AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICA
ISSN Print: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna.2011.2.4.584.590

The antagonism between *Trichoderma viride* and other pathogenic fungal strains in *Zea mays*

Bouziane Z.,*[1] Dehimat L.,[2] .Abdel aziz W.,[2] Benabdelkader M.,[3] Kacem chaouche N[2]

Laboratory of Applied Mycology Department of Biology and Ecology
University Mentouri Constantine - Algeria

ABSTRACT

Fungi such as *Trichoderma* and *Gliocladium* associated with parasitic behavior manifested by a coil around the hyphae of fungi filaments. This study showed the antagonistic effect of *Trichoderma viride* against the different fungal isolates infecting the plant *Zea mays*. The strain of *Trichoderma viride* was isolated from Jijel soil character by humid climate. By different organs (roots, stems and leaves) of plant *Zea mays* have been isolated 28 fungal strains belonging to 17 genus: *Absidia, Alternaria, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Geotrichum, Melanconium, Monileilla, Penicillium, Phoma, Pythium, Scopulariopsis, Scytalidium, Trichoderma* and *Ulocladium*. The test of direct confrontation between *Trichoderma viride* and fungal isolates was made on PDA. The competitive action of *Trichoderma viride* on the pathogen in the presence or absence of zone of inhibition was seen in this test. The growth rate of fungal isolates and *Trichoderma viride* has been determined. *Trichoderma viride* reached the confluence of the Petri dish four days after sowing, so that different fungal isolates occupy a surface of 29% to *Fusarium roseum*, 13% for *Epicoccum sp2*, 44% for *Epicoccum sp3*, 21% for *Monileilla sp*, 15% for *Absidia sp* and 6% for *Trichoderma sp2* which corresponds to an inhibition of mycelia growth of fungal isolates tested.

Keywords: confrontation, *Trichoderma viride*, pathogen, competition, biocontrol

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).

Several mechanisms are important in antagonistic interactions, including mycoparasitism and competition for substrates and sites of infection (Benitez *and al*. 1998). The fungal strains for biological control against plant pathogens must have an activity that is manifested by the ability to use the same *Trichoderma* community resources that pathogenic fungi, *Trichoderma*, but uses this mode of action primarily to occupy the premises before the arrival of events. (Gaetan LeFloch *and al*. 2006)

In order to find alternative control against the phytopathogens. Our study is to demonstrate the capacity and activity of *Trichoderma viride* isolated from Jijel soil character by humid climate where we are cultivated *Zea mays*.

The capacity of *Trichoderma viride* is translated by the inhibition of growth of many fungal strains isolated from different organs (roots, stems and leaves) of the plant.

MATERIALS AND METHODS

Sampling: Plants of maize (*Zea mays*), exist in the region of Jijel showing unhealthy symptoms were completely removed (ex: wilting). The plants have been wiped from moisture, placed in sterile paper bags, and tacked in laboratory. The analysis in leaves, stems and roots infected was realized by the method of (Davet and Rouxel 1997). Various parts of plant have been washed thoroughly with running water, cuted into pieces and placed in becher containing 0.1% HgCl2 for 2 minutes to disinfect surface and then washed three times in succession with distilled water, and finally dried with sterile paper Wattman sterile. These fragments were placed on PDA in Petri dishes (potato 200g, 20g glucose, 20g agar and 1000ml distilled water, (Guraud 1998) and incubated at a temperature of 25c° for five days. After incubation colonies of different fungi grow on culture medium.

Purification of isolates: In order to purifite the purification of fungal isolates, we applied the dilution method which involves taking, using a sterile

platinum wire, friction from the Petri dish containing several colonies and that we putted in test tubes containing 10ml sterile saline. After stirring, we oriented to prepare decimals dilutions until obtained a single spore / ml. Spores (monospores) were seeded in Petri dishes containing water agar (20g agar agar and 1000ml distilled water (Guraud 1998). After incubation in 18h at 25c ° the cutting Mycelia searched using a microscope to be inoculated in a Petri dish containing PDA (Botton and *al*.1985). **Identification:** Microscopic examination of fungal isolates permitted the identification of the micro flora, the identification of fungal species was based on: micro-morphology, aspect and structure of conidies according (Botton *and al*. 1985).

The pathogen: The pathogen used is represented by fungal strains isolated from different organs (roots, stems and leaves) of maize plant infected in Jijel town (North - East) of Algeria. The different fungal isolates were identified in the laboratory of Applied Mycology. The university of Mentouri, Constantina town and conserved for future use.

The antagonistic agent: We used the strain of *Trichoderma viride* isolated from soil of the Jijel town, where we were grown the corn plant. *Trichoderma viride* has been identified in the laboratory of Applied Mycology. University. Mentouri. Constantine. Algeria.

METHODOLOGY

The antagonist activity on PDA of *Trichoderma viride* was studied by the method of (Comporta 1985) ;(Patel and Brown 1969). We used the method of direct confrontation; this method is consisted to put on the same Petri dish containing 15ml of PDA medium, two agar pellets (8 mm diameter) one strain of *Trichoderma viride* and other agent pathogen are positioned along a diametrical axis 3cm away. The control is presented only by the pathogen; incubation is performed at 25c° for six days in the dark.

The evolution of mycelial growth is performed every 24 hours by measuring the diameter of the colony of the pathogen and the antagonist. The valuation of inhibition by *Trichoderma viride* is estimated by calculating the percentage inhibition of mycelia growth by the following formula: I% = (1-Cn/Co) x100)

Cn is the average diameter of colonies of pathogen in the presence of the antagonist and **Co** the average diameter of colonies of control.

RESULTS AND DISCUSSION

All samples analyzed were contaminated with mold. Several fungi have been isolated and identified. Indeed, 28 fungal isolates were isolated belonging to 17 genus: *Absidia, Alternaria, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Geotrichum, Melanconium, Monileilla, Penicillium, Phoma, Pythium, Scopilariopsis, Scytalidium, Trichoderma and Ulocladium*. The results are presented in Table 1.

The majority of detected fungal isolates are principally a field mold, with variable infected percentage 3, 57% to *Verticillium sp,* 7.14% *Fusarium sp*, 10.71% to *Phoma sp 3,* 57% to *Botrytis sp*, 10.71% to *Epicoccum sp*, 10.71% to *Alternaria sp*, 7.14% to *Trichoderma sp*, 3.57%*Pythium sp* with and 7.14% to Penicillium *sp*. Other molds are relatively less frequent*: Absidia sp, Aspergillus sp* and *Melanconium sp* with 3.57% for each isolate.

The isolation from the roots is revealed the presence of three genus *Trichoderma, Pythium* and *Verticillium*. The presence of *Trichoderma* and *Pythium* on root explained by the two nonpathogenic species and by humidity and water is very high in the roots. *Pythium* was present a nombrous interested characters for a biological control agent (Le Floch and *al*.2003a); (Roquebert 1996). It is known to reduce the severity of the pathogen agent, but its survival and propagation in culture is also promoted by the production of oospores. *Pythium* may be inter in competition with the endogenous flora (Gaetan Le Floch *and al*. 2006). Several researchers have reported that *Trichoderma* species are characterized by their mode of action primarily to occupy the site before the arrival of phytopathogenic, and have a good activity mycoparasitic and competitive ability (Chet 1987). *Trichoderma* is a natural fungus that colonizes the soil and plant roots before phytopathogenic, it can play a role in the health of plants (Gams and Bissett 1998). Furthermore, to level the stem were encountered four different genus: *Absidia*, *Alternaria, Fusarium* and *Monileilla*.

On the leaves were isolated twenty fungal strains belonging to 12 genus*: Alternaria, Aspergillus, Botrytis, Cladosporuim, Epicoccum, Fusarium, Geotrichum, Melanconium, Penicillium, Pythium, Scytalidium and Ulocladium at variable frequencies. Fusarium sp, Epicoccum sp, Phoma sp* and *Ulocladium sp* are among the leaf parasites encountered on maize (Frisvad and Samson 1991). Generally *Fusarium, Ulocladium* and *Epicoccum* attack all plant parts (roots, stems and leaves), they are destroyed.

Christensen (1957) has been reported that fungi invade grain fields under development on the growing plant or after the seeds have matured. At this stage, the water content of seeds is high and their tissues are active. The results of the mycological analysis showed a clear dominance seems to be favored by moisture content in equilibrium with a relative humidity of 90% or more (Bouchet *and al*. 2005).

The dominance of the genus *Fusarium, Phoma* and *Ulocladium* contaminating flora in cereals has been reported in several studies (Christensen 1964; Dhingra and Sinclair 1995; Gevers 1975; Marjoline and *al*.2002). Thus, species of *Aspergillus* and *Penicillium* mold are considered storage. The rate of contamination by the two previous genus has revealed the length of storage and high humidity of the grain (Champion 1997; Prusky and Yakoby 2003). The other strains isolated from samples analyzed belong to the genus: *Epicoccum*, *Cladosporium, Absidia, Monileilla, Scytalidium* and *Geotrichum* are naturally present on crops in fields and in soil (Christensen *and al*. 1977).

The results of isolation taken from each organ of the plant show that the leaves are the most contaminated with a percentage of 71.42% followed by the roots and stems with 14.28% for each in figure 1.

The results of antagonism between *Trichoderma viride* and different phytopathogens agent infected plant maize, showed a significant reduction in mycelial growth of fungal colonies of different isolates face the strain *Trichoderma viride* compared to the control table 2. The colony growth of *Monileila sp, Absidia sp, Phoma sp2, Penicillium sp and Botrytis sp*, is stopped on the third days of confrontation, the *Cladosporium sp, Geotrichum sp* and *Ulocladium sp* is stopped during the fourth days when these isolates are in contact directly with *Trichoderma viride*. After six days the colony of *Trichoderma viride* completely covers the colonies of these parasites on which it sporulated figure 2. *Trichoderma viride* showed a power antagonist which is the ability to remotely stop pathogen development *Trichoderma sp2, Verticillium sp*.

According, Antel *and al*. (2000), the test of confrontation in vitro exerted by *Trichoderma* species showed an increase in the colony and the secretion of extracellular enzymes, which destroys the membranes of pathogen hyphae.

These results showed a slowing of mycelial growth of different isolates. Indeed, the average diameter of colonies of *Alternaria sp1, Epicoccum sp3, Epicoccum sp2 and Epicoccum sp1, Trichoderma viride* faces respectively to 26.83 mm, 26.25 mm, 37.25 mm and18, 25 mm instead to 40.33 mm, 46.75 mm, 42.41 mm and 22.41 mm in the control. The inhibition of growth is about 34% to *Alternaria sp1*, 44% to *Epicoccum sp3*, 13% to *Epicoccum sp2* and 19% to *Epicoccum sp1*. *Trichoderma viride* reached the confluence of the Petri dish, after three days of incubation, the box is almost invaded by *Trichoderma viride*, whereas colonies of different isolates occupy a very small area, which corresponds to an inhibition of mycelia growth. In contrast we note the mycelial growth continues to evolve with isolates *Scytalidium sp; Ulocladium sp4; Pythium s; Phoma sp3* and *Melanconium sp* figure 3

Isolates *Scytalidium sp; Ulocladium sp4; Pythium sp, Phoma sp3* and *Melanconium sp* show significant resistance to the substances produced by *Trichoderma viride* with an average diameter of the colony, respectively, 48.46 mm; 49.33 mm; 45.5 mm; 66.5 mm and 53.83 mm, compared with the control 75.83 mm ;70.91 mm ;60.66 mm ;74.25 mm and 72.66 mm figure 4.5.

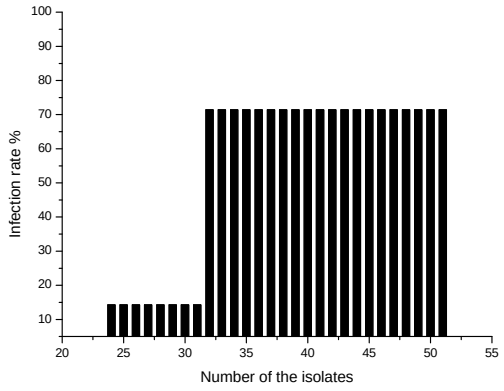


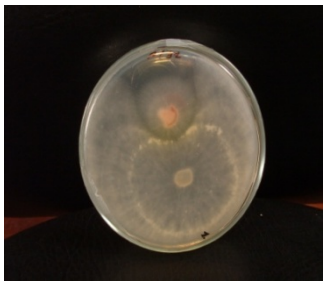
Fig 1: Percentage of infection of various organs of the maize plant

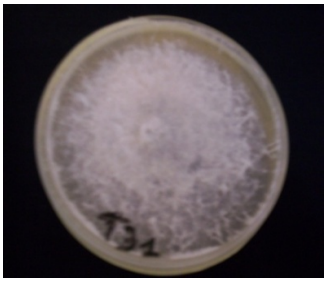
Table 1: Origin of isolates and the infection rate

No. isolates	Fungal isolates	Source	Infection%
24	*Trichoderma sp2*	roots	14.28%
25	*Trichoderma sp3*		
26	*Pythium sp*		
27	*Verticillium sp*		
28	*Alternaria alternata*	stems	14.28%
29	*Monileilla sp*		
30	*Absidia sp*		
31	*Fusarium roseum*		
32	*Penicillium frequentans*	leaves	71.42%
33	*Phoma sp2*		
34	*Penicillium sp*		
35	*Ulocladium sp2*		
36	*Botrytis sp*		
37	*Cladospoium sp1*		
38	*Ulocladium sp3*		
39	*Phoma sp3*		
40	*Alternria alternata*		
41	*Phoma sp4*		
42	*Aspergillus niger*		
43	*Epicoccum sp1*		
44	*Fusarium sp2*		
45	*Epicoccum sp2*		
46	*Geotrichum sp*		
47	*Scytalidium sp*		
48	*Ulocladium sp4*		
49	*Melanconium sp*		
50	*Epicoccum sp3*		
51	*Alternaria sp1*		

Table 2: Average diameter (mm) of colonies of *Trichoderma viride* pathogenic face and the importance of inhibition rate

No. strains	average diameter of pathogen (mm)	average diameter of control(mm)	percentage inhibition%	average diameter of antagonist(mm)
R24	45.25	47.83	6	58.75
R25	44.83	57.16	22	46.83
R26	45.5	60.66	25	48.91
R27	24.33	39	38	63.75
T28	18.66	23.58	21	55.75
T29	10.5	13.25	21	64.58
T30	24.41	28.41	15	48.41
T31	25	34.83	29	60.91
F32	41.16	56.58	28	41.58
F33	28.91	37.83	24	61.5
F34	17.83	24.58	28	55.5
F35	21.41	31.41	33	35.16
F36	15.16	16.83	10	59.5
F37	20.41	26.5	23	44.91
F38	64.41	73.25	13	57.25
F39	66.5	74.25	11	44
F40	14.66	16.5	12	52
F41	39.08	54.81	29	60.16
F42	24.08	28.08	15	55.33
F43	18.25	22.41	19	48.83
F44	15.83	25.08	37	58.66
F45	37.25	42.41	13	46.5
F46	23.9	62.5	62	52.75
F47	48.46	75.83	37	43.58
F48	49.33	70.91	31	59.66
F49	53.83	72.66	26	52.41
F50	26.25	46.75	44	51.08
F51	26.83	40.33	34	50.5

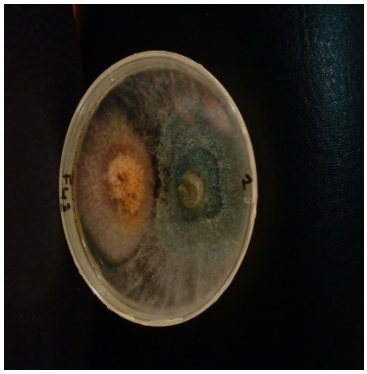


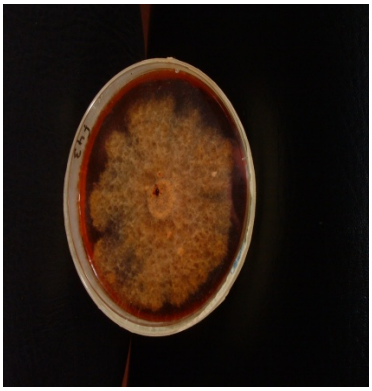


a: colony of *Fusarium roseum* in the presence of *Trichoderma viride*

b: control of *Fusarium roseum* after six days of incubation

Fig 2: Inhibitory effect of *Trichoderma viride* on mycelial growth *Fusarium roseum*





a: colony of *Epicoccum sp1in the* presence of *Trichoderma viride*

b: control of *Epicoccum sp1* after six days of incubation

Fig3: Test on PDA of direct confrontation between *Trichoderma viride* and the strain of *Epicoccum sp1*

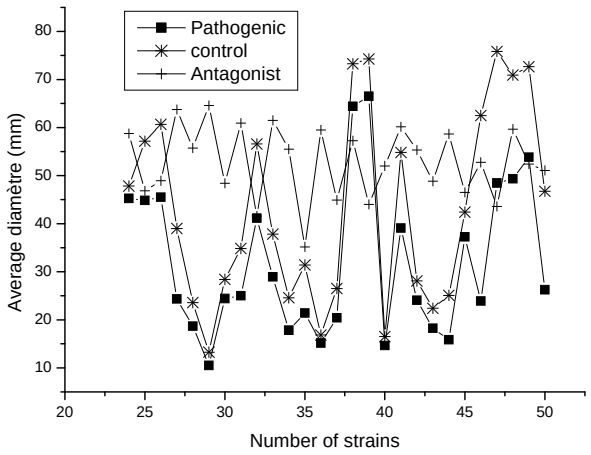


Fig 4: Average diameter (mm) of colonies of pathogens face *Trichoderma viride* Compared with the control after six days of incubation

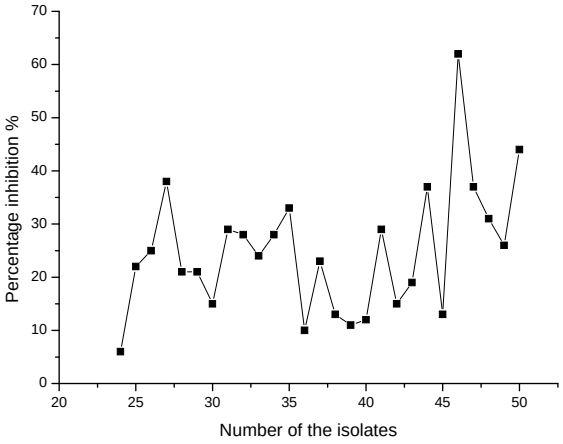


Fig 5: Percentage of inhibition of fungal isolates (technique of direct confrontation)

Similarly, Comporta (1985) demonstrated the inhibitory effect of *Trichoderma* sp between *Rhizoctonia solani*, while Howell (2003) found the mechanism of biocontrol by *Trichoderma sp* used to fight against diseases.

(Cundom *and al*. 2000) observed the invasion of the colony of the pathogen by *Trichoderma harzianum* by performing in vitro competition between the antagonist and *Sclerotina sclerotium*; too (Benhamou and Chet 1997) conducted a direct confrontation between *Trichoderma harzianum* and a soil fungus *Fusarium oxysporium* on a culture medium (PDA). (Harman *and al*. 2004) have written the action of *Trichoderma sp* mycoparasitism on pathogens, it is attached, wrapped around the pathogens and product peptaible which facilitate the entry of hyphae of *Trichoderma sp* in the lumen parasitic mold. Furthermore (Pates *and al*. 1999) found that the strain of *Trichoderma viride* has an important activity to secrete enzymes for end to attack or remove mycotoxins synthesized by the pathogens.

CONCLUSION: Cereals are the raw materials more susceptible to fungal contamination. Our study resulted in isolation and identification of fungal flora affecting various organs of maize grown in Algeria (region of Jijel), with a high prevalence of fungal species from the genus: *Phoma, Ulocladium, Fusarium, Pythium, Epicoccum*, *Melanconium* and *Trichoderma*

Depending on the plant, *Trichoderma* is effective against most pathogens tested. *Trichoderma* grows rapidly on a culture medium, which should benefit during the confrontation. Indeed, tests of *Trichoderma viride* and confrontation between different fungal isolates, which directly on culture medium, we found an inhibition of mycelial growth of the pathogen tested. If there is direct contact between the two fungi, *Trichoderma viride* invaded colonies of fungal isolates sporulated and there even after six days of confrontation.

REFERENCES

Antal, Z ., Manczinger, L ., Szakacs, G ., Tengerdy, RP ., Ferency, L . 2000. Augmentation de la colonie, in vitro antagoniste et secretion d'enzymes de l'extracellulaire dans tensions froid tolérantes d'espèce *Trichoderma Mycol Rés* 104: 545-549.

Benhamou, N., Chet, I. 1997. Cellular and molecular mechanism involed in the interaction between *Trichoderma harzianum* and *Pythium ultimium. Appl. Enviro. Microbial*. 63, p .2095-2099.

Benitez, T., Delgado –Diorama, J., Rincon, A., Rey, M., Limon, C. 1998. Biofungicides: *Trichoderma* as a biocontrol against phytopathogenic fungi. Recent Ress. Dev .*Microbiol,* 1998, 2(1), 129-150

Botton, B., Bretton, A., Fevre, M ., Guy, Ph., Larpent, J. P., and Veau, P. 1985. Moisissures utiles et nuisibles,

Importance industrielle (eds). Masson, Paris, New York, Milan, Mexico.

Bouchet, J. Guignard, J-L ., Pouchus, Y. and Villard, J. 2005. Les champignons: mycologie fondamentale appliquée. Paris : Masson, 2005, pp. 109-112,191p.

Champion, R. 1997. Identifier les champignons transmis par les semences INRA, Paris, 398p

Chase, A.1987. Compendium of ornamental foliage plant diseases. American phytopathological society, 92p

Chet, I.1987. *Trichoderma* application, mode of action, and potential as a biocontrol agent of soil borne plant pathogenic fungi. In: innovative approaches to plant disease control

(Chet I, Ed) New York: John Wiley and Sons. 1987.

Comporta, P. 1985. Antagonisme in vitro de *Trichoderma spp*. vis-à-vis de *Rhizoctonia solani* Kuhn. Agronomies 5, 613-620.

Christensen, C.M. 1957.*Rev*, 23,108.

Christensen, C.M. 1964. In mycotoxin in foods, edited by G.N.Wogan, published by Mit press, Cambridge, Massachusetts.

Christensen, C.M., Microcha, C.J. and Merounuck, R.A. 1977. Molds, mycotoxin and mycotoxicose.

Cundon, M.A., Mazza Mazzanti, S de Castanon and Gutierrez, S.A. 2000. Actividad antagonitica in vitro deaislamientos de *Trichoderma harzianum*, sobre exlerocios de *Sclerotinia sclerotium*. Communication Cientificas y Tecnologicas. Universitad National Del Nordeste, Facultad de Ciencias Agrarias-UNNE. Sargento cabral, corrientes, Argentina.

Davet, P. and Rouxel, F. 1997. Détection et isolement des champignons du sol. Techniques et pratiques. Edition INRA. Paris.

Dhingra, O.D., et Sinclair, J.B. 1995. Basic plant pathology. Methods .2nd edition. Lewis publishers, Boca Raton, 448pp.

Frisvard, J.C. and Samson, R.A. 1991. Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production, Handbook of applied mycology, volume 3, foods and feeds, Dekker, M. New York: Arora, D.K., Mukerji, K. G and Marth. E.H., pp31-68

Gaetan LeFloch., Jessica Vallance., George Barbien., Patrice Rey.2006.Détection de *Pythium oligondrum*, agent de lute biologique, en culture hors sol de tomate12eme colloquée national de la recherché en IVT, Brest, 1-2.Juin 2006.

Gams, W., Bisset, J. 1998. Morphology and identification of *Trichoderma*.In: Kubicek cp, Harman GE (Eds *Trichoderma* and *Gliocladium*, vol1.Taulor and Francis, London, UK pp3-33.

Gevres, H.O. 1975. Anew major gene for resistance to *Helminthosporium turcium* leaf blight of maize. Plant disrep 59:296-300.

Guraud, J.P. 1998. Microbiologie alimentaire. Dunod. Paris. ISBN: 210 0036661.

Harman, GE ., Howel, CR ., Viterbo, UN ., Chet, Je ., Mlorito. 2004. *Trichoderma* espèce- opportuniste, les avirulent plantent des symbiotes. La nature en examene 2 : 43-56.

Howell, CR. 2003.Mécanismes employés par les espèces de *Trichoderma* dans le contrôle biologique de maladies de la plante historique et évolution de concepts courants plantes Dis87 :4-10.

Le Floch ; Rey ,P ; Benizri ,E ; Benhamou ,N .and Tirilly, Y. 2003a. The impact of auxin compounds produced by the antagonistic fungus, *Pythium* group F on plant growth, plant soil, 257:457-470.

Marjoline Verscheure ., Georges Lognay ., Michel Marlier. 2002. Les méthodes chimiques d'identification et de classification des champignons *Bioéthanol .Agron* .Soc .*Environ*-2002 6(3)-131-142.

Pates, E., Meredith, F., Smart, W., Bacon, CW., Jaworski, AJ.1999. *Trichoderma viride* suppresses de fumonisin B1 production by *Fusarium moniliforme*. J Food protects 66:1326-1332(1999).

Prusky, D., Yakoby, N. 2003. Moisissures pathogènes: mener ou a mené par pH ambiant? Mol plant pathol4 :509-510.

Roquebert, M-F.1996. Interaction antagonistes de *Trichoderma sp* dans les systèmes tellurique: systématique compte–rendu de 4 eme rencontre en Toxicologie, Paris, 1996, 13-15.