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APPLICATION OF THE EM TECHNOLOGY IN TOBACCO SEEDLING PRODUCTION

Biljana Gveroska, Gordana Miceska, Miroslav Dimitrieski

University St. Kliment Ohridski – Bitola, Scientific Tobacco Institute -Prilep
Republic of North Macedonia
e-mail: bgveros@yahoo.com

ABSTRACT

Modern EM technology has a particular contribution to environmental management and sustainable agricultural production.
The aim of the research was to determine the effect of the application of the probiotic EMa 5 or the combination EMa 5+ EmFarma, as well as the best model of application (on the soil before sowing; after the emergence of tobacco seedlings; on the seeds) and with/without herbicide application.
Probiotics have a positive effect on tobacco seedlings. The best development of the root system, as well as the total length of the tobacco seedling, was determined in the variant - application of EmFarma Plus + Emma 5 on the soil, before sowing and during the application of herbicide. The length of the root system of the seedling in the standard treatment is lower than that of the seedling of all variants of probiotic application.
Application of probiotics is possible under standard sowing conditions, that is, when herbicide is used.
The results of these researches create a good perspective for their application in the production of tobacco seedlings.

Key words: tobacco, EM technology, probiotics, EmFarma Plus, Ema 5, development, root

ПРИМЕНА НА ЕМ ТЕХНОЛОГИЈАТА ВО ПРОИЗВОДСТВОТО НА ТУТУНСКИ РАСАД

Современата ЕМ технологија има особен придонес во управувањето со животната средина и оддржливото земјоделско производство.
Целта на истражувањето беше да се утврди ефектот од примената на пробиотикот ЕМа 5 или комбинацијата ЕМа 5+ EmFarma, како и најдобриот модел на апликација (врз почвата пред сеидба; по поникнување на тутунскиот расад; врз семето) и со/без примена на хербицид.
Пробиотиците имаат позитивен ефект врз тутунскиот расад. Најдобар развој на кореновиот систем, како и вкупна должина на тутунскиот расад е утврдена кај варијантата - апликација на EmFarma Plus + Ema 5 врз почвата, пред сеидба и при примена на хербицид. Должината на кореновиот систем од расадот при стандардниот третман е помала од таа на расадот од сите варијанти на примена на пробиотици.
Примена на пробиотиците е можна при стандардни услови на сеидба, односно при употреба на хербицид.
Резултатите од овие истражувања создаваат добра перспектива за нивна примена во производството на тутунски расад.

Клучни зборови: тутун, ЕМ технологија, пробиотици, EmFarma Plus, Ema 5, развој, корен

INTRODUCTION

The integrated concept of sustainable agricultural production is based on the principles of ecology and the relationships between organisms and the environment.

EM technology is one of the basic ways to manage the environment in the direction of sustainable agricultural production. The basic principle of this technology is application and increase of the population of efficient and beneficial microorganisms in the soil.

Increasing the population of EM in the soil with application models increases the development of existing soil microorganisms. Thus, the microflora in the soil becomes rich and the soil develops a well-balanced microbial system (Nayak et al., 2020). Degenerative microorganisms, especially soil pathogens, are pushed out. This creates a healthy environment for the plants where the effective microorganisms continue to coexist in the rhizosphere.

But plant roots secrete substances such as carbohydrates, amino and organic acids and active enzymes. Effective microorganisms use these secretions for their development. But, during those processes they also secrete and provide amino and nucleic acids and many vitamins and hormones that are used by plants. Thus, EM coexists with the plant root in the rhizosphere (Higa and Wididana, 1989).

Thanks to all the microbiological processes in which the soil, and especially the rhizosphere microflora participates, the plants are provided with all the nutrients. Thus, they have an outstanding opportunity for proper development.

Modern EM technology, or generally accepted term "probiotics", includes four very important groups of microorganisms - lactic acid bacteria, photosynthetic bacteria, actinomycetes and yeasts, mixed in a carbohydrate medium. Depending on the purpose, certain preparations have a "fortified" formula in which, in addition to the basic components, other bioactive components are represented, e.g., plant extracts. Such is the case with the preparation EMa 5 (SCD Probiotics, 2012).

Application of probiotics doesn't mean only improvement of soil properties and proper development of plants, but also prevention of disease attack. In that regard, the preparation EMa 5 is particularly noteworthy, which is why it is mainly used as a biofungicide.

Therefore, our aim was to investigate the possibility of using probiotics in tobacco seedling production and determine the effect of the application of EMa 5 alone (given its promotion as a biofungicide) or in combination with the probiotic EM Farma. Specifically, our aim was to confirm their multifunctionality and promising role in the production of tobacco seedling.

The examinations also ensure the determination of the best application model. What is most important, it is adapted for application during usual cultural practices in the production of tobacco seedling.

MATERIAL AND METHODS

The examinations were carried out in tobacco seedbeds in 2020. The soil treatment, which was planned when defining the variants, was carried out the previous day. The seedbeds were sown with tobacco seeds of variety P -66-9/7. Seed amount of 6.75 g/10 m^2 was used.

Each variant was tested in three replications, and the area for each replication was 3.33m^2.

The preparation EmFarma was applied in a dose of 1000 ml/100 m^2, i.e., 30 ml / 3.33 m^2.

The preparation Ema 5 was applied in a dose of 300 ml /100 m^2, i.e., 10 ml / 3.33 m^2.

Tobacco seeds (2.25 g/3.33 m^2) were soaked in 3 ml prepared solution and kept for 24 hours before sowing. For this purpose, a solution with EM farm was previously prepared in a dose of 100 ml/10m^2. An overview of the variants with the respective treatments is given in Table 1.

Table 1. Variants and treatments

Application of probiotics	Mark	Variant	Treatment		
	1	Control, without any treatment			
	2	Standard treatment with herbicide, fungicide and supplement			
Seed	3	EmFarma Plus +Ema 5 Herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
Seed	5	Ema 5 Without herbicide		Ema 5	Ema 5
Seed	7	EmFarma Plus+ Ema 5 Without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
After emergence	4	Herbicide Ema 5 after emergence	after emergence Ema 5	Ema 5	Ema 5
After emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	after emergence EmFarma Plus + Ema 5	EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
Seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
Seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5

The herbicide Gamit 4 EC was applied in a dose of 0.07 ml/m^2 in the respective variants.

The treatment after seedling emergence was performed only in the respective variants (variants marked 4 and 6).

The second treatment was performed in all variants. The same day, the second control i.e., standard, received treatment with the fungicides Top M (0.1%) and Ridomil (0.25%). Fertilizing with 15 g/m^2 of ammonium nitrate was carried out the previous day.

The third treatment with probiotics was performed at the beginning of the vigorous growth phase.

During the research, the seedling was observed daily, and the growth and condition of the damping off disease was monitored.

Seedling length was measured twice, first time before the third treatment and second time in the phase of vigorous growth, before pulling for transplanting.

RESULTS AND DISSCUSION

Tables 2 and 3 present the results about the size of tobacco seedling in the three replications of each variant. However, for better conclusions about the impact of probiotics on the development of tobacco seedling, the average values were analyzed (Table 4 and 5).

Seedling have the lowest growth in the control (without any treatment), which is quite expected (Table 4). However, the largest length of the root system, as well as the total length, has the seedling of the 4th variant, i.e., Ema 5 after emergence. They are larger than the dimensions in the standard treatment with herbicide, fungicide and fertilizer. Ema 5 is a probiotic with a fungicidal effect. In addition to the basic formulation characteristic of EM technology, it also contains plant extracts (SCD Probiotics, 2012). According

to the multiple effect (including antagonistic effect against fungal and bacterial pathogens and detoxifying effect on the soil from pesticide residues) it is expected that the composition of all its active components will act very stimulative.

Acting against fungal and bacterial pathogens, it detoxifies the soil of pesticide residues. Starting from the base (probiotic i.e., EM technology) and its additional effect, it is expected that the composition of all its active components will act not only fungicidally, but also stimulating.

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Table 2. Impact of probiotics on the development of tobacco seedling - 1 evaluation

Application of probiotics	Mark	Variant	replication								
			I			II			III		
			root	stalk	Total length	root	stalk	Total length	root	stalk	Total length
	1	Control, without any treatment	3.25	9.50	9.75	2.60	11.50	14.10	2.00	9.16	11.16
	2	Standard treatment with herbicide, fungicide and supplement	2.62	16.37	18.99	2.50	12.50	15.00	3.00	15.33	18.33
on the soi	3	EmFarma Plus +Ema 5 Herbicide	3.12	14.12	17.24	2.66	17.00	19.66	3.33	13.66	16.99
on the soi	5	Ema 5 Without herbicide	2.62	14.00	16.22	3.00	12.33	15.33	3.66	13.16	16.82
on the soi	7	EmFarma Plus+ Ema 5 Without herbicide	3.25	15.00	18.25	3.33	15.33	18.66	3.00	17.50	20.50
after emergence	4	Herbicide Ema 5 after emergence	3.75	15.25	19.00	2.66	13.16	15.82	4.16	18.33	22.49
after emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.00	14.75	17.75	3.33	15.83	19.16	2.66	15.66	18.32
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.33	15.00	18.33	3.16	17.83	20.99	3.16	15.16	18.32
on the seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.25	14.62	17.87	3.33	14.50	17.83	3.00	13.50	16.50

Good development of the whole plant and, what is of particular importance, the root system, has the seedling of variants 7 and 8. Although there are different ways of application (after emergence and on seeds), both variants are without any use of herbicide. It is known that the herbicide has a harmful effect on the soil as well as on the rhizosphere microflora (Ayansina and Oso, 2006). Such a negative effect can be long-lasting and affect the entire microflora-plant system. The soil in which there is no harmful effect of the herbicide is a good substrate for the reproduction and development of the effective microflora and its activity, thus providing the root system with various nutrients.

Table 3. Impact of probiotics on the development of tobacco seedling - 2 evaluation

Application of probiotics	Mark	Variant	replication								
			I			II			III		
			root	stalk	Total length	root	stalk	Total length	root	stalk	Total length
	1	Control, without any treatment	3.50	12.10	15.60	3.10	12.40	15.40	3.30	12.20	15.50
	2	Standard treatment with herbicide, fungicide and supplement	3.37	19.25	22.62	3.16	17.33	20.49	2.50	18.66	21.16
on the soil	3	EmFarma Plus +Ema 5 Herbicide	3.40	14.20	17.60	4.20	18.20	22.40	4.60	35.00	39.60
on the soil	5	Ema 5 Without herbicide	4.25	15.25	19.50	5.50	16.33	21.83	2.66	15.33	17.99
on the soil	7	EmFarma Plus+ Ema 5 Without herbicide	3.87	17.50	21.37	4.66	19.33	23.99	3.66	20.66	24.32
after emergence	4	Herbicide Ema 5 after emergence	4.37	16.75	21.12	4.66	18.66	23.32	4.23	17.13	21.36
after emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.75	21.25	25.00	3.33	19.66	22.99	3.33	21.00	24.33
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.25	18.75	22.00	2.83	15.00	17.83	4.00	23.66	27.66
on the seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	4.50	19.50	24.00	2.83	13.66	16.49	2.83	14.33	17.16

The effect of the seedling treatment with probiotics is best seen in the stage of vigorous growth. According to Table 5, the length of the root system of the seedling in the standard treatment is the smallest. It is even smaller than the control - without any treatment, which can certainly be explained by the special conditions of water supply that favor the development of the root in length. But it lags behind all variants treated with probiotics.

Tobacco seedling of the variants 3 and 7 had the best development, especially of the root system, but also of the whole plant, when EmFarma Plus + Ema 5 was applied to the soil, before sowing.

The obtained results are in accordance with the statements of Ashraf et al. (2004). According to them, the introduction of effective microflora can activate nitrogen fixation, phosphorus solubility, increased release of siderophores, phytohormones, exopolysaccharides, known microbial products that increase plant development and protect them from abiotic stress.

Also, Combant et al (2005) claims that proliferation of microorganisms that are promoters of plant growth in the soil, especially in the rhizosphere, stimulates plant development. At the same time, through the competition for space and food and creation of inhibitory substances, they induce systemic resistance of plants.

Table 4. Impact of probiotics on the development of tobacco seedling - (1 evaluation - average values)

Application of probiotics	Mark	Variant	length		
			root	stalk	whole plant
	1	Control, without any treatment	**2.61**	10.05	11.67
	2	Standard treatment with herbicide, fungicide and supplement	2.70	14.73	17.44
on the soil	3	EmFarma Plus +Ema 5 Herbicide	3.03	14.92	17.96
on the soil	5	Ema 5 Without herbicide	3.09	13.16	16.25
on the soil	7	EmFarma Plus+ Ema 5 Without herbicide	3.19	15.94	19.13
after emergence	4	Herbicide Ema 5 after emergence	**3.52**	15.58	19.10
after emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	2.99	15.41	18.41
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.21	15.99	**19.21**
on the seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.19	14.20	17.40

Increasing soil microbial diversity improves plant health and productivity (Okorski et al., 2008). The same value of the root system length in these two variants (without and with the use of herbicide) confirms the possibility of using probiotics under standard sowing conditions i.e., when using herbicide.

Also, the application of probiotics on the soil confirms the positive effect of probiotics on plant development and at the same time represents a good application model and perspective for their application in the production of tobacco seedling.

The seedling from the variants where the seeds were treated with EmFarma Plus and the soil with Ema 5 have a poorly developed root system and a similar average value of the total length of the plant as the seedling from the standard treatment. There are certain dilemmas whether probiotics applied to seeds increase the percentage of germination, whether they develop a strong secondary root system and a healthy stalk, and whether it is a good application method, (Olle and Williams, 2013). Bharti et al. (2007) present facts that the rhizosphere is a microbial center where there are different but at the same time significant interactions between microorganisms and the soil together with the plants. The nature of these interactions is entirely determined on a molecular basis. These positive beneficial interactions significantly affect plant development and growth, and subsequently result in a progressive impact on yield and production.

Table 5. Impact of probiotics on the development of tobacco seedling - (2 evaluation - average values)

Application of probiotics	Mark	Variant	length		
			root	stalk	whole plant
	1	Control, without any treatment	3.30	12.23	15.50
	2	Standard treatment with herbicide, fungicide and supplement	**3.01**	18.41	21.42
on the soil	3	EmFarma Plus +Ema 5 Herbicide	**4.06**	22.40	**26.53**
on the soil	5	Ema 5 Without herbicide	4.13	15.63	19.77
on the soil	7	EmFarma Plus+ Ema 5 Without herbicide	**4.06**	19.16	23.22
after emergence	4	Herbicide Ema 5 after emergence	4.23	17.13	21.36
after emergence	6	Herbicide EmFarma Plus + Ema 5 after emergence	3.47	20.63	**24.10**
on the seed	8	Seed treated with EmFarma Plus + soil treated with Ema 5 Without herbicide	3.36	19.13	22.49
on the seed	9	Seed treated with EmFarma Plus + soil treated with Ema 5 Herbicide	3.38	15.83	19.21

These data confirm the results of our research that the best development of the root system as well as the whole plant is determined when probiotics are applied to the soil, before sowing (at the same time highlighting it as the best application model). The soil is a natural environment for these groups of microorganisms, in which they best manifest their activity (specific processes). At the same time, early interactions occur in the soil (among microorganisms, but also microorganisms-plants) which lead to an increase in their activity. All this leads to stimulation of plant growth.

CONCLUSIONS

- Probiotics have a positive effect on the development of tobacco seedling, especially on the root system.
- The length of the root system of the seedling with standard treatment was smaller than the length of the seedling treated with all the probiotic variants.
- Tobacco seedling had the best development especially of the root system, but also of the whole plant, when EmFarma Plus + Ema 5 was applied to the soil, before sowing. At the same time, it represents a good application model of probiotics in the production of tobacco seedling.

- Application of probiotics is possible under standard conditions of sowing, i.e., when using herbicide.

- Probiotics have a good perspective for their application in the production of tobacco seedling.

REFERENCES

1. Ashraf M., Hasnain S., Berge O., Mahmood T., 2004. Inoculating wheat seedling with exopolysacharide producing bacteria restricts spdium uptake and stimulates plant growth under salt stress. Biol. Fertil. Soils 40, pp. 157-162.
2. Ayansina AD.V., Oso B.A., 2006. Effect of two commonly used herbicides on soil microflora at two different concentrations. African Journal of Biotechnology, **5**:129-132.
3. Bharti N., Sushil K. Sharma, Saini S., Verma A., Nimonkar Y., Prakash O., 2007. Microbial Plant Probiotics: Problems in Application and Formulation. In book: Kumar et al. (eds.): Probiotics and Plant Health, Springer Nature Singapore Pte Ltd. 2017 317.
4. Combant S., Duffy B., Nowak J., Clement C., 2005.Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases:Principles, Mechanisms of Action, and Future Prospects. Applied and Environmental Microbiology, 71(9):4951-4959.
5. Higa T and Wididana G.N., 1989. Changes in the Soil microflora Induced by Effective Microorganisms. First International Conference, Kyusel nature Farming, Oct. 17-21 1989, pp. 153-162.
6. Nayak N., Sar K., Sahoo KI., Mahapatra P., 2020. Beneficial effecet of effective microorganisms on crop and soil-a review. Journal of Pharmacognosy and Phytochemistry, 9(4), pp. 30370-3074.
7. Okorski A., Olszewski J., Pszczólkowska A., Kulik T., 2008. Effcet of Fungal Infection and the Aplication of the Biological Agent EM 1 on the Rate of Photosynthesis and Transpiration in Pea (Pisum sativum L.) Leaves. Polish Journal of Natural Sciences, Vol. 23 (1): 35-47.
8. Olle M., Williams I.H., 2013. Effective microorganisms and their influence on vegetable production – a review. Journal of Horticultural Science &Biotechnology 88 (4), pp. 380-386.
9. SCD Probiotics, 2012. http://www.scdprobiotics.com