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Original Scientific paper

THE EFFECT OF PROBIOTICS ON SOIL MICROFLORA IN TOBACCO SEEDLINGS PROTECTION FROM DISEASES

Biljana Gveroska

University St. Kliment Ohridski –Bitola, Scientific Tobacco Institute – Prilep

Republic of Macedonia

e-mail: bgveros@yahoo.com

ABSTRACT

The basic principle of EM technology is to apply and increase the population of effective and useful soil microorganisms which will displace degenerative microorganisms, especially soil pathogens.
There are data on the use of probiotics not only in soil improvement but also in prevention of disease attacks. With regard to this, our objectives were to study the impact of probiotics on soil microflora and, more specifically, to determine the number of each group of microorganisms and their influence on certain processes in the soil, even in the control of certain pathogens, to estimate the effect of application of one probiotic or their combination, as well as the best model of application - when usual cultural practices are applied in order to produce healthy and good quality tobacco seedlings.
Investigations included two probiotics - EmFarma Plus and Ema 5 (probiotic with enriched formula and with rather fungicidal effect), and their combinations, as well as the modes of application.
It can be concluded from investigations that probiotics have a positive effect on the number of certain groups of microorganisms.
The best results in increasing the number of actinomycetes were obtained with EmFarma Plus + Ema 5 (with and without herbicide application). The small number of bacteria is compensated by increased number of actinomycetes. A balanced ratio of bacteria and fungi was recorded, too. The application of Ema 5 also confirmed its effects.
It may be concluded that probiotics with their effect on increasing some groups of microorganisms can prevent the attack of soil pathogens.
Keywords: probiotic, EmFarma Plus, Ema 5, actinomycetes, bacteria, fungi

ЕФЕКТОТ НА ПРОБИОТИЦИТЕ ВРЗ ПОЧВЕНАТА МИКРОФЛОРА ВО ЗАШТИТАТА НА ТУТУНСКИОТ РАСАД ОД БОЛЕСТИ

Основниот принцип ЕМ технологијата е апликација и зголемување на популацијата на ефикасни и корисни микроорганизми во почвата, со што се истиснуваат дегенеративните микроорганизми, особено почвените патогени.
Постојат податоци за примена на пробиотиците не само во насока на подобрување на својствата на почвата, но и спречување на нападот од болести. Затоа, нашата цел беше да се испита влијанието на пробиотиците врз почвената микрофлора. Поточно, утврдувањето на бројноста на одделни групи микоорганизми, што би значело нивно влијание врз одредени процеси во почвата, дури и нападот од патогените.
Исто така, да се утврди ефектот од примената само еден или нивна комбинација, како и најдобриот модел на апликација при примена на вообичаените агротехнички мерки, за да на крајот произведиме здрав и

квалитетен тутунски расад.
Испитувањата се извршени при апликација на пробиотиците EmFarma Plus и Ema 5 (пробиотик со зјакната формула и чие дејство е повеќе фунгицидно), при разни нивни комбинации, како и начини на апликација.
Од испитувањата може да се констатира дека пробиотиците имаат позитивен ефект врз бројноста на одделни групи микроорганизми.
Забележителен резултат во зголемување на бројноста на актиномицетите се забележува кај третманите со EmFarma Plus + Ema 5 (без и со примена на хербицид). Малата бројност на бактериите се компензирана со зголемената на актиномицетите. Се забележува и избалансиран однос на бактериите и габите. Примената на Ema 5 исто така го потврди своето дејство.
Може да се констатира дека пробиотиците со своето дејство врз намножувањето на одделни групи микроорганизми, можат да влијаат врз спречување на нападот од почвените патогени.
Клучни зборови: пробиотик, EmFarma Plus, Ema 5, актиномицети, бактерии, габи

INTRODUCTION

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. Effective microorganisms continue to coexist in the rhizosphere and plants grow well, free of pathogens. Therefore, plant growth regulators which exibit no pesticidal activity but instead can promote, inhibit or modify the physiology of plants, are also regulated as biopesticides (BPIA, 2014).

EM farma are bioproducts based on technology of effective microorganisms. Its include four group of microorganisms: lactic acid bacteria, yests, phototrophic bacteria and actinomycetes in carbohydrate medium. Introduction of beneficial microorganisms in any living system will ensure that the healthy microbes dominate the disease-causing populations (ProBioticsPolska, 2015).

There are data on the use of probiotics not only to improve the soil but to prevent the attack from diseases (Higa et al., 1989; Tokeshi et al., 1997; Okorski et al., 2008). The presence of some groups of micro-organisms implies that they have an influence on certain processes in the soil, on conditions for infection provoked by soil pathogens and on development of diseases.

Our main objective was to study the effect of probiotics on some groups of soil microflora, by indirect monitoring of the intensity of damping off disease. Analyses were also made on the effect of biofungicide EMa 5 used alone or in combination with EM farma probiotics, applying all necessary agro-technical measures. Identification of the best model of application of these products will increase the possibility of realization of the basic principle of effective microflora and EM technology will also become applicable from the aspect of seedlings protection from diseases. The ultimate goal is to provide healthy and good quality tobacco seedlings produced by the standards of sustainable tobacco production.

MATERIAL AND METHODS

Trials were set up in tobacco seedbeds and sown with seed of the variety P-66-9/7 at a rate of 6,75 g / 10 m^2.

Treatment of soil in the appropriate variants was carried out the previous day. Variants and treatments are presented in Table 1. Each variant was tested in three replicates and the area of each replicate was 3,33 m^2.

Table 1. Variants and treatments

No.	Variant	Treatment		
1	Check, untreated			
2	Standard treatment (herbicide, funcigide and saltpeter)			
3	EmFarma Plus +Ema 5; herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
4	Ema 5; without herbicide	after emergence of seedlings - Ema 5	Ema 5	Ema 5
5	Ema 5; without herbicide		Ema 5	Ema 5
6	EmFarma Plus +Ema 5 after emergence of seedlings; herbicide	after emergence of seedlings EmFarma Plus + Ema 5	EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
7	EmFarma Plus +Ema 5; without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
8	Seed with EmFarma Plus +soil with Ema5; without herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5
9	Seed with EmFarma Plus +soil with Ema5; herbicide		EmFarma Plus + Ema 5	EmFarma Plus + Ema 5

Treatment of seedlings

EM-farma was applied in a rate of 1000 ml/100m^2, i.e. 30 ml/3,33 m^2.

Ema 5 was applied in a rate of 300 ml/100 m^2, or 10 ml/3,33 m^2.

Tobacco seed (2,25 g/3,33 m^2) was soaked in 3 ml of the prepared solution of EM farma (100 ml/10m^2) and stored 24 hours before sowing.

In variants using herbicide Gamit 4 EC the rate was 0,07 ml/m^2.

The second treatment was after 15 days in all variants (including 4 and 6).

Standard treatment (Variant 2) was applied on the same day, using the fungicides Top M (0,1%) and Ridomil (0,25%) and fertilization with 15 g/m^2 ammonium saltpeter the day before.

Third treatment with preparations and their combinations followed 15 days after the second one.

Monitoring of seedlings and damping off disease was made on daily basis.

Two evaluations of damping off disease were made – the first one prior to the second treatment and the second one - prior to the third treatment.

The percentage of infected area was calculated for the three replicates of each variant and analysis was made on average values.

Microbiological analysis

The number of major groups of microorganisms (bacteria, fungi and actinomycetes) was counted.

Soil samples from all replicates of each variety including the check were collected in sterile paper bags and they were used to make an average sample of 10 g soil.

Applied method of dilution – from the initial 10^{-1} to 10^{-4}. 1ml of each dilution was transferred in 5 petri dishes. For the three groups of microorganisms, the following dilutions were used: 10^{-4} for fungi, 10^{-5} for actinomycetes and 10^{-6} for bacteria.

For each group of organisms, specific nutrient base was used: Capek's agar for fungi, Mesopepton agar for bacteria and Waxmans agar for actinomycetes. The number of colonies was the average of the 5 replicates multiplied by the appropriate dilution..

RESULTS AND DISSCUSION

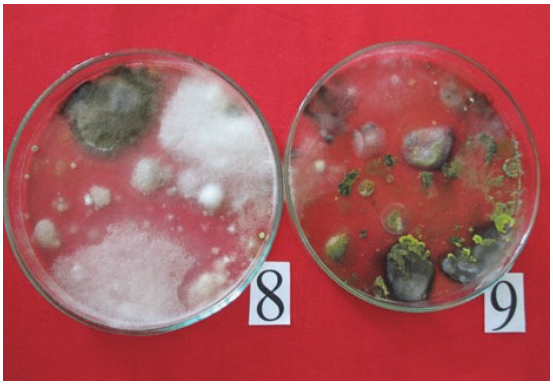
The late treatment with Ema 5 (variant 4) influenced the number of fungi. Therefore, there is the lowest number of fungi in this variant (Table 2). But also, in its application before sowing without herbicide (variant 5), the presence of this group of microorganisms is less than the other two. These data also confirming the effect of Ema 5 as biofungicide.

Table 2. Presence of the main groups of microorganisms in the soil

No.	Group of microorganisms / Variant	Fungi (x 10^4)	Actinomycetes (x 10^5)	Bacteria (x 10^6)
1	Check, untreated	5,7	6,0	4,0
2	Standard treatment (herbicide, funcigide and saltpeter)	3,0	1,7	8,3
3	EmFarma Plus +Ema 5; herbicide	3,0	10,0	3,3
4	Ema 5; without herbicide after emergence of seedlings	2,7	6,3	5,0
5	Ema 5; without herbicide	7,3	11,0	13,7
6	EmFarma Plus +Ema 5 after emergence of the seedlings; herbicide	7,3	10,0	13,3
7	EmFarma Plus +Ema 5; without herbicide	12,0	9,6	18,3
8	Seed with EmFarma Plus +soil with Ema5; without herbicide	5,3	7,6	15,3
9	Seed with EmFarma Plus +soil with Ema5; herbicide	9,0	7	20,3

The greatest numbers is determined in the variant 7 (Ph 1), where is the smallest intensity of damping off disease (Graph 1).
Compared to this variant, there is significantly lower number in the variant 3, which indicates effects of herbicide. However, it is same as the amount of fungi in standard treatment, which favors their application in the use of herbicide.
In application of probiotics on the seed at variant 9 there is almost twice higher value than in variant 8 - without herbicide (Ph 2).

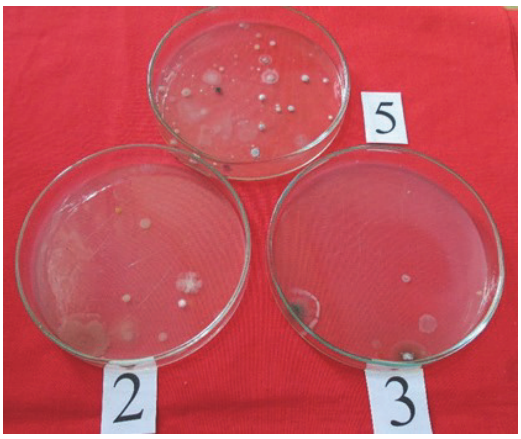
Ph 1. Presence of fungi in Variant 5- Ema 5; without herbicide and 7 - EmFarma Plus + Ema 5; without herbicide



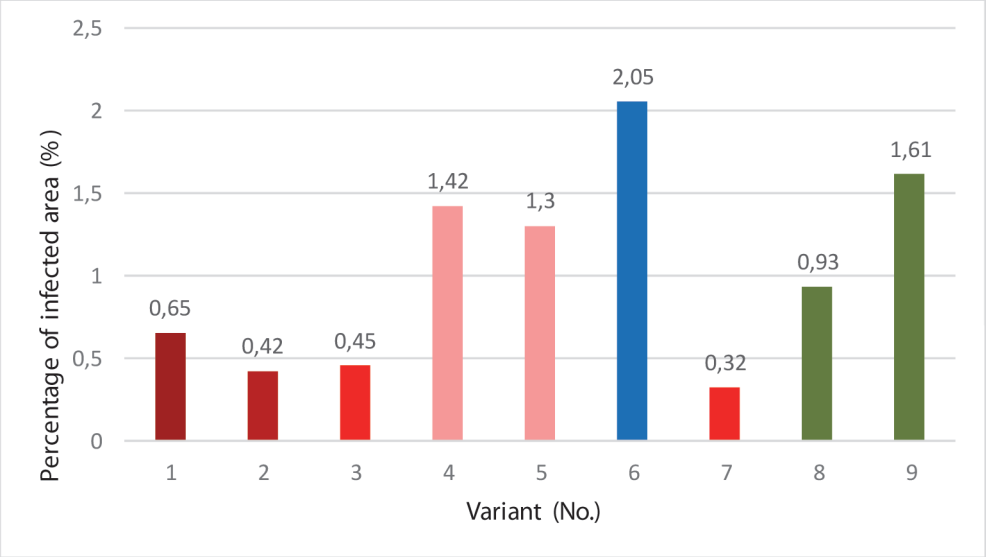
Ph 2. Presence of fungi in variants 8 and 9: seeds with EmFarma Plus + Ema 5; without and with herbicide treatment

The presence of actinomycetes are the smallest in the standard treatment. In the treatment with probiotics, the largest number (11 x 10^5) is determined in the variant 5- with Ema 5 treatment without the use of herbicide (Ph 3). But also they are represented by 10 and 9.6 x 10^5 in treatments EmFarma Plus + Ema 5 (with and without application of herbicide), which is a remarkable result. The same results was in application of EmFarma Plus + 5 Ema, after emergence of seedlings.
Their number is especially important because actinomycetes are active participants in the decomposition of carbohydrate and albuminous complexes in soil (Консулоска, 1999). Hence, increased numbers of actinomycetes along with numerous enzyme complex means providing more nutrients and destroying of pathogens.

Ph 3. Presence of actinomycetes in Variant 5- Ema 5; without herbicide



Ph 4. Presence of bacteria in the variants: 5 - Ema 5 without herbicide, 2- standard treatment and 3- EmFarma Plus + Ema 5; with herbicide



Graph 1. Influence of probiotics on the intensity of damping off disease

Bacteria, as actinomycetes, have the bigest presence in variant 5 (Table 2, Ph 4), followed by variant 9 (20,3 x 10^6). But variant 7 has one of the highest values of the presence of bacteria. This is of great importance for the effect of EM technology on attack of damping off disease. Free living rhizobacteria and endophytic bacteria use some of the same mechanisms to promote plant growth and control phytopathogens (Blomberg, 2001) The small numbers of bacteria in variant 3 is compensated with the number of actinomycetes.

According to the results, variants where the soil is treated with probiotics EmFarma Plus + Ema 5 before sowing, without and with herbicide treatment and two additional treatments, have the smallest intensity of the disease (Graph 1, Ph 5,6).

In these variants, particularly in the application of both probiotics, it is estimated the biggest numbers of fungi (in variant 7) or actinomycetes (in variant 3). At increased presence, their role in the processes influenced by them increased and they have a positive effect on the prevention of attack by pathogens.

The fungi break down highly complex and resistant compounds such as cellulose, starch, gums and lignin (Cinklin, Jr. A. R. 2002, loc cit. Sanko Sangyo Co, 2004). Actinomycetes produce and release in the soil solution antibiotics such as streptomycin, actinomycin and neomycin, and are involved in the decomposition of complex organic compounds such as phospholipids (Cinklin, Jr. A. R. 2002, loc cit. Sanko Sangyo Co, 2004).

The strenghteen formula of EMa 5 certainly contributes to this effect i.e the best results is achieved in their mutual application. Application of these probiotics before sowing as well as aditional treatment unable development and multiplying of beneficial microflora. Therefore, the best result in reducing the damping off disease is achieved when applied in a soil or direct to foliage (Gveroska, 2014a, b).

Higa et al. (1989) emphasize the role of EM on the supression of several destructive soil pathgens, too. Increasing in microbial community – promoters of plant growth are responsible for this effect. The widely recognized mechanisms of biocontrol mediated by plant growth-promoting microorganisms are competition for an ecological niche or a substrate, production of inhibitory allelochemicals and induction of systemic resistance in host plants to a broad spectrum of pathogens (Combant et al., 2005).

According to Okorski et al. (2008), increasing of the microbial diversity of soil im-

proving health and productivity of plants. Sudarma and Suprapta (2011) found that the number of groups of microorganisms like bacteria , fungi and actinomycetes was higher in soil in which there are no symptoms of disease caused by Fusarium, unlike that in which the plants suffer from the disease caused by this pathogen.

Ph 5. Intensity of damping off disease in the check

Ph 6. Seedlings in Variant 3-EmFarma Plus + Ema 5; with herbicide

CONCLUSIONS

- The tested probiotics reduce the intensity of damping off disease.
- Probiotics have a positive effect on the number of some groups of microorganisms and reduce the presence of soil pathogens.
- The lowest intensity of disease was observed in the variants where Ema 5 was applied in combination with EmFarma Plus in the soil before sowing.
- The highest number of fungi was recorded in soil treatment with EmFarma Plus + Ema 5 without herbicide application.
- The highest number of bacteria was recorded by seed treatment with EmFarma Plus + Ema 5, with herbicide application.
- The highest actinomycetes value was obtained during treatment with Ema 5, without herbicide application.
- Significant increase in the number of actinomycetes was recorded in treatments with EmFarma Plus + Ema 5 (with or without herbicide application).
- Increased number of actinomycetes is of major importance because they are active participants in degradation of protein and carbohydrate complexes in the soil.
- Postemergence treatments with biofungicides had no positive effect on microflora or on reducing the intensity of damping off disease.
- The application of bioproducts on the seed before sowing did not give the expected results (with respect to microflora and disease intensity).
- Probiotics with their effect on increasing some groups of microorganisms can prevent the attack of soil pathogens.
- The use of probiotics in the tobacco seedling protection from diseases has good prospects in the sustainable tobacco production.

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