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THE USE OF MICRONUTRIENTS TO CONTROL CHOCOLATE LEAF SPOT AND RUST OF FABA BEAN AND TO ENHANCE ITS GROWTH CHARACTERISTICS AND YIELD UNDER FIELD CONDITION

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ABSTRACT

Under field conditions, in the two successive seasons of 2010/11 and 2011/12 the effect of plant spraying with commercial chelated iron, manganese and zinc on severity of chocolate leaf spot and rust of faba bean cv. Misr 1 was studied. The effects on leaves chlorophyll content and some agronomic characters of the faba bean were also investigated.

Severity of *Botrytis fabae* was significantly decreased on the treated plants and reduced the disease incidence between 7.93% to 36.9% compared with control .Also, microelements decreased rust disease severity between 15.4% to 62.8% in the two growing seasons, respectively .

Chlorophyll (a) and (b) in leaves significant increased in all plants sprayed with micronutrients. Also, results showed that spraying faba bean plants with Fe, Zn and Mn alone or in combination increased plant height, number of pods / plant, 100 seed weight and seed yield / feddan .

INTRODUCTION

Faba bean is an important legume crop in Egypt due to its high nutritive value in both energy and protein contents and it is a primary source of protein in the diet of masses. Therefore, increasing the crop production is one of the most important targets of agricultural policy in Egypt. However, this strategic crop is suffering from many destructive diseases. It is attacked by more than 100 pathogens in the Mediterranean region (Hebblethwaite, 1983).

Chocolate leaf spot caused by *Botrytis faba*e is an important disease worldwide occurring almost in all regions where faba bean is growing (Rahman *et al.,* 2002).

The disease appears as lesions on leaves, flowers and stems with reddish to chocolate brown color, with darker margins that are fairly defined and often of a concentric circular pattern. (Harrison, 1988). The spots led to harmful effects on growth, physiological activities and yield (Khaled *et al*., 1995).

Also rust disease caused by *Uromyces faba*e Pers. Schroet. is an important limiting factor which causes great annual losses and sometimes complete crop failure (Mohamed, 1982, and Hanounik and Bisri, 1991).

The infection by *Uromyces faba*e firstly by appears as minute, slightly raised, white to cream colored spots on leaves and to a lesser extent on stems. As spots enlarge the epidermis rupture, releasing masses of dark brown spores (urediospores) to form characteristic pustules (Uredia). The pustules are often surrounded by a ring of yellow tissue. On highly

susceptible cultivars, rust build up rapidly until most of the leaves are covered with pustules. Severely infected leaves rapidly dry up and premature defoliation may occur (Benier *et al*., 1993)

Biological control of *B. fabae* by different bio-agents is reported by Cook and Baker (1983). Abd-El-Moiety and Abu-Zied (1985) and Omar *et al*. (1987). Essential oil extracts have been considered as natural preservatives or food additives and can be used for controlling pathogens (Naidu, 2000), because of their biocidal effects on bacteria, fungi , viruses, protozoa, insects and plants (Kalemba and Kunicka, 2003).

Many chemicals traditionally used to control chocolate spot and rust disease are becoming less effective (Harrison, 1988), giving only partial disease control despite of the high cost of their use and adverse environmental effects on the accompanying microflora (Khaled *et al.,* 1995). So, faba bean is in a growing need to develop alternative approaches for controlling plant disease. Induced resistance due to foliar application with microelements in some plants against plant disease was reported by (Abd-El-Karem *et al.* 2004, and El-Gamal *et al*., 2007). Microelements were applied successfully in many areas of plant production as a plant growth stimulant (Scheuerll and Mahafee, 2006).

Chlorophyll content was taken as an index for the extent of reduction in effective green area, as, the disease reduces the photosynthetic activity in leaves and ultimately lead to lower yields (Sinha *et al*. 1970). Rahhal (1993) found that microelements Fe and Zn increased concentrations of chlorophyll (a) or (b) and total content of leaves. Also, Abd El-Razek *et al*., (2012) recorded that micronutrients application at rate of 4g/L increased significantly chlorophyll a compared with control. The same trend was found in chlorophyll b content, but values were significantly lower than that of chlorophyll a.

Spraying faba bean by micronutrients under field conditions in Egypt was found to increase the plant height compared with control. This was in agreement with Bozorgi *et al*., (2011). Also, number of pods / plant, 100 seed weight and seed yield/ fadden were increased by spraying micronutrients compared with non treated plants El-fouly *et al*., (2010).

The present study therefore, was amid to study the influence of micronutrients on the alleviation of damage of chocolate spot and rust diseases on faba bean, chlorophyll a and b content and certain growth characteristics, yield and its components.

MATERIALS AND METHODS

Field Experiments :

The experiments were carried out at Etay El- Baroud Agric. Res. St. in the two successive seasons of 2010/2011 and 2011/12 . Faba bean seeds (*Vicia faba*) cv. Misr 1 were sown on 1st November, in 3m- long rows and each four rows represented one replicate. Each treatment was replicated three times in a randomized complete block design. The experiment includes nine treatments which were as the following :

- Spraying plants with water only (control)

- Spraying plants with (Fe 12.5%)
- Spraying plants with (Zn 12.5%)
- Spraying plants with (Mn 12.5%)
- Spraying plants with (Fe+ Mn)
- Spraying plants with (Fe + Zn)
- Spraying plants with (Zn + Mn)
- Spraying plants with (Fe + Zn + Mn)
- Spraying plants with (Diathane M – 45)

Treatments were applied three times , the first one at 45 days after sowing, followed by the other two sprays at 10 days intervals. Commerical chelated Fe, Mn and Zn were used in the experiment at rate of 4 g / L. water while, Diathane M- 45 was used at the at rate of 250 g/ 100 L. water.

Disease assessment : The disease severity of chocolate leaf spot disease was recorded at the 75th day from sowing using the scale of Bernier *et al* . (1993) , as follows :

1 = no disease symptoms or very small specks (Highly resistante)

3 = few small discrete lesions (Resistante).

5= some coalesced lesions with some defoliation (Moderately resastante).

7 = Large coalesced sporulating lesion, 50% defoliation, some dead plants (Susceptible).

9 = extensive lesions on leaves, stems, and pods ;severe defoliation; heavy sporulation; stem girdling; blackieng and death of more than 80% of plant (Highly susceptible) . The formula adopted by Hanounik (1986) was used to estimate the percentage of chocolate spot severity :

$$\text{Disease severity (D.S.) \%} = \frac{\Sigma (NPC \times CR)}{NIP \times MSC} \times 100$$

Where :

NPC = No. of plants in each class rate

CR = Class rate .

NIP = No. of infected plants .

MSC = Maximum severity class rate .

Rust disease severity was recorded at 110 days from sowing according to the standard scale suggested by Bernier *et al*., (1993) as follows:

1= No pustules or very small to non – sporulating flecks (Highly resistant) .

3 = Few scattered pustules covering less than 1% of leaf area, and few for no pustules on stem (Resistant)

5 = Pustules common on leaves covering 1- 4 % of leaf area, little defoliation, and some pustules on stem (Moderatly resistant) .

7 = Pustules very commone on leaves, covering 4-8% of leaf area; some defoliation; and many pustules on stem (Susceptible) .

9 = Extensive pustules on leaves, petioles, and stems covering 8 – 10 % leaf area; many dead leaves and severe defoliation (Highly susceptible) .

Average of chocolate spot and rust were recorded and statistically analyzed .

Leaf chlorophyll determination :

Leaf chlorophyll content was determined spectrophotometically at 645 and 663 nm according to Grodzinsky and Grodzinsky (1973). Chlorophyll (a) and (b) concentrations in mg/g leaves were calculated as follows :

Chl. a = (12.7 EX 663 – 2.69 EX 645) X 0.1

Chl . b = (22.9 EX 645 – 4.68 EX 663) X 0.1

Agronomic traits :

At harvest time(120 days after sowing) ten guarded plants were taken from each plot to estimate the following characters .

1- Plant height (CM)
2- Number of branches / plant .
3- Number of pods / plant .
4- 100 – seed weight (g) .
5- Seed yield / feddan (ton).

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

Statistical analysis

All data were statistically analyzed in two seasons using "MSTAT-C" statistical package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% of probability.

RESULTS AND DISCUSSION

Field experiments :

Effect of some chelated micronutrients on severity of chocolate leaf spot disease on faba bean plants.

Data presented in Table (1) showed that spraying faba bean cv. Misr 1 with microelements three times, on 10 days intervals significantly reduced chocolate spot severity . The highest reductions i.e. 36.9% and 25.6% were recorded when Zn was applied at the to growing seasons , respectively.This was followed by Mn which reduces disease severity by 34.8 % and 28.3 % at the two successive seasons 2010 and 2011, respectively. The disease reduction obtained of however, with the use of Mn was not significantly different of that obtained by the use of the fungicide Diathane M 45.

These results are in agreement with the findings of Rahhal (1993) who found that chocolate spot severity was generally low when Mn was applied as (8 gm/L) and Zn was applied as 8 gm/L. Also, Abd El – Hai *et al*. (2009) found that the application of manganese combined with zinc was more effective than the microelements alone against *Rhizoctonia solani* and *Macrophmmina phaseolina* in sunflower plants .Generally, disease severity decreased through the three heights of the plant assessment treatments.

Table (2) Micronutrients decreased severity of rust disease between 15.4 % to 62.8% in the two successive growing seasons 2010/2011 and 2011/ 2012.

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Also, the high part of plants at 60 cm upper of the land surface was highest affected with disease than at 40 cm or 20 cm. It is clear that in the first season Fe + Zn +Mn as a combination had the best effect, it decreased rust disease to 6.03% compared with un-treated control (16.4%) which resulted in disease reduction of 62.8% followed by Zn (7.47%) alone and Fe+Mn (8.6 %), respectively .However, in the second season 2011/ 2012 the best effect was obtained when Zn applied alone followed by Mn alone and Fe + Zn + Mn where rust severities were 5.1% , 7.6% and 7.9% , respectively .

Diseases decreased when micronutrients used as spray against some diseases of faba bean plants due to stimulating biological activities, *i.e.* , enzyme activity, chlorophyll synthesis , rate of translocation of photosynthetic products and nutrient. (Foilett *et al* ., 1981) .

Effect of spraying by faba bean with micronutrients on the chlorophyll content of leaves :

The effect of micronutrients on the chlorophyll content of leaves was presented in Table (3). The highest concentrations of chlorophyll (a) content of leaves which were 0.86 mg/g leaves and 0.83 mg/g leaves were obtained with foliar applications of Mn alone or(Fe+ Mn) or(Zn + Mn), respectively.Meantime, the highest concentrations of chlorophyll (b) *i.e.* 0.56, 0.51 and 0.51 mg/gm leaves were obtained when faba bean plants were sprayed with Mn or Zn alone and (Fe + Mn), respectively . The total chlorophyll content of leaves showed the highest concentrations in case of Mn alone and(Fe+ Mn) treatments which were 1.42 and 1.34 mg/g leaves, respectively. Al Juburi *et al*. (1992) reported that sprayed mandarin tress with Fe, Mn, Zn and Cu separately or as a smixture as sulphate increased chlorophyll content alternatively without regular pattern during the two growing seasons.

Table (3) : Effect of some chelaled micromutrients as spray treatments on the chlorophyll a , b and the total chlorophyll content (mg / g leaves).

Elements	Chlorophyll (a)	Chlorophyll (b)	Total chlorophyll (a+b)
Fe	0.77	0.45	1.22
Zn	0.81	0.51	1.32
Mn	0.86	0.56	1.42
Fe + Zn	0.80	0.48	1.26
Fe + Mn	0.83	0.51	1.34
Zn + Mn	0.83	0.49	1.32
Fe + Zn + Mn	0.80	0.46	1.26
Diathean M. 45	0.72	0.42	1.14
Check (un - treated)	0.62	0.40	1.02
L.S.D at 0.05%	0.134	0.155	

Values are averages of two seasons.

Correlation between disease severity on faba bean and chlorophyll (a and b).

A considerable positive correlation (r =0.996 and -1) was revealed between disease severity % of chocolate leaf spot and content of chlorophyll (a) in faba bean plant Fig.(1) while, less correlation coefficient of (r = -0.008 and - 0.023) was recorded for the D.S.% and chlorophyll (b) in the two growing seasons 2010 and 2011 respectively, Fig.**(2).**

Also,the disease severity % of rust and content chlorophyll (a)in faba bean plant was highly correlation (- 0.876 and 0.933)whil, less correlation coefficient between rust diseases severity % and chlorophyll (b) in two growing seasons 2010 and 2011, respectively.

Effect of micronutrients on growth characteristics of faba bean :

Data presented in Table (4) showed that, the foliar applications with Fe, Zn and Mn alone or in combination of them enhanced significantly all growth characteristics. Plant height was highly significant affected by foliar application in the combined date (Table 4). The highest plant height (99.7 cm) was obtained with spraying Fe + Zn followed by Fe + Zn + Mn (95.58 cm) and Mn + Zn (94.62 cm), while the lowest heig (83.88 cm) was recorded with un-treated (control). These result are agreement by Bozorgi *et al*. (2011).

The highest number of branches / plant was obtained with spraying faba bean plants with Fe + Zn + Mn (3.2 branches / plant) followed by Fe + Zn (3.0 branches plant) while, the lowest values of number of branches / plant was found in the un – treated plants (control).

The highest number of pods / plant (12.4) was obtained with of Fe + Zn followed by (10.4 pods / plant) when spraing with Fe + Zn. However, spraying with Fe or Zn alone resulted in 9.6 and 8.2 pods / plant, respectively.

These results were the harmony with El – Fouly *et al* . (2010) and Bozorgi *et al*. (2011). On the other hand Amin *et al* ., (1988) and El- Masri *et al* . (2002) found that , two sprays of zinc resulted in the highest number of pods per plant.

Data in Table (4) showed that spraying Fe + Zn recorded the highly significant value (94.06 gm) of 100 seed weight compared with the other treatments and control, Yassen *et al*., (2010) found that, the highest increment (16%) was obtained when plants were sprayed with micronutrients mixture (Fe + Zn + Mn) as compared with control treatment .

Spraying with Fe + Zn exhibited the highest value (3.8 ton / fed.) of seeds yield / fad in the combined data as shown in Table (4), followed by Fe + Zn + Mn (3.7 ton / fed .) and Fe +Mn (3.6 ton / fed .) compared with control for the two growing season of 2010 and 2011. 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.

Abd El- Hai *et al*., (2009) found that microelements were on enhancing the stem and flower head diameters .

Fig.(1).Correlation between disease severity %of chocolate leaf spot and chlorophyll a) content in faba bean plant two growing seasons.

Fig.(2).Correlation between disease severity % of chocolate leaf spot and chlorophyll(b) content in faba bean plant two growing seasons.

Fig.(3).Correlation between disease severity %of rust and chlorophyll(a) content in faba bean plant two growing seasons.

Fig.(4).Correlation between disease severity %of rust and chlorophyll(b) content in faba bean plant two growing seasons.

El.Sayed *et al.* (2011) found that the foliar spraying of pea plants with a mixture of microelements significantly increased yield components expressed as pod length pod weight, number of green seeds (pod. weight of 100 – green seed , seed index, 1000- dry seed weight and chemical constituents.

Table (4) : Effect of foliar application with some micronutrients on growth, yield and yield components of faba bean plants.

Treatments	Plant height (cm)	N. of branches / plant	N . of pods / plant	100 seed weight (g)	Seed yield / faddan (ton)
e	88.22	2.8	9.6	89.32	2.93
e + Zn	99.7	3.0	12.4	94.06	3.8
e +Mn	92.64	2.6	10.4	89.78	3.6
e + Zn + Mn	95.58	3.2	10.2	90.0	3.7
n	91.02	2.6	8.2	87.12	3.2
In + Zn	94.62	2.6	10.0	90.12	3.0
In	89.42	2.6	10.0	88.0	3.1
iathean M 45	91.14	2.8	10.0	90.12	2.9
control	83.88	2.4	7.6	86.04	2.5
L.S.D at 0.05	11.64	Ns	3.5	1.4	1.1

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إستخدام المغذيات الصغرى فى مقاومة تبقع الأوراق الشيكولاتى والصدأ فى الفول وتحسين خواص النمو والمحصول تحت الظروف الحقلية

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أوضحت التجارب الحقلية خلال موسمى الزراعة 2010 / 2011 و 2011 / 2012 تأثير المغذيات الصغرى رشا على نباتات الفول البلدى صنف مصر 1 على تخفيض شدة مرض التبقع الشيكولاتى ما بين 7.93 % الى 36.9 % مقارنة بالغير معامل . كما ادت المعاملة بالرش بالمغذيات الصغرى لنقص الاصابة بمرض الصدأ بنسبة تراوحت ما بين 15.4 % الى 62.8% مقارنة بالغير معامل . كما أدت المعاملة بالعناصر الى زيادة معنوية فى المحتوى الكلورفيلى سواء لكلورفيل أ أو ب أو إجمالى الكلورفيل فى اوراق الفول البلدى . كما أثرت العناصر الصغرى على النباتات المعاملة فى طول النبات وعدد القرون لكل نبات ووزن 100 حبة والمحصول للفدان بالزيادة مقارنة بالغير معامل .

قام بتحكيم البحث

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Table (1) :Severity of chocolate leaf spot in faba bean plants, at three plant heights as affected by spraying with micronutrient under field conditions.

Micronutrients	(Chocolate leaf spot)															
	First season 2010/ 2011								Second season 2011 /2012							
	Disease severity			Mean	Reduction %			Mean	Disease severity			Mean	Reduction			Mean
	60cm	40cm	20cm		60cm	40cm	20cm		60cm	40cm	20cm		60cm	40cm	20cm	
Fe	3.8	3.1	2.4	3.1	35.6	6.1	22.6	21.4	4.4	3.8	2.8	3.7	27.9	7.3	22.2	19.3
Fe+Zn	4.8	3.1	2.6	3.5	18.6	6.1	16.13	13.6	5.6	3.8	3.3	4.2	8.2	7.3	8.3	7.93
Fe + Mn	3.8	3.1	1.9	2.9	35.6	6.1	38.71	26.8	4.6	3.8	2.6	3.7	24.6	7.3	27.8	19.9
Fe + Zn + Mn	4.3	3.1	2.1	3.2	27.1	6.1	32.3	21.8	4.6	3.6	2.6	3.6	24.6	12.2	27.8	21.5
Zn	3.8	2.1	1.9	2.6	35.6	36.4	38.71	36.9	4.3	3.3	2.6	3.4	29.5	19.5	27.8	25.6
Zn + Mn	4.7	2.4	1.9	3.0	20.3	27.3	38.1	28.6	4.8	3.1	2.6	3.5	21.3	24.4	27.8	24.5
Mn	2.9	2.6	2.1	2.5	50.8	21.2	32.3	34.8	3.8	3.3	2.8	3.2	37.7	19.5	27.8	28.3
Diathean M 45	2.6	1.9	1.9	2.1	55.9	42.4	38.1	45.5	3.1	2.6	1.9	2.5	49.2	36.6	47.2	44.3
Cheack(non – treated	5.9	3.3	3.1	4.1					6.1	4.1	3.6	4.6				
L.S.D at 0.05	1.3	n.s	0.1						1.4	n.s	0.7					

***Reduction compared to the untreatment(control).**

Table (2) : Severity of rust disease on faba bean plants, at three plant heights, as affected by spraying with micronutrients under field condition.

Application foliar spray 4 gm / L	First season 2010 / 2011								Seconed season 2011 / 2012							
	% Disease severity			Mean	Reduction %			Mean	% Disease severity			Mean	Reduction %			Mean
	60cm	40cm	20cm		60cm	40cm	20cm		60cm	40cm	20cm		60cm	40cm	20cm	
Fe	15.4	13.9	11.5	13.6	5.5	4.8	37.2	15.9	16.4	11.7	7.9	12.0	6.8	19.9	19.4	15.4
Fe +Zn	12.4	11.1	6.5	10.0	23.9	24.0	64.5	37.5	11.3	9.1	5.7	8.7	35.8	37.7	41.8	38.4
Fe + Mn	10.2	8.7	6.9	8.6	37.4	40.4	62.3	46.7	11.7	10.4	8.7	10.3	33.5	28.8	11.2	24.5
Fe + Zn + Mn	9.8	4.3	4.0	6.03	39.9	70.5	78.1	62.8	8.3	9.1	6.3	7.9	52.8	37.7	35.7	42.1
Zn	14.1	3.7	4.6	7.47	13.5	74.7	74.9	54.4	4.6	5.7	5.0	5.1	73.8	61.0	49.0	61.3
Zn + Mn	14.6	12.8	6.9	11.4	10.4	12.3	62.3	28.3	11.3	10.2	8.3	9.9	35.8	30.1	15.3	27.1
Mn	11.3	9.3	6.1	8.9	30.7	36.3	66.7	44.6	9.8	8.3	4.6	7.6	44.3	43.2	53.1	46.8
Dithane M – 45	5.0	4.6	2.8	4.13	69.3	68.5	84.7	74.2	3.9	5.0	3.7	4.2	77.8	65.8	62.2	68.6
Cheack (non – treated)	16.3	14.6	18.3	16.4	-	-	-		17.6	14.6	9.8	14.0	-	-	-	
L. S. D at 0.05%	3.97	2.45	3.82						3.84	5.4	4.37					

Data are average of three replicates