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INFLUENCE OF APPLIED BIOFERTILIZER ON PRODUCTIVITY ,QUALITY AND NUTRIENTS CONTENT OF SOME SOYBEAN CULTIVARS UNDER SALINE SOIL CONDITIONS.

Manal A. Attia[1,] Amal H. El-Guibali[1]; Kh. A. Shaban [1] and M. I. Abdel Mhosen [2]

1-Soils, Water and Environment Research Institute, Agric. Res. Centre Giza, (Egypt).

2- Food Legumes Dep. Field Crop Res. Inst., Agric. Rese. Center, Giza – Egypt

ABSTRACT.

In order to study the effects of soybean cultivars (Giza 111 ,Giza21 ,Giza22 ,Giza35 and Crawford) as well as to compare inoculation with non-inoculation of PGPR (Plant Growth –Promoting *Rhizobacteria*) as biofertilizers on seed yield ,proline content ,protein% , oil% and the uptake of macro-micronutrients in different cultivars of soybean, two experiments were conducted at El-Quntra Shark Farm, East of Suzie Canal, Ismalia Governorate, The site lies in the north-waste in coast of Sinai, between 32^{o}- 35' and 32^{o} – 45' E and 31^{o}- 00' and 31^{o} - 25^{o} N, (Kaiser, 2009) , Egypt during the two successive summer seasons of 2012 and 2013. The experimental plots were divided into two main groups. The first group was without bio-fertilizer and treated only by one rate of 30kg N fed^{-1} which applied as urea (46 % N). The second group was treated with bio-fertilizer combined with 20 kg N fed^{-1}. The obtained results reported that seeds of Giza 35 and Crawford cultivars without using biofertilizer showed a significant increase in seed yield, weight of pods (Mg/fed) and 100-seed weight (g), concentration and uptake of macro and micronutrients. Also, the untreated seeds significantly affect on proline content, protein and oil percentage; while Crawford cultivar recorded the lowest value of proline and the highest values in protein and oil percentage. The inoculation of seeds for different cultivars of soybean gave the highest values of seed yield, weight of pods, protein and oil percentage and low proline content in comparison to untreated seeds. Also, the seeds treated with biofertilizer recorded a significant increase in the concentration and the uptake of macro and micronutrients. The interaction between cultivars and inoculated seeds indicated that Giza 35 surpassed the other cultivars in seed yield, weight of pods, 100-seed weight and was significant in uptake of Fe, Mn and Zn in the first season, while, in the second season, Crawford cultivar gave the highest values in seed yield, weight of pods and the uptake of Mn and Zn as well as gave significant increase in concentration of Fe, Mn and Zn in both seasons. Proline content was significantly response to inoculation with biofertilizer in Giza35 and Crawford cultivars and gave the lower values than the other cultivars, while protein and oil % were significant increase in the same cultivars. The data reveal that according to the interaction, Giza 22 cultivar recorded highly significantly enhancement for P% only in both seasons and highest uptake of P and K for the second season. The analysis of soil after soybean harvested showed that soil pH and EC values were lowered in all the studied cultivars. Also, the available content of N, P and K were significantly increased, while, the available of Fe, Mn and Zn were non-significant in comparison with untreated seeds.

Therefore, the different cultivars of soybean inoculation with *Rhizobium radiobacter* (PGPR) not only decrease nitrogen fertilizer application, but also improves soybean yield and yield components. We concluded that *Rhizobium radiobacter* (PGPR) could be an eco-friendly alternative for reducing soil pollution caused by fertilizers usage and reduce the impact of soil salinity.

Keywords: Bio-fertilizer, macro-micronutrients, saline soil, soybean varieties

INTRODUCTION

The majority of salt-affected soils in Egypt are located in the northern-central part of the Nile Delta and on its eastern and western sides. Fifty five percent of the cultivated lands of northern Delta region are, twenty percent of the southern Delta and middle Egypt region and twenty five percent of the Upper Egypt regions are salt-affected soils (FAO, 1995). Soil salinity is a worldwide problem, restricting plant growth and production, especially in arid, semiarid and tropical regions through reducing nutrients uptake and increasing osmotic stress of plants, (Abdel-Fattah and Asrar, 2012).

Rhizobium radiobacter could be isolate in high salinity soil. The bacterial growth promoting enhances nitrogenase activity and production of indole acetic acid (IAA), gibberlic acid (GA_3) and abscisic acid (ABA) under osmotic stresses, (Moussa and Youssef, 2012). Rhizobacteria improve plant growth employing a variety of growth promoting mechanisms including nutrient uptake, root growth, proliferation, biocontrol activities, and Indol Acetic Acid (IAA) producing, phosphate solubilizing bacterial strain. Also, that in fully fertilized control plants, biomass was high and grain yield was low while addition of halotolerant PGPR with half fertilization exhibited higher grain yield as compared to biomass. (Rajput *et al.* 2013). Plant growth promoting *rhizobacteria* (PGPR)-induced plants salt stress tolerance has been well studied and is considered to be the cost-effective solution to the problem. PGPR isolated from saline soils improve the plant growth at high level of salinity (Barassi et al., 2006). Egamberdieva and Jabborova (2013) found that the inoculation of seeds of bean with PGPR can result in increased root and shoot growth, dry weight, and seed yield and in enhanced tolerance of plants to salt stress. Figueiredo *et al.* (2007) illustrated that the inoculation of bean with *Rhizobium* and/or PGPR were administered to detect possible changes in the levels of interactions between the phytohormones IAA and cytokinin. Mia *et al.* (2007) reported that inoculation with bacterial biofertilizer may reduce the application of fertilizer N by increasing N uptake by plants. Biswas *et al.* (2000) indicated that inoculation of Rhizobium increased plant growth at different growth stages such as enhanced seed germination, increased shoot length, leaf chlorophyll content, total dry matter, grain yield, N content and yield attributes. Deshwal *et al.* (2013) reported that Rhizobia strains produce plant growth hormones, solubilize phosphorus. Bio-inoculants of rhizobia strains are effectively improve the plant growth and productivity. Hardarson and Atkins (2003) found that nitrogen fixing legumes enhanced protein production, contribute nitrogen to succeeding crops and build soil fertility status. Ramana *et al.* (2011) indicated that the interaction effect of biofertilizers and varieties bean was significant in relation to plant height (cm), number of branches per plant, leaf area (cm^2), test weight of plant and increase availability of N, P and K in soil compared with control might be due to the improvement in soil physical and chemical condition provide for plant growth. Rhizobium seed inoculation alone significantly increased soil nitrogen content and soil available phosphorus compared to the control in both seasons, (Hatim 2013).

Soybean seed is a major source of high-quality protein and oil for human consumption. Soybean is classified as moderately salt sensitive instead of moderately salt tolerant, (Katerji *et al.* 2001). Soybean oil is one of the common vegetable oils containing a significant amount of unsaturated fatty acids: -linolenic acid (omega-3 acid); linoleic acid (omega-6 acid) and oleic acid (omega-9 acid), (Yaklich *et al.* 2002). Soybean (*Glycine max*) is one of the most important summer leguminous crops, extensively successful in many provinces in Egypt and worldwide. It consists of around 20% oil and 40% protein. Therefore, it is an excellent source of food for human and animal consumption, (Abdelhamid and El-Matwally, 2008) and (Essa and Al-ani, 2001). Growth, development and yield of soybean are the result of genetic potential interacting with environment. Soybean seed production may be limited by environmental stresses such as soil salinity (Ghassemi-Golezani *et al.,* 2009). Lehmann *et al.* (2010) reported that the proline is an important multifunctional amino acid and plays a role in carbon and nitrogen metabolism, cell signaling, nutrient adaptation and protection against osmotic and oxidative stresses. Sayari et al (2005) found that the proline accumulation in response to drought or salinity stress has been reported to occur in the cytosol to adjust the osmotic balance. Sessitch *et al.* (2002) found that Rhizobium ssp. i.e rhizobactria and some are endophytes which can produce phytohormones , siderophores , solublitize springly soluble organic and inorganic phosphates and can colnize the roots . Concerning that the importance of soybean in production of oil, its nutritional important and status of biological fertilizers in sustainable agriculture.

This investigation aim to study the effect of with or without using biofertilizers and the interaction between the different cultivars of soybean with using biofertilizers on some yield parameters, the concentrations and the uptake of macro-micronutrients and seeds content of proline, protein and oil percentage. Also, to study the effect of used biofertilizers on some soil properties and its content of available nutrients. As well as, to improve yield and quality of product, effect to provide food and health security and also decrease use of chemical inputs with adverse effects on environmental health.

MATERIALS AND METHODS

Two field experiments were conducted in El-Quntra Shark Farm, East of Suzie Canal, Ismalia Governorate, Egypt during two successive summer seasons of 2012 and 2013. The site lies in the north-waste in coast of Sinai, between 32^o- 35' and 32^o – 45' E and 31^o- 00' and 31^o - 250 N, (Kaiser, 2009) ,to study the productivity and quality of some soybean cultivars (Giza 111, Giza 21, Giza 22, Giza 35 and Crawford) with and without inoculation by biofertilizers under saline soil conditions. Inoculation with biofertilizer was prepared from *Rhiobium radibacter* strain (salt tolerant plant growth promoting rhizobacteria, PGPR) isolated from the rhizosphere soil salinity of Sahl El-Tina location and deposited in Gene bank under number of HQ395610 Egypt by Bio-fertilizer Production Unit, Department of Microbiology, Soils, Water and Enviro. Res. Inst., Agric. Res. Center, Giza, Egypt. *Rhizobia* inoculant was performed through mixing seeds with the appropriate amount of them (750 g/ 30 kg seeds) by using Arabic gum as an adhesive material just prior of sowing. Some physical and chemical properties of the studied soil before planting were determined and shown in Table (1).

Table (1). Physical and chemical properties of soil before planting.

Coarse sand (%)	**Fine sand (%)**	**Silt (%)**	**Clay (%)**	**Soil texture**		**OM (%)**		**$CaCO_3$ (%)**
12.29	70.11	5.88	11.72	Loamy sand		0.52		7.19
pH	EC	Cations (meq/l)				Anions (meq/l)		
(1:2:5)	(dS/m)	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{--}_4
8.12	12.50	15.69	22.50	85.93	0.88	9.14	78.29	37.57
Available nutrients in soil								
Macronutrients (mg/kg)			Micronutrients (mg/kg)					
N	P	K	Fe		Mn		Zn	
33	2.96	175	2.7		1.5		0.8	

In both seasons, each experiment was carried out in a split plot design with three replicates. The used five varieties of soybean were arranged randomly as main plots to study soybean cultivars under saline soil without inoculation, while the bio-fertilizer was distributed randomly as sub plots. The area of each experimental unit plot was 50 m^2 which divided into rows with 50 cm. Soybean (*Glycine max*) verities were (Giza 111, Giza 21, Giza 22, Giza 35 and Crawford) which obtained from Crops Institute Agriculture Research Center, Giza, Egypt. The experimental plots were divided into two main groups. The first group was without bio-fertilizer and treated only by one rate of 30kg N fed^{-1} which applied as urea (46 % N). The second group was treated with bio-fertilizer combined with 20 kg N fed^{-1}. Sowing was carried out on 20 May 2012 and 25 May 2013. Three to four of coated seeds were sown in hole with 5 cm depth. The distance between each two holes was 20 cm. After 30 day of sowing, the plant of each hole was thinned to one plant. Urea fertilizer was added in three equal doses after 30, 45 and 65 days from sowing plant. Calcium super phosphate (15 % P_2O_5) was added at a rate of 31 kg P_2O_5 during soil preparation. Potassium sulphate (48 % K_2O) at rate 75 kg K_2O was added in two equal doses after 30 and 45 days from planting.

Surface soil samples (0- 30 cm) were collected from the used soil after plants harvesting to determine physical analysis (Piper, 1950) and chemical analysis (Black, 1965, Soltanpour, 1985 and Jackson, 1973).

Soybean varieties were harvested on 25 September 2012 and 30 September 2013 and the following characters were recorded: Seed yield (Mg/fed). -Weight of pods (Mg/fed). -100-seed weight (g). The oven dried plant part samples were ground and digested using H_2SO_4 and $HClO_4$ acids mixture according to the methods described by (Chapman and Pratt 1961). The plant contents of N, P, K, Fe, Mn and Zn were determined in plant digestion using the methods described by Jackson (1973) and Cottenie et al (1982). Data were statistically analyzed according to Gomize and

Gomize(1984) Oil seeds content was determined using Soxhlet apparatus and petroleum ether as solvent according to A.O.A.C. (1990). Protein percentage of seeds was calculated by multiplying the nitrogen percentage by the factor 6.25 as described by Hymowitz *et al.* (1972). Proline content was estimated according to the methods described by Bates *et al.,* (1973).

RESLUTS AND DISCUSSION

Effect of biofertilizer application on some crop characters:

I- Yield components

The results in Table (2) showed that the un-inoculated soybean cultivars significantly differed in yield and yield components in both seasons. The data in first season, indicated that plants of Giza35 cultivar had highest weight of seed yield (0.892 Mg fed^{-1}) and weight of pods (1.049 Mg fed^{-1}) while, Crawford cultivar recorded the highest weight of 100 seeds (17.95 g). In the second season, the highest seed yield (0.977 Mg fed^{-1}), weight of pods (1.173 Mg Fed^{-1}) and weight of 100 seed (19.13 g) in Crawford cultivar was significantly higher than of other cultivars. According to the effect of using biofertilizer, the data in Table (2) showed that seed yield, and weight of pods in both seasons significantly response to inoculation plant with biofertilizer in different cultivars of soybean seeds comparing with untreated seeds, except 100-seed weight which reveal that the inoculation with bioferilizer was non-significant effect in the two seasons. In addition, The interaction between cultivars with inoculation and without inoculation by biofertilizers are presented in Table (2) and the data showed that in the first season, inoculated Giza "35" cultivar with biofertilizer significantly increased and achieved high values for seed yield (1.078 Mg/fed), weight of pods (1.392 Mg fed^{-1}) and 100 seed weight (19.74 g) compared to other inoculation cultivars. As for second season, the interaction between Crawford cultivar and inoculated with biofertilizer was significantly affected in seed yield (1.285 Mg/fed) and weight of pods (1.492 Mg/fed), while 100-seed weight was non-significant effect with applied biofertilizer. It can be noticed that inoculation with biofertilizer improved soil microbial through increasing organic matter, microbial activity and in turn increased these parameters over the un-inoculated plants.

These results agree with Kazemi *et al.* (2005) who stated that soybean seed inoculation by rhizobial bacteria significantly increased the yield number of seeds per plant, thousand grain weights and finally the yield of soybean. Also, the increase of seed yield in cultivar received biofertilizer mainly attributed to the effect of microorganisms which can play a very significant role in making available nutrients elements for plants. It is essential by bring about some microbial transformation of both inorganic and organic compounds in the soil to make available of these elements to soybean plants. Better developed root systems and better absorption of nutrient elements in seeds inoculated with biofertilizer may increase seed yield. On the other hand, improvement of photosynthesis by these bacteria may increase seed yield, moreover on increasing vegetative growth. Likely, improve of plant nutrition has led to sufficient photoassimilate being transmitted to seeds in the grain filling stage and seeds have more seed thousand weight of soybean plant (Saleh 2005). Bacteria used in these treatments may be increase seed yield by providing macro and micro nutrients for plant growth, production of stimulate material, development of root system and anti-pathognic effects (Jat and Ahlawat, 2006). Mehasen and Saeed (2005) studied the effects of bacterial inoculation as well as mineral and organic fertilization on the yield and yield components of soybean Giza 22 and Giza 111 cultivars. They concluded that there is a significant effect for the interaction between soybean cultivars and fertilization treatments on seed weight/ plant only. The increase in seed yield as soybean plants that received biofertiliar mainly attributed to the beneficial effect of biofertilizer application to the soil led to improved soil physical, biological properties and chemical characteristics resulting in more release of available nutrients to plant root. These results are in line with Hussein *et al.* (2006) who reported a significant effect on 100 seed weight in both seasons due to the interaction between soybean cultivars and fertilization treatments.

Table (2). Effect of bio-fertilizer and without on yield component of soybean plants:

Varieties	Yield component								
	Seed yield ($Mg\ fed^{-1}$)			**Weight of pod ($Mg\ fed^{-1}$)**			**Weight of 100 seeds (g)**		
	season (2012)								
	Bio-fertilizer								
	without	with	Mean	without	with	Mean	without	with	Mean
Giza 111	0.706	0.896	0.801	0.953	1.296	1.125	16.88	18.25	17.57
Giza 21	0.712	0.954	0.833	0.976	1.357	1.167	17.52	19.57	18.55
Giza 22	0.739	0.947	0.843	0.989	1.388	1.189	17.89	18.69	18.29
Giza 35	0.892	1.078	0.985	1.049	1.392	1.221	17.64	19.74	18.69
Crawford	0.729	0.938	0.843	1.042	1.377	1.210	17.95	19.24	18.59
LSD. 5% V	0.041			0.051			0.774		
LSD. Bio	0.025			0.036			Ns		
V X B	***			***			***		
Season 2013									
Giza 111	0.841	1.056	0.949	1.078	1.328	1.203	16.95	19.47	18.21
Giza 21	0.892	1.183	1.038	1.093	1.388	1.241	18.74	19.86	19.30
Giza 22	0.897	1.250	1.074	1.147	1.425	1.286	18.96	19.46	19.21
Giza 35	0.948	1.279	1.114	1.159	1.476	1.318	18.88	20.18	19.53
Crawford	0.977	1.285	1.131	1.173	1.492	1.333	19.13	20.15	19.64
LSD. 5% V	0.061			0.0820			0..35		
LSD. Bio	0.013			0.0012			ns		
V X B	***			***			ns		

On the other hand, the reduction in seed yield, weight of pods and 100-seed weight on different cultivars without inoculation biofertilizer show that soybean is a salt sensitive crop, but the extent of this sensitivity varies among cultivars. Salinity can severally limit crop production because high salinity lowers water potential and induces ionic stress and results in a secondary oxidative stress (Shanon 1998). In this respect, Han and Lee,(2005) showed that some PGPR are able to produce polysaccharide products ,binding Na^+ in the root zone and hence alleviating the stress of soil on plant and microbial growth and activities .

II- Seeds quality:

With respect to seed quality, the data in Table (3) reveal that proline content and protein percentage significantly response in all cultivars of soybean without biofertilizer in the two seasons, except, oil %, in the first season was not significant. Also, it can be noted that Giza 35 recorded the lowest value of proline (24.19 mg/g), and Giza 111 had the highest protein (18.69%) while Crawford cultivar was achieved the highest value of oil (18.91%) for the first season. On the other hand, in the second season, the Crawford cultivar recorded the lowest value of proline (26.84 mg/g) and the highest values of protein % (19.81%) and (19.15%) for oil%. As for proline content, increasing under salinity stress than the inoculated plants with bioferilizers might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance (Gad 2005). Also, he reported that proline content was much higher in sensitive cultivar of tomato that in salt-tolerant. Some researchers reported an increase in proline (Ibrahim, 2004). Protein and oil contents of grains produced under saline conditions combined with bio-fertilizer were higher than without biofertilizer. Decreasing protein percentage in untreated cultivars with biofertilizers could be attributed to the disturbance in nitrogen metabolism or to inhibition of nitrate absorption. Medhat (2002) reported that salinity stress induce changes in the ion content of plant cell which intern induce changes in the activity of certain metabolic systems that might have serious consequences for protein. Concerning the effect of using biofertilizer in seed quality of soybean cultivars, the data in Table (3) showed that in the first season, protein % significantly affected by using biofertilizer while, proline content and oil% were non significant effect in different cultivars of soybean than that cultivars without using bio-fertilizers.

As well as, in the second season, the proline content was significantly affected by inoculation with biofertilizer of all cultivars and their values were lower than un-inoculated seeds. Also, the protein % and oil % were non significant in seeds of soybean cultivars, compared with untreated plants.

The data in Table (3) cleared the interaction effect and reported that, in first season, Inoculated Giza 35 cultivar with biofertilizer gave the lowest value for proline content (19.40 mg/g), while, the effect of interaction in protein % and oil % didn't significantly affect but their values were still higher in comparison with other cultivars. In the second season, proline content, protein and oil % were significantly response to the interaction effect and Crawford cultivar with biofertilizer recorded low value in proline content (13.29 mg/g) and significant increase in protein (23.31%) and oil (20.93%) in comparison with other cultivars. The increase in seed protein probably due to stimulation of protein biosynthesis processes in soybean plants providing in this way soybean seeds with higher nutritional value. These results are in agreement with Hussein *et al.* (2006) who reported that a significant effect on seed oil content (in the first season), regarding to the interaction effect between soybean cultivars and inoculation with bioferitilzer. On the other hand, these results are in line with Saber *et al.*, (1989) who reported that application of biofertilizer increased oil and protein contents as well as nutrients elements in soybean. Mekki and Ahmed (2005) reported that seed oil % and protein % increased on the soybean plants that treated by biofertilizer. It could be the obtained this result is increase of protein percentage due to the increase in N%.

Table (3) effect of using bio-fertilizer or without using biofertilizer on soybean quality.

Varity	Yield quality								
	Proline (mg/g)			**Protein (%)**			**Oil (%)**		
	(2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	30.14	21.39	25.77	18.69	21.31	18.69	17.95	18.59	18.27
Giza 21	30.57	20.13	25.35	18.13	21.19	18.13	18.68	19.05	18.87
Giza 22	31.19	20.69	25.94	18.44	22.19	18.44	18.70	19.39	19.05
Giza 35	24.19	19.40	21.80	18.50	20.81	18.50	18.88	19.76	19.32
Crawford	25.17	19.86	22.52	18.19	21.13	18.19	18.91	20.18	19.55
LSD.5%V	4.11			0.799			Ns		
LSD. Bio	ns			0.596			Ns		
V X B	*			ns			Ns		
Season 2013									
Giza 111	28.44	18.62	23.53	18.56	21.56	20.06	17.96	19.07	18.52
Giza 21	29.17	17.69	23.43	18.88	22.25	20.56	18.76	19.53	19.15
Giza 22	27.36	15.28	21.32	19.69	22.06	20.88	18.83	19.86	19.35
Giza 35	27.00	14.86	20.93	19.13	22.81	20.94	19.02	20.87	19.95
Crawford	26.84	13.29	20.07	19.81	23.31	21.53	19.15	20.93	20.04
LSD.5%V	3.601			1.22			0.475		
LSD. Bio	0.898			ns			Ns		
V X B	***			**			***		

III- Macronutrients concentration in seeds:

Concerning the macronutrients concentration in seeds of soybean cultivars without biofertilizer , the data in Table (4) revealed that the different between cultivars were non significant in N % and K%, while significant in P % in both seasons .The results showed that Giza 22 cultivar had highest value for P % (0.52%) in the first season and (0.44%) in the second season. The macronutrients concentration in seeds with applying biofertilizer revealed that the P percentage was significantly enhanced with biofertilizer in the two seasons and led to produce more values than in un-inoculated cultivars. On the other hand, from the aforementioned data that N % and K % were not found significant difference with application of PGPR in both seasons but their values were higher as compared with untreated seeds.

Table (4). Macronutrients concentration in soybean seeds as affected with or without bio-fertilizer.

Varity	Macronutrients (%)								
	N			P			K		
	season (2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	2.99	3.41	3.20	0.36	0.27	0.32	2.55	2.84	2.70
Giza 21	2.90	3.39	3.15	0.45	0.33	0.39	2.43	2.74	2.59
Giza 22	2.95	3.55	3.25	0.52	0.29	0.41	2.56	2.82	2.69
Giza 35	2.96	3.33	3.15	0.34	0.30	0.32	2.63	2.77	2.70
Crawford	2.91	3.38	3.15	0.41	0.28	0.35	2.57	2.69	2.63
LSD.5%V	ns			0.038			ns		
LSD. Bio	ns			0.030			ns		
V X B	ns			***			ns		
Season 2013									
Giza 111	2.97	3.45	3.21	0.30	0.46	0.38	2.58	2.88	2.73
Giza 21	3.02	3.56	3.29	0.38	0.49	0.44	2.63	2.93	2.78
Giza 22	3.15	3.53	3.34	0.44	0.53	0.49	2.54	2.94	2.74
Giza 35	3.06	3.65	3.35	0.43	0.52	0.48	2.60	2.69	2.65
Crawford	3.17	3.73	3.45	0.39	0.45	0.42	2.63	2.76	2.70
LSD.5%V	ns			0.050			ns		
LSD. Bio	ns			0.013			ns		
V X B	ns			***			ns		

Sessitch *et al.* (2002) found that Rhizobium ssp. are plant growth promoting rhizobactria and some are endophytes which can produce phytohormones , siderophores , solublitize springly soluble organic and inorganic phosphates and can colnize the roots . The results of interaction in (Table 4) showed that in both season, P % only was highly significant by inoculated Giza 21 (0.33%) with biofertilizer in first season and Giza 22 in the second season compared with other cultivars, while N% and K% were non significant effect from the interaction and their values were still higher with inoculated cultivars compared to un-inoculated plants. Shinde *et al.* (2008) clear that upon application of PGPR, the available nitrogen, phosphate and potassium were increased from 199.0 to 282.0, 14.77 to 27.52 and 366.7 to 448.75 kg/ha respectively. Deshwal *et al.* (2013) reported that Rhizobia strains produce plant growth hormones, increase of N solubilize phosphorus.

In this concern, microorganisms can play a very significant role in availability of phosphorus to plants. These results were supported by the finding of El-Kholy and Gomaa (2000).

IV-Micronutrients concentration in seeds:

The data presented in Table (5) indicated that studied micronutrients concentration (mg/Kg) in the soybean cultivars without using biofertilizer significantly differed in the two seasons. Giza 35 cultivar recorded the highest values of Fe (87.52mg/Kg) and Zn (18.77mg/Kg) concentrations, while, Crawford cultivar achieved the highest values of Mn concentration (52.22mg/Kg) in the first season. In the second season, Crawford cultivar achieved the highest values of Fe and Mn concentrations (88.94 and 58.41mg/Kg) respectively. As well as, Giza 35 had the highest value of Zn concentration (23.47mg/Kg).

Table (5). Micronutrients concentration in seeds soybean

Varity	Micronutrients (mg kg^{-1})								
	Fe			Mn			Zn		
	Season (2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	77.8	83.4	80.6	48.6	55.7	52.1	17.9	22.4	20.2
Giza 21	79.6	90.1	84.8	44.9	58.6	51.7	16.5	23.5	20.0
Giza 22	82.1	88.6	85.4	40.7	61.1	50.9	16.9	27.1	22.1
Giza 35	87.5	92.1	89.8	50.1	60.2	55.2	18.7	25.8	22.3
Crawford	83.6	96.4	90.1	52.2	63.4	57.8	15.9	29.4	22.7
LSD.5%V	3.16			4.45			2.47		
LSD. Bio	ns			ns			1.24		
V X B	***			***			***		
Season 2013									
Giza 111	85.9	96.2	91.1	51.6	66.8	59.2	22.8	23.8	23.3
Giza 21	82.8	97.6	90.2	55.7	68.9	62.3	21.9	28.4	25.2
Giza 22	84.9	95.3	90.2	57.6	67.2	62.4	22.1	30.5	26.3
Giza 35	87.3	98.7	93.0	54.6	69.8	62.2	23.4	28.9	26.2
Crawford	88.9	99.5	94.2	58.4	70.1	64.2	22.6	32.5	27.6
LSD.5%V	2.03			2.79			2.36		
LSD. Bio	ns			ns			0.44		
V X B	***			***			**		

The effect of using biofertilizer on concentration of micronutrients in soybean cultivars is presented also in Table (5) for two seasons. The data reported that the concentration of Zn (mg Kg^{-1}) was significantly enhanced while, Fe and Mn were not found significant (mg Kg^{-1}). Moreover, the effect of interaction between cultivars and inoculated seeds with biofertilizer in micronutrients concentration (mg/Kg) for both seasons are presented in Table (5) and revealed that the concentration of Fe, Mn and Zn were significantly affected by using biofertilizer in different cultivars of soybean. The data reported that inoculated Crawford with biofertifizes recorded highest values for Fe, Mn and Zn (96.46, 63.48 and 29.46mg/Kg) respectively, in the first season, and (99.55, 70.12, 32.54mg/Kg) in the second season respectively, compared with other cultivars inoculation. In generally, pronounced responses were obtained in the concentration of Fe, Mn and Zn when added biofertilizer. This may be due to improved physical and chemical properties of the soil and increased the available nutrients to plant; these results are similar to those found by Nasef *et al.* (2004) and Ashmawy *et al.* (2008).

Also, the increase in concentration of Fe, Mn and Zn is mainly due to the action of biofertilizer that rendered most micronutrients in the available form. Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes; they include mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goel *et al.*, 1999).

Macronutrients content.

The macronutrients content (kg/fed) in seeds of soybean cultivars without adding biofertilizers are presented in Table (6) for both seasons. The results showed that soybean cultivars significantly differ in N, P and K uptake. In the first season, Giza 35 cultivar gave the highest values for N, P and K uptake (31.91, 3.23, 28.35 (Kg/fed), respectively, while in second season, N and K uptake (30.79 and 25.70 Kg/fed) were significantly increased by planting Crawford cultivar which posses the most marked increase compared with the other cultivars, as well as, the highest uptake of P (2.94 Kg/fed) was significantly by planting Giza 21 cultivar.

Table (6). Macronutrients content (kg/fed) in seeds soybean

Varieties	**Macronutrients (kg/fed)**								
	N			**P**			**K**		
	season (2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	26.79	30.55	28.67	2.42	3.23	3.83	22.85	25.45	24.15
Giza 21	27.67	32.34	30.01	3.15	4.29	3.72	23.18	26.14	24.66
Giza 22	27.94	33.62	30.78	2.75	4.92	3.84	24.24	26.71	25.48
Giza 35	31.91	35.90	33.91	3.23	3.67	3.45	28.35	29.86	29.11
Crawford	27.30	31.70	29.50	2.63	3.85	3.24	24.11	25.32	24.72
LSD.5% Varity	1.62			0.65			2.21		
LSD. Bio	3.97			0.030			0.99		
V X B	ns			Ns			Ns		
Season 2013									
Giza 111	24.98	36.43	30.71	2.27	3.80	3.04	21.70	30.41	26.06
Giza 21	26.94	42.11	34.53	2.94	5.32	4.13	23.46	34.66	29.06
Giza 22	28.26	44.13	36.20	2.60	6.50	4.55	22.78	36.75	29.77
Giza 35	29.01	46.68	37.85	2.84	4.35	3.60	24.65	34.41	29.53
Crawford	30.97	47.93	39.45	2.74	5.27	4.01	25.70	35.47	30.59
LSD.5% Varity	2.44			0.58			1.54		
LSD. Bio	4.22			0.47			4.47		
V X B	ns			**			*		

In addition, the Table (6) illustrated that using biofertilizeres significantly affected in the uptake of (N, P and K) for different soybean cultivars as compared to cultivars without using biofertilizers Concerning the effect of the interaction on macronutrient uptake (Kg/fed) in seeds are shown in Table (6). In the first season, inoculation with biofertilizer did not significantly increase the uptake of N, P and K in seeds but their values were still higher than the un-inoculated plants. In the second season, the interaction was not significant effect on N uptake in seeds soybean but P and K uptake were high significant response to the interaction effect and Giza 22 cultivar was recorded higher values(6.50Kg/fed) for P and (36.75Kg/fed) for K by inoculation with biofertilizer than the other inoculated cultivars. Mia *et al.* (2007) reported that inoculation with bacterial biofertilizer may reduce the application of fertilizer N by increasing N uptake by plants. Zarrin et al (2007) revealed that the Rhizobium inoculation of seed soybean led to significantly increased uptake of N, P and K.

Micronutrients content:

Data in Table (7) showed that a signification increase in micronutrient content (g/fed) of seeds without using biofertilizer. In the first season, the results indicated that the uptake by Giza 35 cultivars surpassed the other cultivars in Fe, Mn and Zn uptake and recorded 78.07 ,44.75 and 16.74 (g/fed) respectively. As well as, in the second season, the uptake of Fe was non signification while, Crawford and Giza35 cultivars had highest values in Mn uptake (57.07g/fed) and Zn uptake (22.14g/fed) compared to other cultivars. In addition, the Table (7) showed the effect of using biofertilizeres on micronutrients (Fe, Mn and Zn) uptake in seeds for different soybean cultivars and reveal that a significantly greater uptake of micronutrient (g/fed) in both seasons in comparison with cultivars without using biofertilizes. The simulative effect of Rhizobium on the uptake could be due to their activities on the solubilization of the micronutrients, a phenomenon which requires quantification. Rhizobium inoculation in bean plants significantly increases uptake of Mn , Fe and Zn at the whole plants , (Patrick et al (2011).

Concerning the effect of interaction between cultivars and application with biofertilizers in uptake of micronutrients, Table (7) showed that in the first season, the uptake of Fe, Mn and Zn (g fed^{-1}) were signification increase and inoculated Giza 35 with biofertilizer achieved the high values (99.33, 64.95and 44.75g/fed) for Fe, Mn and Zn respectively, compared with other inoculated cultivars.

In additions, the interaction effect was not significant for Fe uptake while Mn and Zn uptake were highly significant and inoculated Crawford cultivar was surpassed and recorded (90.10 ,41.81g/fed) for Mn and Zn respectively, than the other inoculated cultivars in the second season. Furthermore, the using PGPR as biofertilizer also increases the uptake and efficiency of micronutrients like Zn, Cu, and Fe etc. by secreting the enzymes, organic acids which makes fixed macro and micronutrients mobile and rendered most micronutrients in the available form. PGPR (plant growth promoting rhizobacteria) can also increase Fe solubility and hence its uptake by plant. The difference between studied soybean cultivars in yield components, yield quality, concentrations and uptakes of macro-micronutrients may be attributed to genetic constitution, which may be mannested in lower number and shorter internodes. These results are in accordance with those reported by Abd El-Ghany et al (2010) who found that inoculation with found that Rhizobium increased the uptake of Zn, Mn, Fe, and Cu.

Table (7). Micronutrients content in seeds soybean

Varieties	Micronutrients (g fed^{-1})								
	Fe			Mn			Zn		
	Season (2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	54.96	74.81	64.89	34.36	49.92	42.14	12.67	34.36	23.52
Giza 21	56.70	85.97	71.34	32.00	55.91	43.97	11.78	32.00	21.89
Giza 22	60.70	83.97	72.34	30.13	57.81	43.97	12.56	30.13	21.35
Giza 35	78.07	99.33	88.70	44.75	64.95	54.85	16.74	44.75	30.75
Crawford	60.98	90.48	75.73	38.07	59.54	48.81	11.61	38.07	24.84
LSD.5% Varity	3.41			1.73			1.71		
LSD. Bio	2.97			0.99			3.50		
V X B	*			**			***		
Season 2013									
Giza 111	72.29	101.64	86.97	43.42	70.58	57.00	19.21	25.18	22.20
Giza 21	73.93	115.53	94.73	49.71	81.54	65.63	19.59	33.68	26.64
Giza 22	76.24	119.23	97.74	51.69	84.03	67.86	19.86	38.18	29.02
Giza 35	82.79	126.29	104.54	51.85	89.38	70.62	22.25	37.00	29.63
Crawford	86.89	127.92	107.41	57.07	90.10	73.59	22.14	41.81	31.98
LSD.5% Varity	ns			1.93			1.79		
LSD. Bio	17.14			3.98			3.97		
V X B	ns			**			***		

Effect of biofertilizers on soil pH, EC and macro-micronutrients content:

The chemical analysis of soil pH, EC and the content of N, P, and K are shown in Table (8), low pH and EC values were observed in soybean cultivars. The data showed that the soil pH only varied slightly between the cultivars and with or without using bioferilizers in both seasons. The drop in pH may be attributed to the effect of inoculants on rate of organic matter degradation. Some workers reported that release of organic acids with application of PGPR decreases pH of soil sample.

Also the bacteria that fixed N_2, dissolved P and available K led to decrease in soil pH when added alone and in combination with chemical fertilizers. The obtained data may be explained on the base of some products of added mineral fertilizers transformation in the soil have an acidic effect. Also, most of actives products of the used biofertilizers characterized product acidic effects where these products mainly are weakly acidic compound (Shaban and Omar 2006). According to EC values in soil after harvesting the results showed that the obtained values of EC were decreased in soil for both season as compared with EC in soil before planting. Additionally, the effect of adding biofertilizer was not significant while, the interaction between cultivars and using biofertilizer had significant effect in EC values for two seasons. Hussain *et al.* (2002) showed that salinity was reduced significantly by Rhizobium inoculation, as well as the Rhizobium inoculation of seed was decreased salinity levels from 9.7 2 to 6.68 dSm^{-1}.

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On the other hand, opposite of those observed for pH and EC, concerning the higher soil available N, P and K obtained from soil treated with biofertilizer, they were significant as compared to the soil before planting. These results are confirmed with Berger *et al.,* (2013) who stated that biofertilizers reduced soil pH and increased the contents of N, P and K. The authors added that the biofertilizer may be an alternative for N, P and K fertilization that slows the release of nutrients, favoring long term soil fertility. Moreover, they found that biofertilizer increased the soil organic matter and N,P and K contents and slightly decreased soil EC and pH and showed several benefits over chemical fertilizers and improved fertility of saline soils. Rhizobium seed inoculation alone significantly increased soil available phosphorus compared to the control in both seasons, (Hatim 2013).

According to the available of micronutrients(mg/Kg) in the soil after soybean harvest, the data in Table (9) showed that in the first season, Mn and Zn availability were significantly affected in cultivars of soybean with inoculated by biofertilizers while, Fe content was not-significant. As for second season, the content of available Fe, Mn and Zn (mg/Kg) were in-significantly response as results of using biofertilizers. These results are in agreements with Ipsita and singh (2014) who found that application of PGPR was beneficial showing higher nutrients content in soil.

Table (9). Available micronutrients content in soil after soybean harvest.

Varity	Micronutrients (mg kg^{-1})								
	Fe			**Mn**			**Zn**		
	Season (2012)								
	Bio-fertilizer								
	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 11	2.73	2.87	2.80	1.52	1.58	1.55	0.85	0.88	0.87
Giza 21	2.77	2.80	2.79	1.53	1.63	1.58	0.83	0.96	0.90
Giza 22	2.75	2.77	2.76	1.54	1.55	1.55	0.87	0.95	0.91
Giza 35	2.77	2.82	2.80	1.55	1.60	1.58	0.84	0.95	0.90
Crawford	2.75	2.89	2.82	1.58	1.65	1.62	0.88	0.98	0.93
LSD.5% Varity	Ns			0.040			0.0084		
LSD. Bio	Ns			ns			ns		
V X B	Ns			***			***		
Season 2013									
Giza 11	2.70	2.88	2.79	1.52	1.63	1.58	0.88	1.02	0.95
Giza 21	2.78	2.93	2.86	1.53	1.65	1.59	0.87	1.05	0.96
Giza 22	2.72	2.95	2.84	1.56	1.67	1.62	0.86	0.99	0.93
Giza 35	2.82	2.95	2.89	1.58	1.65	1.62	0.88	0.98	0.93
Crawford	2.83	2.90	2.87	1.52	1.60	1.56	0.82	0.95	0.89
LSD.5% Varity	ns			0.059			0.0124		
LSD. Bio	ns			ns			ns		
V X B	ns			ns			ns		

CONCLUSION

The following are the main conclusions drawn from the study:

Overall, the results of this work appear to indicate that seeds of soybean cultivars inoculation with biofertilizer significantly enhanced the seed yield and its components during the growth period. Oil % and protein %, concentration and uptake of macro-micronutrients were significantly improves in seeds with biofertilizers compared to seeds without using biofertilizers. Giza 35 and Crawford cultivars with biofertilizer achieved the highest values in yield, yield components, oil%, protein %, concentration and uptake of macro-micronutrients. Therefore, when soil is poor, inoculation of seeds by PGPR not only could achieve more yield than full meet of crop nutrition treatment but also completely reserves chemical fertilizer application and leading to plant tolerance improving under saline stress conditions, so that there will be no environmental problems linked to chemical fertilizers.

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تاثير اضافة التسميد الحيوى على انتاجية وجودة ومحتوى العناصر لبعض اصناف فول الصويا تحت ظروف الأراضى الملحية

منال عبد الحكم عطية [1] ، امال حسن الجبالى[1] ، خالد عبده حسن شعبان[1]و محمود ابراهيم عبد المحسن[2]

معهد بحوث الأراضى والمياه والبيئة –مركز البحوث الزراعية- الجيزة – مصر

قسم بحوث البقوليات معهد المحاصيل الحقلية مركز البحوث الزراعية - مصر

اجريت تجربتان حقليتان فى موسمين صيفيين متتالين 2012و 2013 فى منطقة القنطرة شرق –شرق قناة السويس –بمحافظة الاسماعيلية لدراسة تاثير اضافة التسميد الحيوى(مادة PGPR) و عدم اضافتها على نمو أصناف مختلفة من نبات فول الصويا (جيزة 111- جيزة 21- جيزة 22- جيزة 35- صنف كراوفورد) تحت ظروف الاراضى الملحية وتأثير ذلك على محصول الحبوب ومكوناته وكذلك على محتوى البذور من البرولين و نسبة البروتين والزيت -بالاضافة الى تأثير التسميد الحيوى على تركيز ومحتوى العناصر الغذائية (النيتروجين-الفوسفور- البوتاسيوم- الحديد –المنجنيز- الزنك) فى الاصناف المختلفة لنبات فول الصويا.

كان تصميم التجربة منشقة مرة واحده

وقد كانت اهم النتائج المتحصل عليها هى كالآتى :

بالنسبة للنباتات الغير معاملة بالتسميد الحيوى فقد اشارت النتائج الى:

*زيادة وزن كل من محصول الحبوب ووزن القرون (كجم/فدان) وأيضا وزن 100 بذرة(جم) وذلك فى صنفى جيزة 35 وكراوفورد. كما تشير النتائج الى أن صنف كراوفورد قد سجل انخفاض فى محتوى البرولين وزيادة نسبة البروتين والزيت . سجلت النتائج زيادة تركيز ومحتوى العناصر الكبرى والصغرى فى صنفى جيزة 35 وكراوفورد.

فقد اظهرت النتائج المتحصل عليها مايلى:

*زيادة محصول الحبوب ووزن القرون ووزن 100 بذرة وذلك مقارنة بالبذور الغير مضاف لها التسميد الحيوى. *كذلك أظهرت النتائج أنخفاض محتوى البذور من البرولين وزيادة نسبة البروتين والزيت

*أيضا فقد أدت أضافة السماد الحيوى الى الاصناف المختلفة من نبات فول الصويا الى زيادة معنوية فى تركيز ومحتوى كل من العناصر الكبرى (نيتروجين-فوسفور –بوتاسيوم)و العناصر الغذائية الصغرى (حديد-منجنيز-زنك) . كذلك فانه عند دراسة افضل الاصناف استجابة للتسميد الحيوى فقد اظهرت النتائج ما يلى: سجل صنف جيزة 35 اعلى قيم فى وزن محصول الحبوب ووزن القرون فقط مقارنة بباقى الاصناف .

*بينما سجل صنفى جيزة 35 وصنف كراوفورد زيادة نسبة البروتين والزيت وانخفاض محتوى البذور من البرولين مقارنة بباقى الاصناف باستخدام السماد الحيوى . سجل صنف جيزة 22 زيادة معنوية فى تركيز الفوسفور فى خلال موسمى الزراعة وكذلك فى محتوى الفوسفور و البوتاسيوم .

*أشارت النتائج ان اضافة السماد الحيوى الى صنف كراوفورد قد أدى الى زيادة تركيز (حديد- منجنيز – زنك) زيادة معنوية فى كلا الموسمين . كذلك فقد أشارت النتائج الى ان محتوى العناصر (حديد- منجنيز – زنك) قد زاد زيادة معنوية فى صنف جيزة 35 فى الموسم الاول فقط بينما فى الموسم الثانى فقد سجل صنف كراوفورد زيادة معنوية فى محتوى عناصر (المنجنيز –الزنك)

أظهرت تحاليل التربة التى اجريت بعد الزراعة انخفاض رقم الحموضة للتربة ونسبة ملوحة التربة.

*أدى أستخدام التسميد الحيوى الى زيادة تركيز (النيتروجين –الفوسفور – البوتاسيوم) زيادة معنوية بينما لم يكن هناك أى تأثير معنوى على تركيز (الحديد-المنجنيز-الزنك) فى التربة.

-يتضح من النتائج السابقة ان اضافة السماد الحيوى الى الاصناف المختلفة لنبات فول الصويا قد أدى ألى تقليل كمية الاسمدة المعدنية المستخدمة وتحسين أنتاجية محصول فول الصويا .

-لذلك فان البحث يوصى باستخدام التسميد الحيوى الريزوبيم ريدوباكتر المعزولة من التربة الملحية والمحتوية على مجموعة PGPR لتقليل الاثر الناجم عن ملوحة التربة والتلوث الناتج من استخدام الاسمدة المعدنية وزيادة خصوبة التربة وامتصاص النبات. .

Table (8). Soil pH; EC and macronutrients content in soil after soybean harvest.

Varity	pH (1:2.5)		EC (dSm^{-1})			Macronutrients ($mg\ kg^{-1}$) N			P			K		
			season (2012)											
			Bio-fertilizer											
	Without	With	Without	With	Mean	Without	With	Mean	Without	With	Mean	Without	With	Mean
Giza 111	8.07	8.06	8.24	7.98	8.11	37.96	48.63	43.30	3.10	3.22	3.16	180	188	184
Giza 21	8.05	8.03	8.39	6.52	7.46	39.88	52.14	46.01	3.15	3.36	3.26	182	186	184
Giza 22	8.06	8.04	8.05	6.87	7.46	42.18	50.77	46.48	3.18	3.38	3.28	179	193	186
Giza 35	8.04	8.02	7.85	6.25	7.05	41.22	56.10	48.66	3.44	3.85	3.65	188	195	192
Crawford	8.03	8.00	8.60	6.89	7.75	44.63	52.94	48.79	3.48	3.95	3.72	179	193	186
LSD. 5% V	-----		0.64			1.77			0.44			4.73		
LSD. Bio	-------		ns			ns			0.021			2.02		
V X B	--------		**			***			***			***		
Season 2013														
Giza 11	8.06	8.05	8.10	7.52	7.81	41.59	59.82	50.71	3.52	3.74	3.63	188	199	194
Giza 21	8.04	8.01	7.85	6.24	7.05	42.33	55.14	48.74	3.49	3.88	3.69	191	204	198
Giza 22	8.03	8.00	7.60	5.98	6.79	40.89	57.66	49.28	3.53	3.89	3.71	193	206	200
Giza 35	8.00	7.96	7.22	5.71	6.47	45.71	57.98	51.85	3.98	4.10	4.04	199	208	204
Crawford	8.01	7.92	7.18	5.63	6.41	46.62	60.77	53.70	3.99	4.20	4.10	201	212	207
LSD.5% V.	------		0.400			3.29			0.049			5.18		
LSD. Bio	--------		ns			3.42			0.083			ns		
V X B	------		***			***			***			***		