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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

To study the effects of foliar application with micronutrients on yield and yield components of faba bean (c.v. Misr 1), an experimental research was conducted in complete randomized block design by three replications during the two growing seasons of 2009/2010 and 2010/2011 in Etay El-Baroud Agricultural Research Station, El-Behera Governorate. Results shwoed that**,** spraying with Fe+Zn+Mn increased plant height, number of pods/plant, number of seeds/pod, number of seeds/plant, 100 seed weight and seed yield /faddan 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, Altermaria leaf spot incidence on treated plants significantly decreased 18.2% - 60.5% compared with non-treated control plants.

INTRODUCTION

Faba bean (*Vicia faba* L.) is generally regarded as an important source of plant proteins that could help in supplementing the meager amount of animal proteins in the diet of averaged Egyptians. The total area planted for production of dry beans was 336,000 Fad. producing 428,000 t during 1995-1999 Abd El-Salam, (2002). Deficiency of micronutrients, especially Zn, Fe and Mn are widespread in most crops in Egypt either due to low available concentration 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 of micronutrients under field conditions in Egypt was found to increase the yield (Eweida *et al.,* 1980; El- Sheikh, 1981; Fawzi *et al.,* 1983; Abdel Hadi *et al.,* 1985; El-Fouly and Rezk, 1986 ; Gomma *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 *et al* . 1970).In this respect Rahhal (1993) found that Zn 4gm treatment showed the highest concentrations of chlorophyll (a) content of leaves. Meanwhile Zn (8gm) , Mn (8gm) and Fe (4gm) treatments showed the highest concentrations of chlorophyll (b) content of leaves, 0.62 , 060 , and 0.57 mg/g leaves, respectively, while Zn (8gm) , Mn (8gm) , Zn (4gm) and Fe (4 and 8gm) 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 leaves ,

respectively. Also, amount of charbohydrates in leaves and stem significant increased in all teratments compared with control. El-Shahaby and Mohamed (1983) found that all treatments of fungicides on onion plants due to significant increase of amount reducing and non reducing sugars further more, total sugars increased except when cuprosan was sprayed at 3g/L water.

On the other hand Alternaria leaf spot disease caused by *Alternaria alternata* are caused problem of bean plants during growing seasons (Honda *et al* . , 2001) There is a growing need to develop alternative approaches for controlling plant diseases .Induced resistance due to foliar application with micronutrants in some plants against plant diseases was reported by (Abd-El-karem 2004, and El-Gamal *et al*., 2007) micronutrients which can be applied successfully in many area of plant production as a plant growth stimulant (Scheuerll and Mahafee 2006).

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

MATERIAL 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:-

1. Spraying plants with tap-water(Control)
2. with (Fe) at 4 g/L
3. with (Zn) at 4 g/L
4. with (Mn) at 4 g/L
5. with (Fe + Mn) at 4 g/L
6. with (Fe + Zn) at 4 g/L
7. with (Zn + Mn) at 4 g/L
8. with (Fe + Zn +Mn) at 4 g/L
9. with (Diathen M 45) at 250 g/L

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 gm/L. 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.

1- Plant height (cm). 2- Number of branches/plant.
3- Number of pods/plant. 4- Number of seeds/plant.
5- Number of seeds per pod. 6- 100 - seed weight (g).
7- Seed yield/faddan (ton).

whole plants in each plot were harvested and left for air drying, then they we re 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 leaves after 70 days of sowing were calculated as follows:

Chl. a = [(12.7 x O.D. 663)-(2.69 x O.D. 645)] x 0.1

Chl. b= [(22.9 x O.D. 645)-(4.68 x O.D. 663)] x 0.1

Total chlorophyll = Chl. a (mg/g fresh weight of leaves) + Chl. b (mg/g 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 gm 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 minutes. 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 gm calcium carbonate were placed in a beaker and the mixture was boiled for 30 minutes 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 estemated 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 Vakalunkis (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:

Disease severity (D.S)% = $\frac{\varepsilon(NPCXCR)}{NIPXMSC} \times 100$

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

Isolation and dentification

Under field condation were growing faba bean plants, when observet of symptom disease of Altermaria leaf spot at natural infected takeing leaves and isolate fungal on P D A media *in vitro*. Fungal were kept in selant 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 (1980).

RESULTS AND DISCUSSION

1-Effect of micronutrients on growth characters:

Data presented in Table (1) showed that, foliar apllications with Fe, Zn and Mn alone or in comnination of them significantly increased all growth characters.

The highest plant height (99.93 cm) was obtained with sparying 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 sparying 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 sparying 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	3.00ab	8.67ab	3.27ab	30.38ab	88.91ab	2.75ab
Fe+Zn	99.93 a	3.23a	11.07a	3.56ab	37.96ab	93.75a	3.60ab
Fe+Mn	91.77 abc	2.70ab	9.67a	3.70ab	35.28ab	89.97ab	3.19ab
Fe+Zn+Mn	94.27 ab	2.67b	10.00a	4.02a	40.33a	89.75ab	3.66a
Zn	88.57 bc	2.70ab	6.20b	3.57ab	28.76ab	87.34ab	3.11ab
Mn+Zn	85.37 bc	2.90ab	9.87a	3.06b	35.82ab	87.64ab	2.84ab
Mn	89.47 bc	2.63b	9.93a	3.68ab	32.38ab	87.92ab	2.93ab
Diathane 45	90.43 abc	2.70ab	10.87a	3.99a	34.66ab	89.27ab	2.63ab
control	82.20 c	2.63b	6.13b	3.33ab	25.21b	85.86b	2.49b
LSD 0.05	7.35	0.56	2.20	0.86	10.03	4.74	0.82

2-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), Rashed and Ahmed (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, Ewieda *et al.,* 1980; Saber 1980; El-Sheikh, 1981; Abdel Aziz *et al.,* 1982. Fawzi *et al.,* 1983; El-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 sparying 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 compered with the control. This was in harmony with Bozorgi (2011) who found that, the highest 100 seed weight was obtained with 1g/L 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 other Monged *et al.,* (1988), Baza *et al.,* (1992), Monged *et al.,* (1994) and Sabik *et al*., (2001). On the other hand, Azarpour *et al.,* (2011) and Bozorgi (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 micronutriets in Egypt may be due to seceral 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)

3- 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 per g 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 reduing 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. There results are agreement With El – shahaby and Mohamed (1983) as they found that the amount of reducing sugars decreased at the lower rates of fungicides but increased for the 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 3g/L water .

Table (2): Sugar contents (mg / g fresh weight in leaves and stem of faba bean treated with chealte micro-elements 75 days after sowing

Treatments	Sugar contents mg/1g fresh weight					
	Leaves			Stem		
	Reducing	Non-reducing	Total sugars	Reducing	Non-reducing	Total sugars
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 + Mn+ Zn	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
Zn + Mn	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
Diathiane M45	3.1	3.3	6.4	1.9	1.6	3.5
Control (non – treated)	3.3	3.9	7.2	1.3	2.0	3.3
L.S. D at 0.05%	0.3	0.2	0.8	0.34	0.38	0.91

Data are average of three replicates .

4- Effect of micronutrients on the chlorophyll content of leaves:

The results recorded in Table(3) showed that micronutrients application at rate of 4g/L. increased significantly chlorophyll A from 0.84

mg/g leaves treated with micronutrients compared with control 0.6 mg / g 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 (1.97mg / g fresh weight).

Table (3): Effect of some chelated micronutrients as foliar application on the chlorophyll a, b and the total chlorophyll content (mg/ g f.w. leaves) .

Elements	Chlorophyll(a)	Chlorophyll (b)	Total chlorophyll
Fe	0.70 d	0.44 d	1.14
Zn	0.81 b	0.52 a	1.33
Mn	0.74 c	0.42 e	1.16
Fe + Zn	0.66 e	0.39 f	1.05
Fe + Mn	0.82 b	0.48 b	1.97
Zn + Mn	0.64 f	0.42 e	1.06
Fe + Mn + Zn	0.84 a	0.41 e	1.25
Diathane- M45	0.73 c	0.46 c	1.19
Cheak (non- treated)	0.61 g	0.36 g	0.97
L . S . D at 0.05	0.017	0.17	0.29

*** Data are avereg of three replicates .**

These results indicated that spraying micronutrients (Fe and Mn) stimulated the activity of chloroplasts this was followed by Zn (1.33 mg / g fresh weight leaves) while the lowest was found with Fe+ Zn (1.05 mg/ gm 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 gm), Mn (8gm) and Zn (4gm) treatments.

5-Effect of micronutrients on Alternaria leaf spot in feba bean plant:

Results in Table (4) and Fig. (1) 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 4g/L 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 2g L^{-1}) were tested to control of damping- off and charcoal rot diseases of sunflower (varieties Sakha53 and Giza 102) . All treatments of antioxidante and microelement significantly reduced the incidence of charcoal rot disease .

Also, the correlation co-efficient between total sugar and disease severity% in leaves was positive and was 0.319 and 0.289, during the first and second year, respectively.

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

Treatment	First season 2010								Second season 2011							
	Disease severity%			Mean	Reduction %			Mean	Disease severity%			Mean	Reduction %			Mean
	20 cm	40 cm	60 cm		20 cm	40 cm	60 cm		20 cm	40 cm	60 cm		20 cm	40 cm	60 cm	
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	6.7	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	8.3	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
Zn + Mn	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
Diathane –M45	8.33	6.7	1.7	5.6	80.1	78.9	88.7	82.6	6.7	1.7	0.0	2.8	76.3	90.7	100	89.0
Check (un-treated)	41.7	31.7	15.0	29.5	-	-	-	-	28.3	18.3	10.0	18.9	-	-		-
L.S.D at 0.05	9.3	7.1	n.s	6.7					11.6	7.7	n.s	4.4	-	-		-

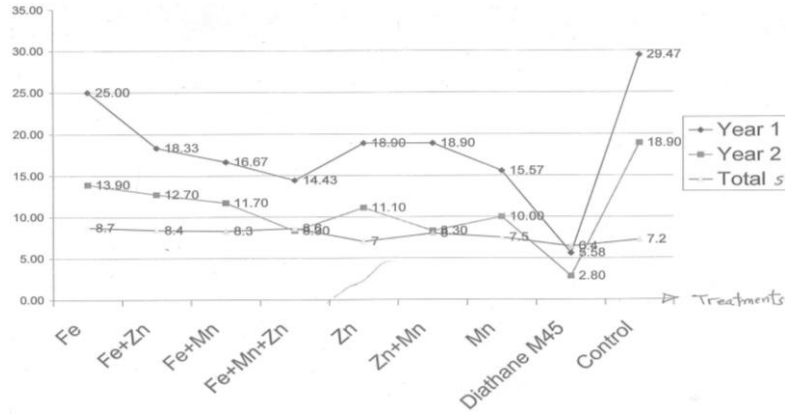

Fig. (1) : Relation between total sugar and disease severity in leaves of faba bean during two seasons 2010 and 2011

CONCLUSION

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

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تأثير بعض العناصر الصغرى على بعض الصفات النباتية والمحتويات الكيميائية ومرض لفحة الأوراق الالترنارى فى الفول البلدى

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تم دراسة تأثير الرش بالعناصر الصغرى (الحديد - الزنك - المنجنيز)على المحصول ومكوناته لمحصول الفول البلدى صنف مصرا. صممت التجربة فى قطاعات كاملة العشوائية حيث احتوت كل معاملة على ثلاث مكررات وذلك خلال موسمين الزراعة ٢٠٠٩ / ٢٠١٠ و ٢٠١٠ / ٢٠١١ بمحطة بحوث ايتاى البارود بمحافظة البحيرة . أوضحت النتائج أن هناك فروق معنوية مختلفة لكل الصفات تحت الدراسة كما أوضحت النتائج أن الرش بالعناصر الصغرى الحديد والزنك والمنجينز أدى لزيادة فى ارتفاع النبات وعدد القرون / نبات وعدد البذور / قرن وعدد البذور / نبات ووزن ١٠٠ بذرة والمحصول / فدان .

كما تمت دراسة المحتويات الكيميائية لكل من الكلورفيل أ،ب والسكريات المختزلة والغير مختزلة وأوضحت النتائج ان الرش بالعناصر الصغرى يزيد كمية الكلورفيل والسكريات لكل المعاملات التى عوملت بالرش بالمقارنة بالغير معامل بالعناصر الصغرى .

وأوضحت الدراسة بأن ظهور مرض لفحة الأوراق الالتزنارى يقل عند الرش بالعناصر الصغرى حيث وصلت نسبة الانخفاض فى المرض ما بين ١٨,٢ % الى ٦٠,٥% مقارنة بالغير معامل .

قام بتحكيم البحث

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Disease severity %

تلصق فى فيجر ١