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Use of Biopreparates in Tobacco Protection – Contribution to Sustainable Agriculture

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Abstract

Tobacco plays an important role in the social and economic life of our country. For that reason, efforts have been made to develop strategies for development and application of the basic principles of sustainable agricultural production. Conventional tobacco production has numerous negative effects on the environment and human health. Therefore, 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. Application of biopreparates is a good way to manage the environment in the process of achieving sustainable tobacco production. In terms of tobacco production, the main objective is application of these products inthe control of economically important pathogens. Among the investigated preparations, the most promising results were obtained withbiocontrol agent*Trichoderma sp.*Multifunctionality of certain microorganisms with stimulating effect on plants, along with some plant extracts, also provides a satisfactory effect in pathogens control. Efforts for the wide application of biopreparates is promoting of their beneficial effect and the greater utilization of resources. Scientific research in the field of tobacco production will continue to develop procedures and instructions for the most appropriate use of biopreparates.

Keywords: Sustainable Agriculture, tobacco production, protection, biopreparates

Introduction

The conventional agricultural production have appreciable consequences: exhaustion of natural resources, increased erosion and loss of natural soil fertility, increased new diseases, collapse in water supply, reduction of biomass production and disruption of natural diversity. Therefore, introduction of a new principle in agricultural production was inevitable.

The integrated concept of sustainability in agricultural production is based on agroecologial concept. Agrosystem is a cornerstone upon which man, with his experience and knowledge, and the results of continuous progress in science and technology can manage soils, plants and animals in order to satisfy the growing and changing human needs for food, without injuring the environment (Basso and Diaz, 2004).

Sustainable agricultural production is the kind of production based on the principles of ecology, studing relationships between organisms and their environment. It can be understand as approaching the ecosystem closer to agricultural production. According to Gold (2007), sustainable production provides "starting point", a sense of direction and urgency, causing excitement and innovative thinking in the world of modern agriculture.

Environmental protection and sustainability are in harmony and sustainability can be achieved only with the protection of natural resources (Malik et al., 2012). But, sustainable production also has un aim to increase food production and reduce the occurrence of diseases and pests to the extent that they will not cause significant damage to the culture. Therefore, there are developed methods to restore the natural mechanisms of the stability of the community.

In the recent years great emphasis is placed on the application of Integrated Pest Management (IPM). In the existence of different understanding and definitions of IPM, emphasis on reducing the use of pesticides is dominant.

Great public concern about the impact of the use of pesticides on the environment places emphasis on the development of agents for the

crops protection with desirable environmental profile and low toxicity to humans and wildlife (Srinivasan, 2006). Regarding this, biopesticides are better alternatives than synthetic pesticides. They contribute to sustainable agricultural production and reducing the environmental consequences. Their application in the production of many cultures, as well as the proved multiple role, reduces confidence in chemical products.

According to United States Environmental Protection Agency (EPA), biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Plant growth regulators (PGRs), which exhibit no pesticidal activity but instead can promote, inhibit or modify the physiology of plants, are also regulated as biopesticides.

In commercial terms, biopesticides include microorganisms that control pests (microbial pesticides), naturally occurring substances that control pests (biochemical pesticides), pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) and biochemical plant growth regulators. Biopesticides are employed in agricultural use for the purposes of insect control, disease control, weed control, nematode control and plant physiology and productivity (BPIA, 2014).

The advantage of using biopraparates consist in: plolution reduction in crops and environment, skip the gain of resistance to chemicals in populations, total security for workers, sustainable use of the beneficial natural rsurces grom the agricultural systems unexploited till now (Constantinescu et al., 2004).

Tobacco production has a tendency to join the modern trends in agriculture. Since its final product-cigarettes are directly related to human health, serious efforts have been made to implement ecological principles in tobacco production.

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.

The insistence on their increased application in tobacco production must be supported by confirmation of their effect on a particular pathogen. Also, their multiple role in the ecosystem has to be proven. Of course, suitable model of application must be found in order to define their overall effect. Therefore, this is the aim of this investigations.

Only this kind of agricultural production will provide a comprehensive long terme profit, proper access and preservation of a healthy environment and quality of life for producers, consumers and the overall community (NIFA – SARE, 2014).

Materials and Methods

Biocontrol agent *Trichoderma* and some bioproducts based on different sources were subject of this research. Survey of performed investigations is given in Table 1.

Investigations were made on some economically important diseases of tobacco, especially on damping off. The fungus that is most often associated with damping-off disease is *Rhizoctonia solani* (along with *Pythium debarianum*) and therefore the investigation usually refers to this pathogen, although some products show the same efficiency in natural conditions (where the presence of both pathogens is possible).

Investigations with *Trichoderma*, EMa 5, Extrasol and Trilogy 70 EK were performed mainly in biological laboratory, with artificial inoculation with pathogens; trials with EM technology were made in natural conditions, in seedbeds.

Investigations of seedlings for each biocontrol agent/ bioproduct were carried out with three replicates at an area of 0,3m^2, sown with 0,5 g seed /m^2. Trials in seedbeds were made on 10 m^2 (about 3.3m^2 for each variant in three replications).

Brown spot disease and powdery mildew was investigated on (15-20 plants per variant) transpanted plants. They were treated during the growing season and there were multiple spraying of the check with water. Also, there are variants with application immediately after occurrence of disease.

- *T. harzianum, T. harzianum and T. aureoviride* were researched. Pure cultures was obtained and maintained in Scientific Tobacco Institute-Prilep. Fungi of the genus *Trichoderma* are proven biocontrol agents and constituent part of many microbiological products. However, such products have not been registered in R. Macedonia yet, although there were some efforts for their registration. This research will certainly be useful in realization of that purpose.

Table 1. Survey of performed investigations

biocontrol agent / bioproduct	Sowing (I treatment) / Transplanted plants	II treatment	III treatment	Inoculation	Intensity
Trichoderma	Seed storage for 48-72 hours in biocontrol agent before sowing Soil treatment before sowing with pure culture of *Trichoderma*	Watering of seedling with suspension of a pure culture of *Trichoderma*	Watering of seedling with suspension of a pure culture of *Trichoderma*	Pure culture of the pathogen	Infested area %
	Spraying of transplanted plants with pure culture of *Trichoderma*	Spraying of transplanted plants with pure culture of *Trichoderma*	Spraying of transplanted plants with pure culture of *Trichoderma*	Pure culture of the pathogen	Pejcinovski scale (1994)
EXTRASOL	Soil treatment before sowing with 2l / ha Seed treatment 48 hours before sowing, with 10% или 20% solution	Spraying of seedlings with 1% solution of the product	Spraying of seedlings with 1% solution of the product	Pure culture of the pathogen	Infested area %
EM farming	Soil treatment with the product, 100 ml/10 m² (in 1/ unchloridated water)	Spraying of seedlings, 100 ml/10 m² (unchloridated water)	Spraying of seedlings, 100 ml / 10 m² (unchloridated water)	*Assessment in seedbeds	Infested area % *Seedling growth
EMa 5	Soil treatment with the product, 30 ml/10 m² (in 1/ unchloridated water) Seed treatment 24 hours before sowing, with 1% solution	Spraying of seedlings, 30 ml / 10 m² (unchloridated water)	Spraying of seedlings 30 ml / 10 m² (unchloridated water)	Pure culture of the pathogen	Infested area %
	Spraying of transplanted plants with 0,3% EMa 5	0,3% EMa 5	0,3% EMa 5	Pure culture of the pathogen	Pejcinovski scale (1994)
Trilogy 70 EK	Spraying of transplanted plants with 1% solution	1%Trilogy 70 EK	1%Trilogy 70 EK	Pure culture of the pathogen	CORESTA scale Pejcinovski scale (1994)

- EM farming is a bioproduct based on technology of effective microorganisms (EM). 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. This bioproducts improved nutritional uptake efficiency of plants and crop performance, when applied in a soil or direct to foliage.

- EMa 5 has strenghteen formula, i.e. beside these four group has some plants extracts.
- Extrasol is a microbiological product that contains active rhizospheric ammonifying bacteria - Bacillus subtillis Ch13. It has stimulating effect on the plants growth but also, a fungicidal effect against plants pathogens.
- Trilogy 70 EK is a bioproduct based on neem oil, derived from the neem tree *(Azadirachta indica). T*his contains several chemicals, including 'azadirachtin', which affects the reproductive and digestive process of a number of important pests. Recent research carried out in India and abroad has

led to the development of effective formulations of neem, which are being commercially produced. Although more than 100 firms are registered to produce neem-based pesticides in India, only a handful are actually producing it.

Results

Damping off disease is of a great importance for tobacco seedlings. Its reduces quantity of plants prepared for transpantation, so, the whole production has a big loss (Fig 1 and 2). Investigations of bioconrol agents (BCA) – fungi of the genus *Trichiderma* have a very good results in reducing damping off.

When the seed was stored in a pure culture, there were the best results, i. e. there was not appearance of a disease (Fig 3). Reducing the infected area in only few percentage is a very satisfing results. Application of the biocontrol agent over the soil before sowing has a good protection from the causing agent, too. But, compared these two application way, seedling (in seed stored in a BCA) has a better development than in the other case (Fig 3).

According the investigated three species of the genus *Trichoderma*, *T. harzianum* seems to has the most promising role in the control of damping off. Compared with *T. aureoviride* and *T. hamatum*, there was a less success in two last mentioned species (data unpublished).

Application of a pure control of *Trichoderma* for control of the brown spot disease has a results, too. But, multiple treatment during the growing season (as is possible- in a short period) has a results, because of specific condition of maintainig the pure culture. Final bioproduct based on these biocontrol agents seems will offer better opotunities in leaf pathogens.

The bioproduct Extrasol was investigated on the two causing agents of damping off. In both cases, this bioproduct has a very goog results in control of disease. We applicated it with spraying the soil before sowing or sowing with stored seed in 10 or 20% solution. Of course, 2-3 spraying are needful, too. Percentage of infected area is smaller (better results) in variants with stored seed. But, result is increasing when we used this bioproduct over the soil and with seed (Fig 4 and 5).

Fig. 1 Damping off - *Rhizoctonia solani*
Pythium debarianum

Trichoderma
Fig. 2 Intensity of damping off disease in the check

Fig. 3 Pre-sowing treatments of soil (1)and seed (6)

EM farming is a modern bioproduct recommended for agriculture and it has a good results in tobacco production, too, The mash of four kind of microorganisms (EM technology) ensure complete needs for nutrients. Beside increased growth, plants are protected from pathogens in rhisosphere. So, efective microorganisms are good way in control of diseases, too.

Table 2. Biocontrol agents / bioproducts efficient in the control of some pathogens in tobacco

Biocontrol agent / biopreparate	Trade name	Disease on seedlings / tobacco plants	Pathogen	The most propriate model of application
Trichoderma harzianum		seedlings	*Rhizoctonia solani*	Seed kept in a culture of Trichoderma48-72 h before sowing + 2-3 treatments of seedlings
Trichoderma aureoviride *Trichoderma hamatum*		plants	*Alternaria alternata*	Multiple treatment during the growing season
Bacillus subtilis	Extrasol	**jbv** seedlings **mv**	*Rhizoctonia solani*	Seed kept in 10% or 20% solution
			Pythium debarianum	Treatment of soil and 2-3 treatments during seedlings growth
Effective microorganisms (EM technology)	EM farming	seedlings	*Rhizoctonia solani*	Spraying the soil before sowing and 2-3 treatments in the curent fase of seedlings
			**increased seedlings growth*	treatment of seeds and 2-3 treatments of seedlings
EM technology – strenghteen formula	EMa 5	seedlings	*Rhizoctonia solani*	Spraying the soil before sowing and 2-3 treatments in the current seedling stage treatment of seeds and 2-3 treatments of seedlings *better combination Ema 5+EM
Neem oil	Trilogy 70 EK	plants	*Alternaria alternata*	Multiple treatment (spraying)
			Erysiphe cichorachearum	Multiple treatment during vegetation
			Alternaria alternata	Spraying immediately after occurrence of disease

Ema 5 with its strenghteen formula has better ability to act as a biofungicide. Treatment of soil has to be with a bioproduct in a recommended dose. Reducing a dose, effect is less, too (Fig 6).

According to brown spot disease, multiple treatment (spraying with 0,3% solution) achieved a good control on the causing agent.

Extrasol

Fig. 4 Intensity of damping off in check (1)

Fig. 5 Intensity of damping off - soil treatment with Extrasol + seed with 20% Extrasol (11)

EMa 5

Fig. 6 Intensity of damping off in treatment with EMa 5 Ø = Check ; 1= Before sowing(100 ml/ 10 m^2); 3= Before sowing (20 ml/ 10 m^2)

Foliar diseases cause a significant losses (Fig 7,9). Artificial innoculation in investigations of biopreparates effectiveness in control of diseases is a good way for estimation (Fig 8, 10).

A bioproduct Trilogy 70 EK applicated as multiple spraying with 1 % solution has the best results in control of brown spot disease, as well as the powdery mildew. It can be applicated several times over the vegetation (in order to prevent disease attack).

But, good results in disease control is achieved when it is applied immediately after the appearance of the first symptoms, too.

Fig. 7 Brown spot disease - *Alernaria alternata*

Fig. 8 Intensity of brown spot disease – artificial inoculation

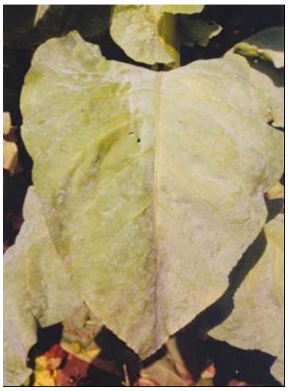

Fig. 9 Powdery mildew - *Erysiphe cichorachearum*

Fig. 10 Intensity of powdery mildew – artificial inoculation

Discussion

The concept of sustainability contains views on what should be maintained, why, what are the

reasons for concern and implicit-presence of substitutability of human-made with natural wealth (Dobson, 2009). In fact, the definitions of sustainable development must allow avoidance of irreversible loss of natural values or compensation of their loss through other natural values (Pirs 1989, loc cit Dobson, 2009).

Sustainable agriculture relies on integrated pest management (IPM) techniques - which combine proven cultural, biological, and chemical methods to control pests - as an environmentally sound approach to crop production. Biopesticides are rapidly entering in IPM programs. It is ensured by their importance and positive impact not only on agricultural production, but also all aspects of human life. The significance of biopesticideite are confirmed by several authors: Basso and Diaz (2004), Constantinescu et al. (2004), Rathod (2009), Gupta and Gipshit (2010), Ansari et al. (2012).

Trichoderma is a fungus which is present in nearly all soils and other diverse habitats. They attack and parasitize other fungi. There are many mechanisms involved in biolocical control of the genus *Trichoderma* (Harman et al., 2004; Harman, 2006).

Biological control of *Trichoderma* spp. on the pathogens in tobacco is confirmed, too (Gveroska, 2013 a). They are used mostly for control of soilborne pathogens. Therefore, it reduces the damping off in tobacco seedlings. *T. harzianum* is the best known anthagonist against its causing agent - *R. solani* (Gveroska, 2011, 2013 b).

The best effect in control of damping off in tobacco seedlings is achieving in the treatment of a soil or storage the seed before sowing together with the biocontrol agent. Conjuction of these ways is also effective. This model of application is confirmed by Harman, (1996, 2004) and Heydari and Pessarakli (2010). The additional effect is increasing the plant growth. So far, preparation of *Trichoderma* biopesticide is cheap and requires only basic knowledge of microbiology (Rathod, 2009; Gupta and Gipshit, 2010). Therefore, introduction of *Trichoderma* biopraparates in tobacco production will be of great interes: it will offer friendly approach and limited chemical usage in the management of tobacco diseases, control of pathogens, effective integrated pest management (IPM) system, increasing tobacco growth, and, of the end, healthy and clean environment.

Trichoderma is effective on leaf pathogens, too. It is confirmed in our investigations. But, fungi require specific environmental conditions to proliferate and their means of affecting the target pest are diverse (BPIA, 2014).

Extrasol is a microbiological product that contains active rhisossphere ammonifying bacteria *Bacillus subtillis Ch13*. This bacteria has multifunctional properties and give Extrasol its stimulating and fungicidal effect on plants. It was determined that the application of Extrasol reduces the intensity of damping off disease. The best results in investigations of the two pathogens are obtained when soil is treated with the preparate and sowing with seed treated with 20 % solution of Extrasol. The fungicidal efect of this preparate on tobacco is confirmed by data presented by НИКТИТТИ (1998). This product supress the root rot in seedlings, infection by bacterial and fungal pathogens in the field. It accelerates the seedling growth, improves the root forming, increase the yield and assortment of a varyety, also. So, this microbiological product has good prospects for its use in IPM program and tobacco production.

EM technology is a promissing strategy for disease control as well as tobacco developing. Investigations in tobacco seedlings confirmed that. It has a beneficial effect not only diseases associated with soil, but leaf diseases, too (Gveroska, 2014). 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 (Combant et al., 2005). But, the strenghteen formula of EMa 5 certainly contributes to this effect. The better effect is achieved in their mutual application.

Foliar diseases have great economic importance for tobacco production. They cause a significant reduction of yield. Appearance of brown spot disaease and powdery mildew means lower quality. At the end, reduction in the overall economic effect. Therefore, introducing biopreparates in control of foliar diseases will contribute to improving the quality in tobacco leaves and getting sustainable production.

Trilogy 70 EK is a bioproduct based on neem oil, derived from the neem tree *(Azadirachta indica)*, this contains several chemicals, including 'azadirachtin', which affects the reproductive and digestive process of a number of important pests. Neem is non-toxic to birds and mammals and is non-carcinogenic (Rathod, 2009). Its demand is likely to increase in agriculture because of its effect on plant disease, too. It reduces the intensity of the brown spot disease and powdery mildewy on tobacco. But, the time of spraying (depending of conditions for disease development as well as maintaining on the leaf area) is a crucial element for application and result in disease attack. Multiple spraying during vegetation is a good prevention of disease occurrence. Bozukov (2005) noted that Trilogy 70 EK is a bioproduct with high effectiveness and prolonged effect on the causing agent of

Peronospora Tabacina Adam and *Erysiphe cichorachearum*.

We consider that presented data for effect of researched biocontrol agent and biopreparates are of great importance in tobacco protecting from some economically important pathogens and contibution to sustainable agricultural production. Methods of application and tobacco treatment offer the proper way to achieving the best effect. We also researched effect of some pesticide mostly used in tobacco production on *Trichoderma* and EM farming (unpublished data) and made a model in application concerning the pesticide used. Bio-pesticides can either substitute for conventional crop protection products or be added to the spray program to increase yields from the same acreage (STK, 2012).

Dealers supply products that support these methods and many biopesticides are specifically designed for use in conjunction with stable traditional chemistries in an IPM program BPIA, 2014).

Use of biopesticides in tobacco production would be solve a question for residues. The problem is especially serious because of the development of resistance to pesticides in important pests and the presence of pesticide residue in agricultural and dairy products.The World Healt Organization restricts use of many pesticedes for tobacco according to effect on risk on the human health and community. Many biopesticides are exempt from residue limits on fresh and processed foods. In addition, biopesticides may be applied in environmentally sensitive areas without residue concerns and may be used to manage residue levels for exported produce (BPIA, 2014).

Hence, use of biopesticides in tobacco production as well as in other crops, is a proved because of their additional benefits – they improve efficacy, enhance yield and are cost effective. They are used in IPM Management, Resistance Management, Residue Management and Harvest & Labor Management (STK, 20126). Generally, biopesticides are a valuable option because of their sustainability, crop quality and environmental safety.

The present paper describes the detailed discussion on the potential of biopesticides in sustainable tobacco production. This makes an effort to understand these effect throught experiments and ensure their way in production. This is in accordance with the statements of another researchers. The effectiveness of biopesticides is measured differently than traditional chemicals. The best way is monitoring pest/disease in-season and evaluate the effectiveness of the product through field performance trials and marketable yield and quality data. Train/educate people on the benefits of bio-pesticides for sustainable agriculture. Help develop and publicize fact that practising these the quality of life and health will be assured (Gupta and Dikshit, 2010). Publicize MRL compliance (STK, 2012)

Further research and development of biopesticides must be given high priority and public in general. All this will lead to a general enlightenment about the benefits of biopesticides and will force governments to make policy decisions in reducing the use of chemical pesticides and increasing the use of a green alternative (Rathod, 2009; Ansari et al., 2012).

Conclusion

Modern tobacco production tends to implement the principles of sustainable agricultural production.

Investigations were made with several biocontrol agents/bioproducts which showed effectiveness in the control of some pathogens on tobacco.

Biocontrol agent *Trichoderma sp*. showed promising results in protection of tobacco seedlings from damping off disease.

B. subtilis also showed good results in the control of *R. solani* and *P.debarianum* - causing agents of damping off disease on tobacco seedlings.

The technology of effective microorganisms (EM) showed satisfactory effect in the control of damping off.

Good results were also obtained with application of bioproduct EMa 5, but in natural conditions of seedling production its effect was much higher when applied together with EM farming.

Bioproducts with soil application, beside their stimulative effect, also provide good level of protection from diseases.

Investigations of a number of biocontrol agents/biopoducts have shown promising results in tobacco control from some diseases.

Scientific research on biological control agents will continue, providing the most adequate ways of their application in disease control.

We consider that the results of several year-investigations are of huge importance in protection of tobacco and a significant contribution to sustainable agricultural production.

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