EFFECT OF FOLIAR MICRONUTRIENTS UNDER SOME BIO-STIMULANTS ON THE PRODUCTIVITY OF FABA BEAN (*Vicia faba* L.)

Ismail, S. A.; Galal, O. A. M.; and Sarhan, M. G. R*

* Soil, Water and Environment, Res. Inst., Agricultural Research Center

ABSTRACT

Two experimental trails were designed to quantify the response of nodulation, growth and yield and its components of faba been plants as well as nutrient content in seeds toward foliar spray twice of zinc (0.3% zinc sulphate), iron (0.6% iron sulphate) and molybdenum (0.05% ammonium molybdedate) under some bio-stimulants, i.e., inoculation with rhizobium and 10 kg humic acid/feddan. The experiments were conducted at the Farm of Sids Agricultural Research Station (ARC), Beni Swif Governorate, Egypt during two successive seasons (2013/2014 and 2014/2015). Number and dry weight of nodules/plant showed positive significant response to iron and molybdenum applicationas well as rhizobia and humic acid treatments comparing with control. The maximum values of these parameters were produced in plots under the effect of molybdenum followed by iron application in combined with bio-stimulants. Plant height, dry weight/plant, number of pods/plant, number of seeds/pod and grain and straw yields were recorded under the treatments of spraying 0.6% iron sulphate in combined with rhizobia inoculation plus humic acid. Higher nitrogen or protein content in seeds were produced under 0.3% zinc sulphate plus rhizobia inoculation plus humic acid, while, Fe and Mo application did not affect nitrogen content. Zinc or molybdenum applications decreased phosphorus content in seeds, while, humic acid enhanced P content. Potassium content was affected only by humic acid. Zn application inhibited Fe content and vice versa iron application decreased zinc content in seeds. Added Zn, Fe, and Mo increased their contents in seeds, respectively. Also, humic acid application improved macro and micronutrients in faba bean seeds.

Key words: Faba bean, foliar spray, zinc, iron, molybdenum, rhizobia, humic acid, nodulation, growth, yield and yield components and nutrient content.

INTRODUCTION

Faba bean (*Vicia faba* L.) is considered as one of the important sources of plant protein for human nutritional and even carbohydrates in many countries, especially Egypt. Faba bean seeds are rich in protein (28-30%), vitamins and mineral salts and so it is considered a daily meal for poor people in many parts in the world. The total world production was 5.1 million tons in 2011. Faba bean produce about 6 t/h under well managed environments (Saxena *et al.*, 1986). As a result of agriculture intensification and cropping systems, soil has become poor in micronutrients content; also micronutrients are inadequately supplied to the soil for different reasons. Therefore, improving plant micronutrient status by foliar application would be very important for sustainable and economic crop production.

Based on studies done by scientists, most micronutrients play a key and vital role in treatment of a lot of mental and physical disorders in humans and animals (Hemmati, 2005). Due to lime soils, high pH, unbalanced use of chemical fertilizers, failure to observe rotation, minimal use of organic fertilizers and lack of the use of fertilizers containing micronutrients in the past, there is deficiency of micronutrients in the soil of most of the farms in Egypt, especially after High Dam bulding. Most importantly, micronutrients are involved in the key physiological processes of photosynthesis and respiration (Mengel *et al.*, 2001) and their deficiency can impede these vital phosiological processes and thus limiting yield gain. For example, iron (Fe) plays a crucial role, being a cofactor of enzymes of the reductive assimilatory pathway (Marschner, 1995). Biochemical changes result in an increased ability to acquire Fe, and include the induction of a plasma-membrane Fe (III)-reductase and an Fe (II) transporter, an enhanced proton extrusion capacity, and the release of low molecular weight compounds such as carboxylates, flavins and phenolic compounds (Abadía *et al.*, 2002). Zinc has important functions in protein and carbohydrate metabolism and activates many enzems; tryptophan synthetase, superoxide dismutase and dehydrogenises. Therefore, low Zn reduces the plant protein content. Molybdenum play an important role in the process of rhizobium symbiosis, which it is a constituent of the nitrogenase enzyme and every bacterium which fixes nitrogen needs molybdenum during the fixation process. Molybdenum has a positive effect on yield quantity, quality and nodule forming in legume crops. Application of molybdenum into the soils has increased the contents of potassium, phosphorus and crude protein (Anonymous, 2005).

Bio-stimulants are organic materials that have been shown to influence several metabolic processes such as respiration, photosynthesis, and nucleic acid synthesis and ion uptake and when applied in small quantities, enhance plant growth and development. They are mixtures of one or more things such as microorganisms, yeast, seaweed extracts, plant growth promoting rhizobacteria (PGPR) and humic acid. They may enhance water-holding capacity, increase antioxidants, and enhance metabolism (Abbas, 2013). Humic acids are characterized as aheterogeneous natural resource, ranging in colour from yellow to black, having high molecular weight, and resistance to decay. Humic acid, as a commercial product contains 44-58% C, 42-46% O, 6-8% H and 0.5-4% N, as well as many other elements (Larcher, 2003). External seed inoculation of rhizobia is also one of another practices to increase the nitrogen fixation potential of the crops since there might be low population of effective indigenous rhizobia or due to higher competitions with non-effective ones (Tolera *et al.*, 2009).

The objective of this study was to determine the effect of some bio-stimulatants, i.e. humic acids and rhizobia inoculant under Fe, Zn and Mo application and their interactions on nodulation and growth, yield and its components as well as nutrient status of faba bean.

MATERIALS AND METHODS

The effect of micronutrients (Zn, Fe, and Mo) and some bio-stimulants, i.e. rhizobium inoculation and humic acid application on faba bean plants (*Vicia faba* L.) was tested in two field experiments at Sids Agricultural Research Station, Beni Sweif Governorate, ARC, Egypt. Some physical (according to Klute, 1986) and chemical properties (according to Page *et al.*, 1982) were determined in a surface soil sample (0.0 – 30 cm) to represent the characteristics of the experimental soil.

The preceeding crop was corn (*Zea mays* L.) in both seasons. Superphosphate (15.5% P2O5) at rate of 31 kg P2O5/feddan and potassium sulphate (48% K2O) at rate of 24 kg K2O/feddan was added before planting. Also, 10 kg N/feddan as ammonium nitrate was added as activator before sowing. The experimental plot (3.0 x 3.5 m was planted with faba bean seeds (variety Giza 843) at rate of one seed in the hill after treated the plots concerned with rhizobium treatment in 20 and 25 October in the two studied seasons, respectively. Inter row spacing was 50 cm and bush spacing on row was 10 cm.

Table 1: Some physical and chemical properties of the experimental soil.

Soil properties	First season	Second season
Physical properties:		
Particle size distribution:		
Clay (%)	53.9	57.1
Silt (%)	33.7	31.4
Sand (%)	12.4	11.5
Texture grade	Clay	Clay
Chemical properties:		
pH (1:2.5 soil-water suspension)	8.0	7.9
EC, soil paste (ds m^{-1})	1.22	1.32
Organic matter (%)	1.31	1.43
CaCO3 (%)	1.51	1.58
Available N (ugg^{-1})	21.2	22.8
Available P (ugg^{-1})	16.7	15.9
Available K (ugg^{-1})	140	149
Available Zn (ugg^{-1})	2.3	1.9
Available Fe (ugg^{-1})	3.8	3.5
Available Mo (ugg^{-1})	0.5	0.47

The experiment consisted of two factors, namely foliar application of micronutrients (without, Zn, Fe and Mo) and bio-stimulants application (without, rhizobium inoculation (RH), humic acid application (HA) and rhizobium inoculation + humic acid application (RH + HA). The experiment was arranged in 4 x 4 treatments with four replications. The spraying of micronutrients took place twice, at 30 days from sowing and 15 days later. The treatments were sprayed at 400 L/feddan of spray solution (or water for control). The foliar spraying of micronutrients was as follows:

_ Without micronutrients with pure water.
_ Spray with zinc at rate of 0.3% as zinc sulphate.
_ Spray with iron at rate of 0.6% as iron sulphate.
_ Spray with molybdenum at rate of 0.05% as ammonium molybdenum.

The bio-stimulants treatments were as follows:

_ Without (without rhizobium inoculation or humic acid application).
_ Rhizobium inoculation (proved by Department of Microbiology, Soil, Water and Environment Institute, ARC) by using Arabic gum and 1% glucose (W/W) for activating the product candidates.
_ Humic acid (humate potassium 86% and potassium oxide 6%) treatment as soil application at rate of 10 kg/feddan.
_ Rhizobium inoculation + humic acid application treatment as mentioned before for each.

All experimental plots received 20 kg N/feddan as ammonium nitrate. All cultural practices for faba bean production were done as in district. At 48 days after planting, 10 randomly selected plants were taken from the two middle rows for nodule count (number of nodules/plant and nodule weight, g). Which plants were dug out with a ball of earth and the soil carfully removed. Roots were carfully washed; nodules were removed, counted then oven dried for 72 h at 70 °C and weghed.

At 90 days after sowing, 10 randomly selected samples were taken from the inner rows for each plot (to minimize the border effect) to determine some growth parameters, namely, plant height (cm), dry weight/plant (g) and number of branches/plant. At maturity, random sample of 10 plants were taken from the middle two ridges for each plot to determine the yield components, e. g. number of pods/plant, number of seeds/pod and 100-seed weight (g). Also, seed (ardab/feddan) and straw (ton/feddan) yields were determined. N, P and K as well as Zn, Fe and Mo in faba bean seeds were determined according to the methods described by Cottenie *et al.* (1982).

Data were subjected to analysis of variance according to Snedecor and Cochran (1980). The treatment means were compared by L. S. D. test at 5% level of probability in both seasons.

RESULTS

1. Effect on nodulation

The results of the effect of micronutrients application along with some bio-stimulant on number and weight of nodules/plant are presented in Table (2). Data show that both number and weight of nodules/plant were significantly affected by the studied treatments in both seasons. The highest number and weight of nodules were recorded for the plants supplied with iron and molybdenum in both seasons, while zinc application did not affect the two studied nodulation parameters. The relative increasing in number and weight of nodules/plant due to iron and molybdenum application reached to 9.2 and 21.1; 16.7 and 26.3 over without micronutrients addition in the first season, respectively. Similar trends were obtained in the second season. It is obvious to notice that Mo application yielded number and weight of nodules/plant higher than those obtained under iron treatment by about 6.8 and 4.3% in the first season and 7.6 and 4.3% in the second one, respectively.

Table (2): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on faba bean nodulation.

Micro-nutrients	Bio-stimulants	No. of nodules/plant		Dry weight of nodules/plant (g)	
		2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	30.1	32.5	0.17	0.17
	Rhizobium inoculation (RH)	34.6	34.6	0.19	0.19
	Humic acid (HA)	33.7	33.9	0.18	0.19
	RH + HA	35.9	37.0	0.21	0.22
Mean		**33.6**	**34.5**	**0.19**	**0.19**
Zinc	0.0	30.3	32.4	0.17	0.17
	Rhizobium inoculation (RH)	34.8	34.7	0.19	0.19
	Humic acid (HA)	33.5	33.8	0.18	0.18
	RH + HA	35.9	36.1	0.21	0.22
Mean		**33.6**	**34.3**	**0.19**	**0.19**
Iron	0.0	33.7	33.7	0.21	0.21
	Rhizobium inoculation (RH)	37.6	37.8	0.23	0.24
	Humic acid (HA)	35.6	35.7	0.22	0.23
	RH + HA	40.0	39.9	0.24	0.25
Mean		**36.7**	**36.8**	**0.23**	**0.23**
Molybdenum	0.0	37.3	37.7	0.22	0.22
	Rhizobium inoculation (RH)	39.6	39.9	0.24	0.24
	Humic acid (HA)	38.2	38.6	0.23	0.23
	RH + HA	41.6	42.0	0.26	0.27
Mean		**39.2**	**39.6**	**0.24**	**0.24**
Mean of bio-stimulant	0.0	**32.9**	**34.1**	**0.19**	**0.19**
	Rhizobium inoculation (RH)	**36.7**	**36.8**	**0.21**	**0.22**
	Humic acid (HA)	**35.3**	**35.5**	**0.20**	**0.21**
	RH + HA	**38.4**	**38.8**	**0.23**	**0.24**
L. S. D. aT 5%					
	A	1.02	1.03	0.02	0.02
	B	0.87	0.89	0.01	0.01
	A X B	N. S.	N. S.	N. S.	N. S.

Concerning the bio-stimulant treatments, the results reveal that both number and weight of nodules/plant were positively responded to rhizobium inoculation and humic acids application and their mixture. It could be arranged the effect of bio-stimulant treatment on values of number and weight of nodules/plant in both seasons in the decendimg order as follow: RH + HA > RH > HA > control. Combined rhizobia inoculation plus humic acid application enhanced number and weight of nodules/plant by about 16.7 and 21.1% as combared with control in the first season. The corresponding increasing in the second season were 13.9 and 26.3% in the abovementioned order.

As for the interaction effect, the obtained results clearly show that the interaction effect between micronutrients and bio-stimulant applications did not effect on plant nodulation. In general, the highest values of number and weight of nodules/plant were recorded under Mo + RH + HA treatment in both seasons. On the other hand, the plants without micronutrients and bio-stimulant yielded the lowest nodulation parameters in both seasons.

2. Effect on growth parameters

The analysis of variance (Table, 3) show that plant height and dry weight/plant were significantly affected by foliar application of Zn, Fe and Mo solutions. Maximum plant height and plant dry weight (113.8 cm and 12.03 g in the first season and 114.3 cm and 12.05 g in the second season) were recorded in those plants h sprayed with 0.6% Fe followed by those sprayed with 0.3% Zn and 0.05% Mo solutions. On the other hand minimum plant height and plant dry weight (108.4 cm and 11.45 g in the first season and 109.8 cm and 11.51 g in the second one) were recorded in control (no spray) plots. The amount of this rises of both plant height and dry weight due to iron application reported 5.0 and 5.1% in the first season and 4.1 and 4.7% in the second one, respectively as compared with control. Non significant differences were observed among the micronutrient treatments in both seasons. On the other hand, number of branches/plant did not affect by micronutrient treatment.

Table (3): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on plant height (cm), dry weight/plant (g) and number of branches/plant.

Micro-nutrients	Bio-stimulants	Plant height (cm)		Dry weight/plant		No. of branchs /plant	
		2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	105.1	107.2	11.13	11.26	5.71	5.72
	Rhizobium inoculation (RH)	109.3	110.6	11.52	11.57	5.73	5.71
	Humic acid (HA)	107.7	108.8	11.38	11.40	5.72	5.72
	RH + HA	111.5	112.7	11.76	11.79	5.70	5.73
Mean		**108.4**	**109.8**	**11.45**	**11.51**	**5.72**	**5.72**
Zinc	0.0	108.6	109.9	11.43	11.46	5.72	5.72
	Rhizobium inoculation (RH)	111.7	112.1	11.81	11.85	5.73	5.72
	Humic acid (HA)	109.5	110.1	11.48	11.52	5.73	5.71
	RH + HA	114.0	114.2	12.06	12.11	5.71	5.73
Mean		**111.0**	**111.6**	**11.70**	**11.74**	**5.72**	**5.72**
Iron	0.0	110.8	111.3	11.73	11.76	5.73	5.72
	Rhizobium inoculation (RH)	114.1	114.7	12.13	12.16	5.72	5.72
	Humic acid (HA)	112.3	112.8	11.79	11.83	5.72	5.73
	RH + HA	118.1	118.3	12.45	12.44	5.71	5.71
Mean		**113.8**	**114.3**	**12.03**	**12.05**	**5.72**	**5.72**
Molybdenum	0.0	108.3	107.9	11.47	11.49	5.72	5.72
	Rhizobium inoculation (RH)	110.9	111.1	11.68	11.73	5.72	5.71
	Humic acid (HA)	109.7	110.2	11.59	11.63	5.73	5.72
	RH + HA	114.5	114.7	12.11	12.18	5.72	5.73
Mean		**110.9**	**111.0**	**11.71**	**11.96**	**5.72**	**5.72**
Mean of bio-stimulant	0.0	**108.2**	**109.1**	**11.44**	**11.49**	**5.72**	**5.72**
	Rhizobium inoculation (RH)	**111.5**	**112.1**	**11.79**	**11.83**	**5.73**	**5.72**
	Humic acid (HA)	**109.8**	**110.5**	**11.56**	**11.60**	**5.73**	**5.72**
	RH + HA	**114.5**	**115.0**	**12.10**	**12.13**	**5.71**	**5.73**
L. S. D. aT 5%							
A		1.1	1.0	0.08	0.06	N. S.	N. S.
B		0.7	0.6	0.04	0.04	N. S.	N. S.
A X B		N. S.	N. S.	N. S.	N. S.	N. S.	N. S.

As for bio-stimulat treatments, the data reveal that both plant height and dry weight were significantly responded to the bio-stimulant treatments in both seasons. It could be arranged the effect of the bio-stimulant treatments on plant height and plant dry weight as the descending order as follow: RH + HA > RH > HA > control. It is obvious to notice that combined rhizobia inoculation with humic acids yielded tallest and heaviest faba bean plant in both seasons, while the plants without bio-stimulant produced the shortest and lightest plants. The bio-stimulants application did not effect number of branches/plant in both seasons.

With regard to the interaction effect, data reveal that the three studied growth parameters were not affected by the interaction between the micronutrients and bio-stimulant treatments (A x B) in both seasons. In general, the highest values of plant height and dry weight were exerted for the plants received with 0.6% iron and supplied with rhizobia inoculation and humic acids. On the other hand the plants without micronutrients and bio-stimulants showed the lowest plant height and dry weight.

3. Effect on yield components

The data regarding yield components of faba bean plants is presented in Table (4). Results show that significant differences were found among various micronutrient treatments for number of pods/plant and number of seeds/pod, while 100-seed weight did not affect in both seasons. However, maximum number of pods/plant (12.7 in both seasons) and maximum number of seeds/pod (3.6 in both seasons) were found in treatment where plants received 0.3% iron sulphate. On the other hand minium number of pods/plant and number of seeds/pod were recorded in control (no zinc, iron and molybdenum) in both seasons. The data also show that both zinc and molybdenum had some positive effect on number of pods/plant and number of seeds/pod comparing with control.

Table (4): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on number of pods/plant, number of seeds/pod and 100-seed weight.

Micro-nutrients	Bio-stimulants	No. of pods/plant		No. of seeds/pod		100-seed weight	
		2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	11.8	11.9	2.7	2.8	71.1	71.3
	Rhizobium inoculation	12.1	12.2	3.0	3.0	71.3	71.3
	(RH) Humic acid	12.0	12.1	2.9	2.9	71.1	71.4
	(HA) RH + HA	12.3	12.5	3.2	3.3	71.2	71.3
Mean		**12.1**	**12.2**	**3.0**	**3.0**	**71.2**	**71.3**
Zinc	0.0	12.2	12.3	2.9	3.0	71.2	71.4
	Rhizobium inoculation	12.6	12.7	3.2	3.3	71.2	71.3
	(RH) Humic acid	12.4	12.4	3.0	3.1	71.3	71.3
	(HA) RH + HA	12.8	12.8	3.3	3.4	71.2	71.3
Mean		**12.5**	**12.6**	**3.10**	**3.2**	**71.2**	**71.3**
Iron	0.0	12.4	12.5	3.3	3.3	71.3	71.3
	Rhizobium inoculation	12.7	12.7	3.7	3.7	71.1	71.3
	(RH) Humic acid	12.6	12.6	3.5	3.5	71.2	71.2
	(HA) RH + HA	12.9	13.0	3.9	3.9	71.2	71.3
Mean		**12.7**	**12.7**	**3.6**	**3.6**	**71.2**	**71.3**
Molybdenum	0.0	12.2	12.3	3.1	3.2	71.2	71.2
	Rhizobium inoculation	12.6	12.7	3.5	3.5	71.2	71.3
	(RH) Humic acid	12.4	12.4	3.3	3.3	71.3	71.3
	(HA) RH + HA	12.8	12.9	3.7	3.7	71.2	71.3
Mean		**12.5**	**12.6**	**3.4**	**3.4**	**71.2**	**71.3**
Mean of bio-stimulant	0.0	**12.2**	**12.3**	**3.0**	**3.1**	**71.2**	**71.3**
	Rhizobium inoculation	**12.5**	**12.6**	**3.4**	**3.4**	**71.2**	**71.3**
	(RH) Humic acid	**12.4**	**12.4**	**3.2**	**3.2**	**71.2**	**71.3**
	(HA) RH + HA	**12.7**	**12.8**	**3.5**	**3.6**	**71.2**	**71.3**
L. S. D. aT 5%							
A		0.06	0.05	0.04	0.05	N. S.	N. S.
B		0.07	0.07	0.04	0.03	N. S.	N. S.
A X B		N. S.	N. S.	N. S.	N. S.	N. S.	N. S.

Regarding the response of yield components to bio-stimulant treatments, the results clearly indicate that number of pods/plant and number of seeds/pod were significantly affected by bio-stimulants application, while 100-seed weight were not affected. It could be arranged the affect of bio-stimulant treatment on the two yield components in the descending order as follow: RH + HA > RH > HA > control (without bio-stimulants application). The relative increasing of RH + HA treatment on number of pods/plant and number of seeds/pod reached 4.1 and 16.7% in the first season, respectively when compared with control. The corresponding values for the second season were 4.1 and 16.1 in the abovementioned respect.

Considering the interaction effect between treatments, the data indicate that the yield components of faba bean plants were not affected by the interaction between the two factors. In general, the highest number of pods/plant and number of seeds/pod were obtained for the plants inoculated with rhizobia and received iron and humic acids, while the plants without micronutrients and bio-stimulants exerted the lowest ones.

4. Effect on grain and straw yields

Results from comparing the mean data show that application of micronutrients had significant effect on both grain and straw yields in the two studied seasons. The highest grain and straw yields were gained from application of iron followed by both zinc and molybdenum application, while the lowest ones were obtained under control (no micronutrients). Iron application in compare with control was superior bu about 5.45 and 5.11% for grain and straw yields, respectively in the first season. Similar trends were obtained for the second season. It is obvious to notice that zinc and molybdenum had significantly the same effect on grain and straw yields, but in lower values.

Table (5): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on grain (ardab/fed) and straw (t/fed) yields.

Micro-nutrients	Bio-stimulants	Grain yield		Straw yield	
		2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	9.72	9.92	4.75	4.82
	Rhizobium inoculation (RH)	10.26	10.44	5.23	5.30
	Humic acid (HA)	10.02	10.31	5.03	5.07
	RH + HA	10.41	10.77	5.35	5.46
Mean		**10.10**	**10.36**	**5.09**	**5.16**
Zinc	0.0	10.20	10.45	5.19	5.23
	Rhizobium inoculation (RH)	10.52	10.87	5.33	5.39
	Humic acid (HA)	10.43	10.69	5.19	5.21
	RH + HA	10.76	10.99	5.46	5.50
Mean		**10.48**	**10.75**	**5.29**	**5.33**
Iron	0.0	10.36	10.65	5.23	5.31
	Rhizobium inoculation (RH)	10.70	10.98	5.39	5.42
	Humic acid (HA)	10.61	10.75	5.26	5.29
	RH + HA	10.94	11.19	5.50	5.61
Mean		**10.65**	**10.89**	**5.35**	**5.41**
Molybdenum	0.0	10.23	10.66	5.13	5.19
	Rhizobium inoculation (RH)	10.51	10.93	5.31	5.44
	Humic acid (HA)	10.44	10.78	5.19	5.23
	RH + HA	10.75	11.20	5.49	5.48
Mean		**10.48**	**10.89**	**5.28**	**5.34**
Mean of bio-stimulant	0.0	**10.13**	**10.42**	**5.08**	**5.14**
	Rhizobium inoculation (RH)	**10.50**	**10.81**	**5.32**	**5.39**
	Humic acid (HA)	**10.38**	**10.63**	**5.17**	**5.20**
	RH + HA	**10.72**	**11.04**	**5.45**	**5.51**
L. S. D. aT 5%					
	A	0.16	0.19	0.08	0.09
	B	0.10	0.11	0.07	0.07
	A X B	N. S.	N. S.	N. S.	N. S.

With respect to the bo-stimulants treatments, the data reveal that both grain and straw yield were significantly responded to bio-stimulants application. It could be arranged the values of grain and straw yields due to bio-stimulant treatments in the descending order as follow: RH + HA > RH > HA > without bio-stimulants application. Comparing with control, inoculated faba bean seeds with rhizobium bacteria plus humic acids addition yielded increasing in grain and straw yields reached to 5.82 and 7.28% in the first season and 5.95 and 7.20% in the second one respectively.

As for interaction effect, the results indicate that the interaction between micronutrients and bio-stimulants application did not affect grain and straw yields in both seasons. The faba bean plants treated with iron + rhizobia inoculation + humic acids recorded the highest values of grain and straw yields. On the other hand, the plants without micronutrients and bio-stimulants recorded the lowest grain and straw yields.

5. Effect on macronutrients content in faba bean seeds

Results of variance analysis (Table,6) show that, the effect of micronutrient treatments on N, P and K concentration in faba bean seeds had no significant differences in 5% probability level, except zinc which positively affect nitrogen content. Also, Zn and Mo treatments decreased P content in faba bean seeds. Plants treated with zinc had nitrogen in their seeds higher than control by about 6.65% and 7.64% in the two seasons, respectively.while, zinc or molybdenum application decreased seed phosphorus content when compared with control by about 17.20% for both treatments in the first season. Same trends were obtained in the second season. It is obvious to mention that zinc application produce higher protein content in faba bean seeds, since protein percentage equal nitrogen percentage in seeds multiplying by 6.25, while other micronutrient treatments did not ulter protein percentage (Fig, 1).

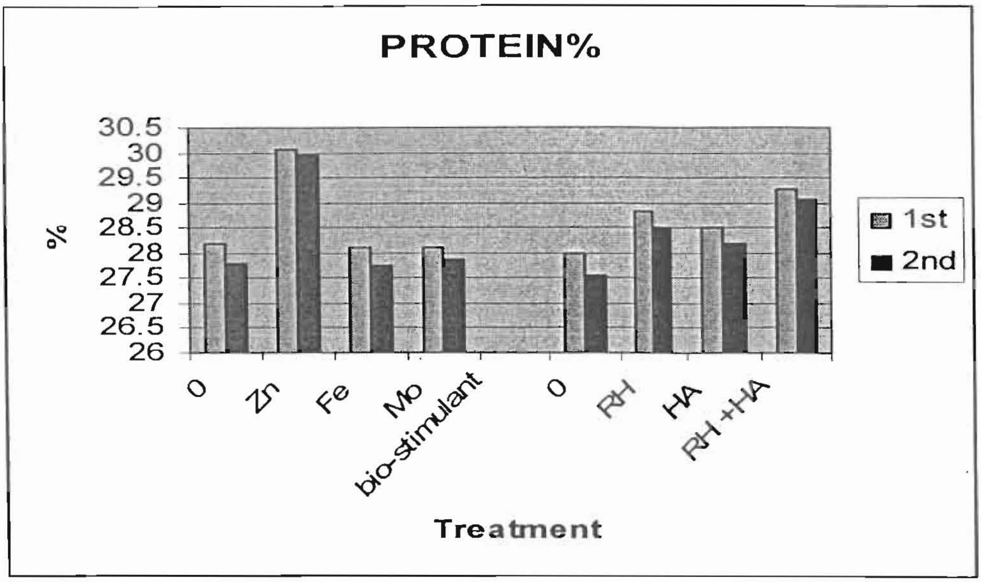
Table (6): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on N, P and K content in faba bean seeds.

Micro-nutrients	Bio-stimulants	N%		P%		K%	
		2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	4.40	4.31	0.43	0.41	3.61	3.52
	Rhizobium inoculation (RH)	4.53	4.48	0.42	0.42	3.63	3.51
	Humic acid (HA)	4.48	4.43	0.52	0.53	3.82	3.76
	RH + HA	4.61	4.57	0.52	0.54	3.82	3.77
Mean		**4.51**	**4.45**	**0.47**	**0.48**	**3.72**	**3.64**
Zinc	0.0	4.71	4.68	0.36	0.35	3.61	3.53
	Rhizobium inoculation (RH)	4.83	4.80	0.36	0.35	3.63	3.52
	Humic acid (HA)	4.79	4.77	0.41	0.41	3.83	3.75
	RH + HA	4.91	4.90	0.41	0.42	3.82	3.76
Mean		**4.81**	**4.79**	**0.39**	**0.38**	**3.72**	**3.64**
Iron	0.0	4.40	4.32	0.43	0.41	3.62	3.54
	Rhizobium inoculation (RH)	4.53	4.47	0.43	0.41	3.62	3.52
	Humic acids (HA)	4.48	4.40	0.51	0.50	3.84	3.76
	RH + HA	4.60	4.57	0.52	0.51	3.82	3.75
Mean		**4.50**	**4.44**	**0.47**	**0.48**	**3.73**	**3.64**
Molybdenum	0.0	4.41	4.34	0.38	0.39	3.63	3.53
	Rhizobium inoculation (RH)	4.53	4.49	0.38	0.39	3.63	3.52
	Humic acid (HA)	4.47	4.43	0.40	0.41	3.83	3.77
	RH + HA	4.60	4.56	0.40	0.40	3.83	3.77
Mean		**4.50**	**4.46**	**0.39**	**0.40**	**3.73**	**3.65**
Mean of bio-stimulant	0.0	**4.48**	**4.41**	**0.40**	**0.39**	**3.62**	**3.53**
	Rhizobium inoculation (RH)	**4.61**	**4.56**	**0.40**	**0.39**	**3.63**	**3.52**
	Humic acid (HA)	**4.56**	**4.51**	**0.46**	**0.46**	**3.84**	**3.76**
	RH + HA	**4.68**	**4.65**	**0.46**	**0.47**	**3.82**	**3.76**
L. S. D. aT 5%							
A		0.12	0.16	0.05	0.06	N. S.	N. S.
B		0.08	0.07	0.03	0.03	0.08	0.09
A X B		N. S.	N. S.	N. S.	N. S.	N. S.	N. S.

Conceming bio-stimulant treatments, the data clearly indicate that nitrogen content or protein percentage in seeds (Table, 6 and Fig, 1) were affected by rhizobia inoculation and humic acid application. The increasing of N or protein percentages due to rhizobia or humic acid treatments reached to 2.90 and 1.19% over control in both seasons, respectively. However, phosphorus and potassium content in seeds were not affected by rhizobia inoculation in both seasons. Moreover, the data reveal that N, P and K content in faba bean were affected by humic acids application,where plants received humic acid contain N, P and K in their seeds higher than control by about 1.79, 19.51 and 6.08% in the first season. The same trends were obtained in the second season.

Ineraction effect of micronutrients and bio-stimulants on N, P and K concentration in seeds was not significant. The highest nitrogen content in seeds (4.91 and 4.90% in both seasons, respectively) were obtained by use of zinc and humic acid and inoculated faba bean seeds before planting with rhizobium inoculation. While, the highest phosphorus content in seeds recorded under the treatments of micronutrients, except zinc in combined with humic acid treatment. Furthermore, the plants treated with humic acids combined with any of micronutrient treatments showed the highest potassium content in plant seeds. On the other hand, the plants without micronutrients or biostimulants produced the lowest nitrogen and potassium content in plant seeds, while the plants fertilized with zinc showed the lowest phosphorus content in seeds.

Figure (1): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on protein percentage.



6. Effect on micronutrients content in faba bean seeds

Data in (Table 7) show that sprayed plants with zinc sulphate at 0.3% twice significantly increased zinc and decreased iron concentration in seeds comparing with control. While, molybdenum concentration did not affect by zinc application. The relative increasing zinc and decreasing iron in seeds due to zinc application reached to 16.7 and 10.9% when compared with control in first season, respectively. The corresponding percentages in the second season were 16.5 and 11.3%. Also, iron application positively and negatively affected iron and zinc in seeds, respectively. The relative increasing of iron and relative decreasing of zinc due to iron application when compared with control were 13.3 and 8.3, respectively in the first season. Same trends were obtained in the second season. Moreover, zinc or iron did not affect molybdenum content in seeds. On the other hand, molybdenum treatment affected only molybdenum content in seeds, where molybdenum content due to molybdenum application surpassed that of control by about 33.8 and 36.5% in both seasons, respectively.

Table (7): Effect of Zn, Fe and Mo along with humic acid and rhizobium inoculation on Zn, Fe and Mo content in faba bean seeds.

Micro-nutrients	Bio-stimulants	Zn (PPm)		Fe (PPm)		Mo (PPm)	
		2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
0.0	0.0	18.1	17.7	35.9	35.6	0.53	0.50
	Rhizobium inoculation (RH)	18.0	17.8	35.8	35.5	0.52	0.50
	Humic acid (HA)	20.2	19.6	39.1	38.7	0.77	0.76
	RH + HA	20.3	19.9	39.4	38.6	0.78	0.76
Mean		**19.2**	**18.8**	**37.6**	**37.1**	**0.65**	**0.63**
Zinc	0.0	21.1	20.7	31.2	30.7	0.55	0.51
	Rhizobium inoculation (RH)	21.4	20.9	31.5	30.9	0.53	0.53
	Humic acid (HA)	23.5	23.0	35.6	35.0	0.76	0.77
	RH + HA	23.4	23.1	35.8	35.1	0.78	0.78
Mean		**22.4**	**21.9**	**33.5**	**32.9**	**0.66**	**0.65**
Iron	0.0	16.5	16.1	40.4	40.1	0.54	0.53
	Rhizobium inoculation (RH)	16.6	16.3	40.7	40.2	0.54	0.55
	Humic acid (HA)	18.7	18.1	44.6	44.3	0.79	0.79
	RH + HA	18.6	18.3	44.7	44.4	0.78	0.80
Mean		**17.6**	**17.2**	**42.6**	**42.3**	**0.66**	**0.67**
Molybdenum	0.0	18.2	18.0	35.8	35.6	0.76	0.77
	Rhizobium inoculation (RH)	18.1	17.8	35.7	35.4	0.76	0.75
	Humic acid (HA)	20.3	20.1	39.0	38.3	0.98	0.97
	RH + HA	20.3	20.2	39.3	38.5	0.96	0.96
Mean		**19.2**	**19.0**	**37.5**	**37.0**	**0.87**	**0.86**
Mean of bio-stimulant	0.0	**18.5**	**18.1**	**35.8**	**35.5**	**0.60**	**0.58**
	Rhizobium inoculation (RH)	**18.5**	**18.2**	**35.9**	**35.5**	**0.59**	**0.58**
	Humic acid (HA)	**20.7**	**20.2**	**39.6**	**39.1**	**0.83**	**0.82**
	RH + HA	**20.7**	**20.4**	**39.8**	**39.2**	**0.83**	**0.83**
L. S. D. AT 5%							
A		1.17	1.13	2.75	2.96	0.14	0.16
B		0.76	0.65	1.39	1.47	0.11	0.12
A X B		N. S.	N. S.	N. S.	N. S.	N. S.	N. S.

Considering the bio-stimulants, the results clearly show that Zn, Fe and Mo concentration in faba bean seeds were positively affected by humic acid treatment, while rhizobia inoculation did not effect micronutrients content in seeds. The relative increasing of Zn, Fe and Mo in seeds due to humic acid application reached to 10.9, 10.6 and 38.3% in the first season and 11.6, 10.1 and 41.4% in the second one, respectively.

Micronutrients content in seeds did not respond to the interaction between treatments in both seasons. In general, the highest Zn, Fe and Mo content were exerted in plants fertilized with Zn, Fe and Mo under humic acid supplying, respectively. On the other hand, zinc application + rhizobia or without bio-stimulants treatments recorded the lowest Fe content in seeds. Also, iron treatment + rhizobia or without bio-stimulants produced the lowest Zn content. Furthermore, the plants without micronutrients and bio-stimulants recorded the lowest Mo in seeds.

DISCUSSION

On the basis of the experimental results, it was stated that number of nodules/plant and dry weight of nodules/plant were positively responded to foliar spray with 0.6% iron sulphate or 0.05% molybdate ammonium solutions applied two times, while zinc application did not effect. In this concern, Kaiser *et al.* (2005) mentioned that, the symbiotic bacterial enzyme nitrogenase is comprised of two subunits one of which is the Mo Fe protein directly involved in reduction of N2 to NH3. Supply of molybdenum and iron to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes. So, application of Fe and Mo are closely correlated with nodule development. Additionally, Haque *et al.* (1979) reported that micronutrients molybdenium and iron are very important for legumes to fix atmospheric nitrogen because molybdenum and iron are essential constituents of nitrogenase enzyme which is responsible for biological nitrogen fixation and their deficiency in soil may affect nitrogen fixation and yield of legumes. Similar results were obtained by Kandil *et al.* (2013) and Ismail *et al.* (2014). On the other hand number of nodules/plant and the dry weight of nodules/plant were positively affected by inoculation of faba bean plants with rhizobium and/or humic acid application. It is worthy to notice that combined rhizobia inoculation with humic acid produced highest number and dry weight of nodules/plant. The improvement of faba bean nodulation due to rhizobia inoculation is mostly due to early nodulation, which can encourage root development. Also, the concentration of bacterial and timing of inoculation contributed to increase plant features (Volpin and Kapulnic, 1994). Also, Tolera *et al.* (2009) mentioned that external seed inoculation of rhizobia is one of another practices to increase the nitrogen fixation potential of the crop since there might be low population of effective indigenous rhizobia or due to higher competitions with non-effecte ones. The promotive effect of humic acid on nodulation status may be due to humic substances lead to a greater uptake of nutrients into the plant roots and through the cell membrane (Tipping, 2002 and Kulikova *et al.*, 2005). It could be observed that humic acid had a synergistic effect on the influence of bacterial inoculation on nodulation status, which combined rhizobia inoculation with humic acid yielded the highest number and dry weight of nodules/plant. This may be due to humic acid can enhance the lateral root development by activating cell membrane and the H pump in the tonoplast of plant cell (Zandonadi *et al.*, 2007). These results are in line with those obtained by Yu *et al.* (2012) and Ismail *et al.* (2014) for rhizobia inoculation; and Akinci *et al.* (2009) and Abbas (2013) for humic acid. According to the interaction effect, the maximum number and dry weight of nodules/plant were recorded under Mo + rhizobia inoculation + humic acid treatment.

Micronutrients application were markedly increased all studied growth and yield parameters and its components of faba bean plants, except number of branches/plant and 100-seed weight. Comparing with control (without micronutrients), sprayed Zn, Fe and Mo were significantly increased plant height, dry weight of plants, number of pods/plant, number of seeds/pod, grain yield and straw yield of faba bean plants. The response of these parameters to micronutrients applications were followed these order: Fe > Zn > Mo > control. Conceming the effect of micronutrients foliar spray on the aforementioned parameters studied in this experiment, the positive marked increases could be due to maintain balanced plant physiology as mentioned in several research studies on their reaction and disturbances caused by their deficiency (Malakouti, 2008). The positive marked effect of sprying micronutrients on the studied parameters may also be due to the stimulating effect of these nutrients on root growth and nutrient uptake by roots as reported by Abdalla and Maburak (1992).

Zinc micronutrient is the main and important component of enzyme system of plant. Zn is a very essential element for generation of energy, regulation of plant growth. It's also important in the protein system of plant (Nadergoli *et al.*, 2011). In addition, zinc has an important role in the production of biomass furthermore. Zinc may be required for chlorophyll production, pollen function and germination (Kaya and Higgs, 2002 and Pandey *et al.*, 2006). Similarly, iron plays a key role in several enzyme systems in which hoem or hoemin functions as the prothectic groub. These haem enzyme systems comprise the catalases, peroxidase and several cytochromes. Cytochromes operate the respiratory metabolism of living cell. Among the haem Fe enzyme is ferredoxin which regulates oxidation reduction reaction its role in photosynthesis, $NO2^-$ and $SO4^{-2}$ reduction and nitrogen assimilation under lines the vital functions iron performs in over plant metabolism (Khan *et al.*, 2014). Molybdenum is required for growth of most biological organisms including plants (Graham and Stangouls, 2005). Generally, molybdenum is an essential micronutrient for plants and bacteria (Williams and Silva, 2002). They added that molybdenum is an essential component of nitrate reductase and nitrogenase, which control the reduction of inorganic nitrate and help in fixing N2 to NH3. Moreover, Katyal and Randhawa (1983) stated that molybdenum is required in the synthesis of ascorbic acid. The results reveal that iron application enhanced faba bean growth and yield and its components than zinc which mainly due to its promotic effect on plant nodulation than zinc. The positive effect of micronutrients application on plant production were reported by many authors such as Reda *et al.* (2014) and Vahedifar and Rahimi (2014) for zinc and iron on faba bean plants and Kandil *et al.* (2013), Khan *et al.*

(2014) and Ismail *et al.* (2014) for molybdenum on common bean, chickpea and soybean plants, respectively.

Cồncerning the effect of rhizobia inoculation and humic acid treatments on the aforementioned parameters studied in these experiments, positive marked increases were observed due to addition of rhizobia and/or humic application. The increment of growth and yield and its components of faba bean plants treated with bio-fertilizer may be due to early nodulation, which can encourage root development and enhanced the number and dry weight of nodules as mentioned before. Tien *et al.* (1979) reported that the increase in nodulation and plant growth of legume plants was attributed to the production of growth promoting substances by bacteria. On the other hand, humic acid has been shown to stimulate plant growth and yield and its components by acting on the mechanisms involved respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities and increase of microbial population (Abbas, 2013). Also, El-Bassiony *et al.* (2010) reported that the beneficial effect of humic acid on plant growth may be attributed to the promoting effects on nutrient uptake and nutritional status especially nitrogen, potassium and phosphorus necessary for plant growth. In addition, it is acting as a source of plant growth hormones, carbohydrates, amino acids and vitamins. The results clearly show that combined rhizobia inoculation with humic acid exerted the highest values of the abovementioned parameters, which is mainly due to the role of humic acid in increasing the microbial population (Abbas, 2013). These results are similar to those obtained by Bhuiyan *et al.* (2008) and Ismail *et al.* (2014) for rhizobia inoculation and Saruhan *et al.* (2011) and Gad El-Hak *et al.* (2012) for humic acid.

Concerning the effect of micronutrients foliar spray on nutrient content in faba bean seeds, the results indicate that added 0.3%zinc sulphate two times as foliar spraying significantly increased nitrogen or protein and zinc content in faba bean seeds, while both phosphorus and iron in seeds were significantly decreased as affected by zinc application. Application of 0.6% iron sulphate two times as foliar spraying was markedly improved iron content in seeds and significantly inhibited zinc in seeds. In addition, molybdenum treatment was significantly increased molybdenum in seeds; however it decreased phosphorus content in faba bean seeds. In this concern, Soepardi (1998) reported that foliar feeding of nutrient may actually promote root absorption of the same nutrient. Also, Abdalla and Mobarak (1992) mentioned that, the positive marked effect of spraying nutrients on nutrient uptake may be due to the stimulating effect of these nutrients on root growth. Similar results were obtained by El-Fouly and El-Sayed (1997) and Oesterhuis (1998). The positive effect of zinc on protein percentage may be due to zinc being an essential component of RNA polymerase enzyme, the deficiency of Zn inactivates RNA polymerase to form m-RNA and reduces RNA levels and ribosome content of cells (Falchuk *et al.*, 1977). These results agree with those obtained by Ahmed *et al.* (1992) who reported that zinc application enhanced protein content in broad bean. The inhibition effect of zinc application on iron content and vice versa is mostly due to Zn and Fe is naturally antagonistic. Each inhibits the uptake of the other (Srivastava and Gupta, 1996). Moreover, Tisdale *et al.* (1997) reported that both inorganic and organic anions such as $MoO4^{2-}$ can compete with P, resulting in decreased adsorption of P. In addition, although the relationship between phosphorus and zinc in plant nutrition has been studied by many workers, many contradicting results were reported. Brown *et al.* (1970) stated that zinc application inhibited phosphorus absorption, while Orabi *et al.* (1981) and Orabi and Abdel-Aziz (1982) showed that the relationship between the two elements was a positive one.

As for the effect of bio-stimulants on nutrient content in seeds, the data reveal that rhizobia inoculation enhanced nitrogen content in seeds (or protein percentage) which may be due to bacteria are able to exert positive effects on plants through several mechanisms, maintaining soil fertility, nitrogen fixation, promoting root elongation (Mady, 2009). On the other hand, humic acid application improved all studied nutrients. The promotive effect of humic acid on nutrient uptake is mainly due the mechanism of possible growth promoting effects which is usually attributed to hormone-like impact, activation of photosynthesis, consequently improved nutrient uptake (Chen and Aviad, 1990). Eyheraguibel *et al.* (2008) stated that humic acid caused increases in length and dry weight of maize plant roots and enhanced the uptake of nitrogen, phosphorus, potassium, zinc and iron. These results are in harmony with those obtained by Akinci *et al.* (2009) and Denre *et al.* (2014) who reported that humic acid application improved nutrient uptake of faba bean plants.

CONCLUSION

In respect of the above results it can be concluded and suggested that the foliar spray of micronutrients in combined with rhizobia inoculation plus humic acid application improve the quality and quantity of faba bean plants under the soils of Middle Egypt.

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تأثير رش العناصر الصغري وأضافة بعض المنشطات الحيوية علي انتاجية الفول البلدي

صفوت احمد اسماعيل واسامة احمد محمد جلال ومحمد جمال رمضان سرحان*

* معهد بحوث الاراضي والمياة والبيئة – مركز البحوث الزراعية.

أقيمت تجربتان حقليتان بمحطة البحوث الزراعية بسدس –مركز البحوث الزراعية – محافظة بني سويف في موسمي النمو ٢٠١٣/٢٠١٤ ، ٢٠١٤/٢٠١٥ لدراسة استجابة عدد ووزن العقد الجذرية ، صفات النمو ، المحصول ومكوناتة ومحتوي البذور من العناصر الصغري الي اضافة العناصر الصغري رشا مع اضافة بعض المنشطات الحيوية مثل التلقيح بالريزوبيا (بكتريا مثبتة للنيتروجين تكافليا) واضافة حامض الهيوميك. وكانت اهم النتائج المتحصل عليها كما يلي:-

- استجاب عدد ووزن العقد الجذرية لاضافة الحديد والمولبدنيوم وكذلك الحقن بالبكتريا واضافة حامض الهيومك. وقد اعطت معاملة المولبدنيوم + الحقن البكتيري + حامض الهيوميك اعلي قيم لعدد ووزن العقد الجذرية يليها معاملة الحديد المضاف اليها المنشطات الحيوية.
- أدي الرش بالعناصر الصغري مع اضافة الحقن بالريزوبيا وحامض الهيوميك الي تحسن في طول النبات ووزنة الجاف وعدد القرون علي النبات وعدد البذور في القرن الواحد ومحصول الحبوب والقش. وقد سجلت معاملة رش الحديد مع اضافة الريزوبيا وحامض الهيوميك الي اعلي قيم محصول البذور والقش وأدي اضافة حامض الهيوميك الي زيادة نسبة الفوسفور في البذور.
- أدت معاملة رش ٣% سلفات زنك + الريزوبيا + حامض الهيوميك الي اعلي قيم النيتروجين (أو البروتين)، بينما لم يتأثر محتوي البذور من النيتروجين بالحديد والمولبدنيوم.
- أدت اضافة الزنك أو المولبدنيوم الي انخفاض الفوسفور في البذور ، بينما ادي اضافة حامض الهيوميك الي زيادة نسبة الفوسفور في البذور.
- تأثرت نسبة البوتاسيوم في البذور باضافة حامض الهيوميك فقط.
- أضافة الزنك أثرت سلبيا في تركيز الحديد في البذور ، والعكس صحيح حيث أدت اضافة الحديد الي انخفاض نسبة الزنك في البذور.
- أدت أضافة الزنك ، الحديد ، المولبدنيوم الي زيادة عناصر الزنك والحديد والمولبدنيوم في الحبوب علي الترتيب.
- أدت اضافة حامض الهيوميك الي زيادة تركيز العناصر الكبري والصغري في بذور الفول البلدي.