POSSIBILITIES FOR APPLICATION OF A BIOPREPARATE EMA 5 IN TOBACCO PROTECTION FROM SOME DISEASES

Biljana Gveroska
University St. Kliment Ohridski-Bitola, Scientific Tobacco Institute-Prilep
7 500 Prilep, Republic of Macedonia
e-mail:gveroska@t-home.mk

ABSTRACT

The principle of effective and beneficial microorganisms (EM) embedded in SCD Probiotics Technology presents an ecological solution for maintaining a healthy environment. They are important not only for their stimulation effect but also from the aspect of pathogens control. Such role is attributed to the bioproduct EMa 5.

The aim of this research is to determine the possibility for application of EMa 5 in protection of tobacco from the damping off and brown spot disease.

The best results in the control of damping off, manifested in the lowest percentage of infected area, was recorded in the variant where the soil was treated with 5 ml Ema 5 diluted in 100 ml of water for an area of 0,5 m^2 and then treated additionally with 0.5 % solution of the product.

The best control of brown spot disease was obtained with multiple treatment of tobacco with 0.3% solution of EMa 5.

EMa 5 bioproduct can be applied in tobacco protection from these diseases. The new courses of investigations will determine the most efficient model of its application in tobacco.

***Keywords**: effective and beneficial microorganisms (EM), EMa 5, application, intensity of attack, protection*

INTRODUCTION

Long-term use of chemicals for plant protection caused various negative effects such as resistance to pathogens, residues, expensive and not always effective protection. Likewise, consumer demands for food without the use of pesticides has lead off the need of finding substitutes for these products in agricultural production (Compant et al., 2005). Introduction of EM technology is one of the basic ways for environmental management in turn to sustainable agricultural production (Sanko Sangyo Co., 2004).

Modern EM technology includes four very important groups of organisms - lactic acid bacteria, photosynthetic bacteria, actinomycetes and yeasts, mixed in carbohydrate medium. Sometimes, certain preparations, depending on the purpose, have "enriched" formula with the basic components and other bioactive components such as plant extracts. Such is the case with product EMa 5 (SCD Probiotics, 2012).

The basic principle of this technology is the application and the increasing population of effective and beneficial microorganisms in the soil. Therefore, degenerative microorganisms, particularly soil pathogens are squeezed. This creates a healthy environment for plants. EM continue to coexist in rhizosphere and plants are growing well, free of pathogens (SCD Probiotics, 2012).

Increasing the microorganisms - promoters of plant growth, especially in the rhizosphere, (besides stimulative function on plant) they express the main mechanisms of biological control: competition for space and food, release the inhibitory substances and induced systemic resistance of plants to more pathogens (Combant, 2005).

There are data of the use of probiotics not only in tendency of improving the soil, but in plant protection from pathogens attack (Higa et al., 1989; Okorski et al., 2008; Tokeshi et al., 1997).

But, for determing the usefulness of certain preparate, it is necessary to examine its influence on a culture in the specific conditions of its cultivation. Therefore, our aim was to examine the impact of the application of the biopreparate EMa 5 on certain pathogens in tobacco and determine the possibility of its use in tobacco protection from pathogenic fungi *Rhizoctonia solani* and *Alternaria alternata.*

MATERIALS AND METHODS

There were no precise methods for application of this biopreparate in available literature, especially for tobacco. Therefore, we used the literature concerning the application of the products of EM program and the biopreparate EMa 5 in other crops.

Rhizoctonia solani

The first sowing was carried out on 13.06.2011. The second was repeated on 20.09.2011. Examined tobacco variety was oriental P23, sowed on area of 0,3 m^2 per variant.

In the second replication we increased the concentration of this biopreparate, to 0.5 %. The addittional treatment with Ema 5 was involved, too. Treatments of variants by date for both replications are given in Table 1.

Only one estimation was made in the first replication, after several days of treatment. It was due to the high temperatures in this period, unusual for tobacco seedlings production. But it is not negligible, because the results are confirmed by the second replication. In the last, the estimation was carried out 7-10 days after the last treatment.

Table 1. Variants and treatments of tobacco seedlings

Variant		date of treatment				
		1 st replication		2nd replication		
		5.07	12.07	5.10	17.10	26.10
Check Ø		-	inoculation	-	inoculation	-
1	Before sowing (5 ml/100 ml/0,5m^2)	0,3% EMa 5	inoculation	0,5% EMa 5	inoculation	0,5% EMa 5
2	With inoculation	-	inoculation + 0,3% EMa 5	-	inoculation + 0,5% EMa 5	0,5% EMa 5
3	Before sowing	0,3%	inoculation	0,5%	inoculation	0,5% EMa 5

Alternaria alternata

Two plants of the variety P 23 was transplanted in 10 pots per each variant and 5 pots for control. Plants were grown and treated by standard procedure. Variants and treatments by dates are given in Table 2.

Treatment (spraying) was performed on the pointed dates. Inoculation was performed by fresh infected material - leaves with typical symptoms of the brown spot disease. Previously, a microscopic examination and isolation of the pathogen was made.

Inoculated plants were covered with polyethylene bags and kept for 10 days. Afterwards, the estimation was performed, i.e. categorization of leaves in the appropriate category of sixgrade scale,

from 0 to 5 (Пејчиновски, 2007). The intensity of the attack disease was calculated by the formula of McKinney.

Table 2. Variants and treatments of plants

Variant		date of treatment				
		14.07	1.08	15.08	21.08	2.09
Check Ø		-	-	-	-	
1	Multiple treatment	0,3% EMa 5	0,3% EMa 5	0,3% EMa 5	-	inoculation
2	Treatment before 48 hours	-	-	-	0,3% EMa 5	

RESULTS AND DISCUSSION

Rhizoctonia solani

Percentage og infected area in the check is 13,33%. In the variant (2) where the Ema 5 is applied immediately after inoculation, it is 7,15%. In variants where the soil is treated before sowing, it is a low occurrence of disease (Table 3).

Table 3. Influence of EMa 5 on intensity of damping off disease

Variant		Percentage of infected area		
		1 st replication	2nd replication	
			1 st estimation	2 nd estimation
Check Ø		13,33	54,05	60,00
1	Before sowing (5 ml/100 ml/0,5m^2)	+	4,00	9,00
2	With inoculation	7,15	34,00	36,00
3	Before sowing (1 ml/100 ml/ 0,5m^2)	+	32,00	42,00

In the second replicatioan, there is a very high intensity of disease in the check (Fig. 1). However, the variant with the recommended dose of application of Ema 5 before sowing (1), there is a very low occurrence of the disease - only 4,00%. This variant has the lowest percentage of infected area in the second estimation, too – 9,00%, (Fig. 2).

According to Combant et al. (2005), increasing in microbial promoters of plant growth in the soil, especially in rhizosphere (besides their stimulation effect on plants) they can express the main mechanisms of biological control: competition for space and food, producing and releasing an inhibitory substances and induced systemic resistance of plants to many pathogens.

The results obtained in these study are in accordance with those of Alli et al. (2013), in which 14 of the tested isolates of probiotics showed 52,3% reduction of *Rhizoctonia solani.* Higa et al. (1989) emphasize the role of EM on the suppression of several destructive soil pathogens, too.

In the variant where the soil is treated before sowing with a reduced dose of the product (3), the intensity of the disease has a high value (Fig. 2). The situation is similar то that of the variant (2), where the product is applied immediately after inoculation. This situation is due of the small number of applications, which unable development and multiplying of beneficial microflora. Therefore, additional treatment has no positive result.

According Okorski et al. (2008), increasing of the microbial diversity of soil improving 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.

The question of need for another treatment remains. This application will shorten the period when the seedling was without biopreparate, by which the possibility of occurrence of the disease. will be reduceed.

Fig 1. Intensity of damping off in the check

Fig 2. Intensity of attack at treatment with EMa 5 in variants 1 and 3

Alternaria alternata

Plants of the check have the highest percentage of infected leaves as well as intensity of a disease (Table 2).

Treatment with EMa 5, 48 hours before inoculation with *Alternaria alternata* has little effect in reducing the intensity of disease, which is consistent with the nature of the this biopreparate and its application.

Table 4. Influence of EMa 5 on intensity of the brown spot disease

Variant		Total number of leaves	Percentage of infected leaves	Intensity of disease
Check Ø		252	73,02	42,79
1	Multiple treatment	167	61,08	26,65
2	Treatment before 48 hours	268	68,28	35,45

The multiple treatment with EMA 5 show an expressive effect, ie, the intensity of the brown spot disease in these plants (variant 1) are 26,65% and almost twice lower than in check.

The results confirm the beneficial impact of microorganisms on the appearance not only of diseases associated with soil, but leaf diseases, too. Examined pathogens of peas, Okorski et al. (2008) came to similar findings.

CONCLUSIONS

- ✓ The lowest percentage of infected area, ie the best results in control of tobacco seedlings from the damping off disease has a variant where the treatment is carried out before sowing the soil with a solution of 5 ml of the product in 100 ml water to 0,5 m^2 with additional treatments with 0,5% solution of EMa 5.
- ✓ Multiple treatment of plants with 0.3% solution of EMa 5 showed the best results in control of the brown spot disease. The disease has manifested with the lowest intensity in these plants.
- ✓ EMa 5 bioproduct can be applied in tobacco protection from these diseases
- ✓ Investigations should be continued to found the most efficient model of application in tobacco.
- ✓ Application of a biopreparate EMa 5 is a modern, environmentally friendly approach to the protection of tobacco, in accordance with global trends in agriculture.

REFERENCES

1. Alli F.S., Saad O.A., Salwa A.H., 2013. Antimicrobial activity of probiotic bacteria. Egypt. Acad. J. Biolog. Sci., 5 (2): 21-34.
2. Sanko Sangyo Co., 2004. Prospects of EM Technology in Agriculture.
3. 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.
4. Higa, T. and G.N. Wididana, 1989: Changes in the Soil Microflora Induced by Effective Microorganisms. First International Conference, Kyusei Nature Farming pp 153 – 162, Oct 17-21, 1989.
5. 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.
6. Пејчиновски Ф., Митрев С., 2007. Земјоделска фитопатологија (Општ дел). Институт за јужни земјоделски култури, Стримица
7. SCD Probiotics, 2012. http://www.scdprobiotics.com
8. Sudarma I.D and Suprapta D.N., 2011. Diversity of soil microorganisms in banana habitats with and without Fusarium wilt symptom. J. ISSAAS, Vol 17, No 1: 147-159.
9. Tokeshi, H., M.J.A. Jorge, A.B. Sanches and D.Y. Harada., 1997: Interaction between microorganisms, soil physical structure and plant diseases. Presented at the 6th EM Technology Conf. Nov. 24-26 1997, Saraburi, Thailand.