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INFLUENCE OF *AGARICUS* SP FUNGUS ON DAMPING OFF DISEASE CONTROL, GROWTH AND MAINTENANCE OF CYPRESS (*CUPRESSUS SEMPERVIRENS*) SEEDLINGS

By

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ABSTRACT

The potential protection of *Cupressus sempervirens* seedlings by aid of *Agaricus* sp as an ectomycorrhizal fungi (EMF) from damping-off (DF) disease caused by *Rhizoctonia solani* was investigated in the Experimental Station and Laboratories of Forestry and Wood Technology Dept., Faculty of Agriculture, Alexandria University in two successive season, 2007 and 2008. Five treatments were used as follows: (i) Seeds inoculated with DF, (ii) seeds inoculated with DF and EMF, (iii) seeds inoculated with DF and treated with fungicide, (iv) seeds inoculated with EMF and (v) uninoculated seeds. After one month of the inoculation with *Rhizoctonia solani,* seedlings of *C. sempervirens* displayed damping off symptoms, while those dually inoculated ones with ectomycorrhizal fungus *Agaricus* sp and *R. solani* displayed no symptoms. The uninoculated seeds were germinated earlier than all treatments (after 12 days). The seedlings inoculated with EM and those dually inoculated with DF (EM+DF) displayed emergence level of 64.5 and 52.15 % in the first season and 70.01 and 50.43 % in the second season, respectively and survival level of 85 and 90.5% in the first season and 90.0 and 90.5% in the second one. The survival after one year averaged 80% for both seasons in case of inoculated seedlings with the ectomycorrhizal fungus. Shoot height and total dry matter of

ectmycorrhizal seedlings was three fold those of the control and recovered plants and about two fold that of amended ones with fungicide. Total dry weight of the ectomycorrhizal seedlings was higher significantly than those on nonmycorrhizal ones. It is suggested that *C. sempervirens* plant is mycorrhiza dependent, not only for protection against pathogenic fungi, but for its survival as well. The highest activity of cellulolytic enzyme was found in case of *C. sempervirens* seedlings infected by *R. solani*, yet the presence of ecomycorrhizal fungus *in situ* act as antagonistic agent and led to impede degradation of cell wall of the host via minimizing cellulolytic enzyme activity of the pathogen. The filtrate of *Agaricus* sp has inhibitory effects on the hyphal growth (expressed as dry weight of hyphae) of the pathogenic fungus *R. solani*. It is recommended to inoculate seeds or seedling of *C. sempervirens* with the EM fungi in the nurseries and forest plantations for tree maintenance and protection against pathogenic soil-borne fungi.

INTRODUCTION

Cypresses are common coniferous evergreen trees, divided by the botanist into two genera, belong to family Cupressaceae , the true cypress (*Cupressus* sp) and the false cypress (*Chamaecyparis* sp). *Cupressus sempervirens* is the common species in many temperate and warm zones and is used in many purposes, amongst which, windbreaks, lumber and timber and planted as specimen to give contrast and winter foliage in flower bed (Procter, 1982).

Mycorrhizae are mainly divided into two groups; the endomycorrhizae (that implied different seven types, amongst which, orchidaceous, ericaceous, monotropoid, arbutiod and vesicular-arbuscular mycorrhizae and ectendomycorrhizae), that infect feeder roots of the most edaphic plants. The second important group is the ectomycorrhizae that infect only feeder roots of trees and some shrubs (Manion, 1981).

Ectomycorrhizae (ECM) is a fungus-host relationship where the fungus surrounds the roots; forming mantle or the pseudoparenchymateous sheath and Hartig's net. These structures are thought to present barriers to invasion of the roots by soil fungi and, therefore, to protect the tree from root diseases (Blanchard and Tattar, 1981). Ectomycorrhizal fungi compete for nutrients in the rhizosphere (Yangui *et al.* 2008). Furthermore, they produce polyphenols and may also antibiotics; impeding the growth of pathogenic fungi (Emmert and Handelsman, 1999 and Niemi *et al*, 1999).

The antagonistic effects of ECM against pathogenic fungi being evident recently from *in vitro* studies (Chakravary and Hwang, 1991; Branzati and Zambonelli, 1994 and Napierala- Filipiak; Werner, 2000 and Wong and Kitts 2006).

This study was carried out to compare the efficacy of the Basidomyceteous fungus; *Agaricus* sp. relative to use fungicide on control of damping-off caused by *Rhizoctonia solani*, as well as its effects on survival and growth of *Cupressus sempervirens* seedlings. The enzymological and antagonistic studies were applied to prove the potential of *Agaricus* sp on disease control and growth and maintenance of the plant.

MATERIALS AND METHODS

1. Preparation of inoculates

1.1. Ectomycorrhizal fungi (EMF)

The ectomycorrhizal fungus sp. was detected on the roots of *Salix safsaf* trees in Damanhour City, Behera, Egypt in March, 2007 in form of fruit bodies (Fig.l). The heads of the fruit bodies were collected in sterilized polyethylene bags then kept at 5°C, thereafter the fungus transformed into black fluid, contained several millions of basidiospores. One ml of such fluid was taken then diluted to obtain approximately 25.000 basidiospores ml"[1] and stored till using.

1.2. Damping-off fungus (DF)

The damping-off causal agent, *Rhizoctonia solani* was previously isolated from diseased *Cupressus sempervirens* seedlings in PDA medium. The most virulent isolate was selected upon the preliminary pathogensty test, subcultured in petri dishes (15 cm diam.) contained PDA medium. From the outermost active hyphal colonies, 10 discs of 0.8 cm diam. were taken via flamed or sterilized cork borer then

incorporated with 100 ml of sterilized water into hyphal suspension using warn blender, then kept at 5°C .

2. Germination of seeds

Fresh seeds, collected from apparently healthy *Cupressus sempervirens* trees of aged about 30 years old, grown in Experimental Station of Faculty of Agriculture, Alexandria, Egypt were surface sterilized with 1% sodium hypochlorite for 30 min., rinsed several times with sterile water, then sown in polyethylene bags containing about 1.0 kg of sterilized soil (5:1 peat: sand).

3. **Artificial inoculation**

Single inoculation (with DF or EMF) and dual inoculation (DF +EMF) have been done by application of hyphal suspension over the seeds then covered with one cm deep of sterilized soil.

4. Application of fungicide

The 0. I g L^{-1} of the fungicide Rizolex was applied both on uninoculated seeds and on the half number of inoculated seeds with DF. Control seeds received only boiled inocula of DF and EMF.

Eventually, there were 5 treatments applied as follows:

i. Seeds inoculated with DF,
ii. Seeds inoculated with DF and EMF,
iii. Seeds inoculated with DF and treated with fungicide,
iv. Seeds inoculated with EMF and
v. Uninoculated seeds.

10 replicates were used for each treatment in an experiment of complete randomized design (CRD). The comparison among treatments had been set using least significant difference (LSD) at 0.05 of probability level according to Steel and Torrie (1980). The experiment was conducted in 2 seasons (on 18 August, 2007 for the first season and 2008, for the second season) in the nursery of Experimental St., Fac. Agric, Alex. Univ., Egypt. The experimental data included rate of 50% emergence (day), emergence (%), survival after one month (%), plant height (cm), stem diameter (mm); root length (cm), number and length of needles(cm) and total dry weight (g) were recorded. Survival of seedlings (Si) was determined one month after inoculation with the pathogenic and symbiotic fungi and after one year as well as mortality (M%) by using of the following equations:

$$S_1 = \frac{L_1}{E} \times 100$$

$$S_2 = \frac{L_2}{E} \times 100$$

$$M = \frac{L_1 - L_2}{L_1} \times 100$$

Where:
L_1: is the number of living seedlings after one month of inoculation,
L_2: is the number of living seedlings after one year of inoculation and
E: is the number of post emerged seedlings.

5. Histological studies of inoculated plants

Feeder root segments of all treated plants were collected to study the incidence of the fungi *(Rhizoctonia solani* and *Agaricus* sp.), washed from soil debris then softened and fixed according to the method described by Phillips and Hayman (1970).

6. Determination of enzyme activity *(in vivo)*

6.1. Cellulolytic enzyme (Cx)

Inoculated *Cupressus sempervirens* seedlings with DF, DF + EMF and uninoculated ones as well as those treated with fungicide (1.0% Rizolex), aged 30 days were removed gently from the soil, washed free from debris then placed in sterilized test tubes which contained the substrate of C_x enzyme. Substrate of C_x was 6 ml of 0.8% carboxymethylcellulose (CMC) and 2 ml of phosphate buffer (pH-6). The volume of root immersed in substrate was adjusted to be 50 mm^3 by moving the seedling up-and downward, then fixed tightly with tube wall by sticker tape. All tubes were kept in an incubator at 25°C for 12 hours. The activity of enzyme was determined adopting to the method described by Hancock *et al.* (1964) using capillary Ostwald viscometer.

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6.2. Polymethylgalocturonase (PMG)

Activity of PMG was similarly assayed, but the reaction mixture (substrate) was comprised 4 ml of 0.8% pectin and 4 ml of buffer phosphate (pH-6) according to the method described by Talboy and Busch (1970). Ten replicates were used for each treatment in CRD design.

7. Antagonistic study

The following procedure was applied to evaluate the antagonistic effect of EMF on the pathogenic fungi; *R. solani; in vitro,*:

0.1 ml of EMF fluid was applied in 100 ml-flasks containing 35 ml of Melin-Norkans nutrient solution that described by Marx (1969), kept in room temp (25 ±_5°C) for 30 days. The resultant fungal growth was filtered then the supernatant was collected in sterilized flasks then kept at 5° C. The supernatant was diluted, by which every ml should comprise 10000 ppm based on the dry weight of fungal growth. Volumes of 0.0,1.0,2.0,3.0,4.0 and 5.0 ml of EMF supernatant were added to 150 ml jars containing 100, 99, 98, 97, 96 and 95 ml of sterilized liquid PD medium to obtain concentrations of 00.0, 100.0, 200.0, 300.0,400.0 and 500.0 ppm based on dry weight of fungal growth; respectively. From 10-day old culture of *R. solani* colonies, one disc of 0.8 mm diam. was picked up then allowed to float over the surface of PD medium that contained the different concentrations of EMF supernatant or; filtrate. The antagonistic effect was expressed as the dry weight (g) of hyphal mat of *R. solani* after 50 days of the inoculation.

RESULTS AND DISCUSION

1. Effects of the inoculation with damping off and mycorrhizal agents

After one month of the inoculation with *Rhizoctonia solani*, seedlings of *Cupressus sempervirens* displayed damping off symptoms, while those dually inoculated ones with ectomycorrhizal fungus *Agaricus* sp and *R. solani* displayed no symptoms. The feeder roots of inoculated seedlings with mycorrhizal fungus displayed the mantle and Hartig's net that characterized ectomycorrhizae (EM). It was reported that the mantle or pseudoparenchymatous sheath

regarded as mechanical barrier besides its role in antibiotic production that act as antagonism against pathogenic fungi (Fuegey *et al.*, 1999).

2. Rate of 50% emergence of seedlings

Table (1 and 2) showed that inoculation with EM fungus (either singly or dually with *R. solani*) led to delay germination of the seeds relative to the other treatments in both seasons. However statistical analysis of variance revealed that the seeds of control were germinated earlier than all treatments (after 12 days). However, the fungicides and fungal exudations (either pathogenic or symbiotic ones) may interfere with physiological process during germination of seeds, in turn led to delay germination.

3. Emergence of seedlings (%)

Although the inoculation with EM fungus delayed germination, it brought about the highest emergence level (E%) , since seedlings inoculated with EM and those dually inoculated with DF (EM+DF) displayed emergence level (%) of 64.5 and 52.15 % in the first season and 70.01 and 50.43 % in the second season, respectively (Tables 1 and 2). The increased E% in inoculated seeds with EM fungus might be attributed to its inhibitory impact of inherent mycelium that may be localized in the endosperm or embryo of the cypress seed. Also, it is possible that the spore germination and extrametrical hyphae of the EM fungus may contribute in solubility and availability of nutritional elements needed during germination process.

4. Survival (%) after one month

After one month of the artificial inoculation with pathogenic and/or symbiotic agents, it was found that the survival of seedlings inoculated only with EM fungus, dual inoculated with *R. Solani* displayed the highest survival levels (85 and 90.5 in the first season and 90.0 and 90.5% in the second season, respectively). Nonetheless the amendment of fungicide had brought about highly survival level (80 and 70% in the first and second season, respectively), it was less efficient than EM fungus in this respect (Tables 1 and 2).

5. Mortality and survival (%) after one year

After one year of seed sowing, it was found that the inoculation with EM fungus either singly or dually with the damping off causal agent, *R. solani* brought about mortality levels (10 and 29% in the first season and 10 and 10% in the second one, respectively) lower than that of the other treatments. On the other hand, the efficacy of

Table 1: Survival (%) and growth parameters of seedlings of *Cupressus sempervirens* inoculated with *Rhizoctonia solani* (RS), with *Agaricus* sp (A), dual inoulation of the both (RS+A), uninouculated and amended with fungicide and control in the first season.

	Cont.	Fungicide	RS	RS+A	A
Rate of 50% emergence (day)	12 [c]	17 [b]	15 [b]	18 [a]	18[a]
Emergence (%)	32.3 [d]	40.4 [c]	20.2[e]	52.15 [b]	64.5[a]
Survival (one month)(%)	74.0 [c]	80.0 [b]	30.0 [d]	90.5 [a]	85.0 [a]
Mortality (%)	70.0	70.0[b]	80.0 [a]	20.0	10.0
Survival (one year) (%)	25.0	34.0	15.0	85.0 [a]	80.0 [a]
Shoot height (cm)	10.7	17.21	11.4	33.15 [a]	35.19 [a]
Stem diameter (cm)	2.2 [b]	2.8 [b]	2.4 [b]	4.6 [a]	4.4 [a]
Dry weight of roots (g)	0.5 [b]	0.7 [b]	0.5[b]	1.1 [a]	1.3 [a]
Dry weight of branchlets (g)	1.3 [bc]	1.6 [b]	1.1 [c]	3.7 [a]	3.5 [a]
Dry weight of stem(g)	1.0 [b]	1. 4 [c]	0.7 [d]	3.1 [a]	2.9 [a]
Total dry weight (g)	2.8 [c]	3.7 [b]	2.3 [d]	7.9 [a]	7.7 [a]

Within row, values of the same postscript litters are not significantly different at 0.05 of the probability.

fungicide application was temporary, since the seedlings treated showed highly mortality (%) level, i.e. similar to that obtained in the control in the first season or lower than it, as it obtained in the second one (Tables 1 and 2). Survival after one year as a parameter may elucidate the positive role of EM fungus in enhancing survival level of the seedlings at the different growth stages regardless the infection with root diseases. However the survival after one year averaged 80% for both seasons in case of inoculated seedlings of *C. sempervirens* with the ectomycorrhizal fungus. The presence of synergistic or symbiotic fungi such as EM ones was emphasized by many workers, amongst which, Whipps (2001) and Weller *et al.*(2002) and its positive role as biological control against pathogenic fungi (Morin *et al*., 1999 and Rudawska, 2000, Martín-Pinto *et al.*, 2006).

Table 2: Survival (%) and growth parameters of seedlings of *Cupressus sempervirens* inoculated with *Rhizoctonia solani* (RS), with *Agaricus* sp (A), dual inoulation of the both (RS+A), uninouculated and amended with fungicide and control in the second season.

	Cont.	Fungicide	RS	RS+A	A
Rate of 50% emergence (day)	12 [b]	13 [b]	12[b]	16 [a]	18[a]
Emergence (%)	31.4 [d]	41.6 [c]	19.4 [e]	50.43[b]	70.01[a]
Survival (one month)(%)	60.1	70.0	30.0	90.5 [a]	90.0 [a]
Mortality (%)	80.0 [a]	70.0[b]	80.0 [a]	10.0 [b]	10.0 [b]
Survival (one year) (%)	20.0 [c]	30.0 [b]	17.0[c]	80.0 [a]	80.0 [a]
Shoot height (cm)	9.1 [e]	15.2 [c]	10.6 [d]	26.12 [b]	33.13[a]
Stem diameter (cm)	1.8 [c]	1.9 [c]	2.6 [b]	5.7 [a]	5.9 [a]
Dry weight of roots (g)	0.4[c]	0.7 [b]	0.6[bc]	2.0 [a]	2.2 [a]
Dry weight of branchlets (g)	1.3 [cd]	1.2 [d]	1.6 [c]	4.1 [b]	4.9 [a]
Dry weight of stem(g)	0.8 [e]	1. 6[c]	0.9 [d]	2.9 [b]	3.5 [a]
Total dry weight (g)	2.5 [e]	3.5 [d]	3.1 [c]	8.9 [b]	10.6 [a]

Within row, values of the same postscript litters are not significantly different at 0.05 of the probability.

6. Growth and yield parameters

After one year of inoculation with EM fungus, shoot height and stem diameter of ectomycorrhizal seedlings (either singly inoculated with EM fungus or dually with R. solani) was higher than those nonmycorrhizal ones (control, recovered and amended seedlings with fungicide) (Tables 1 and 2). However shoot height of ectmycorrhizal seedlings was three fold as that of control and recovered plants and about two fold as that of amended ones with fungicide. Fig. (2) illustrates seedlings growth after 6 months of germination.

Total dry weight (implied branchlets, stem and root dry weight) of the ectomycorrhizal seedlings was higher significantly than those on nonmycorrhizal ones. It worthy noticeable that there were no significant differences between single inoculated seedlings with EM fungus and dual inoculated ones with R. solani in total dry matter in the first season, whereas in the second season the single inoculated ones displayed value higher significantly than that of the dual

Fig 1: Fruit bodies of *Agaricus* sp.

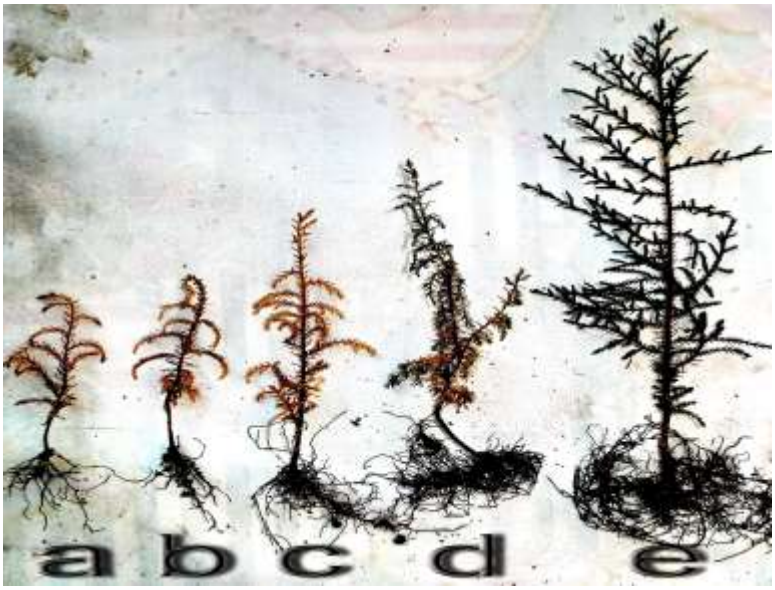

Fig. 2: Cypress (*Cupressus sempervirens*) seedlings, aged 6 months old, recovered from DF disease (a), uninoculated control (b), inoculated with R. solani and amended with fungicide (c), dual inoculated (EM and DF fungi) and inoculated seedling with Agaricus sp (e).

inoculated ones. This may be attributed to dynamic changes of symbiotic and pathogenic fungi and their interaction.

These findings, however, revealed that the *C. sempervirens* plant is mycorrhiza dependent, not only for protection against pathogenic soil- or seed- borne fungi, but for its survival as well, since nonmycorrhizal seedlings showed poor growth rate and less dry matter (about one third that of ectomycorrhizal ones). Furthermore, seedling may fail to survive without infection by mycorrhizal fungi. The lack of root hairs of the feeder roots may interpret why such species regarded as mycorrhiza dependent as it has been evident in many ectomycorrhizal tree species. However, the most of coniferous trees regarded as an ectomycorrhiza- dependent plants and has a positive role in plant biomass (Trappe and Strand, 1969; Branzanti *et al*., 1999; Morin *et al*., 1999 and Singh and Lakhanpal, 2000) and the afforestation using some trees might be doomed to failure due to the absence of ectomycorrhizae.

7. Enzyme activities

The activities of the macerating enzymes that implied cellulolytic enzymes (Cx) and the pectolytic enzyme, polymethylgalacturonase (PMG) were affected by the infection either by the pathogenic and/or symbiotic agent.

The highest activity of cellulolytic enzyme was found in case of *C. sempervirens* seedlings infected by *R. solani* that caused damping off disease, since it was higher significantly than that of the inoculated seedlings with *Agaricus* sp and the dual inoculated ones (Fig. 3). This may interpret the virulent of such fungus as pathogen that capable of degrade the cell wall of feeder roots of the host by aid of such enzymes. Fortunately, the presence of ecomycorrhizal fungus *in situ* act as antagonistic agent, since the dual inoculation with EM led to impede degradation of cell wall of the host via minimizing Cx activity of the pathogen. It is worthy noticeable also that the Cx activity of *Agaricus* sp was not mutually affected by the pathogenic *R. solani*, yet the later only was significantly affected by the first. Hence, it is possible that the exudation of *Agaricus* sp may inhibit the pathogenic fungus. The production of antibiotic substances by ectomycorrhizal fungi was reported by Garza-Ocanas (1993), who found that protected seedlings of pine against root diseases. The inhibition of the

pathogenic agent could also be due to the direct effect of pH altered by the symbiotic agent (Yangui *et al.*, 2008).

Application of fungicide brought about significant reduction in Cx enzyme. This may ascribe to the direct effect on the damping off causal agent *per se* or may interfere with the physiological processes of the pathogenic agent or both and may impede its activity in plant per see as it is shown in Fig (3).

As for PMG enzyme, it was found that *C. sempervirens* seedlings infected by *R. solani* displayed increase in its activity relative to that obtained in control and plants amended with the fungicide. The increased activity of PMG indicated that the pathogenic fungus tend to breakdown the middle lamellae located between cells of the feeder roots of the host. It is noticeable that the activity of PMG increased significantly in inoculated seedlings with EM fungus and slightly enhanced, as an additive impact, as a result of dual inoculation. It is well known that the EM fungi only colonize the middle lamellae of the cortex cells of the feeder roots, and never invade cell cytoplasm; it is of consistent to find such significant increase in PMG activity induced by EM fungi. Besides, it is expected that more enzymes may involved in antagonistic impacts. Jung *et al.* (2003) reported that the hydrolytic enzymes may also play an important role in the control of disease caused by soilborne plant pathogens. In addition, Yangui *et al.* (2008) suggest that suppression of damping-off caused by *R. solani* is associated with hydrolytic enzymes that act alone or synergistically causing hyphal lyses and deformation of fungal cell walls.

8. Effect of filtrate of EM fungus on linear growth of *R. solani*

Fig (4) showed the inhibitory effects of the fungal filtrate obtained from *Agaricus* sp on the hyphal growth (expressed as dry weight of hyphae) of the pathogenic fungus *R. solani*. However, the higher the concentration of the EM fungal filtrates, the lower the dry weight of *R. solani* hyphae. The impacts of microbial filtrate of beneficial microorganisms, particularly ectomycorrhizal fungi were studied by many researchers to prove its biochemical antagonism. In many cases it has been proved the antagonistic impacts of fungal filtrate on hyphal growth and spore germination of the pathogenic fungi (Duchesne *et al*., 1989 ;Chakravarty *et al*., 1990; Branzanti and Sambonelli, 1994 and Kasuya *et al*., 1996 and Chakravarty *et al.*, 2008).

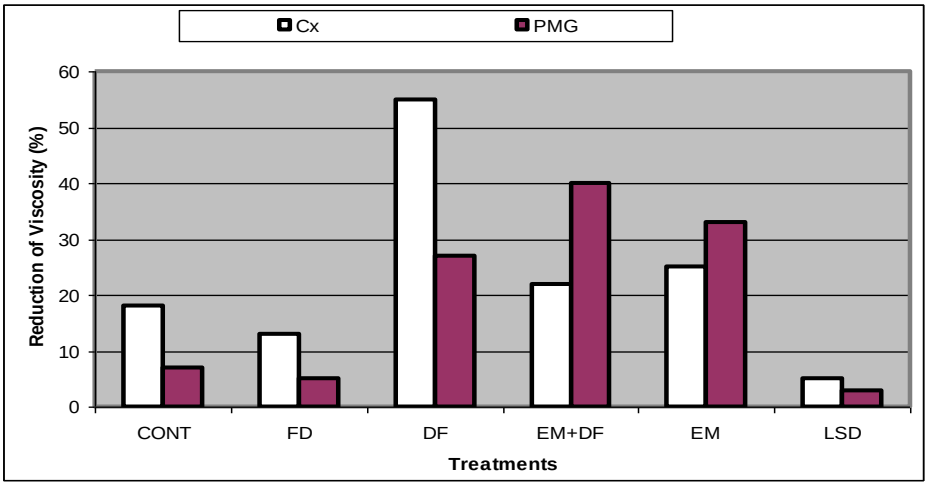

Fig 3: Cellulolytic (Cx) and polygalacturonase (PMG) enzyme activates of uninoculated (CONT), amended with fungicide (FD), inoculated with damping off causal fungus (DF), inoculated with ectomycorrhizal fungus (EM), dual inoculated plants with EM and DF.

Chakravarty and Hwang (2007) reported that the mycorrhizal seedlings of *Pinus banksiana* had significantly higher amount of total soluble phenols than nonmycorrhizal ones. Wong and Kitts (2006) found that phenolic compounds are able to chelate transition metals and also lower the reactivity of metal iron by forming an inert metal–ligand complex. Chelating of transition metals, such as iron and copper, reduces bioavaibility for fungal growth. On the other hand, Ciafardini and Zullo (2003) reported that the growth inhibition of mycelia could be due to phenolic compounds that can potentially impair cellular function and membrane integrity. Furthermore, Bais *et al.*, 2002; demonstrated that the phenolic compounds become tightly linked in the cell wall materials, eventually led to damage it.

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CONCLUSIONS

Inoculation of the seed or seedling feeder roots of *Cupressus sempervirens* with *Agaricus* sp as an ectomycorrhizal (EM) symbiotic agent brought about protection against the damping off causal agent, *Rhizoctonia solani*. The positive impacts of EM fungus expressed as survival against pathogenic or soil borne fungi is sustainable compared with the action of fungicides that may impede important physiological process of the plant). The positive impacts of EM fungus expressed as shoot height, dry weight components (branchlets, stem and root dry matter) of seedlings was obtained compared with those of unmycorrhizal plants that displayed poor growth. It has noticed from this work that the *Cupressus sempervirens* is mycorrhizal dependent. The macerating enzymes determined proved the virulence of the pathogen, yet the antagonistic impact of EM fungal exudation inhibits its efficiency qualitatively and quantitatively. The increased PMG activity owing to infection by EM fungus suggested its tendency to colonize middle lamellae of root cortex as typical ectomycorrhizal fungus. It is recommended, however, to inoculate seeds or seedling's roots of *C. sempervirens* with the EM fungi in the nurseries and man-made forest or plantation for tree maintenance and protection against pathogenic soil-borne fungi. Protection of forest seedlings against this disease must be focused on integrated management for in which biological control is one of the most important tools (Martín-Pinto *et al.*, 2006). Further research are needed to capitalize on more EM fungi with coniferous and EM-dependent hardwood tree species in regeneration or afforestation programs.

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الملخص العربى

تأثير فطر الأجاريكس على مقاومة مرض الذبول الطري ونمو وحيوية شتلات السرو

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تم إختبار قدرة فطر الأجاريكس (كفطر ميكورايزا خارجية) على حماية شتلات السرو من الإصابة بفطر الذبول الطرى المتسبب عن فطر الريزوكتونيا سولانى فى محطة بحوث التجارب ومعامل بحوث قسم الغابات وتكنولوجيا الأخشاب- كلية الزراعة- جامعة الأسكندرية فى موسمى 2007- 2008. أستخدمت 5 معاملات على النحو التالى: (1) عدوى بذور السرو بفطرالريزوكتونيا فقط، (2) عدوى بذور السرو بفطرالريزوكتونيا+ فطر الأجاريكس، (3) عدوى بذور السرو بفطرالريزوكتونيا+ إضافة مبيد الرايزولكس، (4) عدوى بذور السرو بفطر الأجاريكس فقط و (5) عدم عدوى البذور (كنترول). بعد مضى شهر من العدوى، ظهرت أعراض الذبول الطرى على الشتلات التى لقحت قبل إنباتها بفطرالريزوكتونيا فقط ، فى حين أن العدوى الثنائية بفطرالريزوكتونيا+ فطر الأجاريكس منعت ظهور أعراض المرض. وقد أنبتت بذور النباتات الكنترول مبكراً عن المعداة جميعاً، ولكن بنسبة ظهور وحيوية أقل من المعداة. وقد بلغت نسبة الشتلات الظاهرة فوق سطح التربة فى المعاملة الفردية (4) و المعاملة المزدوجة (3) نحو 64% و52% فى الموسم الأول و 70% و 50% فى الموسم الثانى، بنفس الترتيب. وقد بلغت نسبة الحيوية (بعد ظهور البادرات) نحو 85% و 90% فى الموسم الأول و 90% و 90% فى الموسم الثانى، بنفس الترتيب. وقد بلغت عموماً الحيوية أو البقاء بعد عام من الزراعة نحو 80% فى الموسمين ، مع فروق طفيفة بين المعاملتين (4) و (3). وقد أدت العدوى بفطر الأجاريكس بشكل عام إلى زيادة النمو الطولى و الوزن الكلى الجاف بما يوازى 3 أضعاف المتحصل عليه فى النباتات غير المعداة بنفس الفطر(التى لم تكون ميكورايزا خارجية). وهذه النتائج تعزز من أهمية عدوى السرو بفطر الميكورايزا الخارجية ليس فقط لمقاومة أمراض جذور الشتلات، بل ولإستمراريتها فى البقاء. أظهرت الدراسات الإنزيمية حدوث زيادة ملحوظة فى نشاط التحلل (السليوليليز والبولى جلاكتويورنيز) نتيجة الإصابة بفطرالريزوكتونيا مما يدل على قدرته الممرضة والمحللة لمواد الجدار الخلوي للجذور المغذية للعائل، إلا أن فطر الأجاريكس أدى إلى كبح تأثير السليوليليز الناتج منه (فى المعاملة3). ومن ناحية أخرى زاد نشاط إنزيم البولى جلاكتويورنيز فى ذات المعاملة مما يؤيد ميل هيفات فطر الأجاريكس لإستيطان الصفيحة الوسطى لتكوين ما يعرف بشبكة هارتج. وقد أيدت فكرة التأثير المضاد لفطر الميكورايزا تجربة أثر الراشح الناتج عنها على الوزن الجاف لفطر الريزوكتونيا سولانى المنمى صناعياً والذى تأثر تصاعدياً بزيادة التركيز. وقد أوصت الدراسة بضرورة تلقيح جذور السرو بفطريات الميكورايزا الخارجية ليس فقط للحماية من الفطريات الممرضة ولكن لضمان تحسين نموها واستمراريتها.

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