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BIOLOGICAL AND CHEMICAL CONTROL OF ROOT-ROT/WILT DISEASES IN SOME LEGUME CROPS UNDER GREENHOUSE CONDITIONS IN EGYPT

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Abstract

Legume crops (faba bean, lentil, chickpea and lupin) are important human food and animal feed in Egypt. Several soil-borne fungi attack the roots of these crops and cause root-rot and wilt diseases. *Fusarium oxysporum.* and *Rhizoctonia solani* are the most destructive fungi attacking such plants. Under greenhouse conditions, four biocontrol agent namely *Trichoderma harzianum, Gliocladium virens, Paecilomyces farinasus* and *Bacillus subtilis* and the fungicide Rizolex T, were tested for controlling root-rot/wilt diseases of the formentioned crops at Giza Res. Station in 1999/2000.

T.harzianum and *Gliocladium virens* were the most effective bioagents in reducing the root-rot/wilt diseases caused by *Fusarium oxysporum* and *Rhizoctonia solani* in faba bean, chickpea and lupin whereas *T.harzianum* and *Bacillus subtilis* were effective in lentil. The percentage of survival plants were 86.7%; 80.0%; 80.0%; 83.3%; 80.0% and 73.3%, respectively. The respective values were 68.3% 56.7% in lentil as compared with the controls 26.7%, 30.0%, 30.0% and 20.0%, respectively.

Key words: Biological control; *Trichoderma, Gilocladium, Paecilomyces* and *Bacillus*, root-rot/wilt, legume crops.

INTRODUCTION

Legume crops (faba bean, lentil, chickpea and lupin) are important winter pulse crops in Egypt as well as in many other countries. They are important sources of protein for human consumption as well as for animal feed. Damping-off, root-rot and wilt diseases are the most destructive diseases attacking such plants causing serious losses to the yield. The damage caused by such diseases extends to quantity and quality of the crop beside a decrease in plant population which affects atmospheric nitrogen fixation. These diseases are caused by several fungi i.e. *Rhizoctonia solani, Fusarium oxysporume, Fusarium solani, Fusarium moniliforme, Macrophomina phaseolina, Verticillium spp, Pythium spp* and *Sclerotium rolfsii* (Abdallah, 1969; Nene, 1980; El-Garhy, 1994; Hassanein *et al*, 1996, and Abou-Zeid *et al.*, 1997). *Fusarium oxysporum* specialized forms cause wilt on legume crops; namely, *F.oxysporum* f.sp. *fabae* on faba bean, *F.oxysporum* f.sp. *lentis* on lentil, *F.oxysporum* f.sp. *ciceri* on chickpea, *F.oxysporum* f.sp. lupini on lupin (Booth, 1971). Different control methods including varietal resistance, biological and chemical control were attempted under greenhouse and field conditions. Microorganisms have been used as antagonists to the pathogenic fungi as an alternative control method to fungicides. (Abdel Moity and Abou-Zeid, 1988).

There are three general strategies in considering biological control with introduced microorganisms: (a) reduce the population of the pathogen; (b) prevent the pathogen from infecting the plant; and (c) limit disease development after infection (Cook, 1993). The work reported herein aimed at evaluating some biocontrol and chemical agents in an attempt to control or reduce the incidence of such diseases.

MATERIALS AND METHODS

I. *In vitro*:

1. Isolation and identification of pathogens:

Roots and basal stems of diseased plants showing typical symptoms of root-rot and wilt were washed carefully with tap water, dried between two filter papers, then cut into small pieces. Small pieces of infected roots were surface-sterilized in 3% sodium hypochlorite for 2 min., dried between two sterilized filter papers, were directly transferred to Petri dishes containing potato dextrose agar (PDA) medium, then incubated at 25°C for 5 days. The isolated colonies were purified by the single spore and / or hyphal tip techniques and were identified according to Booth (1971), Alexopoulos and Mims (1979) and Nelson *et al.,* (1983). All cultures were stored in the refrigerator for further study.

II. *In Vivo*:

T.harzianum was grown on wheat bran, while *Gliocladium virens, Paecilomyces farinasus , Rhizoctonia solani and Fusarium oxysporum* were prepared on corn sand medium (75:25 w/w). *Bacillus subtilis* was grown on nutrient broth medium for 48hr at 30°C. Field soil and pots were sterilized with formalin solution (5%) and sterilized soil was infested with *T.harzianum, G.virens* and *P.farinasus* at the rate of 10 g/kg soil and *B.subtilis* at the rate 50 ml/pot (5×10^8 cfu / ml.). Seven days later, soil was infested with the pathogenic fungi at the rate of 5% (w:w) of each pathogen per pot (Hassanein *et al.*, 1996). Rizolex T fungicide seed treatment was carried out at the recommended dose (2 g/kg seeds). Ten seeds of lentil (Giza 9), 10 seeds of chickpea (Giza 3), 5 seeds of faba bean (Giza 531) and 5 seeds of lupin (Giza 1) were sown in each pot and 4 replicates were used for each treatment. Data were recorded after 15, 45 and 90 days for pre, post emergence damping – off and survival plants percentage, respectively (Soleman *et al.*, 1988).

RESULTS AND DISCUSSION

Data presented in Table (1) showed, for faba bean, that all treatments significantly decreased the percentage of pre emergence damping-off. The least percentage was obtained with *T.harzianum, B.subtilis* and Rizolex T, with values of 3.3%, 6.7% and 3.3% respectively, followed by *G.virens* at 10%, while *P.farinasus* was the least effective showing pre emergence damping-off of 20.0%, compared with the control 33.3%. In case of post emergence damping-off, *T.harzianum, G.virens* and Rizolex T, were the most effective in reducing the percentage of post emergence damping-off showing 10%, 10% and 3.3% respectively, followed by *B.subtilis* at 13.3%, while *P.farinasus* was the least effective on post emergence damping-off allowing infection of 16.7% compared with the control at 40.0%. The highest percentage of survival plants was obtained with *T.harzianum, G.virens* and Rizolex T, at 86.7%, 80.0% and 93.7% respectively, followed by *B.subtilis* (73.3%), while *P.farinasus* was the least effective of the tested formulations resulting in 63.3% survival plants compared with 26.7% in the control. These results are in agreement with Windham (1986) who reported that the efficacy of different bioagents against root-rot/wilt disease may be due to different modes of action of these fungi, i.e. production of inhibitory substances and mycoparasitism or colonizing the court of infection and preventing the pathogenic fungi from reaching susceptible plant tissues.

Table 1. Effect of antagonistic microorganisms on root-rot/wilt diseases in faba bean under artificial inoculation in greenhouse in 1999/2000 seasons.

Treatments	Damping		Survival %
	Pre	Post	
Trichoderma harzianum	3.3	10.0	86.7
Gliocladium virens	10.0	10.0	80.0
Paecilomyces farinasus	20.0	16.7	63.3
Bacillus subtilis	6.7	13.3	73.3
Rhizolex. T	3.3	3.3	93.3
Control (1) *	33.3	40.0	26.7
Control (2) **	0.0	3.3	96.7
L.S.D at 5%	4.01	2.35	2.35

* Control (1) Infested soil with pathogen only

**Control (2) Non infested soil

Data presented in Table (2) indicated that for lentil, the least percentage of pre emergence damping-off was obtained with *T.harzianum* and Rizolex T resulting in 10% and 8.3% infection respectively, followed by *B.subtilis* and *G.virens* (11.7% and 13.3%), while *P.farinasus* was the least effective against pre emergence damping-off giving 18.3% disease incidence, compared with the control at 25.0%. Also, the least percentages of post emergence damping-off, obtained with *T.harzianum* and Rizolex T, were 21.7% and 16.7% respectively, followed by *G.virens* and *B.subtilis* with 31.7% and 31.7% infection, while *P. farinasus* was the least effective on post emergence damping-off allowing 51.7% infection compared with the control of 55.0%. Therefore, the highest percentage of survival plants was obtained with *T.harzianum* and Rizolex T, (68.3% and 75.0%) followed by *B.subtilis* and *G.virens* (56.7% and 55.0%) while the least survival percentage was obtained with *P.farinasus* at 30.0% compared with the control (20%). Singh and Methrotra (1980) and Hassanein *et al* (1996) reported that *T.harzianum, G.virens,* and *Bacillus spp*, either mixed in soil or applied to seeds provided a good protection against *F.oxysporum* and *R.solani.* Sayed *et al.* (1992) reported that *Gliocladium virens, Trichoderma harzianum* and *Paecilmyces lilacinus* significantly reduced root-rot of lentil caused by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp.

Table 2. Effect of antagonistic microorganisms on wilt/root-rot diseases in lentil under artificial inoculation in greenhouse at 1999/2000 seasons.

Treatments	Damping-off		Survival %
	Pre	Post	
Trichoderma harzianum	10	21.7	68.3
Gliocladium virens	13.3	31.7	55
Paecilomyces farinasus	18.3	51.7	30
Bacillus subtilis	11.7	31.7	56.7
Rhizolex. T	8.3	16.7	75
Control (1) *	25	55	20
Control (2) **	3.3	6.7	90
L.S.D at 5%	7.9	2.33	2.34

* Control (1) Infested soil with pathogen only

**Control (2) Non infested soil

Data presented in Table (3) showed that for chickpea, the least percentage of pre-emergence damping-off was obtained with *P.farinasus* and Rizolex T at 3.3%, followed by *B.subtilis* and *T.harzianum* which resulted in 6.7% and 10% infection, respectively. *Gliocladium virens* was the least effective in reducing the percentage of pre emergence damping-off to 13.3%, compared with the control of 26.7%. In case of post emergence damping-off, disease incidence obtained with *G.virens, T.harzianum, Bacillus subtilis* and Rizolex T were 3.3%, 10%, 13.3 % and 10% respectively, while *P.farinasus* decreased the post emergence damping-off, slightly to 33.3% compared with the control (43.3%). The percentage of survival plants was highly increased with *G.virens* and

Rizolex T to 83.4% and 86.7% followed by *T.harzianum* and *B.subtilis* showing 80% for both. *P.farinasus* treatment had a survival percentage of 63.4% compared with the non treated showing 30.0%. El-Barougy (1997) studied the antagonism between *Streptomyces griseoviridis, T.harzianum* and *G.roseum* and the main pathogenic fungi *F.oxysporum* and *R.solani*. *Streptomyces griseoviridis* was the most potent antagonistic agent against *F.oxysporum* and *R.solani* in alfafa plants.

Table 3. Effect of antagonistic microorganisms on wilt/root-rot diseases in chickpea under artificial inoculation in greenhouse at 1999/2000 seasons.

Treatments	% damping-off		Survival %
	Pre	Post	
Trichoderma harzianum	10	10	80.0
Gliocladium virens	13.3	3.3	83.4
Paecilomyces farinasus	3.3	33.3	63.4
Bacillus subtilis	6.7	13.3	80.0
Rhizolex. T	3.3	10.0	86.7
Control (1) *	26.7	43.3	30
Control (2) **	0	3.3	96.7
L.S.D at 5%	11.1	11.1	5.49

* Control (1) Infested soil with pathogen only

**Control (2) Non infested soil

Data presented in Table (4) showed that for lupin, all treatments significantly reduced the percentage of pre emergence damping-off. The reduction in the percentage of infection was very high with *T.harzianum* and Rizolex T, showing 6.7% and 3.3% respectively, followed by *G.virens* (10%). *P.farmasus* and *B.subtilis* were less effective in reducing pre emergence damping-off giving 16.6% and 16.7% infection, respectively, compared with the control of 26.7%. Also, the least percentage of post emergence damping-off obtained with *T.harzianum* and Rizolex T were 13.3% and 10%, followed by *G.virens* (16.7%), while *B.subtilis* and *P.farinasus* resulted in 23.3% and 26.7% infection, respectively, compared with the control (43.3%). The highest percentage of survival was observed with *T.harzianum* and Rizolex T, at 80% and 86.7%, respectively, followed by *G.virens* at 73.3%, while *P.farinasus* and *B.subtilis* were the least effective resulting in 56.7% and 60% infection, compared with control (30%). These results agree with Abdel-Kader (1977) who reported that *T.harzianum,* as seed treatment, significantly decreased the root-rot incidence of bean. Sesan (1988) used species of *Trichoderma* to control fungal pathogens of lentil. Good results were obtained with *Trichoderma viride* for seed treatment of lentil against pathogens such as *R.solani.* The effect of *Trichoderma* in reducing pre and post emergence damping-off was attributed to the production of a growth regulating factor that increase the rate of seed germination (Windham, 1986).

Table 4. Effect of antagonistic microorganisms on wilt/root-rot diseases in Lupin under artificial inoculation in greenhouse 1999/2000 seasons.

Treatments	% damping-off		Survival %
	Pre	Post	
Trichoderma harzianum	6.7	13.3	80.0
Gliocladium virens	10.0	16.7	73.3
Paecilomyces farinasus	16.6	26.7	56.7
Bacillus subtilis	16.7	23.3	60.0
Rhizolex. T	3.3	10.0	86.7
Control (1) *	26.7	43.3	30.0
Control (2) **	3.3	3.3	93.4
L.S.D at 5%	14.8	13.2	7.14

* Control (1) Infested soil with pathogen only

**Control (2) Non infested soil

It is concluded, based on results obtained, that *Trichoderma harzianum* and *Gliocladium virens* showed acceptable efficacy in controlling root-rot/wilt disease in faba bean and chickpea. *Bacillus subtlis* gave satisfactory control on chickpea and Faba bean. However, the fungicide Rhizolex T always exhibited higher efficiency than the biocontrol agents. It remains to uncover the nature of interaction among different biocontrol agents and its reflection on their efficacy, when used in mixture.

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المقاومة الحيوية و الكيماوية لأمراض عفن الجذور والذبول في بعض المحاصيل البقولية تحت ظروف الصوبة في مصر

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أجريت التجربة لمقاومة عفن الجذور و الذبول في الفول البلدي ،العدس ، الحمص و الترمس الناتج عن الإصابة بفطري رايزوكتونيا سولانى و فيوزاريوم اكسسبورم تحت ظروف الصوبة بالجيزة وتم فيها استخدام بعض الفطريات و البكتيريا المضادة لمقاومة مسببات عفن الجذور و الذبول.

وجد أن الفطر ترايكودرما هيرزيانم كان أفضلهم في مقاومة عفن جذور الفول البلدي المتسبب عن الفطر رايزوكتونيا سولانى يليه الفطر جليوكلاديوم فيرنس بينما كان أقلهم فاعلية الفطر باسيلومايسس حيث كانت نسبة النباتات الباقية (٨٦.٧٪ ، ٨٠٪، ٦٣.٣ ٪) علي التوالي بالمقارنة بمعاملة المقارنة التي أظهرت ٢٦.٧ ٪ كنسبة مئوية للنباتات الباقية و أيضا وجد أن الفطر ترايكودرما هيرزنايم كان الأفضل في مقاومة مرض الذبول في العدس المتسبب عن الفطر فيوزاريوم اكسسبورم تليه البكتريا باسيلس ستلس و أيضا كان أقلهم الباسيلوماسيسس(٦٨.٣ ٪ ، ٥٦.٧٢ ٪ ، ٣٠٪) بينما الكنترول ٢٠ ٪.

وجد أن الفطر جليوكلاديوم فيرنس كان الأفضل في مقاومة المرض في الحمص يليه كل من الفطر ترايكودرما هيرزيانم و البكتريا باسيلس ستلس و أيضا كان أقلهم الباسيلومايسس (٨٣.٤ ٪ ، ٨٠ ٪ ، ٨٠٪ ، ٦٣.٤٪ نباتات باقية على التوالي) بينما كان الكنترول ٣٠ ٪ .

ووجد أن الفطر ترايكودرما كان الأفضل في مقاومة المرض في الترمس يليه الفطر جليوكلاديوم و أيضا كان الباسيلومايسس الأقل في المقاومة حيث كانت نسبة النباتات الباقية (٨٠٪، ٧٣.٣ ٪ ، ٥٦.٧ ٪) على التوالي بينما الكنترول ٣٠ ٪ في جميع الحالات وجد أن كل الكائنات الدقيقة التي تم استخدامها في المقاومة قللت النسبة المئوية لعفن الجذور و كذلك مرض الذبول بنسبة كبيرة و لكن بدرجات متفاوتة مما أدى إلي زيادة النسبة المئوية للنباتات الباقية.

وتتراوح الزيادة في نسبة النباتات الباقية في حالة استخدام المبيد (رايزولكس- ت) ما بين ٣,٣ ٪ إلى ٦,٧٪ وهى زيادة قليلة إذا ما قورنت بما تسببه هذه المبيدات من أضرار سواء للكائنات الدقيقة النافعة في التربة أو تلوث للبيئة أو بما تسببه من أخطار للإنسان.