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Original Scientific paper

INCREASING THE BIOCONTROL ACTIVITY OF Trichoderma spp. WITH THE USE OF AN APPROPRIATE MANURE

Biljana Gveroska

St. Kliment Ohridski University –Bitola,
Scientific Tobacco Institute – Prilep, Kicevska bb, 7500 Prilep
Republic of Macedonia

e-mail: bgveros@yahoo.com

ABSTRACT

The application of a biocontrol agent Trichoderma is an effective and environmentally friendly way in tobacco seedlings protection from the damping off disease. This effect is especially expressed in its greater quantity.
Natural manures have a positive impact on the development of this agent Therefore, the aim of this study was to determine the impact of different manures on the quantitative presence of Trichoderma spp.
The biggest number of the colony units is found in a goat manure (19.21 x 10^4 cfu / g).
Covering the areas sown with tobacco seed with a combination of sheep + goat and sheep + farmyard manure has a significant positive effect. The greatest quantitative presence of Trichoderma spp. is estimated in combination sheep + goat manure (48.20 x 10^4 cfu / g soil).
The right choice of the manure has the positive effect on expression the Trichoderma's biocontrol mechanisms of and of course, increased opportunity for application of biocontrol in tobacco seedlings protection. At the same time, it is a real environmentally friendly way to produce healthy tobacco seedlings.
Keywords: manure, combination, quantity, Trichoderma spp, tobacco seedlings

ЗГОЛЕМУВАЊЕ НА БИОКОНТРОЛНАТА АКТИВНОСТ НА Trichoderma spp. СО УПОТРЕБА НА СООДВЕТНО ЃУБРЕ

Примената на биоконтролниот агенс Trichoderma е ефикасен и еколошки начин за заштита на тутунскиот расад од болеста сечење. Ваквиот ефект е особено изразен при негова поголема застапеност.
Природните ѓубриња имаат позитивно влијание врз развојот на овој агенс Затоа, целта на ова истражување беше да се утврди влијанието на различни ѓубриња врз квантитативната застапеност на Trichoderma spp.
Најголем број на формирани колонии е констатиран кај козјото ѓубре (19,21 x 10^4 cfu / g).
Покривањето на посеаните површини со тутунско семе со комбинација на овчо + козјо и овчо + кравјо ѓубре има значителен позитивен ефект. Најголема квантитативна застапеност на Trichoderma spp. е утврдена во комбинацијата овчо + козјо ѓубре (48,20 x 10^4 cfu / g почва).
Вистинскиот избор на ѓубре за покривање има позитивен ефект врз експресија на биоконтролните механизми на дејство на Trichoderma и секако, зголемена можност за примена на биолошката борба во заштитата на тутунскиот расад. Истовремено, тоа претставува вистински еколошки начин за производство на здрав тутунски расад.
Клучни зборови: ѓубре, комбинација, застапеност, Trichoderma spp, тутунски расад

INTRODUCTION

Biological control is contemporary ecological way for plant protection from diseases which avoids the problems of chemical protection (excessive use of pesticides, resistance to pathogens and negative effects on human health and environment). Therefore, it is incorporated into integrated pest management system (Monte, 2001).

Fungi of the genus *Trichoderma* are among the most famous biocontrol agents. Their effect has been confirmed in numerous pathogens and host plants.

They are present in soil and root ecosystems. Colonizing the root, they use a number of mechanisms by which attack pathogens, but also enhance the development of root and whole plant (Harman, 2004; 2006).

The most *Trichoderma* species are used in plant protection, mainly from soil pathogenic fungi and diseases of seeds, including the causing agents of seed rot, root rot and damping off in plants (Heydari and Pessarakli, 2010).

The main factor for their ecological success is the combination of a very active and efficient mechanisms of defense strategy. There are number of mechanisms involved: mycoparazitizam, antibiosi, competition for food and space, tolerance to stress by increasing root and plant development, improved solubility and adsorption of inorganic substances, induced resistance and enzyme inactivation of the pathogen (Harman, 2000; Monte , 2001).

Many species of the genus *Trichoderma* express their biocontrol effect against pathogenic fungus *Rhizoctonia solani*. *Trichoderma harzianum* is the best antagonistic fungi against to this causing agent of damping off in tobacco seedlings. Its application on a soil before sowing and several times in a growing season of tobacco seedlings has a good result in reducing the intensity of disease (Gveroska and Ziberoski, 2011). Effect is the greater in the larger quantity of a biocontrol agent (Gveroska, 2013). Handelsman and Stabb (1996) estimeted that there is a relation between the population size of biocontrol agent and the suppression degree of the pathogen, too.

Therefore, the development of a suitable technique for the mass production of biocontrol agent is required for each system of biocontrol (Heraux et al., 2005). The commercial application of antagonist *T. harzianum* need maximum production of biomass with minimal economic costs (Jahan et al., 2013).

In general, all studies concerning the use of synthetic substances like glucose, cellulose, starch, etc. According Sargin et al. (2013) solid state fermentation is an effective method for the production of biomass which provides increasing the colony number and production of conidia.

The ability to develop on inexpensive substrates makes Trichoderma isolates suitable for application as biocontrol agents. It actually provides a high degree of ecological adaptability in different environmental conditions and widespread in the world (Harman, 2006).

Many authors examined the possibility of using various "waste" agro-industrial materials (Yadav 2012; Mamo et Alemu, 2012; Duli et al., 2013). However, the use of organic amendments in the culture of the antagonist is considered a very good way to optimize control of plant pathogens through the use of strong potential of conidia producing (Hutchinson, 1999; Heraux et al., 2005; Palanna et al., 2007 , Barakat and Al-Masri, 2009).

Different types of manures influence the development of biocontrol agent *Trichoderma*, which certainly affects its activity. Therefore, the use of certain manures which have a positive effect on the number of colony forming units would have a positive impact on reducing the damping off disease in tobacco seedlings.

The purpose of this research was to determine the influence of different manures on *Trichoderma* spp. quantity.

MATERIAL AND METHODS

Experiment was carried out in a biological laboratory. Soil was prepared in the usual way. 0,3 m^2 was sowed for each variant. The biocontrol agent T. harzianum was previously prepared by sieving the fragment of the pure culture on substrate - rice, according to the method of Soares et al. (2007). Its incubation was performed at 15 days to 25^0C.

Two Erlenmeyer dishes with colony was used by each variant. It was mixed with appropriate manure or their mixture, i.e. combination. Tobacco seed from variety P79 was sown according to the usual sowing norm (0,5 g/m^2). After sowing, soil was covered with appropriate manure or combination (mixing them in an equal ratio). Sheep manure (without application of a biocontrol agent-BCA) was taken as a control, while the same, with application of Trichoderma, as standard. Investigated variants are given in Table 1.

Table 1. Investigated variants

Variants			
Ø	Sheep manure withouth BCA (Check)	5	Sheep + goat manure
1	sheep manure	6	Sheep + farmyard manure
2	goat manure	7	Sheep + poultry manure
3	farmyard manure	8	Goat + farmyard manure
4	poultry manure	9	Goat + poultry manure
		10	Farmyard + poultry manure

The first application of the biocontrol agent was taken with sowing. The second and third application were made in intervals of two weeks. In these cases, biocontrol agent was applied by drenching with suspension prepared from the pure culture.

Total number of colony forming units (cfu/g soil) of *Trichoderma* spp. was recorded 15 days after each application (three estimations).Before sowing, a sample was taken from the soil to determine the possible presence of *Trichoderma* spp.

The medium Rose Bengal Agar with an antibiotic Tetracyclin was used. An average soil sample was taken and made a series dilutions to 10^{-4}. 1ml of the final dilution (10^{-4}) was spilled in petri dishes. 20ml of PDA medium, previously sterilized and

cooled were spilled. For each variant 5 petri boxes were used. They were incubated for 10 days at 28^{0}C. After this period, number of *Trichoderma* spp. colonies was counted. It is expressed in colony forming units in gram of soil (cfu/g soil) multiplied by number of final dilution.

RESULTS AND DISCUSSION

The presence of *Trichoderma* spp. during tobacco seedling vegetation (three estimations) is shown in Table 2. It was presence of 0.3 x 10^4 colonies in the soil sample before sowing.

According to the presented results, the lowest number of Trichoderma spp. colonies at the first estimation is in the check - soil mulched only with sheep manure. This situation is noticeable in the other estimations, too. This is expected because there is no application of biocontrol agent.

Number of colony forming units in the other variants ranged from 2,60 x 10^4 in goat + poultry manure to 27,40 x10^4 in sheep + goat manure and 26,00 x10^4 in sheep + farmyard manure. The number of Trichoderma spp. colonies is the smallest in variants - sheep + poultry, farmyard + poultry and sheep manure.

The highest number of colony forming units was estimated in a combination of sheep + goat as well as in sheep + farmyard manure, followed by goat manure and goat + farmyard combination (Table 2).

Table 2. The presence of *Trichoderma* spp.

Variant	Number of colony forming units x 10^4 (cfu/g soil)		
	1st estimation	2nd estimation	3 rd estimation
Sheep manure withouth BCA (Check)	1,00	1,8	1,8
sheep manure	4,30	8,32	18,48
goat manure	12,2	18,60	19,21
farmyard manure	6,00	10,00	16,34
poultry manure	6,00	7,80	10,23
Sheep + goat manure	**27,40**	**34,60**	**48,20**
Sheep + farmyard manure	**26,00**	**32,44**	**39,62**
Sheep + poultry manure	2,80	7,84	12,63
Goat + farmyard manure	10,60	**24,63**	**28,30**
Goat + poultry manure	2,60	**19,82**	22,67
Farmyard + poultry manure	4,20	16,45	21,40

When considering the results in variants with mulching only with one manure (variants 1-4) (Figure 1), the number of colonies is the largest in soil with goat manure. It certainly influenced on the increasing quantity in combination with sheep, farmyard and poultry manure (Fig 2). The same situation is especially noticeable in the second and third estimation.

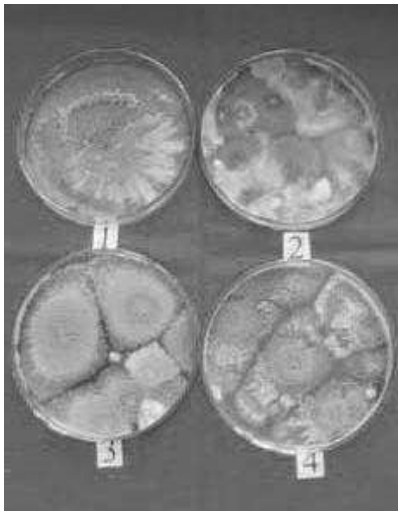

Figure 1. Number of colony forming units in soil mulched with different manures

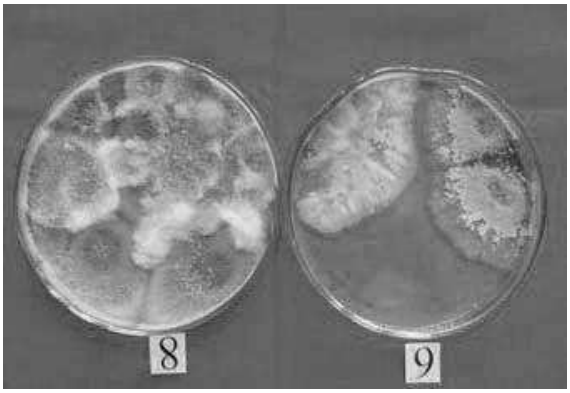

Figure 2. Number of colony forming units in variants goats +farmyard and goat +poultry manure

The number of *Trichoderma* spp. colonies at the second estimation is ranged from 7,80 x10^4 in poultry (and 7,84 x10^4 in sheep + poultry manure) to 34,60 and 32,44 x10^4 in the same variants as in the previous estimation - sheep + goat manure and sheep + farmyard manure.

In all variants, in this estimation there is an increased population of *Trichoderma* than in the first, which is a result of its re-application and the physiological activity of the fungus.

At the third estimation, the quantity of colony forming units is not so distinquished than the previous estimation. There is the highest quantity in the same variants (number 5 and 6). There are noticeable increasing the cfu/g soil in goat +farmyard manure and goat + poultry manure, too. In mulching only with one manure, there is the highest increasing of cgu/g soil in sheep manure, from the lowest value in the first to the second place, in the third estimation. In the sheep manure, the quantity is geting satisfuing value (Table 2).

There is an increasing the quantity of the biocontrol agent over the vegetative period in variants with its application (Table 2). According to Jayalakshmi et al. (2009), Trichoderma spp. are able to use a wide range of substances as a source of carbon or nitrogen and release amount of enzymes which decomposes plant polymers into simple sugars for energy and growth. It allows them utilizing the manures and intensification of development.

According the results, the highest number of colony forming units during the vegetative period was estimated in a combination of sheep + goat as well as in sheep + farmyard manure (Figure 3).

It may be noted that the number of colonies

is the largest when sheep manure is amended, i.e. combined with goat and farmyard manure (Figure 4). Our results are in agreement with the results of Palanna et al., 2007, which examined the effect of five manures and their combinations on *T. viride.* The combination of farmyard + goat has the best effect in increasing the dry mass of the colony, sporulation and fungicidal activity (against *Machropomina phaseolina*). In the case of individual manure, the best effect was noted in farmyard, followed by goat and poultry manure.

In the investigations of Pramodkumar and Palakshappa (2009), the maximum growth (colony diameter and dry biomass) was founded in sterilized farmyard manure. In our investigations, it shows a possitive effect in increasing cfu/g soil, when it is combined with others manures (Table 2, Figure 5).

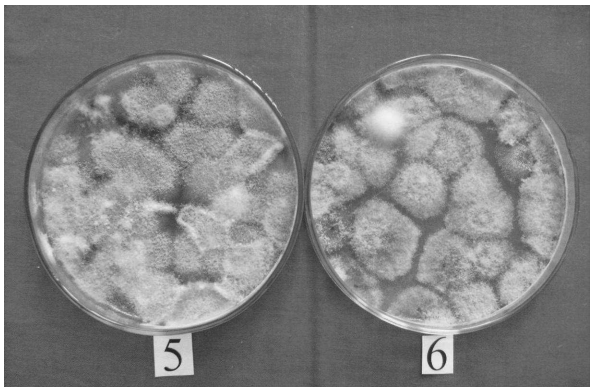

Figure 3.
Number of colony forming units in variants sheep+goat and sheep+farmyard manure

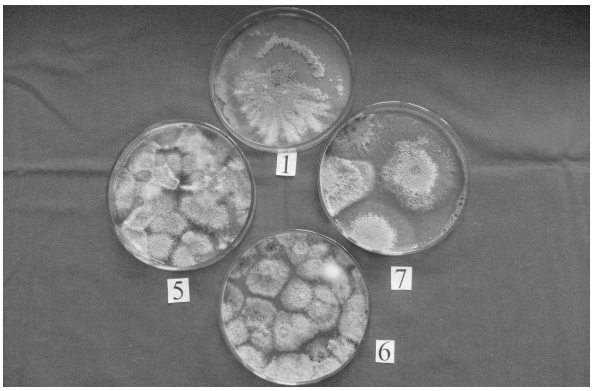

Figure 4.
Number of colony forming units in sheep manure and its combination with other manures

Figure 5.
Number of colony forming units in farmyard manure and its combination

The highest values of cfu/g soil are estimated in combinations sheep + goat and sheep + farmyard manure, as well as goat + farmyard. In combination sheep+poultry manure there was not a positive effect on *Trichoderma* quantity (Figure 4).

In studies of Hutchinson (1999), composted poultry manure inoculated with T.virens can be used as an economical substrate for application of this biocontrol agent in control of weeds. But, in our study, there is not a signifficant result in the application of this manure alone or in combination with others (Table 2).

Quantitative presence of *Trichoderma* spp. during the vegetative period of tobacco seedlings are growing up. There was a low value in the sheep manure at start, but at the end of the vegetation we have a good quantity compared with other varieties. These results are in agreement with those of Barakat and Al-Masri (2009) who stated that the number of *T. harzianum* (10^6 cfu g- 1) significantly increased over time and concentration of organic amendment - sheep manure. It is certainly not disparaging result because increased quantity of *Trichoderma* can be an advantage in rooting of plants during transplantation.

According to Pallana et al., (2007) the use of fertilizers to increase its development is necessary to increase the biocontrol ability of a biocontrol agent. Therefore, the results from these studies filled out an aim - to optimize the control of tobacco seedling from diseases through the application of the biocontrol agent with manure which gives the best effect on increasing its numbers.

CONCLUSIONS

- All investigated manures have a positive effect on increasing the quantitative presence of Trichoderma spp. in soil.
- In the standard way of mulching in tobacco seedbeds, there was a greater increase in the number of biocontrol agent in the third estimation.
- In the case of single manure, there is the highest number of colonies in goat manure.
- Significantly positive effect on increasing the quantity of the biocontrol agent has the mulching of tobacco seed with combinations of sheep + goat and sheep + farmyard manure.
- Increased number of colony forming units has the positive impact on expression of the mechanisms of action in fungi of the genus Trichoderma, which means increased biocontrol activity in plant protection from diseases.
- The application of biocontrol agent in mulching of tobacco seedbeds with manure who gives the greatest effect on increasing its quantity means optimizing the control of tobacco seedlings diseases.
- The real choice of manure also means a bigger possibility for the use of biocontrol in tobacco protection from diseases.
- This way of application the biocontrol agent and use the biocontrol is an ecological way for producin the healtjy tobacco seedlings.

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