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BIOLOGICAL CONTROL OF PRE AND POST-EMERGENCE DISEASES ON FABA BEAN,LENTIL AND CHICKPEA IN EGYPT

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Abstract

Efficiency of four bioagents (i.e. *Trichoderma harzianum, Gliocladium virens, Bacillus subtilis and Paecillomyces sp.*.) and vitavax 200 fungicide, were evaluated at two sowing dates as seed coating for some leguminous crops and planted in a soil naturally infested with *Rhizoctonia solani, Fusarium* spp. and others. The study included faba bean, lentil, and chickpea. The results of the two seasons (1999 and 2000) showed that, seed treatment with all bioagents or Vitavax 200 fungicide significantly decreased damping-off diseases and increased percentage of surviving plants; however, the biocontrol agents were more effective than the fungicide, when compared to untreated seeds of the three leguminous crops, especially in the early sowings, except for lentil where the opposite trend was found.

Bioagents significantly reduced damping-off and increased surviving plants of faba bean and were as effective as vitavax 200, while seed coating with *G.virens* or *T. harzianum* was more effective in controlling pre- and post- emergence damping-off on lentil in 1999 season, whereas *B.subtilis* was superior in 2000. On chickpea, Rhizo-N (*B.subtilis*) gave the best results followed by *G. virens* or *T. harzianum.*

INTRODUCTION

Damping-off, root-rot and wilt diseases of faba bean, lentil, and chickpea crops are considered the most important diseases that affect plant stand causing great losses in the annual seed yield. Some of these diseases are caused by seed and/or soil borne pathogens such as *Rhizoctonia solani, Fusarium oxysporum, Fusarium* spp., *Sclerotinia sclerotiorum* (El-Awadi, 1993 and El-Garhy, 1994).

In order to increase the productivity, the leguminous crops should be protected against root-rot/wilt disease complex and other diseases as well. Integrated control programs of plant diseases are the most successful and economical means to control diseases, especially when all available pertinent information regarding the crops, its pathogens, the environmental conditions expected to prevail, locality, availability of materials and costs are considered in developing the control program.

Biocontrol agents, such as *Trichoderma* spp. and *B. subtilis* were used as seed treatments and were the most feasible and economic method in preventing soil and/or seed– borne diseases (Paulitz and Baker, 1987). The use of *Trichoderma* spp as biocontrol agent is well established (Sivan *et al.*, 1984). *T.harzianum* was effective against *Sclerotium cepivorum*, the causal organism of white rot of onion when added to the soil (Abd El-Moity, 1981). *T.harzianum* was effective in controlling *S.rolfsii* and *R.solani* and there was a positive correlation between the amount of *T. harzianum* preparation and disease reduction (Chet *et al.*, 1979). This work is aimed to evaluate the effect of four bioagents and one fungicide as seed treatment at two sowing dates to control the pre- and post-emergence damping-off diseases of leguminous crops under field conditions.

MATERIALS AND METHODS

a. *In vitro* studies :

For all experiments, a loamy naturally infested soil with *Rhizoctonia solani*, *Fusarium* spp., and other fungi was obtained from Shandaweel locality (Sohag Governorate). The population densities of soil-borne fungi were estimated before planting the tested leguminous crops using the method described by Hussey and Rancadori (1977) .

b. Bioagents and the fungicide :

Trichoderma harzianum, Gliocladium virens, and *Paceillomyces* sp. were provided by Dr. N.M. Abou-Zeid, Plant Pathology Research Institute, Giza, Egypt, and Rhizo-N (*Bacillus subtilis*) from Nasr company of fertilizers and bioagents. One fungicide named vitavax 200 (carboxin { 5.6-dihydro-2methyl-n-phenyl. } 1.4 oxathhiin-3-carboxamide) + thiram (tetramethyl thiuram disulphide) was used to compare its efficiency with the above mentioned bioagents.

c. Bioagents and the fungicide seed coating:

Fifteen ml of suspension of each of *T.harzianum* { 6×10^6 colony forming units (cfu)/ml }, *Golocladium virens* { 5×10^6 (cfu)/ml }, *B.subtilis* {12×10^6 (cfu)/ml }, and

Paceillomyces sp. { 7 x 10^6 (cfu)/ml }, were added to each kg seeds, while the fungicide vitavax 200 was used at a rate of 3g/kg seed. Both bioagents and fungicide were used as a seed coating for each of the tested crops . Wet seeds of the three leguminous cultivars (*faba bean,* Giza 714; *chickpea,* Giza88 and *lentil,* Giza9) were coated with each of the tested bioagent, and shaken several times, then planted immediately in the field, while seeds treated with vitavax 200 were allowed to dry six hr before planting. Seeds for the control treatments were similarly treated, but without any bioagent or fungicide.

d. In field biocontrol studies:

A field experiment, was conducted in two successive seasons (1999-2000). A complete randomized block design was performed to study the effect of four bioagents and one fungicide as seed treatment for the control of pre- and post- emergence damping-off of three leguminous crops . Three replicated plots, 3x3 m. each contained four 3 m long rows, were used for each treatment. Two healthy seeds previously treated or not, as a control, were sown in hills, 20 cm apart. Two sowing dates were used in this experiment: the early date on October 15 and the late date on November 25 for lentil and faba bean crops, while chickpea crop was sown on mid – November and December 25. Recommended agricultural practices for each leguminous crop were followed.

e. disease severity:

Percentage of pre-and post emergence damping-off and surviving plants were recorded 15,30 and 90 days after planting.

RESULTS AND DISCUSSION

Under field conditions, seed coating with the tested bioagents provided a marked protection against seedling diseases of leguminous crops (lentil, chickpea, and faba bean) at the two sowing dates. The protection was significant in seeds treated with *T. harzianum, G. virens,* and *B. subtilis.* However, *Paceillomyces* sp. and vitavax 200 caused less protection against the disease. The effect of the planting date on the disease incidence varied. The optimum time for sowing field faba bean in the area of experimentation was around mid-October; November 25 and October 15 for lentil and chickpea, respectively.

Table 1. Population of soil-borne fungi before planting leguminous crops.

Fungi	Number of propagules in 10gm of dry soil	
	1999	2000
Rhizoctonia solani	57.73 [a)]	60.32
***Fusarium* spp.**	34.13	33.33
Other fungi [b)]	8.14	6.35

a) Each value is the average of ten replicates of soil samples taken from tested field.

b) Other fungi included; *Macrophomina phaseolina, Sclerotioum rolfsii, Aspergillus flavus, Penicillium* spp., *Alternaria* sp., *Helminthosporium* sp., and *Phomopsis* sp.

Faba bean:

Data in Table (2) clearly indicate that the pathogenic fungi in naturally infested soil in 1999 and 2000 growing seasons, varied as regards to the incidence of diseases in the control (pre -and post- emergence damping- off) with time of planting. Bioagents and fungicide applications in the field, as seed treatment, were generally effective against faba bean pre- and post -emergence damping -off diseases in the two-planting dates. All tested bioagents and vitavax 200 decreased pre- and post- emergence damping-off and increased percent of surviving plants as compared with untreated seeds, especially in the earlier sowing (15 th of October). Moreover, the interaction between bioagents was not significant in the two sowing dates. *T.harzianum* or *G. virens* was the most effective in controlling damping-off diseases, when compared with vitavax 200 only. Therefore, it could be concluded that as far as disease control is concerned, all tested bioagents were satisfactorily equal, except for *T.harzianum* or *G.virens* which were more effective in controlling post- emergence damping-off in late sowing (25th of November). In general, early planting resulted in low disease incidence, which is a reflection of environmental factors, particularly temperature.

Results obtained during 2000 growing season were almost the same as in 1999. Minor differences, however, were observed. All tested bioagents was more effective in controlling seedling diseases in 2000 than 1999. However, in case of *T. harzianum* or

G.virens they were the best bioagents during 2000 and 1999 seasons. The results were in accordance with Abu Salih *et al.* (1973), who reported that delaying sowing of irrigated field beans in the Sudan after October greatly lowered the yield of seed, and increased both infestation by aphids and the incidence of Sudanese broad bean mosaic virus (SBBMV). However, the application of *T.harzianum* and *B.subtilis* bioagents was the most effective treatment in controlling white rot disease caused by *Sclerotium cepivorum* on onion and garlic, when added to soil (Abdel Rahman *et al.*, 1998). Also, experiments carried out in the greenhouse and the field indicated that, seed coating of faba bean with *T.harzianum* and *B.subtilis* could be considered as an effective biocontrol for Fusarium root-rot (Yehia *et al.*, 1982). Brown (1974) found that *T. viride* and *B. subtilis* in greenhouse trials were active in the rhizosphere of broad bean plants and inhibited Fusarium root-rot when seeds were treated with the above microorganisms. Present results showed that the bioagents (*T.harzianum* and *G.virens*) as faba bean seed coating coupled with early-sowing (15^{th} of October) could reduce pre- and post-emergence damping-off diseases.

Lentil:

Data in Table (3) Indicate that in 1999 growing season, late-sowing (25^{th} of November) resulted in a decrease in pre-and post- emergence damping-off and increased number of surviving plants than in earlier-sowing (15^{th} of October). Seed coating with *G.virens* was more effective in controlling pre-emergence damping-off as compared with other tested bioagents or vitavax 200, without any significant difference among them. On the other hand , *G. virens* and *Trichoderma harzianum* were more effective in reducing post-emergence damping-off than other treatments at the two-sowing dates. Data also show that percentage of surviving plants 90 day after planting was considerably high in case of seed treated with *T. harzianum* or *G. virens* as compared with the control (un-treated seed) or other treatments in both sowing dates. No significant differences were observed between the remaining bioagent treatments.

Results obtained in 2000 were as in 1999 season, with minor differences. *T. harzianum* or *B. subtilis* were more effective to control pre-and post- emergence damping-off in the late sowing (25 November 2000), as compared with other treatments. However, in case of *G.virens*, the effect was lower in 1999 than 2000 at the two sowing

Table 2. Influence of seed coating with some bioagents or fungicide and planting dates on damping-off and surviving plants of Giza 714 faba bean, grown in naturally infested soil in the 1999 and 2000 season.

Seed treatments by	Dose ,g or ml./kg seed	1999						2000					
		Sowing date and (%) infection				Surviving Plants (%)		Sowing date and (%) infection				Surviving plants (%)	
		(15-October)		(25-November)				(15-October)		(25-November)			
		Pre.	Post.	Pre.	Post.	First date	Second date	Pre.	Post.	Pre.	Post.	First date	Second date
		damping-off [a]		damping-off				damping-off		% damping-off [a]			
Trichoderma harzianum	50 ml (ml/6x10 ^{7} cfu)	7.6 [b]	14.9	18.2 [b]	24.2	77.5	57.6	5.8	9.5	13.7	22.5	84.7	63.8
Gliocladium virens	50ml (ml/5x10 ^{6} cfu)	8.8	12.8	19.2	22.0	78.4	58.8	6.6	9.1	17.9	19.2	84.3	62.9
Rhizo-N (*Bacillus* subtilis)	50ml (ml/12x10 ^{6} cfu)	10.8	13.8	17.3	31.9	75.4	50.8	8.7	10.8	16.6	29.1	80.5	54.3
Paecillomyces sp.	50 ml (ml/7x10 ^{6} cfu)	9.2	16.7	21.5	29.8	74.1	48.7	7.5	13.7	21.2	26.2	78.8	52.6
Vitavax 200	3 9	13.8	25.0	26.3	32.8	61.2	40.9	11.3	19.2	25.0	30.0	69.5	45.0
Control (un-treated) [c]	---	21.3	37.2	30.8	41.3	41.5	27.9	17.9	27.6	29.8	40.3	54.5	29.9

L. S .D at 5% for treatment (t) : 3.31 : 4.93
Sowing dates (s) : 4.41 : 5.23
TxS : 5.23 : 6.61

i) Pre- and post-emergence damping-off of seedlings recorded 15 and 30 days after planting and percent of surviving plants at 90 days after seeding.
b) Each reading is the average of three replicates.
c) Untreated seeds planted in naturally infested soil .

dates. Other treatments showed similar effects. The use of *Trichoderma sp.* as biocontrol agent is well established (Harman *et al.*, 1980 and Sivan *et al.*, 1984). Also, the use of *T. harzianum* as seed treatment significantly decreased the root-rot incidence of bean (Abdel-kader, 1977). In previous studies, *T.harzianum* was considered to be effective against *S.cepivorum*, the causal organism of white rot of onion when added to the soil (Abd El-Moity, 1981). It was also demonstrated that *T.harzianum* was effective in controlling *S.rolfsii* and *Rhizoctonia solani* and there was a positive correlation between the amount of *T.harzianum* preparation and disease reduction. In this respect, EL Neshawy *et al.*, (1999) reported that control of onion neck rot caused by *Botrytis allii* and the reduction of black mould caused by *Aspergillus niger* were obtained on onion with promo (*T.harzianum* and *T.koninjii*). The effect of planting dates in lentil was more pronounced and disease reduction (pre-and post- emergence damping-off diseases) in the late sowing date (25th of November) was higher than in the earlier sowing (15th of October) might be due to soil temperature differences according to Mehrotra and Claudieus (1973). *Gliocladium virens*, as bioagent was the best in field application as a seed treatment. Thus, it could be concluded that *G. virens* and late- sowing date (25th of November) are a promising treatment against pre- and post-emergence damping-off diseases of lentil. Also, *Bacillus subtilis* could produce the same effect depending on the environmental conditions as was observed in the year 2000.

Chickpea:

Data presented in Table (4) show that in early sowing (15th of November), post-emergence damping-off disease of chickpea was less than in later sowing date (25th of December). All tested bioagents and vitavax 200 were generally effective in controlling pre-and post-emergence damping-off diseases in 1999 season compared with the control treatment. At the two planting dates, the effect of bioagents seed treatment, at pre- emergence stage, except *Paecillomyces* were almost similar. *Bacillus subtilis* was more effective in controlling post-emergence damping-off, with no significant differences between *Trichoderma* and *Gliocladium*. Data also show that the percentage of surviving plants were higher with all tested bioagents as compared with both control and vitavax 200 seed- treatment at the two sowing dates. Rhizo-N (*B. subtilis*) gave the highest increase in surviving plants in both dates of sowing followed by *T. harzianum* or *G.virens*, then *Paceillomyces sp*, while vitavax 200 at the recommended dose (3g/kg

Table 3. Effect of seed treated with some bioagents or fungicide and planting dates on damping-off and surviving plants of Giza 9 lentil, grown in naturally infested soil in the 1999 and 2000 seasons, at Shandweel experimental farm.

Seed treatments by	Dose ,g or ml./kg seed	1999						2000					
		Sowing date and (%) infection				Surviving Plants (%)		Sowing date and (%) infection				Surviving plants (%)	
		(15-October)		(25-November)				(15-October)		(25-November)			
		Pre. damping-off a)	Post.	Pre. damping-off	Post.	First date	Second date	Pre. damping-off	Post.	Pre. % damping-off a)	Post.	First date	Second date
Trichoderma harzianum	50 ml (ml/6x10 ^{7}cfu)	14.2 b)	10.8	11.8 b)	10.0	75.0	78.2	11.3	14.0	8.7	13.2	78.1	74.7
Gliocladium virens	50ml (ml/5x10^6 cfu)	8.8	13.8	7.5	13.2	77.4	79.3	14.0	18.3	13.2	15.0	67.7	71.8
Rhizo-N (*Bacillus subtilis*)	50ml (ml/12x10^6 cfu)	14.5	20.0	13.0	19.2	65.5	67.8	11.7	15.0	7.5	11.8	73.3	80.7
Paecillomyces sp.	50 ml (ml/7x10^6 cfu)	16.3	18.3	11.3	16.7	65.4	72.0	14.6	19.0	14.5	18.0	66.4	67.5
Vitavax 200	3 9	15.0	22.8	14.7	20.0	62.2	65.3	18.0	22.0	14.2	21.8	60.0	64.0
Control (un-treated)[c]	- - -	25.9	35.4	24.0	39.1	38.7	36.9	24.7	38.9	24.8	39.1	36.4	36.1

L. S .D at 5% for treatment (t)	: 4.16	: 2.09
Sowing dates (s)	: 3.25	: 3.81
TxS	: 5.14	: 4.02

a) Pre- and post-emergence damping-off of seedlings recorded 15 and 30 days after planting and percent of surviving plants 90 days after planting.
b) Each reading is the average of three replicates.
c) Untreated seeds planted in naturally infested soil .

seeds) decreased pre-and post- emergence damping-off diseases incidence but at a lower efficacy as compared with the bioagent treatments. This may be due to the expected degradation of fungicide when introduced into the soil and exposed to the environmental conditions as reflected by the high post-emergence damping-off. On the other hand, the biocontrol agent establishes itself in the soil and increases its population to a density sufficient to inhibit plant pathogens.

The trend of results obtained during the 2000 growing season was almost similar to 1999 season. All tested bioagents had significant effect on reducing percentage of damping-off, especially in early- sowing date. Seed treated with *G.virens*, *B. subtilis*, and *T. hazianum* could be considered as an effective biocontrol for pre- and post-emergence damping-off ,especially in early planting-date. However, in case of *Paecillomyces* sp., the effect was less in 2000 than in 1999 seasons. These results are in agreement with those obtained by Kommedahl and Mew (1975). In this respect, Schiller *et al.* (1977) suggested that some by-products of microorganisms (*T. viride* and *B. subtilis*) stimulated plant growth and at the same time reduced population density of plant pathogens. Also, they found that reduction in disease severity on some crops may be due to the low inoculum potential of the pathogen in the rhizosphere. The use of the bioagent *B. subtilis* as seed coating and early-sowing date (15^{th} of October) against *R. solani* and *Fusarium .spp* decreased chickpea pre-and post- emergence damping-off .

Generally, it could be concluded that the application of antagonistic microorganisms to seed has been employed not only to control pre- and post-emergence damping-off diseases of the leguminous crop and to promote plant growth and yield, but also to reduce the density of soil-borne pathogens . Also, prospective application of biocontrol technique may help to decrease the use of fungicide and avoid environmental pollution.

Table 4. Effect of seed treated with some bioagents or fungicide and planting dates on damping-off and surviving plants of Giza 88 chickpea, grown in naturally infested soil in the 1999 and 2000 seasons, at Shandweel experimental farm.

Seed treatments by	Dose ,g or ml./kg seed	1999						2000					
		Sowing date and (%) infection				Surviving Plants (%)		Sowing date and (%) infection				Surviving plants (%)	
		(15-October)		(25-November)				(15-October)		(25-November)			
		Pre. damping-off a)	Post.	Pre. damping-off	Post.	First date	Second date	Pre. damping-off	Post.	Pre. % damping-off a)	Post.	First date	Second date
Trichoderma harzianum	50 ml (ml/6x10^7 cfu)	7.5 b)	22.5	14.2 b)	25.4	70.0	60.4	6.6	20.4	13.3	26.4	73.0	60.3
Gliocladium virens	50ml (ml/5x10^6 cfu)	6.7	22.0	14.9	24.3	71.3	60.8	5.5	19.4	14.0	25.2	75.1	60.8
Rhizo-N (*Bacillus subtilis*)	50ml (ml/12x10^6 cfu)	6.7	18.0	14.9	19.0	75.3	66.1	7.7	17.0	14.4	21.1	75.3	64.5
Paecillomyces sp.	50 ml (ml/7x10^6 cfu)	10.0	25.4	18.0	27.9	64.6	54.1	11.0	27.1	17.2	26.9	61.9	55.9
Vitavax 200	3 9	11.7	36.7	17.1	38.3	51.6	44.6	11.0	33.4	18.5	37.4	55.6	44.1
Control (un-treated) c	---	22.1	50.0	25.8	68.8	27.9	5.4	20.1	45.0	26.6	56.7	34.9	16.7

L. S .D at 5% for treatment (t)	: 2.19	: 2.04
Sowing dates (s)	: 5.14	: 7.12
TxS	: 6.04	: 8.09

a) Pre- and post-emergence damping-off of seedlings recorded 15 and 30 days after planting and percent of surviving plants 90 days after sowing.
b) Each reading is the average of three replicates.
c) Untreated seeds planted in naturally infested soil .

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المقاومه الحيوية لأمراض موت البادرات للفول البلدي و العدس و الحمص في مصر العليا

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أستخدمت أربع مستحضرات حيوية (تريكوديرما هارزيانم، جلوكوديرم فيرينز، باسيلس ستلس ، باسيلوميسس) ومطهر فطري (فيتافاكس ٢٠٠) كمعامله بذرة في ثلاث محاصيل بقولية (عدس – حمص – فول بلدي)، و زرعت في تربة ملوثة طبيعيا بفطريات ممرضه لتقييم قدرتها على مكافحة أمراض موت البادرات .

ولقد دلت النتائج علي ما يلي :

١- معاملة البذور بالمستحضرات الحيوية والمطهر الفطري قللت معنويا من أمراض موت البادرات وبالتالي زادت نسبة البادرات السليمة القائمة ، ولكن تأثير المواد الحيوية المستخدمة كانت أفضل من المطهر الفطري مع المحاصيل البقولية محل الدراسة وبالأخص في طور البادرة وأيضا في الزراعة المبكرة ، ما عدا العدس الذي أظهر التأثير السابق .

٢- **الفول البلدى**: المواد الحيوية والمطهر الفطري تشابهت في تأثيرها علي خفض حدوث أمراض موت البادرات

العدس : تغطية البذرة بالمواد الحيوية تريكودرما هارزيانم وجليوكلاديمفيرينز أعطي أعلي تأثير في مقاومة أمراض موت البادرات سواء كان قبل أو بعد الأنبات فوق سطح التربة مع مواعيد الزراعة المستخدمة خلال موسم النمو عام ١٩٩٩ ، أما المواد الحيوية تريكوديرما هارزيانم وباسيلس ستلس كانت افضل تأثير خلال موسم الزراعة عام ٢٠٠٠ .

الحمص : الريزوأن (باسيلس ستلس) أعطت نفس التأثير في خفض المرض بمحصول العدس يليها جليوكلاديوم فيرنز أو تريكودرماهارزيانم خلال موسمي النمو.