Minufiya J. Agric. Res. Vol.36 No. 5:1361-1386 (2011) **"http://www.mujar.net"**

تأثير معدلات مختلفة من التلقيح البكتيرى والعناصر الصغرى على تعقيد الجذور وتثبيت النيتروجين الجوى وإنتاج بعض المحاصيل البقولية

فريد شوقى فريد بدوى – عبد الناصر حسن دسوقى –
عبد الحميد محمود محمد بيومى
قسم بحوث الميكروبيولوجيا الزراعية– معهد بحوث الأراضي والمياه والبيئة –
مركز البحوث الزراعية – الجيزة– مصر.

الملخص العربى

أجريت تجربتى أصص بصوبة وحدة أنتاج الأسمدة الحيوية– معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية وكذلك تجربتين حقليتين بقطاع جنوب التحرير فى أرض رملية طميية خلال الموسم الصيفى ٢٠١٠ والموسم الشتوى ٢٠١١/٢٠١٠ لدراسة تأثير أستخدام معدلات مختلفة من اللقاح البكتيرى مع اضافة بعض العناصر الصغرى على حالة تعقيد الجذور ونشاط أنزيم النيتروجينيز وبعض صفات النمو الخضرى وأنتاجية نباتات فول الصويا والفول البلدى. تم تلقيح بذور فول الصويا بخليط من ***Bradyrhizobium japonicum*** **(USDA-110** و **(USDA-HH303** ***Rhizobium fredii*** ، بينما تم تلقيح بذور الفول البلدى بالـ ***Rhizobium leguminosarum*** **bv.** ***Viceae*** خليط من سلالتين **(ICARDA-441 and ICARDA-481)** وذلك بخلط بذور فول الصويا والفول البلدى قبل الزراعة باللقاحات المضافة للحامل البكتيرى الصلب المعقم بأشعة جاما. وقد تم أستخدام أربع معدلات من التلقيح مع فول الصويا وهى (بدون تلقيح ، ١ ، ٢ ، ٣ جم لقاح/ ١٠٠ جم بذور). وكانت معدلات التلقيح المستخدمة مع الفول البلدى هى (بدون تلقيح، ٠.٧٥ ، ١.٥ ، ٢.٢٥ جم لقاح/ ١٠٠ جم بذور). وجرى تكرار جميع معاملات التلقيح المذكورة فى وجود أو غياب مخلوط من بعض العناصر الصغرى والتى تم اضافتها للتربة بعد ٣٠ يوم من الزراعة. كما تم اضافة جرعة تنشيطية من السماد النيتروجينى (٢٠ كجم ن/فدان) لجميع المعاملات تحت الدراسة. وكانت أهم النتائج المتحصل عليها ما يلى:

تشير نتائج تجارب فول الصويا (تجربتى الأصص والحقل) الى فشل النباتات الغير ملقحة فى تكوين أى عقد جذرية كما أعطت أقل قيم لجيمع صفات النمو الخضرى والمحصول (بذور وقش) وكذلك محتوى البذور والقش من البروتين الخام. أدى تلقيح بذور فول الصويا بمعدلات التلقيح

المختلفة الى حدوث زيادة معنوية فى حالة التعقيد (عدد العقد الجذرية ووزنها الجاف) وكذلك جميع صفات النمو الخضرى (الوزن الجاف للمجموع الخضرى ، المحتوى الكلى من الكلوروفيل فى الأوراق ومحتوى المجموع الخضرى من النيتروجين) و كذلك محصول فول الصويا (بذور وقش) ومحتواهم من البروتين الخام. وأشارت النتائج الى أن أستخدام المعدلين المرتفعين من اللقاح البكتيرى (٢ أو ٣جم لقاح/١٠٠ جم بذور) أظهرت أعلى قيم لحالة التعقيد ونشاط أنزيم النيتروجينيز والنمو وجميع قياسات المحصول لنباتات فول الصويا. ومن ناحية أخرى أشارت النتائج الى حدوث زيادة معنوية لجميع الصفات السابقة نتيجة اضافة العناصر الصغرى مقارنة بعدم اضافتها. كذلك أدت الأضافة المشتركة للمعدلات المرتفعة من اللقاح البكتيرى مع العناصر الصغرى الى الحصول على أعلى قيم لحالة التعقيد ونشاط أنزيم النيتروجينيز والنمو ومحصول فول الصويا مقارنة بأستخدام المعدل المنخفض أو المعاملة الغير ملقحة فى وجود أو غياب العناصر الصغرى.

أخذت نتائج الفول البلدى (تجربتى الأصص والحقل) خلال مرحلة النمو الخضرى والمحصول نفس أتجاه نتائج تجارب فول الصويا. كذلك أكدت النتائج أن حالة التعقيد والنمو ومحصول الفول البلدى تأثر معنويا بأستخدام معدلات التلقيح المختلفة. أيضاً فأن حالة التعقيد وجميع صفات النمو والمحصول لنباتات الفول البلدى أستجابت معنوياً لاضافة العناصر الصغرى. كما أكدت النتائج فعالية استخدام المعدلات المرتفعة من اللقاح البكتيرى مع العناصر الصغرى فى الحصول على أعلى قيم للعقد الجذرية (أعداد وأوزان جافة) ونشاط أنزيم النيتروجينيز والنمو وأنتاجية الفول البلدى.

وعليه تشير النتائج السابقة الى أهمية أضافة المغذيات الصغرى مع المعدلات المناسبة من اللقاح البكتيرى الفعال (٦٠٠ جم لقاح لتقاوى الفدان) وذلك لزيادة حيوية ونشاط الريزوبيا والتى يمكن أن توفر الظروف المناسبة لتحسين نمو وأنتاجية البقوليات. وهذا يؤكد أهمية التلقيح فى الأمداد بالنيتروجين الجوى لأستخدامه فى أنتاج المحاصيل البقولية وجودتها، وكذلك للحد من التسميد الكيماوى ودعم نظم الزراعة المستدامة تحت ظروف الأراضى حديثة الأستصلاح.

EFFECT OF VARIOUS BACTERIAL INOCULATION RATES AND MICRONUTRIENTS ON ROOT NODULATION, BIOLOGICAL NITROGEN FIXATION AND PRODUCTION OF SOME LEGUMINOUS CROPS

F.Sh.F. Badawi, A.H. Desoky and A.M.M. Biomy
Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst., ARC, Giza, Egypt.

(Received: Jul. 12, 2011)

ABSTRACT: ***Greenhouse and field trials were executed on a sandy loam soil during summer and winter growing seasons, to evaluate the effect of varying rates of bacterial legume inoculants and application of some micronutrients on root nodulation, N_2-ase activity, some vegetative growth characters and production of soybean and faba bean crops. Soybean seeds was inoculated with a mixture of Bradyrhizobium japonicum (strain USDA-110) and Rhizobium fredii (strain USDA-HH303), while faba bean was inoculated with Rhizobium leguminosarum bv. Viceae (mixture of strains, ICARDA-441 and ICARDA-481) via blending the soybean and faba bean seeds with gamma irradiated vermiculite-based inoculants before sowing. Four inoculation rates (zero, 1.0, 2.0 and 3.0 g inoculum/100g seeds) were used for soybeans. The inoculation rates used for faba beans were zero, 0.75, 1.5 and 2.25g inoculum/100 g seeds. The treatments was replicated in presence or absence of micronutrients composite, applied to soil 30 days after sowing. All treatments received 20 kg N/ fed as a starter dose of nitrogen.***

Results of soybean pot and field experiments showed that the uninoculated plants failed to form nodules on all soybean roots and had the lowest values of vegetative growth features, yield and its crude protein contents. Inoculation of soybean seeds with the different rates of rhizobial inoculum resulted in significant increases in nodulation originated on the plant roots and crop growth aspects (shoot dry mass, total chlorophyll content and shoot N-content) as well as soybean yield (seeds and straw) and its crude protein content. However, seeds inoculated with higher rates of inoculum, i.e. 2.0 or 3.0g /100g seeds achieved higher values of nodulation and all soybean growth and yield characters. Addition of micronutrients led to a substantial increase in nodulation developed on soybean roots, N_2-ase activity and all soybean growth and yield parameters in comparison to the unamended one. Data obtained exhibited the superiority of using higher inoculum rates in combination with the applied micronutrients in achieving the highest values of nodulation and all soybean growth and yield characters as compared with using lower rate or uninoculated treatment with or without micronutrients.

Results of faba bean pot and field experiments along the two vegetative growth stage and yield behaved similarly to those obtained for soybeans. Data confirmed that nodulation, all growth and yield characters of faba beans significantly improved by various the inoculation treatments. Nodulation, all growth features and yield in both experiments positively responded to micronutrients application. Data reconfirmed the stimulative effect of using higher rates of rhizobial inoculum with micronutrients in achieving the

highest values in number and dry weight of nodular tissues, plant growth and crop production.

Key words*: Bradyrhizobium japonicum, Rhizobium fredii, Rhizobium leguminosarum, Micronutrients, Sandy loam soil, Diazotrophy, Inoculation rate, Soybean, Faba bean.*

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] and faba bean (*Vicia faba*) are the most important food legumes and common diet in Egypt, due to the high nutritional value of their seeds. They are considered as available sources for high energy, protein and other nutrient contents for human and livestock. They are used for human consumption of a wide range of traditional dishes, as well as for animal feed. Expanding legumes cultivation in newly reclaimed lands is considered as an important way to increase their productivity and improve soil fertility through stimulating the activity of beneficial soil organisms.

Biological nitrogen fixation (BNF) is a complex process and considered the second important biological process after photosynthesis. It is a substantial source of N in cropping system since atmospheric N_2 is a renewable resource (Bohlool *et al.,* 1992). BNF is one of the most 'environmentally friendly' approach for obtaining nitrogen in agro-ecosystems since BNF uses energy derived from photosynthesis and does not accumulate excess nitrogen to cause pollution (Jensen and Hauggaard–Nielsen, 2003). This process is accomplished in nature by few genera of prokaryotic organisms that can fix atmospheric nitrogen through different systems, including free living, associative and symbiotic (Tilak *et al.,* 2005).

Application of legume-*Rhizobium* symbiosis system as essential strategy to Egyptian soils is very important practice, particularly under the intensive cropping system to decrease chemical inputs and raise soil quality and sustainability. *Rhizobium*-legume symbiosis system is considered the most efficient and important process in crop production. It plays a role in sustaining long-term soil fertility in different agricultural systems (Amarger, 2001). Leguminous plants in partnership with *Rhizobium* have the ability to convert the atmospheric nitrogen into usable forms (Ndakidemi *et al.,* 2006). The use of *Rhizobium* inoculation meets 50-70% of the crop nitrogen requirement and increases legumes productivity. However, our soils do not have either the proper kind of nodule-forming bacteria or enough of them to really bring about good legume growth (Saleh *et al.,* 2010), which may be due to the absence of effective strains in the soil. Many workers have indicated the necessity of legume inoculation with effective and efficient rhizobial strains (Kandil *et al.,* 2008; Badawi *et al.,* 2011 and Kala *et al.,* 2011).

Increasing the efficient of biological nitrogen fixation is a vital process, which should be available to cultivate legumes and to increase the fertility of

soils (Chen *et al.,* 2002). Inoculants should be used when there is a reason to believe that soil populations of the necessary rhizobia bacteria are low such as new reclaimed soils, which represented the vast area of Egypt. Denton and Peoples (2010) mentioned that the efficacy of inoculant is known to vary according to the presence of soil rhizobia. Many factors contribute to high quality legume inoculant products. Most important is that they contain high numbers of live rhizobia that can nodulate and fix nitrogen on the target host legume, and that the inoculum has little or no contamination by other organisms. Many investigators found that increasing rhizobial inoculant rates (10^5 to 10^7 rhizobial cell $seed^{-1}$) lead to a greater rhizosphere colonization, improved nodulation, N_2-fixation and growth of legumes (Brockwell *et al.*, 1988; Heggo and Barakah, 2004 and Herridge, 2008).

The positive effect of micronutrients on the *Rhizobium*-legume symbiosis might be explained by the essential roles in initiation and development of nodular structure, particularly in sandy soil, which suffers from severe deficiency of such micronutrients. Although micronutrients are needed in relatively very small quantities for adequate plant growth and production, their deficiencies induce a great disturbance in the different physiological and metabolic processes inside the plant (Marschner, 1998). For instance, iron promotes formation of chlorophyll, acts as an oxygen carrier and reactions involving cell division and growth. Manganese functions as a part of certain enzyme systems and aids in chlorophyll synthesis. Zinc aids plant growth hormones and enzyme system, necessary for chlorophyll production, carbohydrate formation, starch formation and seed formation. Improving legumes productivity by combining of rhizobia with micronutrients has been reported by many workers (O'Hara, 2001 and Sajid *et al.,* 2008). More recently, Abdel-Wahab *et al.* (2009) elicited that faba bean plants cultivated in sandy soil showed a high response to applied micronutrients and microbial inoculants, which was reflected by the higher values of the plant vigor (dry matter), photosynthesis capacity (chlorophyll content) and N_2-fixation performance (N-content of plant tissues), as well as faba bean yield and its attributes.

The main target of the current investigation is to evaluate the effect of different legume inoculant rates and micronutrients on nodulation, growth and productivity of soybean and faba bean under newly reclaimed soil conditions.

MATERIALS AND METHODS

Preparation of inocula

***Bradyrhizobium japonicum* strain (USDA-110), *Rhizobium fredii* strain (USDA-HH303) and *Rhizobium leguminosarum* bv. *viceae* strains (ICARDA-**

441 and ICARDA-481) were supplied by Biofertilizer Production Unit, Microbiology Dept., Soils, Water and Environ. Res. Instit., ARC, Giza, Egypt. Such bacterial diazotrophs were grown separately on yeast extract mannitol (YEM) broth medium (Vincent, 1970) at 28°C and shaked for 3-5 days, to have population density of 10^9 cfu/ml culture.

Tested pure cultures of *Bradyrhizobium japonicum*, *Rhizobium fredii* (mixture of slow and fast growing rhizobia) and *Rhizobium leguminosarum* were prepared as solid inoculants via the injection of two bacterial cultures (120 ml) into gamma sterilized carrier (polyethylene bags containing 180g vermiculite supplemented with 10% Irish peat) to satisfy 60% of the maximal water holding capacity, then mixed thoroughly and left for a week for curing.

Greenhouse experiments

Two pot experiments were conducted in the greenhouse of the Biological Nitrogen Fixation Unit; Soils, Water and Environ. Res. Instit., ARC, Giza, during both growth seasons, namely summer 2010 and winter 2010/2011 to study the response of soybean and faba bean crops to inoculation with different rates of rhizobia inoculant (Okadeen) and application of micronutrients composite. Nodulation capacity, N2-ase activity, plant shoot dry weight, total chlorophyll content of leaves and shoot nitrogen content were evaluated.

Soil used was collected from El-Tahrir Province Sector in the West of Nile Delta. The main physical and chemical characteristics of the experimental soil were determined according to Piper (1950) and Page *et al*. (1982) and data are presented in Table (1).

Earthenware pots of 30 cm in diameter, each was filled with 10 kg soil. All pots received the recommended dose of superphosphate (15.5% P_2O_5), at a rate of 200 kg/fed before sowing. Potassium sulphate (48.5% K_2O) at a rate of 50 kg/fed was applied at two equal doses (10 and 30 days of sowing). All treatments received ammonium sulphate (20.5%N) at a rate of 20 kg N fed^{-1} as a starter dose of mineral nitrogen.

Seeds of each of soybean (*Glycine max* cv. Giza 111) and faba bean (*Vicia faba,* cv. Giza 40) were supplied by Legume Crops Res. Dept., Field Crops Res. Instit., ARC, Giza, Egypt. The seeds of soybean were inoculated with a mixture of *Bradyrhizobium japonicum* (strain USDA-110) and *Rhizobium fredii* (strain USDA-HH303), while those of faba bean were inoculated with *Rhizobium leguminosarum* bv. *viceae* (mixture of strains, ICARDA-441 and ICARDA-481) via blending the soybean and faba bean seeds with gamma irradiated vermiculite-based inoculant before sowing using Arabic gum as adhesive materials. The pots were randomized in a split-plot design and five seeds of soybean and faba bean were sown in each pot. The following treatments were executed for both experiments:

The main plots:

1- Without micronutrients composite.

2- With micronutrients composite (200 kg $FeSO_4.7H_2O$ + 100 kg $ZnSO_4.7H_2O$ + 150 kg $MnSO_4.1H_2O$ + 4.5 kg citric acid/ fed.), equal to 2.0, 1.0, 1.5 and 0.045 g/pot, respectively, applied to soil 30 days after sowing. Citric acid was applied to make micronutrients in available form.

Table (1): The main physical and chemical characteristics of the experimental soils (for both greenhouse and field trials).

Property	Value	
	Summer seasson (2010)	Winter season (2010/2011)
Mechanical analysis:		
Sand (%)	67.8	68.0
Silt (%)	25.9	25.6
Clay (%)	6.3	6.4
Texture grade	Sandy loam	Sandy loam
Organic matter (%)	0.62	0.65
SP (%)	39.20	40.20
pH (soil paste)	7.35	7.30
ECe (dS/m)	0.39	0.36
Soluble cations and anions (meq/L):		
Ca^{++}	1.76	1.72
Mg^{++}	0.85	0.91
Na^+	0.74	0.72
K^+	0.38	0.33
$CO^=_3$	0.00	0.00
HCO^-_3	0.86	0.94
Cl^-	0.74	0.88
$SO^=_4$	2.13	1.86
Total-N (%)	0.034	0.036
Total soluble-N (ppm)	36.80	40.20
Available-P (ppm)	7.30	7.65
Available-K (ppm)	194.3	188.4
DTPA-extractable (ppm):		
Fe	3.25	3.62
Mn	3.00	3.25
Zn	1.13	1.26
Cu	0.21	0.34

The subplots :

1- Uninoculated (control).

2- 1.0 g inoculum/ 100 g soybean seeds, equal to 300 g inoculum /30 kg seeds/fed and

0.75 g inoculum/100 g faba bean seeds, equal to 300 g inoculum/40 kg seeds/fed.

3- 2.0 g inoculum/ 100 g soybean seeds, equal to 600 g inoculum /30 kg seeds/fed and 1.5 g inoculum/100 g faba bean seeds, equal to 600 g inoculum/40 kg seeds/fed.

4- 3.0 g inoculum/ 100 g soybean seeds, equal to 900 g inoculum /30 kg seeds/fed and 2.25 g inoculum/100 g faba bean seeds, equal to 900 g inoculum/40 kg seeds/fed.

Each treatment was replicated three times and irrigation was made using a stored tap water (to avoid the harmful effect of Cl_2). Plants were thinned after 15 days of planting to three per pot.

After 60 days from sowing, plants were uprooted and plant parts were separated, then prepared for assaying the nodulation status (number and dry weight of nodules), N_2-ase activity and some growth aspects (shoot dry weights, total chlorophyll content of leaves and shoot N-content).

Field experiments

Two field experiments were carried out in a sandy loam soil at El-Tahrir Province in the West of Nile Delta, during the growth seasons, i.e. summer 2010 and winter 2010/2011, to investigate the response of soybean and faba bean crops to inoculation with different rates of diazotrophic bacterial inoculant (Okadeen) in absence or presence of micronutrients composite, and under using drip irrigation system. Root nodulation, nitrogen fixation, some growth aspects and production of soybean and faba bean crops were evaluated.

Inoculated seeds of both leguminous crops used in the greenhouse trial were introduced to this experiment. Split polt design with three replicates was used with the plot area of 13.5 m^2. The main plots were assigned to the micronutrients composite treatments as follows:

1- Without micronutrients composite.

2- With micronutrients composite (200 kg $FeSO_4.7H_2O$ + 100 kg $ZnSO_4.7H_2O$ + 150 kg $MnSO_4.1H_2O$ + 4.5 kg citric acid/ fed.) applied to soil 30 days after sowing.

The subplots were devoted to rhizobial inoculation rates as follows:

1- Uninoculated (control).

2- 300 g inoculum /30 kg soybean seeds/fed and 300 g inoculum/40 kg faba bean seeds/fed.

3- 600 g inoculum /30 kg soybean seeds/fed and 600 g inoculum/40 kg faba bean seeds/fed.

4- 900 g inoculum /30 kg soybean seeds/fed and 900 g inoculum/40 kg faba bean seeds/fed.

Superphosphate (15.5 % P_2O_5) at a rate of 200 kg/fed was added before sowing. Potassium sulfate (48% K_2O) was added at a rate of 50 kg/fed at two equal doses (10 and 30 days of sowing). All treatments received ammonium sulphate (20.5%N) at a rate of 20 kg N fed^{-1} as a starter dose of chemical nitrogen.

After 60 days of sowing, five plants of each of soybean and faba bean were uprooted from each plot to evaluate the root nodulation status, nitrogenase activity, dry weight of shoots, as well as total chlorophyll content of leaves and shoot nitrogen content. At harvest, seed yield (ton/fed), straw yield (ton/fed) and crude protein content of seeds and straw (%) were assayed.

Nitrogenase enzyme activity in fresh plant roots was measured using acetylene reduction assay procedure as described by Hardy *et al*. (1973). Total chlorophyll content in leaf disks was determined according to Arnon (1949). Nitrogen content in plant materials was determined according to Page *et al*. (1982) and crude protein of seeds and straw of soybean and faba bean was calculated by multiplying the N concentration by the factor 6.25. The experimental data obtained were subjected to analysis of variance (ANOVA) according to the procedures outlined by Snedecor and Cochran (1980), treatment means were compared on the basis of LSD at 5% level of probability.

RESULTS AND DISCUSSION

I. Effect of bacterial inoculation rates and micronutrients on the *Bradyrhizobium*-soybean symbiosis and crop production (pot and field experiments)

a) Nodulation status of plant roots:

Nodulation originated on the soybean roots as affected by different rates of rhizobial inoculation and micronutrients application under greenhouse and field conditions are presented in Table (2). Obtained data showed that uninoculated plants failed to form nodules on roots of the tested soybean plants, which indicated that the experimental soils are devoid from soybean specific indigenous rhizobia. These results are in line with the findings of Heggo and Barakah (2004) and Saleh *et al.* (2010). Irrespective of micronutrients application, it is evident that addition of any rate of rhizobial inoculum in both studied experiments resulted in significant increases in nodulation status as compared to uninoculated treatment. Nodule numbers ranged from 20.00 to 39.30 nodule/plant and from 46.50 to 78.15 nodule/plant in greenhouse and field experiments, respectively. Nodule dry weight fluctuated from 157.00 to 261.80 mg/plant and from 218.30 to 444.90 mg/plant in the same respective order. However, seeds inoculated with higher rates of

inoculum 2.0 or 3.0g/100g seeds in the greenhouse (equal to 600 or 900g/30 kg seeds in the field trial) achieved higher values of nodules number and nodules mass on soybean roots rather than lower one. These results reflected the necessary role of soybean inoculation, when rhizobial populations are low, for establishing a distinct nodulation pattern on soybean roots in sandy soil. These results are in accordance with those obtained by Heggo and Barakah (2004), Denton and Peoples (2010) and Saleh et *al.* (2010).

Table (2): Nodulation pattern of soybean roots grown under greenhouse and field conditions as affected by *Bradyrhizobium* inoculation rates and micronutrients composite (60 days of planting).

Treatments	Number of nodules/ plant			Dry weight of nodules (mg/plant)		
	without micro-nutrients	with micro-nutrients	Mean	without micro-nutrients	With micro-nutrients	Mean
	Pot experiment					
Uninoculated (control)	0.00	0.00	0.00	0.00	0.00	0.00
1.0 g inoculum/ 100g seeds	18.00	22.00	20.00	142.70	171.30	157.00
2.0 g inoculum/ 100g seeds	33.30	39.00	36.15	240.40	283.20	261.80
3.0 g inoculum/ 100g seeds	36.30	42.30	39.30	230.20	261.10	245.65
Mean	21.90	25.83		153.33	178.90	
L.S.D. values at 0.05 :						
Micronutrients	2.70			6.70		
Inoculation	3.80			9.50		
Micronutr. x Inoc.	5.40			13.40		
	Field experiment					
Uninoculated (control)	0.00	0.00	0.00	0.00	0.00	0.00
300 g inoculum/ 30 kg seeds	41.70	51.30	46.50	176.60	260.00	218.30
600 g inoculum/ 30 kg seeds	64.70	88.70	76.70	351.60	449.50	400.55
900 g inoculum/ 30 kg seeds	66.30	90.00	78.15	414.70	475.10	444.90
Mean	43.18	57.50		235.73	296.15	
L.S.D. values at 0.05 :						
Micronutrients	3.80			16.80		
Inoculation	5.40			23.80		
Micronutr. x Inoc.	7.60			33.70		

Irrespective of rhizobial inoculation rate, addition of micronutrients led to a significant increases in the number and dry weight of nodules developed on soybean plant roots in comparison to the unamended one. These increases were 17.95 and 33.16% in the number of nodules and were 16.68 and 25.63% in the dry weight of nodules, above the unamended treatment in both the greenhouse and field experiments, respectively. The positive effect of tested micronutrients on nodulation process could be explained by their essential roles in initiation and development of nodular structure, particularly in the newly reclaimed soils, which suffer from severe deficiency of such micronutrients. These results are quite in line with those of Tang *et al.* (1991) and Abdel-Wahab *et al.* (2009).

With regard to the interaction between inoculation rates and micronutrients, there were significant differences due to their interaction in the values of nodulation parameters, which showed superiority of using higher two rates of rhizobial inoculum in the presence of micronutrients in achieving the highest values of nodulation, comparing to using the lower inoculation rate or the uninoculated treatment with or without micronutrients. In the greenhouse experiment, the amximum numbers of nodules were 39.00 and 42.30 nodule/plant and dry weight of nodules were 283.20 and 261.10 mg/plant, respectively were recorded by using higher rates of inoculum (2.0 or 3.0g /100 g seeds) with micronutrients. In the field experiment, the maximum number of nodules (88.70 and 90.00 nodule/plant) and nodules dry weight (449.50 and 475.10 mg/plant), respectively were achived also by using the same treatments. This might be attributed to that the micronutrients were not only essential for the symbiotic interaction, but also for the host plant and its microbial partner leading to hasten the initiation and performance of nodulation process. Similar findings were reported by Amos and Ogendo (2001) who reported that biological nitrogen fixation system is influenced by many environmental factors such as nutritional status particularly micronutrients and their effect on nodulation, fixed nitrogen and nitrogen fixers organisms.

b) Nitrogenase activity of soybean root nodules:

Effectiveness of N_2-fixation was assessed by measuring the specific nitrogenase activity of root nodules. Data in Table (3) showed that nitrogenase activity behaved the same pattern of the nodulation process in both pot and field experiments in regard to the stimulative effect of inoculation on increasing N_2-ase activity as compared with the uninoculated treatment. All of the tested inoculant rates showed the same effect on improving nitrogenase activity of soybean roots. Application of the low level of inoculum significantly increased N_2-ase activity, which gradually increased with increasing the application rate of inoculant up to 2.0 or 3.0g /100g seeds, with no significant differences between them. The increases were 268.66 and 293.10% in the pot experiment, 252.06 and 293.18% in the field trial by using the middle and higher rates of inoculums above the lower one, respectively. In this respect, Heggo and Barakah (2004), Denton and Peoples (2010) and Saleh *et al.* (2010) reported that plants inoculated with the highest density level of rhizobia produced significantly greater N_2 fixation compared with those inoculated with a low and uninoculated treatment.

Data also exerted that N_2-ase activity in both experiments significantly responded to the micronutrients application. The corresponding increases were 12.22% and 25.48% above the unamended treatment in both greenhouse and field experiments, respectively. These findings reflected the promotive impact of micronutrients on nodule formation and enzymatic system responsible for biological nitrogen fixation process. These results

are in harmony with those obtained by Bell *et al.* (2004) and Abdel-Wahab *et al.* (2009).

Table (3): Activity of nitrogenase enzyme of soybean roots grown under greenhouse and field conditions as affected by *Bradyrhizobium* inoculation rates and micronutrients composite (60 days of planting).

Treatments	N_2-ase activity (µmol C_2H_4/plant /hr.)		
	without micro-nutrients	with micro-nutrients	Mean
	Pot experiment		
Uninoculated (control)	0.00	0.00	0.00
1.0 g inoculum/ 100g seeds	4.28	6.43	5.36
2.0 g inoculum/ 100g seeds	18.43	21.08	19.76
3.0 g inoculum/ 100g seeds	20.80	21.34	21.07
Mean	10.88	12.21	
L.S.D. values at 0.05 :			
Micronutrients	1.23		
Inoculation	1.88		
Micronutr. x Inoc.	2.65		
	Field experiment		
Uninoculated (control)	0.00	0.00	0.00
300 g inoculum/ 30 kg seeds	4.26	6.87	5.57
600 g inoculum/ 30 kg seeds	16.65	22.57	19.61
900 g inoculum/ 30 kg seeds	20.84	22.96	21.90
Mean	10.44	13.10	
L.S.D. values at 0.05 :			
Micronutrients	1.66		
Inoculation	2.35		
Micronutr. x Inoc.	3.32		

Concerning the interaction of rhizobial inoculation rate and micronutrients, obtained data confirmed the superiority of using higher rates of rhizobial inoculum in combination with micronutrients in achieving the highest values of N_2-ase activity, compared to using the lower rate or the uninoculated treatment with or without micronutrients. In the greenhouse experiment, the highest values of N_2-ase activity (21.08 and 21.34 µmole C_2H_4/plant/hr) were recorded by using the higher rates of inoculum (2.0 or 3.0 g/100g seeds) with micronutrients. Using the same treatmnets exhibited the highest values of N_2-ase activity, i.e. 22.57 and 22.57 µmole C_2H_4/plant/hr under the field conditions. It could be deduced that there was a close positive relationship between application of micronutrients and nodulation process and consequently biological N_2-fixation. This finding stands in agreement with Amos and Ogendo (2001) and Eisa *et al.* (2011) who attributed the positive effect of micronutrients application to enhancing the activity of rhizobia and thus increasing the nodule number, size and mass, and in turn higher rate of N_2- fixation.

c) Some growth aspects and N-accumulation in soybean crop:

Some growth features of soybean plants, expressed by shoot dry mass, total chlorophyll content of leaves and shoot N-content as affected by rhizobial inoculation rates and micronutrinets are shown in Table (4). Data exerted that the overall growth of soybean plants was significantly affected by the various inoculation treatments. The uninoculated plants had the lowest values of each of shoot dry weight (8.01 and 17.14 g/plant), total chlorohphyll content (3.78 and 5.95 mg/g leave) and shoot N-content (180.50 and 398.90 mg N/plant) in the pot and the field experiments, respectively. Inoculation with the lower rate of inoculum increased significantly all growth parameters comparing to the uninoculated treatment. This trend was true in both investigated experiments. However, inoculation with the higher doses of inoculum recorded significant increases in all growth parameters as compared with lower inoculation rate. Shoot dry weight increased by 30.57 and 30.30%, total chlorophyll content by 15.08% and 17.29% and shoot N-content by 24.32 and 27.43% above the plants inoculated with the lower rate in the greenhouse experiment, respectively. The corresponding increases in the field trial were 27.60 and 30.59% in shoot dry weight, 5.20 and 6.35% in total chlorophyll content and 39.43 and 40.26% in shoot N-content, respectively. There were no significant differences between inoculation with 2.0 and 3.0g inocula/100 g seeds or 600 and 900g inocula/30 kg seeds for all tested parameters in both pot and field conditions. These increases could be due to the biological role of rhizobia in enhancing plant growth, N_2-fixation and the biochemical characteristics of the plant as reported by Mekhemar *et al.* (2005), Tajini *et al.* (2008) and Bambara and Ndakidemi (2010). In this concern, Denton and Peoples (2010) and Kala *et al.* (2011) showed appreciable increase in the biochemical characteristics of the plant (total chlorophyll content and protein content in leaves and seeds) by increasing the rate of *Rhizobium* inoculation.

Irrespective of inoculation effect, application of micronutrients seems a salient effect on the growth aspects and N-content of soybean plants in comparison to the unamended one. For instance, the percentages increase attained for shoot dry mass were 9.23 and 11.71%, for total chlorophyll content were 8.44 and 16.01% and for shoot N-content were 10.98 and 9.53% above the unamended treatment, in both experiments. The promotion occurred in soybean growth features of micronutrients may be due to their effects as a metal component of some enzymes or regulator for the others and their remarkable role in affecting nodule formation, improving plant growth and promotion of photosynthetic activity (Nassar *et al.,* 2006; Abdel-Wahab *et al.,* 2009 and Eisa *et al.*, 2011).

Table (4): Effect of *Bradyrhizobium* inoculation rates and micronutrients composite on some growth aspects of soybean plants grown under

greenhouse and field conditions (60 days of planting).

Treatments	Shoot dry weight (g/plant)			Chlorophyll content of leaves (mg/g)			Shoot N-content (mg/plant)		
	without micro-nutri.	With micro-nutri.	Mean	without micro-nutri.	With micro-nutri.	Mean	without micro-nutri.	With micro-nutri.	Mean
	Pot experiment								
Uninoculated (control)	7.00	9.02	8.01	3.60	3.96	3.78	156.90	204.10	180.50
1.0 g inoculum/ 100g seeds	10.62	11.56	11.09	4.25	4.77	4.51	299.80	323.20	311.50
2.0 g inoculum/ 100g seeds	14.15	14.81	14.48	4.98	5.39	5.19	371.30	403.20	387.25
3.0 g inoculum/ 100g seeds	14.13	14.77	14.45	5.17	5.41	5.29	381.80	412.10	396.95
Mean	11.48	12.54		4.50	4.88		302.45	335.65	
L.S.D. values at 0.05 :									
Micronutrients	0.62			0.28			19.01		
Inoculation	0.88			0.35			26.89		
Micronutr. x Inoc.	1.24			N.S			38.02		
	Field experiment								
Uninoculated (control)	16.10	18.17	17.14	5.25	6.64	5.95	384.90	412.90	398.90
300g inoculum/ 30kg seeds	20.29	24.63	22.46	7.31	8.45	7.88	610.80	779.90	695.35
600g inoculum/ 30kg seeds	27.37	29.95	28.66	7.85	8.74	8.29	949.40	989.60	969.50
900g inoculum/ 30kg seeds	28.43	30.23	29.33	7.83	8.92	8.38	955.70	994.90	975.30
Mean	23.05	25.75		7.06	8.19		725.20	794.33	
L.S.D. values at 0.05 :									
Micronutrients	1.39			0.83			39.50		
Inoculation	1.97			1.17			55.90		
Micronutr. x Inoc.	2.79			N.S			79.10		

On the other hand, the plant vigor and N-accumulated in shoots of the soybean plants gave a high response to the combined application of rhizobia inoculation and micronutrients. There was significant differences due to their interaction in the values of shoot dry weight and N-content, while total chlorophyll content insignificantly responded to such interaction, which is indicative of the independency of each factor in affecting the different parameters. From these results, it has been elicited that soybean plants exhibited high response to bacterial inoculation and micronutrients addition, which was reflected by the higher values of plant vigor (shoot dry matter), photosynthesis capacity (chlorophyll content) and N_2-fixation performance (N-content of plant shoots), particularly those attained as consequence of joint application of the higher rates of inoculum together with micronutrients. Many workers found that inoculated plants reliant on symbiotic nitrogen fixation need micronutrients more than those supplied with mineral nitrogen, indicating that the effect of micronutrients on nitrogen fixation is direct and the symbiosis has a greater micronutrients requirement. Enhancement caused in soybean growth as a consequence of rhizobial inoculation with micronutrients has been evident by several investigators (Amos and Ogendo, 2001; Daza *et al.,* 2003 and Abdel-Wahab *et al.,* 2009).

d) Soybean yield and its crude protein content :

Influence of rhizobial inoculation rates and micronutrients application on the soybean yield and its crude protein in field trial is presented in Table (5). The uninoculated plants recorded the lowest seed yield (0.861 ton/fed), straw yield (1.300 ton/fed), seed crude protein (24.67%) and straw crude protein

(12.07%). In general, soybean yield and its crude protein content revealed high response to raising rhizobial inoculation rate in a consistency with the trend above mentioned for the vegetative growth. As the inoculation rate increased to 300, 600 and 900g inoculum/30 kg seeds, the seed yield of soybean increased by 44.37, 110.34 and 107.90%, respectively above the uninoculated treatment. The corresponding values attained for the straw yield were 36.08, 70.54 and 87.46%, respectively. Crude protein content of soybean seeds recorded increases up to 14.31, 19.01 and 19.54%, while straw crude protein increased by 10.11, 21.46 and 21.38%, respectively above the uninoculated control. The increased soybean yield and its crude protein content in all inoculated treatments might be attributed to the promoted vegetative growth owing to the availability of nitrogen via diazotrophy. Denton and Peoples (2010) and Kala *et al.* (2011) observed a substantial increase in yield and protein content in response to *Rhizobium* inoculation rate, as a result to the nitrogen fixation potential of legumes.

Table (5): Effect of *Bradyrhizobium* inoculation rates and micronutrients composite on soybean yield and its crude protein content under field conditions.

Treatments	Seed yield (ton/fed.)			Straw yield (ton/fed.)		
	without micro-nutrients	with micro-nutrients	Mean	without micro-nutrients	With micro-nutrients	Mean
Uninoculated (control)	0.850	0.872	0.861	1.160	1.440	1.300
300 g inoculum/ 30 kg seeds	1.233	1.253	1.243	1.629	1.909	1.769
600 g inoculum/ 30 kg seeds	1.770	1.852	1.811	2.058	2.376	2.217
900 g inoculum/ 30 kg seeds	1.731	1.849	1.790	2.291	2.582	2.437
Mean	1.396	1.457		1.785	2.077	
L.S.D. values at 0.05 :						
Micronutrients	0.055			0.182		
Inoculation	0.077			0.256		
Micronutr. x Inoc.	0.110			0.360		
	Seed crude protein (%)			Straw crude protein (%)		
Uninoculated (control)	24.00	25.34	24.67	11.95	12.19	12.07
300 g inoculum/ 30 kg seeds	27.59	28.81	28.20	12.68	13.90	13.29
600 g inoculum/ 30 kg seeds	28.82	29.90	29.36	14.33	14.98	14.66
900 g inoculum/ 30 kg seeds	29.00	29.97	29.49	14.31	14.99	14.65
Mean	27.35	28.51		13.32	14.02	
L.S.D. values at 0.05 :						
Micronutrients	0.93			0.63		
Inoculation	1.10			0.77		
Micronutr. x Inoc.	1.87			N.S		

Regardless of rhizobial inoculation rate, results clearly exerted that more increases occurred in soybean yield and its crude protein content due to micronutrients application. The corresponding percentage increases in seed yield, straw yield, seed crude protein and straw crude protein reached to 4.37, 16.36, 4.24 and 5.26%, respectively above the unamended treatments. These findings may indicate that micronutrient deficiencies can reduce

legume productivity by affecting nodule development and function. In these concern, Ahmed *et al.* (2000), Abdel-Wahab *et al.* (2009) and Eisa *et al.* (2011) found that amending legume plants with a mixture of miconutrients favourably induced the number of effective nodules, plant growth, seed yield and seed content of macro and micronutrients.

Data in Table (5) revealed that increasing the applied inoculum dose in combination with micronutrients resulted in significant increases in soybean yield and its crude protein content. For instance, addition of 600 or 900 g inoculum/30 kg seeds in combination with micronutrients achieved values of seed yiled (1.852 and 1.849 ton/fed), straw yield (2.376 and 2.582 ton/fed), seed crude protein (29.90 and 29.97%) and straw crude protein (14.98 and 14.99%), respectively. In this respect, Amos and Ogendo (2001) and Abdel-Wahab *et al.* (2009) reported that using inoculation with rhizobia and addition of micronutrients gave better nodulation and yield of soybean and faba bean crops comparing to the control treatment. They attributed those results to a positive effect of micronutrients on *Rhizobium* agents and nodulation process and subsequently increased N_2-fixation, leading to higher seed yield.

II. Effect of bacterial inoculation rates and micronutrients on the *Rhizobium*-faba bean symbiosis and crop production (pot and field experiments)

a) Nodulation status of plant roots:

Results of faba bean greenhouse and field experiments as affected by the different rates of rhizobial inoculum and micronutrients addition are presented in Table (6). The uninoculated plants were poorly nodulated as they had 9.00 and 29.00 nodules/plant with dry weights of 101.05 and 242.30 mg/plant in the pot and field experiments, respectively. This was due to the establishment of native rhizobia in the experimental soil. Nodulation process initiated on the faba bean roots was extremely enhanced due to inoculation with any level of rhizobia inoculant rather than native rhizobia (control treatment). The lower inoculum rate (0.75 g /100 g seeds, equal to 300 g/40 kg seeds/fed) in the pot and field experiments possessed about 39.50 and 62.10 nodule/plant having dry weights of nodules 252.52 and 536.40 mg/plant, respectively. The maximum nodules number and dry weight were recorded in the treatments inoculated with 1.5 or 2.25g inoculum/100g seeds in the greenhouse and 600 or 900 g inoculum/fed in the field trial. In other words, raising the inoculum level from 0.75 to 2.25 g/100g seeds (300 to 900g inoculum/40 kg seeds) recorded significant increases in number of nodules ranging form 338.89% to 537.11% and nodules dry weight from 149.90% to 310.54% above the uninoculated treatment (control) in the greenhouse experiment. Under field conditions, increases in nodules number ranged from 114.14 to 209.66% with increasing the nodular mass from 121.38 to

198.79%. Data elicited the prominent role of legume inoculation with rhizobia as an efficient approach for improving the nodulation process. Heggo and Barakah (2004), Karahne and Singh (2009), Denton and Peoples (2010) and Kala *et al.* (2011) showed that there were significant increases in nodulation due to increasing the amount of inoculant on legume seeds, especially when low rates of nitrogen fertilizers were added.

Table (6): Nodulation of faba bean roots grown under greenhouse and field conditions as affected by *Rhizobium* inoculation rates and micronutrients composite (60 days of planting).

Treatments	Number of nodules/plant			Dry weight of nodules (mg/plant)		
	without micro-nutrients	with micro-nutrients	Mean	without micro-nutrients	With micro-nutrients	Mean
	Pot experiment					
Uninoculated (control)	8.00	10.00	9.00	90.60	111.50	101.05
0.75 g inoculum/ 100g seeds	37.00	42.00	39.50	222.23	282.80	252.52
1.5 g inoculum/ 100g seeds	50.00	58.00	54.00	335.70	473.00	404.35
2.25 g inoculum/ 100g seeds	55.67	59.00	57.34	365.20	464.50	414.85
Mean	37.67	42.25		253.43	332.95	
L.S.D. values at 0.05 :						
Micronutrients	3.56			11.66		
Inoculation	5.03			16.50		
Micronutr. x Inoc.	7.12			23.29		
	Field experiment					
Uninoculated (control)	22.00	36.00	29.00	238.40	246.20	242.30
300 g inoculum/ 40 kg seeds	60.60	63.60	62.10	483.60	589.20	536.40
600 g inoculum/ 40 kg seeds	82.30	88.00	85.15	655.03	792.90	723.97
900 g inoculum/ 40 kg seeds	87.60	92.00	89.80	648.10	772.30	710.20
Mean	63.13	69.90		506.28	600.15	
L.S.D. values at 0.05 :						
Micronutrients	5.50			25.80		
Inoculation	6.75			31.57		
Micronutr. x Inoc.	11.02			51.55		

Irrespective of rhizobial inoculation rate, nodule traits of faba bean roots were better as a consequence of micronutrients composite application. The corresponding increases in number and dry weight of nodules were 12.16% and 31.38%, respectively above the unamended treatment of the greenhouse experiment. Under field conditions, the corresponding values attained for nodules number and dry mass were 10.72% and 18.54%, respectively. Encouraging the nodulation capacity with application of micronutrients reflected the vital role of these elements in enhancing nodule formation. These results are in agreement with those obtained by Ahmed *et al.* (2000) and Abdel-Wahab *et al.* (2009).

Concerning the interaction between rhizobial inoculation rates and micronutrients application, data displayed that the superior results were attained by using higher rates of rhizobial inoculum in combination with micronutrients application for the nodulation status rather than using the

higher level of inoculum without micronutrients or using lower level of inoculum with or without micronutrients. This tendency was obtained during both pot and field experiments. These results are presumably due to the vital role of such micronutrients for leguminous plants reliable on N_2-fixation, particularly under sandy soil conditions, which is commonly poor in micronutrients. In this concern, Hegazi *et al.* (1993), Amos and Ogendo (2001) and Abdel-Wahab *et al.* (2009) reported that using inoculation with rhizobia and addition of micronutrients gave better nodulation of soybean and faba bean crops as compared with the control treatment.

b) Nitrogenase activity of faba bean root nodules:

Data of nitrogenase activity of faba bean root nodules as affected by rhizobial inoculation rates and micronutrients composite are presented in Table (7). It is worthy to mention that the activity of nitrogenase enzyme was parallel to the trends obtained for the number and dry weight of faba bean root nodules, as the values tended to increase significantly by increasing the inoculum rate. The rate of acetylene reduction by root nodules (ARA) for the different treatments showed the values (3.59 and 4.18 µmole C_2H_4/plant/hr) for the uninoculated treatment in the pot and field experiments, respectively. The lowest rhizobial inoculation dose (0.75 g/100 g seeds, equal to 300 g/40 kg seeds) increased the rate of acetylene reduction as they recorded 15.32 and 18.28 µmole C_2H_4/plant/hr, respectively. The maximal rates of acetylene reduction (20.64 and 21.69 µmole C_2H_4/plant/hr) were recorded in the treatments inoculated with 1.5 and 2.25g inoculum/100g seeds, respectively. In the field trail, the corresponding higher values of ARA were 22.59 and 22.56 µmole C_2H_4/plant/hr for the treatments inoculated with 600 and 900g/40 kg seeds, respectively with no significant differences between them. Inoculation of faba bean seeds with *Rhizobium* activated nitrogenase enzyme in the root nodules to fix more atmospheric nitrogen. In this concern, Heggo and Barakah (2004), Karahne and Singh (2009) and Denton and Peoples (2010) found that inoculation with a high rate of effective rhizobia inoculum significantly stimulated nodulation and nitrogen fixation, as compared with the uninoculated treatment.

Irrespective of inoculation rate, application of micronutrients to the soil tended to significantly increase the activity of nitrogenase enzyme in comparison with the unamended one. Obviously, ARA increased by 13.53 and 10.92% above the unamended treatment. This emphasized the important role of micronutrients, to be considered as an important practice for increasing root nodulation and biological nitrogen fixation in legumes. These findings are in consistence with those obtained by Daza *et al.* (2003), Bell *et al.* (2004) and Abdel-Wahab *et al.* (2009) who reported that absence of micronutrients, biological nitrogen fixation was strongly inhibited. They added that the declination of nitrogenase activity under micronutrients

deficiency could be partly caused by impaired leghemoglobin synthesis and limited bacteriods proliferation.

Table (7): Activity of nitrogenase enzyme of faba bean roots grown under greenhouse and field conditions as affected by *Rhizobium* inoculation rates and micronutrients composite (60 days of planting).

Treatments	N_2-ase activity (µmol C_2H_4/plant /hr.)		
	without micro-nutrients	with micro-nutrients	Mean
	Pot experiment		
Uninoculated (control)	3.24	3.93	3.59
0.75 g inoculum/ 100g seeds	14.05	16.59	15.32
1.5 g inoculum/ 100g seeds	19.39	21.89	20.64
2.25 g inoculum/ 100g seeds	20.67	22.72	21.69
Mean	14.34	16.28	
L.S.D. values at 0.05 :			
Micronutrients	1.72		
Inoculation	2.63		
Micronutr. x Inoc.	3.89		
	Field experiment		
Uninoculated (control)	3.69	4.67	4.18
300 g inoculum/ 40 kg seeds	17.55	19.01	18.28
600 g inoculum/ 40 kg seeds	21.36	23.81	22.59
900 g inoculum/ 40 kg seeds	21.50	23.62	22.56
Mean	16.03	17.78	
L.S.D. values at 0.05 :			
Micronutrients	1.59		
Inoculation	1.99		
Micronutr. x Inoc.	3.39		

Concerning the interaction, data in Table (7) confirmed that the application of higher rhizobial inoculation rate combined with micronutrients showed a superiority in enhancing the activity of nitrogenase enzyme in both experiments. These findings may indicate that the presence of such micronutrients composite with rhizobial inoculum might act to sustain their action resulting in an enhanced nitrogen fixation performance (Amos and Ogendo, 2001; Abdel-Wahab *et al.,* 2009 and Eisa *et al.,* 2011).

c) Some growth aspects and N-accumulation in faba bean crop:

Response of faba bean plants to rhizobial inoculation rate and application of micronutrients was represented by some growth aspects namely, shoot dry weight, total chlorophyll content of leaves and shoot N-content of plant tissues under greenhouse and field conditions (Table 8). Irrespective of micronutrients application, the uninoculated plants recorded the lowest values of shoot dry weight (4.68 and 11.08 g/plant), total chlorophyll content (4.78 and 5.14 mg/g) and shoot N-content (103.05 and 291.45 mg N/plant) in

the pot and field experiments, respectively. Increasing the applied dose of rhizobial inoculum resulted in gradual increases in all investigated traits in the two experiments. Addition of 1.5 and 2.25g/100 g seeds resulted in 7.50 & 7.74 g/plant shoot dry matter, 7.42 & 7.35 mg/g total chlorophyll content and 226.95 & 226.35 mg N/plant, respectively. The corresponding values obtained in the field experiment were 25.71 & 25.59 g/plant, 7.95 & 8.02 mg/g and 781.85 & 790.97 mg N/plant. There were no significant differences between the treatments with 1.5 or 2.25g inoculum/100g seeds in the pot experiment and 600 or 900g inoculum/40 kg seeds in the field trial. It was clear that *Rhizobium* inoculation is important and plays a crucial role in improving plant growth, N-accumulation and level of chlorophyll content in leaves relative to the uninoculated control. The present results are in agreement with those obtained by Herridge (2008), Bambara and Ndakidemi (2010) and Kala *et al.* (2011) who observed increases in plant dry weight, nitrogen content and total chlorophyll content in leaves of legumes due to the role of rhizobial inoculation.

Table (8): Effect of *Rhizobium* inoculation rates and micronutrients composite on some growth aspects of faba bean plants grown under greenhouse and field conditions (60 days of planting).

Treatments	Shoot dry weight (g/plant)			Chlorophyll content of leaves (mg/g)			Shoot N-content (mg/plant)		
	without micro-nutri.	With micro-nutri.	Mean	without micro-nutri.	With micro-nutri.	Mean	without micro-nutri.	With micro-nutri.	Mean
	Pot experiment								
Uninoculated (control)	4.47	4.88	4.68	4.22	5.33	4.78	96.40	109.70	103.05
0.75 g inoculum/ 100g seeds	6.10	6.67	6.39	5.42	6.50	5.96	188.70	216.00	202.35
1.5 g inoculum/ 100g seeds	7.20	7.80	7.50	6.91	7.92	7.42	220.70	233.20	226.95
2.25 g inoculum/ 100g seeds	7.65	7.82	7.74	6.81	7.89	7.35	221.50	231.20	226.35
Mean	6.36	6.79		5.84	6.91		181.83	197.53	
L.S.D. values at 0.05 :									
Micronutrients	0.33			0.31			13.49		
Inoculation	0.47			0.43			23.33		
Micronutr. x Inoc.	0.67			N.S			32.99		
	Field experiment								
Uninoculated (control)	10.33	11.82	11.08	4.35	5.92	5.14	272.20	310.70	291.45
300g inoculum/ 40kg seeds	21.02	22.81	21.92	6.00	6.65	6.33	623.50	674.70	649.10
600g inoculum/ 40kg seeds	24.57	26.85	25.71	7.62	8.28	7.95	765.80	797.90	781.85
900g inoculum/ 40kg seeds	24.68	26.50	25.59	7.73	8.30	8.02	778.40	803.53	790.97
Mean	20.15	22.00		6.43	7.29		609.98	646.71	
L.S.D. values at 0.05 :									
Micronutrients	1.74			0.52			17.22		
Inoculation	2.13			0.84			21.09		
Micronutr. x Inoc.	3.47			N.S			34.45		

Irrespective of rhizobial inoculation rate, results reconfirmed that the application of micronutrients composite was a distinct practice for improving the faba bean plant vigor. The corresponding increases in shoot dry weight, total chlorophyll of leaves and shoot N-content were 6.76, 18.32 and 8.63% above the unamended one, respectively. In the field experiment, the increases were 9.18, 13.37 and 6.02%, respectively in the same order. These results exhibited the essential role of micronutrients on the faba bean plant

vigor, indicating that many physiological and biological activities of N_2-fixing systems require micronutrients supplement. In this respect, Ahmed *et al.* (2000), Nassar *et al.* (2006), Abdel-Wahab *et al.* (2009) and Eisa *et al.* (2011) reported that the chlorophyll is an indication to photosynthesis process, which mainly fueled the nitrogen fixation process, consequently the increased chlorophyll content could enhance the performance of biological nitrogen fixation and accumulation N in plant tissues.

Data in Table (8) exhibited that all of the tested growth features seemed to highly improved by the application of micronutrients, especially at the highest inoculation rate. The synergistic effect between the higher inoculation rates (1.5 and 2.25 g inoculum/100 g seeds) together with micronutrients addition achieved better values of shoot dry weight (7.80 and 7.82 g/plant), total chlorophyll content (7.92 and 7.89 mg/g) and shoot N-content (233.20 and 231.20.8mg N/plant), respectively. In the field trail, the corresponding values, due to such interaction between 600 and 900 g inoculum/40 kg seeds conjugating with micronutrients were 26.85 & 26.50 g/plant, 8.28 & 8.30 mg/g and 797.90 & 803.53 mg N/plant, respectively. Many investigators mentioned the positive effects of synergy between micronutrients application and rhizobia inoculation on the photosynthetic carbon assimilation and leaf chlorophyll content, as well as their consequences on nitrogen fixation and the quantities of N incorporation in plant organs (Amos and Ogendo, 2001; Abdel-Wahab *et al.,* 2009 and Eisa *et al.,* 2011).

d) Faba bean yield and its crude protein content :

Faba bean yield and its crude protein content as affected by rhizobial inoculation rates and micronutrients addition are reported in Table (9). Irrespective of micronutrients introduction, it is evident that faba bean yield achieved the highest productivity as a result of increasing the bacterial inoculation rate. The uninoculated plants recorded the lowest values of seed yield (0.884 ton/fed), straw yield (1.042 ton/fed), seed protein (23.06%) and straw protein (10.76%). The percentages increase attained in seed yield, straw yield, seed protein and straw protein contents as a function of the lowest rate of inoculum (300g /40 kg seeds) were 40.27, 71.50, 7.20 and 15.71%, respectively above the uninoculated treatment. As the inoculation rate was elevated to 600 and 900g inoculum/40 kg seeds, the seed yield of faba bean increased by 65.61 and 62.44%, respectively above the uninoculated treatment. The corresponding values gained for the straw yield were 104.22 and 104.32%, respectively. Crude protein content of faba bean seeds recorded increases up to 12.75 and 12.92%, respectively. Increases in the straw crude protein were 34.29 and 37.45% above the uninoculated control. However, there were no significant differences in the faba bean yield and its crude protien content between the two higher inoculum rates. These

findings clearly emphasize the stimulatory role of efficient rhizobial strains in survival of such diazotrophs and thus improve the plant growth, N_2-fixation and crop production (Mekhemar *et al*., 2005; Bambara and Ndakidemi, 2010 and Denton and Peoples, 2010).

Table (9): Effect of *Rhizobium* inoculation rates and micronutrients composite on faba bean yield and its crude protein content under field conditions.

Treatments	Seed yield (ton/fed.)			Straw yield (ton/fed.)		
	without micro-nutrients	with micro-nutrients	Mean	without micro-nutrients	With micro-nutrients	Mean
Uninoculated (control)	0.771	0.996	0.884	0.959	1.125	1.042
300 g inoculum/ 40 kg seeds	1.115	1.365	1.240	1.689	1.884	1.787
600 g inoculum/ 40 kg seeds	1.432	1.496	1.464	2.072	2.184	2.128
900 g inoculum/ 40 kg seeds	1.382	1.489	1.436	2.086	2.172	2.129
Mean	1.175	1.337		1.702	1.841	
L.S.D. values at 0.05 :						
Micronutrients	0.125			0.135		
Inoculation	0.165			0.166		
Micronutr. x Inoc.	0.301			0.272		
	Seed crude protein (%)			Straw crude protein (%)		
Uninoculated (control)	22.40	23.71	23.06	10.31	11.20	10.76
300 g inoculum/ 40 kg seeds	24.08	25.36	24.72	12.11	12.79	12.45
600 g inoculum/ 40 kg seeds	25.04	26.96	26.00	14.27	14.63	14.45
900 g inoculum/ 40 kg seeds	25.42	26.66	26.04	14.48	15.10	14.79
Mean	24.24	25.67		12.79	13.43	
L.S.D. values at 0.05 :						
Micronutrients	1.01			0.54		
Inoculation	1.22			0.78		
Micronutr. x Inoc.	N.S			N.S		

On the other instance, addition of micronutrients resulted in significant increases in faba bean yield (seeds and straw) as compared to the unamended treatment. Also, micronutrients behaved the same way in augmenting the contents of seed and straw crude protein. Soil application of micronutrients, under field conditions achieved increases reaching to 13.79, 8.17, 5.90 and 5.00% for seed yield, straw yield, seed and straw protein contents, respectively above the unamended treatment. Many workers documented that the deficiency of micronutrients (Zn, Mn, Fe and Cu) in soils limits crop yield and quality. In this respect, Sajid *et al.* (2008) and Eisa *et al.* (2011) added that the positive marked increases in growth and yield of legumes by application of micronutrients could be due to a maintained balance of plant physiology.

Data in Table (9) elicited that the promotive effect of rhizobial inoculum was magnified by presence of micronutrients, due to enhancing nodulation and nitrogen fixation resulting in a high production of faba bean crop. The highest values of seed yield, straw yield, seed protein and straw protein were 1.496 ton/fed, 2.184 ton/fed, 26.96% and 14.63% were recorded respectively

for the treatment inoculated with 600g/40 kg seeds in combination with micronutrients application. These findings may indicate that such micronutrients composite was able to improve the efficiency of faba bean-*Rhizobium* symbiosis. The synergistic effect of *Rhizobium* inoculation and micronutrients addition on legumes production in newly reclaimed soils has been confirmed by many workers (Hegazi *et al.,* 1993; Abdel-Wahab *et al.,* 2009 and Eisa *et al.,* 2011).

Results of the present study conform the importance of micronutrients application with an appropriate rate of efficeint rhizobial inoculum (600 g/fed) to improve pulse legume growth and development, as well as higher crop yield and quality. Environmentally, such practice supports the sustainable agriculture concept, particularly under newly reclaimed soil conditions.

REFERENCES

Abdel-Wahab, A.F.M., A.M.M. Biomy and W.M. El-Farghal (2009). Co-composting of plant residues and their utility with micronutrients to enhance productivity of faba bean-*Rhizobium* symbiosis under sandy soil conditions. Egypt. J. Appl. Sci., 24: 343-368.

Ahmed, A.Kh., A.M.A. Ali and Wafaa M. El-etr (2000). Effect of some micronutrients spraying on growth, yield and mineral constituents of soybean plant. Ann. Agric. Sci., Moshtohor, 38: 2675- 2686.

Amarger, N. (2001). Rhizobia in the Field. Advances in Agronomy (Ed. D.L. Sparks), Academic Press. 73: 109-167.

Amos, A.O.M. and O.J. Ogendo (2001). Response of common bean to *Rhizobium* inoculation and fertilizers. J. Food Technol. Afr., 6: 121-125.

Arnon, D.J. (1949). Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.

Badawi, F.Sh.F., A.M.M. Biomy and and A.H. Desoky (2011). Peaunt plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizo-microorganims under sandy loam soil conditions. Annals Agric. Sci., Fac. Agric., Ain Shams Univ., 56: 17-25B.

Bambara, Sylvie and P.A. Ndakidemi (2010). *Phaseolus vulgaris* response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. Afr. J. Agric. Res., 5: 1804-1811.

Bell, R.W., B. Dell and L. Huang (2004). Importance of micronutrients in crop nutrition. IFA Inter. Sym. on Micronutrients, 23-25 February, New Delhi, India.

Bohlool, B.B., J.K. Ladha; D.P. Garrity and T. George (1992). Biological nitrogen fixation for sustainable agriculture: A perspective. Plant & Soil, 141: 1-11.

Brockwell, J., D.F. Herridge, L.J. Morthorpe and R.J. Roughley (1988). Numerical effects of *Rhizobium* population on legume symbiosis. In: Nitrogen Fixation by Legumes in Mediterranean Agriculture. ICARDA, Beck, D.P. and Materon, L.A. (Eds.), 179-193.

Chen, L.S., A. Figueredo, H. Villani, J. Michajluk and M. Hungria (2002). Diversity and symbiotic effectiveness of rhizobia isolated from field grown soybean nodules in Paraguay. Biol. Fertil. Soils, 35: 448-457.

Daza, A., C. Santamaria, M. Camacho, D.N. Rodriguez-Navarro and F. Temprano (2003). Influence of micronutrients on biological nitrogen fixation in bean (*Phaseolus vulgaris* L.) under greenhouse hydroponic culture conditions. Spanish J. Agric. Res., 1: 77-80.

Denton, M.D. and M.B. Peoples (2010). The importance of inoculant type or application rate for nitrogen fixation by grain legumes in cropping systems of south-eastern Australia. "Food Security from Sustainable Agriculture". H. Dove and R.A. Culvenor (Eds), Proceedings of 15th Agronomy Conf., 15-18 November, Lincoln, New Zealand.

Eisa, Salwa A.I., M.B. Taha M.A.M. Abdalla (2011). Amendment of soil fertility and augmentation of the quantity and quality of soybean crop by using phosphorus and micronutrients. Inter. J. Academic Res., 3: 800-808.

Hardy, R.W.F., R.C. Burns and R.D. Holsten (1973). Application of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem.,5: 47-81.

Hegazi, M.H., D.N. Abadi and S.A. Genaidy (1993). Effects of some micronutrients and methods of application and *Rhizobium* inoculation on faba bean. Egypt. J. Agric. Res., 71: 21-33.

Heggo, A.M. and F.N. Barakah (2004). Effects of inoculum densities of *Rhizobium meliloti* and different rates of nitrogen fertilizers on alfalfa plants grown in calcareous soil. J. King Saud Univ., Agric. Sci., Riyadh, 16: 161-170.

Herridge, D.F. (2008). Inoculation technology for legumes. In: Dilworth M.J, James E.K, Sprent J.I, Newton W.E (Eds.), Leguminous Nitrogen-Fixing Symbioses. Kluwer, Netherlands, pp. 77–115.

Jensen, E.S. and H. Hauggaard-Nielsen (2003). How can increased use of biological nitrogen fixation in agriculture benefit the environment? Plant & Soil, 252:177-186.

Kala, Christy T., Mary R. Christi and N.R. Bai (2011). Effect of *Rhizobium* inoculation on the growth and yield of horsegram (*Dolichos biflorus linn*). Plant Archives, 11: 97-99.

Kandil., B.A.A., F.Sh.F. Badawi., M.G.M. El-Baz and A.F.M. Abdel-Wahab (2008). Response of peanut yield to inoculation with both *Bradyrhizobium*

sp. and silicate bacteria (*Bacillus Circulans*) under graded levels of feldspar amendment. J. Agric. Sci. Mansoura Univ., 33 : 4463-4477.

Karahne, V. and V.P. Singh (2009). Effect of rhizobial inoculation on growth, yield, nodulation and biochemical characters of vegetable pea (*Pisum sativum*). J. Acta Agronomica Hungarica, 57: 47-56.

Marschner, H. (1998). "Mineral Nutrition of Higher Plants". Harcourt Brace & company. Publishers, London, New York, Tokyo.

Mekhemar, G.A.A., M. Shaaban, A.A. Ragab and A.M.M. Biomy (2005). Response of faba bean to inoculation with *Rhizobium leguminosarum* bv. *Viceae* and plant growth promoting rhizobacteria under newly reclaimed soils.Egypt. J. Appl. Sci.,20: 126−144.

Nassar, K.E.M., Magda A. Ewais and Awatef A. Mahmoud (2006). Raising of the quantity and quality of onion crop by foliar application of phosphorus and sulphur. J. Adv. Agric. Res., Saba Basha Agric., 11: 409-419.

Ndakidemi, P.A., F.D. Dakora, E.M. Nkonya, D. Ringo and H. Mansoor (2006). Yield and economic benefits of common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L. *Merr.*) inoculation in northern Tanzania. Aust. J. Exp. Agric., 46: 571-577.

O'Hara, G.W. (2001). Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation. Australian J. Exp. Agric., 41: 417-433.

Page, A.L., R.H. Miller and D.R. Keeney (1982). “Methods of Soil Analysis”. II-Chemical and Microbiological Properties. Soil Sci. Amer., 2nd Ed. Madison Wisconsin, USA.

Piper. C. S. (1950). "Soil and Plant Analysis". 1st ed. Interscience Puplishers Inc. New York, USA., pp. 30 -229.

Sajid A., A.R. Khan, G. Mairaj, M. Fida and S. Bibi (2008). Assessment of different crop nutrient management practices for yield improvement. Australian J. of Crop Sci., 2: 150-157.

Saleh, S.A.; D.M. Swelim; Elham I. El-Khatib; F.Sh.F. Badawi; A.H. Desoky and S.H. Youseif (2010). Compatibility of local *B. japonicum* isolates with soybean varieties grown in egypt as compared to reference strains of rhizobia. N. Egypt. J. Microbiol., 27: 67-79.

Snedecor, G.W. and W.G. Cochran (1980). "Statistical Methods". 7th Ed., pp.255-269. Iowa State Univ. Press, Am., USA.

Tajini F., J.J. Drevon, L. Lamouchi, M.E. Aouani and M. Trabelsi (2008). Response of common bean lines to inoculation: comparison between the *Rhizobium tropici* CIAT899 and the native *Rhizobium etli* 12a3 and their persistence in Tunisian soils. World J. of Microbiol. & Biotechnol., 24: 407-417.

Tang, C., A.D. Robson and M.J. Dilworth (1991). Inadequate iron supply and high bicarbonate impair the symbiosis of peanut (*Arachis hypogaea* L.) with different *Bradyrhizobium* strains. Plant & Soil. 138: 159-186.

Tilak, K.V.B.R., N. Ranganayaki, K.K. Pal, R.De.A.K. Saxena, C.S. Nautigal, S. Mittal, A.K. Tripathi and B.N. Tohri (2005). Diversity of plant growth and health supporting bacteria. Curr. Sci., 89: 136-150.

Vincent, J. M. (1970). "A Manual for the Practical Study of the Root Nodule Bacteria". I. B. P. Handbook, No. 15, Blackwell Scientific Publishers, Oxpford, UK.

تأثير معدلات مختلفة من التلقيح البكتيرى والعناصر الصغرى على تعقيد الجذور وتثبيت النيتروجين الجوى وإنتاج بعض المحاصيل البقولية

فريد شوقى فريد بدوى – عبد الناصر حسن دسوقى –
عبد الحميد محمود محمد بيومى
قسم بحوث الميكروبيولوجيا الزراعية– معهد بحوث الأراضي والمياه والبيئة –
مركز البحوث الزراعية – الجيزة– مصر.

الملخص العربى

أجريت تجربتى أصص بصوبة وحدة أنتاج الأسمدة الحيوية– معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية وكذلك تجربتين حقليتين بقطاع جنوب التحرير فى أرض رملية طميية خلال الموسم الصيفى ٢٠١٠ والموسم الشتوى ٢٠١٠/٢٠١١ لدراسة تأثير أستخدام معدلات مختلفة من اللقاح البكتيرى مع اضافة بعض العناصر الصغرى على حالة تعقيد الجذور ونشاط أنزيم النيتروجينيز وبعض صفات النمو الخضرى وأنتاجية نباتات فول الصويا والفول البلدى. تم تلقيح بذور فول الصويا بخليط من (USDA-110) *Bradyrhizobium japonicum* و (USDA-HH303) *Rhizobium fredii* ، بينما تم تلقيح بذور الفول البلدى بالـ *Rhizobium leguminosarum* bv. *Viceae* خليط من سلالتين (ICARDA-441 and ICARDA-481) وذلك بخلط بذور فول الصويا والفول البلدى قبل الزراعة باللقاحات المضافة للحامل البكتيرى الصلب المعقم بأشعة جاما. وقد تم أستخدام أربع معدلات من التلقيح مع فول الصويا وهى (بدون تلقيح ، ١ ، ٢ ، ٣ جم لقاح/ ١٠٠ جم بذور). وكانت معدلات التلقيح المستخدمة مع الفول البلدى هى (بدون تلقيح، ٠.٧٥ ، ١.٥ ، ٢.٢٥ جم لقاح/ ١٠٠ جم بذور). وجرى تكرار جميع معاملات التلقيح المذكورة فى وجود أو غياب مخلوط من بعض العناصر الصغرى والتى تم اضافتها للتربة بعد ٣٠ يوم من الزراعة. كما تم اضافة جرعة تنشيطية من السماد النيتروجينى (٢٠ كجم ن/فدان) لجميع المعاملات تحت الدراسة. وكانت أهم النتائج المتحصل عليها ما يلى:

تشير نتائج تجارب فول الصويا (تجربتى الأصص والحقل) الى فشل النباتات الغير ملقحة فى تكوين أى عقد جذرية كما أعطت أقل قيم لجيمع صفات النمو الخضرى والمحصول (بذور وقش) وكذلك محتوى البذور والقش من البروتين الخام. أدى تلقيح بذور فول الصويا بمعدلات التلقيح المختلفة الى حدوث زيادة معنوية فى حالة التعقيد (عدد العقد الجذرية ووزنها الجاف) وكذلك جميع صفات النمو الخضرى (الوزن الجاف للمجموع الخضرى ، المحتوى الكلى من الكلوروفيل فى الأوراق ومحتوى المجموع الخضرى من النيتروجين) و كذلك محصول فول الصويا (بذور

وقش) ومحتواهم من البروتين الخام. وأشارت النتائج الى أن أستخدام المعدلين المرتفعين من اللقاح البكتيرى (٢ أو ٣جم لقاح/١٠٠ جم بذور) أظهرت أعلى قيم لحالة التعقيد ونشاط أنزيم النيتروجينيز والنمو وجميع قياسات المحصول لنباتات فول الصويا. ومن ناحية أخرى أشارت النتائج الى حدوث زيادة معنوية لجميع الصفات السابقة نتيجة اضافة العناصر الصغرى مقارنة بعدم اضافتها. كذلك أدت الأضافة المشتركة للمعدلات المرتفعة من اللقاح البكتيرى مع العناصر الصغرى الى الحصول على أعلى قيم لحالة التعقيد ونشاط أنزيم النيتروجينيز والنمو ومحصول فول الصويا مقارنة بأستخدام المعدل المنخفض أو المعاملة الغير ملقحة فى وجود أو غياب العناصر الصغرى.

أخذت نتائج الفول البلدى (تجربتى الأصص والحقل) خلال مرحلة النمو الخضرى والمحصول نفس أتجاه نتائج تجارب فول الصويا. كذلك أكدت النتائج أن حالة التعقيد والنمو ومحصول الفول البلدى تأثر معنويا بأستخدام معدلات التلقيح المختلفة. أيضاً فأن حالة التعقيد وجميع صفات النمو والمحصول لنباتات الفول البلدى أستجابت معنوياً لاضافة العناصر الصغرى. كما أكدت النتائج فعالية استخدام المعدلات المرتفعة من اللقاح البكتيرى مع العناصر الصغرى فى الحصول على أعلى قيم للعقد الجذرية (أعداد وأوزان جافة) ونشاط أنزيم النيتروجينيز والنمو وأنتاجية الفول البلدى.

وعليه تشير النتائج السابقة الى أهمية أضافة المغذيات الصغرى مع المعدلات المناسبة من اللقاح البكتيرى الفعال (٦٠٠ جم لقاح لتقاوى الفدان) وذلك لزيادة حيوية ونشاط الريزوبيا والتى يمكن أن توفر الظروف المناسبة لتحسين نمو وأنتاجية البقوليات. وهذا يؤكد أهمية التلقيح فى الأمداد بالنيتروجين الجوى لأستخدامه فى أنتاج المحاصيل البقولية وجودتها، وكذلك للحد من التسميد الكيماوى ودعم نظم الزراعة المستدامة تحت ظروف الأراضى حديثة الأستصلاح.