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YIELD AND SOME SEED CONSTITUENTS OF PEA AS AFFECTED BY SOME MICRONUTRIENTS APPLICATION METHODS

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Abstract

Under green-house conditions, an experiment was carried out at the Agricultural Research Centre, Giza on calcareous soil to evaluate the effect of Fe, Zn, Mn, Mo as well as their mixture applied by either seed coating or foliar application on pea yield and seed quality. The obtained results showed that straw and seed yields and 100-seed weight as well as seed content of total soluble sugars, total soluble amino acids, and crude protein were increased to different extents by micronutrients treatments. The mixture of these micronutrients induced the highest values. Seed coating exerts beneficial effects compared to foliar application. Seed coating with Mn and Mo gave the highest protein content, they also gave remarkable increases in the content of the total and individual amino acids of seed protein, comperced with. Fe or Zn and the mixture of the four elements except the amino acid proline which was found to be decreased by different micronutrients treatments. Moreover, seed coating with Mn and Mo recorded more increases than the foliar application of the same two elements. Fe, Zn and Mn content of pea straw and seeds were greatly increased by the application of these micronutrients either individually or in mixtures.

INTRODUCTION

Pea (*Pisum sativum L.*) is one of the important winter pulses in Egypt because of its protein nutrivitive value that reaches in seeds to about 22% on dry weight basis. It is also one of the legume crops that do well in newly reclaimed sandy and calcareous soils which are potential soils for future expansion. Attention has been paid to improve the total production and quality of pea by improving the agronomic practices in such soils such as micronutrients application which is an important factor to impove seed quality. The effeciencies of micronutrient fertilization are mainly dependent on the method of application and source of micronutrient fertilizer.

In a pot experiment, Gangwar and Singh (1986) found that application of Zn to lentil plants either as seed coating of ZnO or as foliar spray of $ZnSO_4$ resulted in increasing seed yield/plant and seed yield/pot. Also, seed yield of pea was found to be increased significantly by zinc application of 10 and 20 kg $ZnSO_4$/ ha (Singh *et al.*, 1988). Kovalevich (1991) on a sandy soil low in available Mo cocluded that optimum Mo rate was 0.5 kg/ha for pea grown for food or fodder and 1.0 kg/ha for those grown for seed. Fawzi *et al.* (1993) showed in a pot experiment that seed yield/ plant of pea and cowpea was increased with foliar application of Fe and Mo or their combinations.

In a field experiment by Gangwar and Singh (1994) on a sandy loam soil (containing 0.72-0.85 ppm DTPA-extractable Zn) lentils received Zn through seed coating (as ZnO) or soil or foliar application as $ZnSO_4$. seed yields from the three application methods were in the order: seed coating> foliar application> soil application. The highest seed yield of 2.40 t/ha was obtained from coating seeds with 0.1% ZnO.

Different organs of broad bean plants showed significant increases in carbohydrate content due to Zn applied either spraying with 0.03% Zn-EDTA or soil application at 0.5 kg Zn-EDTA/fed, (Abdel-Aziz *et al.*, 1987). Foliar spraying of 20 ppm Mn on cowpea also increased total carbohydrate and total soluble sugar content of seeds (El-Mansi *et al.*, 1994).

The qualititative composition of amino acids in the total soluble proteins of pea leaves was not affected by supplementary Mo, but the percentage of some essential amino acids and those active in N metabolism were increased (Lyukova, 1972). Crude protein of pea seeds was increased by Mo application (Jasinska and Kotecki, 1991, and Kovalevich, 1991).

The objective of this study was to evaluate the yield and some biochemical seed components of pea plants grown on calcareous soil as affected by single application of Fe, Zn, Mn and Mo and their mixture either by seed coating method or by foliar application.

MATERIALS AND METHODS

A green-house experiment was carried out in the Plant Nutrition Section, Soil, Water and Environment Research Institute, Agricultural Research Centre (ARC). Pea seeds cultivar victory freezer were sown in pots containing 8 kg of calcareous soil obtained from Nobaria Research Station, ARC. Chemical analysis of the experimental soil is in Table 1.

Table 1. Chemical analysis of the experimental soil.

pH (1:2.5, soil : water)	:	8.1
EC (1:5, soil: water)	:	1.04 (mmhos/cm)
CaCO3	:	31 %
Available P (Olsen extract)	:	7.2 ppm
Available K (NH_4 - acet. ext.)	:	230 ppm
Available N (K_2SO_4 extract)	:	70 ppm
DTPA - extractable,		
Fe	:	3.60 ppm
Zn	:	1.18 ppm
Mn	:	4.20 ppm

Before sowing, each pot received 23 g Ca-superphosphate (15% P_2O_5), 10g K-sulphate (48% K_2O) and 8.5 g ammonium sulphate (20.6% N). The plants were thinned after two weeks of sowing to 4 plants/pot. Each pot received two additional equal doses of ammonium sulphate (8.5 g/pot) after thinning and two weeks later. Pots were regularly watered weekly at the field capacity of the soil.

The experimental pots were divided into two groups one for seed coating and the second for foliar spray treatments. Coating pea seeds with the micronutrients included 6 treatments as follows using Triton-B as a spreader starker:

(1) 6.6 g Fe-EDDHA (6% Fe) / kg seeds.

(2) 2.8 g Zn-EDTA (14% Zn) /Kg Seeds.

(3) 3.1 g Mn-EDTA (13 % Mn) / kg seeds.

(4) 0.04 g molybdic acid/kg seeds.

(5) Mixture of 6.6 g Fe-EDDHA + 1.4 g Zn-EDTA + 1.5 Mn-EDTA + 0.04 g molybdic acid/kg seeds.

(6) Control : Seeds were pre-treated with Triton-B.

The plants of the second group received two sprays of the following micronutrients, 30 and 45 days after sowing using Triton-B as a surfactant (0.5 ml/l):

(1) Fe-EDDHA (6%Fe) at the concentration of 0.5 g/L.

(2) Zn-EDTA (14% Zn) at the concentration of 0.5 g/L.

(3) Mn-EDTA (13% Zn) at the concentration of 0.5 g/L.

(4) Molybdic acid at the concentration of 0.01 g/L.

(5) Mixture of (0.21 g Fe-EDDHA + 0.14 g Zn-EDTA + 0.14 g Mn - EDTA + 0.01 g Molybdic acid) /L.

(6) Control : 0.5 ml Triton-B/L.

Split-plot design with 4 replications was used. The main plots were for micronutrients, while the sub-plots were for methods of application. The statistical analysis was carried out according to the method of Snedecor and Cochran (1967).

Total nitrogen of pea seeds was determined in the digested solution by micro-Kjeldahl method (A.O.A.C., 1970) and the crude protein was obtained by multiplying the percentage of total N by 6.25. Total free amino acid content of pea seeds was detrmined in the ethanolic extract according to Rosen (1957) and total soluble sugar was determined in the same extract by the picric acid reduction method according to Thomas and Dutcher (1924).

The pea seed meals were hydrolyzed and their contents of amino acids were determined by amino acid analyzer (High performance A.A.A. Beckman 7300). One complete analysis took approximately one hour by using Na-nigh performance 25-cm column.

At harvest, pea straw and dry riped seeds were oven dried at 70°C for 24 hours. Seed and straw yielods/pot and 100-seed weight were recorded.

RESULTS AND DISCUSSION

Seed and straw yields :

The results given in Table 2 show that seed and straw yields of pea were significantly increased by micronutrients applications. The increament percentages of the seed yield were found to be 3.6, 12.5, 13.8, 26.9 and 35.7 while they were

Table 2. Effect of some micronutrient application on pea yield and seed contents of soluble sugars (as mg glucose/g dry weight), amino acids (as mg lucine/ g dry weight) and protein.

Treatments		Yield (g/pot)		100-seed wt. (g)	Total soluble sugars	Total soluble amino acids	Crude protein (%)
Micronutrient (A)	Method of application	Straw	Seed				
Control	Coating	11.46	16.89	18.81	61.33	1.03	16.88
	Spraying	13.27	15.47	20.95	60.75	1.15	17.29
	Mean	12.36	16.18	19.88	61.04	1.09	17.09
Fe	Coating	13.34	16.92	19.76	90.83	1.35	19.69
	Spraying	17.18	16.62	22.58	69.33	1.60	18.96
	Mean	15.26	16.77	21.17	80.08	1.47	19.33
Zn	Coating	16.23	16.58	20.28	95.80	1.62	19.69
	Spraying	15.80	19.83	24.70	67.17	2.84	19.48
	Mean	16.02	18.21	22.49	81.49	2.23	19.59
Mn	Coating	14.57	19.02	25.06	88.67	2.41	22.81
	Spraying	14.33	17.82	26.12	64.00	2.22	16.88
	Mean	14.45	18.42	25.59	76.34	2.32	19.85
Mo	Coating	17.63	20.69	24.00	85.58	3.13	22.19
	Spraying	15.17	20.37	25.95	65.08	2.69	16.67
	Mean	16.40	20.53	24.98	75.33	2.91	19.43
Mixture	Coating	18.39	22..86	26.75	98.33	3.60	19.69
	Spraying	17.69	21.03	24.82	71.00	3.33	20.52
	Mean	18.04	21.95	25.79	84.67	3.47	20.11
Means of application method (B)	Coating	15.27	18.82	22.44	86.76	2.02	20.16
	Spraying	15.27	18.52	24.19	66.22	2.31	18.30
LSD at 5 % for:							
Micronutrient (A)		1.74	2.00	1.16	4.17	0.31	1.77
Method of application (B)		NS	NS	0.67	2.41	NS	1.02
(A) x (B)		2.46	3.87	1.64	5.90	0.43	2.51

26.4, 29.6, 16.9, 32.7 and 45.9 for the straw yield over the control treatment due to Fe, Zn, Mn, Mo and their mixture treatments, respectively. This increase might be due to the divergence in soil fertility status, especially for available micronutrients under study. Similar results were obtained by many investigators such as gangwar and Singh (1986), Kovalevich (1991), Kotecki (1991) and Fawzi *et al.* (1993). The results also show that application of Mn and Mo and micronutrients mixture (MM) with seed coating method was effective as regard to pea production on calcareous soil. This may be in part due to that seed coating method was an effective method as regard to pea production on calcareous soil. This may be in part due to that seed coating creates a nutrition environment in the immediate vicinity of the germinating seed and this provides a "boost" for the seedling in its critical early stage of development, which is particularly important under the types of stress conditions found in many land reclamation projects.

100-Seed weight :

Micronutrients treatments gave significant increases in 100-seed weight by 6.5, 13.1, 28.7, 25.6 and 27.7% compared to the control for Fe, Zn, Mn, Mo and their mixture, respectively (Table 2). These data might indicate that application of these elements produced pea seeds with better quality due to the physiological and biochemical effects of these elements on pea growth and yield quality. It was also observed that seed coating with micronutrient mixture recorded the highest 100-seed weight (26.75 g) as shown in Table 2.

Seed content of total soluble sugars :

The results in Table 2 show that total soluble sugars content of pea seeds was greatly affected by micronutrients application. Application of Fe, Zn, Mn, Mo and their mixture significantly increased seed content of total soluble sugars by 31.2, 33.5, 25.1, 23.4 and 38.7%, respectively. These results are in agreement with those obtained by Malewar *et al.* (1990) on mung bean and El-Mansi *et al.* (1994) on cowpea. Meanwhile, seed coating method gave pronounced effect on total soluble sugars of pea seeds compared with the spraying method. Seed coating with the MM under study gave the highest significant content (98.33 mg/g dry weight). These results could be due to the important functions of these elements in plant metabolism and activation of different enzymes of carbohydrate metabolism.

Seed content of total soluble amino acids :

Significant increases in seed content of free amino acids were detected by micronutrient applications as shown in Table 2. The highest content of soluble amino acids was obtained by the MM (3.47 mg/g dry weight) followed by Mo (2.91 mg/g dry weight), Mn (2.32 mg/g dry weight) and Zn (2.23 mg/g dry weight) while Fe treatment recorded the lowest content (1.47 mg/g dry weight). These effects could be due to that the micronutrients under investigation function as parts or cofactors for the enzymes of amino acid synthesis, e.g. transaminase and protein metabolism. It is notable to mention that seed coating with MM recorded the highest content of soluble amino acids (3.6 mg/g dry weight) in pea seeds as compared to other interaction treatments. Similar results were obtained by Nikolov (1974) with Mo in bean leaves.

Seed protein :

Application of Fe, Zn, Mn and Mo individually increased protein content by 13.1 -16.1% over the control while the micronutrient mixture gave higher increase by 17.7% as shown in Table 2. This may be due to that the metabolic active iron compounds in plants are enzymes, containing heme and non-heme iron proteins which take part in oxidation-reduction reactions of photosynthesis, respiration, ribo mucilic acid (RNA) and protein metabolism. Also, Mn plays an important role in protein synthesis by binding amino acid to peptides (Aberger, 1974). Moreover, Mn is an activator of some peptidases, arginase, pyrovate carboxylase. Zn also has functions as part or cofactor for a lot of enzymes such as carbonic anhydrase, dehydrogenase hexokinase peptidases. Moreover, it is necessary for RNA- and protein-synthesis, as they control auxine metabolism via tryptophane and blocks utilization (Vallee and Wacker, 1973).

Regarding methods of application, it was observed that, seed coating method was supperior to foliar application method since seed protein content was increased significantly by 10.2% over the foliar application method. It was also noticed that seed coating with Mn or Mo gave the highest protein content (22.81 and 22.19%, respectively) compared to the other treatments. Similar results were obtained by Kovalevich (1991) with Mo, and Malewar *et al.* (1990) with zinc.

The amino acids of seed protein :

According to the importance of the pea protein in nutrition, a special interest has been directed to study the effect of Fe, Zn, Mn and Mo as well as their mixture on amino acid content of pea seeds. The results in Table 3 illustrate that micronutrient applications increased, to different extents, most of the detected amino acids, except the amino acid protein which was reduced. The most affected amino acids were isolucine, valine, arginine, glutamic acids, leucine, phenylalanine and aspartic acids. It is interesting to note that both Mn and Mo either by seed coating or foliar application gave more prononuced increases in the contents of the individual amino acids than Fe and Zn, and even than the micronutrients mixture. This could be attributed to that, in the amino acid synthesis, the transamination is catalysed by an amino-transferase which is attached to pyridoxyl phosphate in presence of Mn^{2+} ions (Amberger, 1974). Also the reduction of the nitrate after having been taken up by plants to nitrite is catalysed by the enzyme nitrate reductase which contains Mo (Amberger, 1974). For the final reduction step (NH_3 formation) Mn ions are necessary. It was also noticed that seed coating was superior to foliar application and seed coating with Mn and Mo recorded more increases. On the other hand, the amino acid proline was decreased by micronutrients application as shown in Table 3.

The total amino acids content of pea seeds was increased by 27.8, 28.4, 41.7, 40.7 and 27.9% over the control due to seed coating with Fe, Zn, Mn, Mo and their mixture, respectively, while less increases (17.4, 28.2, 28.9 and 23.4%) were detected for foliar application of Zn, Mn, Mo and the micronutrients mixture, respectively. As for foliar application of Fe, no effect was detected.

Seed and straw content of Fe, Zn and Mn :

The results in Table 4 show clearly that Fe content of pea straw and seeds were greatly increased by Fe and micronutrients mixture treatments. Application of Zn recorded the least value of Fe in seeds while Mn treatment gave the least value of Fe in straw. This may be due to the antagonistic effects between Zn and Fe and between Mn and Fe (Tandon, 1989). It is also observed that seed coating with Fe and the micronutrients mixture gave the highest Fe values.

Zince content of pea plants was markedly increased by Zn and the mixture of micronutrients. Mn and Fe applications had less effect on Zn content of pea plants which may be due to the antagonistic effect between Mn and Zn and between Fe and Zn (Tandon, 1989). There was no significant effect of the method of application of micronutrients, however, seed coating with Zn or the MM recorded the highest Zn content of pea plants.

It is also evident from Table 4 that Mn and the MM applications induced marked increases in Mn contents of pea palnts. On the other hand, Mn content was less or not affected by the other micronutrients which may be due to the antagonistic effects.

Table 3. Amino acids content (%) of pea seed protein as affected by micronutrient application.

Amino acid	Control	Coating treatments					Spraying treatments				
		Fe	Zn	Mn	Mo	Mixture	Fe	Zn	Mn	Mo	Mixture
Aspartic acid	1.93	2.57	2.79	3.20	3.10	2.85	1.69	2.31	2.87	3.02	2.43
Threonine	0.76	0.94	0.99	1.12	1.07	1.00	0.64	0.83	0.98	1.03	0.90
Serine	0.91	1.00	1.06	1.16	1.14	1.11	0.69	0.92	1.11	1.03	0.96
Glutamic acid	3.04	4.30	4.47	4.98	4.92	4.51	3.34	3.98	4.44	4.49	4.12
Proline	1.33	0.92	0.83	0.90	0.88	0.82	0.80	0.83	0.82	0.83	0.90
Glycine	0.86	1.00	1.06	1.15	1.12	1.04	0.83	0.90	1.04	1.06	0.95
Alanine	0.92	1.10	1.17	1.24	1.30	1.15	0.90	1.00	1.18	1.18	1.04
Valine	0.74	1.14	1.12	1.25	1.25	1.14	0.95	1.03	1.16	1.17	1.08
Isoleucine	0.61	1.10	1.03	1.10	1.12	1.02	0.87	0.96	1.02	1.04	1.00
Leucine	1.32	1.80	1.76	1.87	1.91	1.73	1.47	1.62	1.73	1.75	1.70
Tyrosine	0.66	0.75	0.72	0.79	0.85	0.73	0.62	0.67	0.69	0.70	0.69
Phenylalanine	0.83	1.12	1.05	1.13	1.17	1.05	0.93	1.01	1.04	1.06	1.06
Histidine	0.40	0.51	0.51	0.57	0.54	0.50	0.45	0.47	0.50	0.50	0.49
Lysine	0.27	1.44	1.10	1.20	1.17	1.05	1.29	1.50	1.10	1.07	1.37
Arginine	1.05	1.57	1.69	1.90	1.86	1.56	1.19	1.49	1.66	1.50	1.48
Total	16.63	21.26	21.53	23.56	23.40	21.27	16.66	19.52	21.32	21.43	20.17

Table 4. Effect of some micronutrient application on seed contents of Fe, Zn and Mn (ppm).

Treatments		Fe		Zn		Mn	
Micronutrient (A)	Method of application	Straw	Seed	Straw	Seed	Straw	Seed
Control	Coating	53.33	116.67	20.00	13.33	20.00	30.00
	Spraying	56.67	100.00	23.33	10.00	23.33	30.00
	Mean	55.00	108.34	21.67	11.67	21.67	30.00
Fe	Coating	93.33	160.00	26.67	23.33	20.00	36.67
	Spraying	88.33	143.33	23.33	33.33	20.00	53.33
	Mean	83.33	151.67	25.00	28.33	20.00	45.00
Zn	Coating	73.33	130.00	46.67	40.00	23.33	60.00
	Spraying	70.00	113.33	43.33	50.00	30.00	40.00
	Mean	71.67	121.67	45.00	45.00	26.67	50.00
Mn	Coating	60.00	143.33	36.70	33.33	36.67	66.67
	Spraying	56.67	133.33	33.33	33.33	33.67	76.67
	Mean	58.34	138.33	35.02	33.33	35.17	71.67
Mo	Coating	66.67	136.67	40.00	40.00	20.00	53.33
	Spraying	56.67	120.00	36.67	36.67	26.67	46.67
	Mean	61.67	128.34	38.34	38.34	23.34	50.00
Mixture	Coating	96.67	166.67	50.00	56.67	40.00	73.33
	Spraying	86.67	160.00	53.33	53.33	33.33	63.33
	Mean	91.67	163.33	51.67	55.00	36.67	68.33
Means of application method (B)	Coating	73.89	142.22	36.67	34.44	53.33	53.33
	Spraying	68.34	128.33	35.55	36.11	27.80	51.67
LSD at 5 % for:							
Micronutrient (A)		7.94	9.93	5.96	6.59	5.96	6.59
Method of application (B)		4.59	5.73	NS	NS	3.44	NS
(A) x (B)		11.23	14.00	8.43	9.32	8.43	9.32

Similar results were obtained by Wallace and Alexander (1973), Shuman *et al.* (1979), and Konno and Foy (1983).

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محصول البسلة وبعض مكونات البذور ومدى تأثرها بطريقة اضافة بعض المغذيات الصغرى

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٢ كلية الزراعة – جامعة القاهرة – الجيزة .

أقيمت تجربة أصص فى مركز البحوث الزراعية بالجيزة على الاراضى الجيرية لتقييم تأثير كل من الحديد والزنك والمنجنيز والموليبدنم بالاضافة الى مخلوط هذه العناصر، المضافة اما بطريقة تغليف البذور أو الرش على المجموع الخضرى، على محصول البسلة وصفات البذورة.

وقد أظهرت النتائج المتحصل عليها كل من البذور والقش ووزن ١٠٠ بذرة بالاضافة الى محتوى البذور من السكريات الذائبة الكلية والاحماض الامينية الذائبة الكلية والبروتين الخام قد ازدادت بدرجات مختلفة باضافة المغذيات الصغرى. وقد اعطى مخلوط هذه العناصر أعلى القيم. هذا وقد أعطت طريقة اضافة العناصر عن طريق تغليف البذور تأثيرات افضل بالمقارنة بطريقة الاضافة بالرش على المجموع الخضرى.

كما أعطت معاملات تغليف البذور بالمنجنيز والموليبدنم أعلى نسبة لبروتين البذور ومن ناحية اخرى فان عناصر المنجنير والموليبدنم أعطت زيادة ملحوظة فى الاحماض الامينية المنفردة فى بروتين البذور أكثر مما اعطت عناصر الحديد والزنك وكذلك مخلوط العناصر الاربعة، فيما عدا الحمض الامينى برولين الذى كان يقل باضافة المغذيات الصغرى المختلفة. زيادة على ذلك فان اضافة المنجنيز والموليندم بطريقة تغليف البذور أعطت زيادة اكثر من الاضافة بطريقة الرش. كما ازداد محتوى البذور من الاحماض الامينية الكلية بإضافة المغذيات الصغرى، واعطى كل من المنجنيز والموليبدنم زيادة ملحوظة ومع ذلك فقد أعطى رش المجموع الخضرى زيادة اقل. كذلك ازداد محتوى البذور والقش من الحديد والزنك والمنجنيز كثيرا باضافة العنصر المناظر ومخلوط هذه العناصر.