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Fungal Control of Pathogenic Fungi Isolated From Some Wild Plants in Taif Governorate, Saudi Arabia

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ABSTRACT

Twenty two plants were collected from Taif Governorate and identified as: *Aerva lanata, Arnebia hispidissima, Artemisia judaica, Artemisia monosperma, Asphodelus aestives, Avena barbata, Capparis dcidua, Eucalyptus globulus, Euphorbia glomerifera, Foeniculum vulgare, Forsskaolea tenacissima, Juniperus procera, Launaea mucronata, Launaea sonchoides, Medicago sativa, Opuntia ficus, Phagnalon sinaicum, Prunus persica, Pulicaria crispa, Punica granatum, Rumex dentatus and Trichodesma calathiforme.* Pathogenic fungi were isolated from some of these plants and identified as *Alternaria alternata, Cephalosporium madurae, Cladosporium herbarum, Fusarium oxysporum, Humicola grisea, Penicillium chrysogenum* and *Ulocladium botrytis.* Four antagonistic isolates were tested, 2 from *Gliocladium fungus* and 2 from *Trichoderma fungus*. We found that all the four antagonistic isolates (*G. deliquescens, G. virens, T. viride* and *T. hamatum*) significantly inhibited the radial growth of the pathogenic fungi tested, with different ratios. The results indicated that the antibiotics produced by the antagonists were more effective than the fungus itself and differ with different fungi. Coating plant stems with antagonists or with antagonist extracts reduce the severity of the disease but not prevent it in all tested pathogens.

Keywords: pathogen, antagonist, antibiotic

INTRODUCTION

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, pathogens cause important losses, fungi being the most aggressive. 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 (Tjamos *et al*., 1992). By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists (Monte, 2001). Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. A considerable role in limiting the populations of these pathogenic fungi inhabiting the aboveground parts of plants is played by antagonistic microorganisms. Such properties are first of all exposed by the fungi *Trichoderma* and *Gliocladium* (Massart and Jijakli, 2007; Sempere and Santamarina, 2007; Bartmanska and Dmochowska-Gladysz, 2006; El-Katatny *et al.,* 2006; Patkowska, 2003; McQuilken *et al.,* 2001; Roco and Perez, 2001; Ahmed *et al*., 2000; Harman, 2000; Kredics *et al*., 2000; Elad and Kapat, 1999; Gupta *et al*., 1999; Yedidia *et al*., 1999; Lumsden *et al*., 1992; Lifshitz *et al*., 1986).

A general description of the vegetation of the western Saudi Arabia has been given by Vesey-Fitzgeraid (1957) and recognized a number of vegetational and ecological types including littoral marshes, coastal desert plain, coastal foothills, mountain ranges and wadies. Batanouny (1979); Fayed and Zayad (1989); Mahmoud and El-Tom (1985) and Montealegre *et al*. (2000) described the vegetation of the Makkah-Taif roads and recognized a number of vegetational and ecological types mostly organized in zones. Referring to the western provinces (Saudi Arabia) flora. Batanouny and Baeshin (1978 and 1982) gave lists of 135 species belonging to 108 genera and 43 families of angiosperms along Jeddah-Makkah road. The distribution of species composition of each in specific ecologically defined habitats would substantiate the fact that such community types are useful as indicators for their habitat characters (El-Shourbagy *et al*., 1987), even under adverse conditions of disturbance agencies encountered in these habitats. In sand plains *Pergularia tomentosa* is widespread; it dominates in cluster III and recorded in four other communities of these habitats.

Plant sap of the annual herbs *Euphorbia glomerifera* is applied in America to warts, corns and indurations of the cornea. *E. hypericifolia* also used as purgative and as a caustic on skin-lesions. *Artemisia monosperma* Seeds (achenes) of certain desert species deposit considerable amounts of fixed carbon into an external polysaccharide pellicle that has a high capacity for holding water and can be hydrated and dehydrated many times. Mossallam and BaZaid (2000) showed that *P. tomentosa* is widespread in Taif and latex of its stem and leaves is irritant to the skin and eyes and can cause

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inflammation and pain, and if ingested can cause stomach cramps and diarrhea. In medicine it is used as expectorant and purgative.

Many medicinal herbs in nature as *Pulicaria crispa, Launaea sonchoides, Forsskaolea tenacissima, Capparis dcidua, Prunus peorsica* and Avena *barbata* may be infected by many disease (Zhenying *et al*., 2004). So the objective of this work is to protect theses plants from fungal diseases by antagonistic fungi.

MATERIAL AND METHODS

Surveying and monitoring of some wild plants in Al Taif area

Wild plants were collected from different regions of Taif Governorate, and identified according to TackHolm (1974); Boulos, and El-Hadidi, (1994) and Boulos (2002).

Isolation and Identification of pathogenic fungi from Taif plants

Pieces of plants that showed symptoms of the disease were submerged in 5% sodium hypochloride for five minutes. After this treatment, they were extensively washed with sterile distilled water and placed on Petri dishes containing potato-dextrose-agar (PDA, Difco) amended with streptomycin sulphate (30 mg/L) and rose bengal (3.3 mL of 1% (w/v)) to eliminate bacterial contamination and incubated at 25 °C for 72 h according to Abou-Zeid *et al.* (2004); Montealergre *et al*. (2003) and Ismail and Aly (1997). The isolated fungal strains were purified and identified, according to Burgess *et al.* (1988); Klich & Pitt (1988); Pitt (1988); Domsch, *et al*. (1980); Alexopouls and Mims (1979); Ellis (1976) and Booth (1977).

Antagonistic microorganisms

Tested fungal bioagents included 2 isolates belonging to *Trichoderma* spp. [*T. viride* (TU*Tv*56) and *T. hamatum* (TU*Th*56)] and 2 isolates belonging to *Gliocladium* spp. [*G. virens* (TU*Gv*58) and *G. deliquescens* (TU*Gd*58)], where TU is abbreviation of Tanta University culture collection .

In vitro evaluation of the antagonistic potential of the fungal bioagents tested

Antagonistic reaction between the causal pathogens and the bioagents were studied *in vitro*. One agar disc (4mm-diam.) with the active mycelium of the bioagents was taken from advancing mat zone of a 3-days-old culture grew on PDA medium and transferred to one side of a Petri plate. The other side was inoculated with active mycelium plug taken from the outer margin of a 3-days-old culture of the causal pathogens (El-Kafrawy *et al*. 2002). This experiment was conducted in 3 replicates per each bioagent and plates were incubated at 25+3 °C for 5 days. Plates containing only pathogen were used as a control. Inhibition percentages of the pathogen were calculated just after overlapping of the two tested fungi according to the following equation:

Inhibition % = P-C/C

Since:
P= Mean diameter of the pathogen growth on the nearby site of the fungus disk which faces the bioagent candidate.
C= Mean diameter growth of the pathogen control.

Production of diffusible antibiotics

PDA plates, covered with a cellophane membrane, were inoculated in the center with 100 uL of a bioantagonistic fungal suspension. After incubation for 72 h at 22 °C, the membrane with the grown organism was removed, and the plate was inoculated in the middle with a 10-mm disk of a pure culture of the pathogen. Plate was further incubated at 22 °C for 48 h and the growth of the pathogen was measured. Control was run as above replacing the antagonisms by sterile distilled water (Montealegre *et al*., 2003).

Control of pathogen by coating plant stems with antagonists

The basal portions of plant stems were coated with each antagonists by soaking the stems for 3 min in fungal antagonist culture + 2% (w/v) carboxymethylcellulose (CMC), then the coated stems planted in sands infected with its pathogen with the rate of 10^6 spore per g dry soil. Control was run as above replacing the antagonisms by sterile distilled water and the percent of inhibition was calculated (Wu *et al.,* 1986 modified).

Control of pathogen by antagonist extracts

Plant cuttings were soaked in fungal antagonist filtrate for 3 min, then planting in sand beds infected with its pathogen with the rate of 10^6 spores per g dry soil. Control was run as above replacing the antagonisms by sterile distilled water, and the percent of inhibition was calculated (Jone and Pettit, 1987 modified).

RESULTS AND DISCUSSION

Many plants were collected and identified from Taif Governorate and listed in Table 1.

The collected plants revealed that the presence of *Artemisia judaica, Capparis dcidua, Eucalyptus globules, Euphorbia glomerifera, Juniperus procera, Launaea mucronata, Medicago sativa, Prunus persica, Punica granatum* and *Opuntia ficus in* Shaffa area. At South road the following plant**s** were collected*, Aerva lanata*, *Arnebia hispidissima*, *Artemisia judaica*, *Asphodelus aestives*, *Avena barbata*, *Foeniculum vulgare, Forsskaolea tenacissima*, *Launae sonchoides, Rumex*

dentatus, Phagnalon sinaicum, Pulicaria crispa and *Trichodesma calathiforme*. Our results supported with that of Abd El-Fattah and Ali (2005), they recoded twenty-three vegetation groups in Taif area, seven groups dominated by *Aerva lanata***,** *, Aizoon canariense, Arnebia hispidissima, Blepharis ciliaris, Capparis decidua Pergularia tomentosa* and *Salsola spinescens,* in the sand plains, *Aerva gavanica, Anthemis melompodina, Bassia muricata, Calotropis procera, Coccinea grandis, Dipterygium gluacum* and *Haloxylon scoparium* in the valleys, *Anvillea gracinii, Dianthis strictus, Ecobolium gymnostachyum, Euryops arabicus* and *Halothammus bottae* in the slopes, and *Capparis sinaica, Centaurea schimperi, Maerua oblongifolia* and *Salsola kali* in the plateaus.

However, some of the species have wide ecological and sociological ranges of distribution. These are *Aerva lanata, Haloxylon scoparium* and *Salsola spinescens*. Where *Aerva juvanica, Anthemis melompodina, Calotropis procera, Coccinea grandis* and *Dipterygium glaucum* are moderately distributed (Abd El-Fattah and Ali 2005**).** In contrast, other species are confined to certain plant communities: *Ehretia obtusifolia* associated with *C. grandis* community in valleys habitat and *Dianthis strictus* associated with *C. schimperi* in plateaus habitat.

Table 1: Plants collected and identified from Taif Governate

Regions of isolation in Taif	
shafa	**Southern route**
Artemisia monosperma	*Aerva lanata*
Capparis dcidua	*Arnebia hispidissima*
Eucalyptus lobules	*Artemisia judaica*
Euphorbia glomerifera	*Asphodelus aestives*
Juniperus procera	*Avena barbata*
Launaea mucronata	*Foeniculum vulgare*
Medicago sativa	*Forsskaolea tenacissima*
Opuntia ficus	*Launaea sonchoides*
Prunus persica	*Phagnalon sinaicum*
Punica granatum	*Pulicaria crispa*
	Rumex dentatus
	Trichodesma calathiforme

Pathogens:

Pathogenic fungi were identified as:

1. *Alternaria alternata*, the cause of spot disease, was isolated from *Avena barbata, Euphorbia glomerifera, Forsskaolea tenacissima, Launae sonchoides* and *Prunus persica.*
2. *Cephalosporium madurae,* the cause of leaf strip (late wilt), was isolated from *Launae sonchoides*
3. *Cladosporium herbarum*, the cause of leaf mold (leaf spot), was isolated from *pulicaria crispa*.
4. *Fusarium oxysporum*, the cause of wilt disease*,* was isolated from *Prunus persica.*
5. *Humicola grisea*, *the* cause of wilt disease, was isolated from *Artemisia monosperma*.
6. *Penicillium chrysogenum*, the cause of wilt disease, was isolated from *Capparis dcidua*.

Ulocladium botrytis, the cause of rot disease, was isolated from *Forsskaolea tenacissima.*

Frequency of pathogenic fungi

From the results we can concluded that *A. alternata* fungus was recorded in 5 plants (*Avena barbata, Euphorbia glomerifera, Forsskaolea tenacissima, Launae sonchoides* and *Prunus persica*), so it is the most frequent fungus. *U. botrytis* and *A. alternata* fungi were infected *Forsskaolea tenacissima* plant, while *Prunus persica* plant was infected by A*. alternata* and F*. oxysporum* fungi, while all the other tested plants were infected by one pathogenic fungus only.

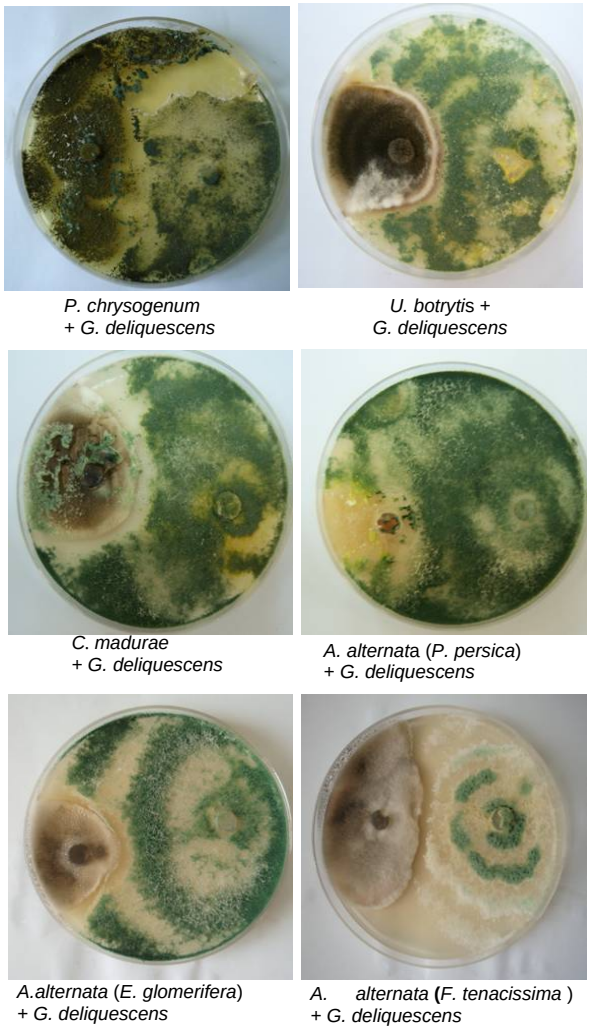

Figure 1: Overgrowth and growth inhibition of some pathogenic fungi by *Gliocladium deliquescens*. Mycelial discs of pathogenic fungus (left) transformand and *G. deliquescens* antagonism were placed at opposite sides (right) of PDA Petri dishes. Plates were incubated and photographs taken after 5 days incubation

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Table 2: *In vitro* antagonistic potential of *Gliocladium deliquescens* and *Gliocladium virens* on the causal pathogens

Pathogen	***Source of Isolation***	***Inhibition percent (I %)***	
		G. deliquescens	***G. virens***
Ulocladium botrytis	*Forsskaolea tenacissima*	*62.12*	*51.97*
Alternaria alternata	*Prunus persica*	*61.54*	*43.08*
Alternaria alternata	*Euphorbia glomerifera*	*46.84*	*42.19*
Alternaria alternata	*Avena barbata*	*54.61*	*47.34*
Alternaria alternata	*Forsskaolea tenacissima*	*52.38*	*53.18*
Alternaria alternata	*Launaea sonchoides*	*48.73*	*42.06*
Cladosporium herbarum	*Pulicaria crispa*	*40.74*	*42.11*
Cephalosporium madurae	*Launaea sonchoides*	*29.75*	*59.44*
Penicillium chrysogenum	*Capparis dcidua*	*63.33*	*65.56*
Fusarium oxysporum	*Prunus persica*	*55.11*	*51.39*
Humicola grisa	*Artemisia monosperma*	*44.58*	*16.67*

Table 3: *In vitro* antagonistic potential of *Trichoderma viride* and *Trichoderma hamatum* on the causal pathogens

Pathogen	**Source of isolation**	**Inhibition percent (I %)**	
		T. viride	***T. hamatum***
Ulocladium botrytis	*Forsskaolea tenacissima*	48.49	62.42
Alternaria alternata	*Prunus persica*	46.15	58.15
Alternaria alternata	*Euphorbia glomerifera*	51.83	67.24
Alternaria alternata	*Avena barbata*	42.48	55.73
Alternaria alternata	*Forsskaolea tenacissima*	62.70	49.18
Alternaria alternata	*Launaea sonchoides*	37.62	49.44
Cladosporium herbarum	*Pulicaria crispa*	7.41	31.71
Cephalosporium madurae	*Launaea sonchoides*	60	59.72
Penicillium chrysogenum	*Capparis dcidua*	55.56	59.44
Fusarium oxysporum	*Prunus persica*	25.7	53.87
Humicola grisa	*Artemisia monosperma*	16.67	22.08

Table 4: *In vitro* antagonistic potential of antibiotics produced by *Gliocladium deliquescens* and *Gliocladium virens* on the causal pathogens

Pathogen	**Source of Isolation**	**Inhibition percent by antibiotics (I%)**	
		G. deliquescens	***G. virens***
Ulocladium botrytis	*Forsskaolea tenacissima*	100	100
Alternaria alternata	*Prunus persica*	100	100
Alternaria alternata	*Euphorbia glomerifera*	100	86.32
Alternaria alternata	*Avena barbata*	100	84.09
Alternaria alternata	*Forsskaolea tenacissima*	100	100
Alternaria alternata	*Launaea sonchoides*	100	82.69
Cladosporium herbarum	*Pulicaria crispa*	100	100
Cephalosporium madurae	*Launaea sonchoides*	46.91	100
Penicillium chrysogenum	*Capparis dcidua*	100	49.44
Fusarium oxysporum	*Prunus persica*	100	48.15
Humicola grisa	*Artemisia monosperma*	100	100

Antagonistic effect of Gliocladium *deliquescens* and *Gliocladium virens* against different pathogens

The antagonistic potential of *G*. *deliquescens* and *G*. *virens* against different pathogens was measured by dual culture method using PDA medium. Data presented in Table 2 and Figure 1 and Figure 2 show that *G*. *deliquescens* and *G*. *virens* significantly inhibited the radial growth of all pathogens tested, when compared with the control. The antagonistic fungus sometimes grew over mycelium of the pathogens. The maximum inhibition for the *G*. *deliquescens* radial growth was reported with *Penicillium chrysogenum* isolated from *Capparis dcidua* which resulted in inhibition of 63.33%, followed by Ulocladium *botrytis* isolated from *Forsskaolea tenacissima* with 62.12% inhibition. The lowest inhibition percent of 29.75 was recorded by Cephalosporium *madurae* isolated from *Launae sonchoides* (Table 2 and Figure 1).

The maximum inhibition for the *G*. *virens* radial growth was reported with *Penicillium chrysogenum* isolated from *Capparis dcidua* which resulted in inhibition of 65.6%, followed by *Cephalosporium* madurae isolated from *Pulicaria crispa* with 59.44% inhibition. The lowest inhibition percent of 16.67 was recorded by *Humicola grisa* isolated from Artemisia *monosperma* (Table 2 and Figure 2).

We can conclude that the inhibition effects of the two *Gliocladium* strains were similar except for *Humicola grisa* isolated from *Artemisia monosperma*, where the inhibition percent was 44.58 for *G. deliquescens* and 16.67 for *G. virens* respectively. Also the *Cephalosporium* madurae was inhibited with 29.75 % by *G. deliquescens* and with 59.44 % by *G. virens* (Table 2). On the other hand the highest inhibition percent was recorded with *P. chrysogenum* by the two *Gliocladium* strains.

Antagonistic effect of Trichoderma *viride* and *Trichoderma hamatum* against different pathogens

The antagonistic potential of *T*. *viride* and *T. hamatum* against different pathogens was measured by dual culture method using PDA medium. Data presented in Table 3 and Figure 3 and Figure 4 show that *T*. *viride* and *T. hamatum* significantly inhibited also the radial growth of all pathogens tested, when compared with the control. The antagonistic fungus sometimes grew over mycelium of the pathogens. The maximum inhibition for the *T*. *viride* radial growth was reported with *Alternaria alternata* isolated from *Forsskaolea tenacissima* which resulted in inhibition of 62.7%, followed by *Cephalosporium madurae* isolated from *Launaea sonchoides* with 60% inhibition. The lowest inhibition percent of 7.41 was recorded by *Cladosporium herbarum* isolated from *Pulicaria crispa* followed by *Humicola grisa* isolated from *Artemisia monosperma* with inhibition percent of 16.67. The maximum inhibition for the *T. hamatum* radial growth was reported with *Alternaria alternata* isolated from *Euphorbia glomerifera* which resulted in inhibition of 67.24%, followed by Ulocladium *botrytis* isolated from *Forsskaolea tenacissima* with 62.24% inhibition. The lowest inhibition percent of 22.08 was recorded by *Humicola grisa* isolated from *Artemisia monosperma*.

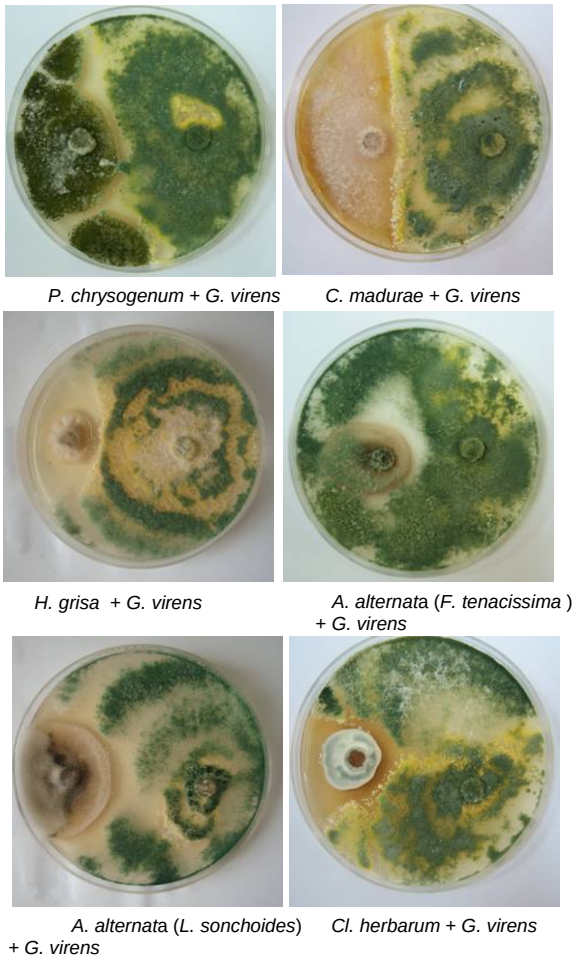

Figure 2: Overgrowth and growth inhibition of some pathogenic fungi by *Gliocladium virens*. Mycelial discs of pathogenic fungus (left) transformand and *G. virens* antagonism were placed at opposite sides (right) of PDA Petri dishes. Plates were incubated and photographs taken after 5 days incubation

So we can conclude that the highest differences between the two strains of *Trichoderma*, (Table 3) were reported with *Cladosporium herbarum* where the inhibition percent was 7.41 by *T. viride* and 31.71% by *T. hamatum* respectively. Also *Fusarium oxysporum* was inhibited with 25.7% by *T. viride* and with 53.87 % by *T. hamatum*. On the other hand the highest inhibition percent was reported with *A. alternata*, and the lowest

percent was found in *H. grisa* by the two *Trichoderma* strains.

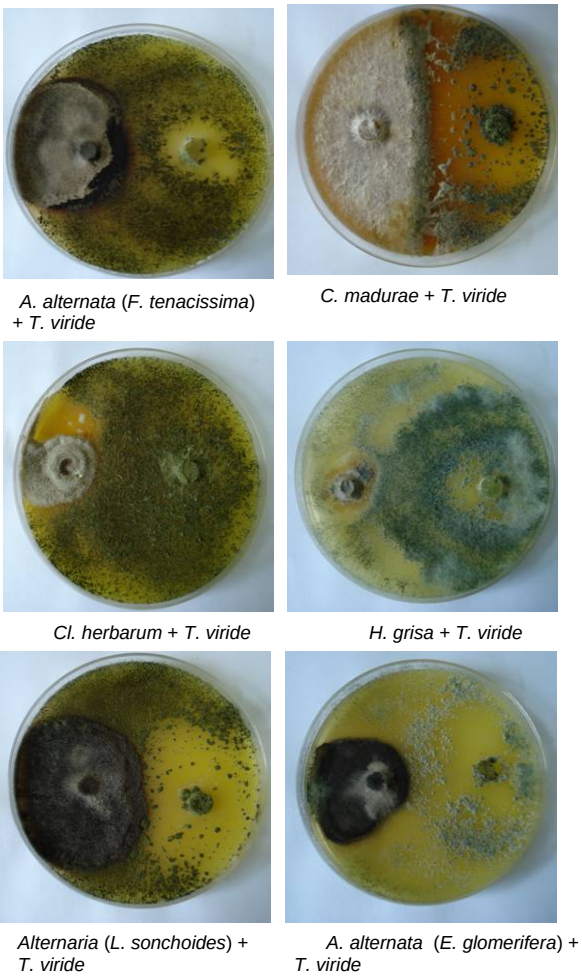

Figure 3: Overgrowth and growth inhibition of some pathogenic fungi by *Trichoderma viride*. Mycelial discs of pathogenic fungus (left) transformand and *T. viride* antagonism were placed at opposite sides (right) of PDA Petri dishes. Plates were incubated and photographs taken after 5 days incubation

With respect to the effects of antibiotics produced by *Gliocladium* spp, as shown in Table 4 the *G. deliquescens* antibiotics were completely inhibited all pathogens growth except for *C. madurae* where the growth was inhibited only by 46.91 %. While the effects of *G. virens* antibiotics were weaker than that of *G. deliquescens. F. oxysporum* was inhibited by 48.15 % and *P. chrysogenum* by 49.44 %, then the three *A. alternata* strains from 82.69 – 86.32 inhibition percent.

The antibiotics produced by *T. viride* were completely inhibited all the pathogens growth (Table 5). The *T. hamatum* antibiotics also inhibited the pathogens growth except for *Cephalosporium madurae* where only 40.45 % of growth was inhibited and 60 % inhibition was detected with *Cl. herbarum*. So we can conclude that the effect of antibiotics produced by the antagonists were more effective than the fungus itself and differ with different fungi. Also the antibiotics produced by *Trichoderma* spp. were more effective than that of *Gliocladium* spp.

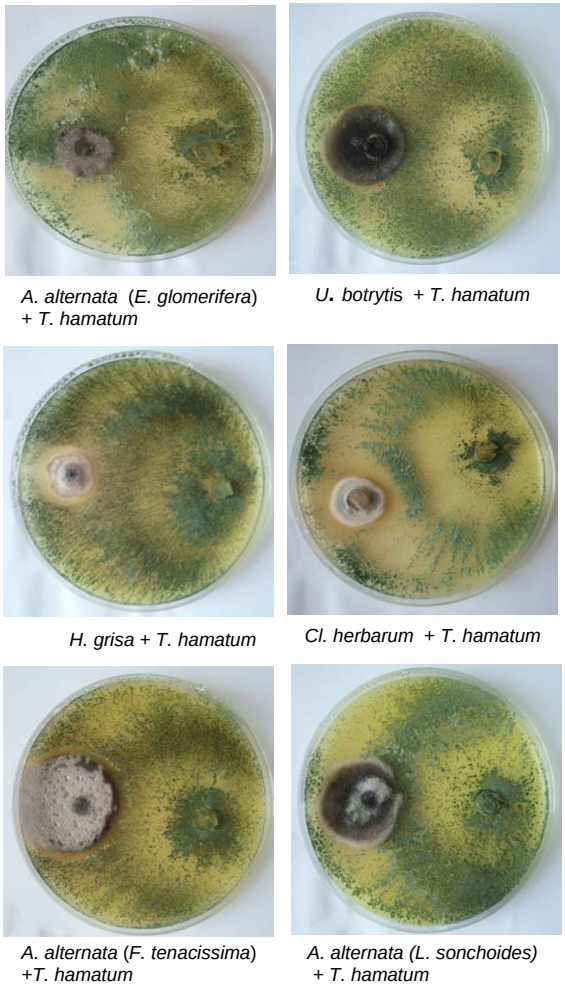

Figure 4: Overgrowth and growth inhibition of some pathogenic fungi by *Trichoderma hamatum*. Mycelial discs of pathogenic fungus (left) transformand and *T. hamatum* antagonism were placed at opposite sides (right) of PDA Petri dishes. Plates were incubated and photographs taken after 5 days incubation

Table 5: *In vitro* antagonistic potential of antibiotics produced by *Trichoderma hamatum* and *Trichoderma viride* on the causal pathogens

Pathogen	Source of isolation	Inhibition percent by antibiotics (I%)	
		T. hamatum	***T. viridae***
Ulocladium botyris	*Forrskaolea tenacissima*	100	100 growth
Alternaria alternate	*Prunus persica*	100	100
Alternaria alternate	*Euphorbia glomenifera*	100	100
Alternaria alternate	*Avena barbata*	100	100
Alternaria alternate	*Forrskaolea tenacissima*	100	100
Alternaria alternate	*Launaea sonchoides*	100	100
Cladosporium herbarum	*Pulicaria crispa*	60	100
Cephalosporium madurae	*Launaea sonchoides*	40.45	100
Penicillium chrysogenum	*Capparis dcidua*	100	100
Fusarium oxysporum	*Prunus persica*	100	100
Humicola grisa	*Artemisia monospema*	100	100

Control of pathogen by coating plant stems with antagonists or with antagonist extracts

We found that coating plant stems with antagonists or with antagonist extracts reduce the severity of the disease but not prevent it in all tested pathogens.

The antagonistic activity of *Trichoderma* and *Gliocladium* spp. could be related to their ability to act as biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Cook, 2000 and Chakraborty *et al.*, 1994). These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the strain, the antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration (Bell *et al*., 1982 and Benítez *et al*., 2004). Most *Trichoderma* strains produce volatile and non-volatile toxic metabolites that impede colonization by antagonized microorganisms; among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl- -pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described (Vey *et al.,* 2001). Strains of *T. virens* are able to produce gliovirin and used to protect cotton seedlings from *Pythium ultimum* (Chet *et al.,* 1997 and Howell, 1998). Also, our results agree with those reported by Mishra and Mukhopadhyay (1997); Larkin and Farvel (1998) and El-Kafrawy *et al*. (2002), they stated that *Trichoderma* spp. and *Gliocladium* spp. controlled *Fusarium oxysporum* f. sp. cucumerinum.

Jaworski *et al*. (1999) isolated a mixture of polypeptides, named trichovirins (TV) from the culture broth of the mold *T. viride* NRRL 5243. Also Krause *et al*. (2005) isolated polypeptide antibiotic suzukacillin (SZ) from the culture broth of the mold *T. viride*, strain 63 C-I. The microheterogeneous alamethicin F30 (ALM F30) was isolated from the fermentation of *T. viride* strain NRRL 3199 (Psurek *et al.,* 2005).

Peptaibols, the products of non-ribosomal peptide synthetases (NRPS), are linear peptide antibiotics produced by *Trichoderma* and other fungal genera (Ada *et al*., 2007). *Trichoderma virens* strain Gv29-8, a well-known biocontrol agent and inducer of plant defence responses, produces three lengths of peptaibols, 11, 14 and 18 residues long, with several isoforms of each.

Metabolites released from *Trichoderma viride*, *T. polysporum*, *T. hamatum* and *T. aureoviride* were tested in culture medium against *Ceratocystis paradoxa*, which causes black seed rot in oil palm sprouted seeds. The *Trichoderma* metabolites had similar fungistatic effects on the growth of *C. paradoxa* except those from *T. aureoviride.* The inhibition varied depending on the *Trichoderma* species producing the metabolites; from 2.0% to 64% in volatile, 0.0% to 74% in non-volatile and 0.0% to 81% from direct-diffusible metabolites (Eziashi *et al*., 2006). This finding supported also our results.

In a laboratory experiment, inoculating Douglas-fir seedlings with *G. virens* (10% w/w) prior to inoculation with *Fusarium* increased survival time when compared to concurrent inoculations of fungi (Dumroese *et al.,* 1996). Highley *et al.* (1996) reported that *Gliocladium virens* has shown good antagonism against decay fungi in agar medium and in wood blocks. Gliotoxin produced by *G. virens* is associated with biocontrol of some plant diseases. Decay was reduced in blocks treated with the culture filtrates but was not completely stopped and this supported also our results.

The results of this study indicated that the tested *Gliocladium* and *Trichoderma* spp. significantly inhibited the growth and reduced the severity of the disease of the pathogenic fungi with different ratios.

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