IN VITRO INTERACTION BETWEEN *TRICHODERMA ASPERELLUM* ISOLATE AND OTHER PATHOGENIC FUNGI ON CORN

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Abstract: *Trichoderma* species are effective agents in biocontrol strategies and identifying their useful isolates for further studies seems to be helpful. The purpose of this study was to assess the *in-vitro using dual-culture technique* biocontrol efficacy of *Trichoderma asperellum* isolate Tdal12 against other important pathogens of corn (*F. oxysporum, F. graminearum, A. flavus*). These antagonistic interactions influence the incidence and severity of the disease caused by the pathogens. Evaluations were conducted in RDIPP, Phytosanitary Risk Laboratory during 2013.

Keywords: *in-vitro*, *Trichoderma asperellum, Fusarium oxysporum, Fusarium graminearum, Aspergillus flavus*

INTRODUCTION

Fungi are among the pathogens that affect foliage plants, causing diseases. A large number of fungi belonging to phycomycetes, ascomycetes and deuteromycetes are responsible for the majority of diseases (wilt, rot and seedlings-off) (Chase 1987).

Chemical compounds have been used to control plant diseases (chemical control), but abuse in their employment has favored the development of pathogens resistant to fungicides.

Unfortunately, the more specific the effect of a chemical on an organism, the greater the probability of decreasing the effect through genetic shifts in the population, whereas fungicides of broad spectrum produce undesirable consequences on non-target organisms. By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists. Moreover, the combination of such biological control agents (BCAs) with reduced levels of fungicide (integrated control) promotes a degree of disease suppression similar to that achieved with full fungicide treatment . Antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications have been carried out with different strains of the fungus *Trichoderma sp*.

Trichoderma sp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes like chitinase and glucanase seems to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal pathogens (Kucuk and Kivanc, 2008).

This study is to demonstrate the capacity and activity of *Trichoderma asperellum* Tdal12 strain isolated from Dobrogea area.

MATERIAL AND METHOD

Biological material has been collected from agricultural area Dobrogea, on plots with different agroweather and soil conditions (SC.SUNMEDEA S.R.L., Ferma Carasuhat-Mahmudia, Jud. Tulcea, 2012).

Fig. 1 Corn samples collected. kernels infected with *Aspergillus flavus*

Pathogens analyzed in research: *Aspergillus flavus*; *Fusarium graminearum, Fusarium oxysporum*

Beneficial microorganisms analyzed in research:

Beneficial fungus *Trichoderma asperellum* Tdal12 isolated on the corn seeds from agricultural Dobrogea area.

The level of antagonism of *Trichoderma asperellum*, Tdal12 strain against two strains isolated during the research from the corn kernels *Aspergillus flavus* and *Fusarium graminearum* highligted by dual culture method (Jouan şi al., 1964).

Tdal12 strain has been isolated from corn seeds coming from Dobrogea area (A. Paica, 2012). To isolate strain has been used water agar culture medium and for growing potato - glucose - agar medium (CGA).

Pathogenic fungi and antagonistic isolate was growing for 7 days on CGA medium in Petri dishes so that when testing the mycelium is developed in as uniform beach. The isolates were seeded on CGA medium by placing a mycelial discs (5 mm) at a distance of 3 cm from each other. Petri plates were seeded such incubated at 25^0C. Were made assessments of the

phenomenon of growth inhibition by measuring the clear zone at 4 and 8 days. The experiment was performed three repetitions.

Assessment of antagonistic action was carried out using a scale for assessing the development of colonies (number of colonies / Petri dish) is shown in Table 1.

Table 1

The scale of assessment of antagonism for the fungi of colony forming

of the *Fusarium* genus

X > 1	antagonism missing, isolated non-antagonistic (N);
X < 1	antagonism (A) the stronger (PA) as the values are closer to the value 0;
X = 1	absence of mutual influences regardless (I);

RESULTS AND DISCUSSION

Tdal12strain of *Trichoderma asperellum* isolated from corn seeds sampled from Dobrogea has a complex spectrum of action against fungi of the *Fusarium* genus manifested by antagonism and competition for food and space. To assess the degree of antagonism by dual culture method was tested on CGA medium the behavior of *T. asperellum* strain Tdal12 against two strains of *Fusarium* and *Aspergillus flavus* strain.

The value of the coefficient X is calculated for *Fusarium oxysporum* is comprised between 0.19 - 0.33 (4 days) with an average of 0.48 and between 0.34 - 0.38 (8 days), with an average of 0.37. These results falling Tdh all2 isolate in the class of antagonism (the coefficient x <1).

Compared with *Fusarium graminearum*, the values of the coefficient x varied between 0.30 and 0.37 (4 days) with an average value between 0.39 and 0.32 to 0.44 (8 days), with an average of 0 35.

For the *A. flavus* isolate, the coefficient x was 0.71 (after four days) and 0.59 (after 8 days).

Our results highlight the antagonist character of Tdh all2 strain against all three isolates subject to tests.

Table 2

Antagonism study of *Trichoderma asperellum* Tdal12 strain and strains of the genus *Aspergillus and Fusarium* isolates from corn

Antagonist	Pathogen test	Coef. X (Average)		Result
		4 days	8 days	8 days
***Trichoderma asperellum* Td al12**	***Fusarium oxysporum***	0,48	0.37	PA
	Fusarium graminearum	0,39	0,35	PA
	Aspergillus flavus	0,71	0,59	A

From the data analysed and direct macroscopic assessments revealed a reduced-moderate antagonism of *Trichoderma asperellum* Tdal12strain against *Aspergillus flavus*.

Trichoderma asperellum Tdal12 strain show a strong antagonism *in vitro* against strains of *Fusarium oxysporum* and *F. graminearum* used in experimentation.

The mechanism of action of *Trichoderma asperellum* Tdal12strain against pathogens can be explained by high sporulation capacity and competition for space and food that has inhibited the development of colonies of *Fusarium oxysporum and F. graminearum* used in experimentation.

Many research has indicated the complexity of the mechanisms for action by the genus *Trichoderma* isolates: antagonism (mycelium inhibit the growth of the pathogen); competition for food and space (release spores grow faster than pathogenic fungi spores and inhibits their development through their colonization) and induced resistance in plants (Rojan et al., 2010, Harman, 2006).

Trichoderma asperellum* Tdal12 and *Fusarium oxysporum

Trichoderma asperellum* Tdal12 and *Fusarium graminearum

Fig. 2 Highlighting the Tdal12 strain antagonist action against pathogens *Fusarium graminearum* (below) and *F. oxysporum* (top)

From the results obtained in vitro is found that *T. asperellum* strain Tdal12 can be classified as BCA (biological control agents), highlighting its capacity of strong antagonistic against isolates of *Fusarium*.

CONCLUSIONS

Our research fall within the thematic area of Phytosanitary Risks Laboratory (RDIPP Bucharest) from identification of new methodologies to reduce primary inoculum level of pathogenic and toxigenic fungi that grow in the soil and on plant debris and new opportunities for system sustainable agriculture by selecting a beneficial microorganism isolated from the seeds of corn antagonistic activity and ability to colonize the roots of corn plants slightly favoring the development of the radicular system, which helps, ultimately, to technology for optimizing the protection of corn.

Strain of *Trichoderma asperellum* Tdh 12 isolated from the corn seed taken from Dobrogea was characterized in that the antagonistic against *Fusarium and Aspergillus* isolates tested *in vitro*.

From the results obtained in vitro is found that *Trichoderma asperellum* strain Tdal12 can be classified as BCA (biological control agents).

Our study contributes to the identification new opportunities for sustainable agriculture in the agricultural Dobrogea area , through selecting a strain of *Trichoderma asperellum*.

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