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Induced Resistance in Faba Bean Plants for Controlling Rust Disease *Uromyces viciae-fabae* (Pers.) Schrot.

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Three nutritional elements, *i.e.* manganese, zinc and calcium as well as fungicide plantvax 20% EC, were evaluated for their efficacy to control rust disease in faba bean. Under artificial inoculation at greenhouse, a significant reduction in rust disease severity was found as a result of spraying faba bean plants with plantvax 20% EC, calcium, zinc and manganese, respectively, compared to the control. Same trend of results recorded in the field experiments conducted under natural infection at four locations, *i.e.* Itay El-Baroud, Tag Elez, El-Gemmeiza and Sers-Ellyan, Nevertheless, minor differences among the locations were noted. Tested treatments improved plant growth (plant height) and yield (number of pods/plant and weight of 100 seeds) over the control. Calcium, zinc and manganese applications increased activity of chitinase and B-1,3-glucanase enzymes. Such increases in enzymatic activities may be responsible for controlling rust disease in faba bean.

Keywords: B-1,3-glucanase, chitinase, crop parameters, faba bean, induced resistance, plantvax 20% EC and rust disease.

Faba bean (*Vicia faba* L.) is considered one of the most economic field crops in Egypt, it is grown mainly for human consumption as green pods or dried seeds for its highly protein content. Rust disease caused by *Uromyces viciae-fabae*(Pers.) Schrot., ranked as a second destructive foliar fungal pathogen (Mohamed, 1982). The disease caused a notable damage to the crop especially in north delta region of Egypt where the environmental conditions favourable development of the disease (Ismail, 1981 and Sarhan, 2006). Different ways for controlling faba bean rust disease were recommended. These including breeding for disease resistance (Rashid and Bernier, 1986), fungicide application (Mansour, 1980 and Al-Zumair, 1994), use of agricultural practices (Abada, 1995), abiotic inducers (Salem *et al.,* 1992), and biotic treatment (Sarhan, 2006). In addition, some macro and/or micro elements showed an enhancement of resistance level in the plant against shoot fungal diseases severity in faba bean as well as increasing plant growth and yield (Omar *et al.*, 1992). Application of calcium (Hahlbrock and Scheel, 1989)**,** manganese (Marschner, 1986) and zinc (Ohki, 1978) or its combination (El-Sayed, 2011), were found to improved disease resistance in treated plants. Recently, Abd El-Hai *et al.* (2007), in laboratory and field experiments, stated that nutritional elements (ferrous, zinc, calcium and manganese) were promising in controlling both rust and chocolate spot diseases in faba bean.

The aim of this research was to evaluate the effect of spraying some microelements, *i.e.* calcium, zinc and manganese, on disease severity in comparison with the fungicide plantvax 20% EC, under greenhouse and field conditions. Plant growth and yield were recorded in spraying and unsprayed treatments. Determination of chitinase and B-1,3-glucanase enzymes activities was also considered.

Materials and Methods

Source of rust urediospores:

Uromyces viciae-fabae (Pers.) Schrot. isolate used in this research was collected from Beheira Governorate.

Greenhouse experiment:

Three microelements, *i.e.* calcium, manganese and zinc at the rate of 4g/l, as well as fungicide plantvax 20% EC at rate of 3.5 g/l, were used to study their effects on inducing resistance against faba bean rust disease.

Faba bean seeds, of highly susceptible cv. Giza-40, were used in this investigation. The seeds were kindly provided by Legume Res. Dept., Field Crops Res. Ins., ARC. Five seeds were sown in 30-cm-diam pot representing one replicate. Three replicates were assigned for each treatment. The experiment conducted at Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt during 2008/2009 growing season. Plants grown in each assigned treatment were sprayed three times, *i.e.* 15, 30 and 45 days after sowing with the tested elements and the fungicide plantvax 20% EC. Plants sprayed with tap water only served as check (control).

Twenty days after sowing, leaves of the growing seedlings were inoculated using a mixture of 10 mg of *U. viciae-fabae* urediospores and 2g of talc powder were added (1:20 v/v). Mixture was prepared immediately before inoculation in 10 ml water, to make slurry then mixed again. The mixture was agitated while drops were applied to leaves with brush. To achieve a uniform inoculation, one drop of the mixture was placed on the abaxial surface and then brushed all over the leaflet (Stoddard and Hearth, 2001). Inoculated plants were incubated for 24 hours in completely dark and covered with transparent plastic film with a water-saturated atmosphere to maintain humidity and enhance spore germination, then the plastic cover was loosened to allow some gas exchange and then pots were transferred to normal greenhouse conditions, under continuous high-output fluorescent lamps, while maintaining high humidity throughout growth. Disease severity was determined 14 days after inoculation (after the 2^{nd} spray) and 28 days (after 3^{rd} spray) using standard scale and formula suggested by Bernier *et al.* (1993).

Disease assessment:

Inoculated faba bean plants were visually inspected after 14 days and disease severity was estimated and calculated according to the standard scale and formula suggested by Bernier *et al.* (1993) as follows:

1= No pustules or very small non-sporulating flecks (highly resistant).

3= Few scattered pustules covering less than 1% of the leaf area, and few or no pustules on stem (resistant).

5= Pustules common on leaves covering 1-4% of leaf area, little defoliation and some pustules on stem (moderately resistant).
7= Pustules very common on leaves covering 4-8% of leaf area, some defoliation and many pustules on stem (susceptible).
9= Extensive pustules on leave, petioles and stem covering 8-10% of leaf area, many dead leaves and several defoliation (highly susceptible).

$$\text{Disease severity (\%)} = \frac{\Sigma\,(\text{NPC x CR})}{\text{NIP x MSC}} \text{ X } 100$$

Whereas: NPC = No. of plants in each class rate.
CR = Class rate.
NIP = No. of tested plants.
MSC= Maximum severity class rate.

Determination of enzyme activities:

Plants were homogenized in mortar with 0.2 M tris HCl buffer (PH 7.8) containing 14 mM of B-mercaptoethanol at the rate of ⅓ w/v. The extracted tissues were filtrated through four layers of cheesecloth. Filtrate was centrifuged at 3000 rpm for 15 minutes at 6°C. The supernatant was preserved in a freezer at –20°C till determination of enzymes (Tuzun *et al.,* 1989).

Chitinase activity:

Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugar. The determination was carried out according to the method of Monreal and Reese (1969). One ml of 1% colloidal chitin in 0.05M citrate phosphate buffer (PH 6.6) in a test tube, then one ml of enzyme extract was added and mixed by shaking. Tubes were kept in a water bath at 37ºC for 60 minutes, then cooled and centrifuged before assaying. Reducing sugar was determined by adding 1 ml of supernatant with 1 ml of dinitrosalicylic acid and 3 ml distilled water in test tubes and the tubes were boiled in water bath for 5 minutes, and then cooled. Optical density was read at 540 nm. Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/gram fresh weight tissues/60 minutes.

β-1,3-glucanase activity:

The method of Abeles and Forrence (1970) was used to determine β-1,3-glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. The method was carried out as 0.5 ml of enzyme extract was added to 0.5 ml of 0.05 M of potassium acetate buffer (PH 5) containing 2% laminarin. The mixture was incubated at 50°C for 60 minutes. The reaction was stopped by adding 1 ml of dinitrosalicylic acid reagent and heating the tubes for 5 minutes at 100°C**.** The tubes were cooled and 3 ml of distilled water were added before assay. The optical density was read at 500 nm. B-1,3-glucanase activity was expressed as mM glucose equivalent released/gram fresh weight tissues/60 minutes.

Field experiment:

Four field experiments were carried out at Itay El-Baroud (Beheira Governorate), Tag Elez (Dakahliya Governorate), El-Gemmeiza (El-Gharbiya Governorate) and Sers-Ellyan (Minufiya Governorate), Egypt during the winter season 2008/2009. The experiment aims to study the effect of foliar spray treatments with the microelements manganese and zinc from chelate at the rate of 4g/l, and calcium from Delta calcium 12% at the rate of 4g/l water. Also, plantvax 20% EC was used at 3.5 g/l water as a control.

A complete randomized blocks design with three replicates was used in the four locations. The experimental plot contained 5 ridges occupying on area of 10.5 m^2 (3.5x3m).

Seeds of faba bean (cv. Giza 40) were sown at the third week in November at the four locations during 2008. All cultural practices were carried out according to the recommendation of Ministry of Agriculture, Egypt. At the age of 45, 60 and 75 days, concentrations plants were sprayed with the aforementioned of the elements and plantvax 20% EC till dripping using small pressure pump. A complete randomized blocks design with three replicates was used in the four locations. The experimental plot contained 5 ridges occupying on area of 10.5 m^2 (3.5x3m). Disease severity was determined using the formula adopted by Bernier *et al.* (1993) after each spraying with 7days, in each location. A random sample of plants was taken from each plot for measuring the following growth parameter, plant height (cm), number of pods per plant and average weight of an hundred seed's (g) per plant were recorded.

Results

Effect of spraying faba bean plants with some microelements on severity of rust disease under greenhouse conditions:

Data in Table (1) show the effect of spraying microelements on faba bean rust disease severity. A significant reduction of the disease was recorded in the plants pre sprayed with the tested treatments compared to control. Plantvax 20% EC ranked the best treatment in reducing rust severity followed by calcium, zinc and manganese respectively. Moreover, reduction of rust disease severity was higher after 28 day than after 14 days.

Effect of spraying faba bean plants with some microelements on severity of rust disease under field conditions:

Data in Table (2) show the efficacy of spraying faba bean plants with the tested elements and the fungicide plantvax 20% EC on the severity of the natural infection by rust disease, 45, 60 and 75 days after sowing, under field condition at four locations during 2008/2009 growing season. The obtained results revealed that all the tested treatments reduced significantly disease severity compared to the control. The third spray showed the best protection against the disease. In this respect, plantvax 20% EC was the most effective treatment, where it recorded the lowest degree of disease severity followed by calcium, zinc, and manganese. All tested substances showed also a significantly protection against the disease over the control.

Table 1. Effect of spraying faba bean plants (cv. Giza 40) with some microelements and plantvax 20% EC on the severity of rust disease under greenhouse conditions

Treatment	Disease severity (%) after 14 days	Reduction (%)	Disease severity (%) after 28 days	Reduction (%)	Mean	
					D.S.* (%)	R.** (%)
Manganese	18.57	44.30	21.44	49.75	20.01	47.03
Zinc	16.22	51.35	19.43	54.46	17.83	52.91
Calcium	14.85	55.46	17.53	58.92	16.19	57.19
Plantvax 20% EC	4.44	86.68	5.22	87.77	4.83	87.23
Control	33.34	-	42.67	-	38.01	-
Mean	17.48		21.26		19.37	
L.S.D at 0.05% for Treatment (A) = 2.13 Day (B) = 0.79 Interaction (A x B) = 1.76						

*D.S.= Disease severity & **R.= Reduction.

Table 2 . Effect of spraying faba bean plants (cv. Giza 40) with manganese, zinc, calcium and plantvax 20% EC. on the severity of rust disease under field conditions at Itay El-Baroud, Tag Elez, Gemmeiza and Sers-Ellyan locations during 2008/2009 growing season

Treatment	Itay El-Baroud			Tag Elez			Gemmeiza			Sers-Ellyan			Mean			General mean
	1st *	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
Manganese	10.9	13.8	15.1	11.1	12.6	14.1	9.3	10.8	12.6	3.6	4.7	6.3	8.7	10.5	12.0	10.40
Zinc	10.6	13.3	14.8	10.7	11.8	13.3	8.8	10.2	12.1	3.3	4.1	5.8	8.3	9.9	11.5	9.90
Calcium	10.6	11.2	12.4	9.8	10.3	11.9	8.1	9.1	10.2	2.4	3.2	4.2	7.7	8.5	9.7	8.63
Plantvax 20%EC	6.4	6.9	7.3	7.6	8.1	8.8	6.3	7.0	7.2	1.3	1.7	2.1	5.4	5.9	6.4	5.90
Control	15.6	19.2	20.3	13.3	15.8	18.6	11.0	12.7	14.6	6.4	8.1	10.8	11.6	14.0	16.1	13.86
Mean	10.8	12.9	14.0	10.5	11.7	13.3	8.7	10.0	11.3	3.4	4.4	5.8	8.4	9.7	11.1	
General mean	12.56			11.84			10.00			4.54						
L.S.D at 0.05% for Treatments (T)= 0.29, Spray (S)= 0.22, Location (L)= 0.26 T x S= 0.50, T x L= 0.57, S x L= .45, T x S x L= 1.00																

* 1st, 2nd and 3rd spray.

Yield component:

Data in Table (3) show that all tested treatments increased significantly faba bean plant height over the control. Calcium treatment was more effective in increasing crop parameters followed by zinc, manganese and plantvax 20% EC respectively. Results were confirmed from the four tested locations with minor differences. On the other hand the application of calcium and zinc increased significantly plant height, number of pods per plants and weight of 100 seed in cv. Giza 40. Calcium was the most effective compared with the plants of non treated control. Significant effect was recorded in the four locations of experiments in plant height, number of pods per plants and weight of 100 seeds.

Table 3. Effect of spraying faba bean plants cv. Giza 40 with manganese, zinc, calcium and plantvax 20% EC on crop parameters under field conditions at Itay El-Baroud, Tag Elez, Gemmeiza, and Sers-Ellyan locations during 2008/2009 growing season

Treatment	Plant Height (cm)					No. of pods plant					Weight of 100 seed				
	Itay El-Baroud	Tag Elez	Gemmeiza	Sers-Ellyan	Mean	Itay El-Baroud	Tag Elez	Gemmeiza	Sers-Ellyan	Mean	Itay El-Baroud	Tag Elez	Gemmeiza	Sers-Ellyan	Mean
Manganese	130.3	132.3	132.2	126.6	130.4	30.1	29.6	28.6	28.3	29.1	64.7	62.2	60.3	60.2	61.9
Zinc	133.3	135.2	135.7	129.8	133.5	32.3	30.3	30.3	30.2	30.8	63.8	63.3	62.6	61.4	62.8
Calcium	138.7	137.6	136.1	132.7	136.3	36.9	33.7	33.2	30.9	33.7	67.3	64.8	64.1	62.1	64.6
Plantvax 20% EC	128.4	129.7	130.6	127.1	128.9	28.2	22.3	28.1	28.1	26.7	59.1	58.1	58.6	60.1	59.0
Control	113.3	117.9	127.7	125.4	121.1	25.3	18.7	26.2	26.1	24.1	53.0	52.3	54.6	57.6	54.4
Mean	128.8	130.5	132.4	128.3		30.6	26.9	29.3	28.7		61.6	60.2	60.0	60.3	
LSD at 5% for Treatment (T)= 2.16 Locations (L)= 1.94 T X L = 4.33						Treatment (T)= 0.69 Locations (L)= 0.62 T X L = 1.38					Treatment (T)= 0.95 Locations (L)= 0.85 T X L = 1.89				

Activity of chitinase and β-1,3- glucanase enzymes:

Three treatments were used to study their effect on chitinase and β-1,3-glucanase activities which play a main role as plant defence mechanisms. Recorded results (Table 4) indicate that all treatments stimulated enzymes activity. The highest activity was obtained with calcium and zinc. They increased chitinase activity by 135 and 109% and β-1,3-glucanase by 176 and 138%, respectively, as compared with control. Meanwhile, manganese show moderate effective increased in chitinase activity by 80% and β-1,3-glucanase by 92%, respectively, as compared with control.

Table 4. Effect of abiotic inducers on the chitinase and β-1,3-glucanase activities of faba bean plants cv. Giza 40 under greenhouse conditions

Treatment	Chitinase *		β-1,3-glucanase**	
	Activity (%)	Increase (%)	Activity (%)	Increase (%)
Manganese	5.6	80	2.5	92
Zinc	6.5	109	3.1	138
Calcium	7.3	135	3.6	176
Control	3.1	-	1.3	-

- Chitinase and β -1,3-glucanase were determined 10 days after the second application of the inducers.

* Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/60min.

** β-1,3-glucanase activity expressed as mM glucose equivalent released/ gram fresh weight/60 min.

Discussion

Manganese, zinc, calcium and plantvax 20% EC. reduced the injurious effects of rust foliar disease on faba bean. Calcium followed by zinc were the most effective in reducing faba bean infection by rust disease, while, manganese was of moderate effective under both of greenhouse and field conditions. In addition, all of treatments used enhanced plant growth productivity and yield as compared with the control.

In the present study, in greenhouse and field condition all the treatments significantly reduced disease severity compared to control (untreated).

Induced resistance against rust disease infection on faba bean by using the microelements, manganese, zinc, calcium due to the increasing the activities of chitinase and β-1,3-glucanase (Schneider and Ullrich, 1994).

Data showed that calcium and zinc increased chitinase by 135 and 109% and β-1,3-glucanase by 176 and 138% of control. Chitinase and β-1,3-glucanase can hydrolyze cell wall of fungi (Skujins *et al.*, 1965 and Mauch *et al.,* 1988). Glucanases are known to play a direct role in plant defence by digesting fungal cell walls, due to their anti-fungal properties *in vitro* either alone or combination with chitinase (Jic Kuc, 1996). Abd El-Hai *et al.* (2007) reported that, treating cowpea plants with zinc, ferrous and manganese caused high reduction in cowpea rust disease and increased seed yield.

Calcium is one of the most elements which enter in middle lamella structure of the cell wall, which is considered the first barrier against plant pathogens invasion. Calcium also increased significantly the pod yield (Saran, 1989). Moreover, (Inanaga *et al*., 1990) found that many immature seeds were produced in soil poor in Calcium, and that seed weight may depend on the quantity of calcium absorbed through the shell. To obtain fill seed, soil must be sufficient rich in calcium during the growth of the fruit.

The role of zinc in enhancement the vegetative growth which followed by stimulation in resistance of faba bean to foliar fungal diseases might be due to that, zinc is known to be essential constituent of three plant enzymes, *i.e.* carbonic anhydrase, alcohol dehydrogenase and superoxide dismutase. In addition, zinc has a marked effect on the level of auxin by it appears to be required in the synthesis of intermediates in the metabolic pathway, through tryptophan to auxin (Ohki, 1978) which is turn encourage the meristemic activity of the plant which resulted in more cell division and cell enlargement (Devlin and Witham, 1983). Auxin may also induce the systemic resistance. Moreover, many researchers concluded that such yield increments might be attributed to the favourable influence of zinc on plant enzymes activity and improving the photosynthetic production and/or mobilization in plants (Sandmann and Bogger, 1983 and Abou Leila *et al*., 1992).

Manganese plays a role in regulating the levels of auxin in plant tissues by activating photosynthesis especially photosystem two (Marschner, 1986). Auxin may induced the systemic resistance and encourage the meristemic activity of the plant which resulted in more cell division and cell enlargement (Devlin and Witham, 1983).

The stimulatory effects of manganese, zinc and calcium on the yield components may be attributed to the increase in plant height, number of pods per plant and average weight of an hundred seed's (Table 3). These findings are in harmony with those reported by many researchers (Ohki, 1977; El-Samnoudi, 1990 and Abd El-Hai *et al*., 2007) who found that nutritional elements significantly increased number of branches/plant and there was a positive relationship between number of pod/plant and value of 100 seed weight and different level of nutritional elements treated.

Based on the previous results and discussion, it is highly recommended to replace fungicides with microelements (manganese and zinc) as well as calcium during the cultivation faba bean as they promising trend in controlling of rust disease of faba bean, where it decreased infection, environmental friendly and coast effective compared with the ordinary fungicides.

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استحثاث المقاومه فى الفول البلدى ضد مرض الصدأ المتسبب عن الفطر *Uromyces viciae-fabae*

قدرى مصطفى محمد مرسى
معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر.

تناول هذا البحث دراسة تأثير بعض العناصر المعدنيه مثل المنجنيز والزنك والكالسيوم بمعدل ٤ جرام / لتر ومبيد البلانتفكس ٢٠% بمعدل ٣.٥جرام / لتر لمقاومة مرض الصدأ فى الفول البلدى. وجد تحت ظروف الصوبة ان هناك نقص معنوى فى شدة الاصابة بالصدأ نتيجة المعاملة رشا بالمبيد بلانتفاكس ٢٠% والكالسيوم والزنك والمنجنيز على الترتيب مقارنة بالكنترول (غير المعدى) . وتحت ظروف العدوى الطبيعية فى اربع محطات بحثية وهى محطة بحوث ايتاى البارود ومحطة بحوث تاج العز ومحطة بحوث الجميزة ومحطة بحوث سرس الليان كانت هناك نتائج متشابه مع تجارب الصوبة. وعموما كان هناك اختلافات بسيطة فى النتائج مابين المواقع الاربعة. وقد وجد ان المعاملة بهذه العناصر ادت الى زيادة فى النمو الخضرى للنباتات (طول النبات) وكذلك زياده فى مكونات المحصول (عدد القرون للنبات الواحد – وزن مائة بذره) مقارنة بالكنترول (غير المعامل). كما وجد ان المعامله بالرش بالعناصر الكالسيوم والزنك والمنجنيز ادت الى زيادة فى نشاط انزيمى الشيتينز وانزيم بيتا ٣.١ جلوكانيز. والزيادة فى نشاط هذه الانزيمات ربما تكون هى المسئوله عن المقاومة لمرض الصدأ فى الفول البلدى.