ISSN 0494-3244
Tutun/Tobacco, Vol.62, N^{o} 7-12, 79-86, 2012
UDC: 633.71-153.037:632.48]:632.937.1
632.937.1:[633.71-153.037:632.48
Original scientific paper

THE USE OF *TRICHODERMA ASPERELLUM* IN THE CONTROL OF PATHOGENIC FUNGUS *RHIZOCTONIA SOLANI* KÜHN

Petre Taskoski
"St. Kliment Ohridski" University – Bitola,
Scientific Tobacco Institute, Kicevski pat bb. 7500 Prilep, R.of Macedonia
e-mail: taskoskip@yahoo.com

ABSTRACT

Fungi of the genus Trichoderma are widely used in production of biological products which show high efficiency in the control of many soilborne pathogens. Laboratory tests with *Trichoderma asperellum* grown together with the pathogenic fungus *Rhizoctonia solani* as dual culture showed high inhibitory effect of this antagonist on the pathogenic fungi. It represses the growth of the pathogen and develops on its colony. The growth of pathogenic fungus grown in the presence of antagonist was reduced by 36.74%, with percentage of inhibition being 63.26%. Biological agent *T. asperellum* was tested for control of *R. solani* on tobacco seedlings in protected conditions. Tobacco seedlings were inoculated both with pure culture of the pathogen and with dual culture, where the pathogen was grown in the presence of the antagonist. Seedlings treated with dual culture were healthier and had a more rapid growth, while those treated with pure culture showed high percentage of infestation.

Key words: tobacco, *Trichoderma asperellum, Rhizoctonia solani*, antagonism

КОРИСТЕЊЕ НА *TRICHODERMA ASPERELLUM* ЗА КОНТРОЛА НА ПАТОГЕНАТА ГАБА *RHIZOCTONIA SOLANI* KÜHN

Габите од родот Trichoderma најдуваат широка примена за изработка на биолошки препарати, кои покажуваат висока ефикасност во контролата на поголем број почвени патогени. Во лабораториските испитувања кои ги направивме со габа *Trichoderma asperellum* одгледувана заедно со патогената габа *Rhizoctonia solani* во двојна култура, се покажа дека овој антагонист има високо инхибиторно дејство врз патогената габа. Таа го инхибира порастот на патогенот, го потиснува и се развива врз неговата колонија. Порастот на патогената габа одгледувана во присуство на антагонистот е намален за 36,74%, додека процентот на инхибирање изнесуваше 63,26%. Биолошкиот агенс *T. asperellum* за контрола на *R. solani* кај тутнскиот расад, беше испитуван во заштитен простор. Тутунскиот расад беше инокулиран со чиста култура од патогенот и со двојна култура, каде патогенот е одгледуван во присуство на антагонистот. Расадот третиран со двојна култура беше здрав и со побрз развој, додека кај расадот третиран со чиста култура од патогенот имаше висок процент на зараза.

Клучни зборови: Тутун, *Trichoderma asperellum, Rhizoctonia solani,* антагонизам.

INTRODUCTION

Phytopathogenic fungus *Rhizoctonia solani* is the major causing agent of root rot disease on tobacco and other crops. Very often, chemical products do not provide full protection of seedlings from this pathogen. Even the most frequently recommended chemicals so far chlorothalonil, thiophanate-methyl and iprodion (12) have shown low effectiveness in the control of root rot in many vegetables. Presently, researchers and growers of agricultural crops, supported by environmentalists, are interested in application of biological means for control of plant pathogenic microorganisms. According to Baker and Cook (1974) (1), biological control in fact reduces the production of inoculum, i.e. the activity of pathogen carried out by one or more organisms.

In phytopathology, the term biological control refers to the use of microorganisms-antagonists of specific pathogens – agents of plant diseases (9) called biological control agents-BCA. In our country, biological control of plants is still recommended as a segment of the integral control.

In literature, a number of mycoparasites are found for biological control of soil pathogens. Some of them have proved to be good antagonists of *R. solani*. The most popular among them are species of the genus Trichoderma. Species belonging to this genus are distributed widely in the nature and can be easily isolated from soil as a pure culture. The rapid growth of the culture on nutrient medium and production of great number of conidia colored with different shades of green are the basic characteristics of fungi of this genus (14). One of the most important features of these species is their ability to parasitize other fungi.

Trichoderma genus includes imperfect fungi which have anamorphic stage of propagation (with no sexual stage) (5, 14). They are optional parasites that parasitize a large number of fungi, but can live as saprophytes, too. Species *Trichoderma spp*. not only parasite the fungal plant pathogens, but can also produce antibiotics, causing systemic or local resistance against the pathogen and improve the development of the plant. The potential of these species to be used as biological agents in the control of plant diseases has been known since the 1930s (16). This activity is due to different mechanisms (14). One of the mechanisms is the competition for food, and the second one, through formation of small groups limits the growth of the pathogen. In both cases the pathogen *R. solani* does not produce sclerotic lesions, which means that Trichoderma species can control the development of pathogen sclerotions in the soil. Thus, Trichoderma species act as mycoparasites, they produce antibiotics and have enzymatic system capable of attacking a wide range of plant pathogens. According to Shalini (13), the mechanism and mode of action of Trichoderma against *R. solani* consists of bending around the hyphae of the pathogen, penetration into their interior and decomposition of cell.

Biopesticides use the beneficial microorganisms or the products of their metabolism in plant protection (6). They act through several mechanisms: direct competition, antibiosis, predation or parasitism and induced resistance in host plant (6). The most common biological agents in the control of soil pathogens are *T. harzianum*, *T. viride* and *T. viriens* (11) and a number of biofungicides based on these agents (*T. harzianum, T. viride*, *T. asperellum*, and *T. polysporum*) can be found in the trade. (1). *T. asperellum* was used as an antagonist to obtain the biofungicide Trifender WP, which showed high efficiency in protection of tobacco seedlings from the pathogen (Taskoski, 15). This biochemical also achieved high efficiency in potato protection from *R. solani* (18).

The use of antagonistic fungus *T. asperellum* and its application in biological control of the pathogen *R. solani* in tobacco seedlings was the main objective of this study.

MATERIAL AND METHODS

Studies were performed *in vitro*, in laboratory conditions, with cultures of the antagonistic and pathogenic fungi grown as dual culture, and *in vivo*, on tobacco seedlings. Antagonistic effect of *Trichoderma asperellum* on pathogenic fungus *Rhizoctonia solani* was

investigated. Pure culture of *R. solani* was obtained from infected tobacco seedlings grown on potato-dextrose agar. The culture of the antagonistic fungus *T. asperellum* was isolated from the biofungicide Trifender WP obtained from the fungus, and than grown on nutrient medium potato-dextrose agar.

Antagonistic ability of the fungus *T. asperellum* against the pathogen *R. solani* was investigated using the dual culture technique described by Dennis and Webster (1971) (14).

Fragments sized 3 mm with mycelia of the pathogenic fungus and the antagonist were placed at 3 cm distance from each other in Petri dishes with 10 cm diameter, on potato-dextrose agar medium. Four trials with three replications were made for this study. Pure culture of the pathogenic and antagonistic fungi set up in triplicate was used as a check. Prepared Petri dishes were incubated in a thermostat at 25^0 C for a period of 10 days. Radial growth of mycelial colony of the pathogen grown in the presence of the antagonist as a pure culture which served as a check was regularly measured in a period of 7 days. The percentage of growth of mycelial colony from the pathogenic fungus grown as a pure culture was calculated by the formula of Siameto (11):

$$\% = \frac{\text{radius of growth in the presence of the antagonist}}{\text{radius of growth in the check}} \text{ x } 100$$

Percentage of inhibition of the pathogen by *T. asperellu,* was calculated by the formula of Mudri (8) and Siameto (11):

$$\% \text{ of inhibition} = (a - b/a) \text{ x } 100$$

where:
a = radial growth of the pathogen in the check
b = radial growth of the pathogen in the presence of the antagonist

Inhibition of colony growth of the pathogen (Zivkovic, 19) can be presented on 0 - 4 scale, where:

0 = no inhibition,
1 = 1-25% inhibition,
2 = 26-50% inhibition,
3 = 51-75% inhibition,
4 = 76-100% inhibition

Biological control of the pathogen *R. solani*, the causing agent of root rot disease on tobacco seedlings was checked in protected conditions. For this purpose two experiments were set up in three replications. Tobacco seedlings of the oriental variety P66 were planted in pots and grown using standard agrotechniques. In the stage when seedlings were 2-4 cm long, inoculation was made with suspension prepared from fungal mycelium. Investigations were performed in three variants:

- Seedlings treated with pure culture of the pathogenic fungus *R. solani*
- Seedlings treated with dual culture of the pathogen and *T. asperellum*
- Check - untreated seedlings

Fungal culture was grown on potato-dextrose agar, in a thermostat at 25^0 C for a period of 10 days. Culture of the pathogenic fungus was grown separately, and dual culture between the pathogenic fungus and the antagonist was placed in other Petri dishes.

Mycelium from one Petri dish was used to inoculate seedlings grown in a 380 cm^2 pot. Mycelial colony was mixed in 200 ml distilled water and the resulting suspension was used for foliar spraying of the seedlings. Inoculation was made on 22.06.2011 in the first trial and on 25.07.2011 in the second trial. Seedlings in the pots which were used as a check were treated with pure water. Health condition of tobacco seedlings was evaluated according to the number of infected plants, i.e. to the percentage of infected area.

RESULTS AND DISCUSSION

The soilborne phytopathogenic fungus *R. solani* can be easily isolated from infected plants. Grown on potato-dextrose agar it has slower growth, creating dirty white mycelial colony which in older cultures gets brighter shade of brown and the appearance of concentric circles (Fig. 1). *T. asperellum* showed similar development as the pathogenic fungus, forming white colony which turned green after a few days, as a result of conidiophores and conidia development (Fig. 2).

The results of laboratory investigations of mycelial colonies in phytopathogenic fungus *R. solani* and antagonistic fungus *T. asperellum* grown both in pure culture and as dual culture were used to calculate the percentage of growth of pathogen colony and the percentage of its inhibition by the antagonist.

Fig. 1. *R. solani* – pure culture

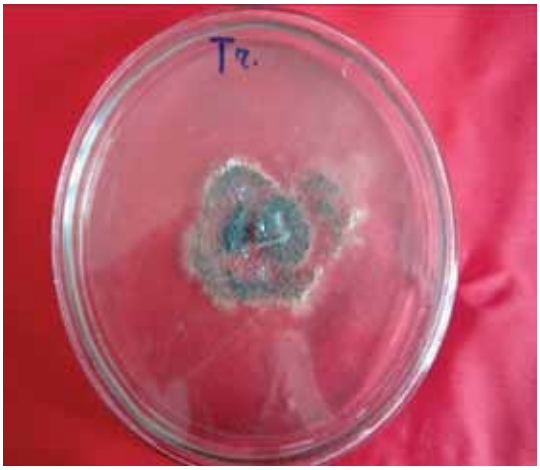

Fig. 2. *T. asperellum* – pure culture

Daily growth of fungi in pure culture and in dual culture is presented in Table 1, through the mean values from the four trials with three replications. .

Table 1 Fungal growth (mm) in the period of incubation by days

Variant	Days of incubation						
	1	2	3	4	5	6	7
Rhizoctonia solani	5.50	15.00	20.00	29.00	38.00	42.50	48.75
R. solani+*T. asperellum*	4.50	11.20	13.00	14.00	15.00	17.00	17.50
Trichoderma asperellum	3.50	14.00	19.00	26.00	38.00	46.00	52.50

Pathogenic fungus *R. solani* grown in pure culture on the nutrient medium showed somewhat slower growth. 24 hours after planting, radius of the mycelial colony was 5.50 mm and on the seventh day, at the end of observation, it measured 48.75 mm. Antagonistic fungus *T. asperellum* grown in pure culture was similar in size with the pathogenic fungus, measuring a radius of 3.50 mm on the first day and 52.50 mm on the seventh day of observation. When grown in dual culture with the antagonistic fungus, the pathogenic fungus showed high inhibitory effect on its growth. On the first day, radius of the colony was 4.50 mm and on the seventh day it reached 17.50 mm. Pure mycelial growth of the pathogenic fungus appeared as a result of the antagonistic effect of *T. asperellum*.

On the seventh observation day, there were no major differences in colony size of the fungi grown in pure culture. The results obtained in all four trials were almost identical (2). *R. solani* reached its maximum colony growth in the first and fourth trial, where a radius of 55.00 mm was measured. Slower growth was

measured in the third and second trial, reaching 40.00 mm and 45.00 mm, respectively. On the seventh day of observation *T. asperellum* reached a radius of 50.00 mm in the first and fourth and 55.00 mm in the second and third trial. Unlike this, the pathogen *R. solani* in dual culture with antagonistic fungus showed a very slow growth, with colony size ranging from 10.00 mm in the fourth to 25.00 mm in the first trial. In the second and third trial the radius measured 15.00 mm and 20.00 mm, respectively.

Table 2. Fungal colony growth on the 7th day of incubation

Variant	Radial growth of the colony in mm by trials				Average, mm
	1	**2**	**3**	**4**	
Rhizoctonia solani	55,00	45.00	40.00	55.00	48.75
R. solani+T. asperellum	25.00	15.00	20.00	10.00	17.50
Trichoderma asperellum	50.00	55.00	55.00	50.00	52.50

In average, *R. solani* grown in pure culture on nutrient medium has 36.74% higher growth of mycelial colony compared to the same pathogen grown in dual culture, in the presence of antagonist (Table 3). Thus, colony growth in the fourth trial was greater for 18.18%, while in the third trial it was 50.00% greater.

Table 3. Percentual growth of the colony of pathogenic fungus *R. solani*

Variant	Radial growth of the colony in the check variant, mm	Radial growth of the colony in the presence of antagonist, mm	Colony growth, %
I Trial	55.00	25.00	45.45
II Trial	45.00	15.00	33.33
III Trial	40.00	20.00	50.00
IV Trial	55.00	10.00	18.18
	Average		**36.74**

Data obtained for radial growth of mycelial colony of the pathogen in the check variant (grown in pure culture) and that grown in dual culture were used to calculate the percentage of inhibition of pathogenic fungus by the antagonist. In our investigation, the percentage of inhibition averaged 63.26% (Table 4). The highest percentage of inhibition of 81.82% was observed in the fourth trial and the lowest of only 50.00% in the third trial. In the first and second trial, the inhibition of the mycelial growth of the pathogen was 54.55% and 66.66%, respectively.

Table 4. Inhibitory effect of *T. asperellum* on *R. solani*

Variant	Radial growth of the colony in the check variant, mm	Radial growth of the colony in the presence of antagonist, mm	Inhibition, %	Index
I Trial	55.00	25.00	54.55	3
II Trial	45.00	15.00	66.66	3
III Trial	40.00	20.00	50.00	2
IV Trial	55.00	10.00	81.82	4
	Average		**63.26**	3

Results of the investigation confirmed the high antagonistic effect of the fungus *T. asperellum* on this pathogen. Its growth on the colony of *R. solani* in dual culture is presented in Fig. 3 and Fig. 4.

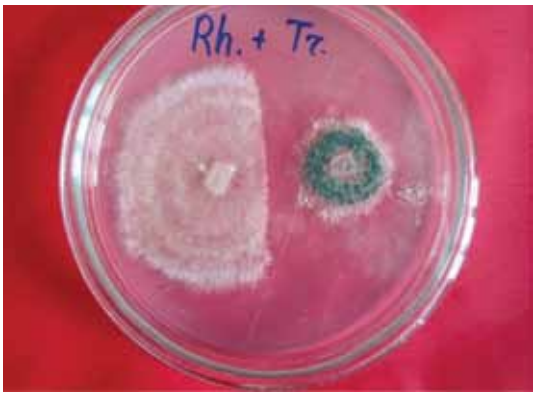

Fig. 3. Dual culture of *R. solani* and *T. asperellum*

Fig. 4. Development of *T. asperellum* on the colony of *R. solani*

Results obtained in this research coincide with those of Soares (12), who reported that Trichoderma species inhibited the growth of *R. solani* for over 60%, and T. koningii – species which produces a huge amount of antibiotics, inhibited the growth for 79-82%. Similar results on antagonistic effect of 15 isolates of *T. harzianum* were reported by Siameto (11) and by Shalini (13), who tested 17 species for their effect on soilborne phytopathogenic fungi grown in dual culture. All isolates showed serious antagonistic effect on mycelial growth of pathogenic fungi, and the maximum inhibition of growth of *R. solani* was 61.55%.

Biological control of *R. solani* was tested under protected conditions, on tobacco seedlings grown in pots. Inoculation was made with a pure culture of the pathogenic fungus and with pathogenic and antagonistic fungi grown in dual culture.

The first symptoms of infection in seedlings treated with pure culture of the pathogen in both trials were observed two days after inoculation. Infection spread rapidly and it only took few days for over half of the seedlings to be destroyed. Unlike this, low percentage of natural infection was observed in the check variant (untreated) and a very small percentage of plants were infected in the seedlings inoculated with dual culture. 10-15 days after inoculation, seedlings inoculated with the pure culture of the pathogen were completely destroyed. In seedlings inoculated with dual culture the spreading of infection was not only stopped, but they had better growth and development compared to the check (Fig. 5 and Fig.6).

Fig. 5. Inoculated seedlings (left-*R. solani*, right- *R. solani*+*T. asperellum*), I trial

Fig. 6. Inoculated seedlings (left-*R. solani*, right-*R. solani*+*T. asperellum*), II trial

The high effectiveness of Trichoderma species in reduction of diseases caused by the pathogen *R. solani* in gardening crops was confirmed in many studies (16). It was observed that these crops were characterized by faster growth and higher yields compared to the check variants. High effectiveness in protection of the lettuce and tomato seedlings from the pathogens *R. solani* and *P. debaryanum* was achieved with biological product TRI 003 based on *T. harzianum*, compared to the standard fungicides Previcur and Dithane (3, 4, 10). In bean plants, the infection by *R. solani* was reduced to 92% when the seed was treated with *T. lignorum* (2). Effectiveness of 89% was obtained in the protection of cucumber from *R. solani*, using a compost prepared from plant waste enriched with the biological control agent *T. asperellum*, isolate T-34 (17). The biofungicide Trifender WP based on *T. asperellum* showed over 90% effectiveness in tobacco seedlings protection from the pathogen *R. solani* (Taskoski, 15). According to the results obtained *in vitro* and *in vivo*, the fungus *T. asperellum* proved to be a good antagonist and a real mycoparasite which inhibits the growth of the pathogenic fungus *R. solani*. It uses its mechanisms of action - antagonism and mycoparasitism to suppress the pathogenic fungus *R. solani* and to prevent its infection on tobacco seedlings.

CONCLUSION

Phytopathogenic fungus *R. solani* grown as a pure culture on potato-dextrose nutrient agar has 36.74% faster growth compared to the same fungus grown with the antagonistic fungus as a dual culture.

Fungus *T. asperellum* showed high antagonistic effect on mycelial colony growth of the pathogenic fungus. The percentage of inhibition of colony growth ranged from 50.00 % to 81.82 %, or 63.26 % in average.

Tobacco seedlings inoculated with a pure culture of the pathogenic fungus was infected and completely destroyed, while in tobacco seedlings treated with inoculum prepared from culture of the pathogen grown together with the antagonist, the percentage of infected plants was very small. The seedlings grown in the presence of the antagonistic fungus had a more raoid growth and better development compared to the check.. *T. asperellum* fungus appeared as a real antagonist and mycoparasite on the fungus *R. solan* and it can be used for biological control of this pathogen in production of tobacco seedlings and other gardening crops.

REFERENCES

1. Anonymous. 2011. Biopesticidi u svetu. http://www.agroservis.rs/biopesticidi u svetu. Agroservis, informativna agencija, noemvri.
2. Aziz N.H., El-Fouly M.Z., El-Essawy A.A. and Khalaf M. A. 1997. Influence of bean seedling root exudates on the rhizosphere colonization by *Trichoderma lignorum* for the control of *Rhizoctonia solani*. Botanical Bulletin of Academia Sinica. Vol. 38, 33-39.
3. Baličević R., Parađiković N., Ćosić J., Rozman V. 2008. Suzbijanje zemljišnih parazita (*Pythium debaryanum, Rhizoctonia solani*) na salati biološkim pripravkom. 43. Hrvatski i 3. međunarodni simpozij agronoma, Opatija, Hrvatska, Zbornik radova 465-468.
4. Baličević R., Parađiković N., Ćosić J., Vrandečić K., Šamota D. 2009. Utjecaj supstrata na biološku zaštitu presadnica salate protiv patogena *Rhizoctonia solani* i *Pythium debaryanum*. 44. Hrvatski i 4. međunarodni simpozij agronoma, Opatija, Hrvatska, Zbornik radova 407-410.
5. Brozova J. (2004): Mycoparasitic fungi *Trichoderma* spp. in plant protection. Plant Protect. Sci., 40: 63–74.
6. Grahovac M., Inđić D., Lazić S., Vuković S. 2009. Biofungicidi i mogućnosti

primene u savremenoj poljoprivredi. Pestic. Fitomed. (Beograd), 24 (4), 245-258.
7. Mudri S., Sušinjak I. 2000. Prototip pripravka za stimulaciju biljnog rasta na osnovu gljive *Trichoderma harzianum*. Studij Bilinogojstvo, usmjerenje zaštita bilja, agronomski fakultet Sveučilišta u Zagrebu, Svetošimunska 25.
8. Pal K.K., Gardener B. Mc S. 2006. Biological control of plant pathogens. The Plant Health Instructor. DOI:10.1094/PHI-A-1117-02.
9. Parađiković N., Baličević R., Vinković T., Parađiković D., Karlić J. 2007. Biološke mjere zaštite u proizvodnji gerbera i presadnica rajčice. Agronomski glasnik 5, 355-364.
10. Siameto E. N., Okoth S., Amugune N. O., Chege N. C. 2010. Antagonism of *Trichoderma harzianum* isolates on soilborne plant pathogenic fungi from Embu District, Kenya. Journal of Yeast and Fungal Research Vol. 1 (3), pp. 47-54.
11. Soares I. M., Faull J. L. 2000. Parasitism of *Rhizoctonia solani* by strains of Trichoderma spp. Sci. agric. Vol. 57, N^{o} 1, Piracicaba jan./mar.
12. Shalini S., Narayan K.P., Lata and Kotasthane A. S. 2006. Genetic relatedness among Trichoderma isolates inhibiting a pathogenic fungi *Rhizoctonia solani*. African Journal of Biotechnology Vol. 5 (8) pp. 580-584.
13. Shalini S. and Kotasthane A. S. 2007. Parasitism of Rhizoctonia solani by strains of Trichoderma spp. Electronic Journal of environmentsl, Agricultural and food chemistry, 6 (8), 2272-2281.
14. Ташкоски П., Чифлигароски З. 2011. Можности за примена на биофунгицидот Trifender WP во заштитата на тутунскиот расад. Заштита на растенија, год. XXII, Vol. XXII, 29-35.
15. Tran N. Ha. 2010. Using Trichoderma species for biological control of plant pathogens in Vietnam. J. ISSAAS Vol. 16, N^{o}. 1:17-21.
16. Trillas M. I., Casanova E., Cotxarrera L., Ordovas J., Borrero C., Aviles M. 2006. Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biological Control 39, 32–38.
17. Zoltan O. 2008. A Trifender alkakmazasa a burgonyaban. Virages Tarsa Bt. 3529 Miskole Szilvas ut 2 II/4.
18. Živković S., Stojanović S., Ivanović Ž., Gavrilović V., Popović T., Balaž J. 2010. *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Arch. Biol. Sci., Belgrade, 62 (3), 611-623.