

SCREENING OF ANTAGONISTIC *TRICHODERMA* FOR BIOCONTROL ACTIVITIES ON PHYTOPATHOGENS

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Abstract

Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly and ecological approach to overcome the problems caused by standard chemical methods of plant protection. *Trichoderma* species are known as biocontrol agents against plant phytopathogens. This finding is a consequence of several key factors, such as faster metabolic rates, anti-microbial metabolites and physiological conformation. In this study, in vitro potential of selected isolates strains of *Trichoderma* were evaluated against well known and virulent phytopathogens, such as *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Botrytis allii*, *Botrytis cinerea* and *Pythium debaryanum*. The selection of bioantagonistic microorganisms was carried out using dual culture method observing the pathogen growth inhibition by biocontrol agent. The inhibition extent varies from strain to strain. *Trichoderma* T27 strain presented the highest bioantagonistic activity and the most sensitive pathogens were *Fusarium graminearum*, *Botrytis cinerea* and *Pythium debaryanum*. The optical microscopy observations revealed changes of morphological characters at pathogens due to biocontrol agent activity. Full exploitation of the biocontrol potential of *Trichoderma* spp. could easily provide growth enhancement of domestic plants, green house plants, and agricultural crops.

INTRODUCTION

Plant pathogens produce significant losses to agricultural products. Traditional chemical control methods are not absolutely efficient to minimize these effects. Biological control of plant pathogens can be highly effective especially with potentials of antagonists on pathogenic fungi. *Trichoderma* spp. is the most widely studied biocontrol agents (BCAs) against plant pathogens. The genus comprises a great number of fungal strains that act as biological control agents [1, 2, 3]. In the present study, in vitro potential of four selected isolates strains of *Trichoderma* were evaluated against well known and virulent phytopathogens, such as *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Botrytis allii*, *Botrytis cinerea* and *Pythium debaryanum*. The selection of bioantagonistic microorganisms was carried out using dual culture method observing the pathogen growth inhibition by biocontrol agent.

MATERIAL AND METHOD

Microorganisms

Potential biocontrol agents *Trichoderma* strains (T27, T36, T57, T83) were cultured on solid PDA medium.

Phytopathogen strains: *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Botrytis allii*, *Botrytis cinerea* and *Pythium debaryanum*. Strains were grown for 7 days on potato dextrose agar (PDA) at 28°C.

Dual solid culture assay

The antimicrobial capacity of the selected strains was evaluated by dual solid culture assay in Petri plates. Agar dextrose-potato-agar medium was inoculated with culture solid disks from biocontrol agent and from fungal pathogen. After 3-5 incubation days, fungal growth was evaluated by measuring the clear inhibition zone around the disks.

The formula to determine the antagonism level is:

$$X = i_A/i_B \times e_B/e_A$$

where i = inner radius; e = outer radius; A = fungus test; B = fungus antagonist; $X < 1$ – antagonism present; $X > 1$ – antagonism absent.

Morphological analysis

The effect of biocontrol action against pathogens was observed using optic microscope Olympus BX 51 (40x photos).

RESULTS AND DISCUSSIONS

In vitro potential of selected isolates strains of *Trichoderma* were evaluated against virulent phytopathogens, such as *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Botrytis allii*, *Botrytis cinerea* and *Pythium debaryanum*. Among the four isolated strains only *Trichoderma* T 36 and T 27 were been of interest as potential biocontrol agents. These strains were evaluated by dual solid culture method (Table 1.).

Table 1. Antagonistic activity of *Trichoderma* strains evaluated by dual solid culture method

Biocontrol	Phytopathogen	Antagonism evaluation (X)*
Trichoderma T36	<i>Rhizoctonia solani</i> ,	0.30
	<i>Fusarium graminearum</i>	0.53
	<i>Sclerotinia sclerotiorum</i>	0.31
	<i>Botrytis allii</i>	0.17
	<i>Botrytis cinerea</i>	0.27
	<i>Pythium debaryanum</i>	0.19
Trichoderma T27	<i>Rhizoctonia solani</i> ,	0.46
	<i>Fusarium graminearum</i>	1.05
	<i>Sclerotinia sclerotiorum</i>	0.38
	<i>Botrytis allii</i>	0.23
	<i>Botrytis cinerea</i>	1.07
	<i>Pythium debaryanum</i>	1.01

* Each value is an average of five replicates.

From the results of dual culture assay it was found that *Trichoderma* T 27 inhibited pathogen more than the other tested strain.

Trichoderma T 27 was particularly very active against *Fusarium graminearum* ($X=1.05$), *Botrytis cinerea* ($X=1.07$) and *Pythium debaryanum* ($X=1.01$). For both *Trichoderma* strains, *Botrytis allii* presented higher resistance to growth inhibition. In this case, the

antagonism level expressed as X was 0.17 for *Trichoderma* T36, and 0.23 for *Trichoderma* T27, respectively.

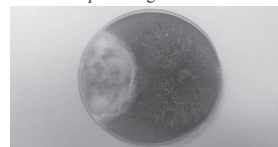
The visual observations of solid dual cultures in Petri places are presented in Fig. 1 and Fig. 2.

From Fig. 1 it can be seen that *Trichoderma* T36 presents higher inhibition against *Fusarium* strain (Fig.1 b).

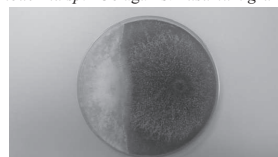
The photographs presented in Fig 1 and 2 were taken 5 days after inoculation.



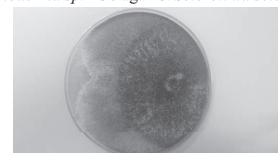
a) *Trichoderma* sp. T36 against *Rhizoctonia solani*



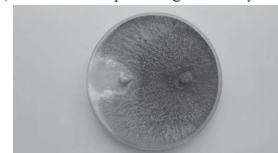
b) *Trichoderma* sp. T36 against *Fusarium graminearum*



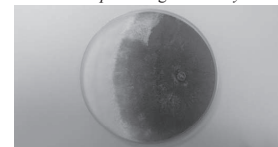
c) *Trichoderma* sp. T36 against *Sclerotinia sclerotiorum*



d) *Trichoderma* sp. T36 against *Botrytis allii*



e) *Trichoderma* sp. T36 against *Botrytis cinerea*



f) *Trichoderma* sp. T36 against *Pythium debaryanum*

Fig. 1. Performance of *Trichoderma* T36 in confrontation as assays on PDA with pathogens

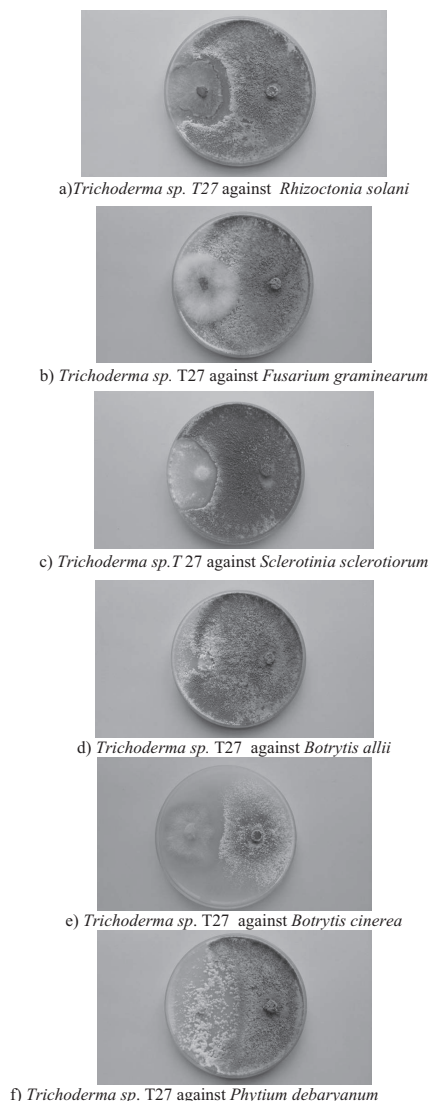


Fig. 2. Performance of *Trichoderma* T27 in confrontation as assays on PDA with pathogens

Fig. 3 presents morphological aspects of microbial cultures taken from contact zone between biocontrol agent and pathogen. The toxic action of *Trichoderma* was evident in various alterations of the hyphal structure of *Rhizoctonia solani* grown together with *Trichoderma*, similar to the effects described in other studies [4, 5].

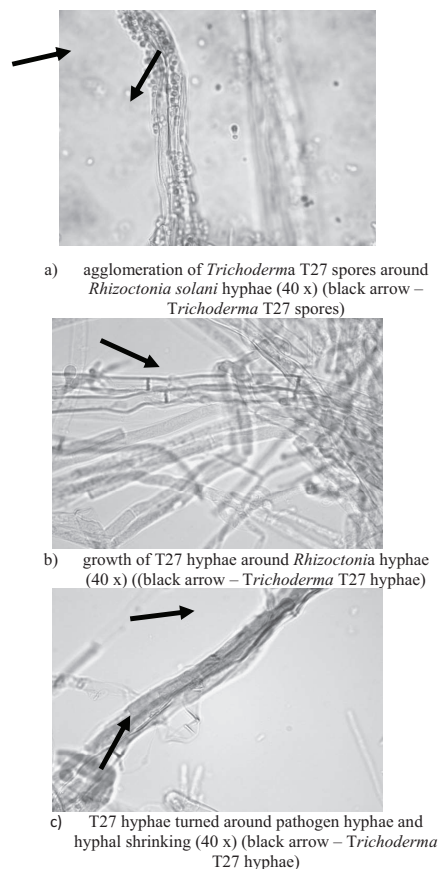


Fig. 3. Morphologicals characteristics between *Trichoderma* T27 and *Rhizoctonia solani*

CONCLUSIONS

This study demonstrated the efficacy of *Trichoderma* T27 in controlling several virulent pathogens, such as *Fusarium graminearum* ($X=1.05$), *Botrytis cinerea* ($X=1.07$) and *Pythium debaryanum* ($X=1.01$).

The result implied that the extent of inhibition by the fungi provides the use of potential antagonists capable of controlling the pathogenicity for sustainable agriculture.

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REFERENCES

1. Monte, E., 2001, Understanding *Trichoderma*: between biotechnology and microbial ecology, Int. Microbiol., 2001, 4, 1-4.
2. Perveen, K., Bokhari, N.A., 2012, *Antagonistic activity of Trichoderma harzianum and Trichoderma viride isolated from soil of date palm field against Fusarium oxysporum*, Afr. J. Microbiol. Res., 2012, 6(13):3348-3353.
3. Schubert, M., Fink, S., Schwarze, F.W.M.R., 2008, *In vitro screening of an antagonistic Trichoderma strain against wood decay fungi*, Arboricultural J., 2008, 31,;227–248.
4. Dennis, C.; Webster, J. *Antagonistic properties of species-groups of Trichoderma. III. Hyfal interaction*, Trans. Br. Mycol. Soc., 1971, 57:363- 369.
5. Brunner, K., Zeilinger, S., Ciliento, R., Woo, S.L., Lorito, M., Kubicek, C.P., Mach, R.L., 2005, Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance, Appl. Environ. Microbiol., 2005, 71(7):3959-3965.