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BIOFUNGICIDES –NEEDS AND PERSPECTIVES IN SUSTAINABLE AGRICULTURAL PRODUCTION

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ABSTRACT

The environmental pollution and the consequences for human health coupled with the development of resistance to pathogens due to excessive use of agrochemicals, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture.

Biofungicides are potential alternatives to synthetic fungicides. Sources of biofungicides are readily available, easily biodegradable, exhibit various modes of action, are less expensive and have low toxicity to humans and non-target organisms. Acting by its base i.e active ingredients, they compete with chemicals with their effectiveness and their use promotes sustainable management and hence contribute towards sustainable agriculture.

This review discusses the current status of knowledge on biofungicides including their sources, production, formulation, commercialization, role in sustainable agriculture and their limitations. It also highlights future directions for strengthening sustainability and the perspectives of their application.

Keywords: biofungicides, ecological, non-toxic, sustainable agriculture

БИОФУНГИЦИДИ - ПОТРЕБИ И ПЕРСПЕКТИВИ ВО ОДРЖЛИВОТО ЗЕМЈОДЕЛСКО ПРОИЗВОДСТВО

Загадувањето на животната средина и последиците по здравјето на луѓето заедно со развојот на отпорност кон патогените поради прекумерната употреба на агрохемикалии, доведе до значителни промени во ставовите на луѓето кон употребата на пестициди во земјоделството.

Биофунгицидите се потенцијални алтернативи на синтетичките фунгициди. Изворите на биофунгицидите се лесно достапни, лесно биоразградливи, покажуваат различни начини на дејство, се помалку скапи и имаат ниска токсичност за луѓето и за нецелните организми. Делувајќи врз основа на својата активна материја тие се натпреваруваат со хемиските со нивната ефикасност. Нивната употреба промовира одржливо управување, а со тоа и придонесува кон одржливо земјоделско производство.

Овој труд го разгледува моменталниот статус на знаења за биофунгицидите, вклучувајќи ги и нивните извори, производство, формулација, комерцијализација, улога во одржливо земјоделство и нивните ограничувања. Исто така ги истакнува идните насоки за зајакнување на одржливоста и перспективите на нивната примена.

Клучни зборови: биофунгициди, еколошки, нетоксични, одржливо земјоделско производство,

INTRODUCTION

Hazards and challenges in the use of conventional pesticides

Plant protection is based mainly on the use of chemical measures i.e. -pesticides. The use of synthetic chemicals is highly accepted due to their efficacy, reliability and quick knock down effect. But, like synthesized compounds, pesticides are of a different chemical composition, toxicological properties, persistence, so that they are polluters of the environment.

They have a health hazards for humans and environment due to their toxicity and pollution (Damallas and Koutrobuas, 2016). Synthetic pesticides also lead to environmental pollution due to the unbiodegradable nature of their constituent compounds. Therefore, Methyl bromide has been banned from agricultural use due to its negative impact on the environment. It is associated with depletion of ozone layer which contributes significantly to climate change. The constituent compounds of chemical pesticides, especially active ingredients contaminate soils (Kumari et al., 2014). They also pollute surface and ground water, killing aqua life after inhalation and consumption.

Continuous use of synthetic pesticides leads to development of resistant plant pathogen strains leading to their resurgence. Farmers apply more chemicals in control of the causing agents. In the process of managing target pests, synthetic pesticides kill non-target beneficial organisms and thereby disrupting biodiversity. After application, the active compounds of the synthetic pesticides are taken up and retained by crops.

Intermediate product of degradation are often more persistent than the starting compound, they remain for a long time in soil or water (groundwater), which can have consequences for subsequent plants in the crop rotation (Djordjevic, 2008, loc cit Grahovac et al., 2009).

Exposure to pesticides adversely affects the human population, directly or indirectly. Consumption of such crops poses chronic health problems to humans due to the accumulated toxic chemical residues. Also, continuous exposure to pesticides cause gene mutations, genetic damages, reproductive health problems and chronic diseases such as asthma, hypertension and cancer. (Kumari et al., 2014). They also reduce resistance of animals to disease-causing pathogen infections (Maksymiv, 2015).

The strong negative effect of pesticides on humans and the environment strengthened the first - people's awareness and then, interest and activity of the dominant and the most competent world organizations. Various other organizations are formed whose work is in the direction of human health and sustainable agricultural production. The World Health Organization starts the strong fight against pesticides in the direction of protecting human health and the environment. The European Union (EU) set out strict regulations regarding registration of active ingredients (especially for the toxicology requirements). There is strengthened application of Good Agriculture Practice.

Tobacco purchasing companies also take the strong care about safety of the raw material exported to their markets (lowered MRLs in residue level). Therefore, they use the active ingredient from the more safety classes according to WHO classification (STIP, 2019). There are a lot of a.i banned by EU. There are efforts to withdrawal the another and substitute by the other (MUCF, 2019).

NEEDS FOR THE USE OF THE BIOFUNGICIDES

Unknowledge in the application of some pesticides by operator and consumer leads to failure in solving some diseases and rapid emergence of resistant individuals in the population of some harmful agents. Also, the ban on the use of synthetic pesticides at the time of ripening and harvesting (especially in protected space), led to increased interest and publicity for

introducing alternative measures in plant protection, where the deserved place finds biological preparations so and other non-pesticide measures (Damallas and Koutrobuas, 2016).

The development of integral protection system will include all methods and means to control the harmful agents with minimum effect on the environment and with no economic consequences. Creating unfavorable conditions for the development of the disease by application agro-technical measures, the application of biological products and antagonistic organisms, individually, or integrated with less risky pesticides are technologies the protection they are tending today (Gold, 2009). Strengthening the induced resistance is also one of the strategic goals of the plant protection.

Application of biopreparates is a good way to manage the environment in the process of achieving sustainable agriculture production (as well as tobacco). Environmental protection and sustainability are in harmony and sustainability can be achieved only with the protection of natural resources (Malik et al., 2012).

Trends in tobacco protection are in accordance with the basic requirements for sustainable agricultural production and in line with modern standards. Bioproducts are important part of the complex of measures for integrated pest management. Efforts for the wide application of biopreparates is promoting of their beneficial effect and the greater utilization of resources. Hence, scientific research in the field of tobacco production will continue to develop procedures and instructions for the most appropriate use of biopreparate (Gveroska, 2014).

Biofungicides are potential alternatives to synthetic pesticides. Sources of biofungicides are readily available, easily biodegradable, exhibit various modes of action, are less expensive and have low toxicity to humans and non-target organisms.

Back ground of biofungicides

The first bacterium called *Agrobacterium radiobacter* strain K 84 was registered with the United States Environmental Protection agency (EPA) for the control of crown gall in 1979. Ten years later the first fungus *Trichoderma harzianum* ATCC 20476 was registered with the EPA for the control of plant diseases. Currently a total of 14 bacteria and 12 fungi have been registered with the EPA for the control of plant diseases. Most of these are sold commercially as one or more products. The technology of commercialization is still in its initial phase. 65% of the EPA registered organisms have been registered within the past 10 years while the remaining 36% registered over the past 5 years. Many technological problems were overcome and shifts in thinking occurred for these products to reach the shelves. There are a number of biological plant protection agents that are available on the market.

In the world, a large number of countries and experts are involved in this field of research, both in education and in commercialization biopesticides. Today in the world are registered 185 biopesticidal preparations, of which 72 are active substances of bacteria, 47 mushrooms, 40 entomopathogenic nematodes, 24 viruses and two protozoa. They are applied to different plant species - vegetables, fruits, cereals, fodder plants and others cultivated species. The distribution of biopesticide was carried out according to the type of organisms it suppresses, on: bioinsecticides, biofungicides, bioherbicides, and others, or can be classified according to groups of living organisms (mushrooms, viruses, bacteria, nematodes) that are in the function of the active substance biopreparates (Djordjevic, loc.cit Grahovac et al., 2009).

In our country, there is the biofungicide Trianum P, based on alive spores of *T. harzianum* T22 Gveroska, 2017).

The ability of biofungicides to protect hosts from pathogens, take place on different plants in different conditions are the basis of their commercial success (Harman, 1996).

BIOFUNGICIDES - NATURALLY RESOURCES BASED

Biofungicides are products and by-products of naturally occurring substances. There are several categories such biocontrol agents especially antagonists, products of metabolism, botanicals, growth promoters etc. Among that, plants and microorganisms are the major sources of biopesticides due to the high components of bioactive compounds and antimicrobial agents.

Biofungicides include the use of useful microorganisms or their structures, products of their metabolism, the use of herbal extracts and essential oils in protection plants. Products metabolism mentioned above microorganisms are toxins, crystals, spores and antibiotics, which protect the plants by acting antagonistic to the causative agents diseases, harmful insects, nematodes and weeds, where abouts are harmless to humans and environmentally safe. Also, useful microorganisms produce both vitamins, enzymes, and plant hormones that can act on the immune system of plants, increasing their resistance (Grahovac et al., 2009).

The active compounds in plants include phenols, quinones, alkaloids, steroids, terpenes, alcohols and saponins. Different plant families have varied antimicrobial bioactive compounds which include oil components such as α - and β -phillandrene, limonene, camphor, linalool, β -caryophyllene and linalyl acetate depending on the plant family.

Compost teas are filtrates of compost extracts and are similarly used as biopesticides.

Microbial biofungicides include bacteria species such as *Pseudomonas*, *Bacillus*, *Xanthomonas* and *Serratia* or fungi such as *Trichoderma*, *Verticillium* and *Beauveria* species. Biopesticides exhibit different modes of action against pathogens such as hyperparasitism, competition, lysis, induced resistance etc.

Plant growth promoting rhizobacteria protect plants from biotic and abiotic stresses and they also enhance plant growth and enhance formation of root hairs. The most common species of plant growth promoting rhizobacteria include *Bacillus*, *Agrobacterium*, *Microbacterium*, *Rhizobium*, *Pseudomonas*, *Chryseobacterium* and *Rhodococcus*. They colonize the environment around the plant roots, fix nitrogen, increase phosphate solubilisation and result in general increase in plant yield. Species of *Pseudomonas* and *Bacillus* have been used as biofertilizers with reports showing increase in plant growth. They also convert unreachable forms of certain chemical elements (P, Fe), turning them into readily available, therefore enlarge the yield of crops.

MICROORGANISMS AS SOURCES OF BIOPESTICIDES

Microorganism-based biocontrol agents form the bulk of commercialized bio-pesticides and they include bacteria, viruses, fungi, nematodes and protozoa (Koil, 2011). There are up to 175 reported microbial based biopesticide active agents and they have been used in management of pathogens, weeds, insects and nematodes (Singh, 2014). Majority of the microbial biofungicides are used to manage soil borne pathogens. The mechanisms of action exhibited by microorganisms against plant pathogens include hyperparasitism, competition, secretion of volatile compounds, antibiosis and parasitism (Howell, 2003; Suprpta, 2012). The most known biofungicides are based on the fungi of genus *Trichoderma* (Harman, 1996, 2004, 2006, Gveroska, 2013).

The major sources of microorganisms with pesticidal activity are agricultural fields where they coexist with other microorganisms including pathogens and beneficial species. The rhizosphere is usually concentrated with various classes of important microorganisms. According to Alizadeh et al. (2013), rhizobacteria are ideal biocontrol agents since they inhabit the rhizosphere and provide front-line defense against attack by pathogens.

Pathogens encounter antagonism from rhizobacteria before and during infection of the root. Formulation of the microbial fungicides has a great contribution to the effectiveness of the resultant product and it is usually dependent on the substrate used. The activity was attributed to the high number of spores produced by the fungus. Gveroska (2016 a) estimated the greater biocontrol activity by the bigger cfu/g soil depending of the used manure.

Various strain of *Bacillus* has the biocontrol activity - there are reports for late blight (*Phytophthora infestans*) on tomato. There is a report for *Bacillus subtilis* Ch13 to damping off in tobacco (Gveroska, 2016 b). Also, *B. subtilis* QST 713 has the big opportunities to become the widely used in tobacco.

EM (effective microorganisms) technology is one of the main modes of environmental management aimed at establishing a sustainable production. The basic principle of this technology is the application and increase of the population of effective and beneficial microorganisms in soil which eliminate degenerative microorganisms, especially soil pathogens, thus creating a healthy environment for plants. There are data on the use of probiotics not only to improve the soil but to prevent the attack from diseases. Effective microorganisms continue to coexist in the rhizosphere and plants grow well, free of pathogens.

These studies indicate that microbial biopesticides can be incorporated in integrated pest management for sustainable agriculture (Gveroska 2014).

Mechanisms of action of biofungicides

Biofungicides act by:

- Direct Competition;
 - Antibiosis;
 - Hyperparasitism;
 - Induced resistance of the host plant;
 - Plant Growth Promotion
- Direct competence implies that the root of the plant (the host rhizosphere) must inhabit the organism that is used for the biological suppression of the causing agent disease or Biological Control Agent (BCA) has to be before the infection by pathogen. The rhizosphere is the source nutrients. In general, the organism that is being applied in biological control must be present in large number for competing with a pathogen.
 - BCA should have antibiotic and antagonistic properties. They produce the low molecular weight compounds or an antibiotic by microorganisms that have a direct effect on the growth of plant pathogen *In situ* production of antibiotics by several different biocontrol agents has been approved despite the effective quantities are difficult to estimate because of the small quantities produced relative to the other, less toxic, organic compounds present in the phytosphere. An efficient bio control agent is one that produces sufficient quantities of antibiotics in the vicinity of the plant pathogen (Junaid et al., 2013).
 - Parasitism implies that the organism that is being applied for biological control attacks pathogenic organism and it is fed. With such a mechanism of action, the biological agent must be present before the pathogen attack.
- Production of enzymes, toxins or antibiotics (antibiosis) are involved in this process, as well as contribute for capturing the territory
- Induced resistance occurs when attacked plants activate the defense system and do not immune, but an internal fight to slow down the infection. Host plants are deliberately

inoculated to induce this type resistance. A biological fungicide is a trigger for this phenomenon type of resistance.

- Antibiosis: The biological activity of biofungicides can be designed on the production of numerous metabolites that act antifungal and antibacterial. Bio agents are known to produce different types of antibiotics which act in different ways to suppress the diseases or plant pathogens. Bio agents are known to produce three types of antibiotics viz., nonpolar/volatile, polar/ non-volatile and water soluble. Several bio-control strains are known to produce multiple antibiotics which can suppress one or more pathogens (Junaid et al., 2013). For example, *B. subtilis* is in the world used to prepare many preparations. Produces antibiotics (bacilysin and fengymycin, dithididine and oxydifidine, bacitracin, bacillin and balomycin b and iturin) that act on many types of aerobic and anaerobic bacteria.

The products of metabolism, lipopeptides, act on different cell wall components, preventing adhesion of the pathogen on plant organs, and the enzyme subtilin blocks development pathogen (Klokočar-Šmit et al., 2006). *Bacillus* products for disease control have the mode of action of biopesticides: fungicidal lipopeptides production (and mimic cell membrane lipid, insert deeply into membrane and disrupt packing of membrane lipids) and plant defence induction. There is the good shelf life, also (Bayer, 2017).

- Plant Growth Promotion: Bioagents can reduce the disease incidence of crops by increasing their growth at least during the early stages of the life cycle by the way of disease escape. The best example of this is the resistance of damping off of *Solanaceous* crops with advance of age. Bioagents both fungal and bacterial help in managing the plant diseases by promoting the growth of plants through increased solubilisation of nutrients, increased nutrient uptake with enhanced root growth and sequestration of nutrients.

USE OF THE BIOFUNGICIDES

Biofungicides are used: for the treatment of seeds, tubers potatoes before planting or before storage, foliar, for sinking or spraying seedlings before planting, watering plants after planting, planting plants, immersion of seedlings etc.

The application of this and similar biofungicides must be very common in order to provide a satisfactory fungal biomass antagonists and protect the seedlings or plants. Gveroska (2013, 2016 a, 2017) highlights the best way of application of *T. harzianum* in tobacco seedlings. She recommended the sinking of seed 24-48h in the culture of the biocontrol agent or in suspension of the current bioproduct.

For the full benefit and sustainable usage, we have to understand mode of action; use as protectant: good coverage, protectant intervals; use under low to moderate disease pressure for best results; use in an integrated program, mixing or rotating with different modes of action; consider additional benefits in addition to efficacy (Bayer, 2017).

Possible combinations are three successive measures: thermal seed treatment, then application of growth stimulator (*Azospirillum brasilense*) and treatment with streptomycin sulphate for which increased efficiency is achieved, for example Better suppression of the cause of bacterial parasites (*Pseudomonas syringae* pv. tomato) (Bashan and Bashan, 2002).

Maximum allowed number of treatments for biofungicides is standard for every plant species. Number of cultivated plants which are protected by biofungicides is quite large, from vegetable, vegetable, fruit, decorative, medicinal and spice herbs (Grahovac et al., 2009).

Despite the huge perspective, ecological and sustainable approach, biofungicides are however faced with challenges of formulation, registration, commercialization, acceptance and adoption. Therefore, these are some aspects of biopesticide development, including their sources, production, formulation, commercialization, efficacy and role in sustainable agriculture.

COMMERCIALIZATION OF BIO CONTROL PRODUCTS

Although the number of biocontrol products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture (Fravel, 2005). In recent years there are enlarged commercial production of bio control agents resulting into the entry of various biocontrol products into the world market.

Commercialization of biocontrol products is a multi-step process involving a wide range of activities:

- a) Isolation of micro- organism from the natural ecosystem;
- b) Evaluation of bio-agent both in vitro and under glass house conditions
- c) Testing of the best isolate under field conditions
- d) Mass production
- e) Formulation
- f) Delivery
- g) Compatibility
- h) Registration and release

The ultimate success of commercialization depends firstable on the base – isolation and the searching and screening process. Also, it depends from the target pathogen, the crop and the cropping system, presence or absence of the right isolate - antagonistic to the pathogen, real formulation, its stability etc.

FORMULATIONS OF BIOPREPARATES AND APPLICATION POSSIBILITIES

The formulation of biological preparations requires very good knowledge of the interaction of microorganisms and objects suppression. It is essential that the preparations can be produced on a liquid or semi-solid substrate and in sufficient quantities, then staying vital during storage and afterwards.

Application of the preparation, under selective pressure in the laboratory conditions do not lose the importance of biological importance suppression (vitality, variability, selectivity), as well as compatibility with application technology. This is it exceeds the use of avoided, nutritious and connective tissue matter or sticker and carrier as a major part in the formulation.

They are applied in the form of a polunative culture, or formulated in the form of a powder, a wettable powder, water-soluble granules, granules, pellets, microcapsules, gels and emulsifiers liquids. The short term storage of biopreparates exceeds by encapsulation of microorganisms, or their products, into the matrix of organic polymers. Biopreparaes may contain one or more microorganisms as active ingredients. Preparation Polyversum is based on *Pythium oligandrum*, and Trichoshield on the basis of *Trichoderma harzianum* + *T. lignorum* + *Gliocladium virens* + *Bacillus subtilis* (Klokočar-Šmit et al., 2006).

Unlike chemical, the content of active substance in the preparation of biofungicide is expressed in millions or billions of living cells and spore / g (*B. subtilis* isolates), or $2.5 \cdot 10^{10}$ cells / ml (*Pseudomonas fluorescence* isolates), or 5 billion spores/g *Penicillium vermiculatum*) (Grahovac et al., 2009).

Existing formulation technologies suitable for biopesticides

Formulation needs to take into account not only that efficacy will be particularly dependent on environmental conditions, but that it will also be necessary to ensure stability of living organisms in distribution and storage.

Among formulation objectives for biopesticides, Dr Knowles lists: maintaining stability of the a.i. during processing and on storage and application; protecting the ai against degradation or loss of effectiveness on exposure to UV light; maintaining viability and effectiveness of the a.i. in the presence of other formulation components, especially for living organisms; and improving effectiveness of the a.i. under low humidity conditions, especially for living systems (Agrow, 2013).

Commercial biopesticides should be economical to produce, have persistent storage stability, high residual activity, be easy to handle, mix and apply, and provide consistently effective control of target pests. Different formulations of biopesticides should be introduced to overcome problems relating to their efficacy and their degradation and to be convenient during handling and application (Gašić and Tanović, 2013).

WHY BIOFUNGICIDES IN SUSTAINABLE AGRICULTURAL PRODUCTION ADVANTAGES VS. DISADVANTAGES OF BIOFUNGICIDE

Disadvantages of biological pesticides:

- They are more difficult to implement;
- Have a narrower spectrum of action;
- They act more slowly than chemical agents; act preventively, never eradically;
- Biopreparates have a shorter shelf life and therefore seem they are more expensive;
- They may be incompatible with other fungicides or bactericides
- Require repeated use and lowering the thresholds

Advantages of biological pesticides:

- The most powerful characteristic is that biopesticides are safety products both for the producer (applicant), the consumer and the environment. They have very short reentry intervals which guarantee safety for the applicant. They have no toxicity (Damalas and Koutroubas, 2015)
- Although it is believed that the application of biopesticides in plant disease control requires more human labor and is less effective of chemical pesticides.
- Biofungicides are natural products. Biocontrol agent are natural, resident of the current ecosystem. They are non-toxic.
- They reduce the need for chemicals;
- Biofungicides do not cause resistance in pathogens. So, they are extremely important in the preparation of an anti-persistent strategy and controlling the susceptibility of suppressed species to chemical pesticides; they are safer for use from chemicals;
- They are eco-friendly since they are easily biodegradable and therefore do not pollute the environment (Leng et al., 2011). They have more specific action on the target organism from chemical preparations; are and hence do not affect the beneficial organisms.
- They can take participation in decontamination of agricultural soils through introduction of beneficial microbial species and some products; reduced environmental impact; increased biodiversity (Anonymous 14).

- Have shorter working withdrawals and no quarantine and can be applied in various types of plant production (organic, integral, etc.); Consumer tastes and preferences fluctuate over time and following the demand for organically produced food, this makes biopesticides suitable alternatives to synthetic pesticides. Biopesticides have very short pre-harvest intervals and therefore are safe to use on fresh fruits and vegetables. Some of them are used for other purposes like food and feed. Additionally, they can take a higher premiums for the production and sale of organic product (excluding pesticide residues);
- Availability of their source materials makes them inexpensive to attain since they are found within the natural environment.
- They compete with pesticides with their effectiveness and their use promotes sustainable management.
- Application of biological agents in programs of integral protection of plants allows the development of sustainable agricultural production;

Development and implementation of the new plant protection program it will take and will require significant investments. Key the factor is the education of workers about handling with a new "tool" – biological preparations and presentation of the benefits. (Anonymous 14).

Biopesticides have sufficient efficacy to be able to be launched separately as products

Agrow (2013) highlights important data, which in some way are the directions of the chemical companies -magnates in the production of pesticides in terms of biofungicides:

Biologicals will have a meaningful role to play in helping growers to protect and enlarge their yields. It points out the challenges, such as limited shelf-life (dealing with living organisms), but it sees a high potential for future innovation in these areas. "We expect to see biologicals in the market with sufficient efficacy as stand-alone products as well as biologicals tailored for applications in combination with classic chemistries," the company adds (BASF, 2013).

The use of biopesticides in a variety of scenarios offering varying degrees of efficacy. Some are designed for speciality production models. Some of them can be used alongside chemical crop protection products (in tank mixes or alternation programmes) to deliver comparable performance to standard and compete very effectively against chemical crop protection products in terms of disease control and yield benefits (Bayer, 2013).

Performance of biopesticides has improved over the years and they can be launched as individual products. When used in combination with chemicals, highquality seeds and established good agronomic practices, their performance can be significantly enhanced. Thus, the role of biopesticides in plant protection is ensured and solidified (Syngenta, 2013).

CONCLUSIONS

Use of synthetic chemicals has raised numerous concerns due to their negative effects on the environmental, human health, natural enemies and ecosystem balance. Global requirements for the need to reduce the use of chemicals pesticides enforced the use of harmless, new, sustainable strategies in plant protection.

Biofungicides are alternative to synthetic fungicides due to their low toxicity, biodegradability and low persistence in the environment. The base materials for biopesticides are readily available and inexpensive. They are real alternative to chemicals, despite the many challenges facing their adoption.

Formulation needs to be efficient, no dependent on environmental conditions, but that it will also be necessary to ensure stability of living organisms in distribution and storage.

Application modes need the knowledge of biocontrol agent which will enable the achievement of the best expression of biocontrol mechanisms.

Globally, researchers have conducted studies on effectiveness of natural plant protection products with significant results, in field conditions. Further research is recommended dedicate to close the gaps in formulation of biofungicides. They should work together with the industry as well as farmers to provide stable, durable formulations of biopesticides. Development and implementation of the new plant protection program it will take significant investments and education manufacturers, workers of forecasting and reporting services. Any research is recommended to dedicate to all points of sustainability. This will strengthen and emphasize their role in sustainable agricultural production.

REFERENCES

1. Agrow World Crop Protection News, 2013. Biopesticides, Informa UK Ltd, www.agrow.com
2. Anonymous, 2000. Fungal Biological Control Agents,
3. Alizadeh O, Azarpanah A, Ariana L., 2013. Induction and modulation of resistance in crop plant against disease by bioagent fungi (arbuscular mycorrhiza) and hormonal elicitors and Plant Growth Promoting Bacteria. *International Journal of Farming and Allied Sciences*, 2(22): 982-998.
4. Damalas, C.A. and Koutroubas, S.D., 2015 Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. *Toxics*, 1, 1-10.
5. EU Minor Uses Coordination Facility (MUCF), 2019. www.minoruses.eu
6. Fravel D R., 2005. Commercialization and implementation of bio control. *Annual review of phytopathology*. 43: 337-359.
7. Gašić S., Tanović B., 2013. Biopesticide Formulations, Possibility of Application and Future Trends, *Pestic. Phytomed.* (Belgrade), 28(2), 97–102
8. Grahovac M., Indić D., Lazić S., Vuković S., 2009 Biofungicidi i mogućnosti primene u savremenoj poljoprivredi, *Pestic. Phytomed.* (Belgrade), 24(4), 245-258.
9. Gold M., 2009. Sustainable Agriculture: Definitions and terms, U.S. Department of Agriculture, Alternative Farming Systems Information Center, Agricultural Research Service, Beltsville.
10. Gveroska B., 2013. Relation of *Trichoderma* spp. quantity in soil to reducing the damping off disease in tobacco seedlings. *Bulgarian Journal of Agriculture Science*, 19 (No.4) pp.671-679.
11. Gveroska B., Miceska G., Dimitrieski M., Korubin-Aleksoska A., 2014. Use of biopreparates in tobacco protection-Contribution to Sustainable Agriculture. *Turkish Journal of Agricultural and Natural Science*, Vol I, Special Issue I (ISSN: 2148-3647), TJANS-157, pp. 1509-1517
12. Gveroska B., 2016 a. Increasing the biocontrol activity of *Trichoderma* spp. with the use of an appropriate manure. *Тутун / Tobacco*, Vol. 66, № 7-12, pp. 27-34, Scientific Tobacco Institute – Prilep.
13. Gveroska B., 2016 b. Application of *Bacillus subtilis* Ch13 in the control of damping off disease in tobacco seedlings. *International Journal of Development Research*, Vol. 06, Issue 09, pp.9322-9326.
14. Gveroska B., 2017. Fungicidal and stimulating effect of biopreparation TRIANUM P on tobacco seedlings. *Тутун / Tobacco* Vol. 67, № 1-6, pp. 48-55, Scientific Tobacco Institute-Prilep.
15. Harman, G.E., 1996. *Trichoderma* for Biocontrol of Plant Pathogens: From Basic Research to Commercialized Products. Conference on Biological Control, Cornell community, April 11-13.
16. Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M., 2004. *Trichoderma* species –opportunistic, avirulent plant symbionts, *Nature Review Microbiology*, 2(2004), pp. 43-56.
17. Harman, G. E., 2006. Overview of Mechanisms and Uses of *Trichoderma* spp., *Phytopathology* 96, pp. 190-194.
18. Howell C.R., 2003. Mechanisms employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. *Plant Disease*, Vol 87, No 1, pp. 4-10.
19. Klokočar-Šmit Z., Šovljanski R., Indić D., 2006. Biopreparati – alternativa u zaštiti plodovitog povrća. *Biljni lekar*, XXXIV(1): 19-30.
20. Kumari A., Kumar K. N. R., Rao C. H. N., 2014. Adverse Effects of Chemical Fertilizers and Pesticides on Human Health and Environment. Proceedings National Seminar on Impact of Toxic Metals, Minerals and Solvents leading to Environmental Pollution. *Journal of Chemical and Pharmaceutical Sciences*, 3, 150-151.

21. Koul O., 2011. Microbial Biopesticides: Opportunities and Challenges. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 6, 056.
22. Leng P., Zhang Z., Pan G., Zhao M., 2011. Applications and Development Trends in Biopesticides. *African Journal of Biotechnology*, **86**, 19864-19873.
23. Malik A., Rahman M., Ikram M.A., Masood F., Grahman E., 2012. Environmental Protection Strategies for Sustainable Development. Springer Science + Business Media BV.
24. Maksymiv I., 2015. Pesticides: Benefits and Hazards. *Journal of Vasyl Stefanyk Precarpathian National University*, 1, 70-76.
25. Junaid J.M., Dar N.A., Bhat T.A., Bhat A. H., Bhat M.A., 2013. Commercial Biocontrol Agents and Their Mechanism of Action in the Management of Plant Pathogens, *International Journal of Modern Plant & Animal Sciences*, 2013, 1(2): 39-57
26. Scientific Tobacco Institute-Prilep, 2019. Programme for Tobacco protection from disease, pests and weeds.
27. Singh H.B., 2014. Management of Plant Pathogens with Microorganisms. *Proceedings of the Indian National Science Academy*, 2, 443-454.
28. Srijita D., 2015. Biopesticides: An Eco-friendly Approach for Pest Control. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6, 250-265.
29. Suprpta D.N., 2012. Potential of Microbial Antagonists as Biocontrol Agents against Plant Fungal Pathogens. *International Society for Southeast Asian Agricultural Sciences Journal*, 2, 1-8.