SCREENING OF DIFFERENT *TRICHODERMA* ISOLATES AS ANTAGONISTS OF VARIOUS MAIZE PHYTOPATHOGENS

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Abstract

The increasing use of chemical plant protection agents in recent years has become a serious problem. Environmental pollution, non-selectivity, the emergence of resistant species, and chemicals in the food chain, has led to need to find new agents with improved characteristics. One of the solutions is certainly the use of beneficial microorganisms. The genus *Trichoderma* is genetically very diverse with a number of capabilities among different strains with agricultural and industrial significance. Maize is an agricultural crop that is susceptible to infections by various phytopathogenic fungi, producers of mycotoxins harmful to humans and animals. Since this agricultural crop has an important place in the human diet, its health safety is very important. In this work, the screening of antagonistic activity of three *Trichoderma* isolates, isolated from the environment, against the most common maize phytopathogens, was examined using dual culture technique. The results showed that there is a statistically very significant difference between the applied *Trichoderma* isolates. Using the Scheffe test, it was determined that the isolate of *Tricoderma harzianum* shows the best effect on the tested phytopathogens of maize by forming Radial Growth Inhibition (RGI) of 100% for *Penicilium* sp., 53.67% for *Helminthosporium carbonum*, 52.33% for *Fusarium graminearum* and 35% for *Aspergillus flavus*. Considering that *Trichoderma* spp. isolates were considered as effective when RGI exceeded 50%, from the obtained results it can be concluded that *T. harzianum* shows a very significant effect on all phytopathogenic isolates. It shows slightly weaker activity only on isolate *A. flavus*. This result once again confirms the great antagonistic potential of *Trichoderma* isolates and their use in biological control would contribute to the development of sustainable agricultural production and would affect the healthier environment.

Introduction

Maize is one of the most important grain crops, with its global production exceeding 1×10^9 t [1]. Also, maize is used for the nutrition of livestock, as well as for the nutrition of adults, children and babies, and the health safety of this food is extremely important. Maize grains can be infected by a variety of toxigenic fungi and the most common are fungi of the genus *Fusarium*, *Aspergillus*, and *Penicillium* [2]. In addition to ear infections, stalk infections can also significantly affect yield reductions.

F. graminearum typically start at the tip of the ear and produce a pink to red fungus growth moving toward the base of the ear. This fungus produces two mycotoxins–vomitoxin and zearalenone, both have detrimental effects on livestock. *Aspergillus* infections may be scattered on the ear and appear as green or gray-green fungal growth. The fungus produces aflatoxin in hot, dry conditions, which harms livestock and humans [3]. In addition, *Penicillium* spp. also belongs to the significant phytopathogens of maize, producers of mycotoxins [4]. On the other hand, phytopathogens of stalks can cause stalk lodging which reduces the yield of this important crop and the genus *Helminthosporium* and *Fusarium* are mentioned as the most common phytopathogen of maize stalk [5].

Protection of the environment is one of the most important issues of politics in many countries. The increasing use of chemical plant protection agents in recent years has become a serious problem. Environmental pollution, non-selectivity, the emergence of resistant species, and chemicals in the food chain, has led to necessity of finding new agents with improved characteristics [6]. One of the solutions is certainly the use of beneficial microorganisms. The largest generator of active agents are living organisms whose natural products have been in human use for thousands of years, and they certainly represent a source that is far from exhausted. *Trichoderma* species are naturally occurring fungi found in soils worldwide, which have been studied mostly for their plant associated biocontrol and growth promotion properties, but also for their important roles in bioremediation. The mechanisms of mycoparasitism and antibiosis are the most important biocontrol mechanism for *Trichoderma* isolates, when direct confrontation with pathogen occurs [7]. All this beneficial effects indicate enormous potential for *Trichoderma* species application in eco-friendly agriculture production.

Therefore, finding an environment-friendly way in maize fungal pathogens control is significant. In this study, we screened the antagonistic activity of three *Trichoderma* isolates against four phytopathogenic fungi (*F. graminearum, A. flavus, H. carbonum* and *Penicillium* sp.) isolated from maize plants. This step is very important because not all *Trichoderma* isolates have the same efficacy, and some do not show it at all. Considering that, it is very important to test their performance and select the best one.

Experimental

Antagonists. As antagonists, three *Trichodema* isolates, *T. harzianum*, *T. citrinoviride* and *T. capillare,* were used. Microorganisms are stored in the Microbial Culture Collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

Test phytopathogens. In the present work phytopathogenic isolates *F. graminearum, A. flavus, H. carbonum* and *Penicillium* sp., were used as test microorganisms. Phytopathogens were isolated from maize plants that showed symptoms of infection. Microorganisms are stored at the PDA (Potato Dextrose Agar) medium in the Microbial Culture Collection of Faculty of Technology Novi Sad.

Dual culture technique. Dual culture technique was used for screening antagonistic effect of three *Trichoderma* isolates on four different pathogens *in vitro*. Each *Trichoderma* isolate plug of 7 days old culture (5 mm^2) was confronted with the pathogens isolate plug in 90 mm Petri plates at the 60 mm distance on PDA (Potato Dextrose Agar) in three replicates. Antagonistic abilities of *Trichoderma* isolates were registered periodically on 7^{th}, 14^{th}, and 21^{st} day of incubation in dark at 25 °C. After 14^{th} day of incubation, Radial Growth Inhibition (RGI) was calculated according to Moya et al. [8].

Statistical analysis. The obtained data were processed by factorial ANOVA using Software Statistica, version 13.0 (StatSoft Inc., USA). Scheffe multiple range test was used to test significance of differences ($p \leq 0.05$) between mean values of measured diameter of inhibition zones.

Results and discussion

Since the obtained results measured after 14^{th} days of incubation and 21^{st} day of incubation did not show a statistically significant difference, the results measured after 14^{th} days of incubation were chosen for this study.

Results presented in Table 1. show significant differences between RGI observed as the result of activity of different antagonists, different tested phitopathogenic fungal isolates as well as interaction between these two factors. However, the biggest source of variation of RGI was activity/sensitivity of different test fungi isolates. This was expected given that tested fungi isolated from maize belong to different genera, so it can be assumed that this is the reason for their different sensitivity to applied *Trichoderma* antagonists. On the other hand, the results of Tančić Živanov et al. (2017), showed that differences in sensitivity to applied *Trichoderma* antagonists can exist within the same genus [7].

Table 1. Results of factorial analysis of variance

Source of variation	SS	Degr. of - Freedom	MS	F-value	p-value
Antagonist	1015.8	2	507.9	117.44	0.0000
Test isolate	24839.2	3	8279.7	1914.56	0.0000
Antagonist*Isolate	1160.6	6	193.4	44.73	0.0000
Error	103.8	24	4.3		

SS – sum of squares; MS – mean square

The significant influence of *Trichoderma* isolates and test fungal isolates on RGI was confirmed with $p<0.01$ and $p<0.01$, respectively. Also, the mutual influence of these two factors shows statistical significance ($p<0.01$). In order to obtain more information on the differences in the significance of the obtained RGI (%) resulting from the interaction of antagonist and phytopathogen, a more detailed post-hoc analysis was performed using the Scheffe test.

By applying Scheffe test, it was determined that the isolate of *Tricoderma harzianum* shows the best effect on the tested phytopathogens of maize (Table 2). Given that *Trichoderma* isolates were considered as effective when RGI exceeded 50%, from the obtained results it can be concluded that *T. harzianum* shows a very significant effect on all phytopathogenic isolates compare to *T. citrinoviride* and *T. capillare*.

Therefore, *T. harzianum* shows the best antagonistic activity by forming Radial Growth Inhibition (RGI) of 100% for *Penicilium* sp., 53.67% for *Helminthosporium carbonum*, 52.33 for *Fusarium graminearum* and 35% for *Aspergillus flavus*. Based on this results, it can be concluded that *T. harzianum* shows slightly weaker activity only on isolate *A. flavus*.

On the other hand, for isolate *T. citrinoviride* significant RGI was registrated on isolates *Penicillium* sp. (90.49%) and *H. carbonum* (53.55%). Efficacy on *F. graminearum* is weaker and has value of 31.78 % while on *A. flavus* shows the weakest activity of 25.33 %. At the same time, according to results, it can be concluded that isolate *T. capillare* show the weakest effect on the tested isolates. Certainly, the highest value of RGI is observed in isolates *Penicillium* sp. of 95.08%. The activity on other isolates is not significant, and the obtained mean RGI values are: *F. graminearum* 47.33%, *H. carbonum* 40% and *A. flavus* 9.67%.

Table 2. Mean values of RGI (%) after 14 days of incubation

Antagonist	Test isolate	RGI (%)
Trichoderma harzianum	*Penicillium* sp.	100.00 [g]
	H. carbonum	53.67 [e]
	F. graminearum	52.33 [e]
	A. flavus	35.00 [c]
Trichoderma citrinoviride	*Penicillium* sp.	90.49 [f]
	H. carbonum	53.55 [e]
	F. graminearum	31.78 [bc]
	A. flavus	25.33 [b]
Trichoderma capillare	*Penicillium* sp.	95.08 [fg]
	H. carbonum	40.00 [cd]
	F. graminearum	47.33 [de]
	A. flavus	9.67 [a]

*Results are means. The mean values with the same lowercase letters in the column RGI (%) are not significantly different at 5% level of probability (Scheffe test).

Observing the results, it can be noticed that all *Tricoderma* isolates show the weakest efficiency on phytopathogenic isolate *A. flavus* while, at the same time, the most sensitive phytopathogenic isolate is *Penicilium* sp.

Conclusion

In vitro results, obtained in this study, have shown that *Trichoderma harzianum* can be successfully used in the biological control of selected phytopathogenic fungi, the causative agents of maize diseases. Additionally, the results confirmed the fact that not all *Trihoderma* isolates have the same activity on certain phytopathogens. Certainly, the use of *Trichoderma harzianum* instead of synthetics agrochemicals in biocontrol of the most common phytopathogens of maize would contribute to the development of sustainable agricultural production and healthier environment.

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