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IMPACT OF MICRONUTRIENTS AND DINITROGEN FIXERS ON FABA BEAN PLANT GROWTH IN ALLUVIAL AND CALCAREOUS SOILS

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ABSTRACT: *A greenhouse pot experiment was carried out to study the effect of certain micronutrients and N_2-fixing bacterial agents on plant growth of the legume crop faba bean (Vicia faba) cultivated in alluvial clay loam and calcareous sandy soils. Micronutrients were added as a mixture composing of sulphate salts of Mn + Zn +Cu, at two levels of each element in the composite (M1 < M2). Rhizobium "B1", either ralone or together with Azotobacter"B2" + Azospirillum"B3", were used to inoculate the bean seeds.*

Application of micronutrient composites and /or diazotrophs improved the tested parameters of plant growth in both soils, at two growth periods (45 & 60 days). The higher level of micronutrients mixture (M2) combining with the triple inoculant (B1 + B2 + B3) showed the greatest results. Such positive response was verified by the values obtained for the number of rhizobial root nodules, fresh and dry mass of plant roots and shoots, as well as the plant shoot contents of both the macronutrients N, P & K and the micronutrients Mn, Zn & Cu. The alluvial soil largely excelled the calcareous soil in all cases of the study.

Key words: *Nutritional elements,legumes,diazotrophy,Rhizobium,Azotobacter,Azospirillum.*

INTRODUCTION

It is a well-known fact, and has been established along decades, that the role of micronutrients is important and necessary in plant growth and crop production (Mengel *et al.,* 2001 and Zeiger and Taiz, 2010).Micronutrients often act as co-factors in enzyme systems and participate in redox reactions, in addition to having several other vital functions in plants and microorganisms. Most importantly, micronutrients are involved in the key physiological processes of photosynthesis, respiration and biological nitrogen fixation (Marschner, 1998 and Poole, 2013).

Biological nitrogen fixation (diazotrophy) drives from the activity of certain soil microorganisms, mainly bacteria, that absorb atmospheric nitrogen gas and convert it into ammonium. This process offers an economic, attractive and ecological advantage by reducing external nitrogen input. Mineral elements, generally, and micronutrients in particular, can influence N_2-fixation in legumes at various stages of the symbiotic process and host plant growth. Micronutrients are imperative module of soil fertility and they manipulate crop productivity (Ahmad *et al.,* 2013).

Plant growth promoting rhizobacteria (PGPR) are a class of beneficial microorganisms in the soil ecosystem. These bacteria significantly affect plant growth by increasing nutrient cycling, suppressing pathogens by producing antibiotics and bacterial and fungal antagonistic substances and/or producing biologically active materials such as auxins and other plant hormones. A diverse away of these bacterial agents had been known (Frankenberge rand Arshad, 1995). *Azotobacter* and *Azospirillum*, beside being diazotrophs, they have been found to play a major role in promoting nodulation in legumes induced by *Rhizobium*, via producing growth stimulators, i.e. auxins and cytokinins (Rodelas *et al.,* 1999 and Dobbelaere *et al.,* 2003). Hence, co-inoculation of legumes with the specific symbiotic *Rhizobium* plus the non-symbiotic N_2-fixing free- living and associative bacteria, is highly recommended (Chebator *et al.,* 2001).

The present work was designed to declare the effect of a number of essential micronutrients, together with certain diazotrophs, on plant growth of a major food and feed leguminous crop, namely faba bean (*Vicia faba*), cultivated in two arid soils of Egypt, i.e. alluvial (respresenting the agricultural Nile alluvium) and calcareous (representing the recently reclaimed desert land).

MATERIALS AND METHODS

This investigation was performed in order to study and comparatively evaluate the efficiency of biological nitrogen fixation (diazotrophy) in alluvial and calcareous soils treated with mixtures of certain important micronutrients ,i.e. manganese + zinc + copper, at two application levels. Faba bean was the test legume, which its seeds were inoculated with certain N_2-fixing bacteria (symbiotic, free-living and associative). Fresh and dry mass of the growing plants and their elemental composition, mainly contents of nitrogen, phosphorus, potassium, manganese, zinc and copper, were chemically determined at two growth periods.

1. Materials Employed

1.1. Soils:

Two different arid soils of Egypt were selected to achieve the purpose of this study, namely alluvial of the Experimental Farm, Faculty of Agriculture, Minufiya University (Shibin El-Kom), Minufiya Governorate and calcareous of El-Nobariya, El-Behiera Governorate. Surface soil samples (0-30 cm-depth) were collected from the assigned locations. The samples of each soil were air-dried, ground, mixed well and sieved through a 2 mm- sieve. The sieved soils were subjected to initial laboratory analyses for pertinent physical and chemical properties and contents of some macro-and micronutrients, following the standard methods described by Page *et al.* (1982) and Klute (1986).The obtained data are recorded in Table (1).

1.2. Plant:

Faba Bean (*Vicia faba*, c.v. Giza 3 mohassan), as a winter legume crop(deep tap rootted plant), was tested.

1.3. Micronutrients:

Sulphate salts of Manganese "Mn", Zinc "Zn" and Copper "Cu", were added to each soil, in two mixtures (composites) varying in the concentration of each element (Table 2). Levels of each element were assigned within the permissible limits, referring to the literature concerned (AOAC, 1995; Marschner, 1998; Alloway, 2008; Farooq *et al.*, 2012 and Bajgiran, 2013).The micronutrient additions were considered as a major treatment "M" in this study.

1.4. Diazotrophs:

Inocula of *Rhizobium leguminosarum* "B1" (symbiotic), either alone or together with *Azotobacter sp.* "B2" (free – living)+ *Azospirillum sp.* "B3" (associative),supplied by the Dept. of Agric. Microbiol. of the Soil, Water& Environ. Inst. of the Minis. of Agric., were used. Such N_2-fixing bacterial agents were applied to the faba bean seeds, at the time of sowing.The diazotrophs inoculation was considered as a co-treatment "B" in this study.

2. Layout

The study was undertaken in a greenhouse pot experiment, using plastic pots of 30 cm– diameter and 25 cm– depth. Each pot was filled with 5 kg of the soil. The pots, allocated for each soil, were divided into main groups, sub- groups and sub- sub-groups, representing the sampling periods, levels of the micronutrient composite and bacterial inoculation, respectively, as to satisfy the requirements for the planned objective. The experimental treatments were randomly arranged in a block design, and performed in six replicates for each. Controls without both micronutrient and diazotroph applications (double controls "M0 B0"), as well as each of them alone alternatively (single controls" M0 / B0"), were involved.

Table (1): Initial physical and chemical properties and nutrient contents of the alluvial and calcareous soils under study.

Properties	Units	Alluvial Soil	Calcareous Soil
Particle size distribution:	%		
Sand		34.7	79.7
Silt		23.6	10.2
Clay		41.7	10.1
Textural grade		Clay loam	Sandy
Organic matter	%	1.9	0.6
pH, 1:2.5(soil/ water) suspension		7.2	8.2
E.C, 1:5(soil:water) extract(TSS)	dSm^{-1}	0.6	1.1
Soluble cations:	meq /100g		
Na^{+}		1.4	3.2
K^{+}		0.2	0.5
Ca^{++}		0.9	1.2
Mg^{++}		0.5	0.6
Soluble anions:	meq /100g		
Cl^{-}		1.8	4.1
HCO_3^{-}		0.4	0.7
CO_3^{--}		0.0	0.0
SO_4^{--}		0.8	0.7
Total CaCO3	%	2.9	15.7
Total N	%	0.15	0.06
Total P	%	0.10	0.05
Total K	%	0.60	0.08
Available N	mg / kg	58.1	14.0
Available P	mg / kg	9.2	1.57
Available K	mg / kg	270	60.1
Total Mn	mg / kg	134.0	68.0
Total Zn	mg / kg	37.0	29.0
Total Cu	mg / kg	89.0	58.0
DTPA extractable :	mg / kg		
Mn		9.2	3.1
Zn		7.5	2.1
Cu		4.2	0.7

2.1. Greenhouse work:

This experiment was performed, during the usual winter growth season, on faba bean plants cultivated in either soil concerned. Table (2) reveals the treatments introduced to this investigation.

All the potted soils were fertilized, before planting, with superphosphate (15.5% P_2O_5), at a rate of 200 kg/fed. (1.0g/pot). The diazotrophic bacterial inocula, namely *Rhizobium* "B1", *Azotobacter* "B2" and *Azospirillum* "B3" were applied to the seeds directly before sowing. Five faba bean seeds were planted in each pot. After 12 days of sowing, seedlings of each pot were thinned to 4 plants. All pots were supplied with potassium sulphate (48%K_2O) at a rate of 100 kg/fed. (0.5g/pot) and ammonium nitrate (33% N), at a rate of 50 kg/fed. (0.25g /pot). Worth mentioning that, the calcareous soil received 1.5 times of the NPK fertilizer amounts as much as those applied to the alluvial soil. The assigned micronutrient mixtures(M1&M2),potassium sulphate and ammonium nitrate were introduced together with irrigation water. After 45 days of sowing,whole plants of three replicates were randomly uprootted (the first sampling), washed well and carefully with tap water to remove the soil particles attached to the plant roots, then the plants were again washed with distilled water,and separated into roots and shoots. Each organs were weighed to obtain the fresh weight. Nodules formed on the fresh roots were counted .The plant materials were oven-dried at 70 ^{o}C for 48 hrs, to record the dry weight of roots and shoots, and the data were then statistically analyzed"LSD"(Gomez and Gomez,1984).Samples of the dried shoot materials were finely ground and kept for chemical analysis. Plants of other three replicates were thus uprootted after 60 days of sowing (the second sampling) for the same assessments.

2.2. Plant analysis:

A sample weighing 0.2 g of the dried fine materials of faba bean shoots were digested with a mixture of 10 ml concentrated H_2SO_4 and $HClO_4$ (at a ratio of 3:1),on a sand hot plate(at approximately 270 ^{o}C), until the digest become clear .The digest was diluted to 100 ml with distilled water. The contents of N, P & K (%) and Mn, Zn & Cu (ppm) in the diluted digest were determined, following the standard methods stated by Cottenie *et al.* (1982).

RESULTS AND DISCUSSION

1. Number of Rhizobial Nodules Formed on Plant Roots

The number of rhizobial nodules formed on the roots of faba bean plants was greatly affected by the studied treatments, properties of the soil tested and growth period (Table, 3). As the level of micronutrients composite increased, the number of bacterial root nodules increased as well, but at lower extents of encouragement with the higher level (M2), particularly for the calcareous soil. On the other hand, the bacterial inoculation with the symbiotic dinitrogen fixer *Rhizobium* "B1" largely contributed to initiate the formation of root nodules, which in turn was favoured by the other diazotrophs (the free-living *Azotobacter*+ the associative endophyte *Azospirillum*) included within the triple inoculum (Rodelas *et al.,*1999), as well as by combination with the micronutrient mixtures. Related results were reported by Abdel-Wahab *et al*. (2009) and Ahmad *et al*. (2013). The alluvial clay loam soil superpassed the calcareous sandy one in which the growing plants suffered from a miserable situation of root nodulation, as they formed severely low numbers of nodules.

2. Fresh and Dry Matter Yields of Plants

Data presented in Tables(4&5) show that, the fresh and dry matter yields of faba bean plants grown on the alluvial and calcareous soils, significantly increased, at both sampling times (45 & 60 days after sowing), by micronutrients mixture (Mn+ Zn+ Cu) additions, with the highest values appearing at the higher dose (M2). Hence, ratios of the relative changes "RC" referring to the double control treatments (M0 B0), of both fresh and dry weights of roots and shoots were positive for all variables of the study, with the root figures being greater than the corresponding ones of the shoots. Results obtained for the alluvial soil were, expectidly, higher than those for the calcareous one. Elapse of growth time, logically, resulted in accumulation of plant materials at the latter stage, resulting from proceeding of photosynthesis, N_2 – fixation and nutrients absorption, to build up bio-organic substances. Noteworthy that, accumulation extents of the shoots mass were higher than those of the roots. The "RC" values got for the plant organs were higher at the first sampling time than at the second one, due to hastening the uptake of nutrients to push up the plant growth.

Table (2): The experimental treatments for either soil planted with faba bean.

Treatment No.	Trace Elements Composing The Mixtures (composites): Mixtures"M" (Exp. Simpols*)	Mn	Zn	Cu	Bacterial Inoculation"B" (Exp. Simpols**)
		Concentration of each element in the mixtures (mg/kg soil)			
1a	M0	0	0	0	B0
1b					B1
1c					B1+B2+B3
2a	M1	100	80	25	B0
2b					B1
2c					B1+B2+B3
3a	M2	300	200	75	B0
3b					B1
3c					B1+B2+B3

* M0 = Control (no addition), M1 = Lower rate, M2 = Higher rate of micronutrients.
** B0 = Control (uninoculated), B1 =*Rhizobium*, B2 = *Azotobacter*, B3 = *Azospirillum*.

Table (3): Effect of the experimental treatments on the number of nodules formed on the roots of FABA BEAN plants grown on the ALLUVIAL and CALCAREOUS soils, at two growth periods.

Treatments*: Levels of Micronutrients Mixture"M"	Bacterial Inoculation "B"	Nodule No. / 4 plants: Alluvial Soil, Sampling time (days) 45	Alluvial Soil 60	Calcareous Soil 45	Calcareous Soil 60
M0	B0	83	135	3	4
	B1	108	204	15	18
	B1+B2+B3	113	212	18	20
M1	B0	95	192	5	7
	B1	131	214	17	20
	B1+B2+B3	140	238	20	23
M2	B0	102	197	7	9
	B1	165	248	19	22
	B1+B2+B3	196	279	21	25

*- Micronutrients mixture "M" levels (ppm): M0 (control-no addition),M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).
- Bacterial inoculation "B": B0 (control-uninoculated), B1(*Rhizobium*),B2 (*Azotobacter*), B3(*Azospirillum*).

Table 4

Table 5

It was generally detected that the ratios between the dry weights to the fresh weights of the plant roots were narrower than those for the shoots in most cases of the present trial. This is refered to the higher presence of sap within the above - ground vegetative plant organs. These results come along with the earlier ones noticed by a number of research workers recognising the favourable role of micronutrients in plant growth (Marschner, 1998; Yu and Rengel, 1999 and Alloway, 2008).

Introduction of the diazotrophic bacterial inocula stimulated the faba bean plant growth, as represented by the increases occurred for both fresh and dry masses of plant roots and shoots. This was right for all of the experimental variables (Tables 4&5).The treatments including *Rhizobium* "B1" together with both *Azotobacter* "B2" + *Azospirillum* "B3", i.e. "B1+ B2+ B3", excelled those of "B1" alone. Nevertheless, such increases were not too much if compared with the high shift which was observed between the uninoculated controls "Bo" and the treatments with *Rhizobium* "B1" singly. Addition of the micronutrients mixture augmented the outcomes, in a positive correlation with the composite level (M2> M1). Prolonging the growth period of the plants favoured the proliferation and activity of the bacterial agents, to be in turn reflected on the plant growth being expressed by greater root and shoot weights. The above – noted comments were true for both soils tested, but with the alluvial soil revealing better results than the calcareous one in all cases, to be affected by soil properties, which are poor for the calcareous soil (Table 1). The mentioned findings agree with those reviewed by Chebator *et al.* (2001); Farooq *et al.* (2012) and Bajgiran (2013).

Data listed in Tables (4&5) also display the effect of the combined treatments of micronutrients composite and bacterial inoculation ("M1/M2" plus "B1/B1+B2+B3") on the fresh and dry weights of both roots and shoots of faba bean plants grown on the alluvial and calcareous soils at two growth periods. The tabulated results point out that, the major and co-experimental treatments led to significant elevations of the dry matter yields of faba bean plant organs. The higher level of micronutrients mixture (M2) together with the multi-inoculum of diazotrophs (B1+B2+B3) produced the best figures of all measurements for both soils at the two periods of plant growth, again with the calcareous soil being inferior to the alluvial one. Related results have been reported by Subramaian*et al.* (2009); Eleiwa *et al.* (2012) and Bajgiran (2013).

3. Macronutrient Contents in Plant Shoots

Data noted in Tables (6&7) reveal that, concentration and uptake of the macronutrients,i.e. nitrogen "N", phosphorus "P" and potassium "K" in the shoots of faba bean plants grown on the alluvial and calcareous soils at both growth periods, generally increased with increasing the level added of the micronutrient mixtures (M2> M1), on one hand, and with the bacterial inoculations "B1 + B2 + B3", on the other. Values of the relative changes "RC" of macronutrient contents, showed the highest response of "P" and followed descendingly with "N" and "K", indicating a sequence of absorption capacity of the plants for such elements, being elucidated by raising the "P" availability brought about in soil through the biochemical activities of the inoculating beneficial bacteria. The calcareous soil exhibited low values for the macronutrient assessed, where N came first, P second and K last.

Introduction of the micronutrients composite, via its activation of the anabolic processes taken place in plant tissues, enhanced the uptake of nutrients by the plants. The biological N_2– fixation by the triple means used, namely the symbiotic *Rhizobium* "B1"+ free–living *Azotobacter* "B2"+ associative *Azospirillum* "B3", actually contributed to the highest contents of plant nitrogen at each of the micronutrients level and growth stage. Reports of Vessey (2003); Omar *et al.* (2007); Ahmed *et al.* (2013) and Weisany *et al.* (2013) are supported by the present results in most cases.

Table 6

Table 7

4. Micronutrient Contents in Plant Shoots

Concentration and uptake of each of the micronutrients, i.e. managanese "Mn", zinc "Zn" and copper "Cu" in the shoots of faba bean plants grown on the alluvial and calcareous soils, amended with micronutrients composite and bacterial diazotrophs were estimated at the two stages of plant growth, and the data obtained appear in Tables (8 & 9). The tabulated results demonstrate that, application of the micronutrients mixture (Mn+ Zn+ Cu) led to raising the contents of the determined elements in the plant shoots, in a direct correlation with their concentration in the composite applied. In the alluvial soil, zinc attained the highest response to the treatments applied, and followed by copper and manganese, respectively, as declared by the calculated "RC" values of the micronutrient contents assessed. However, the calcareous soil revealed a different order among the micronutrients estimated in the plant shoots, where the "RC" values were for Mn> Zn> Cu. Inoculation with *Rhizobium* "B1" singly improved the situation of such micronutrients in the plant shoots, however, the triple inoculant gave better results. The combined treatments of both micronutrients and diazotrophs ("M 1/ M2" plus "B1 / B1+ B2+ B3") exhibited the highest results in either case of growth interval and soil examined. Advancement of growth accumulated the nutrients in plant tissues. High pH value and $CaCO_3$ content and low organic matter and nutrient contents of the calcareous soil made it less fertile, and thus came below the alluvial soil. Marschner (1998); Farooq *et al.* (2012) and Bajgiran (2013) reported similar conclusions.

The present results, got for the various treatments and parameters of faba bean plants grown on the two different soils, proved that, soil properties had a prime effect on the plant growth criteria (Wild and Russell,1988). Particularly, the higher values of each of pH and $CaCO_3$, and lower contents of each of clay, organic matter and plant nutritients of the calcareous soil (Table 1) were behind its lower performance than that of the alluvial soil.

The applied micronutrients are known of their functions in the metabolic processes in both plants and microorganisms, thus correction of their inadequacy in soil certainly provides a better life for such living beings for instance, manganese "Mn" is a part of certain enzyme systems and contributes to chlorophyll synthesis .Zinc "Zn" promotes plant growth hormones and enzyme activities necessary for chlorophyll and carbohydrate formation, as well as plant seed production. Copper "Cu" is a constituent of several key enzymes and other proteins in both plants and microorganisms (Zeiger and Taiz, 2010 and Poole, 2013). However, such micronutrients face some complications affecting their availability in soil, as in the case of the calcareous soil, due mainly to the high $CaCO_3$ content.

Inoculation with the symbiotic *Rhizobium* agent insures the formation of active and effective nodules on the roots of such leguminous crop. Participation of the diazotrophs *Azotobacter* and *Azospirillum* biologically increased the combined nitrogen input for the benefit of the plants. Noteworthy that, contribution of the diazotrophs is not confined only to their role in dinitrogen fixation, but also to their ability to produce growth – promoting substances, i.e. indole acetic acid "IAA", vitamines, gibberellins, organic acids and other metabolites, that favour the flourishment of both soil microorganisms and plants in general, and consequently a better plant growth (Pandy and Kumar, 1989; Bohlool *et al.,* 1992; Arshad and Frankenberger, 1998 and Bajgiran, 2013). Moreover, such rhizobacteria have the ability to increase the infection sites for *Rhizobium* in favour of the nodulation process (Srinivasan *et al.,* 1997).

Table 8

Table 9

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تأثير المغذيات الصغرى ومثبتات النيتروجين الجوى
على نمو نباتات الفول البلدى فى أراضى رسوبية وجيرية

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الملخص العربى

فى تجربة أصص بالصوبة درس تأثير مغذيات صغرى معينة ومثبتات بكتيرية لنيتروجين الهواء الجوى على نمو نباتات الفول البلدى المنماه على أرضين رسوبية طينية طميية وجيرية رملية . وأضيفت المغذيات الصغرى كمخلوط يحتوى على منجنيز + زنك + نحاس (فى صورة كبريتات)، وبتركيزين لكل عنصر فى المخلوط (M2>M1). واستخدم الريزوبيم"B1" منفرداً أو مع الأزوتوباكتر"B2" + الأزوسبيريللم"B3" كلقاحات بكتيرية لبذور الفول قبل الزراعة مباشرة.

وقد أدى استخدام تلك المعاملات منفردة أو مشتركة الى تحسين جميع قياسات نمو النباتات فى الأرضين وعلى مدى مرحلتى النمو (45 ، 60 يوماً من الزراعة). وأظهر المعدل الأعلى من مخلوط المغذيات الصغرى "M2" أفضل النتائج باشتراكه مع التلقيح الثلاثى"B3 + B2 + B1" . وتأكدت هذه الاستجابة فى زيادة اعداد العقد البكتيرية على الجذور، وفى إرتفاع قيم كل من الأوزان الطازجة والجافة للجذور والسوق ، وكذلك فى محتويات السوق من كل من المغذيات الكبرى (نيتروجين ، فوسفور ، بوتاسيوم) والصغرى (منجنيز ، زنك ، نحاس). وتفوقت الأرض الرسوبية على تلك الجيرية فى جميع حالات الدراسة .

Table (4): Fresh and dry matter yields and their relative changes (RC)* of roots and shoots of FABA BEAN plants grown on the ALLUVIAL soil as affected by the studied treatments, at the first and second samplings.

Sampling Periods	Treatments**		Roots				Shoots				Whole plants			
			Fresh		Dry		Fresh		Dry		Fresh		Dry	
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation"B"	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %
First (45 days)	M0	B0	5.93	0	1.30	0	59.82	0	4.56	0	65.75	0	5.86	0
		B1	11.14	88	2.18	68	68.84	15	6.64	46	79.98	22	8.82	51
		B1+B2+B3	11.99	102	2.36	82	70.37	18	7.41	63	82.36	25	9.77	67
	M1	B0	8.78	48	1.79	38	66.93	12	6.23	37	75.71	15	8.02	37
		B1	13.10	121	2.46	89	71.06	19	7.59	67	84..16	28	10.05	70
		B1+B2+B3	13.91	135	2.72	109	73.18	22	7.85	72	87.09	33	10.57	80
	M2	B0	10.36	75	2.07	59	68.06	14	6.63	45	78.42	19	8.70	49
		B1	16.48	178	2.95	127	76.76	28	7.90	73	93.24	42	10.85	85
		B1+B2+B3	20.42	244	3.89	199	81.78	37	8.55	88	102.2	55	12.44	112
	LSD, at 0.05		—	—	0.53	—	—	—	1.32	—	—	—	—	—
Second (60 days)	M0	B0	23.38	0	2.96	0	109.42	0	10.75	0	132.80	0	13.71	0
		B1	39.72	70	4.18	41	122.66	12	12.57	17	162.38	22	16.72	22
		B1+B2+B3	43.58	86	4.33	46	127.11	16	13.17	23	170.69	29	17.50	28
	M1	B0	27.49	18	3.71	25	113.72	4	11.69	9	141.21	6	15.40	12
		B1	43.03	84	4.64	57	133.94	22	13.57	26	176.97	33	18.21	33
		B1+B2+B3	48.65	108	4.98	68	137.31	26	13.71	28	185.96	40	18.69	36
	M2	B0	32.97	41	4.06	37	119.29	9	12.43	16	152.26	15	16.49	20
		B1	49.35	11	5.12	73	138.32	26	14.24	33	187.67	41	19.36	41
		B1+B2+B3	55.60	138	5.49	86	143.71	31	15.87	48	199.31	50	21.36	56
	LSD, at 0.05		—	—	0.58	—	0	—	0.69	—	—	—	—	—

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.

**- Micronutrients mixture "M" levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).

- Bacterial inoculation "B": B0 (control-uninoculated), B1 (*Rhizobium*),B2 (*Azotobacter*), B3 (*Azospirillum*).

Table (5): Fresh and dry matter yields and their relative changes (RC)* of the first and second samplings of roots and shoots of FABA BEAN plants grown on the CALCAREOUS soil as affected by the studied treatments, at the first and second samplings .

Sampling Periods	Treatments**		Roots				Shoots				Whole plants			
			Fresh		Dry		Fresh		Dry		Fresh		Dry	
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation"B"	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %	g / plant	RC %
First (45 days)	M0	B0	3.17	0	0.47	0	12.75	0	1.63	0	15.92	0	2.10	0
		B1	5.33	68	0.69	47	17.21	35	2.15	32	22.54	42	2.84	35
		B1+B2+B3	5.61	77	0.78	66	18.70	47	2.32	42	24.31	53	3.10	48
	M1	B0	4.35	37	0.55	17	15.07	18	1.93	18	19.42	22	2.48	18
		B1	6.15	94	0.90	92	20.11	58	2.49	53	26.26	65	3.39	61
		B1+B2+B3	7.59	139	0.99	111	21.96	72	2.83	74	29.55	86	3.82	82
	M2	B0	4.70	48	0.62	32	16.11	26	2.04	25	20.81	31	2.66	27
		B1	7.83	147	1.09	132	23.91	88	2.98	83	31.74	299	4.07	94
		B1+B2+B3	8.30	162	1.26	168	25.26	98	3.29	102	33.56	111	4.55	117
	LSD, at 0.05		—	—	0.11	—	—	—	0.45	—	—	—	—	—
Second (60 days)	M0	B0	8.58	0	1.05	0	21.08	0	2.88	0	29.66	0	3.93	0
		B1	10.42	21	1.46	39	27.91	32	3.57	24	38.33	29	5.03	22
		B1+B2+B3	10.90	27	1.74	66	29.39	39	3.68	28	40.29	36	5.42	38
	M1	B0	9.17	7	1.13	8	23.83	18	3.03	5	33.00	11	4.16	6
		B1	11.42	33	1.77	69	31.27	48	3.85	34	42.69	44	5.62	43
		B1+B2+B3	11.69	36	1.88	79	32.65	55	3.97	38	44.34	50	5.85	49
	M2	B0	9.42	10	1.32	26	27.42	30	3.20	11	36.84	24	4.52	15
		B1	14.17	65	2.10	100	35.87	70	4.15	44	50.04	69	6.25	59
		B1+B2+B3	14.33	67	2.36	125	40.40	92	5.07	76	54.73	85	7.43	89
	LSD, at 0.05		—	—	0.26	—	—	—	0.43	—	—	—	—	—

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.
**- Micronutrients mixture "M" levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu)
- Bacterial inoculation "B": B0 (control-uninoculated), B1 (*Rhizobium*),B2 (*Azotobacter*), B3 (*Azospirillum*).

Table (6): Effect of the experimental treatments on macronutrients concentration and up take and the relative changes of elements concentration (RC)* in the shoots of FABA BEAN plants grown on the ALLUVIAL soil, at two growth periods.

Sampling Periods	Treatments**		N			P			K		
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation "B"	Conc. (%)	Uptake (mg/plant)	RC (%)	Conc. (%)	Uptake (mg/plant)	RC (%)	Conc. (%)	Uptake (mg/plant)	RC (%)
First (45 days)	M0	B0	1.24	72.66	0	0.36	21.10	0	1.00	58.60	0
		B1	1.82	160.52	47	0.60	52.92	67	1.25	110.25	25
		B1+B2+B3	1.94	189.54	57	0.66	64.48	83	1.25	122.13	25
	M1	B0	1.54	123.51	24	0.54	43.31	50	1.00	80.20	0
		B1	2.05	206.03	65	0.69	69.35	92	1.50	150.75	50
		B1+B2+B3	2.36	249.45	90	0.84	88.79	133	1.75	184.98	75
	M2	B0	1.70	147.90	37	0.57	49.59	58	1.25	108.75	25
		B1	2.15	233.28	73	0.81	87.89	125	1.75	189.88	75
		B1+B2+B3	2.59	322.20	109	0.99	123.16	175	1.75	217.70	75
Second (60 days)	M0	B0	1.66	227.59	0	0.42	57.58	0	1.00	137.10	0
		B1	2.24	374.53	35	0.69	115.37	64	1.25	209.00	25
		B1+B2+B3	2.36	413.00	42	0.72	126.00	71	1.25	218.75	25
	M1	B0	1.91	294.14	15	0.57	87.78	36	1.00	154.00	0
		B1	2.50	455.25	51	0.78	142.04	86	1.50	273.15	50
		B1+B2+B3	2.75	513.98	66	0.90	168.21	114	2.00	373.80	100
	M2	B0	2.17	357.83	31	0.63	103.89	50	1.25	206.13	25
		B1	2.66	514.98	60	0.90	174.24	114	1.50	290.40	50
		B1+B2+B3	2.94	627.98	77	1.14	243.50	171	2.00	427.20	100

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.
**- Micronutrients mixture "M" levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).
- Bacterial inoculation "B": B0 (control-uninoculated), B1 (*Rhizobium*),B2 (*Azotobacter*), B3 (*Azospirillum*).

Table (7): Effect of the experimental treatments on macronutrients concentration and uptake and the relative changes of elements concentration (RC)*, in the shoots of FABA BEAN plants grown on the CALCAREOUS soil, at two growth periods.

Sampling Periods	Treatments**		N			P			K		
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation "B"	Conc. (%)	Uptake (mg/plant)	RC (%)	Conc. (%)	Uptake (mg/plant)	RC (%)	Conc. (%)	Uptake (mg/plant)	RC (%)
First (45 days)	M0	B0	0.54	11.34	0	0.05	1.05	0	0.30	6.30	0
		B1	0.70	19.88	30	0.22	6.25	340	0.38	10.79	27
		B1+B2+B3	1.20	37.20	122	0.22	6.82	340	0.40	12.40	33
	M1	B0	0.60	14.88	11	0.15	3.72	200	0.42	10.42	40
		B1	0.75	25.43	39	0.24	8.14	380	0.45	15.26	50
		B1+B2+B3	1.61	61.50	198	0.28	10.70	460	0.50	19.10	67
	M2	B0	0.93	24.74	72	0.20	5.32	300	0.51	13.57	70
		B1	1.54	62.68	185	0.24	9.77	380	0.58	23.61	93
		B1+B2+B3	1.75	79.63	224	0.30	13.65	500	0.70	31.85	133
Second (60 days)	M0	B0	0.80	31.44	0	0.22	8.65	0	0.60	23.58	0
		B1	0.84	42.25	5	0.26	13.08	18	0.65	32.70	8
		B1+B2+B3	1.52	82.38	90	0.26	14.09	18	0.70	37.94	17
	M1	B0	0.86	35.78	8	0.24	9.98	9	0.72	29.95	20
		B1	0.90	50.58	13	0.28	15.74	27	0.75	42.15	25
		B1+B2+B3	1.80	105.30	125	0.30	17.55	36	0.80	46.80	33
	M2	B0	0.98	44.30	23	0.26	11.75	18	0.65	29.38	8
		B1	1.92	120.00	140	0.28	17.50	27	0.70	43.75	17
		B1+B2+B3	2.20	163.46	175	0.32	23.78	46	0.80	59.44	33

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.
**- Micronutrient mixture "M" Levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).
- Bacterial inoculation "B": B0 (control-uninoculated), B1 ***(Rhizobium),***B2 (*Azotobacter*), B3 (*Azospirillum*).

Table (8): Effect of the experimental treatments on micronutrients concentration and uptake and the relative changes of elements concentration (RC)*, in the shoots of FABA BEAN plants grown on the ALLUVIAL soil, at two growth periods.

Sampling Periods	Treatments**		Mn			Zn			Cu		
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation "B"	Conc. (ppm)	Uptake (mg/plant)	RC (%)	Conc. (ppm)	Uptake (mg/plant)	RC (%)	Conc. (ppm)	Uptake (mg/plant)	RC (%)
First (45 days)	M0	B0	5.30	0.03	0	0.17	0.00	0	0.18	0.00	0
		B1	5.50	0.05	4	0.83	0.01	388	0.62	0.01	244
		B1+B2+B3	5.55	0.05	5	1.17	0.01	588	0.64	0.01	256
	M1	B0	5.60	0.04	6	1.17	0.01	588	0.77	0.01	328
		B1	5.75	0.06	9	2.50	0.03	1371	0.82	0.01	356
		B1+B2+B3	6.75	0.07	27	3.67	0.04	2059	1.05	0.01	483
	M2	B0	5.60	0.05	6	1.83	0.02	977	0.78	0.01	333
		B1	6.15	0.07	16	3.00	0.03	1665	1.02	0.01	467
		B1+B2+B3	6.85	0.09	29	4.33	0.05	2447	1.08	0.01	500
Second (60 days)	M0	B0	5.55	0.08	0	0.33	0.00	0	0.45	0.01	0
		B1	5.70	0.10	3	2.00	0.03	506	0.73	0.01	62
		B1+B2+B3	5.95	0.10	7	2.50	0.04	658	0.75	0.01	67
	M1	B0	6.15	0.09	11	3.00	0.05	809	0.79	0.01	76
		B1	6.55	0.12	18	4.50	0.08	1264	0.92	0.02	104
		B1+B2+B3	6.65	0.12	20	7.00	0.13	2021	1.02	0.02	127
	M2	B0	6.20	0.10	12	3.17	0.05	861	1.13	0.02	151
		B1	6.60	0.13	19	4.67	0.09	1315	1.30	0.03	189
		B1+B2+B3	7.00	0.15	26	7.50	0.16	2173	1.41	0.03	213

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.
**- Micronutrients mixture "M" levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).
- Bacterial inoculation "B": B0 (control-uninoculated), B1 (*Rhizobium*),B2 (*Azotobacter*), B3 (*Azospirillum*).

Table (9): Effect of the experimental treatments on micronutrients concentration and uptake and the relative changes of elements concentration (RC)*, in the shoots of FABA BEAN plants grown on the CALCAREOUS soil, at two growth periods.

Sampling Periods	Treatments**		Mn			Zn			Cu		
	Levels of Micronutrients Mixture"M"	Bacterial Inoculation "B"	Conc. (ppm)	Uptake (mg/plant)	RC (%)	Conc. (ppm)	Uptake (mg/plant)	RC (%)	Conc. (ppm)	Uptake (mg/plant)	RC (%)
First (45 days)	M0	B0	2.63	0.01	0	0.10	0.00	0	0.05	0.00	0
		B1	2.85	0.01	8	0.41	0.00	310	0.05	0.00	0
		B1+B2+B3	2.90	0.01	10	0.62	0.00	520	0.06	0.00	20
	M1	B0	3.58	0.01	36	0.90	0.00	800	0.07	0.00	40
		B1	11.33	0.04	331	1.69	0.01	1590	0.10	0.00	100
		B1+B2+B3	41.58	0.16	1481	2.68	0.01	2580	0.11	0.00	120
	M2	B0	9.35	0.02	256	0.98	0.00	880	0.08	0.00	60
		B1	41.33	0.17	1472	2.18	0.01	2080	0.11	0.00	120
		B1+B2+B3	45.68	0.21	1637	4.68	0.02	4580	0.16	0.00	220
Second (60 days)	M0	B0	3.25	0.01	0	0.47	0.00	0	0.08	0.00	0
		B1	5.25	0.03	62	0.52	0.00	11	0.10	0.00	25
		B1+B2+B3	9.73	0.05	199	0.70	0.00	49	0.11	0.00	38
	M1	B0	9.30	0.04	186	0.72	0.00	53	0.10	0.00	25
		B1	40.75	0.23	1154	2.64	0.01	462	0.13	0.00	63
		B1+B2+B3	43.30	0.25	1232	3.53	0.02	651	0.14	0.00	75
	M2	B0	15.55	0.07	379	2.12	0.01	351	0.12	0.00	50
		B1	42.30	0.26	1202	2.88	0.02	513	0.13	0.00	63
		B1+B2+B3	47.75	0.35	1369	5.70	0.04	1113	0.20	0.00	150

*RC = The difference between the value of a particular treatment and the control (M0 B0), calculated as percent of that control.
**- Micronutrients mixture "M" levels (ppm): M0 (control-no addition), M1 (100Mn+80Zn+25Cu), M2 (300Mn+200Zn+75Cu).
- Bacterial inoculation "B": B0 (control-uninoculated), B1 (*Rhizobium*), B2 (*Azotobacter*), B3 (*Azospirillum*).