

BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI IN TOBACCO SEEDLINGS WITH SOME TRICHODERMA SPECIES

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ABSTRACT

Tobacco production has intentions to introduce a biological control as modern and environmentally friendly plant protection measure.

Rhizoctonia solani is a very destructive pathogen, the causing agent of damping off in tobacco seedlings. The application of biological control is a good way of control, according to all moments that make difficulties in control of this pathogen.

Biocontrol activity of some *Trichoderma* species (*T. aureoviride*, *T. hamatum* and *T. harzianum*) was investigated *in vitro* - by dual culturing technique and *in vivo* - in greenhouse.

The relative development of the pathogen ranged from 33.10% for *T. harzianum* to 41.64% for *T. hamatum*. The percentage inhibition of radial growth of the pathogen has no big differences, however, the highest value (66.91%) is in *T. harzianum*.

In conditions of artificial inoculation with the pathogen, the intensity of a disease was reduced applying the investigated biocontrol agents.

T. harzianum has the best reducing effect on the damping off in all application ways of this biocontrol agent. The intensity of disease was only 5.56% in the application 15 days after sowing, while in the application before sowing and mixed with seeds, there was no disease incidence.

Application of these *Trichoderma* species by seeds has confirmed as the best application way of the biocontrol agent. Biocontrol activity of these fungi enables them to be used in the biological control against *R. solani* in tobacco seedling protection.

INTRODUCTION

Fungal diseases are among the most important factors that cause serious losses of yield, quality and economic effect each year. Damping off is a very destructive disease in production of tobacco seedlings. The most common causing agent of the disease is *Rhizoctonia solani* - pathogenic fungus that attacks the lower part of the stalk, after which the tissue becomes necrotic and dies, making further growth of the plant impossible. Considering the importance of good quality seedlings, the damage to the total production of tobacco is enormous.

Protection from the disease includes a small number of fungicides. The long-term use of chemicals, however, leads to resistance to the pathogen and very often chemical control is inefficient and uneconomic. They also cause adverse effects on human health and the environment. Therefore, scientific approach to disease control is directed toward finding alternative means.

Application of biological control against *R. solani* is a contemporary measure of protection, against all other preventive measures which proved unsuitable and inefficient due to the large number of hosts of this pathogen, the ability to survive in soil, etc.

Fungi of the genus *Trichoderma* are one of the most popular biocontrol agents. Living in the soil and in root systems, they activate numerous biocontrol mechanisms to attack the pathogen. It is believed that antibiosis, mycoparasitism and competition for food are the main mechanisms in biological control. On the other hand, they have a stimulating influence on root and plant development.

Many *Trichoderma* species have antagonistic effect against *R. solani* and are successful in biological control of this pathogen. The aim of this work is to evaluate the impact of some *Trichoderma* species on pathogen's growth at *in vitro* conditions, and also their effect in reducing the severity of the damping off disease.

MATERIAL AND METHODS

Pathogenic fungus *Rhizoctonia solani* was isolated from infected plant material. *Trichoderma* species were obtained from the collection of Scientific Tobacco Institute - Prilep.

In vitro investigations were conducted by the method of dual cultures. 5 mm fragments both from the 10-day culture of the pathogen and from *Trichoderma* species 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.

The experiment was set up in three replications, with five Petri dishes for the check and dual cultures. Incubation was performed at 25° C and the diameter of the colony was measured each day during the 7-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. The percentage of reduction of pathogen's growth was determined according to the formula of Mishra (2010). 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 fifth day.

In vivo investigations were made in biological laboratory, using seedlings of tobacco variety P23. Nine pots were sown for each variant of the three biocontrol agents. The trial was set up in two replications.

The biocontrol agent was applied in three ways:

- through soil, prior to sowing
- by seed (stored 48 hours with the biocontrol agent)
- 15 days after sowing
- check (inoculation with the pathogen only)

Inoculation was carried out before the rapid growth stage (along with the third application of biocontrol agent), with suspension made of pure culture of the pathogen (one Petri dish in 100 ml distilled water per pot). Variants and the applied treatments are presented in Table 1.

The occurrence and growth of the disease was followed daily, and the percentage of infected area was evaluated 10 days after inoculation.

Table 1. Treatments and variants

Variant	Period of growth of tobacco seedlings		
	Sowing	15 days after	Inoculation
Before sowing	T	T	T + R
With seed	T	T	T + R
After sowing	-	T	T + R
Ø Check	-	-	R

T- *Trichoderma*
R- *R. solani*

RESULTS AND DISCUSSION

Table 2. Growth of colonies during incubation (mm)

Variant	Diameter (mm)						
	1	2	3	4	5	6	7
<i>R. solani</i> in <i>T. harzianum</i>	17,2	21,6	35,0	36,0	36,4	38,4	39,8
<i>R. solani</i> in <i>T. hamatum</i>	24,0	29,6	37,4	40,0	45,8	46,0	46,3
<i>R. solani</i> in <i>T. aureoviride</i>	19,8	22,6	32,8	38,6	40,3	41,7	42,8
Ø <i>R. solani</i>	19,8	46,4	67,6	88,2	110,0	110,0	110,0
Ø <i>T. harzianum</i>	13,0	53,2	108,0	110,0	110,0	110,0	110,0
Ø <i>T. hamatum</i>	13,8	58,4	105,4	110,0	110,0	110,0	110,0
Ø <i>T. aureoviride</i>	13,8	51,6	108,2	110,0	110,0	110,0	110,0



Fig. 1. *R. solani* - pure culture

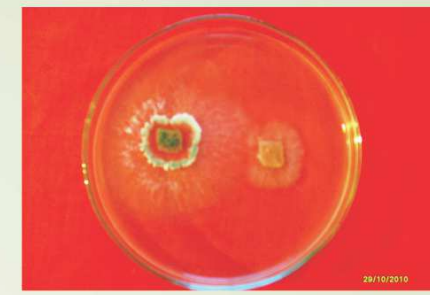


Fig. 2 and 3- Dual culture of *R. solani* and *T. harzianum* (on the third day and at the end of incubation)

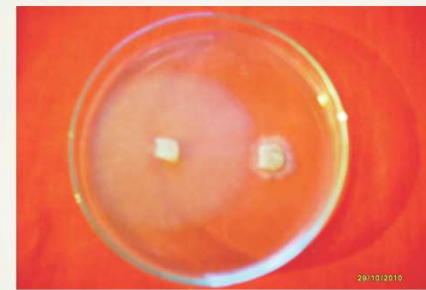


Fig. 4 and 5- Dual culture of *R. solani* and *T. hamatum* (on the third day and at the end of incubation)

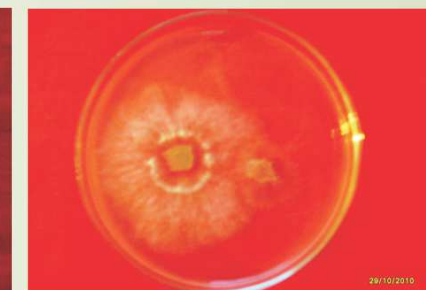


Fig. 6 and 7- Dual culture of *R. solani* and *T. aureoviride* (on the third day and at the end of incubation)



Table 4. Intensity of disease attack depending on *Trichoderma* species and mode of application

Variant	Biocontrol agent		
	<i>T. hamatum</i>	<i>T. aureoviride</i>	<i>T. harzianum</i>
Before sowing	11,37	9,34	-
After sowing	16,34	12,41	5,56
With seed	9,27	6,24	-
Ø Check	21,28		



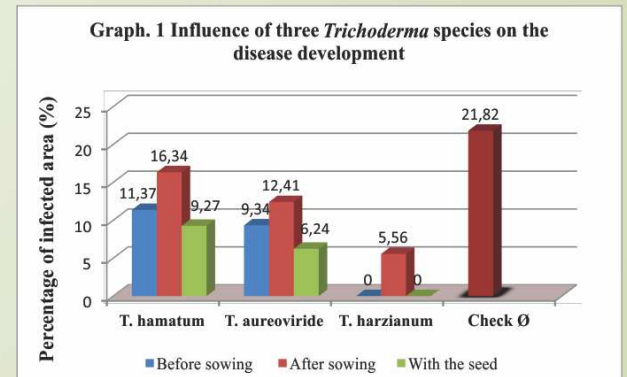
Fig. 8 - Symptoms of Damping off disease in tobacco seedlings



Fig. 9 - The effect of *T. harzianum* on the intensity of Damping off disease (left - check; right - with seed)



Fig. 10 - The effect of the application of *T. harzianum* upon attack intensity (left - prior sowing; right: after sowing)



CONCLUSIONS

- Relative growth of *R. solani* at *in vitro* conditions ranged from 33.10% in the presence of *T. harzianum* to 41.64% with *T. hamatum*.
- Percentage of reduction of pathogen's growth ranged 58.36% - 66.91%.
- *T. harzianum* showed the highest inhibition of growth of *R. solani*.
- In conditions of artificial inoculation with the pathogen, the intensity of disease attack was reduced by application of biocontrol agent.
- The percentage of infected area in *T. hamatum* ranged from 9.27% to 16.34%, while in *T. aureoviride* from 6.24% to 12.41%, depending on the mode of application of biocontrol agent.
- By application of *T. harzianum* 15 days after sowing, the disease intensity was only 5.56%. In application with seed (stored 48 hours in a culture), there were no symptoms of disease.
- All three *Trichoderma* species showed the poorest results when applied 15 days after sowing.
- Seed stored 48 hours in culture of the biocontrol agent proved to be the best way of application.
- *T. harzianum* had the highest reducing effect on the occurrence of damping off disease in all modes of application of biocontrol agent. It can be used in the biological control against *R. solani* in tobacco seedling protection.