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EFFECT OF SEED COATING WITH SOME MICRONUTRIENTS ON FABA BEAN (*Vicia faba L.)*

I- EFFECT ON PHOTOSYNTHETIC PIGMENTS, MICRONUTRIENTS CONTENT AND PLANT GROWTH CHARACTERS

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ABSTRACT

Two field experiments were carried out at the farm of El-Gemmeiza Agric.Res. Station, Agric.Res. Center, during the two successive growing seasons; 1998/99 and 1999/2000 to study the effect of Zn, Mn and Fe on plant growth, photosynthetic pigments and some micronutrients content of faba bean leaves at 70 days age. Zn, Mn and Fe were added by seed coating method at a rate of 0.3gm of Zn or Fe and 0.15 gm of Mn in chelating form per one kilogram seed, individually or in all possible combination. Yet, the relations between the content of photosynthetic pigments and micronutrients in leaves were considered.

The mean results of the two growing seasons showed that plant height, number of both leaves and branches as well as dry matter of various organs seemed to be improved by the different treatments. Moreover, photosynthetic pigments and micronutrients content in leaves were also significantly increased as a result of micronutrients application. In this respect, addition of Zn,Mn and Fe simultaneously induced the highest values for all aforementioned parameters followed by the dual combinations, whereas the individual treatments recorded the least increments compared with the control treatment.

On the other hand, photosynthetic pigments in faba bean leaves were positively correlated by micronutrients content at different treatments of micronutrients as the following descending order: Mn< Zn < Fe.

The aforesaid relations can be expressed as the following multiple equations:

$Y_1 = 3.37 + 38.18X_1^{**} + 28.42X_2^{**} + 1.63X_3^{**}$ $(r^2 = 0.999)$

$Y_2 = 1.61 + 3.82X_1^{**} + 17.35X_2 + 1.21X_3^{*}$ $(r^2 = 0.989)$

Where; Y_1, Y_2, X_1, X_2 and X_3 indicate the mean values of Chl.(a+b), carotene, Zn,Mn and Fe content in leaves, respectively in (mg/plant).

* significant at 0.05 level ** significant at 0.01 level

keywords: (Faba bean- seed coating- photosynthetic pigments- micronutrients)

INTRODUCTION

Faba bean is one of the most important legume crops in Egypt. It owns its importance chiefly to its high protein content that reaches in seeds to about 24%. It is also well supplied with P and Ca. In addition, it is relatively not expensive crop to produce and it promises high return when properly grown.

In recent years, some evidences have been developed that application of micronutrients to legume plants had resulted in better

growth and more yield under Egyptian conditions (Thalooth *et al*., 1981; El-Gayar *et al*., 1988 and Waly,1996).

Since micronutrients affect directly or indirectly on photosynthesis, respiration, synthesis….etc. The presence or absence of any nutrient during a specific stage of plant growth might affect the growth pattern through alteration in metabolic processes. In addition, Eissa *et al* (1992) and Rashid and Fox(1992) stated that rates of photochemical reaction, activities of the carboxylation enzymes i.e. phosphoenol pyruvic (PEP); carboxylase; ribulose biphosphate (RBP) and carbonic anhydrase were increased by the application of Zn, Mn, and Fe, B, Mo and Cu in a descending order compared with the control. Numerous studies showed that chlorophyll a, b as well as carotene contents were closely related to micronutrients supply (Garg, 1987; Cakmak and Marschner, 1993; Waly, 1996 and Nassar,1997).

Seed coating method to supply plants with micronutrients is considered one of the most efficient means of correcting micronutriens defficiencies through creating a nutritional environment in the immediate vicinity of the germinating seed. This provides a “boost” for the seedling in its critical early phase of development, which is very important especially under the stress conditions found in soil (Shams El-Din,1993; Waly,1996 and Nassar,1997).

Thus, the present work was undertaken to study the effect of some micronutrients, namely Zn,Mn and Fe adding by seed coating individually or in all possible combinations on the growth and contents of photosynthetic pigments and micronutrients of faba bean leaves (fourth upper blade leaf) taken into consideration the relations between the contents of the above-mentioned nutrients and photosynthetic pigments in leaves.

MATERIALS AND METHODS

Two field experiments were carried out at El-Gemmeiza Agric. Res. Station, Gharbia Governorate during the two growing seasons; 1998/99 and 1999/2000. The general characteristics of the soil under consideration are given in Table(1).

Table (1): Physical and chemical analysis of the investigated soil amples.

a- Physical analysis:

Season	Organic matter(%)	Total $CaCO_3$(%)	Particle size distribution (%)				
			Coarse sand	Fine sand	Silt	Clay	Soil texture
1998/1999	1.65	3.33	3.14	20.28	24.95	51.63	Clayey
1999/2000	1.80	4.12	2.26	14.58	28.75	54.41	Clayey

b-Chemical analysis:

Season	pH*	EC** dS/m At 25C°	Soluble ions,meq/100g soil(1:5)extract							
			Cations				Anions			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
1998/1999	7.9	1.25	1.10	0.9	4.1	0.2	-	1.4	4.0	0.9
1999/2000	8.1	1.58	1.50	1.3	4.7	0.4	-	1.7	5.0	1.2

*** pH (1 soil : 2.5 water suspension)**
**** EC (1 soil : 5 water extract)**

C-Available nutrients

Season	Available nutrients (ppm)					
	Macro			Micro		
	N	p	K	Zn	Mn	Fe
1998/1999	21.2	8.0	440	1.0	7.5	8.7
1999/2000	25.0	9.6	498	1.3	8.9	11.0

*Organic matter, total $CaCO_3$, pH as well as available N and K were determined according to Jackson (1973).

*Available phosphorous was determined according to Watanabe and Olsen (1965).

*Available Zn, Mn and Fe were extracted by DTPA extraction (Lindsay and Norvell,1969) and determined using the atomic absorption spectrophotometer (Chapman and Pratt,1961).

*Partical size distribution and soluble cations and anions were determined according to Richards (1954).

Each experiment consisted of eight seed coating treatments as the following:

1- Control
2- coating with Zn
3- coating with Mn
4- coating with Fe
5- coating with Zn + Mn
6- coating with Zn + Fe
7- coating with Mn + Fe
8- coating with Zn + Mn + Fe.

The above mentioned elements were applied in the form of EDTA compounds, i.e. Zn-EDTA (14%Zn), Mn-EDTA (13%Mn) and Fe-EDTA (6%Fe) at a rate of 0.3 gm for both Zn and Fe and 0.15gm for Mn per one kg of seeds, before sowing. “Triton B” was used as a speader starter for all treatments.

The experimental plots were 10.5 m^2(1/400 feddan). They were planted with faba bean seeds (Giza 2 variety) at 20 cm distance and rows 60 cm apart, in a randomized complete block design. The L.S.D. was used for comparing the differences between means of treatments according to Gomez and Gomez (1984).

As for the other fertilizers; superphosphate (15% P_2O_5)were applied, before planting, at a rate of 200 kg/fed.; whilst ammonium sulphate (20.6%N) were added at a rate of 75 kg /fed., before the first irrigation, as a starter dose.

At 70 days age, ten plants were taken randomly from each plot for studying morphological characteristics, i.e. plant height (cm), number of branches and leaves per plant, dry matter of various plant organs (g/plant). Photosynthetic pigments (chlorophyll a, b and carotene) were extracted from the fourth upper blade leaves of faba bean plants at 70 days age using aqueous solution of 85% acetone; determined spectrophotmetrically and calculated in mg/L using Wettstein’s formula (Wettstein,1957) then converted into mg/plant.

Samples from the fourth leaves of faba bean plants at 70 days age were also taken, dried, ground and digested using H_2SO_4- $HclO_4$ acid mixture. Zn,

Mn and Fe were determined in the acid digestion resultant using the atomic absorption spectrophotmeter (Chapman and Pratt, 1961).

RESULTS AND DISCUSSION

1-Effect on photosynthetic pigments in leaves:

Data concerning the effect of Zn, Mn and /or Fe adding as seed coating on the content of photosynthetic pigments (mg/plant) in faba bean leaves at 70 days age are presented in Table (2) and illustrated by Fig. (1). These results showed that all photosynthetic pigments (Chl. a & b and carotene) were significantly increased by different treatments. In this respect, mixture additions (Triple and duble) attained the highest increments compared to the control treatment followed by the individual treatments.

The enhancing effects of micronutrients addition on the content of photosynthetic pigments of faba bean leaves could be explained through its benefical effects on the number of chloroplast per cell or by forming chloroplast with high chlorophyll content, (Hassan, 1996). On the other hand, the superiority impact of Fe-treatments might be due to the essential roles of Fe in the redox reactions of chloroplasts, in the mechanism of photosynthetic electron transfer and also in the formation of heme and nonheme proteins that is concentrated in chloroplasts. Numerous investigators obtained the same previous conclusion such as Iturbe-Ormaetxe *et al* (1995) on maize and pea; Waly (1996) on pea and Nassar (1997) on wheat.

Table(2): Effect of investigated micronutrients treatments on the content of hotosynthecic pigments(mg/plant) of faba bean leaves at 70 days age.

Treatments	Chl. (a)			Chl. (b)			Chl. (a+b)			Carotene		
	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	mean	1st season	2nd season	mean
Control	3.93	10.13	7.03	2.03	5.49	3.76	5.96	15.62	10.79	2.87	7.70	5.29
Zn	7.70	11.44	9.57	4.23	6.17	5.20	11.93	17.61	14.77	6.19	9.15	7.67
Mn	5.89	10.81	8.35	3.26	5.78	4.52	9.15	16.59	12.87	4.66	8.33	6.50
Fe	8.17	11.77	9.97	4.45	6.75	5.60	12.62	18.52	15.57	6.74	9.88	8.31
Zn+Mn	11.58	12.98	12.28	6.49	7.45	6.97	18.07	20.43	19.25	9.65	11.04	10.35
Zn+Fe	11.24	13.88	12.56	6.27	7.91	7.09	17.51	21.79	19.65	9.18	11.71	10.45
Mn+Fe	9.14	13.15	11.15	5.29	7.42	6.36	14.43	20.57	17.50	7.79	11.26	9.53
Zn+Mn+Fe	17.51	17.41	17.46	9.62	10.36	9.99	27.13	27.77	27.45	13.36	14.31	13.84
L.S.D.at 5%	1.75	0.80	1.11	1.37	0.63	1.65	2.73	1.17	1.86	2.22	0.64	1.12

2-Effect on micronutrients content in leaves :

Regarding the effect of micronutrents treatments under investigation on its content of faba bean leaves at 70 days age, Table (3) and Fig.(2) indicated that all treatments significantly increased Zn, Mn and Fe contents of leaves .

Table (3): Effect of investigated micronutrients treatments on micronutrients content (mg/plant) of faba bean leaves at 70 days age.

	Zn	Mn	Fe

Treatments	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	0.031	0.070	0.051	0.042	0.113	0.078	1.04	2.96	2.00
Zn	0.064	0.109	0.087	0.086	0.133	0.110	2.13	4.06	3.10
Mn	0.043	0.078	0.061	0.077	0.134	0.106	1.46	3.52	2.49
Fe	0.061	0.098	0.080	0.086	0.135	0.111	2.47	4.83	3.65
Zn+Mn	0.100	0.131	0.116	0.161	0.183	0.172	3.19	4.82	4.01
Zn+Fe	0.103	0.136	0.120	0.121	0.150	0.136	3.70	5.84	4.77
Mn+Fe	0.068	0.112	0.090	0.123	0.165	0.144	2.90	5.29	4.10
Zn+Mn+Fe	0.179	0.178	0.179	0.213	0.246	0.230	5.67	7.44	6.56
L.S.D.at 5%	0.006	0.008	0.004	0.009	0.012	0.007	0.26	0.36	0.20

Generally, all treatments containing any element had the superiority effect on increasing its content of leaves. In this respect, triple treatment gave the highest increments of Zn,Mn and Fe contents followed by the double and single treatments in a descending order. These results are in agreement with those obtained by Osman *et al*.(1993) on corn; and Nassar (1997) on wheat. Moreover, Table(3) and Fig.(2) also showed that the addition of Zn, Mn and /or Fe promote the uptake of others. Baza *et al*(1989) on faba bean and Nassar(1996) on wheat also observed the positive effect of Zn on Fe and Mn uptake. Yet, the enhancing effect of Mn on Zn and Fe uptake was shown by Monged and Basha(1986) and Nassar(1997) on wheat and Azer *et al*(1992) on faba bean.

3- Relations between the content of micronutrients and photosynthetic pigments of leaves:

Fig.(3) reveals that there were strong relations between the content of both micronutrients and photosynthetic pigments of faba bean leaves at 70 days age under all investigated micronutrients treatments as the descending order : Mn < Zn ≤ Fe. The correlation coefficients between the contents of chl.(a+b) and Zn, Mn and Fe were 0.99, 0.97 and 0.99, respectively. For carotene, the corresponding correlation coefficients were 0.98, 0.96 and 0.99.

The simple regression equations were as follows:

$Y_1 = 4.95 + 125.37\ x_1$ $\qquad$ $Y_2 = 2.68 + 64.41\ x_1$

$= 1.67 + 53.92\ x_2$ $\qquad$ $= 3.05 + 104.36\ x_2$

$= 3.59 + 3.56\ x_3$ $\qquad$ $= 1.89 + 1.85\ x_3$

Where; Y_1 and Y_2 indicate the mean values of chl. (a + b) and carotene, respectively in (mg/plant).

x_1, x_2 and x_3 indicate the mean values of Zn, Mn and Fe content, respectively in (mg/plant).

The multiple regression equations were as follows:

$Y_1 = 3.37 + 38.18\ x_1^{**} + 28.42\ x_2^{**} + 1.63 x_3^{**}$ $\quad (r^2 = 0.999)$

$Y_2 = 1.61 + 3.82\ x_1^{**} + 17.35\ x_2 + 1.21 x_3^{*}$ $\quad (r^2 = 0.989)$

The above- mentioned strong relations between the contents of both photosynthetic pigments and micronutrients of faba bean leaves can be explained, as aforesaid, on the basis of the important roles of these elements on the regulation of chlorophyll biosynthesis. Yet, Zn affect on the rates of photochemical reduction, chloroplast structure, photochemical electron transfer and net photosynthesis, Romheld and

Morschner, 1991. They also added that Mn is responsible on the stability of the chloroplast structure, protects the photosynthetic apparatus against the deleterious action of oxygen radicals and plays an essential role in the photosynthetic evolution of O_2 and incorporation of CO_2 in chloroplasts.

4- Effect on plant growth :

Number of branches and leaves /plant and plant height as well as dry matter yield of various organs were used as sake of expressing the growth of faba bean plants at 70 days age. As shown in Tables (4,5) and

Fig1+2

Fig3

illustrated by Fig.(4), it can be observed that all aforementioned plant growth characters were significantly affected by different micronutrients treatments. Triple treatment (Zn + Mn + Fe) recorded the highest increments compared with the other treatments , whereas the individual treatments gave the least increases over control. Among the dual treatments, (Zn+Fe) and (Zn+Mn) surpassed the treatment of (Mn+Fe). Likewise, Fe and Zn gave values higher than those obtained with Mn. The response of faba bean plants to Fe,Zn and Mn may be due to the important roles of these elements in enzymes activation and hormones regulation, in metabolism of carbohydrates, proteins and auxins and also in multiple processes, development, division and differentiation of cells, Romheld and Marschner,(1991). In addition, Fe is characteristic by its ability to undergo oxidation-reduction reactions and to form a component of chlorophyll (Fig.1 and 3). Zn also has an essential role in the synthetase and metabolism of tryptophane. Yet, Mn influences directly in indole acetic acid balance in plants responsible on plant height (Moore and Patrick,1988).

Table (4): Effect of investigated micronutrients treatments on some plant growth characters of faba bean plants at 70 days age.

Treatments	No. of branches/plant			No. of Leaves/plant			Plant height (cm)		
	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	2.54	2.53	2.54	5.00	6.63	5.82	28.7	36.4	32.6
Zn	2.73	2.85	2.79	7.22	7.94	7.58	34.0	39.9	37.0
Mn	2.65	2.75	2.70	5.93	7.30	6.62	30.5	39.6	35.1
Fe	2.75	2.89	2.82	8.10	8.09	8.10	33.1	40.0	36.6
Zn+Mn	2.93	3.22	3.08	8.26	8.37	8.32	35.3	42.5	38.9
Zn+Fe	3.00	3.37	3.19	9.17	9.80	9.49	37.3	43.1	40.2
Mn+Fe	2.83	3.04	2.94	8.89	9.33	9.11	34.2	40.7	37.5
Zn+Mn+Fe	3.13	3.94	3.54	12.00	10.48	11.24	38.7	43.5	41.1
L.S.D.at 5%	0.70	0.94	0.48	3.49	3.79	2.50	4.12	1.56	1.9

Table (5): Effect of investigated micronutrients treatments on dry matter of various organs of faba bean plants (g/plant) at 70 days age.

Treatments	Stem			Leaves			W.plant		
	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	2.20	3.40	2.80	1.14	2.25	1.70	3.34	5.65	4.50
Zn	2.65	3.75	3.20	1.87	2.46	2.17	4.52	6.21	5.37
Mn	2.44	3.47	2.96	1.49	2.34	1.92	3.93	5.81	4.88
Fe	3.34	3.93	3.64	1.96	2.52	2.24	5.30	6.45	5.88
Zn+Mn	4.25	4.09	4.17	2.75	2.66	2.71	7.00	6.75	6.88
Zn+Fe	4.46	4.18	4.32	2.46	2.68	2.57	6.92	6.86	6.89
Mn+Fe	3.80	3.97	3.89	2.14	2.63	2.39	5.94	6.60	6.27
Zn+Mn+Fe	4.91	4.63	4.77	3.40	3.16	3.28	8.31	7.79	8.05

L.S.D.at 5%	1.01	0.41	0.62	0.20	0.20	0.12	1.06	0.43	0.51

Fig.(4): Effect of investigated micronutrients tretments on dry matter of various organs of faba plants (g / plant).at 70 days age (mean values of two seasons).

From the aforementioned results, it could be attributed the enhancing effect of trace elements on dry matter yield of various faba bean organs to its effective physiological roles in increasing the values of plant growth characters (Table, 4) and increasing the content of photosynthetic pigments of faba bean leaves (Table 2 and Fig. 1&3) which in turn encourages plant to convert light energy to metabolities. Consequently, increasing the corresponding values of dry matter yield of faba bean plants (Table 5 and Fig.4). These results were in agreement with those obtained by El-Fadaly(1992) on cucumber; Gangwar and Singh (1994) on lentil, Waly (1996) on pea and Nassar (1997) on wheat.

Finally, it can be concluded two important points : first; seed coating method with micronutrients enables the plants to grow well and absorb more nutrients from the soil. Second; application of Zn, Mn and Fe simultaneously attains suitable balance between them required to obtain the best growth, strong and healthy plants in comparison with application of these elements singly or in dual mixtures.

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تأثير تغليف البذور ببعض العناصر الصغرى على الفول البلدى
1-التأثير على نمو النبات ومحتواه من صبغات التمثيل الضوئى والعناصر الصغرى

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أجريت تجربتين حقليتين بمحطة البحوث الزراعية بالجميزة خلال موسمى النمو 1999/1998 ، 2000/1999 بغرض دراسة تأثير تغليف بذور الفول البلدى بعناصر الزنك والمنجنيز والحديد على نمو النباتات ومحتوى الاوراق من صبغات التمثيل الضوئى والعناصر الصغرى مع ألاخذ فى الاعتبار العلاقات بين محتوى الاوراق من كل من صبغات التمثيل الضوئى و العناصرالغذائية الصغرى.

غلفت بذور الفول البلدى قبل الزراعة بالعناصر الصغرى (الزنك والمنجنيز والحديد) فى صورها المخلبية (بمعدل 0.3جم عنصر/كجم بذور فى حالتى الزنك والحديد و بمعدل 0.15 جم منجنيز / كجم بذور)وقد اضيفت هذه العناصر فى صور منفردة أو فى صوره مخاليط ثنائية أو ثلاثية.

وقد أظهرمتوسط النتائج لكلا موسمى النمو تحسن مقاييس نمو النبات بصفة عامة (ارتفاع النبات، عدد الاوراق والفروع /نبات) وكذلك ألاوزان الجافة لأجزاء النبات المختلفة عند عمر 70يوم (الساق، ألأوراق) نتيجة لاضافة معاملات العناصر الصغرى. علاوة على ذلك فقد أدت اضافة العناصر الصغرى عند معاملاتها المختلفة الى الزيادة المعنوية لمحتوى الاوراق من صبغات التمثيل الضوئى والعناصر الصغرى. وتجدر الاشارة الى أن اضافة العناصر الصغرى الثلاث معا (زنك + منجنيز+ حديد) ادت الى الحصول على أعلى القيم بالنسبة لجميع مقاييس النمو السابقة وكذلك محتوى الاوراق من صبغات التمثيل الضوئى والعناصر الصغرى يليها معاملات المخاليط الثنائية للعناصر الصغرى بينما أدت اضافة هذه العناصر بصورها المنفردة الى الحصول على أقل الزيادات بالمقارنة بمعاملة الكنترول.

وقد أشارت نتائج هذا البحث الى العلاقة الوثيقة لمحتوى الاوراق من صبغات التمثيل الضوئى ومحتواها من العناصر الصغرى عند معاملات العناصر الصغرى المختلفة وسجلت معاملات ارتباط قوية كان أعلاها مع الحديد ثم مع الزنك ثم المنجنيز... ويمكن التعبير عن العلاقات السابق الاشارة اليها بمعادلات الانحدار المركبة الاتية-

الكلورفيل (أ+ب) = 38.18+3.37زنك** + 28.42 منجنيز** +1.63حديد**(معامل الارتباط=0.999)

الكاروتين = 1.61+3.82زنك** + 17.35 منجنيز +1.21حديد * (معامل الارتباط=0.989)

حيث أن محتوى الكلورفيل (أ + ب)، الكاروتين، الزنك، المنجنيز، الحديد محسوب فى الاوراق على صورة (ملليجرام/نبات).