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Effect of Certain Micronutrients on Some Agronomic Characters, Chemical Constituents and *Alternaria* Leaf Spot Disease of Faba Bean

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ABSTRACT
Two field experiments were carried out at Etay El-Baroud Agricultural Research Station, El-Behera Governorate during 2009/2010 and 2010/2011 seasons to study the effects of foliar application with micronutrients on yield and yield components of faba bean (c.v. Misr 1). A randomized complete blocks design with 3 replications was used. Results showed that, spraying with Fe+Zn+Mn increased yield and yield components, while, the highest no. of branches/plant were found with Fe+Zn, in the combined data. Also, chemical constituent, chlorophyll A, chlorophyll B, reducing sugars and the non-reducing sugars significantly increased in all treatments spray with micronutrients compared with non-treated (control). Meanwhile, Alternaria leaf spot incidence on treated plants significantly decreased 18.2-60.5% compared with non-treated control plants. The results concluded that, spraying Fe, Zn and Mn alone or combination could be recommend to control leaf spots and to enhance yield and quality of faba bean under the environmental conditions.

Key words: Faba bean, micronutrients, *Alternaria* leaf spot

INTRODUCTION
Faba bean is one of the main pulse crops grown and it is a primary source of protein (Hussein *et al.*, 1991). The total area planted for production of dry beans was 336.000 producing 428.000 t/faddan during 1995-1999 (Abd El-Salam, 2002). Most of Egyptian crops deficiency of micronutrients particularly Zn, Fe and Mn due to low availability in the soil, high demand of crops, nutrient imbalance or unfavorable agronomic practices (Amberger, 1980; Wallace, 1980; El-Fouly, 1983; El-Fouly *et al.*, 1984; Fawzi *et al.*, 1987). Spraying with micronutrients increase yield, under Egypt conditions (Eweida *et al.*, 1980; El-Sheikh, 1981; Fawzi *et al.*, 1983; Hadi *et al.*, 1985; Fouly and Rezk, 1986; Gomaa *et al.*, 1986).

Chlorophyll content can be taken as an index of the extent of reduction in effective green area, so it is obvious that the disease reduces the photosynthetic activity in leaves and ultimately lead to lower yield (Sinha and Ghildiyal, 1971). In this respect, Rahhal (1993) found that, Zn 4 g treatment showed the highest concentrations of chlorophyll (a) content of leaves. Meanwhile Zn (8 g), Mn (8 g) and Fe (4 g) treatments showed the highest concentrations of chlorophyll (b) content of leaves, 0.62, 0.60 and 0.57 mg g^{-1} leaves, respectively, while Zn (8 g), Mn (8 g), Zn (4 g) and

Fe (4 and 8 g) treatments showed the highest concentrations of the total chlorophyll content of leaves, 1.50, 1.45, 1.39, 1.37 and 1.37 mg g^{-1} leave, respectively. Also, amount of carbohydrates in leaves and stem significant increased in all treatments compared with control. El-Shehaby and Mohamed (1985) found that all treatments of fungicides on onion plants due to significant increase of amount reducing and non-reducing sugars furthermore, total sugars increased except when cuprosan was sprayed at 3 g L^{-1} water.

On the other hand Alternaria leaf spot disease caused by *Alternaria alternata* are caused problem of bean plants during growing seasons (Rahman *et al.*, 2001) There is a growing need to develop alternative approaches for controlling plant diseases Induced resistance due to foliar application with micronutrients in some plants against plant diseases was reported by (Abd-El-Karem *et al.*, 2004) micronutrients which can be applied successfully in many area of plant production as a plant growth stimulant (Scheuerell and Mahaffee, 2006).

The present study therefore was conducted to investigate potential of certain micronutrients to control *Alternarial* leaf spot and to enhance yield and quality of faba bean.

MATERIALS AND METHODS

Two field experiments were carried out at Etay El-Baroud Agricultural Research Station, El-Behera Governorate during 2009/2010 and 2010/2011 seasons to study the effect of Fe, Zn, Mn and their combination on the yield of faba bean C.V (Misr 1).

A-Field studies: The field experiments were laid out in complete randomized block design included ninth treatments as the follows:

- Spraying plants with tap-water (Control)
- With (Fe) at 4 g L^{-1}
- With (Zn) at 4 g L^{-1}
- With (Mn) at 4 g L^{-1}
- With (Fe+Mn) at 4 g L^{-1}
- With (Fe+Zn) at 4 g L^{-1}
- With (Zn+Mn) at 4 g L^{-1}
- With (Fe+Zn+Mn) at 4 g L^{-1}
- With (Diathen M 45) at 250 g L^{-1}

Treatments applied twice, the first at 45 days after sowing, followed by the second at 15 days later. Commercial Fe 12.5%, Mn 12.5 and Zn 12.5% were used in the experiments. Micronutrients were spray at 4 g L^{-1}. water and Dithen M-45 was used at rate of 250 g/100 L. water after 45 days of sowing. However, other agricultural practices were performed as commonly followed in the district. *Alternaria* leaf spot severity was recorded 7 days after last spray (75 days of sowing).

The tested growth characteristics: At harvest time (75 days of sowing), ten guarded plants were taken from each plot to investigate the following characters:

- Plant height (cm)
- No. branches/plant
- No. pods/plant
- No. of seeds/plant
- No. of seeds per pod

- 100-Seed weight (g)
- Seed yield/faddan (ton)

Whole plants in each plot were harvested and left for air drying, then they were threshed and the seeds (which were at 12 % moisture) were weighted (kg).

Determination of Leaf chlorophyll: Leaf chlorophyll content was determined spectrophotometer at 645 nm and 663 nm wavelengths) according to Grodzinsky and Grodzinsky (1973).

Chlorophyll (a) and (b) concentrations in mg g^{-1} leaves after 70 days of sowing were calculated as follows:

$$\text{Chl. a} = [\,(12.7\times\text{O.D. }663)-(2.69\times\text{O.D. }645)]\times 0.1$$

$$\text{Chl. b} = [\,(22.9\times\text{O.D. }645)-(4.68\times\text{O.D. }663)]\times 0.1$$

Total chlorophyll = Chl. a (mg g^{-1} fresh weight of leaves)+Chl. b (mg g^{-1} fresh weight of leaves).

These equations were adapted to optical density measurements of acetone- water extracts of the chlorophyll in a 1 cm vial.

Determination of soluble sugars: Each sample of faba bean leaves was cut of small pieces and 5 g was taken and transferred to beaker of 500 cc, in which 400 mL of distilled water and 3 mg of calcium carbonate were added, and the mixture was boiled for 30 min. After cooling, the mixture was filtered with filter paper Watman No. 2. the clarification occurred by adding lead acetate 10% and swirled till appearance of the precipitate. After filtration, potassium oxalate was added to the filtrate to precipitate the extra amounts of lead acetate. The resulting filtrate was transferred into volumetric flask of 500 cc and completed to the mark, to prepare a blank, 400 mL of distilled water with 3 g calcium carbonate were placed in a beaker and the mixture was boiled for 30 min and filtered. The extracts were kept in a refrigerator until the determination of total, reducing and non-reducing sugars in each sample of extract.

Total soluble sugars: The soluble sugars were estimated calorimetrically according to DuBois *et al.* (1956).

The sugar content was calculated as glucose from a standard curve prepared for it. Determinations of total, reducing and non-reducing sugars were calculated as milligrams of glucose per 100 g fresh weight.

Disease assessments of *Alternaria* leaf spot: Average disease severity of Alternaria leaf spot occurred naturally on faba bean in the field recorded according to Vakalunakis (1990).

Disease scale from 0 to 4 for the leaf area infected was used as follows:

0 = No leaf lesions
1 = 25 or less
2 = 26 to 50%
3 = 51 to 75%
4 = 76 to 100% infected leaf area

The formula adopted by Hanounik (1986) as follow:

$$\text{Disease severity (D.S)\%} = [2(\text{NPC}\times\text{CR})/\text{NIP}\times\text{MSC}]\times 100$$

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

Isolation and densification; Under field condition were growing faba bean plants, when observed of symptom disease of *Alternaria* leaf spot at natural infected taking leaves and isolate fungal on PDA media *in vitro*. Fungal were kept in sealant until identification in Agricultural Research Centre, Giza, Egypt.

Statistical analysis: All data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the complete randomized block design in two seasons and then the combined analysis was achieved by Gomez and Gomez (1984) using means of "MSTAT-C" computer software package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Effect of micronutrients on growth characters: Data presented in Table 1 showed that, foliar applications with Fe, Zn and Mn alone or in combination of them significantly increased all growth characters.

The highest plant height (99.93 cm) was obtained with spraying Fe+Zn followed by Fe+ Zn+Mn (94.27 cm) and Fe+Mn (91.77 cm), while the lowest plant height (82.20 cm) was recorded with spraying water (untreated control). This was in agreement with Bozorgi *et al.* (2011) who reported that, the highest plant height was obtained with foliar zinc spraying. The highest number of branches/plant was obtained with spraying Fe+Zn. While, the other treatments were not significantly different from the untreated control for the number of branches per plant. The lowest values of number of branches/plant (2.63 branch/plant) recorded with spraying Fe+Zn+Mn, only Mn and control which there were no significant differences between them.

Table 1: Effect of foliar application with Fe, Zn and Mn on growth, yield and yield components of faba bean plants in the combined data

Treatments	Plant height (cm)	No. of branches/plant	No. of pods/plant	No. of seeds/pod	No. of seeds/plant	100 seed weight (g)	Seed yield/ faddan (ton)
Fe	85.83^{bc}	3.00^{ab}	8.67^{ab}	3.27^{ab}	30.38^{ab}	88.91^{ab}	2.75^{ab}
Fe+Zn	99.93^{a}	3.23^{a}	11.07^{a}	3.56^{ab}	37.96^{ab}	93.75^{a}	3.60^{ab}
Fe+Mn	91.77^{abc}	2.70^{ab}	9.67^{a}	3.70^{ab}	35.28^{ab}	89.97^{ab}	3.19^{ab}
Fe+Zn+Mn	94.27 ^{ab}	2.67^{b}	10.00^{a}	4.02^{a}	40.33^{a}	89.75^{ab}	3.66^{a}
Zn	88.57^{bc}	2.70^{ab}	6.20^{b}	3.57^{ab}	28.76^{ab}	87.34^{ab}	3.11^{ab}
Mn+Zn	85.37^{bc}	2.90^{ab}	9.87^{a}	3.06^{ab}	35.82^{ab}	87.64^{ab}	2.84^{ab}
Mn	89.47^{bc}	2.63^{b}	9.93^{a}	3.68^{ab}	32.38^{ab}	87.92^{ab}	2.93^{ab}
Control	82.20^{c}	2.70^{ab}	6.13^{b}	3.33^{ab}	25.21^{b}	85.86^{b}	2.49^{b}
Diathane 45	90.43^{abc}	2.63^{b}	10.87^{a}	3.99^{a}	34.66^{ab}	89.27^{ab}	2.63^{ab}
LSD 0.05	7.35	0.56	2.20	0.86	10.03	4.74	0.82

Effect of micronutrients on yield and yield components: Table 1 showed that, all treatments (except for Fe alone and Zn alone) significantly increased the number of pods/plant. Spraying with Fe or Zinc did not exhibit a significant effect compared to control in the combined data. This was generally in agreement with El-Gizawy and Mehasen (2009), El-Fouly *et al.* (2010), El-Masri *et al.* (2002), Rashad and Hanafy (1997), Rizk and Abdo (2001) and Bozorgi *et al.* (2011) as they reported that, the lowest pods per plant were found without spraying zinc. On the other hand, (Saber, 1980; El-Sheikh, 1981; Abdel-Aziz *et al.*, 1982; Fawzi *et al.*, 1983; Fouly and Rezk, 1986; Gomaa *et al.*, 1986; Ibrahim and El-Labban, 1986; Amin *et al.*, 1988; El-Masri *et al.*, 2002) they found that, two sprays of zinc without Fe resulted in the highest number of pods per plant, either spating Fe decreased number of pods per plant.

Concerning the number of seeds/pod the different treatments did not exhibit a significant effect in this respect compared to the control. However El-Masri *et al.* (2002) found that, spraying with zinc increased number of seeds/plant. Genotypic differences for the character were also recorded by Abdalla *et al.* (2000), whereas Ashmawy *et al.* (1998) found insignificant differences for this character.

Also, Table 1 showed significant effect on number of seeds per plant. The highest values were recorded with spraying Fe+Zn+Mn, while, the other treatments were not significantly different from the control.

It is clear from the data given in Table 1 that, spraying with Fe+Zn recorded the highly significant value of 100 seed weight compared with the control. This was in harmony with Bozorgi *et al.* (2011) who found that, the highest 100 seed weight was obtained with 1 g L^{-1} foliar zinc spaying. However, Yassen *et al.* (2010) found that, the highest increment (16%) was obtained when plants sprayed with micronutrients mixture (Fe+Zn+Mn) as compared with control treatment.

Also, it is evident from Table 1 data that Spraying with Fe+Zn+Mn showed the highest values of seeds yield/faddan in the combined data, while the lowest value recorded with spraying Zinc. Allam *et al.* (2004) reported that, the foliar spray with the combination of (Fe:Mn:Zn) showed a highly significant increase in broad bean seed yield. Similar results were confirmed by Monged *et al.* (1988), Baza *et al.* (1992), Monged and Baza (1994) and Sabik *et al.* (2001). On the other hand, Azarpour *et al.* (2011) and Bozorgi *et al.* (2011) they found that, the lowest seed yield per plant found without use of zinc spraying.

Such need of broad bean, and other crops, to micronutrients in Egypt may be due to several reasons; the great nutritional need of the high productivity of the crops, the discontinuity of the precipitation of the Nile mud, the intensive cropping system used and high pH value of most Egyptian soils which hinders the utilization of most of micronutrients by plants (Hamissa and Abdel-Salam, 1999).

Effect of micronutrients on sugar content: Results presented in Table 2 showed clearly that the lowest amount of reducing sugars in leaves and stem of faba bean plants being 3.1 and 1.2 mg g^{-1} fresh weight, respectively, when compared with Fe, the highly amount reducing sugar content 3.9 and 2.0 mg, respectively. Also, Zn showed the lowest amount reducing sugars in leaves and stem 3.4 and 1.2 mg, respectively. On the other hand, the other micronutrients gave intermediate amount in reducing sugars in leaves and stems. Leaves from all treatments with micro-elements contained higher amounts of reducing sugars than that in stem of the some treatments. These results are agreement With El-Shehaby and Mohamed (1985) as they found that the amount of reducing sugars decreased at the lower rates of fungicides but increased for the

Table 2: Sugar contents (mg g^{-1}) fresh weight in leaves and stem of faba bean treated with chelate micro-elements 75 days after sowing

	Sugar contents mg/1g fresh weight					
	Leaves			Stem		
Treatments	Reducing	Non-reducing	Total sugar	Reducing	Non-reducing	Total sngar
Fe	3.9	4.8	8.7	2.0	3.9	5.9
Fe+Zn	3.6	4.8	8.4	1.8	2.8	4.6
Fe+Mn	3.7	4.6	8.3	1.7	3.5	5.2
Fe+Zn+Mn	3.8	4.8	8.6	1.3	2.1	3.4
Zn	3.4	3.6	7.0	1.2	2.0	3.2
Mn+Zn	3.7	4.3	8.0	1.9	2.1	4.0
Mn	3.5	3.9	7.5	1.3	2.0	3.3
Control	3.3	3.9	7.2	1.3	2.0	3.3
Diathane 45	3.1	3.3	6.7	1.9	1.6	3.5
LSD 0.05	0.3	0.2	0.8	0.34	0.38	0.91

Data are average of three replicates

Table 3: Effect of some chelated micronutrients as foliar application on the chlorophyll a, b and the total chlorophyll content (mg g^{-1} fresh weight leaves)

Elements	Chlorophyll (a)	Chlorophyll (b)	Total Chlorophyll
Fe	0.70	0.44	1.14
Fe+Zn	0.81	0.52	1.33
Fe+Mn	0.74	0.42	1.16
Fe+Zn+Mn	0.66	0.39	1.05
Zn	0.82	0.48	1.97
Mn+Zn	0.64	0.42	1.06
Mn	0.84	0.41	1.25
Control	0.61	0.36	0.97
Diathane 45	0.73	0.46	1.19
LSD 0.05	0.017	0.17	0.29

Data are average of three replicates

higher rates. On the other hand, amount of non-reducing sugars increased for all treatments. Furthers more, total sugars increased in all treatments except when cuprosan was sprayed at 3 g L^{-1} water.

Effect of micronutrients on the chlorophyll content of leaves: The results recorded in Table 3 showed that micronutrients application at rate of 4 g L^{-1}. increased significantly chlorophyll A from 0.84 mg g^{-1} leaves treated with micronutrients compared with control 0.6 mg g^{-1} leaves).

The same trend was found in chlorophyll b content, but values were significantly lower than that of chlorophyll A. On the other hand, total chlorophyll increased significantly by the micronutrients and Diathane-M45 treatments. The most pronounced increase was found in the leaves of plants treated with Fe combined with Mn (1.97 mg g^{-1} fresh weight).

These results indicated that spraying micronutrients (Fe and Mn) stimulated the activity of chloroplasts this was followed by Zn (1.33 mg g^{-1} fresh weight leaves) while the lowest was found with Fe- Zn (1.05 mg/ g fresh weight of leaves). These results are agreements with those of Rahhal (1993) who found that the total chlorophyll content of broad bean leaves showed the highest concentrations with Zn (8 g), Mn (8 g) and Zn (4 g) treatments.

Table 4: *Alternaria* leaf spot in faba bean plants as affected by foliar application with micronutrients under field condition

	First season 2010								Second season 2011							
	Disease severity (%)				Reduction (%)				Disease severity (%)				Reduction (%)			
Treatments	20 cm	40 cm	60 cm	Mean	20 cm	40 cm	60 cm	Mean	20 cm	40 cm	60 cm	Mean	20 cm	40 cm	60 cm	Mean
Fe	36.7	28.3	10.0	25.3	10.6	10.7	33.3	18.2	21.7	15.0	5.0	13.9	23.3	17.0	50.0	30.4
Fe+Zn	28.3	20.0	6.7	18.3	23.1	36.9	55.3	41.4	20.0	13.3	5.0	12.7	29.3	27.3	50.0	35.5
Fe+Mn	25.0	18.3	8.3	16.7	40.0	42.3	55.3	45.9	16.7	11.7	6.7	11.7	41.0	36.1	33.0	36.7
Fe+Zn+Mn	20.0	15.0	6.7	14.4	52.0	52.7	44.7	49.8	13.3	8.3	3.3	8.3	53.0	54.6	67.0	58.2
Zn	26.7	23.3	6.7	18.9	36.0	26.5	55.3	39.3	16.7	11.7	5.0	11.1	41.0	36.1	50.0	42.4
Mn+Zn	25.0	21.7	10.0	18.9	40.0	31.5	33.3	34.9	13.3	10.0	1.7	8.3	53.0	45.6	83.0	60.5
Mn	25.0	16.7	5.0	15.6	40.0	47.3	66.7	51.3	18.3	8.3	3.3	10.0	35.3	54.6	67.0	52.4
Control	41.7	31.7	15.0	29.5	-	-	-	-	28.3	18.3	10.0	18.9	-	-	-	-
Diathane 45	8.33	6.7	1.7	5.6	80.1	78.9	88.7	82.6	6.7	1.7	0	2.8	76.3	90.7	100	89.0
LSD 0.05	9.3	7.1	Ns	6.7	-	-	-	-	11.6	7.7	ns	4.4	-	-	-	-

Effect of micronutrients on *Alternaria* leaf spot in faba bean plant: Results in Table 4 indicated that most treatments significantly decreased the *Alternaria* leaf spot severity of faba bean plants during the two successive seasons. The most effective treatments was Mn at rate of 4 g L^{-1} water which reduced the disease incidence by 51.3% during season 2010 while reduced disease incidence by 60.5% during season 2011 in combination with Zn, followed by (58.2%) with Fe+Zn+Mn when spray as a combination in same season.

Also, *Alternaria* leaf spot was more severe at the lower parts of the plant. This results are agreement Abd El-hai *et al.* (2009) who found that seed soaking method or foliar spray of micronutrients (Mn and Z at 2 g L^{-1}) were tested to control of damping- off and charcoal rot diseases of sunflower (varieties Sakha 53 and Giza 102). All treatments of antioxidant and microelement significantly reduced the incidence of charcoal rot disease.

CONCLUSION

According to the obtained results from this study, it can be concluded that, spraying Fe,Zn and Mn alone or combination could be recommend to control leaf spots and to enhance yield and quality of faba bean under the environmental conditions of Etay El-Baroud Research Station Farm, El-Behera Governorate.

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