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Original Research Article

Assays *In Vitro* of the biological control by using three species of *Trichoderma* against various species of *Fusarium* Agent of Fusarium

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A B S T R A C T

Three isolates of *Trichoderma* are isolated from the ground of different regions in Algeria. The antagonist effect of these isolates has been studied on seven isolates of *Fusarium* , agent of Fusarium Head Blight, according to the method of direct confrontation and to distance. The results obtained revealed that the isolates of *Tichoderma* were able to inhibit the mycelial growth of *Fusarium* of more than 65% compared to the witness, and this after four days of incubation at 25^0C. In addition, in the sixth day the *Trichoderma* invades the colonies of *Fusarium* on which it wetspored itself, thus revealing its high myco-parasitic capacity. The obtained results of the indirect confrontation show a slowdown of the mycelial growth of *Fusarium* strains induced by *Trichoderma*sp with a percentage reached 43% compared to the witnesses. This study conclude that the antagonistic strains can exert an inhibitory effect on the pathogenic colonies development.

Keywords

Trichoderma sp, Fusarium sp; mycelial growth; inhibition; antagonism.

Introduction

Several types of telluric mushrooms are capable of infecting the roots of wild and cultivated plants and cause significant damages. It is particularly about *Rhizoctonia*, *Verticillium* and *Fusarium* species ; the latter causes some diseases with important economic losses as the wilting vascular or root rot and the neck among the plants cultivated in fields and greenhouses (Fravel and al. , 2003).

In spite of the economic losses they cause, the control of these pathogens is still limited to Prophylactic measures; soil disinfection is never complete, first because of the difficulty of its achievement and on the other hand, because of the induction of resistant strains (Benhamou et al. 1997).

The biological control, precisely by using micro-organisms, is a very promising alternative to synthetic pesticide, because of the specificity and the effectiveness of the antagonist agent's action, the ubiquity of these natural agents in ecosystems, their large variety, their easy release and their persistence in the environment.

This study mainly concentrates about the interaction between seven species of *Fusarium* causative agent of fusarium head blight and three species of *Trichoderma* an antagonist agent active in biological control on various pathogens (Elad et al., 1982; Daami-Remadi, El Mahjoub, 2001).

Materials and Methods

Biological Material

The isolation of the pathogen agent "*Fusarium*" has been carried out from different organs of infected plants, samples were taken from several areas representative of different wilayas in Algeria (North, East and South). Small pieces of each organ were disinfected superficially by dipping in absolute ethanol for five minutes, rinsed then thoroughly with sterile distilled water to eliminate air contaminants (Benhamou et al., 1997).

After drying, the pieces were placed aseptically in Petri dishes containing sterile the medium PDA (Potato Dextrose Agar) ; followed by incubation of the Petri dishes in a drying oven set at 28 degrees Celsius for six days. The Subculturing of the strains was realized just after their appearance, in new dishes containing the PDA medium, to be purified, then listed (table 1) and maintained on PDA tilted, and on liquid medium (distilled water + glycerol), for future uses. Isolates identification is based on the macro and microscopic observations.

Isolation of the antagonist agent

All of the manipulations on the study of antagonist capacity against *Fusariumsp*. Have been carried out, using three strains of *Trichoderma* an antagonist agent active in biological control on various pathogens .

The three strains of *Trichoderma* used in this study were isolated from agricultural soil of different Wilayas in Algeria by using the method of suspension- dilution (Davet 1996 ; Davet and Rouxel, 1997). Strains identification was carried out by basing on their morphological characters. The strains were purified and listed (Table 2) and then kept on PDA tilted, and in liquid medium (distilled water + glycerol) for future use.

Antagonist activity *in vitro* of the *Trichoderma sp.* vis-to-screws of *Fusarium* isolates

The antagonistic activity *in vitro* of *Trichoderma* has been studied according to two methods. :

Confrontation technique by direct contact on culture medium

This technique consist to place, in the same Petri dish containing a PDA medium, two agar pellets (6 mm in diameter), one with the *Trichodermasp*. and the other contains the isolate of *Fusarium*. The two pads are placed following a radial axis to 3 cm and equidistant from the center of the dish (Figure 2); the transplanters is carried out at the same time (Benhamou and Chet, 1996). The incubation is carried out at 25°C for six days.

Notations concerning the diametric growth inhibition of *Fusarium* colonies species and their flooding by the mycelium of *Trichoderma* are carried out every 24 hours. In addition, the microscopic

Table.1 Origin and characterization of pathogenic isolates

Designations	Origins	Species
F1 (grain but)	Constantine (2011)	*Fusarium sp.1*
F2 (grain but)	Constantine (2011)	*Fusarium sp.2*
F3 (rod of olivier)	Setif (2012)	*Fusarium sp.3*
F4 (grain of the bean)	Constantine (2012)	*Fusarium sp.4*
F5 (palm leaves)	Biskra (2010)	*Fusarium sp.5*
F6 (palm leaves)	Biskra (2010)	*Fusarium sp.6*
F7 (roots of palm trees)	Ghardaya (2012)	*Fusarium sp.7*

Table.2 Origin and characterization of isolates antagonistic

Designations	Origins	Species
T1 (agricultural soil)	Ghardaya (2012)	*Trichoderma sp.1*
T2 (agricultural soil)	Collo (2012)	*Trichoderma sp.2*
T3 (agricultural soil)	Constantine (2012)	*Trichoderma sp.3*

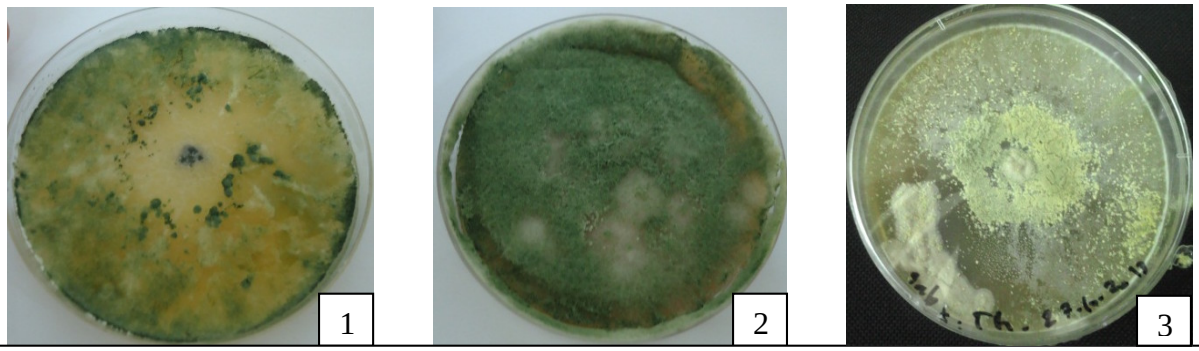


Figure.1 *Trichoderma*species, 1: *Trichoderma sp.1* aged 6 days on PDAmedium, 2: *Trichoderma sp.2* aged 6 days on PDA medium, 3: *Trichoderma sp.3* aged 6 days on Saboureaudmedium.

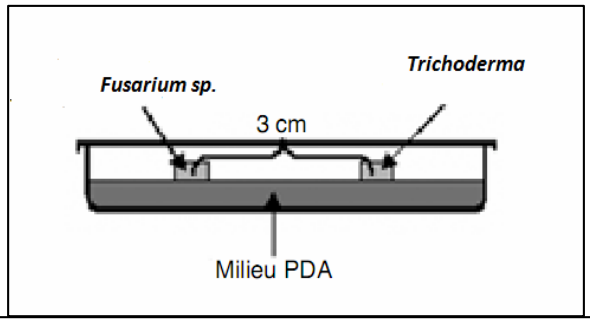


Figure 2. Confrontation equidistant from *Fusarium sp*. And *Trichoderma spp.* By direct contact on PDA medium

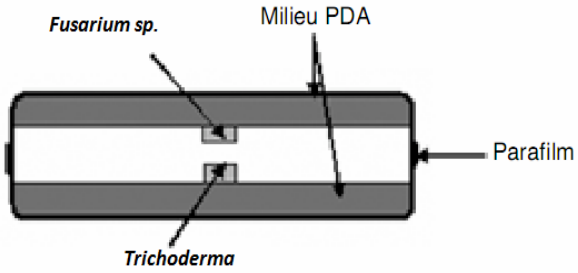


Figure.3 Confrontation to distance between *Fusarium spp. and Trichoderma sp.*

observations related to the antagonist agent direct effect on the state of the mycelium of *Fusarium* were carried out. The witness is constituted by a subculture of the pathogenic only in the center of the dish.

Confrontation to distance

This method consist to inoculate the antagonist and the pathogen in two separate dishes; subsequently, an assembly is achieved by superposition of two dishes, the *Trichoderma* at the bottom and the *Fusarium* in the top (Figure 3). The junction between the two dishes is ensured by layers of Para-film in order to avoid any loss of volatile substances (Daami-Remadi , El Mahjoub, 2001). The culture conditions are identical to those of the confrontation by direct contact on culture medium. Mycelial growth evaluation is realized every 24 hours by measuring the diameter of the mycelia colony of the pathogenic fungus.

The measures of the mycelial growth are taken daily and the test is achieved when one of the settlements will have covered the whole of the box. The evaluation of the inhibition exerted by *Trichoderma* is estimated by calculating the percentage of inhibition of the mycelial growth according to the following formula (Hmouni et al. , 1996) : I (%) = (1- Cn/Co) x 100 or Cn is the average diameter of the colonies in the presence of the antagonist and Co the average diameter of the colonies witnesses.

Results and Discussion

The results obtained show that the mycelial growth of strains witnesses is more important comparing to that obtained with the different confrontations (Pathogen - antagonist). After 144 hours of incubation, a inhibitory action exerted by the three species of *Trichoderma* against the mycelial growth of seven isolates of *Fusarium* was observed. We have seen the emergence of a zone of inhibition followed by a stop of growth for all the strains of the pathogen (Figure 4). The calculation of the inhibition rate has confirmed these results. All strains of *Fusarium* are inhibited with a percentage of more than 55 % and this regardless of the antagonist used. the F2 is determined to be the more sensitive strain with a rate of inhibition of 72% for T1, 69% for T2 and 68% for T3 (Figure 5).

On the qualitative side, the tests of direct confrontation have shown that, all three strains of *Trichoderma* have no significant or differential action on the *Fusarium*mycelial growth of the seven strains. At the end of fourth day of incubation, the box is totally invaded by the antagonist, whereas the *Fusarium* isolates occupy only a surface of 20 mm to 28 mm diameter; which corresponds to an inhibition greater than 65% of the mycelial growth. The witness of *Fusarium* cultivated separately occupies a surface diameterof 55 mm to 60 mm (Figure 6 to 12). Beyond this period and at the end of the sixth day, *Trichoderma* invades the colonies of *Fusarium* and wetspored even on the *Trichoderma*itself, thus revealing its highmyco-parasitic capacity (Benhamou, Chet, 1996; Daami-Remadi, 2001; Daami-Remadi, El Mahjoub, 2001). The envahissement of the pathogen mycelium by *Trichoderma* has also been observed by Benhamou and Chet (1997) by performing a direct confrontation on culture medium between this antagonist and another telluric fungus, the *Pythium ultimum* and this is in the end of fourth to fifth day after inoculation .

The microscopic observations carried out at the contact area between *Trichoderma* and *Fusarium*, show a winding of the *Trichoderma* mycelium on the *Fusarium* mycelium (Figure 13). Similar results were obtained with *T. Lignorum* which is able to wrap on the mycelium of the *Rhizoctonia solani* causing cytoplasm dissolution to the pathogen (Howell, 2003). *Trichoderma* isolates have been also tested by Daami-Remadi (2001) on *F. Solani* var *coerulum F.* Var, *roseum sambucinum* and *F*. Var*roseum gr aminearum*, responsible for dry rots on potato tubers toward which they induce also important lysis on the mycelium of these pathogens. Similarresults were observed for *Pythium sp* in the presence of the same antagonistic (Daami-Remadi , El Mahjoub, 2001). The *Trichoderma* are known for a long time for their antagonistic activities in respect of numerous fungi, *Botriti scinerea* (DUBORDIEU, 1983); *Armilaria obscura* and *Armilaria mellea* (LANUSSE et al. , 1983); *Rosellina nectarix* and *Phomopsis viticola* (BESSELAT, 1985); *Phytophtora citrophtora* and *Phytophthora parasitica* (CHET, 1984). DENNIS and WEBSTER (1971) highlighting the antibiotics secreted by *Trichoderma*, soluble in chloroform and extractable from the medium culture. Corresponding to COMPORATA (1985), this interpretation favors the enzymes action (β1-3) gluconase- chitinase which leads to the lysis of the pathogen mycelium.

In addition to the action of antibiotics, *Trichoderma* develops more rapidly comparing with *Fusarium* by colonising the nutrient medium and using its nutrients, it is the phenomenon of competition (ALABOUVETTE et al., 1983; DUBOT, 1985 ; DAVET, 1996).

Tests of confrontation to distance

The results of this test show a slowdown of the mycelial growth of *Fusarium* strains exerced by *Trichoderma sp* compared to the witnesses. Apparently, despite of the absence of a direct contact between the isolates of *Fusarium* and *Trichoderma* tested, the latter was able to exert an inhibitory effect on the development of *Fusarium* colonies, this effect is evaluated by measurement of *Fusarium* colonies diameters grown in the

presence and in the absence of the antagonist.

The estimation of the inhibition percentages of pathogenic strains by the three antagonist strains is demonstrated in figure 14. Unlike the test of direct confrontation, we note that the mycelial growth continues to evaluate with time (Figure 15 to 21). After 120 hours of incubation, the strain F5 seems to be the least sensitive to substances produced by the three strains of *Trichoderma* with an inhibition rate of 2% with T1 and 8% with T2 and T3. During the same period, the mycelial growth of the strain F4 is significantly more inhibited with an inhibition rate of 43% with T 1 and 44% with T2, the strain F6 shows a considerable sensitivity of 43% with T3. In general, the effect of volatile substances emitted by the three species of *Trichoderma* is significantly low. *Trichoderma sp1* is considered to be the most efficient with highest rates of inhibition (Figure 14).

This can be explained by the ability of *Trichoderma* to produce volatile substances which are able to limit and even stop the development of the pathogen agent (Hibar ready et al. 2005).

According to MESLOUHI (1989), DAVET (1983 and 1983) this inhibitory action is due to substances of chemical nature liberated by the strains of *Trichoderma* (antibiosis phenomenon). The ability to produce such substances varies according to the isolates of the same species or of different species. According to DENNIS and WEBSTERS (1971), the *Trichoderma* emit toxic chemicals which are derivatives of the hydrazine present under important forms of volatile substances.

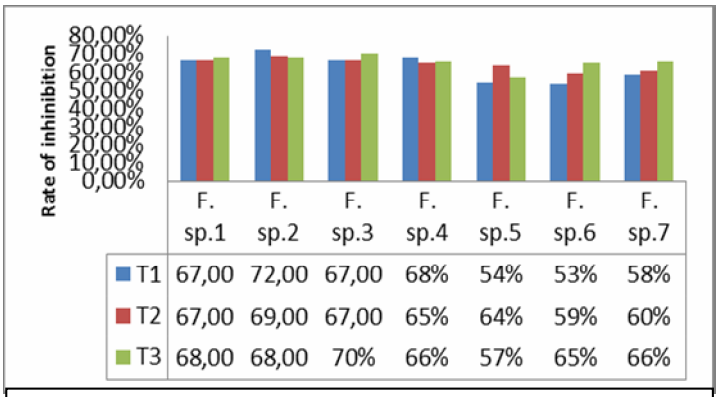
The aim of the present study is to test the effect *in vitro* of three species of *Trichoderma* on the mycelial growth of seven isolates from the genus*Fusarium*, one of the most pathogenic mushrooms of cultivated plants. Effectively, the tests of confrontations between the pathogenic isolates of *Fusarium* and the antagonists T1 , T2 and T3, either in a direct manner on culture medium or well from a distance, have shown inhibition of the mycelial growth of these tested pathogens. In the case of a direct contact between the two fungi, the antagonists show a capacity to attack the pathogens via different modes of action:

The antibiosis: which results from the production of substances that act as "antibiotics" and which inhibit the growth of the pathogenic agent remotely by forming a zone of lysis;

The competition: which is manifested by the rapid development of *Trichoderma* comparing to *Fusarium* in the process of colonizing nutrient medium and using its nutrients,

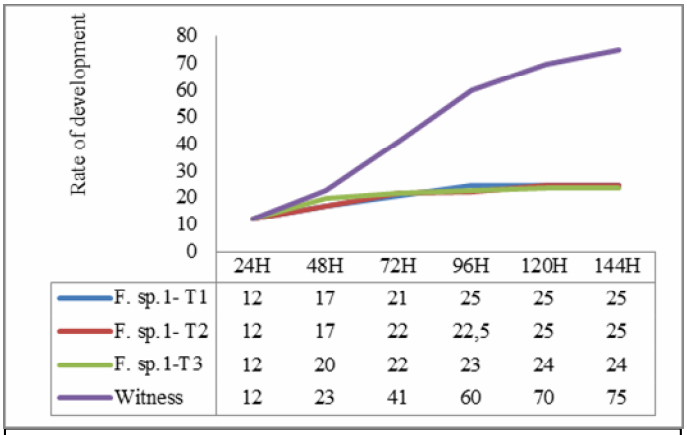
The parasitism:which is manifested by the destruction of the pathogenic agent when the antagonist is wounding around the latter.

In the case of confrontation to distance, despite the absence of direct contact between the pathogen and the antagonists, a decrease of coloniesdiameter of *Fusarium* is observed compared to the



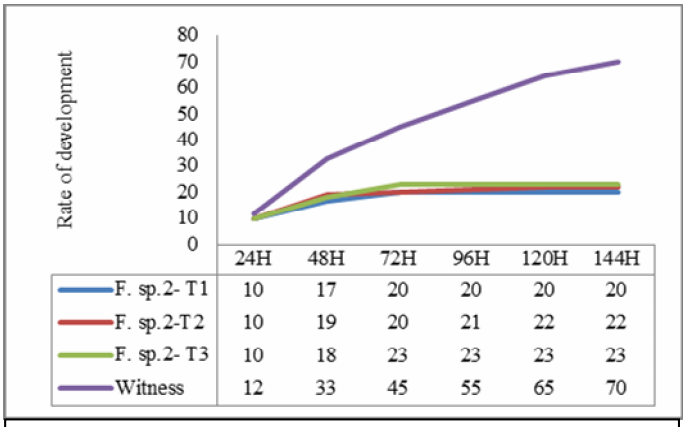
	F. sp.1	F. sp.2	F. sp.3	F. sp.4	F. sp.5	F. sp.6	F. sp.7
T1	67,00	72,00	67,00	68%	54%	53%	58%
T2	67,00	69,00	67,00	65%	64%	59%	60%
T3	68,00	68,00	70%	66%	57%	65%	66%

Fig.5 Estimate of the percentages of inhibition of pathogenic strains by the three strains antagonistic



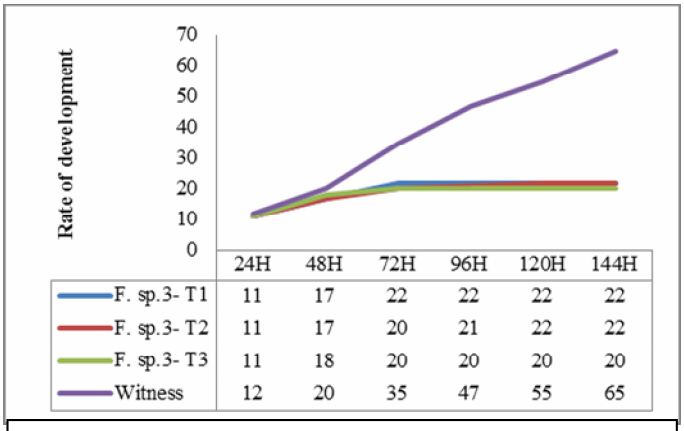
	24H	48H	72H	96H	120H	144H
F. sp.1- T1	12	17	21	25	25	25
F. sp.1- T2	12	17	22	22,5	25	25
F. sp.1-T3	12	20	22	23	24	24
Witness	12	23	41	60	70	75

Fig.6 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.1* (a technique of direct confrontation).



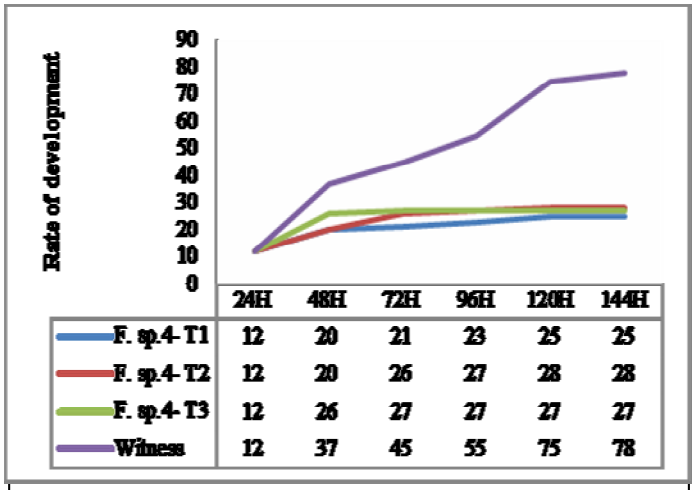
	24H	48H	72H	96H	120H	144H
F. sp.2- T1	10	17	20	20	20	20
F. sp.2-T2	10	19	20	21	22	22
F. sp.2- T3	10	18	23	23	23	23
Witness	12	33	45	55	65	70

Fig..7 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.2* (a technique of direct confrontation).



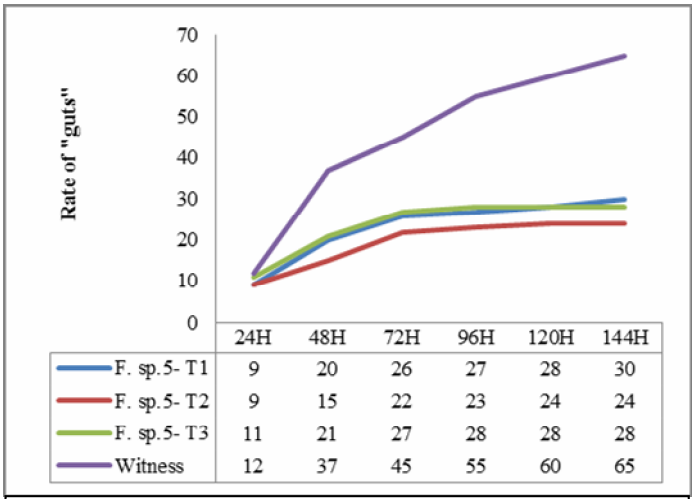
	24H	48H	72H	96H	120H	144H
F. sp.3- T1	11	17	22	22	22	22
F. sp.3- T2	11	17	20	21	22	22
F. sp.3- T3	11	18	20	20	20	20
Witness	12	20	35	47	55	65

Fig.8 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.3* (a technique of direct confrontation).



	24H	48H	72H	96H	120H	144H
F. sp.4- T1	12	20	21	23	25	25
F. sp.4- T2	12	20	26	27	28	28
F. sp.4- T3	12	26	27	27	27	27
Witness	12	37	45	55	75	78

Fig.9 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.4* (a technique of direct confrontation).



	24H	48H	72H	96H	120H	144H
F. sp.5- T1	9	20	26	27	28	30
F. sp.5- T2	9	15	22	23	24	24
F. sp.5- T3	11	21	27	28	28	28
Witness	12	37	45	55	60	65

Fig.10 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.5* (a technique of direct confrontation).

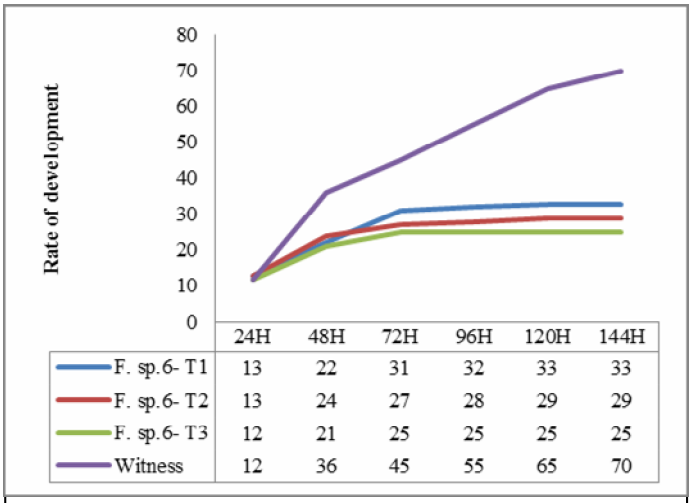


Fig.11 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.6* (a technique of direct confrontation).

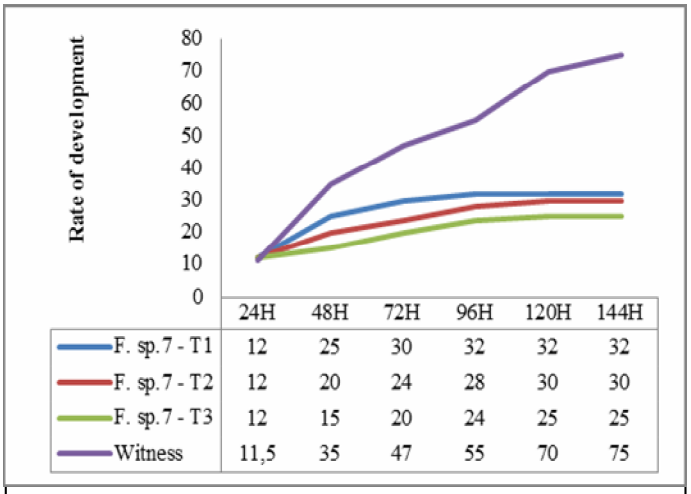


Fig.12 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.7* (a technique of direct confrontation).

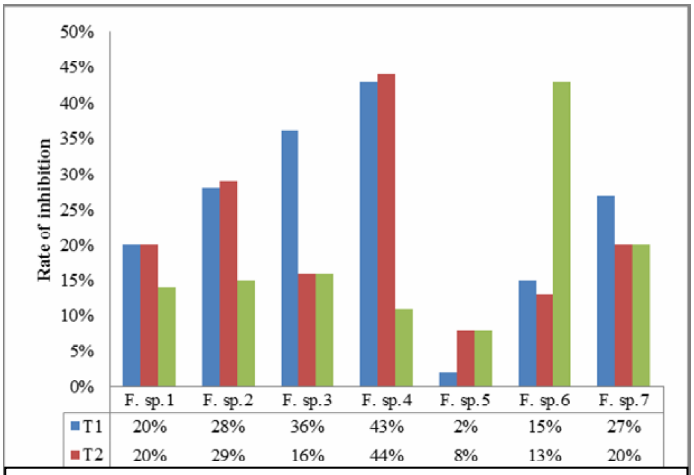


Fig.14 Estimate of the percentages of inhibition of pathogenic strains by the three strains antagonistic

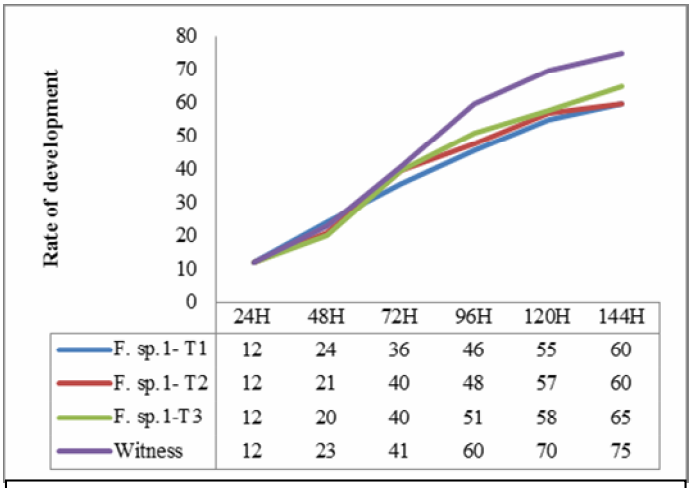


Fig.15 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.1* (a technique of direct confrontation).

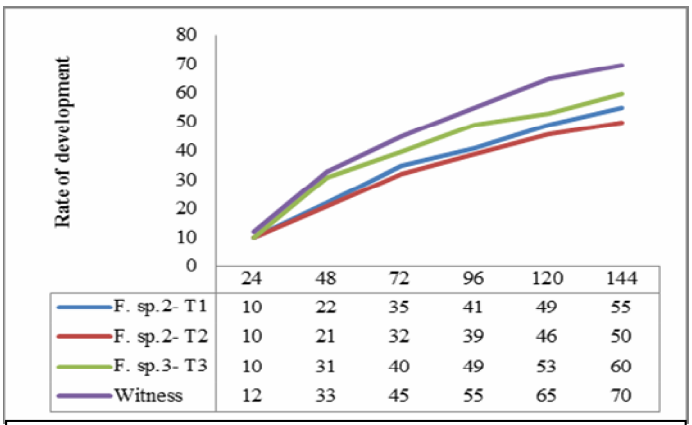


Fig.16 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.2* (a technique of direct confrontation).

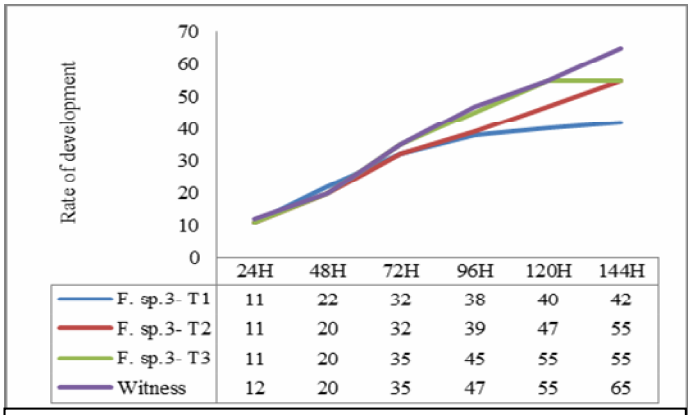
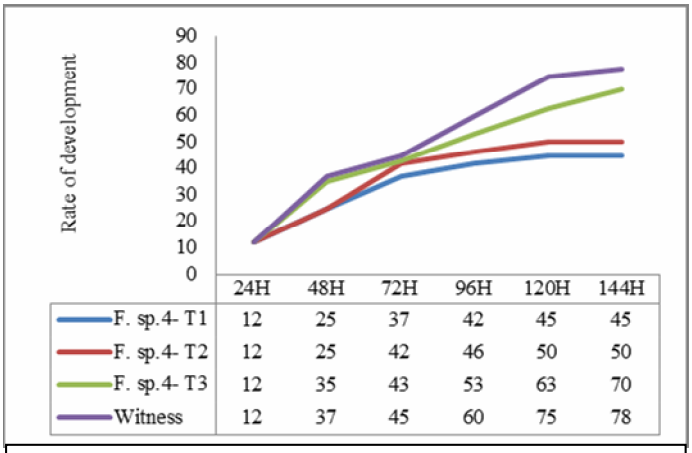
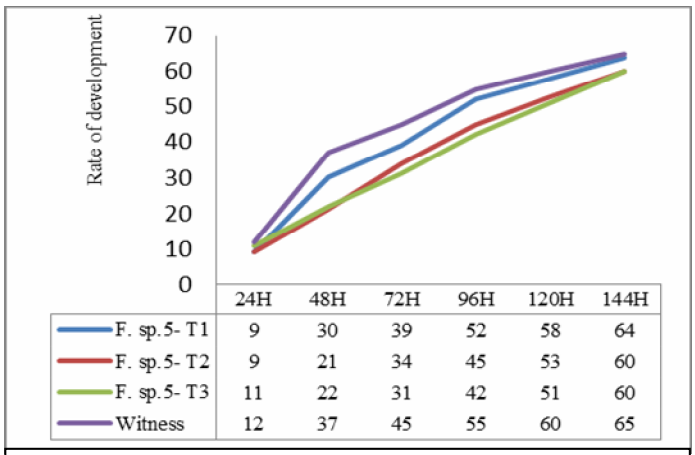


Fig.17 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.3* (a technique of direct confrontation).



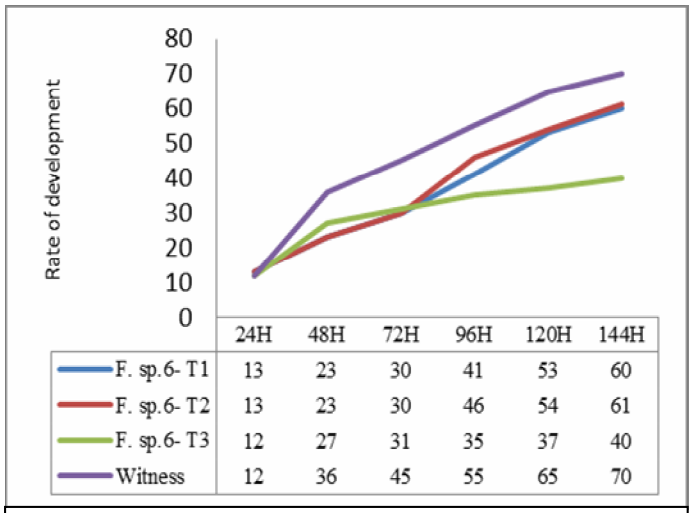
	24H	48H	72H	96H	120H	144H
F. sp.4- T1	12	25	37	42	45	45
F. sp.4- T2	12	25	42	46	50	50
F. sp.4- T3	12	35	43	53	63	70
Witness	12	37	45	60	75	78

Fig.18 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.4* (a technique of direct confrontation).



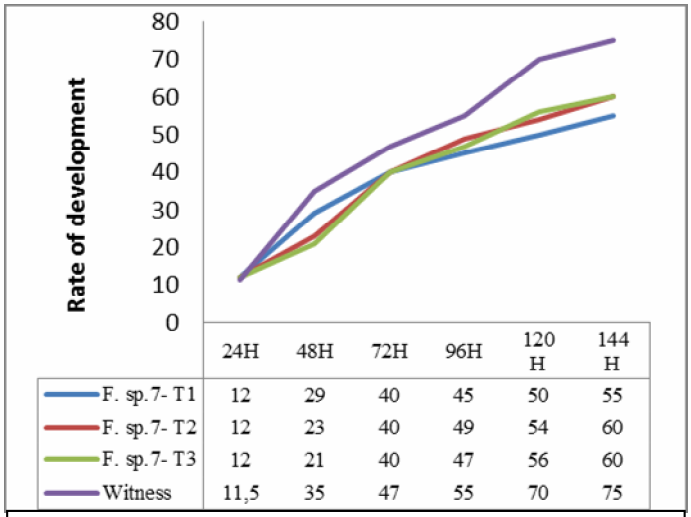
	24H	48H	72H	96H	120H	144H
F. sp.5- T1	9	30	39	52	58	64
F. sp.5- T2	9	21	34	45	53	60
F. sp.5- T3	11	22	31	42	51	60
Witness	12	37	45	55	60	65

Fig.19 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.5* (a technique of direct confrontation).



	24H	48H	72H	96H	120H	144H
F. sp.6- T1	13	23	30	41	53	60
F. sp.6- T2	13	23	30	46	54	61
F. sp.6- T3	12	27	31	35	37	40
Witness	12	36	45	55	65	70

Fig.20 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.6* (a technique of direct confrontation).



	24H	48H	72H	96H	120 H	144 H
F. sp.7- T1	12	29	40	45	50	55
F. sp.7- T2	12	23	40	49	54	60
F. sp.7- T3	12	21	40	47	56	60
Witness	11,5	35	47	55	70	75

Fig.21 Inhibitory effects of T1, T2 and T3 on the mycelial growth (mm) of *Fusarium sp.7* (a technique of direct confrontation).

untreated control, especially for the isolate F4. This proves that, in addition to its mycoparasitic capacity, *Trichoderma* may react by secreting volatile substances which are capable to decrease the development of the pathogen. At the end of this study, it can be interesting, on the one hand to extend the experimentation with the other species of *Fusarium* , and on the other hand to measure the antagonist effect *in situ* of *Trichoderma sp*. as a biological control agent against the genus *Fusarium*, especially as the active chemicals against this pathogen are relatively few.

References

Box, G. E., W.G. Hunter and Hunter, J.S.2005. "Statistics for Experimenters: Design, Innovation, and Discovery", 2nd Edition, Wiley.

Box, G. E. P., W.G. Hunter and Hunter, S. J.1978. "Statistics for Experimenters", John Wiley & Sons, Inc, New York, 1978.

Choi, H., S.R. Al-Abed, D.D. Dionysiou, E. Stathatos and Lianoss, P.2010. "TiO2 Based Advanced Oxidation Nanotechnologies for Water Purification and Reuse", Sustainability Sci. Engineer. 2: 229-254.

Jun Wang, W., Z. Zhaohong Zhang , Y. Rui Xu and Xiangdong, Z.2007. "Treatment of nano-sized rutile phase TiO2 powder under ultrasonic irradiation in hydrogen peroxide solution and investigation of its sonocatalytic activity", ultrasonic sonochemistry.

Manmohan Lal Kamboj, 2000. "Studies on the degradation of industrial waste water using heterogeneous photocatalysis", Department of biotechnology and environmental sciences, Thapar University, Patiala, pp.1-67.

Naresh, N., Mahamuni and Aniruddha B. Pandit. 2006."Effect of additives on ultrasonic degradation of phenol", Ultrasonic Sonochem.13:165–174.

Rajeshwar, K., J.G. Ibanez and Swain, G.M.1994. "Electrochemistry and the Environment", J. Appl. Electrochem. 24:1077.

Sandra Contreras Iglesias.2002. "Degradation and biodegradability enhancement of nitrobenzene and 2, 4-dichlorophenol by means of advanced oxidation processes based on ozone", Barcelona, pp.1-22.

Suneetha Parameswarappa, Chandrakant Karigar and Manjunath Nagenahalli. 2008. "Degradation of ethylbenzene by free and immobilized", Biodegrad.19:137–144.

US EPA.,1996. "Priority pollutants", Code of Federal regulations, Title 40, Chapter 1, Part 423, Appendix A. Environmental Protection Agency, Washington, DC.