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Effect of Seed Coating with Some Micronutrients on Faba Bean (*Vicia faba* L.) I- Effect on Photosynthetic Pigments Micronutrient Contents and Plant Growth Characters

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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 0.3g of Zn or Fe and 0.15 g of Mn in chelating form per one kilogram seed, individually or in all possible combinations. The relations between the content of photosynthetic pigments and micronutrients in leaves were studied.

The mean results of the two growing seasons showed that plant height, number of both leaves and branches as well as the dry matter of various organs seemed to be enhanced by the different treatments. Moreover, photosynthetic pigments and the content of micronutrients in leaves 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 showed the least increments compared with the control treatment.

On the other hand, photosynthetic pigments in Faba - bean leaves were positively correlated with the micronutrients content for different treatments of micronutrients, in the following descending order: Mn< Zn <Fe.

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

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

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

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).

Keywords: Seed coating , faba bean, micronutrients, photosynthetic pigments.

Faba - bean is one of the most important legume crops in Egypt. It ows its importance chiefly to its high protein content reaching 24% in seeds. It is also containing high concentrations of P and Ca. In addition, it is relatively cheap 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 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 and b as well as carotene contents, are closely related to micronutrients supply (Garg, 1987; Cakmak and Marschner, 1993; Waly, 1996 and Nassar,1997).

A seed coating method to supply plants with micronutrients is considered to be one of the most efficient means of correcting micronutriens deficiencies, by 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 soils (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 added by seed coating individually or in

* significant at 0.05 level ** significant at 0.01 level

all possible combinations on the growth and contents of photosynthetic pigments and micronutrients of Faba - bean leaves (fourth upper blade leaf) taking into consideration the relations between the contents of the above-mentioned nutrients and photosynthetic pigments in leaves.

Material 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 samples.

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 phosphorus 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:

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 g for both Zn and Fe and 0.15g 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) was applied, before planting, at a rate of 200 kg/fed., whilst ammonium sulphate (20.6%N) was 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 an aqueous solution of 85% acetone, determined spectrophotometrically 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 a H_2SO_4- $HClO_4$ acid mixture. Zn, Mn

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

Results and Discussion

1-Effect on photosynthetic pigments in leaves

Data concerning the effect of Zn, Mn and Or Fe, added 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 double) attained the highest increase compared to the control treatment, followed by the individual treatments.

The enhancing effect of micronutrients addition on the content of photosynthetic pigments of Faba - bean leaves could be explained by its benefical effects on the number of chloroplasts per cell or by forming chloroplasts with a high chlorophyll content (Hassan, 1996). On the other hand, the superior 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, concentrated in chloroplasts. Numerous investigators came to the same conclusion, *e.g.* 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 photosynthetic 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

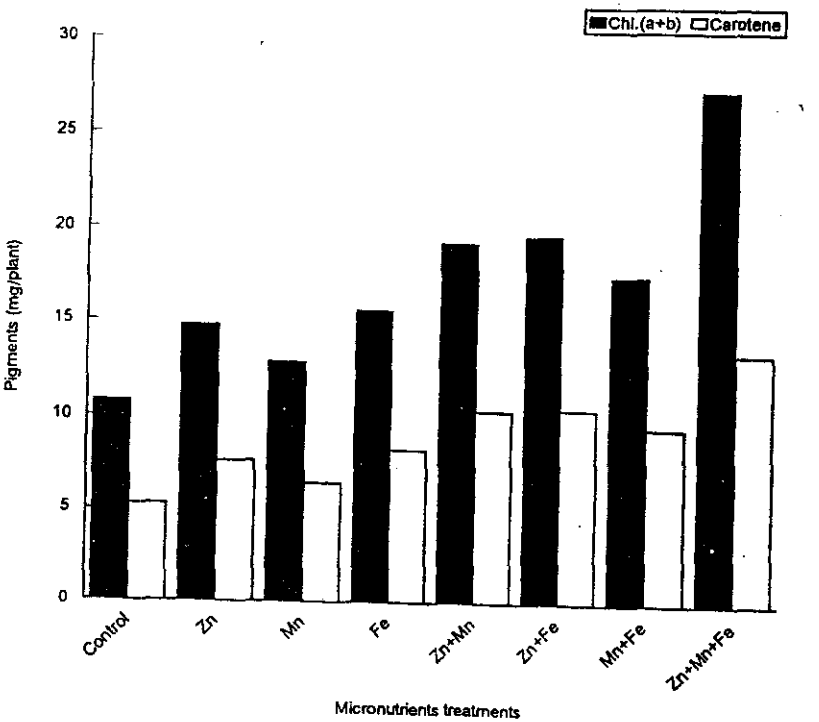

Fig.1. Effect of investigated micronutrients treatments on the content of photosynthetic pigments (mg/plant) of Faba-bean leaves at 70 days age (mean values of two seasons).

2-Effect on micronutrients content in leaves

Regarding the effect of the micronutrients treatments under investigation on the 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.

Generally, all treatments containing any element had a superior effect on increasing its presence in leaves. In this respect, triple treatment gave the highest increase 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 showed that the addition of Zn, Mn and or Fe promotes the uptake of other nutrients. Baza *et al.* (1989) on Faba-bean and Nassar (1996) on wheat also observed the positive effect of Zn on Fe and Mn uptake. The enhancing effect of

TABLE 3. Effect of investigated micronutrients treatments on micronutrients content (mg/plant) of Faba- bean leaves at 70 days age.

Treatments	Zn			Mn			Fe		
	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

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 pigents of leaves

Figure 3 reveals that strong relations exist between the content of both micronutrients and photosynthetic pigments of Faba-bean leaves at 70 days age under all investigated micronutrients treatments, in 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:

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

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

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

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

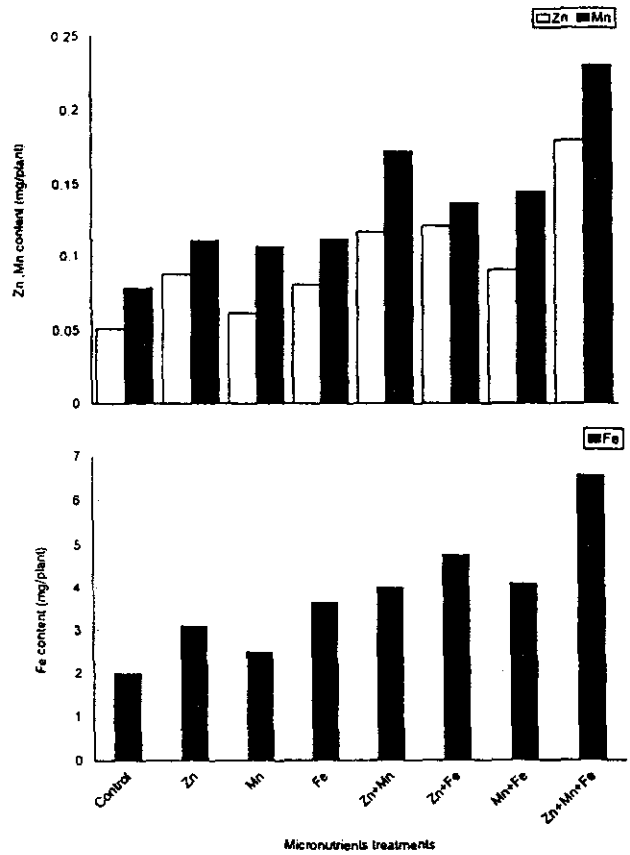

Fig. 2. Effect of investigated micronutrients treatments on micronutrients content in Faba-bean leaves (mg/plant) at 70 days age (mean values of two seasons).

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:

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

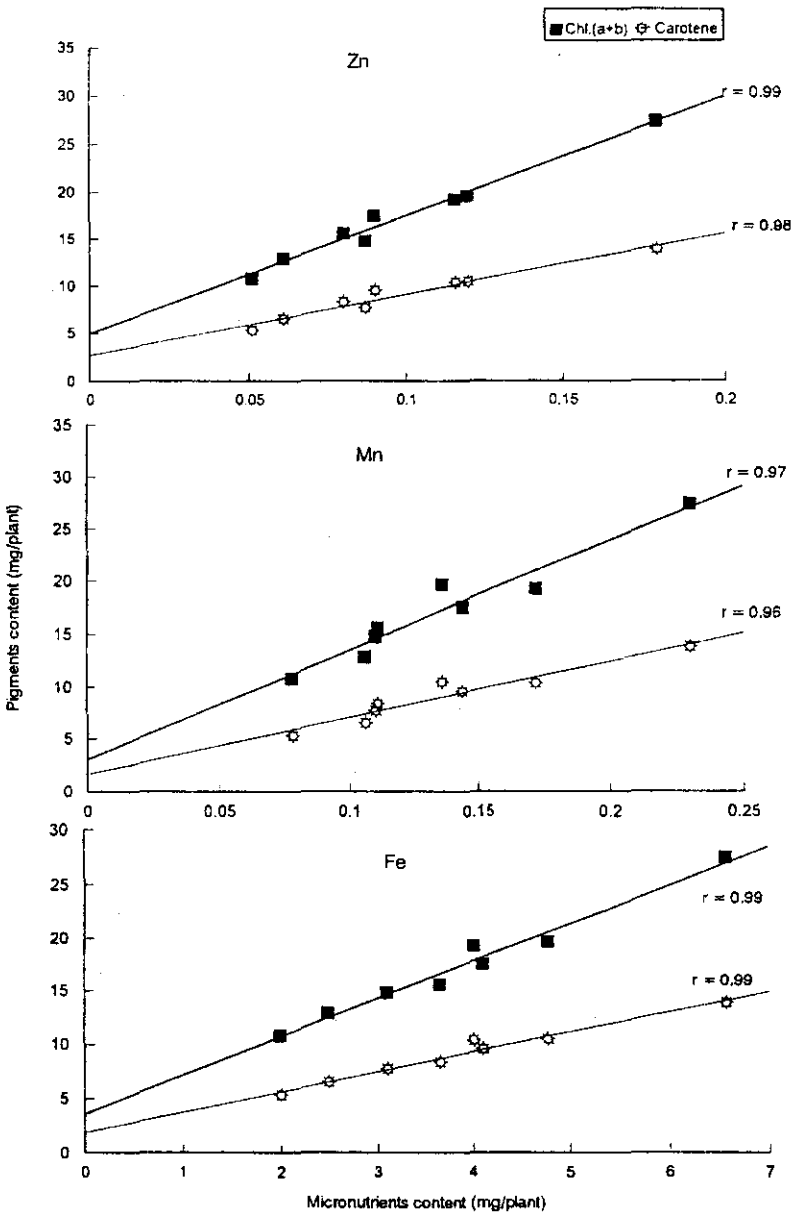

Fig. 3. Photosynthetic pigments content in Faba-bean leaves at 70 days age (mg/plant), as affected by micronutrients content under different treatments of micronutrients (mean values of two seasons).

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

The above-mentioned strong relations between the contents of both photosynthetic pigments and micronutrients of Faba- bean leaves can be explained, as aforementioned, on the basis of the important roles of these elements in the regulation of chlorophyll biosynthesis. Zn affects photochemical reduction, chloroplast structure, photochemical electron transfer and net photosynthesis (Romheld and Machner, 1991). These authors also added that Mn is for the stability of the chloroplast structure. It protects the photosynthetic apparatus against the deleterious action of oxygen radicals and it plays an essential role in the photosynthetic evolution of O_2 and incorporation of CO_2 in chloroplasts.

4- *Effect on plant growth*

The number of branches and leaves per plant and the plant height, as well as the dry matter yield of various organs, were used to express the growth of Faba-bean plants at 70 days age. As shown in Tables 4,5 and illustrated by Fig.4, it can be observed that all aforementioned plant growth characteristics were significantly affected by the different micronutrients treatments. Triple treatment (Zn + Mn + Fe) showed the highest increments, compared with the other treatments , whereas the individual treatments gave the lowest 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 on 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 for its ability to undergo oxidation-reduction reactions and to form a component of chlorophyll (Fig. 1 and 3). Zn also plays an essential role in the synthetase and metabolism of tryptophane. Mn influences directly the indole acetic acid balance in plants, responsible for plant height (Moore and Patrick, 1988).

From the aforementioned results, the enhancing effect of trace elements on dry matter yield of various Faba - bean organs can be attributed to their effective physiological roles in increasing the values of plant growth characteristics (Table 4) and increasing the content of photosynthetic pigments of Faba-bean leaves (Table 2 and Fig. 1 and 3). In turn this encourages the plant to convert light energy to metabolities and consequently, to increase the corresponding values of dry matter yield of Faba-bean plants (Table 5 and Fig.4). These results are in

TABLE 4. Effect of investigated micronutrients treatments on some plant growth characters of Faba- bean leaves 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	2 nd season	Mean	1 st 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

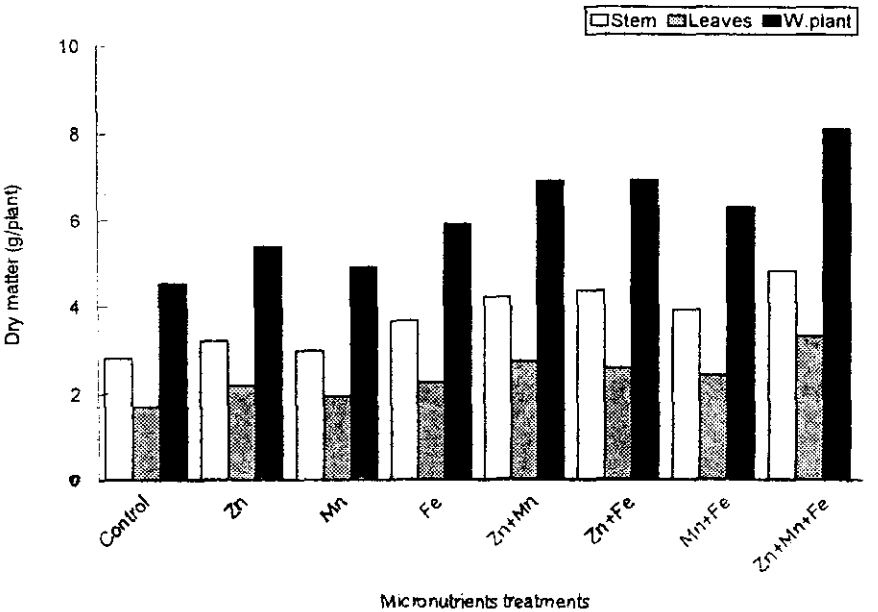

Fig. 4. Effect of investigated micronutrients treatments on dry matter of various organs of Faba-bean plants (g/plant) at 70 days age (mean values of two seasons).

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 leads to two important conclusions. Firstly, the seed coating with micronutrients enables the plants to grow well and to absorb more nutrients from the soil. Secondly, application of Zn, Mn and Fe simultaneously attains a suitable balance between them, which is required to obtain the best growing, strong and healthy plants in comparison with application of these elements individually or in dual mixtures.

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تأثير تغليف البذور ببعض العناصر الصغرى على الفول البلدي. ١- التأثير على نمو النبات ومحتواه من صبغات التمثيل الضوئى والعناصر الصغرى

كرم السيد نصار ، صلاح عبد المجيد رضوان *وعادل عبده رحمو
معهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية - الجيزة. *وكلية الزراعة -جامعة المنوفية - شبين الكوم - مصر.

أجريت تجربتان حقليتان بمحطة البحوث الزراعية بالجيزة خلال موسمى النمو ١٩٩٨/١٩٩٩، ١٩٩٩/٢٠٠٠ بغرض دراسة تأثير تغليف بذور الفول البلدى بعناصر الزنك والمنجنيز والحديد على نمو النباتات ومحتوى الأوراق من صبغات التمثيل الضوئى والعناصر الصغرى مع الأخذ في الإعتبار العلاقات بين محتوى الأوراق من كل من صبغات التمثيل الضوئى والعناصر الغذائية الصغرى.

غلفت بذور الفول البلدى قبل الزراعة بالعناصر الصغرى (الزنك والمنجنيز والحديد) فى صورها المخلبية (بمعدل ٠,٣ جم عنصر /كجم بذور فى حالتى الزنك والحديد وبمعدل ١,٥ جم منجنيز/ كجم بذور) وقد أضيفت هذه العناصر فى صور منفردة أو فى صورة مخاليط ثنائية أو ثلاثية.

وقد أظهر متوسط النتائج لكلا موسمى النمو تحسن مقاييس نمو النبات بصفة عامة (ارتفاع النبات، عدد الأوراق والفروع/ نبات) وكذلك الأوزان الجافة لأجزاء النبات المختلفة عند عمر ٧٠ يوما (الساق، الأوراق) نتيجة لإضافة معاملات العناصر الصغرى. علاوة على ذلك فقد أدت إضافة العناصر الصغرى عند معاملاتها المختلفة إلى الزيادة المعنوية لمحتوى الأوراق من صبغات التمثيل الضوئى والعناصر الصغرى وتجدر الإشارة إلى أن إضافة العناصر الصغرى الثلاث معا (زنك + منجنيز + حديد) أدت إلى الحصول على أعلى القيم بالنسبة لجميع مقاييس النمو السابقة وكذلك محتوي الأوراق من صبغات التمثيل الضوئي والعناصر الصغرى يليها معاملات المخاليط الثنائية للعناصر الصغرى بينما أدت إضافة هذه العناصر بصورها المنفردة إلى الحصول على أقل الزيادات بالمقارنة بمعاملة الكنترول.

وقد أشارت نتائج هذا البحث إلى العلاقة الوثيقة لمحتوى الاوراق من صبغات التمثيل الضوئى ومحتواها من العناصر الصغرى المختلفة وسجلت معاملات ارتباط قوية كان أعلاها مع الحديد ثم مع الزنك ثم المنجنيز ... ويمكن التعبير عن العلاقات السابق الإشارة إليها بمعادلات الانحدار المركبة الآتية.

الكلورفيل (أ+ب) = ٣٧و٣ + ١٨و٣٨ زنك ** + ٤٢و٢٨ منجنيز ** +٦٣و١ حديد ** (معامل الإرتباط = ٩٩٩ ر.)

الكاروتين = ٦١و١+٨٢و٣ زنك ** + ٣٥و١٧ منجنيز + ٢١و١ حديد* (معامل الإرتباط =٩٨٩ ر.)

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