trends impose the need to find new possibilities for increasing the production of medicinal herbs according to the principles of organic production (Jovović et al., 2017a).

Immortelle is a perennial aromatic semi-bush that naturally grows on stony terrains of the Mediterranean (Britvec et al., 2013). It has very specific healing properties and has a wide range of applications. Due to the increased global demand and uncontrolled exploitation in its natural habitats, many natural populations are now in the extinction or are seriously endangered.

The high demand for products based on immortelle, guaranteed trade, excellent quality of produced drugs and very good income are the main reason why immortelle is grown in our area. Today, the immortelle is grown on about 80 ha in Montenegro, with the tendency of further spreading of growth areas.

As in recent years, the demand for raw materials of immortelle for organic production in the world has increased significantly; the aim of this research was to investigate the efficiency of the new liquid organic fertilizer Chap liquid on the productivity of the immortelle plantation.

Material and methods

Study of the impact of the new liquid organic fertilizer Chap liquid on yield and other parameters of immortelle productivity was carried out during 2016 in the vicinity of Podgorica, on terra rossa terrain.

Field experiment was carried out in a commercial plantation of immortelle, in a completely random block system, in 4 replications. The planting was done in early November 2015. The surface of the elemental plot was 10 m^2.

The experiment consisted of two variants of fertilizer: liquid organic fertilizer Chap liquid and organic-mineral fertilizer Sapro elixir and non-fertilized control variant. Chap liquid and Sapro elixir were applied twice: first time on March 1st and the second, 15 days later - on March 15th. Chap liquid was applied in an amount of 100 l. ha^{-1} per treatment, and the Sapro elixir with 100 $kg.ha^{-1}$ per treatment. The basic data on the applied fertilizers are given in Table 1.

Harvest of immortelle was carried out on June 25th, when a number of shoots per plant and their height were determined. After harvest, the yield of herb was measured. The total yield of herbs per hectare is calculated based on the theoretical density of crops of 40000 plants.

The soil on which the experiments were carried out belongs to the type terra rossa (tab 2). It is characterised by deeper ploughing layer depth, and in the texture it is light clay. It is distinguished by the medium humus content, low

carbonate content and slightly acidic reaction. Phosphorus and potassium are well supplied. Meteorological conditions during the experiment are shown in Table 3. From the given data it is obvious that 2016 was very favorable for immortelle production in Podgorica.

The analysis of variance was calculated according to randomize complete block design. The significant differences among the means were evaluated according to least significant difference (lsd) test.

Table 1. Main characteristics of studied fertilizers

Chemical content	Chap liquid	Sapro elixir
Ash content (%)	29,5	less than 30%
Organic substance content in dry matter (%)	70,5	at least 15%
Full nitrogen (N), % (m/m)	3,62	5,9
P_2O_5 (%)	0,95	7,69
K2O (%)	4,67	11,08
Ca (%)	0,75	
Mg (%)	0,40	
Fe (%)	0,77	
Water solution pH (v/v)	7,5	5,2-7,5

Table 2. Chemical characteristics soil on experimental field

Depth (cm)	pH		CaCO3 %	Humus %	Soluble mg/100 g	
	H_2O	nKC			P_2O_5	K_2O
40	6.68	5.51	0.86	3.22	16.3	19.6

Table 3. Meteorological conditions in Podgorica in 2016

Month												Average
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Air temperature (^{0}C)												
6,5	10,8	11,5	17,2	18,6	24,7	28,3	27,6	22,1	15,9	10,4	5,3	16,6
Amount of rainfall (mm)												
240,1	273,3	316	82,6	268,2	158,7	78	3,8	84,4	223,8	264,1	0,7	1993,7

Results and discussion

Immortelle, like other medicinal plants requires high quality fertilization. The fertilization with manure is not recommended, because the manure of poor quality can lead to an unwanted increase of weediness (Pohajda et al., 2015). As demands for organic immortelle are increasing from one year to another, it is essential to create adequate fertilizing systems based on fertilizers licensed for organic production. The results of the impact assessment of the certified organic

fertilizer Chap liquid on yield and other productivity parameters of immortelle are given in Table 4.

Table 4. Research results

Fertilizer variant	**Studied parameter**			**Yield per ha**
	Average number of shoots per plant	Average shoot height (cm)	Herb yield per plant (g)	
Control	31.5	36	183.3	7.3
Chap liquid – applied once	39.4	41	276.7	11.1
Chap liquid – applied twice	42.2	45	366.7	14.7
Sapro elixir – applied once	38.5	41	293.3	11.7
Sapro elixir – applied twice	41.8	46	346.7	13.9

	Lsd 005	Lsd 005
Average number of shoots per plant	6.302	8.746
Average shoot height (cm)	3.228	4.481
Herb yield per plant (g)	47.428	65.824

Results of the measurements given in Table 4, show that all variants of fertilization had a significant effect on increased number of shoots compared with control. The largest number of floral shoots was found in the variants in which Chap liquid and Sapro elixir were applied twice (42.2 and 41.8), while the lowest number of shoots per plant had a control variant (31.5). All other differences in the number of shoots were of no statistical significance.

The applied fertilizers showed the same effect when it comes to the average height of the shoots. Highest plants were measured on variants with two applications of fertilizer (Sapro elixir 46 and Chap liquid 45 cm), while the lowest plants were recorded in fertilizer-free variants. A significant increase in the height of the plant was determined in variants with two applications of fertilizers in comparison with those on which fertilizers were applied once (41 cm in both variants).

The same tendencies were also seen in the case of herb yield per plant. The highest yield of the inflorescence was measured on variants in which Chap liquid and Sapro elixir were applied twice (366.7 and 346.7 g), and the lowest in control (183.3 g). The yield of inflorescence on variants with single application of fertilizers (Sapro elixir 293.3 and Chap liquid 276.7 g) were significantly lower compared to the yield obtained from two applications of the studied fertilizers.

In all variants of application, organic fertilizer Chap liquid exhibited significantly greater influence on the increase in the number and average height of the shoots, as well as the yield of inflorescence per plant, or per unit area (ha).

The differences in the values of the studied yield parameters between the liquid organic fertilizer Chap liquid and the conventional fertilizer Sapro elixir were of no statistical significance. The same tendencies were established by Jovović et al. (2017b) studying the efficiency of liquid organic fertilizer Chap liquid in the production of immortelle seedlings (plant height, root weight, mass of the above-ground part of the plant).

The results of these studies have shown that the certified liquid organic fertilizer Chap liquid represents a high-quality organic source of nutrients and can be used with success in organic production of immortelle.

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Habitat loss through development

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Abstract: Experimental investigations of the relationship between biodiversity and ecosystem functioning (BEF) directly manipulate diversity then monitor ecosystem response to the manipulation. While these studies have generally confirmed the importance of biodiversity to the functioning of ecosystems, their broader significance has been difficult to interpret. The main reasons for this difficulty concern the small scales of the experiment, a bias towards plants and grasslands, and most importantly a general lack of clarity in terms of what attributes of functional diversity (FD) were actually manipulated. We review how functional traits, functional groups, and the relationship between functional and taxonomic diversity have been used in current BEF research.

Key words: Biodiversity offsets; Ecological compensation; Environmental impact assessment.

Introduction

The study of the ecosystem consequences of biodiversity loss represents a synthetic field of ecological research that seeks to understand how changes in species composition, distribution, and abundance alter ecosystem functioning. As changes in biodiversity are widespread, findings from this research have received considerable attention, but these findings have been and continue to be difficult to interpret. Synthesis and consensus are emerging, however, and the central challenges are being identified for biodiversity and ecosystem functioning (BEF) research to become a predictive science. The question for BEF research is no longer whether biodiversity matters, but how it matters.

Material and methods

Habitat loss is the first cause of biodiversity loss (Balmford and Bond, 2005), especially through accelerating urbanization and infrastructure development (McKinney, 2006).

Although measures can and should not replace stringent actions to reduce threats to biodiversity, several countries require that developers first avoid biodiversity impacts, then minimize the impacts that cannot be avoided and, if there are any residual impacts, offset these through actions that generate an equivalent biodiversity gain, there or elsewhere. This hierarchy of avoiding > reducing > offsetting impacts is known as the mitigation hierarchy (Fig. 1).

Although not a new instrument, the mitigation hierarchy has seen increased enforcement in the last decades, not least because it appeals to policy makers looking to involve the private sector in nature conservation through market-based instruments (ten Kate et al., 2004; McKenney and Kiesecker, 2009; BBOP, 2009; EFTEC and IEEP, 2010).

The potential contribution of such mitigation policies to nature conservation are well established (Kiesecker et al., 2010) but conservationists have warned against such an approach giving incentives for developers to downplay or ignore the requirement to first avoid and reduce their impacts under the false impression that any impact can be compensated for.

In fact, offsets can offer only poor or incomplete replacement for the loss of biodiversity which is very location-specific or which was generated by long-term ecological dynamics such as peatlands or mature woodland. Mitigation policies have also been criticized for their poor track record of effective implementation and monitoring of offsets (e.g. Strange et al., 2002; Robertson, 2004; Burgin, 2008).

Given these limitations, and under the requirement that offsets come after avoidance and reduction measures have been taken, appropriate assessments of the ecological equivalence between biodiversity losses and gains expected from offsets is necessary in order for offsets to effectively contribute to minimizing development impacts on biodiversity (Robertson, 2004; ten Kate et al., 2004; Norton, 2008; McKenney and Kiesecker, 2009; Wissel and Wätzold, 2010).

In this paper, we address the need for robust assessments of ecological equivalence by investigating how key considerations for equivalence are incorporated in a selection of procedures and methods that have been developed for assessing ecological equivalence in contrasting regulatory contexts.

We then discuss how the solutions offered by these methods can be combined into consistent approaches for designing and sizing offset requirements. We then highlight the inevitable technical choices associated with offset policies.

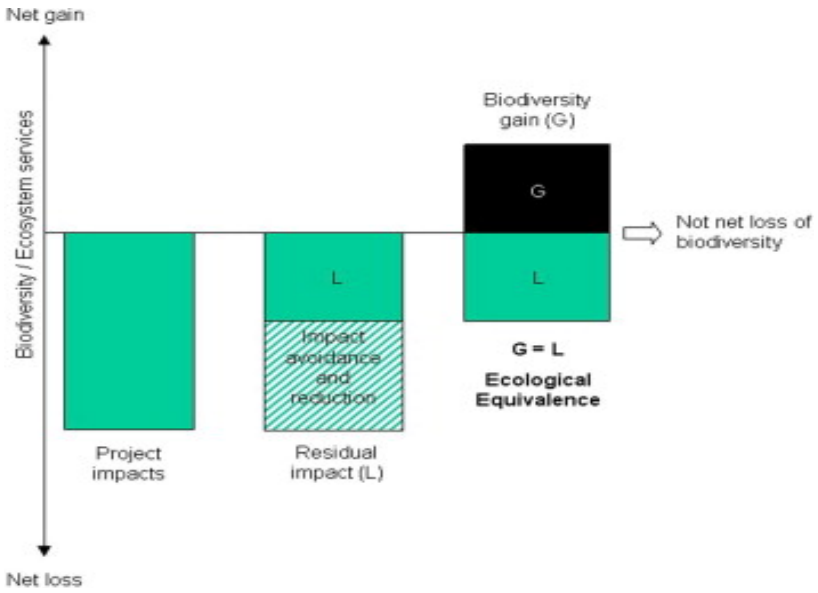

Fig.1 Graphical Representation of biodiversity offsets as part of the mitigation hierarchy and of ecological equivalence in the context of no-net-loss.

Results and discussion

The issues include their scope (i.e. which components of biodiversity or ecosystems are concerned), the question of additionality and in particular when and how protecting existing biodiversity can be considered a gain, as well as possible requirements concerning location (on-site or off-site), timing (pre- or post-impact) and duration of offsets.

While these issues are essential in the design and implementation of offset policies they do not solve the tricky question of assessing the ecological equivalence between losses caused by impacts and the gains that offsets should aim to provide. identified several key points that require particular attention: (i) the definition of detailed target components of biodiversity and ecosystems (animal and plant populations, particular assemblages of species, community types, ecosystem properties, ecosystem services, etc.), (ii) the development or selection of appropriate indicators (including landscape-level processes) and scoring procedures, (iii) the identification of appropriate baselines for calculating losses and gains as well as the need to address, (iv) time-related issues (e.g. delays between losses and gains) and (v) uncertainties in both assessment and offset outcomes. Fig. 2 illustrates how these considerations can be incorporated into an overall procedure for designing and sizing offsets.

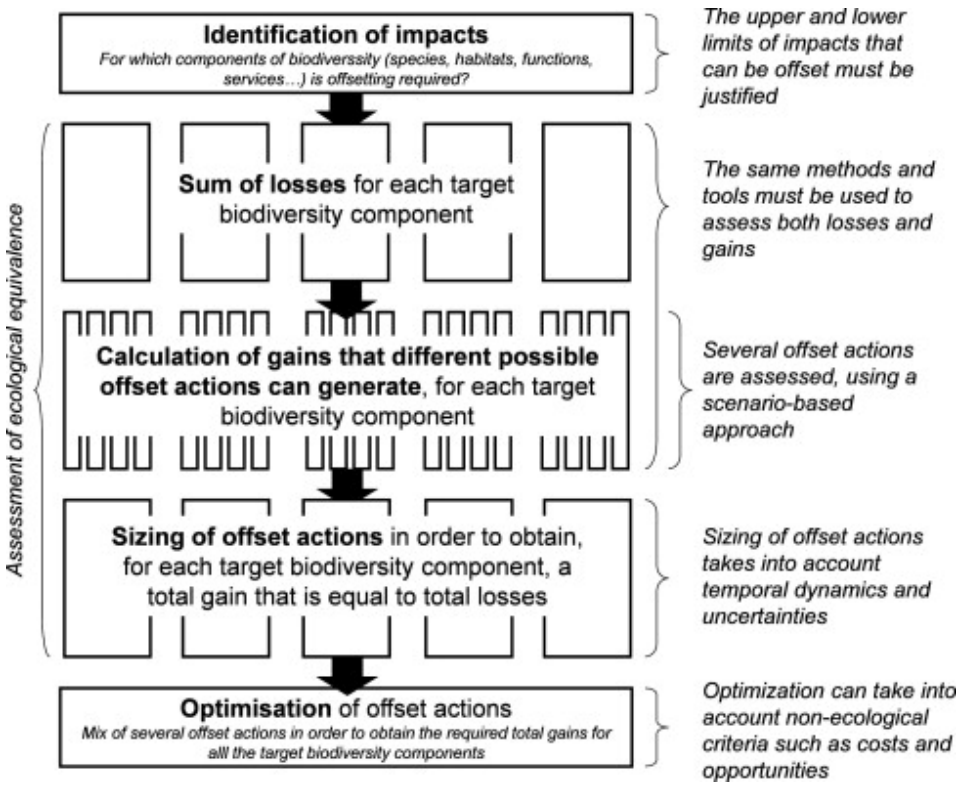

Fig. 2.How the three-step assessment of ecological equivalence fits into an overall procedure for designing, sizing and optimizing offsets measures.

Conclusions

Fig. 1 illustrates how assessing ecological equivalence requires that losses due to impacts and gains generated by offsets be measured using the same metric. If the assessment of losses, gains and their equivalence is to focus on components of biodiversity and ecosystems then these must be clearly defined, ideally in the scoping phase of project appraisal.

Although species richness or evenness are often used in communicating biodiversity issues to the lay public or policy-makers, these are not appropriate for designing and sizing offsets as they do not adequately capture the complexities of biodiversity, either ecological (species interactions) or social (not all species are considered "equal" –Noss, 1990).

Assessing each target component of biodiversity and ecosystems separately does not imply that synergies – for example between a habitat type considered

per se (e.g. a wetland) and considered as habitat for a protected species (e.g. waders) – must be ignored. In fact, offset actions that build on these synergies to generate simultaneous gains for several targets will likely be more successful and more cost-effective. We considered this optimization of offset actions as a separate and final step in the overall design and sizing of offsets, that is not to be confused with the assessment of ecological equivalence (Fig. 2).

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Aspects concerning the grasslands management in Western Romania. A descriptive case.

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Abstract: The permanent grasslands are very important in Europe and in Romania, representing a life place for the most important endemic species and also are a part of the traditional agriculture from a long time ago. The partnership between agriculture and nature protection must to face a lot of challenges concerning grasslands management. For the mountain grasslands the most important remain the productive function in a direct relation with the load of animal per hectare, but in present days this function are related with other aspects as: the grasslands biodiversity management, livestock decreasing in mountain conditions, climatic changes, etc. In these conditions a lot of surfaces are abandoned covered by shrubs or wooded vegetation and each year the production level and quality decrease. For these reasons a correct management in the mountain grasslands are important to maintain a continued use of this ecosystem, to assure a source for life in the rural communities. In last ten years the Romanian farmers in order to apply a correct management forms for grasslands and to increase the livestock seek for support, including finance, from European society.

Key words: grassland management, livestock, production, animals.

Introduction

In Romania the principal legislation concerning the grasslands exploitation is represented by the law (OUG) nr.34/2013, where the surfaces cannot be reduced, and are operate only under a management plan. Also very important for farmers the time period for renting is between 7 and 10 years.

Grassland management and environmental are integrated in present time in rural policies in Europe. Elements of the grasslands management as reducing fertilizer inputs, management intensity, stocking rates, and is central to these sustainable rural policies (Marriot et al., 2004). A great part of the earth's grasslands are over used and poorly managed after Oldeman (1991) cited by Conant, 2011.

A correct grassland management is important to keeps the vegetal sward healthy in order to provide long-term conservation benefits (http://mda.state.mn.us/protecting/ conservation/practices/ grassmgmt.aspx). First function for the grassland ecosystem is the forage production, this function is related with the quantity of organic matter from soil (SOM) the most influenced component by the management (Conant, 2001; Marusca, 2014).

The guidelines for the optimum management of the different grassland types, also depend on the specific goals and has as principal points: timing and technique of cutting, number of grazing animals per hectare in different types of grasslands, chemical imputs according with soil type, the presence of weeds or invasive species like *Pteridium aquilinum*, the presence of the specific fauna, etc (Masé, 2011).

Material and methods

The investigated grasslands was located in the South- West Romania in the Almaj Valley, between the Semenic Massif in the North, the Almăjului Mountains in the South, at altitudes between 312 and 1440 meters, with different exhibitions to the Southwest, South-East and North-East. As a rule the surfaces are inclined below 10% and in the alpine area there are identified slopes with a slope of over 15%.

From the climatic point of view, the region is in the IBp3 climatic formula, and the Koppen system in CFBX with Mediterranean influences. The aridness index of De Martone is 37.5.

The identified phyto climate zones and sub-zones were: The hilly floor of querts with GO CE GI and the lower limit beams. This makes the grassland type predominantly *Agrostis capilalris- Festuca rupicola*, the participation in the composition of the species Festuca rupicola is higher on the shady exhibitions (Bărbulescu and Motcă, 1983; Cernelia and Bistriceanu, 1977). The hilly floor of hornbeam, beech and sessile oak-beech (FD 3) is found on narrow, main and secondary hillsides and slopes.

In the topographical base existing and elaborated for Law 165/2013, the permanent grassland area under the administration of the Prigor Local Council is 3868.9858 hectares. The management plan is made for a number of 57 grasslands Fitting Units (AU) consisting of 174 plots.

For the satellite image processing and for the creation of thematic maps were used: Global Mapper; ArcGIS, WinGIS. Orthophotomap images (with the extension ".tif" and ".sid"), made by the Landsat satellite system, namely Landsat 5, have been used (Barliba, 2011), (Figure 1).

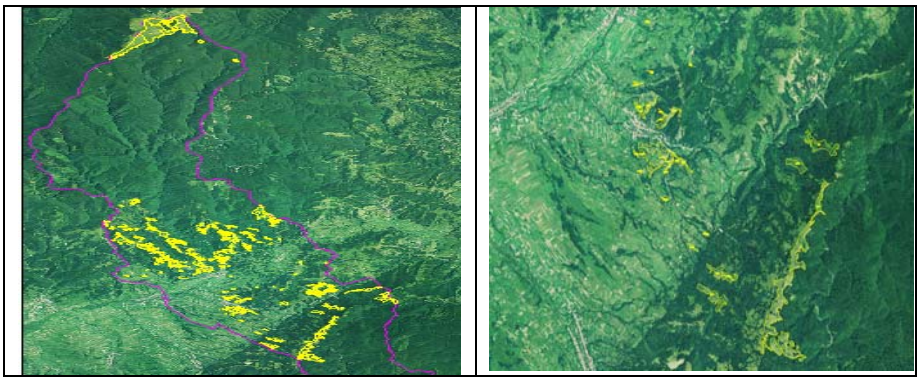

Figure 1. Investigate graslands distribution (yellow color)

For the floral characterization of the meadows the phytosociological (geobotany) method and the double meter method were used to determine the production and the loading with animals. The method of repeated mowing in grazing cages was used (Samfira et al., 2011; Bostan and Samfira, 2014). Determination of pedo-agrochemical characteristics of grassland soils in order to establish the fertilization plan was carried out by OSPA Timis.

For all the studied areas, it is possible to opt for financing from European funds for Measure 10 - Agro-environment and climate foreseen in NRDP 2014-2020 (with funding from EAFRD allocation 2014-2020), Package 1 (P1) - High value grassland Natural or Package 2 (P2) - Traditional agricultural practices.

Results and discussion

Fitoclimatic fitting

As a general appreciation the studied grasslands are exploited in the extensive system with an open and permanent grazing. The improvement measures poorly applied on small surfaces. As a result for this management the vegetal sward are degraded and dominated by vegetal association with low quantity and quality level. Can be noticed the abundant presence of wood species, shrubs and invasive plants as *Pteridium aquilinim*.

Starting from this descriptive level we can follow in the further time the dynamic of the development for the grasslands productive features under the correct management.

The studied territory was altitudinal between 312 and 1440 meters altitude. In the case grasslands Series, Types and Subtypes were identified and classified according to the methodology in force as follows: Series - *Agrostis capillaris - Festuca rubra* (specific in West Romania), The dominant type is *Agrostis capillaries- Festuca rubra, Agrostis capillaries- Festuca rubra; Agrostis capillaries- Festuca rubra- Lolium perenne; Nardus stricta- Festuca rubra*. Characteristic vegetation: *Agrostis tenuis, Anthoxanthum odoratum, Dactylis glomerata, Festuca rubra, Poa pratensis, Festuca ovina, Phleum pratense, Coronilla varia, Trifolium montanum, Hypericum perforatum, Prunella vulgaris, Rumex acetosa, Achillea distans, Campanula serata, Gentiana praecox, Hypericum maculatum, etc*

In function of the altitude gradient between 318 and 1440 meters and the type and subtype of the dominant vegetation the investigated grasslands surface were divided in three altitudinal levels: low and high hills and mountain area.

For the low hills grasslands situated under 500 m altitude can be observed as dominants the vegetal associations like: *Agrostis capillaris +Festuca rupicola+Lolium perenne*; *Botriochloa ischaemum +Agrostis capillaris +Calamagostris villosa.* The dominant soils were the luvisol strong eroded stagnic and the moderate stalled eroded albic luvisol. Under these pedoclimatic conditions the productive grasslands features recorded an average pastoral value (52.11), the production level was also low at 6.5-7.00 tons green mass/ha and the livestock unit at 0.61-0.66 LU/ha (Table 1.).

Regarding to the data obtained on the high hills situated under 900 m altitude can be noticed the dominant vegetal associations: *Agrostis capillaris+ Festuca rubra; Agrostis capillaris +Botriochloa ischaemum+ Juncus effusus; Nardus stricta+ Festuca rubra; Agrostis capillaris +Holcus lanatus+Botriochloa ischaemum.* As a firs regards can be noticed the presence of the *Nardus stricta* at low altitude.

The soils fertility potential decrease with the altitude and the most important are: the moderate stalled albic luvisol, litosol distric eroded 50% and 50% in areas with steep slopes, the litosol distric, typical districambosoil. At this altitudinal level we don't observed production differences correlated with the altitudinal gradient for the vegetal association erected by *Agrostis capillaris+ Festuca rubra.* For the other twe vegetation type *Nardus stricta+ Festuca rubra; Agrostis capillaris +Holcus lanatus+Botriochloa ischaemum* the production decresed at 4-5 tons green mass/ha and also decreased the livestock unit at 0.37-0.42 LU/ha (Table 1.).

The grasslands locate din the mountain area above 900 m altitude are erected by vegetal associations *Agrostis capillaris +Festuca rupicola+Botriochloa ischaemum* and *Nardus stricta + Festuca rubra.* The most present soils are: moderate lytic eroded districambosoil and moderate eroded districambosoil overshadowed gleyic. Starting from 900 m altitude we determinate a low

production level at 4.5 tons green mass/ha associated with a low livestock unit at 0.42 LU/ha (Table 1.).

Establishing of the grazing period

One of the most important parts of the grasslands management is the grazing period (Roath et Krueger**,** 1982.

Starting from the permanent grazing first regulation are related by the period of grazing. The length of the grazing season was established as follows: - For low-grassland grassland grazing, grazing will take place between 1 May and 30 September (to be completed three weeks prior to the first frosty days). Sheep grazing will take place between 20th April and 30th September, respectively 170-180 days. The length of the grazing season for altitude lawns will be in the period 20 May -30 September and 120-130 days respectively.

Table 1.Grasslands production features related by altitude and soil type

Altitude, m	*The grasslands dominant type and subtype*	*Productive features (pastoral value, green mass production, livestock unit LU/ha)*	*Soil type*
320-635	type *Agrostis capillaris+Festuca rupicola*, subtype *Agrostis capillaris +Festuca rupicola+Lolium perenne,*	PV= 52.11;. 6500 kg.gm/ha, GC = 0.61 LU/ha	Luvisol strong eroded stagnic
450-572	type *Agrostis capillaris+Festuca rupicola*, subtype *Botriochloa ischaemum +Agrostis capillaris +Calamagostris villosa,*	PV= 24.00; 7000 kg. gm /ha, GC = 0.66 LU /ha	Moderate stalled eroded albic luvisol
608-895	type *Agrostis capillaris+ Festuca rubra*, subtype *Agrostis capillaris+ Festuca rubra; Agrostis capillaris +Botriochloa ischaemum+ Juncus effusus*	PV= 25.11; 6500 kg. gm/ha, GC = 0.61 LU /ha	Moderate stalled albic luvisol Litosol distric eroded 50% and 50% in areas with steep slopes.
630-870	type *Agrostis capillaris+ Festuca rubra*, subtype *Nardus stricta+ Festuca rubra,*	PV= 15.15; 4000 kg. gm/ha, GC = 0.37 LU /ha	Litosol distric
300-720	type *Agrostis capillaris+Festuca rupicola*, subtype *Agrostis capillaris +Holcus lanatus+Botriochloa ischaemum,*	PV= 37.22; 4500 kg. gm /ha, GC = 0.42 LU /ha	Typical districambosoil
715-1050	type *Agrostis capillaris+Festuca rupicola*, subtype *Agrostis capillaris +Festuca rupicola+Botriochloa ischaemum,*	PV= 31.22;. 4500 kg. gm /ha, GC = 0.42 LU /ha	Moderate lytic eroded districambosoil
1100-1440	type *Agrostis capillaris+ Festuca rubra*, subtype *Nardus stricta + Festuca rubra,*	PV= 19.15; 5000 kg. gm /ha, GC = 0.47 LU /ha	Moderate eroded districambosoil overshadowed gleyic

Establishing of the fertilization plan

According with the type and subtype of the vegetal association correlated with the soil characteristics and the altitude gradient was established the fertilization plan.

For the grassland situated on the low hills, the basic fertilization applied once at three years and represented by N:P:K 300 kg/ha applied in the fall or in early spring. For the late fall was recommended organic fertilizer 30-40 t/ha. Yearly fertilization is based on N 150 kg/ha divided after each grazing or cutting cycle.

For the grassland situated on the high hills, the basic fertilization is the same with the contribution of the sheep holding once at three or four years. Yearly fertilization is based on N 150 kg/ha divided after each grazing or cutting cycle.

In the case of the mountain grasslands the most important is the sheep holding or also the organic fertilizer applied 30-40 t/ha and correlated with the soil slope.

For the surfaces used for hay production the basic fertilization are the same but for nitrogen the quantity (150 kg/ha) is divided with 100 kg/ha in early spring and 50 kg/ha after the first vegetation cycle in order to stimulate the plants growth.

Invasive species control

On the surfaced invaded with invasive species as a consequence of the absence of the management plan the herbicide use assure an optimum control.

The most important invasive species on the investigated grasslands was *Ptheridium aquilinium* in special on the area with a low animal presence or quasi abandoned. A good efficacy was obtained with herbicides as Asulox 6 l/ha, applied two years), Arsenal (6 l/ha) and Glean (50 g/ha), applied at full foliar development for *Pteridium a*. After the herbicides application the surfaces can be sown a mixture with *Dactylis glomerata* (6 kg/ha), *Lolium perenne* (5 kg/ha), *Poa pratensis* (6 kg/ha), *Phleum pratense* (4 kg/ha), *Festuca pratensis* (3 kg/ha), *Trifolium repens* (3 kg/ha) and *Lotus corniculatus* (3 kg/ha).

Conclussions

The descriptive study investigate 3868.9858 hectares of grasslands, structured in 57 grasslands Fitting Units (AU) and consisting of 174 plots. These surfaces was exploited in the extensive system with improvement measures poorly applied.

As a result for this poor management the vegetal swards are dominated by the associations with a low production potential and a low quality. Also the grazing capacity (GC) expressed in livestock units (LU) in between 0.37-0.66 LU/ha.

First two altitude levels (318 -900m) low and high hills don't have clear differences regarding the grasslands productive features. Above 1000 m altitude the soil productive potential decreased and can be noticed the presence of the low quality species *Festuca rubra* and *Nardus stricta* as dominant.

For all altitude levels can be noticed the abundent presence of wood species, shrubs and invasive plants as *Pteridium aquilinim*.

The management plan with all his components can improve in the further the stability of the grasslands ecosystem, increase the vegetal and animal production and also increase the prosperity of the rural community.

Acknowledgements

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Ampelotechnical Measures for Producing of Dessert Wine's Raw Material from Vranec Cultivar In Skopje Wine District's Condition

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Abstract: Possibilities of producing dessert wine's raw material from Vranec cultivar has been researched in the Skopje vineyard district climate condition in the period 201-2016. Cultivar Vranec is the most prevalent in red wine varieties in R.Macedonia. The following treatments were applied: pruning (16, 20 and 24 buds/vine) and thinning of the cluster (without thinning, 1 cluster/shoot and 1 cluster/2 shoot). In three moments of ripening (full ripe, 10 days after, and 20 days after full ripe stage) the yield and content of sugar in must has been researched. The yield and content of sugar in must statistically differ. Depending of the treatment raw material for different types of dessert wine has been obtained (217-241 g/dm^3 sugar in full ripe, 251-281 g/dm^3 10 days after and 282-326 g/dm^3 after full ripe stage. Value of content of sugar is obtained from must of all grapes from one vine. Highest value of content of sugar is possible to obtain with picking of raisin grape.

Key words: vranec, pruning, cluster thinning, dessert wine.

Introduction

The research of quality and high quality wines of Vranec cultivar has shown that for the optimal yield, which do not disturb the vine biology, the content of sugar in the must is 210 g/dm^3 – 230 g/dm^3.

According to the research of Božinović Z. (1996), the cultivar origins from Montenegro and has been widely spread in Macedonia since 1954. It belongs to the ecological and geographical group of *pontica*, subgroup *balcanica.* It ripens

late, and it commonly self-fertilizes with a high percentage (32%). It has regular and good productivity. The cane are pruned to leave 3 – 4 buds, but better yield is achieved with the Guyot system – leaving 8 buds per cane, without decreasing the quality. This is the warm climate cultivar. The cluster is of average weight of 250 grams, with participation of 97% of the berries, and 86% of pulp. The must contains 210 g/dm^3 – 230 g/dm^3 sugars, and 6 g/dm^3 – 6,5 g/dm^3 total acids. It over-ripens fast, increasing the amount of sugar to over 250 g/dm^3. The wines are medium strong to strong, reach in alcohol, extraction, and colouring substances. They are very harmonious in flavour with strong aroma and potability. According to this author, the Vranec cultivar is the most important for red wine production in the Povardarie region. The grape quality is especially influenced by agrotechnical and ampelotechnical measures implemented for establishment and determination of the yield and quality of the grapes and the wine.

Božinović Z. (1985) determines average yield of 15 t/ha in Kazanavlev system, a modified Vranec cultivar. The must sugar content for fully ripen grapes is about 210 g/dm^3, or, theoretically, about 12,6%vol. alcohol.

Reynolds A.G. (2001) evaluated the influence of the minimal pruning upon the chemical composition of the must. In his experiment he used 3 types of pruning: hand pruning, This experiment shows the necessity of ampelotechnical measures of pruning and thinning of grapes and berries. This is also important in pest and diseases control management.

Van Schalkwyk G. (1999), considers there are various opinions about the influence of the yield on the quality, but there also is a general acceptance that high production reduces the quality of the grapes and vice versa. According to him, this opinion does not take into account the cultivation practices (conditions), such as plant spacing, trellis system, irrigation, canopy management and the balance between yield and vegetative growth. The exact production norm for a certain wine category should not be employed for all cultivars.

Material and methods

The research was conducted on 25-year-old Vranec cultivar of the Povardarie region, Skopje vineyard district, especially the area of the foothill of Skopska Crna Gora mountain (41˚51’ N latitude, 21˚38’ E longitude, 340 m. altitude).

The Vranec cultivar is grafted onto *Berlandieri x Riparia Teleki 8 B,* planted 2,8 m x 1,2 m and trellised 100 m with north – south direction. The number of vines per hectare is 2976.

Regarding the ampelotechnical measures, three treatments were applied: differential pruning with three variants and thinning canes with three variants. Each variant has 4 consecutive repetitions or 32 altogether in the experiment.

The differential pruning treatment, leaving certain number of buds (shoots) per grapevine, was set in three variants of double Guyot system. More buds than needed were left out. After shooting, the number of the shoots was corrected.

-	Variant -24	2 h 10 + 2 h 2 = 24	Shoots/vine
-	Standard -20	2 h 8 + 2 h 2 = 20	Shoots/vine
-	Variant -16	2 h 6 + 2 h 2 = 16	Shoots/vine

Three rows of plants were provided for each variant, totalling 9. The experiment was conducted on 0,29 ha.

The next treatment, thinning – removal of fruit clusters, actually regulated the fertility in three variants: control (no thinning) variant, 0,5 variant, and 1 cluster/cane variant. Damaged and infected clusters, or clusters, atypical for the cultivar, were removed.

These ampelotechnical measures were employed ten days after flowering.

There were total of three variants, together with the control.

The dynamics of ripening from full ripeness until reaching the point of over-ripening (raw material for dessert wine producing), was based on average value of three analyses performed at: the moment of full ripeness, 10 days after the full ripeness, and late harvest.

The cluster yield per grapevine was determined by division of the total crop mass with the number of the plants. Production is usually expressed by multiplying the number of the plants per unit surface (hectare) with the yield per production unit (grapevine). The sugar and total acid content were determined by standard methods of **OIV**.

Results and discussion

The following tables show the results from the research. The yield between the variants 16, 20, and 24 buds of one vine, considered at the same level of thinning is statistically important in relation with 16 buds compared to 20 and 24 buds. The deviation between 20 and 24 buds is statistically irrelevant. Decrease in the yield is noted with the increase of the number of buds, from 20 to 24, in the control variants without thinning and with thinning of one cluster per shoot (V – 1.0). But, variants with thinning of clusters – one cluster per two shoots (V – 0.5), showed increase in the yield with the increase of the number of buds with pruning. The yield varies within the groups with 16, 20, and 24 buds per vine with thinning of the clusters. The yield decreased from 12.4 t/ha to 5.0 t/ha (variant 16 buds), from 15.1 t/ha to 6.5 t/ha (variant 20 buds), and from 14.3 t/ha to 8.3 t/ha (variant 24 buds/vine).

Table 1. Influence of the treatments – pruning and thinning of clusters upon the yield in two moments of harvest

Treatments and variants	Full ripeness	20 days after full ripeness
16 buds/vine-St	12.4	8.8
16 buds/vine -1.0	10.0	7.7
16 buds/vine -0.5	5.0	3.8
20 buds/vine -St	**15.1**	**12.6**
20 buds/vine -1.0	**12.0**	**8.5**
20 buds/vine - 0.5	**6.5**	**4.4**
24 buds/vine -St	14.3	11.8
24 buds/vine -1.0	11.6	10.4
24 buds/vine -0.5	8.3	7.1

Table 2. Influence of treatments –upon the sugar content in the must at three points of harvesting

Treatments and variants	Full ripeness	10 days after full ripeness	20 days after full ripeness
16 buds/vine-St	217	251	289
16 buds/vine -1.0	229	265	304
16 buds/vine -0.5	237	273	317
20 buds/vine -St	**220**	**261**	**282**
20 buds/vine -1.0	**233**	**266**	**305**
20 buds/vine -0.5	**241**	**281**	**326**
24 buds/vine -St	215	253	286
24 buds/vine - 1.0	234	265	295
24 buds/vine -0.5	237	271	313

At all points of ripeness, the sugar content in the must is standardised between the variants of 16, 20, and 24 buds at the same level of thinning, the statistical procedure has not indicated significant difference.

The sugar content in the must strongly varies within the group with 16, 20 and 24 buds per vine, where thinning of the clusters has been employed.

At the moment of full ripeness, the content of sugar increases from 217 g/dm^3 to 237 g/dm^3 (variant 16 buds/vine), from 220 g/dm^3 to 241 g/dm^3 (variant 20 buds/vine), and from 215 g/dm^3 to 237 g/dm^3 (variant 24 buds/vine).

Ten days after the full ripeness, the sugar content in the must increases from 251 g/dm^3 to 273 g/dm^3 (variant 16 buds/vine), from 261 g/dm^3 to 281 g/dm^3 (variant 20 buds/vine), and from 253 g/dm^3 to 271 g/dm^3 (variant 24 buds/vine).

Twenty days after the full ripeness, the sugar content in the must increases from 289 g/dm^3 to 317 g/dm^3 (variant 16 buds/vine), from 282 g/dm^3 to 326

g/dm^3 (variant 20 buds/vine), and from 286 g/dm^3 to 313 g/dm^3 (variant 24 buds/vine). Various types of dessert wines produced in Portugal, Spain, France, and Italy, use as raw material must with different sugar content as a starter for the wine. The lowest limit of sugar content for some dessert wine is 190 g/dm^3, 235 g/dm^3 for the wine porto, or 254 g/dm^3 for the natural dessert wines of the south of France.

Raw material for dessert wines is obtained from the researched variants in full ripeness with addition of alcohol. Ten days after the full ripeness, the raw material may be used for natural dessert wines with addition of alcohol and concentrated must. Twenty days after the full ripeness, the raw material produced is favourable for natural dessert wines with minimal addition of alcohol, concentrated must, or may be used for production of wines from raisins.

Conclusion

1. Vranec cultivar of Skopje vineyard district obtained raw material with sugar content of over 210 g/dm^3 or over 12.5 %vol. alcohol in all variants.
2. Differential pruning influences the yield and quality of the grapes. Optimal load is 20 buds per vine. Increasing the number of buds per vine decreases the quality of the grapes.
3. Thinning of the clusters influences the yield and quality of the grapes. The influence of the thinning factor is stronger than the differential pruning factor and it results in high reduction of the yield and increase of the sugar content.
4. Researched variants of ampelotechnical measures influence the possible use of the raw material. The raw material for production of liqueur dessert wines is obtained in full ripeness in all variants of differential pruning. The raw material for production of natural dessert wines is obtained ten days after the full ripeness in all researched variants. The raw material for production of natural dessert wines from over-ripened grapes is obtained in the late ripeness in all variants with employment of thinning of the clusters.

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Effective size calculation for domestic breed Busha cattle

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Abstract: Busha cattle is an autochthonous breed in the R. of Macedonia. It belongs to a group of primitive shorthorn cattle (*Bos Brachyceros Europaeus*). In Macedonia this breed has officially been classified as triple purpose breed (for meat, milk and work) but considering its low productive capabilities it is more similar to some primitive working breeds. This breed of cattle used to be dominant and most important breed in almost all Balkan countries until 50s and 60s of the XX century but today in lowland areas with intensive farming they are already replaced with more productive and specialized cattle breeds. Today these cattle are no longer used for work but because of absence of systematic cattle improvement program these animals have retained their poor milk and meat production capability. This breed is well adapted to the very harsh feeding and housing conditions that exist in the rural areas of the Macedonian mountains and is resistant to diseases. It is still the most significant milk and meat resource for these areas where the more productive cattle breeds cannot thrive successfully. After thorough phenotypic characterization of cattle classified as "Busha type" using FAO guidelines for phenotypic characterization and literature data merits for this breed, total number of 720 pure breed Busha cattle were recorded in the herd book for this breed in year 2015. Based on the population structure, estimated effective population size of Busha cattle in the R. of Macedonia in 2015 was 239,80 and according this figure and the total actual number of breeding females, this population could be classified as population in risk.

Key words: Busha, cattle, domestic breed, effective population size.

Introduction

Cattle production in Macedonia consists of three sectors: small-scale farmers (around 90%) keeping 1-3 cow and mainly producing for home consumption; medium-scale farmers (5%) keeping 10-15 cows with annual production of 4000-5000 kg milk/head and specialized commercial farms (around 5%) with more than

50 heads that produce annually over 7.000 kg milk intended solely for the market (Trajkovski, Bunevski, 2006, AHV, 2014). In the mountain rural regions of Macedonia the dominant type of cattle are the crosses of the Busha breed. This breed of cattle, also known as Illyrian cattle, is autochthonous breed of the Balkan Peninsula. It has been bred for centuries in this area and belongs to a group of primitive short horned cattle (*Bos Brachyceros Europaeus*) (Rako et all, 1955, Bunevski 1994 and 2004). In Macedonia this breed is officially classified as triple purpose breed (for meat, milk and work) but considering its low productive capabilities it is more similar to some primitive working breeds. The main strength of this breed is that survives well under minimum levels of management, has low feeding requirements and thrives on natural grazing. This shorthorn cattle used to be dominant and most important breed for marginalized rural areas of Macedonia, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Albania, Bulgaria and Greece until the middle of the XX century and is still a crucial breed in some mountainous rural parts of the Balkans. In the past, several imports of more productive dual-purpose breeds have been made in Macedonia with intention to improve the production capabilities of the native Busha cattle. But this improvement has been carried out without adequate control and record keeping which in turn resulted in drastic reduction of the number of indigenous Busha animals.

Today Busha cattle are no longer used for work but because of absence of systematic cattle improvement program these animals have retained their poor beef and dairy production capability. It could be said that the Busha's genome is very elastic since this breed in unfavorable conditions easily achieves better milk production and bigger body weight. Having in mind that this breed is well adapted to the very harsh feeding and housing conditions that exist in the rural areas of the Macedonian mountains and is resistant to diseases, it is still the most significant beef and sometimes milk resource for these areas where the more productive cattle breeds cannot thrive successfully (Kume et all, 2014).

In the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha.

Busha strains in the R. of Macedonia

There are two main classifications of Busha strains in the R. of Macedonia:

- **Classification of strains according to locality:** Povardarie strain, Polog strain, Ograzden strain, Prespa (Ohrid) strain, Mariovo strain, etc.
- **Classification of strains according to color:** Black strain, Brown strain, Red strain, Gray strain, and Tiger strain.

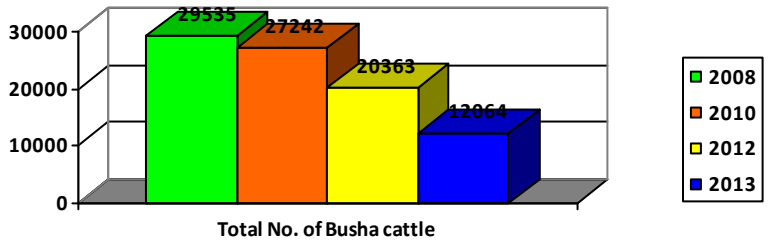
In our country the following varieties on the basis of their coat color can be found: black Busha which is reared in Debar, Tetovo and Gostivar region (Polog strain), red Busha (Metohija strain), grey Busha (Povardarie strain and Prespa or Ohrid strain), brown strain (Ograzden strain) and sometimes the so called "tigar" strain (Ilkovski et all, 2000, Bunevski 1994).

Actual situation of cattle breeding in the R. of Macedonia

According to the official statistical data there are totally 238.000 cattle in the R. of Macedonia (FAO, 2014), from which 12.064 heads of Busha cattle or 5,6% (Agency of Veterinary and Food-AFV, 2014), but according to field reality, there are approximately 1000-2000 heads of Busha cattle in our state.

Table 1 Cattle breed distribution according the official data in the R. of Macedonia (AVF, 2014)

Breed	2008		2010		2012		2013	
	No. of heads	In %	No. of heads	In %	No. of heads	In %	No. of heads	In %
Busha cattle	29535	12,12	27242	7,80	20363	10,11	**12064**	**5,64**
Crosses of Busha	89707	36,82	104961	43,56	113720	38,95	98958	46,30
Total No. of cattle	243667	100,0	269443	100,0	261073	100,0	**213747**	100,0



Graph. 1 Total No. of Busha cattle in RM (AFV, 2014)

State policy and legislative for conservation of Busha cattle

In 2008 a new Low of animal production is in function in the R. of Macedonia, where several articles are regulating the activities related to the protection of biodiversity of different species including autochthonous breeds of cattle in Macedonia. In the bovine species, the only autochthonous breed is Busha cattle.

In 2010 a Common Livestock Breeding Programme was prepared from the Ministry of Agriculture of RM, for the period from 2010 to 2020, where Busha

cattle and it's further breeding strategies, conservation approaches and sustainability use as a breed was addressing . According to this program, a Separate Breeding Programmes for each species and breed have been prepared, for the duration of 5 years (Kume et all, 2014).

In 2011 the Programme for Protection of Livestock Biodiversity was prepared by the Ministry of Agriculture of RM, for the period 2011-2017, with the main activities:

- Inventorization,
- Characterization, monitoring of the trends and risks
- Phenotype analyses,
- Recording of production traits,
- Recording of reproduction traits,
- DNA characterization, and
- Establishment of a gene bank of semen, blood and hair.

Joining the goals of both programmes the overall objective of the National Breeding Program for Busha cattle is to implement the national conservation plan and sustainable use of Busha cattle.

Material and methods

After the previous phenotypic characterization and inventorization of Busha cattle based to the published literature data for breed standards adopted in the National Breeding Program for Busha cattle, the second phase was to collect samples of blood, semen and hair from the adult Busha male and female cattle for the gene bank, in property of the Ministry of Agriculture, Forestry and Water Economy. Herd book recording was established and based on these herd book data estimation of the effective size of the population of Busha cattle was a need as a tool for monitoring the overall status of the population and also rate of increasing of the inbreeding in the population.

Most important, general phenotypic traits (body weight, height, milk yield, age at first calving…), on several herds of Busha cattle were recorded as a part of the activities in the program. Animals were reared in semi-nomadic system, during winter period from the end of October till the middle of April in the mountain village areas, and after higher spring temperatures arrive they were migrated on the mountain pastures surrounding the water sources and good pasture yield areas. The controlled Busha cattle are mainly kept for beef production, but some of them were milked by hand only for the purposes of collecting some milk for the farmers, not rarely selling some quantities.

Estimation of effective size of population of Busha cattle in the R. of Macedonia is very important information for further activities in the Breeding program toward genetic improvement of Busha cattle through

selection procedures. This parameter reflects the state of the population more precisely and considers the quantity and quality of bulls aimed for reproduction (Figure 1).

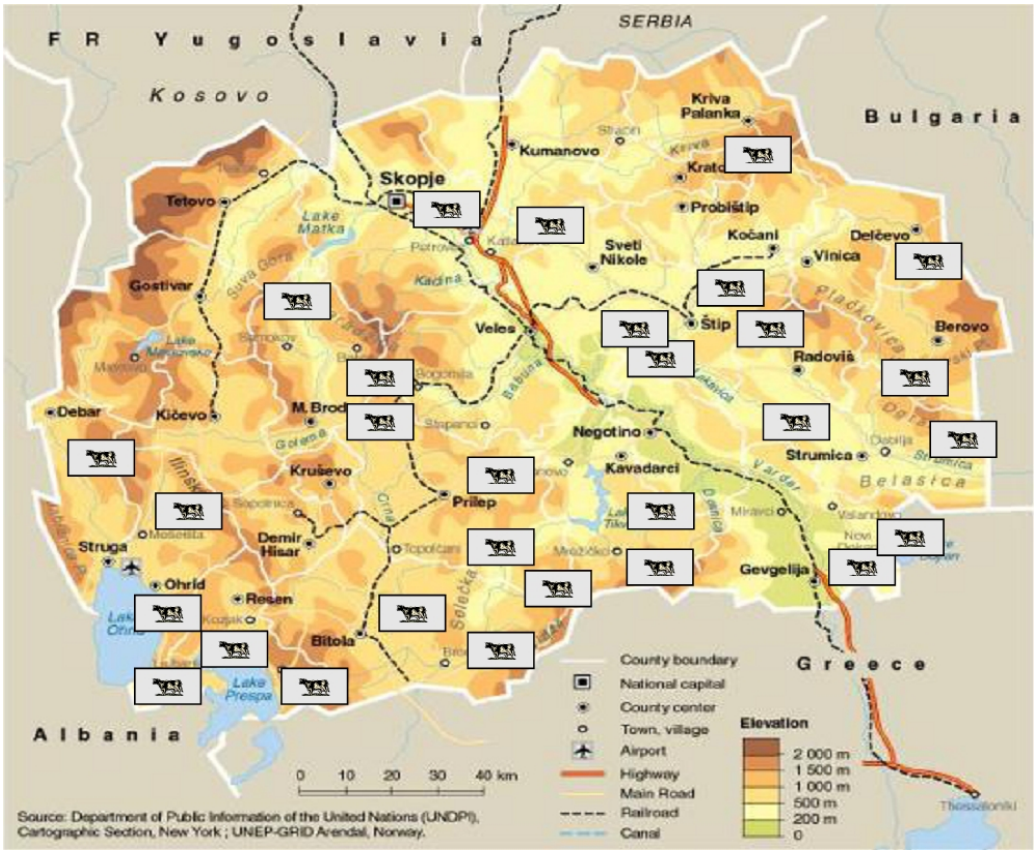


Figure 1. Locations with Busha bulls registered in the book recording in the R. of Macedonia in 2015

Table 2. Male breeding animals (bulls) from Busha breed in the R. of Macedonia in 2015

No.	Name and surename od breeder	No. of bulls
1	Mirjana Shojkarovska	3
2	Atanasa Shojkarovska	2
3	DOOEL ECOSIR	2
4	Petre Stojcev	25
5	Risto Andonovski	3
6	George Oreshkov	1
7	AK Agromihajlo	1
8	Aleksandar Deskovski	6
9	Ali Molaliev	2
10	Ajdin Smailov	1
11	Ljutvie Mustafova	2
12	Enver Sam	3
13	Jovan Stokovski	2
14	Aliti Musli	2
15	Vasil Jakov	3
16	Amet Alilov	4
17	Ali Alilov	1
18	Novo Baloski	3
Total:		66

The above mentioned male individuals of Busha bulls are selected and they are fundament for semen collection for the gene bank from the Ministry of Agriculture, Forestry and Water utilization in the R. of Macedonia. They are distributed in the different locations and regions, but mainly in the hill and mountain areas it the R. of Macedonia (Figure 1).

The estimation of the effective size of the population of Busha cattle in the R. of Macedonia in 2015 is performed using data from the herd book recordings that passed the individual preselection phase on individual phenotypic characterization (720 heads in 2015). According to the data of the official system of identification of cattle, 5,2% of the total number of cattle (250000) are characterized as Busha cattle mainly based on their small body size and expressions “look like primitive cattle”, in 2015. This figure does not correspond with the reality on the field where trained experts using objective guidelines tools specially developed for the purpose of phenotypic characterization according the breed typical merits and standards individually checked all cattle previously classified as Busha and finally only typical representative of the breeds were herd book recorded. Figure of 720 heads that has clear phenotype of Busha without signs of crossing was recorded in 2015.

The calculation of Ne was made after the formula by Wright:

Wright $Ne = (4 \times Nm \times Nf) / (Nm + Nf)$

Where *Nm* and *Nf* are numbers of reproduction male and female breeding animals.

Results and discussion

According to the previous phenotypic characterization and inventorization of Busha cattle, and recorded male and female animals of Busha aimed for reproduction, the effective size of Busha population (Ne) was calculated for 2015.

Table 2. Effective size of Busha cattle in the R. of Macedonia in 2015

Values				CALCULATIONS			
Different No. of male and female cattle				Effective size of the population	Rate of increasing of the -inbreeding	Rate of increasing of the inbreeding	Rate of increasing of the inbreeding
	N	Male	Fe-male	Ne = (4 x Nm x Nf) / (Nm + Nf)	DF =1/(2 x Ne)	DF = (1/(8 x Nm)) + (1/(8 x Nf))	DF = 1-(1-(1/(2 Ne)))
FARM 1	64	3	61	11,44	0,0437	0,0437	0,0437
FARM 2	17	2	15	7,06	0,0708	0,0708	0,0708
FARM 3	18	0	18	0,00	0,00	0,00	0,00
FARM 4	31	0	31	0,00	0,00	0,00	0,00
FARM 5	40	0	40	0,00	0,00	0,00	0,00
FARM 6	18	2	16	7,11	0,0703	0,0703	0,0703
FARM 7	64	25	39	60,94	0,0082	0,0082	0,0082
FARM 8	17	3	14	9,88	0,0506	0,0506	0,0506
FARM 9	11	0	11	0,00	0,00	0,00	0,00
FARM 10	6	0	6	0,00	0,00	0,00	0,00
FARM 11	9	0	9	0,00	0,00	0,00	0,00
FARM 12	15	1	14	3,73	0,1339	0,1339	0,1339
FARM 13	33	0	33	0,00	0,00	0,00	0,00
FARM 14	12	1	11	3,67	0,1364	0,1364	0,1364
FARM 15	13	0	13	0,00	0,00	0,00	0,00
FARM 16	36	6	30	20,00	0,0250	0,0250	0,0250
FARM 17	23	2	21	7,30	0,0685	0,0685	0,0685
FARM 18	18	1	17	3,78	0,1324	0,1324	0,1324
FARM 19	5	0	5	0,00	0,00	0,00	0,00
FARM 20	12	0	12	0,00	0,00	0,00	0,00
FARM 21	7	0	7	0,00	0,00	0,00	0,00
FARM 22	5	0	5	0,00	0,00	0,00	0,00
FARM 23	5	0	5	0,00	0,00	0,00	0,00
FARM 24	27	2	25	7,41	0,0675	0,0675	0,0675
FARM 25	31	3	28	10,84	0,0461	0,0461	0,0461
FARM 26	6	2	4	5,33	0,0938	0,0938	0,0938
FARM 27	32	2	30	7,50	0,0667	0,0667	0,0667
FARM 28	14	0	14	0,00	0,00	0,00	0,00
FARM 29	11	0	11	0,00	0,00	0,00	0,00
FARM 30	11	0	11	0,00	0,00	0,00	0,00
FARM 31	14	3	11	9,43	0,0530	0,0530	0,0530

Values				CALCULATIONS			
Different No. of male and female cattle				Effective size of the population	Rate of increasing of the -inbreeding	Rate of increasing of the inbreeding	Rate of increasing of the inbreeding
	N	Male	Fe-male	Ne = (4 x Nm x Nf) / (Nm + Nf)	DF =1/(2 x Ne)	DF = (1/(8 x Nm)) + (1/(8 x Nf))	DF = 1-(1-(1/(2 Ne)))
FARM 32	7	0	7	0,00	0,00	0,00	0,00
FARM 33	6	0	6	0,00	0,00	0,00	0,00
FARM 34	11	0	11	0,00	0,00	0,00	0,00
FARM 35	5	0	5	0,00	0,00	0,00	0,00
FARM 36	5	0	5	0,00	0,00	0,00	0,00
FARM 37	7	0	7	0,00	0,00	0,00	0,00
FARM 38	5	0	5	0,00	0,00	0,00	0,00
FARM 39	26	4	22	13,54	0,0369	0,0369	0,0369
FARM 40	5	1	4	3,20	0,1563	0,1563	0,1563
FARM 41	8	0	8	0,00	0,00	0,00	0,00
FARM 42	10	3	7	8,40	0,0595	0,0595	0,0595
TOTAL POPULATION	720	66	654	239,80	0,0021	0,0021	0,0021
					Falconer, 1980	Wright, 1969	Soule 1980

Table 3 Effective population sizes of Busha cattle population in R. Macedonia in 2015

		Different No. of male and female cattle		Effective size of the population
	N	Male	Female	Ne = (4 x Nm x Nf) / (Nm + Nf)
Year 2015	720	66	654	239.80

Table 4 Level of endangerment of the breeds in cattle according to the No. of purebred female animals in the book recording

LEVEL OF ENDENGERED OF THE BREED	CATTLE
1. CRITICAL	< 150
2. ENDENGERED	< 250
3. VULNERABLE	< 450
4. IN RISK	< 750
5. NOT IN RISK	< 1500

The value of Ne in the controlled population of Busha cattle in 2015 was 239,80, and comparing to 2013 when was 154,53, and in 2014 when was 117,504 (Table 3).There are some differences in Ne according to the year but it mainly can be attributed to the better systematic control and new herds and new individuals are characterized as “busha pure breed” and enter the busha herd book. This means that there is a big variation of number of animals in the herds, depending on the price of cattle live weight at the start of autumn and availability of hay, roughage and other feed for the winter period and this situations affects especially the total number of bulls that varies dramatically

through the years. Obtained data for Ne for population of Busha cattle in the R. of Macedonia according to the international and FAO criteria, lead to the statement that this population could be characterized as a population IN RISK (only 720 heads of Busha are book recorded), but is not far from NOT ENDANGERED species if official data from the identification system is considered, where more than 1500 breeding heads of busha cattle exists.

Conclusions

Busha cattle are autochthonous breed on the Balkan Peninsula. This breed is a part of the National Biodiversity Program for conservation of the indigenous breeds of animals in the R. of Macedonia. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha cattle. The aim of the research was to estimate the effective size (Ne) of cattle breed Busha in 2015. The estimated figure of Ne in the controlled population of Busha cattle in 2015 was 239,80. Population of Busha cattle in the R. of Macedonia according to the international and FAO criteria, according to the obtained results, can be characterized as a population IN RISK - 720 heads of Busha are under book recording in the whole country in 2015.

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Pesticide residues in cabbage

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Abstract: Our investigation comprised seven cabbage samples for the analysis. They had been treated by the formulatuins "Actara 25 WG" (a.m. thiamethoxam), "Saturn 7,5 GR"(a.m. chlorpyrifos), "Perfekthion" (a. m. dimethoate) and "Decis 2,5 EC" (a. m. deltamethrin).

Cabbage samples LC-MS/MS analysis points at the detection of thiamethoxam whose values were below the MRL of 5 mg/kg.

Key words: pesticide residues, cabbage, LC-MS/MS

Introduction

The intensification of the total production and quality of food is the basis of modern agricultural production which is one of the most significant factors in the environment, necessary for the normal development and human health (Lončarić i sar., 2015). Its significance is emphasiyed by the fact that the natural capacity of soil is limited and the number of population is constantly growing. In the total food production the production of vegetables and their products is prominent (Lazić i sar., 2001).

Vegetable growing makes the intensive use of soil and irrigation systems possible by the rotation of two or three species during a year on the same field or protected areas. The vegetable production is one of the most intensive branches of plant production, which is shown by the yield per area unit, the amount of money earned and by the participation of human labour (Lazić i sar., 2001).

The cabbage is used fresh in nutrition which increases the possibility of the intake of pesticides into human organism. The danger of pesticide residues intake gets higher if the producers in agriculture do not follow good agricultural

practice (GAP - Good Agricultural Practice). It is often the case that the treatment is carried out with a higher concentration than prescribed, that the number of treatments is bigger and that the PHI is not observed. All of it can jepretize human health and that is why the need for the control of health safety of food is admant (Bursić, 2011). Aiming at its control there is a value MRL i.e. maximum pesticide residue amount in the agricultural producs. Off. gazz. RS 29/2014 is the value MRLs harmonized with the valid MRLs in the European Union - Regulation EC No 396/2005.

The aim of the paper was to carry out, by QuEChERS method, the extraction of thiamethoxam, chlorpyrifos, dimethoate and deltamethrin from the cabbage samples and then to quantify their content by the validated method of liquid chromatography with tandem mass spectrometry (LC-MS/MS) with the aim to determine health hazards of the studied samples regarding the content of pesticides, by comparing the pesticides detection with the allowed MRLs by Off. gazz. RS 29/2014 and Regulation EC 396/2005.

Material and methods

Sample preparation

Within the project of the Ministry of Education, science and Technological Development TR 31036, the protection of cabbage was carried out by the application of various plant protection agents. The experiment was carried out during 2016 at the locality Futog on the family farm which belongs to Miloš Trninć (Figure 1).

Figure 1. Experiment locality

The preparation "Actara 25 WG" active matter thiamethoxam was added into the poll for seedling preparation in the concentration of 10 mL/m^3. The preparation "Saturn 7,5 GR" (a m. chlorpyrifos) in the dose of 1 kg/200 m^2 was applied in the soil before planting. By watering the plants the preparation "Perfekthion" (20/05/2016 and 27/05/2016) (a. m. dimethoate) in the concentration of 5 mL/5 L of water was applied. After the prescribed time, the preparation, "Decis 2,5 EC" (03/06/2016 and 15/06/2016) (a. m. deltamethrine) was applied foliarly in the dose of 0.5 L/ha.

The cabbage sampling was carried out on 07/07/2016. The samples were placed into plastic bags and immediately transported to the laboratory. At the laboratory each sample was homogenized and kept in the refrigerator at the temperature of -10 ºC until being analyzed (SANTE/11945/2015).

There were seven cabbage samples in all.

Chemicals and apparatus

All reagents used were of analytical grade, >95% purity. Acetonitrile was purchased from Fisher Chemical (Leics, UK) and methanol (Ultra Gradient HPLC Grade) was purchased from J.T. Baker (Deventer, the Netherlands). Water was deionized (>18 M/cm) by the Elga Maxima system. QuEChERS Extraction Packets, EN Method (BondElute, P/N 5982-7550, Agilent Technologies) was used for extraction and Dispersive SPE, Fruits and Vegetables packets (BondElute, P/N 5982-5056, Agilent Technologies) for cleanup samples.The certified pesticide analytical standards of formetanate-hidrohlorid, spirotetramat, spinosad, dimethomorf, metalaxil-M and mandipropamid were purchased from Dr. Ehrenstorfer. The standard stock solutions were prepared in acetonitrile (1.0 mg/mL), while the working standard was in concentration of 10 μg/mL. This solution was used as spiking solution and also to prepare the standard solutions to obtain the calibration curves, by dilution with mixture of methanol and water (50/50,V/V; with 0.1% formic acid).

The LC-MS/MS analysis was performed on the Agilent 1200 HPLC system (Agilent Technologies, Waldronn, Germany) with an automatic degasser, a binary pump and an auto sampler connected to the Agilent 6410B Triple-Quad LC/MS system. The chromatographic separation was performed on the Zorbax XDB C18 analytical column of 50×4.6mm and 1.8 μm particle size (Agilent Technologies, the USA), which was maintained at 30 °C. The LC flow was maintained at 0.4 mL/min, the injection volume was 5 μL. The mobile phase gradient program started at 90% of B (water with 0.1% formic acid) held for 2 min, then decreases to 10% at 15 and 5% at 17 min, held for 3 min. The mobile was returned to the initial composition at 5.0 min and equilibrated for another 5 min before the next injection. Electrospray ionisation was performed in the positive mode with the following parameters: resolution Q1 and Q3-wide (0.3 units) spray voltage-2000

V, gas temperature-325 °C, vaporizer-220 °C, gas flow (N_2)-5 L/min; nebuliser gas (N_2)-40 psi; the MassHunter software (version B.04. QQQ Agilent Technologies) controlled the LC-MS/MS system and processed the data. The data acquisition was in multiple reactions monitoring (MRM) mode.

Validation

Linearity study, LOD and LOQ determinations: The evaluation of the analytical curves' linearity was done based on injections of the standard solutions prepared in organic solvent (mixture methanol and water) and also in blank union extract, atthe concentrations 10, 25, 50, 100 and 200 ng/mL, where this sequence was injected three times (n = 3). The corresponding range of pesticide concentrations in union extract were from 0.01 to 0.20 mg/kg. The limit of detection (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software. The real LOQ was based on the accuracy and precision data, obtained via the recovery determinations and was defined as the lowest validated spike level meeting the requirements of a recovery within the range 70–120% and RSD≤20%.

Accuracy and precision (recovery experiments): The main goal of the recovery experiments is to determine the method accuracy, via comparison of the real concentration of each pesticide measured by performing the complete procedure with the known pesticide concentration initially added to the matrix. The method precision is expressed as the repeatability (RSD%) of the recovery determinations at the four different spiking levels (10, 50, 100 and 200 mg/kg).The spiking procedure with 6 pesticides, added to blended and homogenized union, was done three times (n=3) at each spike level and also the blank cabbage matrix analysis was performed twice. This blank extract was also used for the preparation of standard solutions in matrix.

Extraction procedure

10g sample + 10mLMeCN + 100µL ISTD (10 µg/mL Carbofuran-D3)

↓ Shake vigorously for 1 min

Add 4g $MgSO_4$, 1g NaCl, 1g Na_3Citrate dihydrate, 0.5g Na_2HCitrat sesquihydrate
Shake tube immediately for 1 min

↓ Centrifuge for 5 min at 3000 g

Transfer 5 ml of the extract into a PP tube contained $MgSO_4$,PSA
Shake for 30s

↓ Centrifuge for 5 min at 3000 g

Transfer 200 µL into a vial, evaporate to dryness
Reconstitute in 200 µL of mobile phase →LC-MS/MS

Results and discussion

The validated method which uses the LC-MS/MS provides appropriate linearity, a very high sensitivity, good repeatability and can be applied with the high reliability to the analysis of pesticide residues in trace levels (Vuković, 2011). Before the calibration and quantification of pesticides it was necessary to set an acquisition method. The determination of the acquisition method comprises: setting chromatographic conditions, determining the precursor and product ion so called monitoring mode of ion transfer (MRM or SRM), determining the fragmentation energy (Frag.) and the energy of collision cell (CE). For setting the MRM MassHunter Optimizer Softvere Version B03.01 (Agilent Technologies 2010) and Agilent G1733AA MassHunter Pesticide Dynamic MRM Database were used.

Table 1. Retention times (Rt), MRM, CE and Frag

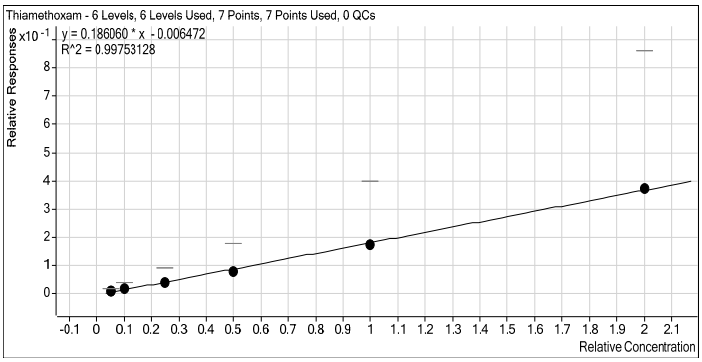
Pesticide	Formula	M (g/mol)	Precursor ion (*m/z*)	Product ion (m/z)	Frag (*V*)	CE (*V*)	Rt (min)
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	503	502.1	280.9	80	28	3.89
			502.1	93.2	80	12	
Dimethoate	$C_5H_{12}NO_3PS_2$	229	229	125	130	20	17.4
			229	199	130	10	
Chlorpyrifos	$C_{11}H_{15}Cl_2O_3PS_2$	360	360	154.9	100	10	20.20
			360	98.8	100	30	
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	291.7	292	211	150	10	9.25
			292	118	150	10	

Validation parameters

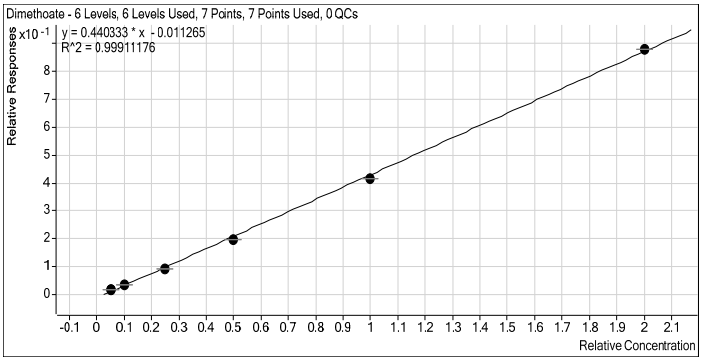
Linearity – To analyze the linearity of the method the MS response for the calibration standards for pesticides mixtures ranging from 0.02 to 0.20 μg/mL, which corresponds with the pesticides concentration in the sample of 0.02 to 0.20 mg/kg.

Due to the significant matrix influence on the studied pesticides which can appear at LC-MS/MS, the quantification of the detected pesticides was carried out by "matrix-matched calibration" (MMC).

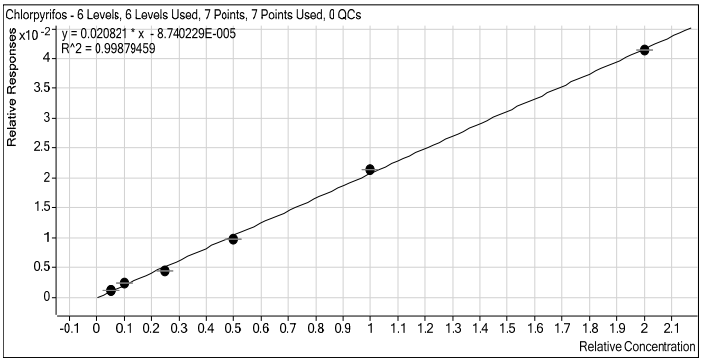
Thiamethoxam, chlorpyrifos, dimethoate and deltamethrin had the calibration curve coefficient (R^2) above 0.99 (Graphic 1- 4).



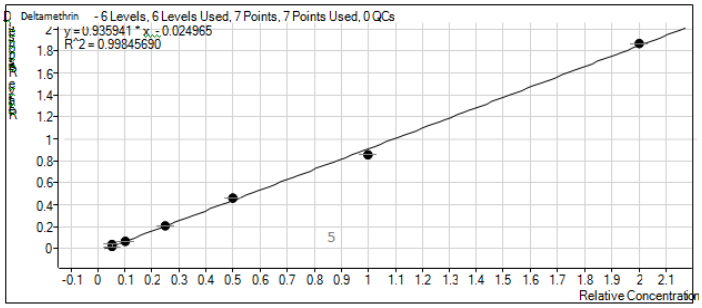
Graphic 1. Calibration curve of thiamethoxam



Graphic 2. Calibration curve of dimethoate



Graphic 3. Calibration curve of chlorpyrifos



Graphic 4. Calibration curve of deltamethrin

LOD and LOQ – The values of the detection limits (LODs) were calculated by the calculator "Calculate Signal-to-Noise"within the Qualitative MussHunter B.03.01 program (Agilent Technologies, 2010) based on the relation of the standard deviation (SD, %) of peak height and noise height in the chromatograms for the lowest values of the calibration standard of the pesticides mixture (5 ng/mL) in the cabbage matrix.

For all the examinated pesticides the LOQ (limit of quantification) of 0.01 mg/kg was set while the
LOD values were calculated mathematically.

Accuracy and precision – As an accuracy parameter the recovery (Rec, %) was studied by spiking the control cabbage sample with two levels of spiking (0.05 i 0.1 mg/kg) in three replications.

The obtained recoverys were in the range of 73.8% (deltamethrin) to 101.4% (thiamethoxam), while the values of the RSDs (%) were below 20% (SANTE/11945/2015).

Analysis of cabbage samples

In the analysed samples only thiamethoxam was detected which the detections of dimethoate, deltamethrine and chlorpyrifos were below the LOQ (Figure 2).

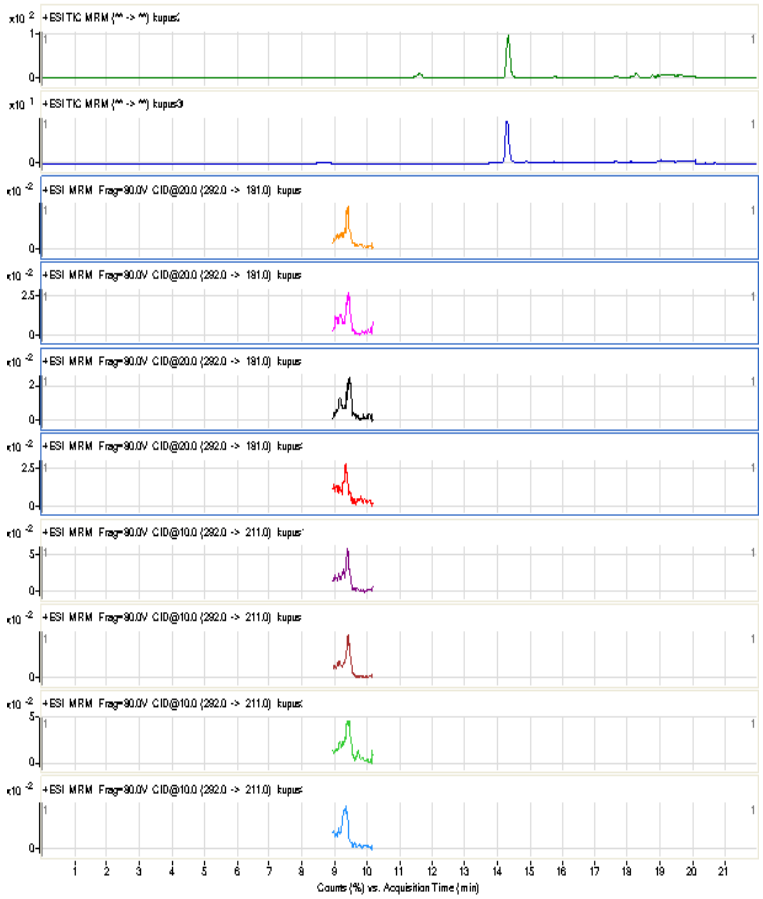


Figure 2. TIC chromatograms and MRM chromatograms of thiamethoxam in all samples

The quantifications of thiamethoxam arepresented Table 2.

Table 2. Detected thiametoxam residues

Sample	**Detected thiamethoxam residues (mg/kg)**
1	0.0039
2	<LOQ
3	0.0047
4	<LOQ
5	0.0053
6	0.0036
7	<LOQ

The Off. gazette RS 29/2014 defines the MRL of pesticides in accordance with the valid MRLs in the European Union (Regulation EC No 396/2005). The analysis of cabbage samples point to the detecten of thiamethoxam, the values of which were below the MRL of 5 mg/kg.

*In our experiment the preparation "*Actara 25 WG" *whose active substance is thiamethoxam, was added in the pool for cabbage production. The PHI for thiamethoxam is 14 days (Aleksić i sar., 2016). Although more than 30 days passed since the time when the thiamethoxam based formulation was used, its residues were detected significantly in the quantities well below the MRL.*

The assumption may refer to the fact that the other pesticides in the analysis were not detected at all as more time passed than the PHI for cabbage samoling really is.

Conclusion

Our investigation comprised seven cabbage samples for the analysis. They had been treated by the formulatuins "Actara 25 WG" (a.m. thiamethoxam), "Saturn 7,5 GR"(a.m. chlorpyrifos), "Perfekthion" (a. m. dimethoate) and "Decis 2,5 EC" (a. m. deltamethrin).

Cabbage samples analysis points at the detection of thiamethoxam whose values were below the MRL of 5 mg/kg.

*In our experiment the preparation "*Actara 25 WG" *whose active substance is thiamethoxam, was added in the pool for cabbage production. The PHI for thiamethoxam is 14 days. Although more than 30 days passed since the time when the thiamethoxam based formulation was used, its residues were detected significantly in the quantities well below the MRL. It can be assumed that the other pesticides comprised by the analysis were not detected because more time passed than the registered PHI for cabbage sampling is long.*

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Službeni Glasnik RS broj 29/2014 Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja.

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