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BIOLOGICAL CONTROL OF ROOT-ROT AND WILT DISEASE OF FABA BEAN USING SOME BIOAGENTS

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ABSTRACT

In this study *Trichoderma harzianum, Trichoderma album, Bacillus subtilis* and *Bacillus megaterium* were antagonistic to the growth of *Rhizoctonia solani, Fusarium oxysporum* and *F. solani*, the causal pathogens of faba bean root-rot and wilt, with different degrees of inhibition. *T. harzianum* was the most effective followed to *T. album, B. subtilis* and *B. megaterium*, respectively. *B. megaterium* was the least effective.

Under greenhouse conditions, it was found that the radial growth of the fungus and significantly decreased root-rot and wilt and increased plant height, fresh weight and dry weight. Plant guard gave the highest inhibition followed by Bio-zeid, Rhizo-N and Bio-ARC, respectively.

In field experiments, results indicated that all antagonists offered a highly significant protection comparing with the untreated. Plant guard was the best followed by Bio-zeid, Rhizo-N and Bio-ARC, respectively.

Keywords: Faba bean, damping-off, soil-borne fungi and bio-agents.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a legume crop with high nutritional value. Yehia *et al.* (1988) proved the antagonistic effect of *Trichoderma viride* against *Fusarium solani* of faba bean. Nelson (1992) reported that *Trichoderma* spp. are specific biocontrol agents against fungal pathogens (from *Pythium* to *Rhizoctonia*) according to the type of antibiotic produced. Condrej (1993) reported that *F. oxysporum* f. spp. Faba caused wilting of faba bean growing at the breeding station, Czech Republic in 1990 and 1991. Akem and Bellar (1999) found though survey in the main faba bean growing regions of Syria, that the wilt/root-rot complex (*Fusarium oxysporum* and *Macrophomina phaseolina* dominating the complex were the most important and widespread fungal diseases observed at all locations, Metwaly (2004) revealed that pathogenicity test of isolates *Rhizoctonia solani* exhibited that *R. solani* isolated from Kafr El-Sheikh governorate was the most virulent isolate from Sharkia governorate. El-Sayed (2006) found that *F. oxysporum, R. solani, F. maniliforme* and *V. alboatrum* caused post-emergence damping-off infection % ranged between 3.3 – 23.3%. Baker (1987) emphasized that antibiotics produced in direct content between microorganisms may function at low concentration and be less subject to biotic decomposition. Sas-Piotrowska and Dorszewski (1996) studied the effect of *Trichoderma harzianum, T. viride, T. koningii* and *Gliocladium roseum* and 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*. Zheng and Sinclair (2000) showed that *Bacillus megaterium* is a potential bacterial biocontrol agent against *Rhizoctonia solani*.

Lewis and Lundsen (2001) cleared that *T. harzianum* and *T. viride* isolates reduced damping-off of different plants caused by isolate R-23 of *R. solani*. Cigdem and Kivane (2003) showed that the filterates of *T. harzianum* was effective against plant pathogens, *F. oxysporum, F. moniliforme, Rhizoctonia solani* and *Sclerotium rolfsii*. Santamarine and Rosello (2006) showed that a *Trichoderma harzianum* was antagonistic; *in vitro*, to *Rhizoctonia solani* and *Verticillium dehliae* and may be considered a potential biocontrol agent.

MATERIALS AND METHODS

Isolation and identification of the causal pathogen:

Naturally diseased faba bean plants, showing root-rot and wilt symptoms were collected from different localities of Sers El-Layian Agricultural Research Station, Minufiya Governorate in 2010 growing season. They were cut in small pieces, thoroughly washed with tap water, surface sterilized for two minutes with 2% sodium hypocholorite solution, then rinsed several times in sterilized distilled water and dried between folds of sterilized filter papers. The surface sterilized samples were plated onto potato Dextrose Agar (PDA) medium and incubated at 27°C. After 4 – 5 days incubation period, the developed fungal colonies were purified by hyphal tip and single spore isolation techniques. Identification of the fungal isolates was carried out by the Department of Mycology and Plant Disease Survey, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Also, another four bioagents namely; *Trichoderma harzianum* (plant guard) contains 10×10^6 spore / g, *Trichoderma album* (Bio-Zeid) contains 10×10^6 spore / g, *Bacillus magaterium* (Bio-ARC) which contains (29×10^6 cfu/g) and *Bacillus subtilis*, contains (3×10^7 cfu/g).

1. *In vitro*:

T. harzianum and *T. album* were grown on PDA medium, while *B. megaterium* and *B. subtilis* on nutrient agar medium (NA).

In vitro antagonistic effect of fungal and bacteria on pathogenic fungus. Antagonistic effect on the linear growth of *Rhizoctonia solani, Fusarium solani* and *F. oxysporum* of faba bean root-rot and wilt disease, the following method was used. Petri-dishes (9 cm in diameter), each contained 10 ml, of PDA medium were inoculated with discs (5 mm in diameter) of any of the tested pathogens, taken from 7 day-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* and *T. album*. Three plates were used as replicates for each treatment. Antagonistic effect of *B. megaterium* and *B. subtilis* on the linear growth of the same pathogens were tested *in vitro*. The tested bacteria were streaked on PDA plate near of the edge of each Petri-dish, while the inoculation with the tested pathogens was done as mentioned before in the second half of each dish. All plates were incubated at

28°C until the growth in the control treatment reached the edge of the plates. Reduction percentage of fungal growth was calculated according to the followed formula:

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

Table (1). Biopesticides used as seed treatment trade name, bioagent and rate of application.

Trade name	Bioagent	Recommended dose	
		Per 1 medium	Per kg seed
Bio-arc-1	*Bacillus megaterium*	2.5 g	2.5g
Bio-zeid	*Trichoderma album*	2.5 g	2.5 g
Rhizo-N	*Bacillus subtilis*	4.0 g	4.0 g
Plant guard	*T. harzianum*	4.0 ml	4.0 ml

2. *In vivo*:

Effect of the tested biopesticides on the disease incidence in pots:

This experiment was carried out in sterilized pots (20 cm diam.) containing sterilized clay soil in the green house. Both pots and soil were sterilized with 5% formalin solution and then left for two weeks for formalin evaporation.

Discs (5 mm in diam.) of 7 days old culture of the isolated fungi (*Fusarium solani, F. oxysporum* and *Rhizoctonia solani*) were placed in 500 ml conical flasks containing autoclaved sorghum sand medium, flasks were then incubated at 28°C for 15 days.

Soil infestation was carried out by adding the inocula of the fungi and combination between the tested fungi genera to the sterilized soil at the rate of 3.5% soil weight (w/w) (El-Sayed, 2006). The fungal inocula were mixed thoroughly with the infested soil, which was watered for 7 days to enhance fungal growth. The control was treated with the same amount of autoclaved sorghum-sand medium without fungal inocula.

Faba bean 'Giza 3' seeds were treated with the tested biopesticides, Rhizo-N, Bio-ARC, Bio-Zeid and plant guard at (recommended dose) after treated with 4% solution of carboxymethyl cellulose (CMC) as sticker. The same aforementioned methods were used without biopesticides as control. Five seed were sown per pot, and three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off, survival respectively using the next formula according to El-Helaly *et al.* (1970), and healthy survival, plant height, fresh weight and dry weight were recorded.

Statistical analysis:

Data were analyzed in a randomized complete block design according to Gomez and Gomez (1984).

3. In field conditions:

Effect of bio-control agents on root-rot, wilt and some growth characters:

These experiments were conducted in naturally infested soil at Sers El-Layian Agric. Res. Stat., at the mid of November during 2008/2009 –

2009/2010 growing seasons. The experimental layout was in randomized complete block design. The plot was divided into equal sub-plots each one consisting of 5 rows (3 × 3.5 m).

Seed treatment:

Faba bean 'Giza 3' seeds with the four treatments in form of plant guard, Bio-Zeid, Rhizo-N and Bio-ARC were applied as mentioned before faba bean 'Giza 3' seeds without bio-control agents were used as control. Three replicates were used for each treatment. Disease assessment and yield components were recorded and estimated as mentioned before was assessed. Pre-emergence damping-off was assessed after 15 days from sowing post-emergence damping-off after 30 days from sowing and survival plant. Yield components were recorded as mentioned before.

RESULTS

1. Effect of antagonists on fungal linear growth:

As shown in Table (2) the tested antagonists significantly reduced linear growth of all tested fungi compared to control. In general, *T. harzianum* and *T. album* were the most effective bioagents followed by *B. subtilis*, respectively. While *B. megaterium* was the lowest bioagents with all the tested pathogens. On the other hand, there were variations among the tested fungi in relation to their reaction to *T. harizanum* where the higher reduction % was recorded *Rhizoctonia solani* followed by *F. oxysporum* and *F. solani*, respectively.

Table (2). *In vitro* effect of the bioagents on mycelia growth of the three pathogens of faba bean wilt and root-rot disease.

Tested fungus	*T. harzianum*		*T. album*		*B. megaterum*		*B. subitlus*	
	Grown (cm)	Red. (%)	Grown (cm)	Red. (%)	Grown (cm)	Red. (%)	Grown (cm)	Red. (%)
R. solani	2.00	77.77	5.00	44.44	6.30	30.00	5.50	38.88
F. oxysporum	3.20	64.44	6.00	33.33	7.00	22.22	6.00	33.33
F. solani	4.00	55.55	6.40	28.88	7.00	22.22	6.20	31.11
Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00
L.S.D at 0.05	0.42	-	0.22	-	0.20	-	0.11	-

2. Effect of antagonists damping-off pathogens:

Results in Table (3) indicate clearly that the four tested antagonists reduced significantly pre-emergence damping-off infection of faba bean plants infected with the causal pathogens comparing with control treatment. However, there was a variation in efficacy of the four tested antagonists where *T. harzianum* was the best followed by *T. album* and *B. subtilis*, respectively. Meanwhile, *B. megaterium* was the least effective one. All tested antagonists reduced significantly the impact of the pathogenic fungi. As for pre- and post-emergence damping-off *T. harzianum* was the best bioagent followed by *T. album* and *B. subtilis* while *B. megaterium* was the least effective one. Nevertheless, the best effective treatment was *T. harzianum* against *R. solani, F. oxysporum* and *F. solani*. In this respect,

the highest percentage of healthy survived plants was in case of treated seeds before sowing with *T. harzianum*, *T. album*, *B. subtilis* and *B. megaterium*, respectively compared to the control (untreated seeds).

Table (3). Influence of biocontrol agents on faba bean seedling under greenhouse conditions.

Treatment	*R. solani*			*F. oxysporum*			*F. solani*		
	Preemergence (%)	Postemergence (%)	Survival (%)	Preemergence (%)	Postemergence (%)	Survival (%)	Preemergence (%)	Postemergence (%)	Survival (%)
T. harzianum	11.00	10.00	79.00	9.12	8.00	82.88	6.70	6.70	86.60
T. album	14.30	12.15	73.55	11.12	13.00	75.88	10.00	10.00	80.00
B. subtilis	18.14	16.17	65.69	15.30	16.00	68.70	13.33	13.67	73.00
B. megaterium	21.40	19.23	59.37	20.00	20.00	60.00	17.67	15.33	67.00
Control (blank)	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00
Control pathogen	33.33	20.00	46.67	24.13	20.12	55.75	20.00	23.33	56.67
L.S.D at 0.05	0.23	0.42	0.67	0.30	0.49	0.70	0.35	0.52	0.75

Results in Table (4) indicate to the effect of antagonists on some plant growth characters of faba bean plants infected with wilt and root-rot pathogens under greenhouse conditions. Application of the biocontrol agent led to increase their growth characteristics over the treatment with the pathogen alone. In this respect, plant height, fluctuated between 26 – 40 cm of the four tested antagonists without clear trend to compare with the control treatment.

Table (4). Influence of biocontrol agents on faba bean seedling growth characteristics under greenhouse conditions.

Treatment	*R. solani*			*F. oxysporum*			*F. solani*		
	Plant height (g)	Fresh weight plant (g)	Dry weight plant (g)	Plant height (g)	Fresh weight plant (g)	Dry weight plant (g)	Plant height (g)	Fresh weight plant (g)	Dry weight plant (g)
T. harzianum	29.40	13.28	2.00	32.17	16.50	2.13	38.33	14.27	1.89
T. album	28.00	11.05	1.40	30.08	14.00	2.00	35.07	12.53	1.70
B. subtilis	28.00	10.63	1.22	30.00	12.73	1.78	30.50	11.28	1.50
B. megaterium	26.20	10.50	1.15	27.18	10.00	1.60	30.00	8.48	0.94
Control (blank)	32.80	14.00	1.70	35.14	17.00	2.14	40.00	16.17	2.32
Control pathogen	22.13	6.00	0.85	25.06	8.25	0.94	27.33	7.47	0.78
L.S.D at 0.05	3.18	1.20	0.35	3.73	1.40	0.60	2.94	1.38	0.65

3. Factors affecting damping-off on faba bean under field conditions:
Effect of treating faba bean plants with antagonists on damping-off infection and some growth characters:

Data in Table (5) clearly show the influence of antagonists treatment on damping-off disease of faba bean under field conditions during two seasons. The results revealed that antagonistic application reduced significantly pre- and post-emergence damping-off compared to un-treated plants (control). In this respect, *T. harzianum* (plant guard) gave a

considerable protection against root-rot infection followed by Bio-Zeid, Rhizo-N and Bio-ARC, respectively. *T. harzianum* was the best in reducing pre- and post-infection and consequently increased survival of plants comparing to the other tested bio-control agents whereas, Bio-ARC impact this shown in the two seasons (2008 – 2009) and (2009 – 2010).

Table (5). Effect of treating faba bean seeds with antagonists on incidence of damping-off during two seasons under field condition.

Treatment	Season 2008-2009			Season 2009-2010		
	Pre- (%)	Post- (%)	Survival (%)	Pre- (%)	Post- (%)	Survival (%)
Plant guard	10.00	6.70	83.30	10.00	10.00	80.00
Bio-Zeid	13.00	10.00	77.00	13.33	16.67	70.00
Rhizo-N	15.00	10.00	75.00	18.00	18.00	64.00
Bio-ARC	18.30	20.00	61.70	22.22	25.0	52.78
Control	28.00	25.00	47.00	30.00	25.00	45.00
L.S.D at 0.05	9.13	10.08	13.00	11.03	12.00	14.05

As for the effect of antagonists on growth characters and yield component of faba bean under field conditions, the obtained data in Table (6) reveal a significant effect antagonistic isolates over the control where these were shown in case of fresh weight, dry weight, plant height after harvest, number of pods, and 100 seed weight. The obtained results were confirmed from the two experiments conducted in 2008/2009 – 2009/2010 seasons, respectively. However, the great effect on plant growth and yield component was given by Bio-control agents application than that given the control. On the other hand *T. harzianum* gave a good performance followed by *T. album*, *B. subtilis* and *B. megaterium*, respectively.

Table (6). Effect of treating faba bean seeds with antagonists on growth characters and yield component/plant under field conditions.

Treatment	Season 2008-2009					Season 2009-2010				
	Fresh weight (g)	Dry weight (g)	Plant height after harvest	No. of pods	100-seed weight (g)	Fresh weight (g)	Dry weight (g)	Plant height after harvest	No. of pods	100-seed weight (g)
Plant guard	83.22	29.33	103.22	30.00	75.00	86.00	31.21	107.00	33.00	73.00
Bio-Zeid	77.21	25.12	100.27	27.53	73.33	82.27	29.15	102.00	31.00	71.00
Rhizo-N	73.11	20.00	97.67	25.55	71.15	77.16	28.00	98.14	27.0	69.17
Bio-ARC	70.00	18.00	96.32	24.18	70.00	73.00	23.00	95.00	25.18	65.00
Control	67.90	16.37	86.30	20.00	55.00	70.33	20.00	88.08	21.63	57.00
L.S.D at 0.05	11.00	3.02	7.13	3.34	1.55	9.11	2.85	6.27	3.05	2.0

DISCUSSION

Faba bean (*Vicia faba* L.) is a leguminous crop with high nutritional value. It is infected with many fungal pathogens causing considerable yield losses. The obtained data showed that the tested bioagents were effective against the tested causal pathogens of faba bean wilt and root-rot diseases *T. harzianum* and *T. album* were the most effective bio-agents followed by *B. subtilis* and *B. megaterium* (Yehia *et al.*, 1988 and Nelson, 1992). All the tested antagonists reduced the growth of the fungus. Moreover, application of the antagonistic under bioagents greenhouse conditions, reduced significantly pre- and post-emergence damping-off infection and increased healthy survived plants of faba bean plants comparing with control treatment with clear variation in the efficacy of the four tested antagonists. Bioagents affected resulted in an increase of some plant growth characters of faba bean plants infected under greenhouse conditions. The activity of biocontrol agents against soil-borne disease is important to achieve successful control activity (Hye-Sook *et al.*, 2009).

Application of the tested effective antagonists under field and naturally inoculation conditions significantly decreased damping-off disease of faba bean. Consequently plant survival was much better than those of untreated beds. Seedling characteristics *i.e.*, plant height, fresh and dry weights were more satisfactory. Baker (1987) emphasized that antibiotics produced in direct contact between microorganisms may function at low concentration and be less subject to biotic decomposition.

REFERENCES

Akem, C. and M. Bellar (1999). Survey of faba bean (*Vicia faba* L.) diseases in the main faba bean-growing regions of Syria. Arab Journal of Plant Protection, 17: 113 – 116.

Baker, K. F. (1987). Involving concepts of biological control of plant pathogens. Ann. Rev. Phytopathol., 25: 67 – 85.

Cigdem, K. and M. Kivane (2003). Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turk. J. Biol., 27: 247 – 253.

Condrej, M. (1993). The occurrence of the fungus *Fusarium inlexum* Schneider & Dalchow on broad bean in Czech Republic. Ochrana Rostlin, 29: 227 – 230. (C.F. CABI Data base Abstracts).

El-Helaly, A. F.; H. M. Elarosi; M. W. Assawah and M. T. Abol-Wafa (1970). Studies on damping-off and root-rots of bean in UAR (Egypt). Egypt. J. Phytopathol., 2: 41 – 57.

El-Sayed, Sahar A. (2006). Use of intercropping and other treatments for controlling faba bean diseases. Ph.D. Thesis, Fac. Agric. Benha University.

Gomez, K. A. and A. A. Gomez (1984). Statistical Procedures for Agricultural Research. Second Ed. A Wiley-Inter. Science Publication, John Wiley & Sons. Inc. New York, p. 680.

Hye-Sook, K.; M. K. Sang; I. S. Myung; S. C. Chun and K. D. Kim (2009). Characterization of *Bacillus luciferensis* strain KJ_2Cl_2 from pepper root a biocontrol agents of phytophthora blight of pepper. Plant Pathol. J., 25 (1): 62 – 69.

Lewis, J. A. and R. D. Lundsen (2001). Biocontrol of damping-off greenhouse grown crop caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. Crop Protection, 22 (9): 49 – 56.

Metwaly, M. M. M. (2004). Resistance induction against diseases of faba bean crop. Ph.D. Thesis, Plant Pathology Dept., Fac. Agric., Suez Canal Univ.

Nelson, E. B. (1992). Rapid germination of sporangia of *Pythium* species. Phytopathology, 77: 1108 – 1112.

Santamarine, Maria P. and J. Rosello (2006). Influence of temperature and water activity on the antagonism of *Trichoderma harzianum* to *Verticillium* and *Rhizoctonia.* Crop Protection, 25 (10): 110 – 121.

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

Yehia, A. H.; S. A. El-Hassan and A. H. Bahdali (1988). Biological seed treatment to control Fusarium root-rot of broad bean. Egyptian Journal of Phytopathology, 14 (1-2): 59 – 66.

Zheng, X. Y. and J. B. Sinclair (2000). The effect of traits of *Bacillus megaterium* on seed and root colonization root-rot of soybean. Biocontrol, 45 (2): 223 – 243.

المقاومة الحيوية لأمراض أعفان الجذور والذبول فى الفول البلدى باستخدام بعض العوامل الحيوية

رانيا زكى الشناوى

قسم بحوث أمراض البقوليات والعلف – معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

- أظهرت الدراسة أن النتائج المتحصل عليها فى المعمل أن *Trichoderma harzianum* و *T. album* و *Bacillus subtilis* و *B. megaterium* لها نفس الفعل المضاد المؤثر على نمو الفطريات المُسببة لمرض عفن الجذور والذبول فى الفول البلدى وهى *F. oxysporum* و *R. solani* و *F. solani* وقد وجد أن فطر *T. harzianum* وهو الفطر الأعلى تأثيرا على النمو الميسليومى للفطريات المُسببة للمرض تحت الدراسة ثم *T. album* و *B. subtilis* ويليه البكتريا *B. megaterium* وكان تأثيرها على النمو الميسليومى للفطريات الممرضة ضعيفا .
- تحت ظروف الصوبة وجد أن جميع العوامل الحيوية المستخدمة قللت النمو الميسيليومى للفطريات الممرضة المُسببة للمرض وكان العامل الحيوى Plant guard قد أعطى أعلى تثبيط للنمو وأقلهم Bio ARC وقد أعطى Plant guard أقل نسبة موت للبادرات وزيادة فى طول النباتات وزيادة فى الوزن الغض والوزن الجاف .
- أظهر استخدام العوامل الحيوية تحت ظروف الحقل أثراً ملحوظاً فى خفض نسبة موت البادرات قبل وبعد الظهور فوق سطح التربة إذا ما قورنت بالنباتات غير المعاملة وكان أفضل هذه العوامل هو Plant guard (*T. harzianum*) وبوجهٍ عام كانت هذه العوامل الحيوية ذات حماية أكثر إذا ما قورنت بالكنترول وقد ظهر ذلك على قياسات طول النبات والوزن الطازج والجاف وعدد القرون ووزن الـ ١٠٠ بذرة .

قام بتحكيم البحث

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