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Antagonism capability in vitro of *Trichoderma harzianum* against some pathogenic fungi

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ABSTRACT

The aim of this study is to clarify the antagonism capability in vitro of the antagonistic fungus *(Trichoderma harzianum*) against the pathogenic fungus, four isolates of pathogenic fungus associated internally with the solid wheat seeds (*Triticum durum*)Desf , follower for species: *Botrytis cinerea*, *Cladosporium sp*, *Stemphylium botryosum* and *Alternaria sp*, were brought. The antagonistic sample (*Trichoderma harzianum*) was isolated from the wheat plant soil. The results showed that:The direct confrontation of *Trichoderma harzianum* against the different fungus isolates in vitro on PDA medium, showed in the third day of the experiment an inhibition in the pathogenic mycelia growth, with a different ratios, it was equal to: 41.66% and 50% for *Stemphylium botryosum* and *Cladosporium sp*, respectively, and amounted in the fourth day to 56.52% and 57.14% , for *Botrytis cinerea* and *Alternaria sp*, respectively, did not show any growth of the different pathogenic fungi when re-planting a disk from the interaction hyphal areas between the antagonistic fungus and the pathogenic fungus from the different dual cultures, while the antagonistic fungus was grown. The microscopic observations of the different interactions hyphal showed that the antagonistic fungus was affected on the pathogenic fungi with a several biological forms: Decomposition phenomenon (Lyses): the antagonistic fungus was analyzed the mycelia and spores of *Cladosporium sp*, while was analyzed the mycelia and damped the spore formation in the other pathogenic fungi, compared with control. Parasitism phenomenon (Mycoparasitism): it was found that the hyphae of *Trichoderma harzianum* has formed Haustoria on the cell walls of *Stemphylium botryosum* hyphae and they penetrated within them. The remote confrontation showed that the volatile metabolic substances of the antagonistic fungus affected the growth of the pathogenic fungi , with a different rates over the seven days of treatment, it peaked after two days of treatment to reach 13.33% and 50% in *Botrytis cinirea* and *Cladosporium sp*, respectively, and decreased to 08.33% in the fourth day in *Botrytis cinirea* and scored in the fifth day a ratio equal to 25.42% for *Cladosporium sp*, and was scored the maximum ratio in the third day in *Stemphylium botryosum* to 23.07%,and lowered to 07.93% in the seventh day, but in the *Alternararia sp* has recorded the lowest inhibition percentage to 05.55% in the third day and 05.76% in the seventh day. The microscopic observations Noted that the volatile metabolic substances of the antagonistic fungus was affected the pathogenic fungi with a several modes, their were with the mycelia analysis and prevent the spore formation in the *Alternararia sp*, while was analyzed and aggregated the spores in the *Cladosporium sp*, and stopped only the spore formation in both *Botrytis cinirea* and *Stemphylium botryosum*, compared with control.

Key words: metabolic volatile substances, wheat varieties, antagonistic, isolates, *Cladosporium*, *Trichoderma harzianum*.

INTRODUCTION:

The wheat seeds were be a favorable medium for the pathogenic mycoflore and carrying them, that causing a decrease in both seed vitality, and nutritional value, morever the excretion of mycotoxins. *Cladosporium cladosporioides*,is met on the leaves ,the fruits or the many vegetal grains, it settles there in the form of black spots. *Stemphylium*

botryosum is at the origin of the abnormal germs and germination lacks. It is especially a parasite of cycle end which is installed on the pods of leguminous plants, the beet clusters, the ears of cereals. *Botrytis cinerea* is a fungus which one meets on many seeds and the most varied plants .It is the origin of the seed damping off disease and a significant losses of output in the wet years ,it can be present in all the parts of the plant (stems,sheets,flowers,pods,ears,flowerheads).*Altern aria* genus contains a great number of species plus of sixty, parasites or saprophytes, there effect announced on seeds. *Alternaria* parasites are the origin of the germination lacks, sowing dissolution and there are significant inoculum sources of the adult plants. (Rémi, 1997). The use of the chemical fungicides to reduce or eliminate fungal diseases in many cases decreased at the same time the seed vitality (Moreno et al, 1981). The chemical pesticides cause a significant damage to the public health, environment and groundwater pollution; it is uneconomical, so that scientists went recently to the biological control. In bibliographical study for more than 200 research carried out by Mausam and al (2007) confirmed that the *Trichoderma sp* plays an important role in biological control, and it represents 60% of all other biofungicides include bacteria, nematode and virus, and was used as a pesticide, and herbicide, and use of this fungus contributes to the improvement of plant growth, also found. Jegathambigai and al (2009), developed the treatment of *Crossandra infundibuliformis* var.Danica with *Trichoderma viride* and *T.harzianum* decreased the wilt diseases caused by *Fusarium oxysporium* , and increased the plant growth in the field trials and in laboratory alike, and their study strongly suggests that *Trichoderma* isolates,especially, *T.viride* can be exploited for the biological control of wilt disease at field level. Also the use of *Trichoderma viride* and *T.harzianum* in the striving against the fungi associated with seeds, including *Aspergillus flavus* and *Fusarium moniliforme*, and also was used an anti-fungal against *Lasiodiplodia theobroma* and *Diplodia natalensis*, *Botryodiplodia theobromae*, *Rhizoctonia sp*, *Aspergillus niger*, *A.tamarii*, *Penicillium oxalicum*, *P.sclerotinum* [Calistru and al, 1997, Calistru and al, 1997,. Thangavelu and al, 2004, Okigbo and Okediugwu, 2000, Moreno and Paningbatan, 1995, Mortuza and Ilag, 1999].

The *Gloicladium* and *Trichoderma* have been mostly used as biofungicides agents; they showed a high inhibitory effect against certain fungal diseases exceeded the effect of some chemical pesticides, or same as them, [Illipronti and al, (1993).Harman and al, (1980)].

In this investigation, one antagonistic local isolate of *Trichoderma harzianum*, was evaluated against four isolates of pathogenic species: *Botrytis cinerea*, *Cladosporium sp*, *Stemphylium botryosum* and *Alternaria sp*.

MATERIALS AND METHODS:

Fungus material: Four isolates of pathogenic fungi, follower for species : *Botrytis cinerea*, *Cladosporium sp*, *Stemphylium botryosum*, *Alternaria sp*,were isolated from wheat seeds. and other sample of antagonist *Trichoderma harzianum* / *Hypocrea lixii* was isolated from the soil of wheat plant.All samples were identified in Laboratory of Microbiology ,University of Oum-elbouaghi(Algeria),but *Trichoderma* identification was confirmed by Professeur Thonart Philipe,Microbial biotechnology,Walloon Center of Biology Industrial,University of Liege,Belgium. to purposed the following studies:

1:Evaluation of the antagonistic capability in vitro of *Trichoderma harzianum* against the pathogenic fungi ,on PDA medium (direct confrontation): Dual culture technique: For study the confrontation vis-à-vis between the antagonistic fugus and the other pathogenic fungi, Two discs (8mm in the diameter) of one week old culture on (PDA), one carrying the stock of the antagonistic agent (*Trichoderma harzianum*)and the other the pathogenic agent were then placed at the periphery of Petri plate (9cm in diameter) at the same distance on PDA medium. One disc of each pathogenic agent was maintained as control (alone culture). Each replicates has three plates. Both the dual and alone cultures were incubated at 25°c for four days, and measurement of radial mycelia of the fungus were taken every 24 hours. The percentage growth inhibition (I) was calculated using the formula given below

[I (%) = (1 – T /C) x 100].

Where: I=Percentage inhibition of pathogen by antagonists. C=Radial growth in control.T=Radial growth in the treatment.

The speed of the fungal colony growth (V) was measured using the formula given below (Rapilly, 1968): V = [(L2-L1) + (L3-L2) ... (Ln-Ln-1] / n-1, V = the speed of growth (mm / day), L =mycelia growth (mm), L1 = growth in the first day. Ln = growth in the last day. D = (D1 + D2) / 2. L = D-d / 2. L = the

growth of the fungal mycelia (mm), D = diameter of the fungal colony (mm), d = diameter of the initial fungal disk. [1], [22], [10], [11].

Effect of the volatile substances of *Trichoderma harzianum* on the growth of the pathogenic fungi on the PDA medium (remote confrontation : This method consists in mending the antagonist fungus and the pathogenic one, in two plates separated thereafter, an assembly is carried out by the superposition of two plates , antagonist in bottom and the pathogenic one in top, the junction between the two plates is ensured by layers of parafilm in order to avoid all loss of volatile substances.

for study the effect of the volatile substances of *Trichoderma harzianum* on the growth of the pathogenic fungi on PDA medium, two discs (8mm in the diameter) of one week old culture on (PDA), one carrying the stock of the antagonistic agent (*Trichoderma harzianum*)and the other the pathogenic agent, were then placed at the center of Petri plates(9cm in diameter)containing PDA medium. The lids are removed aseptiquement, then the bottom of each plate containing the antagonist tested is placed below that containing the pathogenic fungus, the two juxtaposed bottoms are closed by layers of Parafilm. For the control, a bottom of plate containing the medium alone is invested below a bottom plate containing the pathogenic fungus. Each replicates has three plates, figure(1). Both the dual and the alone cultures were incubated at 25°c,in the darkness for four days, and measurement of radial mycelia growth were taken every 24 hours. The percentage growth inhibition (I) was calculated like previously.[10] and [11].

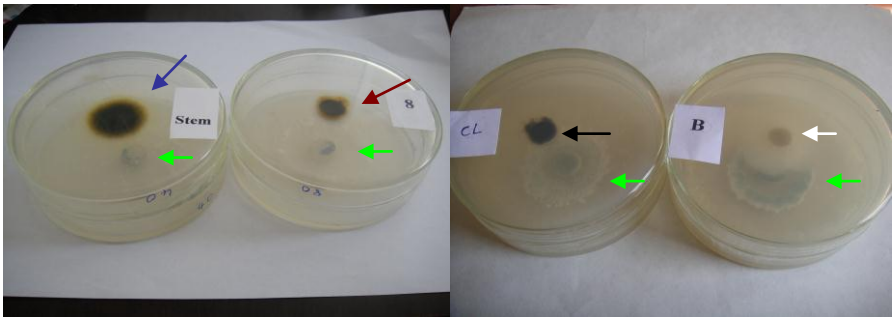


Fig 1: Method used in the impact study of the volatile substances of the antagonistic fungus *Trichoderma harzianum* (green arrows). Against the pathogenic fungi used in this study. (Stem) = *Stemphylium* (blue arrow).,(8)= *Alternaria* (brown arrow).. (CL)=*Cladosporium* (black arrow). (B) =*Botrytis* (white arrow).

RESULTS:

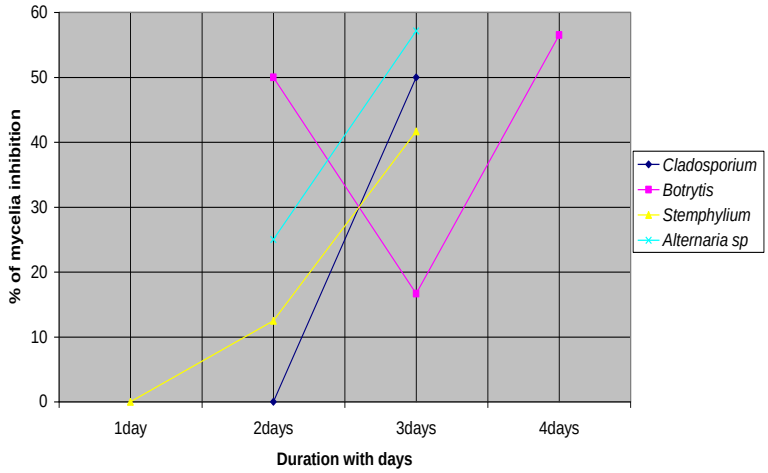
The antagonistic capability in vitro of *Trichderma harzianum* against the pathogenic fugi:The results showed that :

1- The direct confrontation of *Trichoderma harzianum* against the different fungal isolates in vitro on PDA medium, showed that when the mycelium of both the cultures came in contact with each other the hyphal growth of the pathogenic fungus were found to be inhibited by the hyphae of *Trichoderma harzianum*.This inhibition with a different ratios, it was equal in the third day of the experiment to: 41.66% and 50% for *Stemphylium botryosum* and *Cladosporium sp*, respectively, and amounted in the fourth day to 56.52% and 57.14% , for *Botrytis cinerea* and *Alternaria sp*, respectively .Table (01) and figures (2), (3-1A),(3-2A),(3-3A)and (3-4A). The results did not show any growth of the different pathogenic fungi, when re-planting a disk from the interaction hyphal areas between the antagonistic fungus and the pathogenic fungi from the different dual cultures, while the antagonistic fungus was grown. Figures (3-1B),(3-2B),(3-3B),(3-4B).

Table 1: Effect of *Trichoderma harzianum* on the mycelia growth of the pathogenic fungi, and fungus mycelia growth speed in the dual cultures, on PDA medium.

Test number	Fugul spieces	Percentage of the mycelia growth inhibition after:				speed fungus growth in dual cultures
		1 day	2 days	3 days	4 days	
01	*Botrytis cinerea*	/	50	16.66	56.52	0.8
	Trichoderma harzianum	/	/	/	/	4.8
02	*Cladosporium sp*	/	00	50	/	0.2
	Trichoderma harzianum	/	/	/	/	05
03	*Stemphylium botryosum*	00	12.5	41.66	/	0.8
	Trichoderma harzianum	/	/	/	/	04.8
04	*Alternaria sp*	/	00	25	57.14	0.8
	Trichoderma harzianum	/	/	/	/	04.8

Figure.2: Effect of *Trichoderma harzianum* on the mycelia growth of the pathogenic fungi in vitro(dual cultures) on PDA medium.



The microscopic observation of the different interactions hyphal displayed that the antagonistic fungus affected the pathogenic fungi with several biological forms:

A - Decomposition phenomenon (Lyses): the antagonistic fungus was analyzed the mycelia and spores of *Cladosporium sp*, figure (4 -1B), while was analyzed the mycelia and damped the spores formation in the other pathogenic fungi, compared with control. Figures [(4-2B), (4-3B) and (4-4B)].
B - Parasitism phenomenon (Mycoparasitism): it was found that the hyphae of *Trichoderma harzianum* has formed Haustoria on the cell wall of *Stemphylium botryosum* hyphae and were penetrated within them.Figure (5).

Effect of the volatile substances of *Trichoderma harzianum* on the growth of the pathogenic fungi on the PDA medium: The remote confrontation showed that the volatile metabolic substances of the antagonistic fungus affected the growth of the fungi studied, with different rates over the seven days of treatment, it was peaked after two days of treatment to reached 13.33% and 50% in *Botrytis cinirea* and *Cladosporium sp*, respectively, and decreased to 08.33% in the fourth day in *Botrytis cinirea* and scored in the fifth day a ratio equal to 25.42% for *Cladosporium sp*, and was scored the maximum ratio in the third day in *Stemphylium botryosum* to 23.07%,and lowered to 07.93% in the seventh day, but in the *Alternararia sp* has recorded the lowest inhibition percentage to 05.55% in the third day and 05.76% in the seventh day. [Table (02) and figure (6)]. The microscopic observations Noted that the volatile metabolic substances of the antagonistic fungus affected the pathogenic fungi with several modes, their were with the mycelia analysis and prevent the spores formation in the *Alternararia sp*, figure (4-4C), while was analyzed and aggregated the spores in the *Cladosporium sp*, figure(4-1C), and stopped only the spore formation in both *Botrytis cinirea* and *Stemphylium botryosum*, compared with control, figures [(4-2C) and (4-3C)].

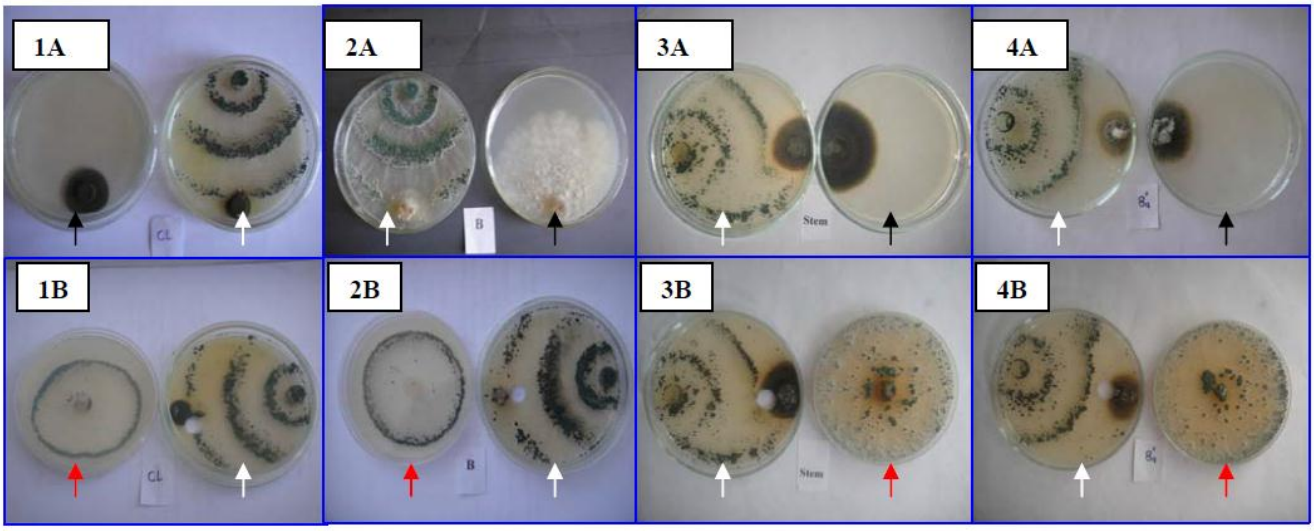


Fig.3: Direct confrontation on PDA medium, between the *Trichoderma harzianum* (green colony), and the pathogenic fungi, on (dual cultures) (white arrows). Compared with the alone cultures (controls) (black arrows). (1)=*Cladosporium*. (2)=*Botrytis*. (3)=*Stemphylium*. (04)=*Alternaria*. (A)= First test. (B)=After replanting a disc from the interaction areas, where the growth of the *Trichoderma* only appear (green colony) (red arrows).

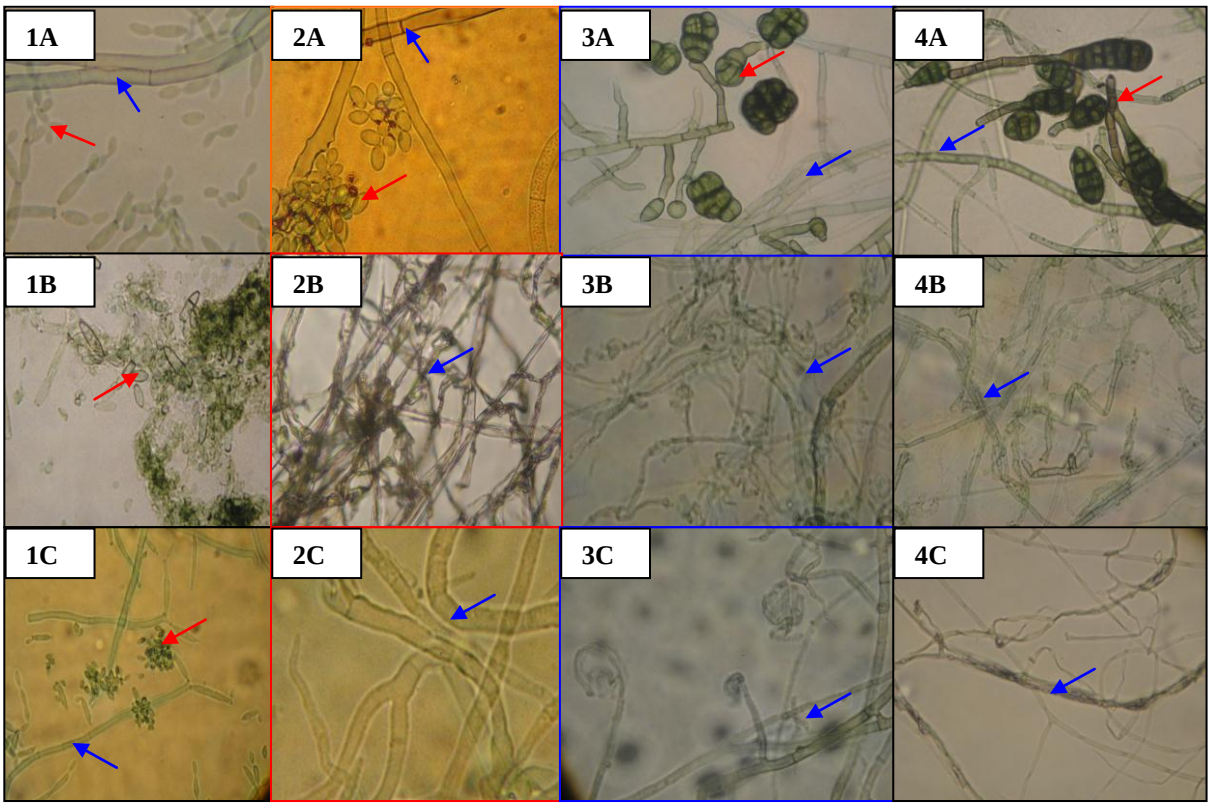


Fig 4 :Microscopic observations showed the affect of the *Trichoderma harzianum* and its volatile metabolite substances in the mycelia (blue arrows)and spore formation(red arrows) of the pathogenic fungi compared with controls. (1)=*Cladosporium*. (2)=*Botrytis*. (3)=*Stemphylium*. (4)=*Alternaria*. (A)=Controls. (B)= Direct confrontation affect.(C)=Affect of the volatile substances. 40 x.

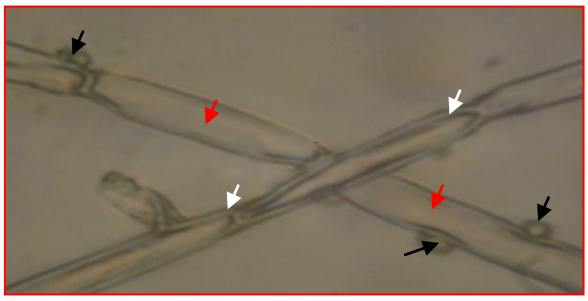
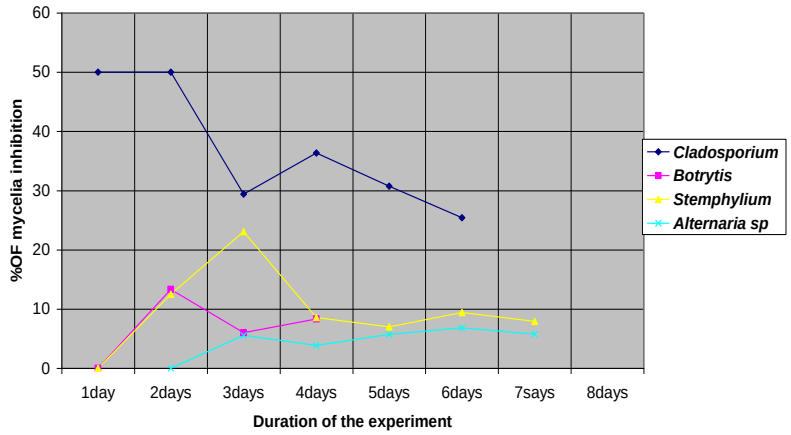


Fig 5: Microscopic observation of haustoria formation (black arrows) and penetration within the large hyphae of *Stemphylium botryosum* (red arrows) by the smaller hyphae of *Trichoderma harzianum* (white arrows). 100 x

Table 2: Percentage of the mycelia growth inhibition of the Pathogenic fungi by the volatile substances of the *Trichoderma harzianum*, in the dual cultures , on PDA medium.

Fungus species	Percentage of the mycelia growth inhibition after:							
	1ST day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
Botrytis cinerea	00	13.13	06	8.33	/	/	/	/
Cladosporium sp	50	50	36.36	30.76	25.42	/	/	/
Stemphylium botryosum	00	12.5	23.07	08.57	06.97	09.43	07.93	/
Alternaria sp	/	00	05.55	03.84	05.71	06.81	05.76	/

Figure 6 : Inhibition of the volatile substances of *Trichoderma harzianum* on the myelia growth of the pathogenic fungi onPDA medium



DISCUSSION:

The results of this study revealed that the antagonistic fungus(*Trichoderma harzianum*) has a high inhibitory effect against the different pathogenic fungi, with several biological modes. Competition, with his growth in the dual cultures where is a faster than the growth of the different pathogenic fungi (Table 1). Mycoparasitism, (Figure 5), lyses (figure 4), volatiles substances (Table 2) and [Figures 4(1C, 2C, 3C)].This results has been reported and confirmed by Fadwa and al [11], when studying the effect of the antagonism in vitro between six isolates of the antagonistic fungus *Trichoderma harzianum* and *T. viride* against four pathogenic isolates of *Bipolaris* and found that the *Trichoderma harzianum* inhibit the pathogenic growth with a different rations, including the following: 68.55-72% and 69.52-73.32% for each of *B.maydis* and *B.sorghicola* respectively, and 67.02-70.02% for each of *B.sorokiniana* and *B.tetramra*,and *Trichoderma viride* was inhibited the pathogenic fungi *B.maydis* and *B.sorghicola* and *B.sorokiniana* and *B.tetramera* respectively as follows: 67.55-74.48% and 69.52-82.85% and 68.12-73.61% and 71.22-76.66%, and concluded the inhibited spore formation in different proportions ,and the volatile metabolic substances of different antagonistic isolates were affected the mycelia growth and spores formation of the pathogenic fungi with a different rates, with recording a different

degrees of parasitism of various antagonistic fungi at the pathogenic isolates. Hibar and al [13] that the antagonism in vitro of *Trichoderma harzianum* against Fusarium *oxysporium* showed inhibited growth of pathogenic fungus more than 65%, and volatile metabolism substances of the antagonism reduced the growth of the pathogenic fungus by 63% compared with controls. Through the work done by Comporota [10] studied the antagonism in vitro, between 28 biological isolation of *Trichoderma* ,follower for species: 14 isolates of *T.harzianum*, 5 isolates of *T.hamatum*, 3 isolates of *T.viride*, one of *T.koningii* and 5 non-specific type , on 3 isolates of the pathogenic fungus *Rhizoctonia solani* Kuhn,and the different antagonistc isolates showed a different effect on the pathogenic fungus which affected the mycelia growth with a different degrees, and their volatile metabolism substances inhibited the

mycelia growth and spores formation of the pathogenic fungi . And also including by Larralde et al [15], when the choice(9) fungal isolates of *Trichoderma*: 2 of *T.atroviride*, 2 of *T.longibrachiatum* and 1 of each *T.reesi* and *T.koningiopsis* and *T.citrinoviride* and 02 did no- specific type from 30 isolates of *Trichoderma*, where they inhibited the growth of pathogenic fungus *Macrophomina phaseolina* with proportions higher than 50% during the antagonism study, and the microscopic observations in the hyphal interaction showed that the fungal antagonistic fungus has an ability to analyze the hyphae and sclerotes of the pathogenic fung, the analysis of the metabolic substances of these antagonistic fungi in laboratory revealed that there is a positive correlation between the strength of inhibition of these fungi with the high quantity of enzymatic production of B-1, 3glucanase and N-acetylhexosaminade .Azza and Aly [2]. discovered that the *Trichoderma sp* isolates have a strong antagonism against wilt diseases caused by *Fusarium sp*, in vitro ,on potato dextrose agar medium, when decreased his growth with the following proportions: (88%) , (86%) and (80%) for *Trichoderma harzianum*, *T.hamatum* and *T. viride* respectively. Ramsy and al [6]. Found that the *Rhizopus stolonifer* and *Trichoderma harzianum* and *T. viride*, inhibited the mycelia growth,and lowered the proportion of spore germination and spore tube lengths of *Bipolaris oryzae* and *Pyricularia oryzae* .The treatment of cowpea seeds with spore suspension of *Trichoderma viride* has protected them against the brown blotch disease caused by *Colletotrichum truncatum* and found that the *T.viride* produce a volatile and non-volatile organic compounds, Viridin and antibiotics, biofungicides. (Bankole and Adebanjo, [2]). Also found that the isolate (T60) of *Trichoderma viride* Used as a commercial biopesticide against *Coniophora puteana* and *Postia placenta* and *Serpula lacrymans* has a multiple effects, there are with a volatile and non-volatile organic compounds, Lytic enzyme and soluble antibiotics in the water, and nutrient competition. (Brown and Bruce,[5]. Brown and al[7]).

These results showed that a high efficacy of this local isolate of *Trichoderma harzianum*, against a few pathogenic seed borne fungi dangerous to the plant and the consumer, this study strongly suggests that this *Trichoderma harzianum*, isolate can be exploited for the biological control of seed diseases at the field level.

REFERENCES:

[1] -Azza, A.Tawfik. and D.A.Allam. 2004.Improving cumin production under soil infestation with Fusarium Pathogen 1-screening of biocontrol agents .Ass. Univ. Bull.Environ.Res.2, 35-45.

[2]- Bankole,S.A.,A.Adebanjo.1996.Biocontrol of brown blotch of Cowpea caused by *Colletotricum truncatum* with *Trichoderma viride* .Crop Prot.15:633-636.

[3]- Booth, C.1977. Fusarium laboratory guide to the identification of the Major species .Commonwealth Mycological Institute. England.58p.

[4] - Botton,B.,Breton,A.,Fevre,M.,Gauthir,S.,Larpent,J.P.,Gay,P.H., Reymond,P.,Sanglier,J.J.,VayssierY.and Veau,P.1990. Moisissures utiles et nuisible importance industrielle 2 ème Ed .Masson, Paris. Milan, Barcelone, Mexico, 512pp.

[5]- Brown,H.L.,A.Bruce.1999.Assessment of the biocontrol Potential of a *Trichoderma viride* isolate Part I: Establishmen of field and fungul cellartials .Int.Biodeterior Biodegard.44:219-223.

[7]-Brown,H.L.,A.Bruce.,H.J.Staines.1999.Assessment of the Biocontrol Potential of a *Trichoderma viride* isolate PartII: Protection against soft rot and basidiomycetes decay.Int. Biodeterior .Biodegad.44:225-231.

[8]- Calistru,C.,M.McLean,.P.Berjak.1997.In vitro studies on the Potential for biological control of *Aspergillus flavus* and

Fusarium moniliforme by Trichoderma species.A study of the

Production of extracellular metabolites by Trichoderma species Mycopathologia.137:115-121.

[9]- Calistru,C.,M.McLean,.P.Berjak.1997.In vitro studies on the Potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by Trichoderma

species1. Macro- Scopical and microscopical observations of fungal interactions Mycopathologia.139:115-121.

[10]- Camporota,Pierre.1985.Antagonisme in vitro de *Trichoderma spp* vis-à-vis de *Rhizoctonia solani* Kuhn.Agronomie,5(7):613-620.

[11]-Fadwa. Berber,Amina,Ouazzani ,Touhami.,Alain,Badoc.,Allal,Douira.2009. Antagonisme in vitro ET in vivo de deux Trichoderma à l'Egard de quatre espèces de Bipolaris pathogens sur le Sorgho.Bull, Soc.Pharm, Bordeaux, 148:93-114.

[12]-Fernandez, A., Stroshine, A.and Tuit, J. 1984.Mold growth And carbon dioxide production during storagee of high Moisture corn Cereal Chemistry, 62:137-144.

[13]-Hibar,Khaled.,Mejda,Daami-Remadi.,Haifa,Khiareddine.,Mohamed,

Elmahjoub. 2005.Effet inhibiteur in vitro et in vivo du *Trichoderma harzianum* sur *Fusarium oxysporium* f.sp.radicis lycopersici. Biotechnol.Agron.Soc.Environ.9(5):163-171.

[14]- Jegathambigai,V.,R.S.Wilson.Wijeratnam and R.L.C.Wijesundera 2009.Trichoderma as a seed treatment to control Helminthosp- orium leaf spot disease of *Chrysalidocarpus lutescens*.World Journal of Agricultural Sciences.5(6):720-728.

[15]-Larrade-Corona C.P.,M.R.Santiago-Mena.,A.M.Sifuentes-Rincon.,I.C.Rodriguez-Perez.,K.Shirai.,J.A.Narvaez-Zapata.(2008).Biocontrol potential characterization of novel native Trichoderma strains against Macrophomina phaseolina isolated from sorghum and commonbean.Appl.Microbiol Biotechnol:167-177.

[16]-Mausam,Verma.,Satinder.K.Brar.,R.Y.Surampalli.,J.R. Valero.

2007.Antagonistic Fungi, *Trichoderma spp*.:Panoply of Biological control.Biochemical Engineering Journal,37:1-20.

[17]-Moreno,M.E. and G.Vidal-Gaona.1986.Preserving the viability of Seed with fungicides. Plant Disease, 65:260-261.

[18]- Okigbo,R.N.,F.E.Oikediugwu.2000.Studies on biological control of postharvest rot in yams Dioscorea spp using *Trichoderma viride.* J.Phytopathol.148:259-263.

[19]-Moreno,L.S.,R.A.Paningbatan.1995.Biological control of mango Stem-end rot caused by *Diplodia natalensis* with *Trichoderma Viride* .Philippine Phytopathol.31:103-116.

[20]-Mortuza,H.G.,L.L.Ilag.1999.Potential for biocontrol of Lasiodip- lodia theobromae (Pat) Griff.and Maubl.in banana fruits by Trichoderma species.Biol.Control.15:235-240.

[21]- Ramsy,M.R.1991.Studies on rice diseases in Egypt Ph.D.Thesis, Faculty of Agric. Alexandria University.

[22]- Rapilly, F.1968.Les techniques de mycology en pathologie Végétale .Annales Epiphyties, INRA.Paris, 210p.

[23] - Rémi, Champion.1997.Identifier les champignons transmis par les semences. INRA. France, 399p.

[24]-Thangavelu,R.,A.Palaniswami.,R.Velazhahan.2004.Mass product ion of *Trichoderma harzianum* for managing fusarium wilt of banana .Agric.Ecosys.Environ.103:259-263.