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Biological Control of Root-Rot Disease of Plum by using some Bioagents and A Vesicular Arbuscular Mycorrhizal Fungus

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In this study both *Trichoderma harzianum* and *Bacillus megaterium* were antagonistic to the growth of *Fusarium chlamydosporum*, *Macrophomina phaseolina* and *Phytophthora parasitica*, the causal pathogens of plum root rot, with different degrees of inhibition. *T. harzianum* was the most effective in this regard compared with *B. megaterium* in the laboratory experiment. Also, *B. megaterium* was the least effective, especially with *M. phaseolina*. The same trend was observed in greenhouse experiment. The percentage respective disease incidence was significantly decreased by tested VAM isolate compared with the control check. Also, using *T. harzianum*, *B. megaterium* (Bio-ARC) and *Trichoderma album* (Bio-Zeid) decreased the percentage of disease incidence. *F. chlamydosporum* was the most sensitive to all bioagents followed by *P. parasitica* and *M. phaseolina*. On the other hand, the tested VAM fungus showed the highest effect against all tested root rot pathogens especially with *F. chlamydosporum*.

Keywords: Biological control, plum, root-rot and vesicular arbuscular mycorrhiza.

Plum (*Prunus domestica* L.), belongs to the family Rosaceae, is cultivated world wide. Under Egyptian conditions during 2007 growing season, the cultivated area with plum in Egypt reached about 2725 feddans which yield about 2648 ton as fresh fruits (Agric. Statistic Dept., Min. of Agric., Egypt). Plum is liable to infection by several fungal, bacterial and viral diseases, in addition to physiological disorders (Psarros, 1980; Ravelonandro, *et al.*, 1997 and Mohamed, 2004). Fungal diseases, especially root rot disease caused by *Phytophthora parasitica*, *Macrophomina phaseolina* and *Fusarium chlamydosporum*, are the most important fungal pathogens. Sas-Piotrowska and Dorszwski (1996) studied the effect of *Trichoderma harzianum, T. viride, T. album, T. koningii* and *Gliocladium roseum* on seven species of *Fusarium* and found that *T. koningii* was the most antagonistic to the tested pathogens. Also, *T. viride* and *T. harzianum* exhibited an antagonistic activity more than that recorded for *T. album* and *G. roseum.* The ability of vesicular arbuscular mycorrhizal fungi to suppress root rot disease, caused by soil borne pathogen, has been intensively studied in the last few decades. Fungal root pathogens are inhibited by mycorrhizal inoculation in the case of *F. oxysporum* on different crops (Filion *et al.*, 1999). The influence of the arbuscular mycorrhizal fungus, *Glomus clarum*, on the development of root rot caused by *Rhizoctonia solani* under greenhouse conditions was determined. In cowpea root necrosis the number of sclerotia in the rhizosphere produced by pathogen was significantly reduced by this fungus (Abdel Fattah and Shabana, 2002). The vesicular arbuscular mycorrhizal fungi are particularly, important in organic and/or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant pathogens. An arbuscular mycorrhizal fungus plays an important role in the suppression of crop pests and diseases, practical soil-borne fungal diseases (Gosling *et al.*, 2006). Of particular importance is the bio-protection conferred to plants against many soil-borne pathogens such as species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotium*, *Verticillium* and *Thielaviopsis* and various nematodes by VAM fungal colonization of the plant root (El-Batanony *et al.*, 2007). Mycorrhizal inoculation had greater effect on development of both diseases studied. The use of mycorrhizal inoculum reduced rosette virus disease upon 38.8% and *Cercospora* leaf spots upon 54.4% (Ambang *et al.*, 2008). This work was planned to test some bioagents to control root rot disease of plum.

Materials and Methods

During the isolation trials, an isolate of *Trichoderma harzianum* was isolated from plum showing root rot disease. Meanwhile, *Bacillus megaterium* was obtained from Plant Pathol. Res. Inst. Effect of these bioagents against the causal pathogens of plum root rot disease, *i.e. Fusarium chlamydosporum*, *Macrophomina phaseolina* and *Phytophthora parasitica*, these pathogenic fungi were isolated from infected plum roots and tested *in vitro* and *in vivo*. Also, another two bioagents namely; *Bacillus megaterium* (Bio-ARC) which contains $25X10^6$ cfu /g and *Trichoderma album* (Bio-Zeid) contains of $10X10^6$ spore/g were provided by Plant Pathol. Res. Inst., ARC, Giza. In addition, the vesicular arbuscular mycorrhizal (VAM) fungus (*Glomus* sp.) was obtained as a formulation from the Mycology and Plant Dis. Survey Dept., ARC, Giza. All bioagents were evaluated against the tested pathogens.

1. In vitro trials:

T. harzianum was grown on PDA medium, while *B. megaterium* on nutrient agar medium (NA).

To test the antagonistic effect of the two bio-agents under study *in vitro* on the linear growth of the causal pathogens of plum root rot disease, the following method was used. Petri dishes (9 cm in diameter), each contained 20 ml., of PDA medium were inoculated with discs (5 mm in diameter) of any of the tested pathogens, taken from 7day-old cultures. The discs were placed near of the edge of each Petri-dish. At the same time plates were inoculated with equal discs of *T. harzianum*. Three plates were used as replicates for each treatment. Antagonistic effect of *B. megaterium* on the linear growth of the same pathogens was tested *in vitro*. The tested bacterium was streaked on PDA plate near of the edge of each Petri dish, while the inoculation with the tested pathogen was done as mentioned before in the second half of each dish. All plates were incubated at 28±1°C until the growth in the control treatment reached the edge of the plates. Reduction percentage of fungal growth was calculated according to the following formula:

$$\text{Reduction (\%)} = \frac{\text{Control - treatment}}{\text{Control}} \times 100$$

2. In vivo trials:

These trials were carried out under greenhouse conditions. Sterilized plastic pots (25-cm-diameter) filled with autoclaved sandy clay (1:1 w/w) soil were utilized to evaluate the efficiency of the bioagents *T. harzianum*, Bio-ARC (*B. megaterium*), Bio-Zeid (*T. album*) and VAM vesicular arbuscular mycorrhizal fungus (*Glomus* sp.), for controlling pathogenic fungi associated with plum root rot disease. The vesicular arbuscular mycorrhizal was obtained as mentioned before.

The inoculum of *T. harzianum* (the fungal isolate) was prepared by growing on autoclaved corn-meal sand medium (100 g corn-meal, 50 g sand and 100 ml distilled water in 500 ml bottles. The inoculation was carried out with 5mm in diameter fungal disks taken from the margin of 7 days old culture. The inoculated bottles were incubated at 28±1°C for 15 days, *T. harzianum* was added to the soil at the rate of 15 g / kg soil before inoculating the pathogen (Abd El-Ghany, 2007). On the other side, all pathogens were added to the soil, at the rate of 5% (Abo-Rehab, 1997) then the pots were planted with transplant of Myrobalan 29C, as being the most susceptible rootstock, and three pots were used. Each pot was for each treatment planted with three transplants of plum. The pots were irrigated for one week before soil infestation with the tested pathogens to stimulate the bioagents growth and their homogenous distribution. Also a similar number of pots was inoculated with the bioagent, each alone, and the same number was left free from the pathogens and the bioagent as control treatment. All pots were kept under greenhouse conditions.

In the second method, the bioagents were added to the soil after transplanting the transplants and before adding the tested pathogens. While, Bio-ARC (*B. megaterium*) and *T. album* were added to the soil as suspensions at the rate 5g / liter water.

In the third method corn roots colonized by the vesicular arbuscular mycorrhizal (VAM) fungus *Glomus* sp., were added to the soil at the time of transplanting and before adding of the pathogen at the rate of 10 g root segments as a layer 3 cm under the plum nurslings roots (Shaltout, 2002).

Data were statistically analyzed using complete randomized design in factorial experiment according to Gomez and Gomez, (1984). The averages were compared by least significant differences (L.S.D) test at level 5%.

Results

a. Effect of bio-agents in vitro:

The results in Table (1) show that *T. harzianum* and *B. megaterium* were antagonistic to the tested casual pathogens of plum root rot disease. The obtained data showed that *T. harzianum* was more effective than *B. megaterium* with all the tested pathogens. In this respect, the most affected fungus by these treatments was *F. chlamydosporum* followed by *P. parasitica*. While no antagonistic action was recorded by *B. megaterium* to *M. phaseolina*.

Table 1. *In vitro* effect of the bio agents *Trichoderma harzianum* and *Bacillus megaterium* on mycelial growth of the three pathogens of plum root rot disease

Tested fungus	*T. harzianum*		*B. megaterium*	
	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
F. chlamydosporum	2.0	77.77	2.5	72.22
M. phaseolina	3.2	64.44	9.0	0.00
P. parasitica	2.5	72.22	3.0	66.66
Control	9.0	0.00	9.0	0 00

b. Effect of bio-agents in vivo:

Data presented in Table (2) revealed that, *T. harzianum* was the most effective bioagent against the tested pathogens of plum root rot disease followed by *T. album* (Bio-Zeid). While *B. megaterium* (Bio-ARC) was the lowest bioagents with all the tested pathogens especially, with *M. phaseolina*, where the percentage of disease reduction was (20 %). On the other hand, most fungi affected by these bioagents were *F. chlamydosporum*, followed by *P. parasitica* and *M. phaseolina*, with *T. harzianum*. On the other hand, *M. phaseolina* was the least affected fungus with *T. album*, where the percentage of disease reduction was 40%.

Table 2. Effect of bioagents on disease incidence under greenhouse conditions

Pathogenic fungus	Bio-agents	Dis. incid. (%)	Reduction (%)
P. parasitica	*T. harzianum*	22.22	66
M. phaseolina		22.22	60
F. chlamydosporum		11.11	75
P. parasitica	*B. megaterium* (Bio-ARC)	44.44	33
M. phaseolina		44.44	20
F. chlamydosporum		33.33	25
P. parasitica	*T. album* (Bio-Zeid)	33.33	50
M. phaseolina		33.33	40
F. chlamydosporum		22.22	50
P. parasitica	No bioagent	66.66	
M. phaseolina		55.55	
F. chlamydosporum		44.44	
Control		0.0	

L.S.D. at 5 % for: Pathogenic fungi (P) = 1.61, Bioagents (B) = 1.86, B×P = 3.22

Data presented in Table (3) indicated that the arbuscular mycorrhizal *Glomus* sp. was the most effective in reducing disease incidence, with *F. chlamydosporum* and *Glomus* sp., application led to decrease of the disease incidence from 66.66 to 33.33% with *P. parasitica,* while with *F. chlamydosporum* the percentage was from 44.44 to 11.11%, but with *M. phaseolina* the percentage was reduced from 55.55 to 44.44%. Also, adding mycorrhizal fungus to plum roots stimulated the growth of root hairs and thus healing damaged root and thus reducing disease incidence.

Table 3. Effect of vescular arbuscular mycorrhizae (*Glomus* sp.) on incidence of plum root rot disease under greenhouse conditions

Treatment	Dis. incid. (%)	Reduction (%)
F. chlamydosporum + VAM	11.11	75
M. phaseolina + VAM	44.44	20
P. parasitica + VAM	33.33	50
F. chlamydosporum	44.44	
Macrophomina phaseolina	55.55	
Phytophthora parasitica	66.66	
Control with VAM only	0.0	
Control	0.0	

L.S.D. at 5%= 3.96

Discussion

Root rot disease incited by *Phytophthora parasitica*, *Fusarium chlamydosporum*, and *Macrophomina phaseolina* is one of the diseases which attack plum rootstocks causing severe damage, (Psarros, 1980). Biological control is widely used in controlling root rot disease in many fruit crops (Davis and Menge, 1980; McMillan, 1983 and Jefferson *et al.*, 2000). The obtained data showed that the tested bioagents were effective against the tested causal pathogens of plum root rot disease. *In vitro* studies proved *Trichoderma harzianum* isolated from plum rhizosphere was the most effective bioagent against the tested pathogens followed by *Bacillus megaterium*. In this respect, the most affected fungus by these treatments was *F. chlamydosporum* followed by *P. parasitica* and *M. phaseolina* . No reduction in the mycelial growth of the latter fungus occurred by *B. megaterium*. *In vivo* studies also showed that *T. harzianum* the most effective bioagent against the tested pathogens followed by VAM then *T. album* (Bio-Zeid). While *B. megaterium* (Bio-ARC) was the least effective bioagent against all the tested pathogens, especially *M. phaseolina* . On the other hand, the most affected fungus by these bioagents was *F. chlamydosporum*, followed by *P. parasitica* and *M. phaseolina* respectively. These results are in agreement with Rachniyom and Jaenaksorn (2008) who found that, *Trichoderma* spp., are common inhabitants of the rhizosphere as biocontrol against soil-borne plant pathogens. Commendable amount of researches have been focused on the mycoparasitic nature of genus *Trichoderma* and its contribution to plant health. Several mechanisms have been considered to be key factors in antagonistic interactions, *i.e.* lysis of host cell walls, antibiosis, competition for nutrients, induced resistance in plants and inactivation of host enzymes. Thus, strains of *T. harzianum* have been commonly used as bioagents for pathogenic fungi such as *Rhizoctonia solani*, *Lentinus liibideus*, *Fusarium solani*, *Botryosphaeria berengeriana* f.sp. *piricola*, *Phytophthora capsici* and *Pythium aphanenidermatum*. Symbiotic association between mycorrhizal fungi and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne diseases, improved resistance to drought, tolerance to heavy metals and better soil structure. VAM fungi are known to enhance plant uptake of phosphorus and other mineral nutrients. This may lead to disease escape or to higher tolerance against soil-borne pathogens. *B. megaterium*, *B. cereus* and *B. subtilis* have been used for the biocontrol purpose. The activity of biocontrol agents against soil-borne disease is important to achieve successful control activity (Hye-Sook *et al.*, 2009).

References

Abd El-Ghany, M. 2007. Pathological studies on apricot root rot in the new reclaimed lands in Egypt. Ph.D. Al-Azhar, Univ. Fac. Agric. 82pp.

Abdel Fattah, G.M. and Shabana, Y.M. 2002. Efficacy of the arbuscular mycorrhizal fungus(*Glomus clarum)* of cowpea plants against root-rot pathogen *Rhizoctonia solani. Zeitschrift Fur Pflazenky UND. Planzeschutz, J. of Plant Dis. and Protection*, **109**(2): 207-212.

Abo-Rehab, M. E. (1997). Studies on root-rot of persimmon and its control in the new reclaimed in Egypt. M.Sc. Thesis, Fac. Agric., Cairo Univ., 94 pp.

Ambang, Z..; Beime, N.B.; Dooh, N.; Yalen, M. and Godswill, N. 2008. Effect of mycorrhizal inoculum and urea fertilizer on diseases development and yield of groundnut crops (*Arachis hypogaea*). *African J. Biotechnol.*, 7(16): 2823-2827.

Davis, R .M. and Menge, J.M. 1980. Influence of *Glomus fasiciculatus* on Phytophthora root rot of citrus. *Phytopathology*, **70**: 447-452.

El-Batanony Nadia, H.; Massoud, O.N.; Mazen, M.M. and Abd El-Monium, M.M. 2007. The inhibitory effects of cultural filtrates of some wiled *Rhizobium* spp., on some faba bean root rot pathogens and their antimicrobial synergetic effect when combined with arbuscular mycorrhiza (AM). *World J. Agric. Sci.*, 3(6): 721-730.

Filion. M.; M .St-Arnoud and J.A. Fortin 1999. Direct interaction between the arbuscular mycorrhizal fungus, *Glomus intraradics* and different rhizisophere microorganisms. *New Phytopathologist*, **141**: 525-533.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultrural research.John Wiley and Sons, Inc.New Yourk, USA.

Gosling, P.; Hodge, A.; Goodlass, G. and Bending, G.D. (2006). Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems and Environment, 113: 17-35.

Hye-Sook, K.; Sang, M.K.; Myung, I.S.; Chun, S.C. and Kim, K.D. 2009. Characterization of *Bacillus luciferensis* strain KJ2C12 from pepper root a biocontrol agents of Phytophthora blight of pepper. *Plant Pathol. J.*, **25**(1): 62-69.

Jefferson, L.; Costa, S.; Menge, J.A. and Casale, W.L. 2000. Biological control of Phytophthora root rot of avocado with microorganisms grown in organic mulches. *Brazilian J. Microbiol.*, **31**: 239-246.

McMillan, IR., R.T. 1983. Control of citrus foot rot and root rot. Proc. Fl. State, *Hort. Soc.*, **96**: 221-222.

Psarros, E.F. 1980. Recent isolation of species of *Phytophthora* from deciduous fruit trees in central Macedonia. *Phytopathology*, Volume, 19XIX. Abstract.

Rachniyom, H. and Jaenaksorn, T. 2008. Effect of soluble silicon and *Trichoderma harzianum* on the *in vitro* growth of *Pythium aphanidermatum*. *J. Agric. Technol.*, 4 (2): 57-71.

Ravelonandro, M.; Scorza, R.; Bachelier, J.C.; Labonne, G.; Levy, L.; Damsteegt, V.; Callahan, A.M. and Dunez, J. 1997. Resistance of transgenic *Prunus domestica* to plum pox virus infection. *Plant Dis.*, **81**: 1231-1235.

Mohamed, Rabab E. 2004. Pathological and biological studies on crown gall diseases of stone fruits. M.Sc. Thesis, Fac. Agric., Cairo Univ., 97pp.

Sas-Piotrowska, B. and Dorszewski, J. 1996. Relationship between potato pathogens and *Trichoderma* spp., and *Gliocladium roseum,* (Link)Thoms, Phytopathology Polonica. *Plant Pathol.*, **11**: 93-96.

Shaltout, Abeer M.A. 2002. Use mycorrhizal fungi and some other bioagents to control root-rot and stalk-rot of corn. Ph.D. Thesis, Fac. Agric. Cairo Univ., 127pp.

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المقاومة الحيوية لمرض عفن جذور البرقوق بإستخدام بعض الكائنات المضادة والميكروهيزا الداخلية

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أظهرت الدراسة أن النتائج المتحصل عليها في المعمل أن *Trichoderma harzianum* and *Bacillus megaterium* لهما نفس الفعل المضاد المؤثر على نمو الفطريات المسببة لمرض عفن جذور البرقوق وهى *Fusarium chlamydosporum, Phytophthora parasitica* and *Macrophomina phaseolina* وقد لوحظ أن الفطر المضاد

T. harzianum كان الأعلى تأثيراً على النمو الميسيليومي للفطريات المسببة للمرض تحت الدراسة يليه البكتيريا *B. megaterium* حيث وجد أن تأثيرها على النمو الميسيليومي للفطريات المسببة لمرض عفن جذور البرقوق كان ضعيفاً ومع فطر *M. phaseolina* لم يلاحظ لها أي تأثير تضادي وكانت النتائج متشابهة تحت ظروف الصوبة حيث كان أعلى الكائنات تأثيراً من حيث الفعل المضاد والقدرة على تقليل الإصابة بالفطريات الممرضة فطر *T harzianum* يليه *Glomus* sp. ثم *T. album* وأخيراً *B. megaterium* وكان أكثر الفطريات المسببة لمرض عفن جذور البرقوق تأثراً بفعل الكائنات المضادة الفطر *F. chlamydosporum* ثم *P parasitica* بينما كان اقل هذه الفطريات تأثراً هو الفطر *M. phaseolina*