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Effect of some Micronutrients on the Incidence of Pea Damping-Off

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Damping-off disease of pea is one of the most dangerous diseases most dangerous diseases attacking pea plants. It reduces the plants. It reduces the number of grown plants and significantly decreases the pod yield. Pea plants can be protected from this disease chemically, biologically and by using certain doses of micro or macronutrients. In this work, zinc, calcium and manganese were used and applied at different doses to pea plants in field experiments. In this research work, different microelements were used at four concentrations, *i.e.* 1, 2, 3 and 4 g for each treatment. The effect of the different microelements was compared to the effect of Rhizolex-T50 under laboratory condition where the effect of different treatments on the fungal linear growth was tested. Under field conditions, the three elements were tested and showed significant effect on disease incidence during two seasons 2010/11–2011/12, zinc gave 85.57% and 87.79% survival plants, respectively, in comparison with the control treatment 78.00% and 12.34%, respectively, while manganese gave 82.12% and 83.35% and calcium gave 84.23% and 86.66%, respectively, in comparison with the control treatment. On the other hand yield components, *i.e.* mean number of pods / plant, average weight of green pods and yield/plot (kg) were also determined and recorded significant increase.

Keywords: Calcium, damping-off, manganese, pea and zinc.

Root-rot disease of pea is one of the most dangerous diseases which reduce the number of plants. It consequently reduces the number of pods per plants leading to a significant reduction in yield. The disease can be controlled by several methods, *i.e.* chemical control, biological control, using micro-organisms and a balanced nutrition of different nutritive elements.

In Egypt, *Fusarium oxysporum* and *Rhizoctonia solani* were the most frequent pathogens isolated from the infected pea plants Abada *et al.* (1992). Different methods of disease management were applied to reduce the incidence of wilt and root-rot of pea plants. These methods were biological control, chemical control and integrated control (Kloapper *et al.,* 1992 and Ragab *et al.,* 2009). However, the use of plant extracts, antioxidant materials and bioagents seemed to be the most save methods for controlling different soil borne pathogens (Abada, 1996). Calcium content of the host cell wall may be associated with increased resistance to the pathogen (Pateman and Lumsden, 1965). Conway *et al.* (1991) and Biggs *et al.* (1997) showed the effectiveness of calcium chloride application in reducing incidence of peach canker disease. Application of calcium compounds after transplanting significantly suppressed the incidence of *Phytophthora stemrol* of soybean and delayed the onset of the disease. Calcium amendments increased plant height, number of nodes and pods and consequently seed yield. Also, zinc and manganese showed the same results.

Zinc is one of most important nutritive microelements to the higher plants. Deficiency causes inhibition of growth and damage to some cell compounds. It plays a significant role in defence system against some agents. Emara *et al.* (2004) found that zinc sulphate treatments to peanut pods were correlated with increasing zinc concentration. Fahim *et al.* (2006) stated that mixture of microelements (Cu, Fe, Zn and Mn) reduced damping-off and wilt of peanut. To clarify the effect of Zn, Fe, Cu and Mn, Ziadi *et al*. (2001); Cakmak and Marschner (1992) and Ragab *et al*. (2009) studied the activity of the oxidative enzymes to find out an explanation of the effect of some nutrient elements of plant resistance.

Therefore, the objectives of the present study were designed to study the effect of zinc, calcium and magnesium on root-rot disease of pea, in order to reducing the number of rotted plants and increasing the number of survival plants. Moreover, the oxidative enzymes and the protein were determined in order to explain the effect of the different treatments.

Materials and Methods

The present study dealt with root-rot disease of pea management under greenhouse and field using some nutritive microelements.

Source of inocula:

The concerned disease caused by *Rhizoctonia solani* kühn. This pathogen was obtained from infected roots of pea collected from different locations of Egypt. The used isolates were purified and identified following the methods adapted by Sneh *et al.* (1992). In this research, different experiments were carried out in a randomized complete plot design with four replicates for each treatment using cv. Master pea.

Laboratory experiments:

Different microelements, *i.e*. zinc, magnesium and calcium were used in comparison with the fungicide Rhizolex-T50. These elements were used at different concentration, *i.e*. 0, 1, 2, 3 and 4 g/l in comparison with Rhizolex-T50 WP. All tested materials were separately added to100 ml PDA before solidification, poured in Petri dishes. Three plates were used as replicates for each treatment. The plates were inoculated with fungal discs cut from the periphery of 5 days old culture of tested fungus. The plates were incubated at 27°C. The linear growth of the tested pathogenic fungi was measured when the fungal growth covered the surface of any plate.

Determination of the oxidative enzymes:

Extracts of the different plants were prepared by grinding the sample in 0.1 M sodium phosphate buffer at pH 7.1 (2 ml buffer / g sample) in mortar and kept in the refrigerator until used. The extracts were used for assaying biochemical change associated with the tested treatments of chemical inducers and Rhizolex-T50, the activities of peroxides enzyme (Allam and Hollis, 1972) and polyphenoloxidase enzyme (Snell and Snell, 1953) were determined.

Protein extraction:

1- Protein extraction of samples:

One ml of extraction protein buffer was added to each sample (1g) then grind in prechilled mortar and pestle with liquid nitrogen. The homogenate was centrifuged at 14000 rpm for 15 min the concentration of protein of each sample in the supernatant was determined according to the method of Bradford (1976).

2- SDS-polyacrylamide gel electrophoresis:

Electrophoresis of total protein was carried out on a vertical gradient gel form (5-15% polyacrylamide gel) containing sodium dodecyl sulphate (SDS) according to method of Bollag and Edelstein (1993). Contents of separating gel were acrylamide and N-methylene bisacrylamide (30: 0.8) and tries HCl buffer (pH 8.8).

The running buffer composed of 0.025 M-tries and 0.129 M glycine, pH 6.3 and 10% SDS. Each well was loaded with 20 ul of protein sample and subsequently run at 2 mA. Gel documentation (AAB advanced American Biotechnology) system was used then to cluster the protein patterns.

3- Staining of protein bands and densitometer scanning:

Protein bands were visualized by silver staining method described by Giulian *et al.* (1983). After staining, the gels were washed with glass distilled water and stored in 7% acetic acid until photographed. Then they were dried using a gel drier and scanned in a recording gel densitometer (Gs 365 w-Hoefer Scientific Instruments).

Field experiment:

Field experiment was carried out at Sers El-Lyain Research Station, Minufiya Governorate, Egypt, during the two growing seasons, *i.e.* 2010/11 and 2011/12. The experiment aimed to study the effects of some chemicals, *i.e.* zinc (3 g/l) in zinc shelate, manganese (3 g/l) in manganese shelate, calcium (3 g/l) in the form of delta calcium 12% and Rhizolex-T 50% W.P. at the rate of 3 g/kg seeds as protection material against root rot disease of pea. Zinc, manganese and calcium treatments were applied as seed soaking. Meanwhile, Rhizolex-T 50% was used as seed coating. Pea seeds cv. "Master pea" was soaked for 20 minutes in each tested chemical inducers. The wetted seeds were left until air dried before sowing in 4 rows under natural infection. A complete randomize block design with three replicates was used in this experiment. Pre-emergence damping-off was assessed 21 days after sowing. Post-emergence damping-off was determined 35 days after sowing, when survival plants was also estimated. Root-rot percentages and yield components were also determined. Data were statistically analyzed according to Snedecor and Cochran (1972).

Results

Laboratory experiments:

1- The effect zinc, manganese, calcium and Rhizolex-T50 on the linear growth of Rhizoctonia solani:

This experiment was carried out to study the effect zinc, manganese, calcium and Rhizolex-T50 at four concentrations (1, 2, 3 and 4 gm/l for each) on the linear growth of the tested fungus (*Rhizoctonia solani*). Data presented in Table (1) show significant reduction in fungal linear growth as a result of using the microelements. Moreover, increasing concentrations of the different treatments decreased the fungal linear growth gradually of the tested pathogenic isolate to reach the maximum reduction at the higher concentration. In this respect, at 3 and 4 g/l Rhizolex-T50, no growth was recorded for tested isolate. Rhizolex-T50 was the most effective in this respect followed by zinc, calcium and manganese microelements in descending order.

Table 1. Effect of microelements and Rhizolex-T50 on the linear growth (cm) of *Rhizoctonia solani*- the causal organism of pea root-rot

Treatment	Concentration (gm/l)	Mycelia growth (cm)	Reduction (%)
Zinc	1	7.22	19.77
	2	5.13	43.00
	3	2.99	66.77
	4	3.12	65.33
Manganese	1	8.17	9.22
	2	6.35	27.44
	3	4.46	50.44
	4	4.90	45.55
Calcium	1	3.50	16.66
	2	6.13	31.88
	3	3.40	62.22
	4	3.33	58.55
Rhizolex-T50	1	6.00	53.33
	2	3.18	64.66
	3	0.00	100.00
	4	0.00	100.00
Control (Treatment free)		9.00	0.00
L.S.D at 5%	---	0.59	---

2- Effect of treating pea plants (cv. Master pea) with different microelements and Rhizolex-T50 on root-rot incidence:

Data presented in Table (2) show the influence of using microelements on root-rot disease of pea under field conditions during two successive seasons (2010/11 and 2011/12). Recorded results revealed that the used microelements and Rhizolex-T50

Table 2. Effect of different microelements and Rhizolex-T50 on the incidence of root-rot of pea (cv. Master pea) during two seasons under field conditions

Treatment	Season / damping off (%)							
	2010/11				2011/12			
	Pre-emergence	Post-emergence	Root-rot	Survival	Pre-emergence	Post-emergence	Root-rot	Survival
Zinc	6.66	7.77	12.12	85.57	5.55	6.66	13.50	87.79
Manganese	8.88	9.00	16.65	82.12	8.88	7.77	18.00	83.35
Calcium	7.77	8.00	13.34	84.23	6.67	6.67	10.30	86.66
Rhizolex-T50	0.00	0.00	3.37	100.00	0.00	3.33	2.50	96.60
Control (treatment free)	10.00	12.00	53.60	78.00	13.30	12.22	53.60	74.45
L.S.D. at 5%	7.48	8.00	4.90	10.85	8.00	6.67	4.90	12.34

application reduced significantly pre- and post-emergence damping-off compared to the untreated plants (control). Rhizolex-T50 was the most effective in reducing pre- and post-infection and consequently increased the survival plants compared to the used microelements; manganese ranked the lowest effective nutrient and reduced disease impact in the two seasons (2010/2011 – 2011/2012). On the other hand, the tested treatment reduced significantly root-rot disease compared to untreated control.

3- Effect of some microelements on the green pod yield of cv. Master pea under field conditions during 2010/11 – 2011/12 growing seasons:

Results presented in Table (3) reveal a significant effect of all treatments over the control treatment. Moreover, the number of pods and weight of green pods yield / plot (kg) was improved. However, the great effect on plant growth and yield component was given by microelements. The effect of zinc was more significant among the tested microelements. On the other hand, zinc gave a good performance than microelements in improving pea plant growth and yield components of the cv. Master pea.

4- Effect of some microelements and Rhizolex-T50 on polyphenoloxidase and peroxidase enzyme activities:

Data presented in Table (4) indicate that seeds soaked in the solutions of different microelements and Rhizolex-T50 resulted in an increase of polyphenoloxidase activity and peroxidase compared to the untreated control. Zinc caused the highest increment in polyphenoloxidase activity (23.33) followed by calcium (21.30) and Rhizolex-T50 (19.23) compared to the control treatment. Furthermore, zinc gave the highest increment in peroxidase activity (16.50 activity / min) followed by calcium (14.93 activity/min), Rhizolex-T50 and manganese (13.27and 10.10 activity/min), respectively, compared to the untreated control.

Table 3. Effect of different microelements and Rhizolex-T50 on the pod yield of pea plants (cv. Master pea) during 2010/11 – 2011/12

Treatment	Season			
	2010/11		2011/12	
	Mean number of pods/plant	Average weight of green pods yield/plot (kg)	Mean number of pods/plant	Average weight of green pod yield/plot (kg)
Zinc	17.30	23.60	16.70	21.70
Manganese	11.00	17.80	10.20	16.11
Calcium	16.20	21.50	15.00	20.30
Rhizolex-T50	14.20	19.40	13.50	18.80
Control (treatment free)	9.30	12.30	8.80	10.60
L.S.D. at 5%	0.90	2.50	1.10	2.90

Table 4. Polyphenoloxidase and peroxidase activities in pea seeds (cv. Master pea) treated with different microelements and Rhizolex-T50

Treatment	Concentration	Polyphenoloxidase activity (activity min)	Peroxidase activity (activity min)
Zinc	3 gm/l	23.33	16.50
Manganese	3 gm/l	17.33	10.10
Calcium	3 gm/l	21.30	14.93
Rhizolex-T50	3 gm/l	19.23	13.27
Control (treatment free)		12.2	8.53
L.S.D. at 5%		0.47	0.39

According to the pattern of protein of SDS (PAGE) presented in Table (5) and illustrated in Fig. (1), the number of separated protein bands were 12, 15, 13, 12 and 7 for control, zinc, Rhizolex-T50, calcium and manganese treatments, respectively. All used treatments as well as the control gave 121 and 20 kda bands. Calcium and Rhizolex-T50 treatments gave 6 bands at 121, 39, 28, 20, 19 and 16 kda, respectively.

Table 5. Number of separated bands (pk), related molecular weight in peaks (Kda) and area (%) in the five treatments of micronutrients

Molecular weight (kda)	Control*		Manganese		Zinc		Rhizolex-T50		Calcium	
	PK	%	PK	%	PK	%	PK	%	PK	%
121	1	5.69	1	18.36	1	12.42	1	19.24	1	19.42
100	2	7.63								
72							2	3.29		
71	3	4.64			2	4.77				
65							3	2.24		
52	4	9.09								
50									2	38.92
49	5	12.28			3	17.71	4	32.84		
47			2	36.08						
45	6	5.53			4	11.32				
42	7	3.48								
39			3	13.02			5	11.69	3	15.02
38	8	4.22			5	6.36				
36	9	1.46								
34	10	2.97			6	2.15			4	2.22
33					7	2.45	6	4.28		
32	11	1.80			8	0.67				
31	12	1.92							5	5.1
30			4	8.04	9	6.21	7	4.47		
29	13	3.91							6	1.17
28	14	1.33			10	4.49	8	1.54	7	2.34
27	15	4.99								
26							9	0.31		
25					11	3.28				
24	16	5.14								
22	17	1.36			12	1.94				
21	18	3.98							8	0.29
20	19	4.93	5	5.03	13	10.03	10	1.01	9	0.61
19			6	3.51	14	1.97	11	1.68	10	0.23
18	20	3.79			15	14.22	12	15.69		
17	21	9.91	7	15.97					11	12.75
16							13	1.7	12	1.9

* Refer to Fig. (1) for explanation.

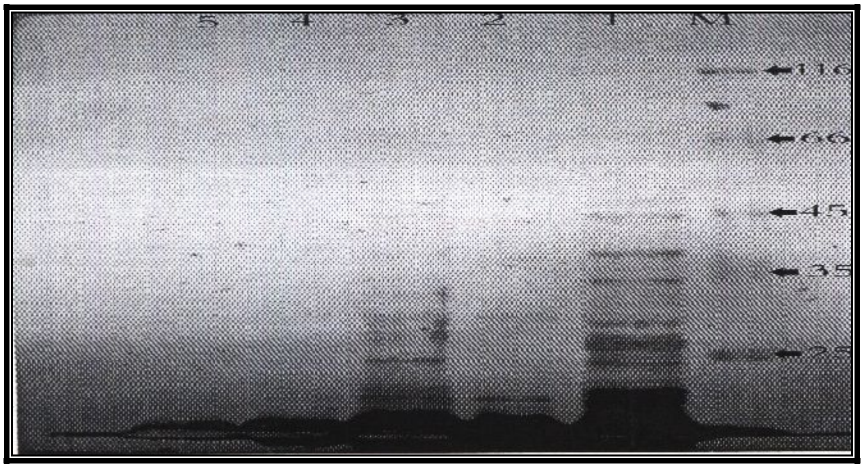

Fig. 1. M-Protein marker. 1- Check treatment, 2- Plant treated with manganese, 3- Plant treated with Zinc, 4- Plant treated with Rhizolex-T50 and 5- Plant treated with calcium.

Discussion

Pea is a major vegetable legume crop in Egypt and in the world. It is contains high percentage of protein (Anonymous, 1995). In Egypt, it is liable to be attacked by several soil borne diseases which cause severe losses in seed yield (Abada *et al.,* 1992). Obtained results of the present study indicated that resistance to pea root-rot could be induced by microelements zinc, manganese and calcium in laboratory and field conditions (Cakmak, 2000 and Cakmak and Marschner, 1992). Applications of zinc, manganese and calcium significantly reduced the pre- and post-emergence damping-off as well as root-rot diseases and consequently increased germination percentage and healthy plants of the pea cv. Master pea. In additions, seed soaking treatments significantly increased the yield components. Giri and Saran (1989) reported that calcium application significantly increased the pod yield of peanut. Sandman and Begger (1983) reported that such yield increase might be attributed to the influence of zinc on plant enzyme activity. Tested chemicals might stimulate some defence mechanisms such as phenolic compounds, oxidative enzyme and some metabolites (Ragab *et al.,* 2009). In the present work, the activity of peroxidase and polyphenoloxidase were obviously higher in plant growth from treated seed compared to the untreated. Sandman and Begger (1983) reported that such yield increase might be attributed to the influence of zinc on plant enzyme activity. The tested microelements might stimulate some defence mechanisms such as phenolic compounds, oxidative enzyme and some metabolites, possible roles of zinc in protecting plant cells from damage by reactive oxygen. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate, peroxidase and glutathione reductive in bean leaves (Cakmak and Marschner, 1992 and Ragab *et al.,* 2009). In the present work, the activity of peroxidase and polyphenol oxidase was obviously higher in plants produced from treated seed compared to the untreated. Regarding the results of SDS (PAGE), six new protein bands were detected in untreated infected plants. One protein band was detected at 19 kda. These new proteins with the same or very close molecular weight detected with different resistance inducers which could lead to the explanation that a very limited number of gens control the root-rot disease under the effect of inducers. These new proteins could be created as a response to calcium, Rhizolex-T, zinc and manganese treatments. Thus, it could be suggested that in resistance induced plants, the accumulated intercellular proteins from the first line of defence to challenge pathogen and they are implicated in plant defence because of their antifungal and/or antibacterial activities (Van-Loon *et al.*, 1994). On the other hand, Hammerschmidt *et al.* (2001); Ziadi *et al.* (2001) and Hassan *et al.* (2006) concluded that the induction of plant resistance with chemical inducers was associated with the accumulation of pathogenesis-related proteins. It can be concluded that the use of balanced amount of nutritive elements is recommended to reduce the level of root rot incidence of pea. The improvement of plant defence against root rot disease could be explained by the change in oxidative enzyme activities and protein pattern.

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تأثير المعاملة ببعض العناصر الصغرى على حدوث اعفان الجذور فى البسلة

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أجريت هذه الدراسة فى الحقل بغرض استحثاث المقاومة فى نباتات البسلة صنف ماستر بى ضد مرض عفن الجذور المتسبب عن الفطر ريزوكتونيا سولانى . ولقد تم استخدام بعض العناصر الصغرى (كالسيوم ، منجنيز ، زنك) مقارنة بمبيد الريزولكس. وكانت نتائج الدراسة كالتالى:

فى تجربة الحقل كانت المعاملة بالريزولكس-تى هى الأكثر إيجابية فى تقليل موت البادرات تلتها المعاملة بالزنك ثم الكالسيوم والمنجنيز وقد وجد أيضاً أن تغطية البذور بالريزولكس-تى أعطت أعلى تأثير فى تقليل الإصابة بمرض عفن الجذور .

وقد أعطت المعاملة بالزنك أعلى تأثير فى زيادة عدد القرون ووزن المحصول تليها المعاملة بالكالسيوم ، وقد أوضحت هذه الدراسة أن المعاملة بالمستحثات الكيميائية والمبيد الفطرى ريزولكس-تى أدت إلى زيادة فى إنزيم البيروكسيديز والبولى فينول أوكسيديز كما أدت إلى استحثاث تكوين بروتينات جديدة فى النباتات المعاملة.