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CONTROL OF FABA BEAN ROOT ROT DISEASE BY USING MICRO-ELEMENTS AND THE FUNGICIDE, RHIZOLEX-T

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ABSTRACT

Some microelements, namely zinc, manganese and calcium were used singly or in combination with typical fungicide, Rhizolex-T 50 to investigate their effect towards the control of faba bean root-rot caused by *Rhizoctonia solani*. Laboratory, greenhouse and field studies were conducted during 2008/2009 & 2009/2010 seasons using the faba bean cultivar, Giza 40. Results of *in vitro* studies showed that microelements or Rhizolex-T significantly inhibited the mycelial growth of the pathogen, *R. solani* when compared with the untreated control.Under greenhouse conditions, all isolatedfungi proved to be pathogenic and caused pre- and post-emergence damping-off while *R. solani* being the most virulent.

All some microelements or the fungicide tested as seed treatments for field experiments led to an increase in the plant height . Rhizolex-T 50 followed by zinc were the most effective treatments in reducing the incidence of faba bean damping-off. Calcium followed by zinc were the most effective in increasing the number of pods per plant, the weight of 100 seeds, and seed yield / plot. The most effective seed treatment in reducing the root-rot disease incidence was Rhizolex-T followed by zinc and then calcium.

Keywords: Faba bean, root-rot, *Rhizoctonia solani*, microelements, zinc, manganese, calcium, Rhizolex-T.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a legume crop with high nutritional value. It contains about 18.5 and 37.8% protein (El-Syed *et al.,* 1982). Faba bean plants could be infected with many fungal pathogens, which can causes considerable yield losses (Mahmoud, Nagwa 1996). In this respect, root-rot disease is among the most important fungal disease affecting faba bean production in Egypt. Hussein (1985) reported that root-rot / wilt complex and mosaic are the major diseases of faba bean in the Sudan. Omar (1986) tested several pathogens, and found that *Rhizoctonia solani* was the most virulent in causing root disease. Susceptibility increased with increasing inoculums level and decreased with increasing plantage. The fungus was pathogenic to several legume crop and its saprophytic ability was low in soil previously cultivated with maize and soybean. El-Morsy *et al.* (1997), Akem and Bellar (1999), and Hugar (2004) isolated *R. solani* and *Fusarium oxysporum* from wilted and rotten roots of faba bean in different parts of the world and considered them the most important and wide spread fungal diseases observed at all locations. In this respect also, Metwaly (2004) found that *R. solani* isolated from Kafr El-Sheikh governorate was more virulent than that of Sharkia governorate. Control of the root rot disease depends mostly on using chemical fungicides (Marschner, 1986 and Hanounik and Bisri, 1991). The resistance to diseases depended on the host plant physical or chemical barriers activated by biotic or abiotic agents (Kloepper *et al.,* 1992). The resistance can be systemically induced by chemical substances (Gamil, 1995). El-Baz and Shaltout (2007) revealed that adding microelements, either manganese or zinc, increased all growth parameters of the host plant when compared with the control treatment infested with the pathogen.

The objective of the present work is to investigate the effect of some microelements and fungicide Rizolex-T in controlling root rot of faba bean under laboratory, greenhouse and field conditions.

MATERIALS AND METHODS

Disease survey of faba bean root-rot in different governorates:

Survey was carried out in three governorates in Egypt, namely Minufiya, Gharbia and Kafr El-Sheikh. Diseased plants of faba bean showing root-rot symptoms were collected from growing fields and greenhouses. The average percentage of disease incidence was calculated as the number of rotted plants in relative to the total number of examined plants.

Isolation, purification and identification of fungi associated with faba bean diseased plants:

Infected roots were cut into small fragments, washed thoroughly with tap water, then sterilized with sodium hypochlorite solution (1%), then dried between two sterilized filter papers. Fragments were then placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 25°C for 7 days and observations were recorded (Christensen, 1957). Hyphal-tips and single sporea of grown fungi were transferred individually to new PDA plates (Riker and Riker, 1936) and then identified according to their morphological and microscopically characters as described by Jenset *et al.* (1991). Identification was confirmed by the Department of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

Pathogencity tests:

Pathogencity tests were carried out under greenhouse conditions at Sers El-Layian Agricultural Research Station in 2008 growing season. At first, all fungal isolates which were isolated from rotten roots of faba bean were tested for their pathogenic potentialities on the susceptible cultivar, Giza 40, under greenhouse conditions in order to select the highly pathogenic isolates.

Pots (25–cm-diameter) were sterilized by dipping into 5% formalin solution for 5 min and then left in open air till dryness. Soil was sterilizedusing 5% formalin solution by mixing throughtly. Then the treated soil was covered with plastic sheet for one week and then the plastic sheet was removed in order to allow complete formalin evaporation (Whitenhead, 1957). Soil infestation with each individual fungal pathogen was carried out at the rate of 3% of soil weight (Metwelly, 2004). Fungi were individually grown on sand-barley (SB) medium (25 g clean sand, 75 g barley and enough water to cover the mixture). Flasks containing sterilized medium were inoculated with each fungus and incubated at 25°C for two weeks. Potted soil was watered daily for a week to enhance fungal growth. Soil of control pots was mixed with the same amount of sterilized fungus-free sand-barley (SB) medium. Ten faba bean seeds were surface sterilized using 5% sodium hypochlorite for 2 min., washed several times with sterilized water, and then sown. Three replicate pots with a total of 30 seeds were used for each treatment (Farahat, 1970).

$$\text{Pre-emergence (\%)} = \frac{\text{Number of non germinated seeds}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{Number of dead seedling}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Survival plant (\%)} = \frac{\text{Number of survived plant}}{\text{Total number of sown seeds}} \times 100$$

Disease assessment:

Percentages of pre- and post-emergence damping-off as well as healthy survival plants in each treatment were determined 15 and 30 days after sowing, respectively using the formula according to El-Helaly *et al.* (1970).

Laboratory experiment:

This experiment was conducted to investigate the effect of zinc, Manganese and calcium (3 g / l medium; the recommended dose)and the fungicide Rhizolex-T 50 in its full dose (3 g / l medium) on the mycelia growth of *R. solani*. These substances were used singly or in combination to be added to the PDA medium before solidification. Rhizolex-T was used at 1/2 strength (1.5 g / l medium) when combined with microelements (El-Baz, 2007).Three replicates (culture plates) for each substance/combination were inoculated with a fungal disc cut from the periphery of a 5-days-old culture of *R. solani*. The plates were incubated at 25°C. The linear growth of the tested pathogen was measured when the fungal growth of the control treatment filled the Petri plate.

Greenhouse experiment:

Greenhouse experiment was carried out to evaluate the effect of the microelements, zinc, manganese and calcium as well as the chemical fungicide, Rhizolex-T 50 (used at 50% of the recommended doze) on the disease incidence of faba bean root-rot in pot experiment. The microelements were tested singly or in combination with a half dose of Rhizolex-T as seed treatment. Sterilized pots (25 cm diam.) filled with autoclaved sandy-clay soil were used. *Rhizoctonia solani* inoculum was produced on sand-barley (SB) medium as previously described and used to infest the sterilized soil at a rate of 3% (w/w). The inoculum was mixed thoroughly with the upper layer of the soil, then watered and left for one week to ensure the distribution of the inoculum and establishment of the pathogen. Zinc, manganese and calcium treatments were applied as seed soaking treatment. While, Rhizolex-T 50% was used as seed coating. Faba bean seeds (c.v. Giza 40) was soaked for 20 min in each tested substance (El-Baja, 2007). Three pots (as replicates) were used for each treatment and three pots were left without pathogen infestation to serve as control. Ten seeds of faba bean were sown in every pot. Disease incidence was recorded as the percentage of pre- and post-emergence damping-off as well as healthy/survived plants after 15, 30 and 35 days from sowing, respectively.

Field experiment:

Field experiment was carried out at Sers El-Lyain Research Station, Minufiya Governorate, Egypt in two growing season (2008/2009 & 2009/2010). The experiment aimed at studying the effect of the microelements, zinc (3 gm / l) in zinc chelates, calcium (3 gm / l) and manganese (3 gm / l), and the fungicide, Rhizolex-T 50% w.p at the rate of 3 gm / kg seed against the faba bean root rot disease. Faba bean seeds (c.v. Giza 40) were soaked for 20 min. in the tested microeliment singly or in combination with the fungicide Rhizolex-T in half and full doses. The wetted seeds were left until air dried before sowing. A split plot design with three replicates was used in this experiment. The area of each sub-plot was 10.5 m^2 (3.5 × 3 m). Three hundred seeds were sown in each plot.

The seed germination percentage, and the pre- and post-emergence damping-off were determined after 15 and 30 days from sowing. Also, healthy/survived plants were counted and expressed as percentage, 35 days after sowing. In addition, faba bean plant samples were taken after harvest to measure plant height, number of branches/plant, number of pods/plant, weight of 100 seeds and weight of seed yield/plot. The disease severity of root-rot disease was determined after 55 days from sowing according to Soleman *et al.* (1988).

Statistical analysis:

All data were subjected to the proper statistical analysis of variance (ANOVA) of randomized complete block design or split plot design as appropriate, with three replicates (Gomez and Gomez, 1984).

RESULTS

Isolation and identification of the causal organisms:

As shown in Table (1), two hundred and five isolates of different soil-borne fungi were isolated from rotten roots of faba bean plants (cv Giza 40) cultivated in three Egyptian governorates, namely Minufiya, Gharbia and Kafr El-Sheikh.

These isolates were identified as *R. solani* (54 isolates), *Macrophomina phaseolina* (31 isolates), *Fusarium moniliforme* (30 isolates), *Fusarium solani* (28 isolates), and *Fusarium semitectum* (22 isolates). Meanwhile, 40 isolates stayed without identification from the three governorates and named unknown fungi. As for the frequency of the isolated fungi in the three governorates, *R. solani* followed by *Macrophomina phaseolina* were the most frequent fungi recovered in the three governorates.

Table (1). Fungi isolated from rotten roots of faba bean and their frequency at three different governorates.

Fungus	Gharbia		Kafr El-Sheikh		Minufiya		Total
	*F	**F %	F	F%	F	F%	
M. phaseolina	13	19.12	8	12.12	10	14.08	31.0
F. solani	10	14.71	8	12.12	10	14.08	28.0
F. semitectum	5	7.35	9	13.63	8	11.27	22.0
F. moniliforme	10	14.71	10	15.15	10	14.08	30.0
R. solani	18	26.47	16	24.24	20	28.17	54.0
Unknown fungi	12	17.65	15	22.72	13	18.31	4.0
L.S.D at 0.05	68	-	66	-	71	-	205

*** F: frequency** **** F %: frequency %**

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Pathogencity tests:

Four different isolates of *R. solani* were selected in each governorate for carrying out the pathogenicity test.

Data in Table (2) show that all tested isolates of *R. solani* were pathogenic but varied in their pathogenicity on faba bean plants. Data indicate that isolate "R_2 M" (from Minufiya) was the most pathogenic isolate causing 33% of pre- and post-emergence root-rot (Table 2). On the other hand, the least virulent isolate was "R_2 G", which was obtained from Gharbia governorate.

Table (2). Pathogencity tests of four selected *R. solani* isolates (from each governorate) on faba bean plants under greenhouse conditions.

Source of isolate / Governorate	Isolate No.	Pre-emergence damping off (%) after 15 days	Post-emergence damping off (%) after 30 days	Plants survived (%)
Gharbia	R_1 G	13.33	10.00	76.67
	R_2 G	3.33	16.67	80.00
	R_3 G	6.67	23.33	70.00
	R_4 G	10.00	16.67	73.33
Kafr El-Sheikh	R_1 K	23.33	16.67	60.00
	R_2 K	20.00	10.00	70.00
	R_3 K	10.00	20.00	70.00
	R_4 K	16.67	13.33	70.00
Minufiya	R_1 M	13.33	20.00	66.67
	R_2 M	33.33	33.33	33.34
	R_3 M	16.67	20.00	63.33
	R_4 M	23.33	16.67	60.00
Check	-	0.00	0.00	100.00
L.S.D at 0.05		2.17	2.30	4.07

Laboratory experiment:

Effect of microelements and Rhizolex-T on the linear growth of *Rhizoctonia solani*

Data in Table (3) show that the microelements Zn, Mn and calcium that used alone inhibited the mycelial growth by 72, 56 and 62%, respectively, while the fungicide Rhizolex-T caused 97% inhibition to *R. solani* mycelial growth. Data also reveal that using the combinations of microelements with a half dose of Rhizolex-T 50%, has increased the level of mycelia inhibition to be 79, 72, and 86% for Zn, Mn and calcium combined with 50% Rhizolex-T, respectively.

Table (3). Effect of some microelements and Rhizolex-T on the linear growth of *Rhizoctonia solani*.

Treatment	Linear growth (colony diameter)	
	Linear growth (cm)	% Growth inhibition
Zn	2.5	72.22
Mn	4.0	55.55
Calcium	3.5	61.60
Rhizolex-T (RT)	0.3	96.66
Zn + Mn	3.2	64.44
Zn + RT (50% rd)	1.9	78.89
Mn + RT (50% rd)	2.5	72.22
Calcium + RT (50% rd)	1.3	85.55
Untreated control	9.0	0.00
L.S.D at 0.05	2.9	-

Greenhouse experiment:

Effect of micro-elements and Rhizolex-T 50% on the incidence of faba bean root-rot under greenhouse conditions:

A greenhouse experiment was conducted to test if adding microelements (Zn, Mn and calcium) singly or combined with a half dose of Rhizolex-T (the recommended dose) can control the root-rot disease of faba bean. Data in Table (4) show that using Zn, Mn and calcium alone had negative effect on faba bean plants where they induced the pre-emergence damping-off by 30, 36, and 33%, respectively. However, using microelements combined with recommended half dose of Rhizolex-T apparently increased the percentage of survival plants over the pathogen-alone treatment (Table 4).

Table (4). Effect of micro-elements and Rhizolex-T 50% on the incidence of faba bean root-rot under greenhouse conditions.

Treatment	Pre-emergence damping off (%)	Post-emergence damping off (%)	Plants survived (%)
Zn (no pathogen)	30.00	20.00	50.00
Mn (no pathogen)	36.33	27.76	35.91
Calcium (no pathogen	33.33	21.22	45.45
Rhizolex-T 50% (no pathogen)	0.00	0.00	100.00
Zn + *R. solani*	40.00	25.00	35.00
Mn + *R. solani*	43.00	30.00	27.00
Calcium + *R. solani*	30.00	25.00	45.00
Zn + *R. solani* + Rhizolex-T 50% r.d	6.70	10.00	83.30
Mn + *R. solani* + Rhizolex-T 50% r.d	10.00	13.33	76.67
Calcium + *R. solani* + Rhizolex-T 50% r.d	10.00	10.00	80.00
R. solani	60.00	20.00	20.00
Control	0.00	0.00	100.00
L.S.D at 0.05	17.00	15.32	19.67

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Effect of seed treatment with micro-elements and Rhizolex-T 50% on the incidence of faba bean root-rot under field conditions:

The effects of Zn, Mn and Ca^{++} as well as Rhizolex-T (applied as seed treatment) on pre- and post-emergence damping-off of faba bean plants are presented in Table (5). The results revealed that these micro-elements and Rhizolex-T significantly reduced pre- and post-emergence damping-off when compared to the untreated plants (control). Rhizolex-T produced the highest level of the disease control with the highest number of survived plants, whereas, manganese was ranked as the least effective micro-element minimizing the disease incidence in both seasons (2008/2009 and 2009/2010).

Table (5). Effect of treating faba bean seed with micro-elements and Rhizolex-T on the incidence of root-rot in two growing seasons under field conditions.

Treatment	Season 2008/2009			Season 2009/2010		
	Pre-emergence damping off (%)	Post-emergence damping off (%)	Survival plants (%)	Pre-emergence damping off (%)	Post-emergence damping off (%)	Survival plants (%)
Zn	10.00	10.00	80.00	8.33	7.66	84.01
Mn	13.33	13.33	73.34	10.00	10.00	80.00
Calcium	12.00	13.33	74.67	9.33	8.33	82.34
Rhizolex-T	3.67	6.67	89.66	0.00	0.00	100.00
Zn + Rhizolex-T(100%)	0.00	0.00	100.00	0.00	0.00	100.00
Zn + Rhizolex-T(50%)	0.00	3.33	96.67	0.00	0.00	100.00
Mn + Rhizolex-T(100%)	0.00	3.33	96.67	0.00	0.00	100.00
Mn + Rhizolex-T(50%)	3.33	3.33	93.34	3.33	6.67	80.00
Ca + Rhizolex-T(100%)	3.33	0.00	96.67	0.00	0.00	100.00
Ca + Rhizolex-T(50%)	6.70	3.33	89.97	3.33	3.33	93.34
Control	20.00	20.00	60.00	12.18	4.33	76.49
L.S.D at 0.05	9.55	10.12	13.21	9.80	11.22	17.32

As for the effect of some micro-elements and Rhizolex-T on disease incidence, growth and yield component of faba bean under field conditions, the obtained data in Table (6) revealed significant positive effects of micro-elements and Rhizolex-T 50% when compared with the control where they lowered the disease incidence while increased the plant height, number of pods per plant, 100-seed weight, and seed yield / plot. The results of the first season were confirmed by those of the 2nd one. Rhizolex-T was the best among all treatments, while calcium gave a better performance than Zn and Mn in inhancing faba bean plant growth and yield components.

Table (6). Effect of treating faba bean seeds with micro-elements and Rhizolex-T on plant growth characters and some yield components under field conditions.

Treatments	Season 2008/2009					Season 2009/2010				
	Disease incidence	Plant height (cm)	No. of pods / plant	100-seed weight	Seed yield / plant	Disease incidence	Plant height (cm)	No. of pods / plant	100-seed weight	Seed yield / plant
Zn	12.22	110.0	17.50	75.20	3.20	11.11	100.0	17.00	73.13	3.00
Mn	14.33	108.0	16.30	73.30	3.10	13.33	98.0	15.33	70.22	2.90
Calcium	15.66	107.0	21.40	78.85	3.90	14.44	103.0	18.00	75.00	3.85
Rhizolex-T	11.20	108.0	18.35	76.13	3.70	10.00	105.0	17.55	74.14	3.50
Zn + Rhizolex-T (100%)	5.33	115.0	19.50	78.00	3.40	6.66	108.0	18.00	75.18	3.50
Zn + Rhizolex-T (50%)	7.77	110.0	19.00	76.00	3.30	6.66	107.0	16.85	75.00	3.40
Mn + Rhizolex-T (100%)	9.99	112.0	18.00	74.00	3.20	10.00	108.0	17.00	73.33	3.15
Mn + Rhizolex-T (50%)	11.11	108.0	18.00	73.00	3.10	10.90	105.01	16.00	72.12	3.10
Ca + Rhizolex-T (100%)	10.00	110.0	22.70	82.00	4.00	9.99	08.0	21.34	81.14	3.90
Ca + Rhizolex-T (50%)	12.22	106.0	21.80	80.00	3.90	11.11	107.0	20.00	78.00	3.70
Control	23.33	102.0	13.50	70.00	2.90	26.66	95.0	11.00	68.00	2.80
L.S.D at 0.05	7.18	6.33	2.13	1.70	1.20	8.02	6.00	2.70	2.00	1.50

DISCUSSION

Faba bean (*Vicia faba* L.) is a legume crop with high nutritional value. Soil borne diseases including root rot cause considerable yield losses . In the present investigation, extensive surveys were conducted throughout three Egyptian governorates to determine the occurrence and frequency of various fungal pathogens associated with diseased faba bean plants. The surveys showed differences in the frequency of the isolated fungi similar results were early reported by El-Morsy *et al.* (1997), Akend and Bellar (1999), Hugar (2004) and Metwaly (2004).

The present investigation indicated that faba bean seeds pre-treated with Rizolex-T and Zn, Mn and Ca singly reduced root-rot disease incidence. Moreover, combined Rizolex-T and the tested microelement reduced significantly the disease impact. This finding was confirmed under greenhouse and field trails over two successive seasons. On the other hand, plant growth characters and seed yield were also increased.

It can be explained that Zn, Ca or Mn application improved plant growth and that may-partily-increased defense mechanism of the plant to soil borne infection and also to same extent increase plant growth parameters. This finding was previously reported by Soleman *et al.* (1988) and Omar *et al.* (1992). They found that application of microelements gave a renearkeble protection against faba bean fungal diseases.

Manganese activates some of the enzyme reactions in tricarboxylic acid and dehydrogeneres (Marschener, 1986). The results of this study revealed the appearance zinc and calcium as root-rot for faba bean plants. Both treatments have greatly reduced of root-rot disease and also, increased seed yield.

In short, use of Rhizolex-T and / or some microelement can improve disease resistance of faba bean to root-rot disease and increase growth and yield of the plant.

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تأثير العناصر الصغرى ومبيد الريزولكس – تى على مرض عفن الجذور فى الفول البلدى

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- أظهرت نتائج الدراسة المعملية أن الزنك والمنجنيز والكالسيوم وكذلك مبيد الفطرى "ريزولكس- تى" لها تأثير مثبط على فطر الريزوكتونيا سولانى المُسبب لمرض عفن الجذور فى الفول البلدى ، فقد أدى إضافة هذه المواد لبيئة النمو إلى تثبيط النمو الميسليومى للفطر المُمرض ريزوكتونيا سولانى مقارنةً بالكنترول الغير معامل .
- وتحت ظروف الصوبة وجد أن هذه العناصر أدت إلى انخفاض فى نسبة موت البادرات وبالتالى زيادة عدد النباتات الناجية من الإصابة مقارنةً بالكنترول المُمرض الغير معامل بهذه العناصر .
- وتحت ظروف الحقل وجد أن معاملة البذور بالعناصر الصغرى أوبالمبيد الفطرى ريزولكس- تى أدت إلى انخفاض نسبة موت البادرات وكذلك زيادة فى عدد القرون ووزن الـ 100 بذرة وزيادة فى كمية البذور فى قطعة التجربة ، وكانت المعاملة بمبيد الريزولكس- تى أعلى المعاملات تأثيرا فى خفض نسبة موت البادرات أما بالنسبة للصفات الخضرية وكمية المحصول فكان الكالسيوم أفضل العناصر يليه الزنك ، لذا ننصح باستخدام العناصر الصغرى بدلا من استخدام المبيدات لمقاومة مرض عفن الجذور الرايزوكتونى فى الفول البلدى .

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