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Original scientific paper

FUNGICIDAL AND STIMULATING EFFECT OF BIOPREPARATION TRIANUM P ON TOBACCO SEEDLINGS

Biljana Gveroska

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

st. "Kicevska " bb, 7500 Prilep, Republic of Macedonia

e-mail: bgveros@yahoo.com

ABSTRACT

Fungi of the genus Trichoderma are biological control agents applied in the control of pathogens, but they are also multifunctional and have a stimulating effect on root and plant growth.
Trianum P is biopreparation which active ingredient are living spores of Trichoderma harzianum T22.
The aim of this study was to determine the effect of this preparation on tobacco seedling, the most effective model of application and the possibility of its application in the standard mode of planting.
The highest effectiveness in the control of damping off disease in tobacco seedling was recorded in variants where the seed was treated, with and without herbicide application.
The highest stimulating effect of Trianum P on tobacco seedling was recorded in the variant where the seed was stored in suspension of the preparation and two additional treatments, characterized by the highest total length and root length.
Trianum P with its fungicidal and stimulating effect on tobacco seedling has a good perspective in the sustainable production of tobacco.

Keywords: Trianum P, damping off disease, fungicide effect, development, stimulating effect

ФУНГИЦИДНО И СТИМУЛАТИВНО ДЕЈСТВО НА БИОПРЕПАРАТОТ TRIANUM P ВРЗ ТУТУНСКИОТ РАСАД

Габите од родот Trichoderma се биоконтролни агенси кои имаат значајна примена во биолошката борба, но исто така се карактеризираат со мултифункциско дејство и дејствуваат стимулативно врз развојот на коренот и растението.
Trianum P е биопрепарат чија активна материја се живи спори од Trichoderma harzianum T22.
Целта на ова истражување е да се утврди влијанието на препаратот врз тутунскиот расад, најефикасниот модел на апликација и можноста за примена при стандарднен начин на сеидба.
Најдобра ефикасност во контролата на болеста сечење кај тутунскиот расад покажаа варијантите со третирано семе, со и без употреба на хербицид.
Стимулативното дејство на Trianum P врз тутунскиот расад е најизразено кај варијантата каде семето е чувано во суспензија од препаратот и два дополнителни третмани, што се карактеризира со најголема вкупна должина и должината на коренот.
Биопрепаратот Trianum P со своето фунгицидно и стимулативно дејство врз тутунскиот расад има добра перспектива во одржливото производство на тутун.

Клучни зборови: Trianum P, сечење, фунгициден ефект, развој, стимулативен ефект

INTRODUCTION

Fungi of the genus Trichoderma are antagonists and biocontrol agents of pathogens and their use is of particular importance, but they also have multifunctional effect on the plant.

They are found in soil and root system, constantly multiplying stimulated by root exudates and activating numerous biocontrol mechanisms which attack the pathogen. In the complexity of actions, antibiosis, mycoparasitism and food competition are considered the main mechanisms in biological control. On the other hand, these agents have a stimulating effect on root and plant growth.

Trichoderma spp. are found in soil and root system and are beneficial to plants (Goes, 2002; Contreras-Cornejo et al., 2009). The most important benefit of Trichoderma is that they directly attack and control the causing agents of diseases, i.e. they have direct effect on plants (Chet et al., 2006).

They are opportunistic, avirulent plant symbionts as well as parasites of other fungi. Colonizing the root, Trichoderma spp. activate numerous mechanisms that attack pathogens and stimulate the growth of plants and roots (Harman, 2004, 2006; Benitez et al., 2004; Howell, 2003). Trichoderma is effective in the control of numerous plant pathogens (Harman, 2004, 2006). The biocontrol agents are effective, eco-friendly and low-cost agents that minimize the harmful effects of chemicals (Benitez et al., 2004).

Contemporary research data indicate that plant growth and biochemical processes are strongly affected by Trichoderma species. The colonization with Trichoderma spp. increases plant growth, crop yield, resistance to abiotic factors and nutrient uptake and utilization (Saba et al., 2012).

The beneficial effects of Trichoderma are elaborated in the research of Hermosa et al. (2012).

According to Shoresh et al. (2010), some species have direct effect on plants, increasing the growth potential through nutrient uptake, fertilization, seed germination and stimulation of defense mechanisms against biotic and abiotic factors. T. harzianum can solubilize several nutrients (Altomare et al., 1999) and colonization of root with T. asperellum increases the availability of P and Fe (Yedidia et al., 2001). Such effects of Trichoderma species on plant growth were also reported by Harman (2000).

The antagonistic activity against pathogens and the stimulation of soil fertility makes Trichoderma species a good alternative to harmful fumigants and fungicides (Monte, 2001). Today, there are advanced technologies for their application in agriculture. There are numerous preparations, but the best known among those that use antagonistic microorganisms are the preparations based on various strains of Trichoderma.

T. harzianum strain T-22 is widely accepted for disease protection instead of chemical fungicides because it is more secure for the farmers, provides longer and cheaper protection and allows better growth of root system compared to pesticides application (Harman, 2004).

The T-22 strain has the following characteristics: efficient control of a large number of soil diseases, improved growth and development of plant root and increased absorption of water and nutrients from the soil, rapid growth on roots of all cultivated plants, good development in various soil types, tolerance to wide temperature and pH range and compatibility with a large number of active ingredients.

Bandjo (2016) also reported that Trichoderma harzianum T22 provides good resistance to soil diseases, strengthens the plant and stimulates its growth and provides long-lasting protection. It is efficient in various substrates and safe for humans, wild life and environment.

Trichoderma harzianum T-22 in a form of

water soluble powder or granules for direct use is registered as a biostimulator and plant protection agent in many countries, including the Republic of Macedonia, under trade name Trianum P.
The purpose of this study is to determine the effect of T. harzianum T22 on tobacco, i.e. the effect of the biofungicide Trianum P in the control of damping off disease and its impact on development of tobacco seedling. The research should also identify the most effective application model in the standard way of sowing.
The results of these investigations will determine its place in the sustainable tobacco production.

MATERIAL AND METHODS

Investigations on the influence of biopreparation Trianum P on tobacco were carried out in Scientific Tobacco Institute-Prilep, in seedbed conditions, with the following variants:

Ø Check - standard treatment with herbicide and nutrition, without fungicide application
1. Treatment after 15 days
2. Treatment after 15 days (post-sowing - herbicide)
3. Pre-sowing
4. Pre-sowing (post-sowing - herbicide)
5. Seed treated with Trianum P
6. Seed treated with Trianum P (post-sowing - herbicide)

Seed amount of 6.75 g/10 m2 i.e. 0.67 g/m2 was used for planting. All variants were set up in 3 replications and each replication was 3.33 m2.
Since the purpose of this research was to determine not only the effect of the preparation, but also the possible application in standard way of sowing (with herbicide), all the variants were repeated, i.e. set up with the use of herbicide.
Heriticide Gamit (a.i. clomazone) in a rate 0.07 ml/m2 was used after sowing.

Application of the biopreparation Trianum P

Trianum P biological fungicide was applied at a recommended rate of 15 g/10 m2, i.e. 1.5 g/m2 using 2.5-5l water/m2.
- Soil in variants 3 and 4 was treated prior to sowing with Trianum P at the same rate, i.e. 5 g/3.33m2 using 10 l of water. According to the instructions, the preparation was previously dissolved in 1: 5 ratio and added to the water by mixing.
- Seed for varieties 5 and 6 was prepared for sowing the previous day. 5 g of the product was dissolved in 25 ml water. The seed was stored in the suspension for 24 hours.
Variants 1 and 2 were treated 15 days after sowing, at the same rate as other variants.
During the growing season, two more treatments were carried out on the seedling at 15-20 day intervals, by which all stages of seedling growth were covered. Thus, seedling of the variants 1 and 2 received 2 treatments and that of other variants 3 treatments.
Health condition of tobacco seedling was monitored throughout the growing season.
Assessment on the intensity of damping off disease was made 10 days after the third treatment, by measuring the infected area in each replication of the variants. The results are expressed in percentage of infected area.
Seedling size was measured after the treatments: 10 plants from each replication and each variant were randomly selected and total length of seedlings and roots was measured. The value of the corresponding parameter from each replication is presented, as well as the average replication value.

RESULTS AND DISSCUSION

Influence of Trianum P on the intensity of damping off disease

High intensity of attack was observed in the check variety (Table 1), which was not treated with fungicides, but the nitrogen nutrition led to increased susceptibility to pathogens and spread of infection. It was confirmed by Gveroska (2016).

Delayed treatment with Trianum P showed negative effect on multiplication of T. harzianum T22 and on the expression of biocontrol mechanisms, resulting in highest percentage of infected area, which was even higher in variant 2 than in the check. Gveroska (2013), comparing the Trichoderma applications prior to sowing and 15 days after sowing, determined the advantage of the first two models over the third one.

In other variants investigated, the intensity of attack was lower as a result of the two additional treatments with biofungicide. The variants where treatment began 15 days after had one treatment less, which certainly had some influence on creating the conditions for growth and multiplication of the biocontrol agent population.

Table 1. Influence of Trianum P on the intensity of damping off disease

Mark	Variant	Percentage of infected area			
		Replication			
		I	II	III	Mean value
Ø	Check (herbicide, saltpeter; without fungicide)	7,15	4,50	9,91	7,19
1	Sowing without herbicide; Treatment after 15 days	4,12	2,15	8,23	4,83
2	Sowing with herbicide; Treatment after 15 days	18,02	3,04	4,61	8,56
3	Sowing without herbicide; Pre-sowing soil treatment	6,42	1,46	0,59	2,82
4	Sowing with herbicide; Pre-sowing soil treatment	1,50	2,22	1,28	1,67
5	Sowing without herbicide; Seed treated with Trianum P	1,35	0,79	1,22	1,12
6	Sowing with herbicide; Seed treated with Trianum P	0,59	0,77	1,19	0,85

The lowest values for disease attack were found in the variants where seed was stored in suspension of the preparation and with two additional treatments. In the variant with treated seed and with herbicide use, the values were insignificant (Table 1). This situation is affected by the greater possibility of development and multiplication of Trichoderma population due to the possibility of seed colonization and monitoring the growtht of root system, stimulated by root exudates (Handelsman and Stabb, 1996; Harman 2000; Howell et al., 2000).

Disease attack values were also lower in

variants where the preparation was applied in soil, compared to the check and the variants treated after 15 days (Table 1).
However, with regard to variants 5 and 6 and 3 and 4, higher intensity of damping off disease was observed in those treated with herbicide (variants 4 and 6).
T. harzianum is multiplied in the soil as a natural environment where the possibility of herbicide influence is lower because it is bound to the organic matter from the fertilizer. Such results for herbicide effect on the population of T. harzianum are in agreement with previous studies (Gveroska, 2014), according to which the small digression of the population caused by herbicide application is compensated or even increased during the additional treatments.
The biopreparation Trianum P, containing Trichoderma harzianum T22, has fungicidal effect on the causing agent of damping off disease on tobacco seedling. This is in agreement with the results of Chacon et al. (2006) and Devaki et al. (2008) who reported antagonistic action of T. harzianum on Pythium sp. It also has biocontrolling effect on Rhizoctonia solani, as confirmed by Monte (2001), Harman (2000, 2004, 2006), Chet et al. (2006) and Wilson et al. (2008).

The influence of Trianum P on development of tobacco seedling

According to the data presented in Table 2 and 3, it can be concluded that Trianum P, despite its strong fungicidal effect, promotes the development of tobacco seedling. Thus, root length ranges from 5.26 cm in variant 1 (first treatment of seedlings carried out 15 days after sowing, without herbicide) to 14.51 cm in variant 6 - seed stored in suspension of the preparation, with herbicide application).

Table 2. Influence of Trianum P on seedling length (cm)

Mark	Variant	Replication			Mean value
		I	II	III	
Ø	Check (herbicide,saltpeter; without fungicide)	12,58	13,47	11,85	12,63
1	Sowing without herbicide; Treatment after 15 days	5,75	5,60	4,42	5,26
2	Sowing with herbicide; Treatment after 15 days	11,80	11,98	10,38	11,38
3	Sowing without herbicide; Pre-sowing soil treatment	8,65	6,39	5,28	6,94
4	Sowing with herbicide; Pre-sowing soil treatment	12,5	12,61	11,8	12,30
5	Sowing without herbicide; Seed treated with Trianum P	7,80	8,75	7,90	8,09
6	Sowing with herbicide; Seed treated with Trianum P	15,00	15,25	13,28	14,51

It can be seen that tobacco seedling in variants treated with herbicide is larger compared to the variants without herbicide application. This situation is expected, knowing the importance of the competitive relation of weeds and plants.

Among variants 2, 4 and 6, where herbicide was applied after sowing, the longest seedling was observed in variant 6, where the seed was stored in suspension of the preparation.

Among variants without herbicide application - 1. 3 and 5, the seedling with the smallest length was observed in variant 1, treated 15 days after sowing and the highest length was measured in variant 5, where the seed was stored in suspension of the preparation.

Still, the application of biofungicide together with the seed directly affected the multiplication of Trichoderma harzianum T22 and its multifunctional activity significantly affected the growth of the seedling. These fungi are commonly used in treatment of seed, to improve its health condition and to allow a long-term improvement of plant quality. If properly quantified, they can represent a powerful accelerating system of the physiological processes of plant (Saba et al., 2012).

From the results in Table 3 the same conclusions can be drawn as for the seedling length, i.e. appropriate application and further treatment of the seedling can affect the root length.

Table 3. Influence of Trianum P on the length of seedling's root (cm)

Mark	Variant	Replication			Mean value
		I	II	III	
Ø	Check (herbicide,saltpeter; without fungicide)	2,73	3,13	1,92	2,59
1	Sowing without herbicide; Treatment after 15 days	1,75	1,65	0,62	1,34
2	Sowing with herbicide; Treatment after 15 days	2,33	3,58	2,26	2,72
3	Sowing without herbicide; Pre-sowing soil treatment	2,43	1,75	1,3	1,83
4	Sowing with herbicide; Pre-sowing soil treatment	2,58	2,45	2,38	2,47
5	Sowing without herbicide; Seed treated with Trianum P	2,05	2,28	2,43	2,25
6	Sowing with herbicide; Seed treated with Trianum P	3,73	3,30	3,10	3,38

In variants where herbicide was applied after sowing, higher root length was measured compared to the variants without herbicide application.

With regard to the model of application, the maximum root length was measured in the variant where seed was stored 24 hours in suspension of the preparation, with two additional treatments (Table 3).

Additional treatments were also made in variants 3 and 4, with pre-sowing soil treatment, but the root length was still lower.

Delayed treatment with the preparation disabled the stimulating effect, which is understandable given the reduced ability of multiplication of Trichoderma population, as compared to that in other treatments.

Results on the stimulating effect of Trianum

P are in accordance with the claims of Harman (2000) and Contreras-Cornejo (2009) that Trichoderma enhances root growth and increases crop yield, proliferation of secondary roots, fresh mass and leaf surface. Yedidia et al. (2001) also reported increase of dry matter, stalk length and leaf surface.

The correct application model of Trianum P is important not only for achieving the fungicidal effect but also for the stimulating effect. Sowing with seed stored for 24 hours in suspension of the preparation is the best application model that will provide a double effect.

The results of our research confirmed the conclusion of Saba et al. (2012) that Trichoderma spp. are endophytic plant symbionts widely accepted in seed treatment for disease control, improved plant growth and increased yield.

CONCLUSIONS

- Trianum P biofungicide confirmed its fungicidal effect.
- The highest effectiveness in the control of damping off disease in tobacco seedling was recorded in variants with treated seed, with and without application of herbicide.
- Pre-sowing soil treatment can be, to a certain extent, an alternative to the treated seed.
- The herbicide application did not show any adverse effects in these variants.
- Delayed treatment (first treatment of the seed after emergence) gives the worst results. In variants where first treatment was made 15 days after sowing, the lowest fungicidal effect was found.
- The best model of application is to treat the seed with the preparation (storage in suspension) before sowing and at least two additional treatments of the seedling.
- The Trianum P biofungicide has stimulating effect on tobacco seedling growth.
- The length of the root system, as well as the total length of seedling depends on the model of biofungicide application.
- In all models of Trianum P application, the values are higher with the use of herbicide.
- The highest length of seedling is measured in the variant where seed was stored in suspension of the preparation, with two additional treatments.
- When the preparation was applied in the soil, also with additional treatments, the seedling reached the same length as in the check variant (with saltpeter nutrition).
- Seedlings of the variant where seed was stored in the suspension of the preparation with two additional treatments of seedling had the highest length of the root.
- The other two models of application did not cause significant change in root length of the check variant.
-Trianum P with its fungicidal and stimulating effect on tobacco seedling has a good perspective in the sustainable tobacco production.

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