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Impact Spraying of Some Microelements on Growth, Yield, Nitrogenase Activity and Anatomical Features of Cowpea Plants

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Abstract: This experiment was carried out at the Experimental Farm of El-Kassasin Horticultural Research Station (30°11 N, 31°18 E), Ismailia Governorate, during two summer seasons of 2011 and 2012, to study the effect of foliar spray with mixture of Fe, Zn, Mn, Mo and B at different concentrations (T0 = control (sprayed tap water), T1= mixture of Fe, Zn, Mn, Mo and B at rates of 50, 50, 50, 5 and 5 ppm, respectively, T2= mixture of Fe, Zn, Mn, Mo and B at rates of 100, 100, 100, 10 and 10 ppm, respectively, T3= mixture of Fe, Zn, Mn, Mo and B at rates of 150, 150, 150, 15 and 15 ppm, respectively, T4= mixture of Fe, Zn, Mn, Mo and B at rates of 200, 200, 200, 20 and 20 ppm, respectively, T5= mixture of Fe, Zn, Mn, Mo and B at rates of 250, 250, 250, 25 and 25 ppm, respectively and T6= mixture of Fe, Zn, Mn, Mo and B at rates of 300, 300, 300, 30 and 30 ppm, respectively) on growth, yield, chemical constituents and anatomical feature of cowpea (*Vigna unguiculata* (L.) Walp.) cv. Cream 7 under sandy soil conditions. It is obvious that growth, yield, chemical constituents were promoted with all mixtures of microelements as compared to the control. Growth, yield, chemical constituents were significantly increased gradually with increasing microelements rates from T0 up to T3. Meanwhile, there was no significant difference between T3, T4 and T5 and then reduced with increasing rates (T5 and T6). Foliar spray of cowpea plants with mixture of some microelements (T3) caused improving in microscopical counts and measurements of certain histological characters of stem and leaflet blade tissues.

Key words: Cowpea · Microelements · Growth · Yield · Nitrogenase Activity · Anatomical features

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is considered one of the most vegetable legumes. The pods were harvested either at green stage for fresh market or at mature stage for dry seeds. Cowpea seeds are a nutrition component in the human diet as well as a nutritious livestock feed. The protein in cowpea seeds is rich in lysine and tryptophan amino acids compared to cereal grains. The cultivated area of cowpea in Egypt was about 8381 fed. in 2011, yielding about 1.088 ton [1].

It is well known that, sandy soil is infertile and has very small amounts of microelements and high in soil pH. Micronutrients deficiencies are more frequent in many soil types; i.e. alkaline, calcareous and sandy soils [2]. The availability and uptake of micronutrients by plants decrease with increasing soil pH [3]. The mobility of microelements in soil, plant and their translocation to plants as well as interaction among them selves or with microelements in the soil and plant play an important role in plants nutrition [4]. The deficiency of microelements that recognized as essential to higher plants impaired the important metabolic functions are impaired and resulted in poor growth and yield of crops [2].

Micronutrients have considerable significant effects, as limiting factors, on the productivity of legumes; spraying cowpea plants with Zn + Mn + Fe at 100 ppm of each increased dry matter, yield/plant and number of pods/plant. Also foliar spray with Fe, Mn and Zn at 200 + 100 + 100 ppm individually or together in mixture solution significantly supported the measured growth characters of garlic plants [5].

Foliar spray with Zn + Mn + Fe at 100, 100 and 200 mg/l. respectively as sulphate salts increased dry weight/plant, yield/plant as well as yield/fed. and NP contents in garlic cloves [5]. Spraying pea plants with

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Mn + Zn + Fe at rates 0.25, 0.25 and 0.50 g/l. respectively, increased NPK contents in leaves and green pod yield and at 0.5, 0.5 and 1 g/l. respectively, increased NPK contents in seeds [6]. Spray of micro-elements such as Fe, Mo and B stimulated the root system parameters and root nodules in legumes [7]. Also, spraying pea plants with Fe, Mo and B at 100, 50 and 25 ppm, respectively, significantly increased vegetative growth, dry weight, mineral content and uptake of N,P and K as well as total yield compared with the control [8,9,5]. Microelements as Fe and Mo play a vital role in synthesis of chlorophyll and chloroplast formation. Iron, molybdenum and boron play a vital role of enzymes activity as nitrogenase, catalase and peroxidase. Molybdenum also, is very important for N_2- fixation [4]. Mohamed and Saif El-Yazal [10] using Fe + Zn + Mn at concentration of 0.08% on cotton plants, Ali *et al.* [11] using zinc at concentrations of 100 and 200 ppm on *Vicia faba* L. plants and El-Tantawy and Eisa [12] using boron at a concentration of 25 ppm on table beet plants, recorded favorable anatomical changes in leaf and stem anatomy due to the effect of micronutrients mixture. Therefore, the aim of this work was to study the effect of mixture of some micro-elements such as Fe, Zn, Mn, Mo and B on cowpea plants under sandy soil conditions.

MATERIALS AND METHODS

The present investigation was conducted at the Experimental Farm of El-Kassasin Horticultural Research Station (30°11 N, 31°18 E), Ismailia Governorate, during two summer seasons of 2011 and 2012, to study the effect of foliar spray with mixture of Fe, Zn, Mn, Mo and B on growth, yield, chemical constituents and anatomical feature of cowpea (*Vigna unguiculata* (L.) Walp.) cv. Cream 7 under sandy soil conditions.

Soil samples were randomly taken from the experimental field at 30 cm depth to determine physical and chemical properties before sowing and were analyzed. Standard methods were followed to determine pH, EC, organic matter content, total N and available P and K as described by Jackson [13]. Available Fe, Zn, Mn and Mo were extracted by DTPA (Diethylene triamine penta acetic acid). Available B was extracted by hot water [14]. The abovementioned microelements were determined with atomic absorption apparatus (FAAS Perkin Elmer HGA 4000 programs)

This Experiment Included 7 Treatments as Follows:

To : Control (sprayed tap water).
T1 : Mixture of Fe, Zn, Mn, Mo and B at rates of 50, 50, 50, 5 and 5 ppm, respectively.
T2 : Mixture of Fe, Zn, Mn, Mo and B at rates of 100, 100, 100, 10 and 10 ppm, respectively.
T3 : Mixture of Fe, Zn, Mn, Mo and B at rates of 150, 150, 150, 15 and 15 ppm, respectively.
T4 : Mixture of Fe, Zn, Mn, Mo and B at rates of 200, 200, 200, 20 and 20 ppm, respectively.
T5 : Mixture of Fe, Zn, Mn, Mo and B at rates of 250, 250, 250, 25 and 25 ppm, respectively.
T6 : Mixture of Fe, Zn, Mn, Mo and B at rates of 300, 300, 300, 30 and 30 ppm, respectively.

The sources of Fe, Mn, Zn, Mo and B were ferrous sulphate, manganese sulphate, zinc sulphate, molybdic acid and boric acid, respectively.

Seeds were sown in hills at 15 cm apart on one side of dripper line (2 seeds/hill) on 20^{th} and 25^{th} March in 2011 and 2012 seasons, respectively. Seeds were successive washed and inoculated with root nodule bacteria (*Rhizobium leguminosarum*) at dose of 5 g/kg seeds. Arabic Gum 20% was used as adhesive agent. The inoculated seeds were left in a shade place for one hour before sowing.

The studied treatments were arranged in a randomized complete block design with three replications. The experimental unit area was 24 m^2 which contained 8 rows with 5 m length and 60 cm width for each, the four inner rows were possessed for yield determination, whereas the four outer rows were used in measuring plant growth characters. One row was left between each two experimental plots to avoid the overlapping.

Plants were sprayed three times at 20, 35 and 60 days after sowing. Each experimental unit received 2 litre solutions of each concentration using spreading agent (Super film at 1 cm/l.) to improve adherence of the spray to the plant foliage for increasing absorption by the plants [3]. The untreated plants (check) were sprayed with tap water and spreading agent. The normal agricultural practices of cowpea production under drip irrigation system of this area were followed according to the recommendations of Ministry of Agriculture.

Data Recorded: Samples of ten plants from each experimental unit were randomly taken at 70 days after sowing and the following data were recorded.

Table 1: The physical and chemical properties of the soil.

	Chemical properties					
Properties	pH	E.C. (dsm^{-1})	*OM (%)	Total N %	Available N (ppm)	Available P (ppm)
1st season	8.00	2.21	0.05	0.14	12.86	10.03
2nd season	7.99	2.03	0.07	0.13	12.63	9.91
Properties	Available K (ppm)	Available Zn (ppm)	Available Mn (ppm)	Available Fe (ppm)	Available B (ppm)	Available Mo (ppm)
1st season	38.12	1.93	1.12	3.60	0.28	0.01
2nd season	31.71	1.97	1.98	3.37	0.21	0.01
			Physical properties			
Properties	Sand (%)	Silt (%)	Clay (%)	Texture	----	----
1st season	95.00	3.46	1.54	Sandy	----	----
2nd season	95.69	3.03	1.28	Sandy	----	----

* OM: Organic matter

Plant Growth Parameters
Vegetative Characters:

- Plant height (cm).
- Number of branches/plant.
- Number of leaves/plant.

Root System: The roots of plants were carefully separated by washing sand from them and then placed in a flat glass dish containing a little amount of water. Roots were straightened with forceps, so that they can not overlap and were held in position, according to Helal and Sauerbesk [15] and the following data were recorded per root:

- Main root length (cm).
- Second order of roots number.
- Third order of roots number.
- Number of nodules.

Dry Weight: Different plant organs, i.e. leaves, branches and roots were oven dried at 70°C till constant weight and the following data were recorded:

- Dry weight of leaves/plant (g).
- Dry weight of branches/plant (g).
- Dry weight of roots/plant (g).
- Dry weight of whole plant (g).
- Relative dry weight (calculated based on the dry weight/plant (g) of each treatment in proportion to the control).

Photosynthetic Pigments: Ten disc samples from the fourth upper leaf of the plant tip from each experimental unit were randomly taken after 50 days from sowing, to determine both chlorophyll a and b as well as total chlorophyll (a+b) and carotenoids, according to the method described by Wettestein [16].

Yield and its Components: Dry pods of each plot were harvested and the following parameters were recorded: Average number of seeds/pod, number of pods/plant, weight of seeds/pod (g), seed yield/plant (g), seed yield/fed. (kg) and relative seeds yield/fed. (calculated based on the seed yield (kg)/fed. of each treatment in proportion to the control).

Chemical Contents: Total nitrogen, phosphorus and potassium were determined in dry seeds according to A.O.A.C. [17].

Crude protein was calculated based on total N concentration according to A.O.A.C. [17].

Total Carbohydrates were determined colorimetrically using the method described in A.O.A.C. [17].

Nitrogenase activity as an indicator for N_2-fixation activity in root nodules was assayed, after 70 days from sowing, according to Hardy *et al.* [18] by using Gas Chromatography System (Model Dani 1000). The results are presented as ì moles C_2H_4/ g dry weight of nodules/hr.

Microelements: Iron, manganese, zinc and molybdenum were determined by Atomic Absorption Spectrophotometer (FAAS Perkin Elmer HGA 4000 Programs) as described by Evenhuis and Dewaard [19]. Boron was determined according to the method of Gupta and Stewart [20].

Anatomical Studies: For anatomical studies, specimens of selected treatments of cowpea plants were taken at 90 days after sowing during the second season of 2012, specimens from the middle part of the 5th upper internode of the main stem as well as from the terminal leaflet of the corresponding leaf for examination. These specimens (1 cm long) were killed and fixed for 24 hours at least in plant fixative which is known as FAA (formalin acetic alcohol) represented by the following formula: 50 ml. ethyl alcohol (95%), 5ml. glacial acetic acid, 10 ml. formaldehyde (37- 40%) and 35 ml distilled water. Then the specimens were washed and dehydrated in ascending concentrations of ethyl alcohol series, then cleared in transferring concentrations of xylene and absolute alcohol. Specimens were embedded in pure paraffin wax of melting point (52-54°C). Sections were prepared using EPMA a rotary microtome at 14 microns. Paraffin ribbons were mounted on slides and sections were stained in safranin and light green. Sections were mounted in Canada balsam [21]. Selected sections were examined to detect histological manifestations of the chosen treatments using light microscope (Olympus) with digital camera (Canon power shot S80) connected to computer; the photographs were taken by Zoom Browser Ex Program. The dimensions of sections were measured by using Corel Draw program ver. 11.

Statistical Analysis: The obtained data were subjected to statistical analysis of variance according to Snedecor and Cochran [22] and means separation was done according to LSD at 5 % level of probability.

RESULTS AND DISCUSSIONS

Plant Growth Parameters

Vegetative Characters: Data in Table 2 show the effect of spraying cowpea plants with mixtures of microelements (Fe, Zn, Mn, Mo and B) at different concentrations, viz, T0, T1, T2, T3, T4, T5 and T6 on vegetative growth. It is obvious that vegetative growth was promoted with all mixture of microelements as compared to the control (T0); plant height was significantly increased gradually with increasing mixture of microelements rates from T0 up to T4 and then reduced with increasing rates (T5 and T6). Meanwhile, there was no significance difference between T3, T4 and T5. In the same trend, number of branches and leaves/plant were significantly increased with increasing microelements rates from T0 up to T5 and then reduced with increasing the rates (T6). This high concentration might be toxic and caused inhibition for growth plant.

These results may be due to the effect of these microelements in plant physiology; Fe plays a prominent role in several vital processes in plant such as photosynthesis consequently affecting plant growth; Zn is important for ^{14}C fixed in the primary photosynthetic process [23]. Zn increased photosynthetic efficiency, which was reflected as simulative effect on vegetative growth plant and also zinc is a component of a variety of enzymes such as dehydrogenase, proteinase, peptidase and phosphohydrases (metabolism of carbohydrates, protein and phosphate) and Zn is known to stimulate plant resistance to dry and hot weather [4]. Zn is also well known to be directly involved in biosynthesis of IAA hormone which induces cell division and cell elongation; Mn is involved in the evolution of O_2 in photosynthesis (Hill reaction). It is a component of several enzyme systems. It has also function in chloroplast as a part of electron-transfer (oxidation-reduction) reactions and electron transport system [2]. Mo is a part of nitrate reductase, which is involved in reduction of NO_3 to NH_4 after its absorption by plants. Also, it is structural component of nitrogenase, which involved in nitrogen fixation of N_2 into the ammonium form in a symbiotic relationship with legumes [24,25]. B is involved in transport of sugars and in synthesis of cell wall materials, cell division and differentiation, carbohydrate metabolism, formation and synthesis of protein [4]. Micro-elements as Fe and Mo play a vital role in synthesis of chlorophyll or chloroplast formation [23]. Obtained results were in harmony with those reported by Atia and Bardisi [9], EL-Mansi *et al.* [7] and Abou El-khair *et al.* [5].

Root System: Results given in Table 3 testify the effect of mixtures of microelements (Fe, Zn, Mn, Mo and B) at different concentrations, viz, T0, T1, T2, T3, T4, T5 and T6 on main root length, number of second order, third order of roots and number of nodules. Data showed that all spraying treatments greatly improved the root system of cowpea plants compared with control (T0); main root length and number of nodules were significantly improved with increasing mixture of microelements concentrations from T0 up to T4 and then reduced with increasing concentrations. Meanwhile, there was no significant difference among T2, T3, T4 and T5 in most cases. In the same trend, number of second order and third order of roots were significantly increased with increasing mixture of microelements concentrations from T0 up to T3 and then reduced with increasing the concentrations (T4, T5 and T6). However, the effect of T3, T4, T5 and T6 did not differ in numbers of second order and third order of roots in most cases.

It is well known that B is required for normal development and function of root nodules in legumes via activation of nitrogenase [26]. Molybdenum is an essential element; it is a constituent of the nitrogenase enzyme and every bacteria which fixes nitrogen needs molybdenum during the fixation processes. Molybdenum has a positive effect on yield, quality and nodules forming in legume crops. The functions of molybdenum in leguminous plants include nitrate reduction, nodulation, nitrogen fixation and general metabolism [27]. Also, molybdenum was required for normal plant growth, reduction supply with molybdenum to the growth medium decreased activities of nitrate reductase and glutamine

Table 2: Effect of foliar spray with mixture of microelements on vegetative growth of cowpea plants during the two growing seasons.

	Plant height (cm)		Number of branches		Number of leaves	
Treatments	2011	2012	2011	2012	2011	2012
T0	49.30c	42.23e	5.05c	3.91e	28.60d	25.58f
T1	54.88b	44.18d	5.83b	4.15d	33.15c	28.78e
T2	56.21b	47.23c	6.07ab	4.86c	36.48b	34.40d
T3	58.20ab	52.72b	6.28ab	5.69b	38.94ab	37.58c
T4	59.91a	55.60a	6.33ab	6.14a	40.32a	39.29b
T5	59.08a	55.51a	6.56a	6.30a	41.45a	41.40a
T6	56.78b	53.43b	6.12ab	6.00ab	39.50a	40.76a

T0 = Control

T1 = Mixture of Fe, Zn, Mn, Mo and B at rates 50, 50, 50, 5 and 5 ppm, respectively.

T2 = Mixture of Fe, Zn, Mn, Mo and B at rates 100, 100, 100, 10 and 10 ppm, respectively.

T3 = Mixture of Fe, Zn, Mn, Mo and B at rates 150, 150, 150, 15 and 15 ppm, respectively.

T4 = Mixture of Fe, Zn, Mn, Mo and B at rates 200, 200, 200, 20 and 20 ppm, respectively.

T5 = Mixture of Fe, Zn, Mn, Mo and B at rates 250, 250, 250, 25 and 25 ppm, respectively.

T6 = Mixture of Fe, Zn, Mn, Mo and B at rates 300, 300, 300, 30 and 30 ppm, respectively.

Table 3: Effect of foliar spray with mixture of microelements on root system of cowpea plants during the two growing seasons

	Main root length (cm)		Number of second order roots		Number of third order roots		Number of nodules	
Treatments	2011	2012	2011	2012	2011	2012	2011	2012
T0	38.58d	36.46e	21.68d	19.18d	55.31e	50.57e	7.56f	5.75d
T1	41.37c	38.94d	23.80c	21.96c	59.91d	56.00d	12.30e	11.53c
T2	46.08a	42.50bc	26.17b	23.52b	65.05c	62.54c	14.53d	12.67c
T3	46.91a	43.67ab	28.91a	27.17a	70.02a	65.71a	17.32c	16.46b
T4	47.00a	44.15a	28.39a	26.91a	68.94ab	65.17a	18.91ab	18.37a
T5	46.08a	43.00abc	26.86b	26.35a	68.92ab	64.44ab	20.09a	18.97a
T6	44.29b	41.62c	26.55b	25.67a	68.07b	63.35bc	18.28bc	18.66a

See footnote of Table 2.

Table 4: Effect of foliar spray with mixture of microelements on dry weight of cowpea plants during the two growing seasons

	D.W of leaves (g)		D.W of branches (g)		D.W of roots (g)		D.W of whole plant (g)		Relative D.W (%)	
Treat.	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
T0	6.70f	5.76e	2.86d	2.29e	1.90c	1.80e	11.52e	9.94e	100	100
T1	7.34e	6.31d	3.16c	2.88d	1.99b	1.88d	12.54d	11.07d	109	111
T2	7.90d	7.18c	3.42b	3.04c	2.02ab	1.98b	13.34c	12.19c	116	123
T3	8.31c	7.79b	3.52b	3.26b	2.07a	2.05a	13.90b	13.11b	121	132
T4	9.18a	8.55a	3.72a	3.31ab	2.01ab	1.96bc	14.90a	13.82a	129	139
T5	9.32a	8.74a	3.76a	3.38a	1.96bc	1.90cd	15.04a	14.02a	131	141
T6	8.79b	8.44a	3.64a	3.23b	1.92c	1.87d	14.35b	13.55ab	125	136

See footnote of Table 2.

synthetase involved at initial steps of nitrate assimilation [28]. The results indicated that micro-elements were very necessary for improving root system and vegetative growth of cowpea plants compared to control treatment. The previous results are in agreement with those reported by EL-Mansi *et al*. [7] and Gad and Kandil [29].

Dry Weight: Data in Table 4 illustrate that all levels of foliar spraying treatments increased significantly the dry weight compared with control (T0); increasing levels of mixtures of microelements up to T5 significantly increased dry weight of leaves, branches, whole plant and relative dry weight then decreased at T6. However, there was no significant difference between T4 and T5. In the same trends, dry weight of roots was greatly increased up to T3 and then reduced with increasing concentrations; the results proved that the treatments T2, T3 and T4 did not differ.

Table 5: Effect of foliar spray with mixture of microelements on photosynthetic pigments (mg/g dry weight of leaves) of cowpea plants during the two growing seasons

	Chl. A		Chl. b		Chl. a+ Chl. b		Carotenoids	
Treat.	2011	2012	2011	2012	2011	2012	2011	2012
T0	1.82e	1.70e	1.49e	1.32d	3.30e	3.03e	3.07e	2.90e
T1	1.96d	1.87d	1.58d	1.39c	3.54d	3.26d	3.09d	3.03d
T2	2.06cd	1.97c	1.66a	1.50b	3.72bc	3.47c	3.17c	3.12bc
T3	2.22a	2.12a	1.69a	1.57a	3.91a	3.68a	3.33a	3.23a
T4	2.13b	2.07ab	1.67ab	1.56ab	3.80b	3.63ab	3.28ab	3.19a
T5	2.08bc	2.03bc	1.64bc	1.53ab	3.72bc	3.56bc	3.22bc	3.14ab
T6	2.02d	1.97c	1.61cd	1.50b	3.64c	3.47c	3.18c	3.05cd

See footnote of Table 2.

Table 6: Effect of foliar spray with mixture of microelements on yield and its components of cowpea plants during the two growing seasons

	No of seeds/pod		No of pods/plant		Seeds weight/pod		Seed yield /plant (g)		Seed yield/ fed.(kg)		Relative yield/fed. (%)	
Treat.	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
T0	5.74e	4.96f	9.08d	7.53d	1.76d	1.63c	16.05c	12.28e	641.89c	491.27e	100	100
T1	5.92d	5.21e	10.10bd	9.08c	1.84c	1.75b	18.62b	15.92d	744.80b	636.90d	116	130
T2	6.22bc	5.49d	11.05ab	10.47b	2.00b	1.86a	22.15a	19.47bc	885.91a	778.92c	138	159
T3	6.44a	6.22a	11.46a	11.61a	2.09a	1.89a	23.92a	21.94a	956.60a	877.71a	149	179
T4	6.26b	6.14ab	11.29a	11.42a	2.06ab	1.85a	23.25a	21.14a	950.16a	845.59ab	148	172
T5	6.19bc	6.07bc	11.68a	11.10ab	2.03ab	1.77b	23.74a	19.66ab	949.83a	786.41bc	148	160
T6	6.12c	5.99c	11.70a	10.97ab	2.00b	1.70b	23.40a	18.64c	936.00a	745.73c	146	152

See footnote of Table 2.

The positive effect of Fe, Zn, Mn, Mo and B on dry weight could be attributed to that Fe, Zn and Mn have vital roles in plants as follows: Iron is a component of many enzymes associated with energy transfer, nitrogen reduction and fixation and lignin formation. Iron associates with sulfur in plants to form compounds that catalyze other reactions [3]. Zinc is a component of many enzymes such as dehydrogenase, proteinase, peptidases and phosphohydrolase important for metabolism of carbohydrate, protein and phosphate [2]. Manganese involves in the evolution of O_2 in photosynthesis (Hill reaction). It is a component of several enzyme systems. It has also, function in chloroplast as a part of electron-transfer (oxidation-reduction) reactions and electron transport system [2].

These results are in conformity with those obtained by Attia and Bardisi [9] and Abou El-khair *et al.* [5].

Photosynthetic Pigments: Results in Table 5 attain that spraying cowpea plants with mixtures of microelements (Fe, Zn, Mn, Mo and B) at different concentrations; viz, T0, T1, T2, T3, T4, T5 and T6 had a significant effect on photosynthetic pigments. It is evident that chlorophyll a, b, total chlorophyll (a + b) and carotenoids were increased with increasing concentrations from T0 up to T3 and then reduced after that, with increasing the concentrations.

Microelements as Fe and Mo play a vital role in synthesis of chlorophyll or chloroplast formation [4].

Yield and its Components: Data in Table 6 appear the effect of spraying with mixtures of microelements (Fe, Zn, Mn, Mo and B) at different concentrations on yield and its components (number of seeds/pod, number of pods/plant, seeds weight/pod, seed yield/plant, seed yield/fed. and relative yield). It can be concluded that yield and its components were significantly increased gradually with increasing concentrations from T0 up to T3 and then decreased at the highest concentrations.

The increase in total yield owed directly to the increase in vegetative growth (Tables 2, 3 and 4) and high photosynthesis capacity expressed in leaf pigments (Table 5). These increases might be ascribed to the favorable role of the used micronutrients in pigments formation, photosynthesis activation and carbohydrates assimilation diverted to the seed [25]. The results are in conformity with those obtained by Abou El-khair *et al.* [5] and Gad and Kandil [29].

Chemical Contents and Enzyme Activity: Data presented in Table 7 showed that, applying different microelements concentrations greatly improved the elemental composition (NPK) and carbohydrates contents in seeds

Table 7: Effect of foliar spray with mixture of microelements on NPK, protein and carbohydrates contents in seeds and enzyme activity (nitrogenase) in nodules roots of cowpea plants during the two growing seasons

	N %		P %		K %		Total crude protein %		Total carbohydrates %		Nitrogenase (µ mole/g DW nodules/hr)	
Treat.	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
T0	2.99g	2.91d	0.41c	0.38d	1.31d	1.30e	18.69g	18.17d	28.06e	27.61d	96.57e	91.17e
T1	3.03f	2.99c	0.45a	0.41bc	1.35c	1.33d	18.96f	18.69c	29.37d	29.35cd	167.25d	164.90d
T2	3.09d	3.04bc	0.47a	0.44ab	1.38b	1.37b	19.33d	19.02b	32.06c	31.10bc	190.05c	182.74c
T3	3.21a	3.11a	0.47a	0.46a	1.43a	1.40a	20.04a	19.42a	35.04a	33.22a	201.88b	196.06b
T4	3.18b	3.06ab	0.47a	0.43abc	1.39b	1.36bc	19.85b	19.10b	33.71b	31.82ab	216.21a	213.78a
T5	3.12c	2.99c	0.45a	0.42bc	1.37b	1.34cd	19.52c	18.71c	32.56bc	30.31bc	217.52a	215.08a
T6	3.07e	2.98c	0.40b	0.40c	1.31d	1.30e	19.19e	18.63c	32.41c	30.11bc	215.19a	212.03a

See footnote of Table 2.

Table 8: Effect of foliar spray with mixture of microelements on Zn, Mn, Fe, Mo and B contents in seeds of cowpea plants during the two growing seasons

	Zn (mg/kg D.W)		Mn (mg/kg D.W)		Fe (mg/kg D.W)		Mo (mg/kg D.W)		B (mg/kg D.W)	
Treat.	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
T0	4.33g	4.23f	3.50f	3.51e	6.18g	6.11g	0.014f	0.013e	1.86f	1.84d
T1	4.40f	4.36e	3.69e	3.62d	6.27f	6.18f	0.021e	0.017de	1.92e	1.92d
T2	4.50e	4.43de	3.85d	3.78c	6.35e	6.36e	0.024de	0.022cd	2.05d	2.02c
T3	4.60d	4.52cd	4.01c	3.89b	6.48d	6.44d	0.027cd	0.026bc	2.15c	2.08c
T4	4.68c	4.61c	4.04c	3.96b	6.64c	6.51c	0.030bc	0.030ab	2.27b	2.22b
T5	4.81b	4.72b	4.11b	4.07a	6.82b	6.67b	0.033ab	0.032ab	2.37a	2.29ab
T6	4.94a	4.90a	4.21a	4.11a	6.97a	6.92a	0.037a	0.036a	2.41a	2.35a

See footnote of Table 2.

and nitrogenase activity in roots of plants compared with control in both seasons. NPK and carbohydrates contents were increased with increasing the concentration and reached the highest values at T3 then decreased again to low values at T6.

Nitrogenase activity in roots was significantly increased gradually with increasing the concentration from T0 up to T4, with no significant difference among T4, T5 and T6.

The applied Zn improves its concentration within leaves of the treated plants. In turn, it might protect plasma membrane and its linked transporter enzymes against the harmful effects of higher temperature/oxidative stresses [4]. Thereby, improves its transportation functions for other elements and solutes. Also, zinc is a component of many enzymes such as dehydrogenase, proteinase, peptidases and phosphohydrolass important for metabolism of carbohydrate, protein and phosphate [2]. Molybdenum is a part of nitrate reductase, which is involved in reduction of NO_3 to NH_4 after its absorption by plants. Also, it is structural component of nitrogenase enzyme, which involved in nitrogen fixation of N_2 into the ammonium form in a symbiotic relationship with legumes. Iron, molybdenum and boron play a vital role of enzymes activity as nitrogenase, catalase and peroxidase. Iron is essential component of all those enzymes systems and electron carriers in which a heam prosthetic group is present (peroxidase and catalase enzymes). It is also, present in non heam compounds such as nitrate reductase [2].

Data presented in Table 8 showed clearly the effect of spraying plants with mixtures of microelements (Fe, Zn, Mn, Mo and B) at different concentrations; viz, T0, T1, T2, T3, T4, T5 and T6 on Fe, Zn, Mn, Mo and B contents in seeds. It is obvious that Fe, Zn, Mn, Mo and B contents were promoted with all spraying concentrations as compared to control. Fe, Zn, Mn, Mo and B contents were significantly increased gradually with increasing the concentration from T0 up to T6.

Anatomical Studies: This investigation aimed to explain the internal structure of vegetative growth which showed the most noticeable response to tested treatments. The above mentioned findings in this work concerning the morphological characters of vegetative growth, physiological and yield characters in the first growing season of cowpea plants exhibited that foliar spray with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at

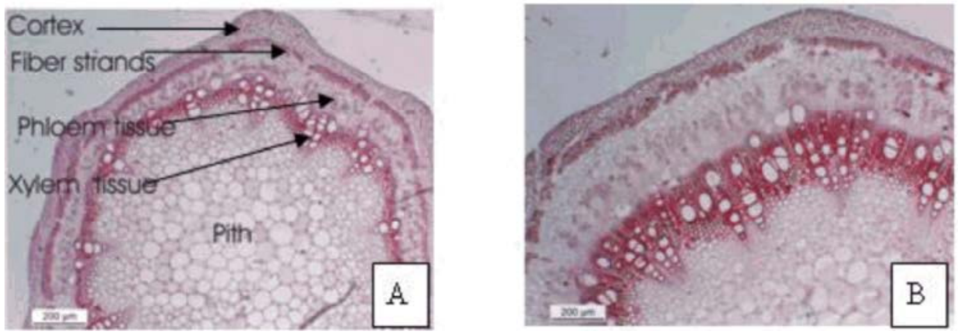

Fig. 1: Transverse sections of cowpea main stem as affected by foliar spray with mixture of microelements

A: Untreated (control).

B: Sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo, and B at rates 150, 150, 150, 15 and 15 ppm, respectively

Table 9: Effect of foliar spray with mixture of microelements on measurements in microns of certain histological features in transverse sections through the middle part of the five upper internode of the main stem of cowpea plants grown during 2012 season

	Treatments		
Characters	A	B	± % to control
Stem diameter (µ)	3330.20	5264.20	58.10
Cortex thickness (µ)	104.20	117.20	12.50
Fiber strands thickness average (µ)	92.40	98.80	7.00
Vascular tissue thickness average (µ)	416.80	1211.00	190.60
Phloem tissue thickness (µ)	135.60	551.20	306.70
Xylem tissue thickness (µ)	268.60	686.00	155.40
Number of xylem rows in Vascular cylinder	48.00	100.00	108.30
Diameter of secondary vessel average (µ)	47.60	67.40	41.50
Pith diameter (µ)	2082.40	2366.80	13.70

A: Untreated (control)

B: Sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at rates 150, 150, 150, 15 and 15 ppm, respectively)

concentrations 150, 150, 150, 15 and 15 ppm, respectively) reflected high increasing in the studied characters among various tested concentrations for mixture of some micronutrients, therefore this level of micronutrients concentration was chosen for studying the internal structure of cowpea plants cv Cream 7.

Microscopical counts and measurements of certain histological characters of the upper five internode which resembled the median internode of the main stem as well as from the terminal leaflet of the corresponding leaf of cowpea plants sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at concentrations 150, 150, 150, 15 and 15 ppm, respectively) and those of control.

Stem Anatomy: It is obvious from Table 9 and Figure 1 that foliar spray with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at concentrations 150, 150, 150, 15 and 15 ppm, respectively) increased diameter of the main stem of cowpea plants by 58.1% more than the control. The increase in stem diameter, due to foliar spray with mixture of some micronutrients, could be attributed mainly due to the prominent increase in all included tissues. The thickness of cortex, fiber strands, vascular tissue and pith diameter were increased by 12.5, 7, 190.6 and13.7% more than those of the control, respectively.

It is also clear that the prominent increase which was observed in the thickness of vascular tissue of the main stem as affected by foliar spray with mixture of some micronutrients could be attributed mainly to the increase in thickness of both phloem and xylem tissues by 306.7 and 155.4% over the control, respectively. Moreover, number of xylem rows in vascular cylinder and secondary vessel diameter were also increased by 108, 3 and 41.5% over the control, respectively. These results agreed with those obtained by Ali *et al*. [11], on *Vicia faba* L. cv. Kassasin l, using 100 and 200 ppm Zn as well as by Mohamed and Saif El-Yazal [10] using Fe, Zn and Mn on cotton plants. They recorded favorable anatomical changes in stem structure due to the effect of micronutrients.

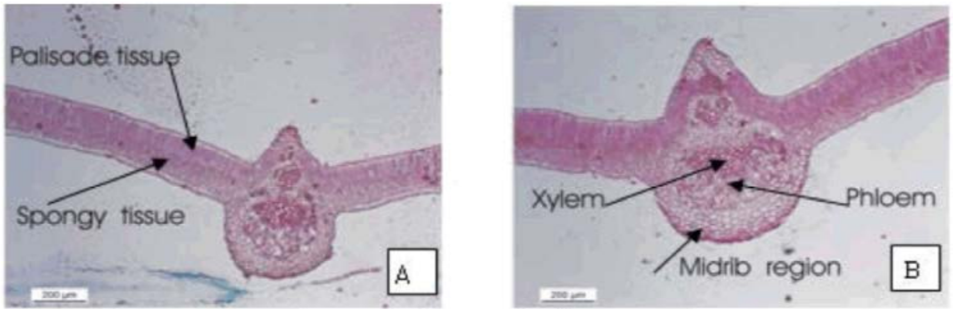

Fig. 2: Transverse sections of cowpea blade of terminal leaflet of the compound leaf on the main stem as affected by foliar spray with mixture of microelements during the second growing season (2012)

A. Untreated (control).
B. Sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo, and B at rates 150,150, 150, 15 and 15 ppm, respectively

Table 10: Effect of foliar spray with mixture of microelements on measurements in microns of certain histological features in transverse sections through the blade of the terminal leaflet of the five compound leaf upper on the main stem of cowpea plants grown during 2012 season

	Treatments		
Characters	A	B	± % to control
Midrib thick (µ)	624.00	829.70	33.00
Midrib width (µ)	425.10	654.10	53.90
Midvein bundle thick (µ)	134.50	183.00	36.10
Midvein bundle width (µ)	181.50	325.70	79.40
Phloem tissue thick (µ)	33.80	60.30	78.40
Xylem tissue thick (µ)	92.20	115.10	24.80
Number of xylem rows in midvein bundle	7.00	9.00	28.50
Number of xylem vessels in midvein bundle	26.00	39.00	50.00
diameter of xylem vessel average (µ)	19.10	23.60	23.60
Blade thick. (µ)	198.50	229.40	15.60
Palisade tissue thick. (µ)	94.30	102.40	8.60
Spongy tissue thick. (µ)	66.70	112.60	68.80

A: Untreated (control) B: Sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at rates 150, 150, 150, 15 and 15 ppm, respectively)

Leaf Anatomy: Microscopical counts and measurements of certain histological features in transverse sections through the blade of the terminal leaflet of the upper five corresponding leaf on the main stem of control plants of cowpea plants and of those sprayed with mixture of some micronutrients (Fe, Zn, Mn, Mo and B at concentrations 150, 150, 150, 15 and 15 ppm, respectively) are presented in Table 10. Likewise, microphotographs illustrating these treatments are shown in Figure 2. Data showed that increases in thickness of both midvein and lamina of leaflet blades of cowpea plants by 33.0 and 15.6 %, respectively compared with the control. It is noted that the increase in lamina thickness was accompanied with 8.6 and 68.8 % increments in thickness of palisade and spongy tissues, respectively compared with the control. The main vascular bundle of the Midvein increased in size as a result of spraying mixture of some micronutrients, the increment was mainly due to the increase in bundle thickness by 36.1 % and in width by 79.4 % more than the control. Also, number of xylem rows and average number of vessels per midvein bundle were increased by 28.5 and 50 % more than the control; respectively. Moreover, xylem vessels had wider cavities being 23.6% more than the control.

The positive effects of micronutrients (Fe, Zn and Mn) applied either alone or in combination on cotton stem or leaf blade tissues may be due to the role of micronutrients as co-factors for many enzymes that included in metabolic processes and synthesis of auxins, which stimulate cell division and expansion [10].

These results are in harmony with those obtained by Mohamed and Saif El- Yazal [10] using Fe + Zn + Mn at concentration of 0.08% on cotton plants, El-Tantawy and Eisa [12] using boron at a concentration of 25 ppm on table beet plants and Ali *et al.* [11] using zinc at concentrations of 100 and 200 ppm on *Vicia faba* L. plants. They recorded favorable anatomical changes in leaf anatomy due to the effect of micronutrients mixture.

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