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EVALUATION OF SOME *TRICHODERMA* ISOLATES FOR BIOCONTROL EFFECT ON *RHIZOCTONIA SOLANI*

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ABSTRACT

Tobacco production is affected by the need to reduce the use of pesticides due to strict standards in recent years. Therefore, all methods and means to control the harmful agents with minimal environmental impact and economic consequences are included.

Biological control is a modern, environmentally friendly approach in plant protection, which is easily incorporated into the system of Integrated Pest Management. In plant pathology, the term biocontrol usually is concerning to the use of microbial antagonists to suppress diseases.

Trichoderma strains are the most known biocontrol agents, mostly against many soil pathogens. *Rhizoctonia solani* is a very destructive pathogenic fungus, the causing agent of a damping off in tobacco seedlings. Therefore, our aim was to examine the impact of several *Trichoderma* isolates obtained from rhizosphere of tobacco plants against this pathogen.

Investigations were carried out under in vitro conditions, using the method of dual cultures. Relative growth of the pathogen in the presence of biocontrol agent and the percentage of reduction of the radial growth of the pathogen were estimated. The relative growth was the weakest in PT1 and PT2 isolates (40.8 and 40.3%). These isolates showed the best results with the percentage reduction of pathogen 59.2 and 59.7%. PT3 and PT4 showed smaller effect (49.4 and 54.6% reduction).

These investigations confirmed the role of this biocontrol agent control of the pathogenic fungus *R. solani*. Further research should be lead to the true determination of the species, as well as intended biocontrol effect on this pathogen. We believe that this research open the way for the application of Trichoderma species, with mass multiplication or commercial products.

Key words: biocontrol, *Trichoderma* sp., *R. solani*, relative growth, inhibition of radial growth

ОЦЕНА НА БИОКОНТРОЛНИОТ ЕФЕКТ НА НЕКОИ *TRICHODERMA* ИЗОЛАТИ ВРЗ *RHIZOCTONIA SOLANI*

Производството на тутун поради строгите стандарди, во последниве години е засегнато од потребата за намалување на употребата на пестициди. Затоа, се вклучуваат сите методи и средства за контрола на штетните агенси со минимално влијание врз животната средина и економски последици.

Биолошката борба претставува современ, еколошки пристан во растителната заштита, која лесно се инкорпорира во системот на интегрална заштита. Во фитопатологијата, терминот биолошка борба најчесто се однесува на употреба на микробни анатагонисти за сузбивање на патогените.

Trichoderma видовите се најпознати биоконтролни агенси, најчесто против бројни почвени патогени. *Rhizoctonia solani* е мошне деструктивна патогена габа, предизвикувач на болеста сечење која тутунскиот расад. Затоа, нашата цел беше да се испита влијанието на неколку *Trichoderma* изолати добиени од

ризосферата на тутунски растенија врз овој патоген.
Испитувањата беа вршени во in vitro услови, по методот на двојни култури. Одредуван беше релативниот развој на патогенот во присуство на биоконтролниот агенс, како и процентот на редукција на радијалниот развој на патогенот. Релативниот развој беше најслаб кај двата изолати ПТ1 и ПТ2 (40,8 и 40,3%). Тие два изолати покажаа најдобри резултати, со процент на редукција 59,2, односно 59,7% во споредба со ПТ3 и ПТ4 (49,4 и 54,6%).
Со овие истражувања се потврди улогата на овој биоконтролен агенс во сузбувањето на патогената габа *R. solani*. Понатамошните истражувања треба да водат кон точната детерминација на видовите, како и одделниот биоконтролен ефект врз овој патоген. Сметаме дека со овие истражувања се отвора патот на примена на Trichoderma видовите, со масова продукција или како комерцијални препарати.

Клучни зборови: биоконтролен ефект, *Trichoderma* sp., *R. solani*, релативен развој, инхибиција на радијалниот развој

INTRODUCTION

Crop production bears the great losses because of a number of diseases caused by various pathogens. Tobacco production is also affected by several economically important diseases. Among them, the diseases caused by pathogenic fungi have a great part.
Concerning to damages, diseases of tobacco seedlings are of a greatest importance, especially the damping off caused by the pathogenic fungus *Rhizoctonia solani*. The importance of a healthy and quality tobacco seedlings for total production is known, and hence, the losses caused by this pathogen are huge. Also, *R. solani* is known for its great destructiveness specific to soil pathogens and a wide range of host plants (Nunez, 2005).
From the above, it is obvious the need for protection from the disease. In practice there are a limited number of fungicides, which are also used for a long time. The extended and excessive use of pesticides cause pathogen resistance and the control is not always efficient (Benitez et al, 2004; Hajieghrari et al., 2008). It also causes harmful effects to human health and environmental safety (Monte, 2001).
The total production of food, including agriculture and tobacco production in recent years is affected by the strict standards that require reducing the use of pesticides. Therefore, all methods and means of control of harmful agents with minimal impact on the environment are involved.
Biological control is a modern, environmentally friendly approach in crop protection, which can be easily incorporated into the Integrated Pest Management System. It stands out among the leading components in the development of many systems for sustainable agricultural production (Monte, 2001). According to Brimmer and Boland (2003), it is an alternative to synthetic pesticides because it provides higher level of security and minimal impact on the environment.
Biological control i.e. application of specific microorganisms that interfere with plant pathogens and pests is a natural, environmentally friendly approach to overcome the problems caused by the standard method of plant protection (Chet et al., 2006). In phytopathology, the term biological control often refers to the use of microbial anatagonists for control of pathogens.
Bacterial and fungal biocontrol agents with strong antagonistic abilities have the power to control many plant pathogens (Szekeres et al., 2006). Fungi of the genus *Trichoderma* are the most popular biocontrol agents. The success of *Trichoderma* species as biocontrol agents is due to their strong reproductive capacity, ability to survive in very unfavorable conditions, the efficiency of utilization of nutrients, the capacity to modify rhizosphere, stong aggressiveness

against phytopathogenic fungi and efficiency in stimulating the growth of the plant and its defense mechanisms. These properties make this genus an unique inhabitant with a high population densities in many life unions (Benitez et al., 2004).

As soil inhabitants, they live in the area of root system where they activate numerous biocontrol mechanisms that affect pathogen. Antibiosis, mycoparasitism and competition for food and space are the main in numerous mechanisms of biocontrol. These are complex, and what can be defined as a biocontrol, presents final result of various mechanisms that act synergistically to achieve protection from a disease (Howel, 2003).

But various biotic and abiotic environmental factors may influence the efficiency of *Trichoderma* spp. against phytopathogens (Handelsman and Stabb, 1996; Jaworska and Dlużniewska, 2007). Therefore, the various isolates show different biocontrol activity. Local isolates have the greatest antagonistic activity towards the pathogen in the many cases.

The first and quickest way for determining of mycoparasitism and producing of antibiotics is method of Petri boxes (Harman, 2006). Therefore, our aim was to investigate biocontrol activity of several local isolates of *Trichoderma* spp., to pathogen *R. solani* at *in vitro* conditions. It would allow selection of the best isolate for further application as biocontrol agent in tobacco protection from the damping off disease in tobacco seedling.

MATERIAL AND METHODS

Pathogenic fungus *Rhizoctonia solani* was isolated from infected plant material.

Trichoderma isolates were obtained from the root zone of the rhizosfere of healthy tobacco plants from region of Prilep, using the method of dilution. 1ml of dilution of 10^{-4} was was thrown into Chapeck agar as the most suitable medium for fungi. Reisolation and the maintainance of the pure cultures were on potatto medium.

In vitro investigations were conducted by the method of dual cultures. 5 mm fragments both from the 10-day culture of the pathogen and *Trichoderma* isolates were placed in the center of each half of the Petri dish on PDA (potato dextrose agar) as nutrient medium.

Pure cultures of *R. solani* and of each *Trichoderma* control agent were used as a check. Biocontrol effect of the four isolates (PT1-PT4) was researched.

The experiment was set up in three replications, with five Petri dishes for the check and dual cultures. Incubation was performed at 25º C and the diameter of the colony was measured each day during the 10-day incubation interval.

Relative growth of the pathogen was calculated by the method of Mello (2000), based on the values of pathogen's diameter in the presence of biocontrol agent.

$$RD = [(GP \text{ in the presence of BCA}) / (GP \text{ in the control})] \times 100$$

RD = relative development of a pathogen in a presence of biocontrol agent (%)

GP = growth of the pathogen

BCA = biocontrol agent

The percentage of reduction of pathogen's growth was determined according to the formula of Mishra (2010).

$$PIRG = [(C-T) / C] \times 100$$

PIRG = percentage inhibition of radial growth of the pathogen (%)

C = radial growth of pathogen in the absence of biocontrol agent (control)

T = radial growth of pathogen in the presence of biocontrol agent

Estimation was made by taking the values for diameter of pathogen's colony in the presence of biocontrol agent at the time of placing the pathogen in the control Petri dishes, i.e. on the sixth day. Evaluation was continuing to 10th day.

RESULTS AND DISCUSSION

Damping off disease in tobacco seedling causes significant economic losses. It is manifested by the appearance of infections in the small group of plants. Spreading of the disease, the percentage of infected area is increasing (Fig. 1). It is caused by the pathogenic fungus *R. solani* (Fig. 2).

Fig. 1 Symptoms of damping-off disease in tobacco seedlings

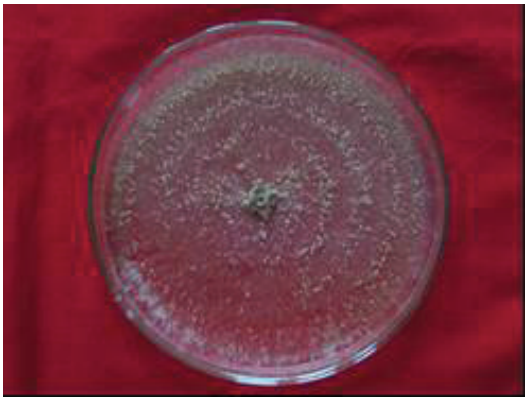


Fig. 2 Causing agent of damping off – R. solani (pure culture)

Biocontrol agent *Trichoderma* shows extremely fast radial growth (Table 1). It has be seen in all isolates. The poorest development is shown by the isolate PT3. Sporulation (beginning of forming the spores and intensity) is the lowest in the same isolate (PT3) (Fig. 3)

PT1 PT2 PT3 PT4

Fig 3. Pure cultures of the biocontrol agent Trichoderma – isolates PT1, PT2, PT3 and PT4

Table 1. Growth of colonies during incubation (mm)

Variant	Diameter (mm)									
	Days									
	1	2	3	4	5	6	7	8	9	10
R. solani in PT1	14,4	29,6	34,5	43,8	44,2	44,9	45,8	48,5	48,5	48,5
R. solani in PT2	13,8	30,0	41,9	43,7	44,0	44,3	45,0	45,1	45,1	45,1
R. solani in PT3	15,9	32,2	45,1	51,4	54,4	55,7	56,0	56,0	56,0	56,0
R. solani in PT4	14,9	28,7	39,5	43,6	45,6	49,9	52,4	52,5	52,5	52,5
Ø R. solani	12,9	45,8	59,8	83,7	106,6	110,0	110,0	110,0	110,0	110,0
Ø PT1	20,6	67,0	110,0	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT2	13,0	54,1	109,1	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT3	14,4	57,7	106,7	110,0	110,0	110,0	110,0	110,0	110,0	110,0
Ø PT4	19,4	62,1	109,1	110,0	110,0	110,0	110,0	110,0	110,0	110,0

In a pure culture, *R. solani* radially develops and fills Petri box on 6th day (Table 1). But in the dual cultures, in the presence of the biocontrol agent, its non inpediment development is seen only the first day. On the second day the contact of both cultures occurs (Fig. 4a-7a). From that moment the colony gets distorted form and development of the pathogen is difficult. The diameter of the colony is nearly 30% lower compared to that of control (Table 1). The pathogen continues to grow slightly, but measuring its diameter is nearly impossible, because of fulfilled Petri box by *Trichoderma. R. solani* colony seemed to "trapped by biocontrol agent. This situation is observed in the presence of tested four *Trichoderma* isolates.

Biocontrol agent continues to develope smoothly despite the presence of the pathogen. The first, its surrounds the pathogen and then „passes" through it, destroying and deforming his mycelia (Fig. 4b-7b). At the end of incubation, the Petri box is completely filled by the colony of Trichoderma (Fig. 4c-7c).

While the colony of pathogen in the check has got the maximum at 6th day, it is more than 50% lower in dual cultures, i.e. in the presence of the biocontrol agent. Thus, all the tested *Trichoderma* isolates showed the biocontrol activity against *R. solani*. The relative growth of the pathogenic fungus is the smallest in the presence of the isolate PT2, and the greates in the presence of isolate PT3 (Table 2).

Therefore, the percentage reduction of the of *R. solani* growth in the presence of *Trichoderma* ranges from 49.4% for the isolate PT3 to 59.7% for T2 isolate. Therefore, isolate PT3 showed the weakest, while isolate PT2 the strongest reducing effect on the development of *R. solani.*

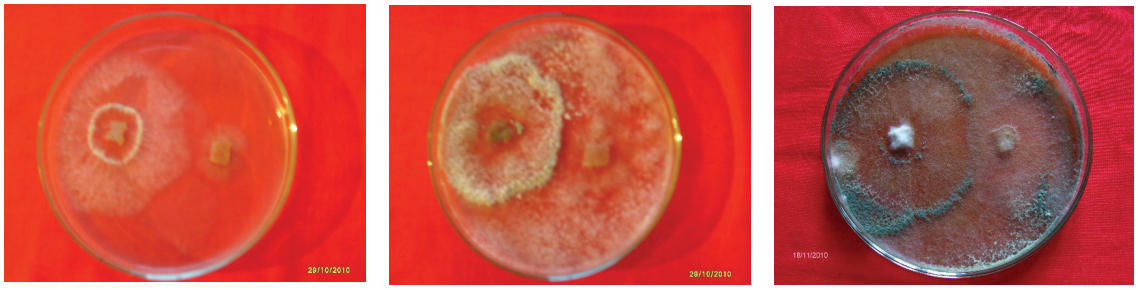
Table 2. Reduction of growth of *R. solani* with four *Trichoderma* isolates

Variant	Relative growth of the pathogen in the presence of *Trichoderma*	Percentage reduction of patho-gen's growth in the presence of *Trichoderma*
R. solani in PT1	40,8	59,2
R. solani in PT2	40,3	59,7
R. solani in PT3	50,6	49,4
R. solani in PT4	45,4	54,6

The results obtained in our investigations are in accordance with those of Rini and Sulochana (2007), in which there is a difference in the percentage inhibition of *R. solani*. Among examined 26 isolates of *Trichoderma*, 11 have efficacy in the control of the pathogen. In these studies, only *T. harzianum* TR 20 is characterized as a class 1 on the 6 th day of incubation. Despite these data, our tested isolates are included in class 1 of the mentioned scale in that paper, which is a good assessment of biocontrol activity of our local isolates.

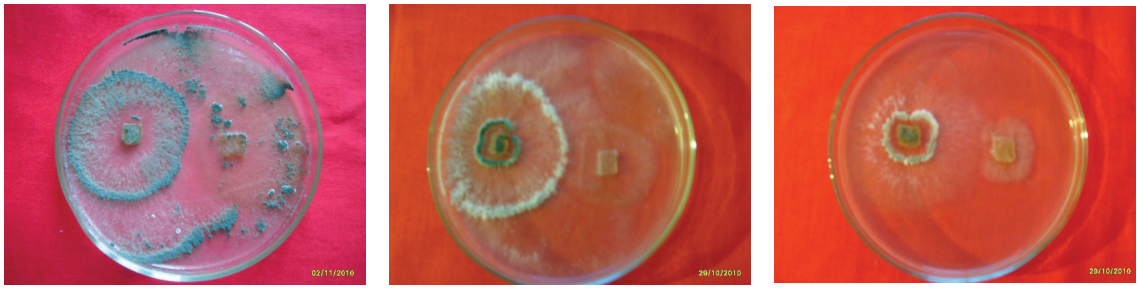
Data for differences between isolates is presented by Foroutan (2013), in which is highlightened the different inhibition of mycelial development of *Fusarium graminearum* by different isolates as *T. harzianum*, as well as the *T. viride*. Also, the percentage inhibition of radial development of *Pythium aphanidermatum* is different by *Trichoderma* species, but different isolates of the same species, too. For eg. percentage of reduction among isolates of *T. harzianum* ranges from 52,2 to 72,0% (Mishra, 2010). According to Grondona et al. (1997), in a practical situation of biocontrol, differentiation is required to define the population in a range of species. Also, it is necessary to make a selection of the most effective isolate for each patosistem.

Mishra et al. (2011) pointed that *T. viride* isolate Tr8 due expressed antagonistic properties can be used for commercial purposes in local climatic conditions. According to the results of these investigations, the isolates PT2 and PT1 can be used for mass propagation and involvement in the system of integrated protection of tobacco from diseases.



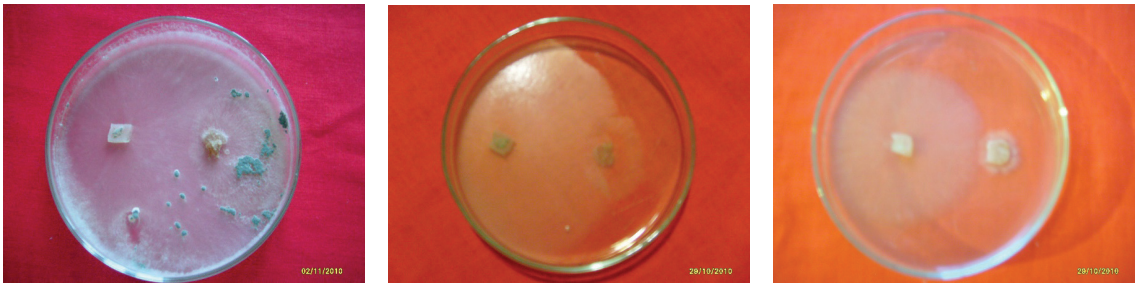
a **b** **c**

Fig. 4 Development of R. solani in a dual culture with Trichoderma - isolate PT1 (a= on the third, b=fourth day, c= the end of incubation)



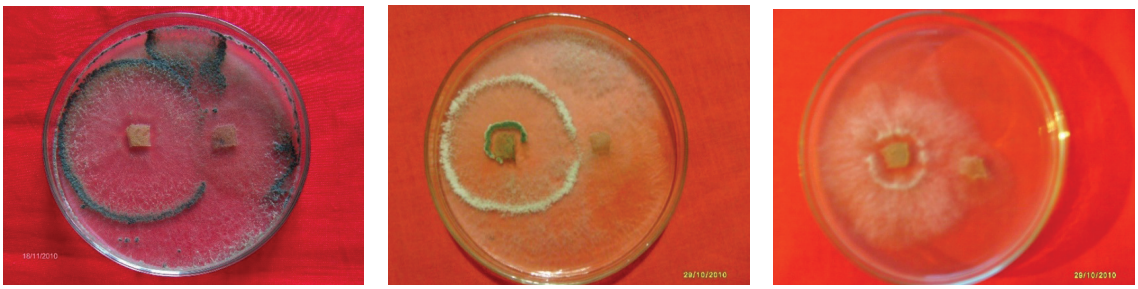
a b c

Fig. 5 Development of R. solani in a dual culture with Trichoderma - isolate PT2 (a= on the third, b=fourth day, c= the end of incubation)



a b c

Fig. 6 Development of R. solani in a dual culture with Trichoderma - isolate PT3 (a= on the third, b=fourth day, c= the end of incubation)



a b c

Fig. 7 Development of R. solani in a dual culture with Trichoderma - isolate PT4 (a= on the third, b=fourth day, c= the end of incubation)

CONCLUSIONS

- Four investigated *Trichoderma* isolates showed the biocontrol effect against the causing agent of damping off in tobacco seedlings- *R. solani*.
- Relative growth of *R. solani* at *in vitro* conditions ranged from 40,3% in the presence of isolate PT2 to 50,6% in the isolate PT3.
- Percentage of reduction of pathogen's growth ranged from 49,4% (PT3) to 59,7% (PT2).
- Isolate PT2 showed the highest inhibition of growth of *R. solani.*
- Isolate PT2 had the highest reducing effect on development of pathogenic fungus *R. solani*.
- Isolate PT1 had the good reducing effect, too.
- They can be used in the biological control against *R. solani* in tobacco seedling protection.

- Identification of Trichoderma species is needful for further development of methods of mass propagation.
- Preparations on the basis of the isolate with the best biocontrol effect have the biggest opportunities to use them in biological control of damping off in tobacco seedlings.

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