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Evaluation of Bio- and Mineral Phosphorus Fertilizers Accompanied with Foliar Application of Micronutrients on Quantity and Quality of Soybean (*Glycin max* L.) Yield

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A FIELD experiment was carried out to investigate the effect of different levels of phosphorus fertilizer application P_0 (without P fertilizer), P_1 (15 kg P_2O_5 /fed), P_2 (22.5 kg P_2O_5/fed) and the recommended dose P_3 (30 kg P_2O_5 /fed), either alone or in combination with phosphorus biofertilizer (phosphorus solubilizing bacteria *Bacillus megatherium*) and foliar spray of micronutrients (Fe + Mn + Zn at mixture rate 1:1:1) on yield, yield components and seed quality of soybean. The results showed that the yield and its components were significantly increased with the levels of mineral P-fertilizers, biological P-fertilizers and foliar spray of micronutrients alone or in combination. Also, the results disclosed that the highest values of yield components were obtained with combination of P rate of 22.5 kg P_2O_5/fed with inoculation of P-biofertilizer and foliar spray of micronutrients. Application of phosphorus at the rate of 22.5 kg P_2O_5/fed in combination with phosphorus dissolving bacteria significantly increased the uptake of macro-, micro-nutrients, protein and oil contents. Moreover, the combined application of mineral phosphorus and foliar micronutrients application had positive effect on N, K, Mn, as well as protein and oil content. The interaction between phosphorus level at 22.5 kg P_2O_5/fed, phosphorus biofertilizer and foliar micronutrients had significant influence on uptake of K, Fe and Mn in seeds of soybean plants. Therefore, it could be concluded that, biofertilizers could replace a portion of the chemical fertilizers in soybean production reducing environment problems linked to the mineral fertilizers.

Keyword: Biofertilizer, Phosphorus, Micronutrients, Soybean.

Soybean (*Glycine max* L.) 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). Soybean oil is one of the common vegetable oils containing a significant amount of unsaturated fatty acids including linolenic acid (omega-3 fatty acid), linoleic acid (omega-6 fatty acid) and oleic acid (omega-9 fatty acid) (Yaklich *et al.,* 2002). Its dietary composition is reported to be the best in the legume family containing vitamins good for diabetics (Agwu *et al.*, 2009).

The use of fertilizer is considered to be one of the most important factors in increasing crop yield. Phosphorous has been shown to be an essential element and its application has been shown to be important for growth, development and yield of soybean (Kakar *et al.*, 2002). Phosphorus is an important plant nutrient involved in several energy transformation and biochemical reactions including biological nitrogen fixation. Phosphorus fertilizers have low efficiency of utilization due to chemical fixation in soil (MeviSchutz *et al.*, 2003). Phosphorus deficiency can cause a limit nodulation in legumes and P fertilizer application can also overcome this deficiency (Carsky *et al.,* 2001). The use of phosphate solubilizing bacteria is able to change insoluble phosphorus in soil into the absorbed soluble form (Turan *et al*., 2006). El-Gizawy and Mehasen (2009) showed that application of mineral phosphorus fertilizer with phosphate- solubilizing bacteria had a significant effect on bean seed yield and its components, nitrogen, phosphorus and zinc content in seeds. Seeds inoculated with biofertilizers in combination with foliar application of micronutrients gave higher values of all estimated parameters of pea bean than plants fertilized with chemical fertilizers in the presence or absence of foliar application (Zaghloul *et al.*, 2015). In all cases, fertilizers are major factors in maintaining soil fertility, but using too much of them, greatly reduce the amount of soil microorganisms. Environmental problems caused by irregular application of chemical fertilizers have harmful effects on biological cycles and destroyed farming stability systems; these factors altogether encourage the application of biofertilizers (Kannayan, 2002). Integration of chemical and biofertilizers is one of the ways to increase production in sustainable agriculture (Ali *et al.*, 2008). From otherwise, one biological way to increase the productivity in the agricultural sector is the effective use of beneficial microorganisms that have more ability to enhance plant growth and yield.

Micronutrients application led to enhance plant productivity, leaf area and grain yield as result of increasing the enzymatic system of plants (Zayed *et al.*, 2011). Micronutrients foliar application treatment showed a significant effect on the produced the highest values of plant height at harvest, number of branches per plant, number of pods per plant, 100 seed weight, seed yield per plant, seed yield (kg ha^{-1}), oil content, oil yield, protein content and its yield of soybean compared with control treatment (El-Haggan, 2014).

Therefore, the objective of this study was to evaluate the role of phosphate solubilizing bacteria in soybean production as well as to study the possibility of decreasing rate of mineral P-fertilizer in presence of these bacteria as a growth motive in increasing the solubility and absorption of phosphate fertilizers.

Materials and Methods

A field experiment was carried out during two summer seasons (2013 and 2014) in Gemmeiza Agri. Res. Station, Gharbia Governorate located at lat. 30.47, long 31.00 and 14.80 m above the sea level to evaluate the effect of different levels of mineral P-fertilizer combined with or without phosphorus solubilizing bacteria (*Bacillus megatherium*) and foliar spray of micronutrients on yield and its attributes, nutrients uptake and quality of soybean. Soil samples were taken for physical properties according to KLute (1986). Soil samples were subjected to the chemical properties including determination of the available N, P and K as outlined according to Page *et al.* (1982). The physical and chemical properties of the experiment soil were presented in Table 1.

TABLE 1. Physical and chemical properties of the soil used.

Property	Value	Property	Value
Particle size distribution			
Clay %	54.25	Soluble ions ($cmol_c$ kg^{-1}) in soil paste	
Silt %	30.50	Na^+	21.00
Sand %	15.25	K^+	0.75
Textural class	Clay	Ca^{++}	7.01
EC (dSm^{-1})	3.80	Mg^{++}	9.24
in soil paste extract		Cl^-	20.12
pH [Soil suspension 1:2.5]	7.81	HCO_3^-	5.22
Organic matter (g kg^{-1})	2.05	$SO_4^=$	12.56
$CaCO_3$ (g kg^{-1})	3.22		

Available macro- and micro-nutrients (mg kg^{-1} soil)					
N	P	K	Fe	Mn	Zn
56.70	7.21	354	9.99	4.11	3.23

Experiment was conducted in split split plots design in complete randomized blocks with three replicates. The main plots included non-inoculated plant (B_0) and inoculated plant (B_1), sub-plot was considered four levels of mineral P-fertilizers (P_0, P_1, P_2 and P_3), while sub-sub plot represented in presence (F_1) and absence (F_0) of foliar application of micronutrients. Concerning Bio-fertilizer, phosphorin (*Bacillus megaterium* var. phospaticium), was supplied by Bio-fertilizers Production Unit, Soil Microbiology Dept. Soils, Water and Environment Res. Inst., Agric. Res. Center. Giza, Egypt. Inoculation with phosphorin as a bio-fertilizer was performed through mixing seeds with appropriate amount of this inculator (200 g / fed).

Soybean (cv. Giza 111) obtained from Crops Institute, Agriculture Research Center, Giza, Egypt. Seeds were inoculated with biofertilizer using Arabic gum as an adhesive material just prior to sowing. The micronutrients treatments were a combination in the form of EDTA compound of Fe-EDTA (12%), Zn-EDTA (14%) and Mn-EDTA (12%) with a ratio of 1:1:1 (using 0.2 g of each element/L). Foliar spray solution from the mixture of the chelated compounds was applied at a rate of 200 L/fed in two times, the first was 40 days after sowing and the second was a month later.

The four levels of mineral phosphorus namely P_0 (without P fertilizer), P_1 (15 kg P_2O_5 /fed), P_2 (22.5 kg P_2O_5/fed) and the recommended dose P_3 (30 kg P_2O_5/fed) were added before planting in the form of calcium super phosphate (15.5% P_2O_5). The recommended doses of N (50 kg/fed) as urea (46.5% N) and K (24 kg/fed) as potassium sulphate (48% K_2O) were applied to all treatments at the proper time. Sowing of soybean seeds was carried out on the 20th and 25th of May in the first and second seasons, respectively. At harvest, seed yield and its components were estimated. Seed oil percent was age estimated according to

AOAC (1990). Crude protein content was also calculated by multiplying N % by 6.25 as described by Hymowitz *et al.* (1972). Yield data were statistically analyzed according to Snedecor and Cochran (1990).

Results and Discussion

Seed yield and its attributes

Data revealed that increasing levels of P fertilizers were significant increases over that of P_0 (control) in 100 seed weight, pod, straw and seed yields (Table 2). Also, increasing the rate of P-fertilizers up to 75% (P_2) from (30 kg P_2O_5) caused significant increases in 100 seed weight and pod yield, and up to 100% (P_3) in case of seed yield. These data were confirmed with results considering that leguminous crops require more phosphorus than other crops to attain optimum growth and productivity (Gitari and Murith, 2003). However, the results are in agreement with that obtained by Tomar *et al*. (2004) and Qasim *et al*. (2009) who stated that increased levels of P had a positive effect on 100-seed weight, number of pods and seed yield of soybean. Similar results were obtained also by Zarei *et al*. (2012). In addition, significant increases were achieved in faba bean yield and its attributes by increasing phosphorus fertilization rate up to 46 kg P_2O_5 / fed (El-Gizawy and Mehasen, 2009). The increase in seed yield might be associated with high number of pods/plant, 100-seed weight and seed yield. The results are in accordance with those obtained by Ahmed and El-Abagy (2007).

Application of bio-fertilizer or micronutrient spraying individually caused significant superiority in the mentioned estimations to that without any of them. Regarding the effect of micronutrients spray, the results showed that, treating seeds with micronutrients as foliar application significantly improves the yield and its components rather than without using foliar application. Such increase indicates the efficiency of applying micronutrients by foliar method in correcting micronutrients deficiency that improve nutrients balance, use of NPK fertilizers and yield in turn. Thus, bio-fertilizer and/or micronutrients spraying raised the applied P rate use efficiency. It can be noted that, plants treated with P biofertilizer and foliar application of micronutrients were more effective than plants without P biofertilizer and foliar application of micronutrients recording 55%, 33%, 24% and 29% for weight of 100-seeds, pods, straw and seed yield, respectively. In this regard, Amara (1998) found that spraying with micronutrients resulted in a large increase in total microbial counts in rhizosphere and increased nodule numbers and weights on bean yield. Imtiaz *et al*. (2003) stated that lack of just one micronutrient can greatly reduce plant yield. Furthermore, data in Table 2 reveal that treating seeds with P- biofertilizer significantly increased the yield and its components compared with non-inoculated seeds. Bio-fertilizer results are in accordance with those obtained by Mekail *et al.* (2005) and Yilmaz (2008). Also, IIbas and Sahn (2005) in their work on soybean showed that inoculation with phosphorus has significant effect on 1000-seed weight.

TABLE 2. The effect of all treatments used on yield components and yields.

Growth characters	P levels	Without Bio-P-Fert.(B_0)		With Bio-P-Fert.(B_1)		P Mean	Mean of P.F		Mean of P.B		L.S.D at 5%
		F_0	F_1	F_0	F_1		F_0	F_1	B_0	B_1	
Weight of 100 seeds(g)	P_0	9.32^{k}	11.38^{j}	14.07^{h}	15.84^{g}	12.65^{c}	11.69^{g}	13.61^{f}	10.35^{f}	14.92^{e}	P 0.57 B 0.35 F 0.22 P.B 0.70 P.F 0.43 B.F 0.31 P.B.F 0.60
	P_1	12.82^{i}	15.84^{g}	17.08^{ef}	19.8^{bc}	16.37^{b}	14.95^{e}	17.80^{d}	14.31^{e}	18.44^{c}	
	P_2	16.75^{f}	17.59^{e}	20.03^{b}	23.60^{a}	19.36^{a}	18.39^{c}	19.54^{b}	17.17^{d}	21.82^{a}	
	P_3	18.19^{d}	18.74^{d}	20.18^{b}	20.35^{b}	19.49^{a}	19.19^{b}	20.60^{a}	18.46^{c}	20.27^{b}	
	Mean	14.27	15.84	17.84	19.90	17.00	16.41	17.89	15.06	18.86	
Pods yield kg/fed	P_0	1742^{h}	2097^{g}	2406^{f}	2591^{f}	2209^{d}	2074^{g}	2344^{f}	1919^{f}	2498.8^{e}	P 92.93 B 79.33 F 107.95 P.B 158.67 P.F 215.89 B.F 158.67 P.B.F 305.32
	P_1	2573^{f}	2700^{ef}	3073^{d}	3224^{cd}	2892^{c}	2823^{ed}	2962^{d}	2636^{e}	3148.82^{c}	
	P_2	2706^{ef}	2993^{de}	3440^{c}	5046^{a}	3366^{b}	3073^{cd}	3528^{b}	2850^{d}	4243.33^{a}	
	P_3	3015^{d}	3195^{cd}	3393^{c}	3862^{b}	3546^{a}	3204^{c}	4020^{a}	3105^{c}	3627.83^{b}	
	Mean	2509	2746	3078	3681	3004	2794	3214	2630	3380.0	
Straw yield kg/fed	P_0	1306^{k}	1686^{jk}	2120^{hi}	2133^{hi}	1811^{d}	1713^{e}	1910^{e}	1496^{f}	2126.67^{e}	P 199.40 B 160.42 F 138.76 P.B 320.84 P.F 277.52 B.F 196.24 P.B.F 392.48
	P_1	2066^{ij}	2573^{fg}	3780^{e}	$3960d^{e}$	3095^{c}	2923^{d}	3266^{c}	2320^{e}	3870.0^{c}	
	P_2	$2233g^{hi}$	4173^{cd}	4764^{b}	5893^{a}	3941^{b}	3498^{c}	4433^{b}	3203^{d}	5328.83^{a}	
	P_3	2360^{gh}	4180^{cd}	$4540b^{c}$	4686^{b}	4266^{a}	3450^{c}	5033^{a}	3270^{d}	4613.33^{b}	
	Mean	1991	3153	3801	4168	3279	2896	3661	2573	3985.0	
Seed yield kg/fed	P_0	505^{l}	535^{kl}	570^{k}	757^{fgh}	592^{d}	537^{f}	646^{e}	520^{f}	663.61^{e}	P 15.16 B 19.98 F 12.49 P.B 39.95 P.F 24.98 B.F 39.95 P.B.F 35.43
	P_1	633^{j}	706^{i}	766^{fgh}	1013^{c}	779^{c}	699^{d}	860^{b}	670^{e}	889.83^{c}	
	P_2	$740h^{i}$	$749g^{h}$	949^{d}	1214^{a}	886^{b}	$844b^{c}$	956^{a}	744d	1081.83^{a}	
	P_3	787^{f}	779^{fg}	845^{e}	1134^{b}	913^{a}	816^{c}	981^{a}	783^{d}	989.50^{b}	
	Mean	666	692	782	1029	793	724	861	680	906	

F_0 = without foliar application F_1= with foliar application P_0=without P fertilizerP_1= 15 kg P_2O_5/fed P_2= 22.5 kg P_2O_5/fed P_3=30 kg P_2O_5/fed

Many investigators obtained similar results of the interaction between the P rates of bio-fertilizer and spraying micronutrients including (1) due to mineral P application and P-bio-fertilization (Singh *et al*. 2008; Selvakumar *et al*. 2012 and Uddin *et al.* 2014), (2) due to mineral P application and micronutrients foliation (Kashturikrishna and Ahlawat, 1999) and (3) due to P bio-fertilization and micronutrients spraying (Amara, 1998). This might be due to combined stimulating effect of phosphorus dissolving bacteria and P fertilizer levels in supplying the growing plants with their phosphorus requirements.

Concerning the trible interaction between the treatments of $P_2B_1F_1$ was significantly the best one. The relative increase for weight of 100 seeds, pods, straw and seed yield were 30%, 67%, 150% and 54%, respectively, compared with P_3 (30 kg P_2O_5) without bio-fertilizer or micronutrients spraying in the two studied seasons .This may be attributed to the plant growth promoting substances produced by the bio-fertilizer, in addition to the reasonable quantity of atmospheric nitrogen fixed by *Azotobacter chrococcum*. These reactions saved more available nutrients for enzymes required to build up the different organs compounds and consequently for better growing soybean plant. The general physiological status of the plants as indicated by the dry weight always exhibited positive response to use bio-fertilizer addition. These results are in accordance with Hamed (2003) in his work on faba bean and El- Gizawy and Mehasn (2009) who concluded that adding 40 kg P_2O_5 mixed with phosphate dissolving bacteria (PDB) under spraying of 0.04% Zn EDTA (14% Zn) may be the recommended treatment for improving the productivity of faba bean crop. Thus, it could be recommended that using P_2 (*i.e.,* 75% from the recommended P application rate) in presence of P bio-fertilization and micronutrients spraying gave the best significant results for 100 seed weight, pod, straw and seed yields of soybean under the experiment condition as compared to applying higher inorganic P fertilizer levels, which exert a massive cost on the farmer (Dutta and Prohit, 2009).

Nutrients uptake

Macronutrients

As indicated by the effect of phosphorus levels, the data in Table 3 showed that not only the positive effect of P application on P uptake, as expected, but also its significant positive effect on N and K uptake. Also, raising the rate of P application up to 100 % (P_3) achieved the highest values for the aforementioned parameters. Therefore, the application of phosphorus has a dramatic effect on legumes when it applied to soils low in phosphorus. Phosphorus helps legumes plants in root nodulation, efficient use of nutrients, N_2 fixation and efficient partitioning of photosynthates from vegetative to reproductive parts (Gitari and Mureith, 2003). These results are in conformity with those that report a significant increase in N, P and K content and uptake in the leaves or straw due to P application in soybean (Fatima *et al.*, 2007).

TABLE 3. The effect of all treatments used on macronutrients uptake in seeds of soybean plants.

Macronutrients (Kg fed^{-1})	P levels	Without Bio-P-Fert. (B_0)		With Bio-P-Fert. (B_1)		P Mean	Mean of P.F		Mean of P.B		L.S.D at 5 %
		F_0	F_1	F_0	F_1		F_0	F_1	B_0	B_1	
N	P_0	37.78	42.09	42.90	58.78	45.39^{c}	40.34^{c}	50.44^{ab}	39.93^{b}	50.84^{a}	P 19.98 B 15.16 F 12.49 P.B 39.95 P.F 17.67 B.F N.S P.B.F N.S
	P_1	42.91	46.71	53.07	69.71	53.10^{b}	41.99^{bc}	58.21^{ab}	44.81^{a}	61.39^{a}	
	P_2	48.50	50.21	66.49	83.02	61.96^{a}	57.49^{abc}	66.62^{a}	49.36^{a}	74.76^{a}	
	P_3	52.39	52.96	63.51	78.78	62.06^{a}	57.95^{abc}	65.87^{a}	52.67^{a}	71.14^{a}	
	Mean	45.40	48.0	56.50	72.57	55.63	50.94	60.29	46.7	64.53	
P	P_0	2.17	2.78	2.96	3.48	2.85^{d}	2.57	3.13	2.48^{e}	3.22^{d}	P 0.25 B 0.11 F 0.18 P.B 0.50 P.F N.S B.F N.S P.B.F N.S
	P_1	3.19	3.35	3.58	4.22	3.58^{c}	3.38	3.78	3.27^{d}	3.90^{c}	
	P_2	3.41	3.90	5.44	6.31	4.61^{a}	4.43	5.11	3.66^{cd}	5.88^{a}	
	P_3	3.67	4.38	4.65	5.75	4.17^{b}	4.16	5.07	4.03^{c}	5.20^{b}	
	Mean	3.11	3.60	4.16	4.94	3.80	3.64	4.27	3.36	4.55	
K	P_0	9.78^{h}	11.13^{gh}	11.56^{g}	14.29^{f}	11.70^{c}	10.64^{f}	12.71^{e}	10.46^{f}	12.94^{e}	P 0.63 B 0.63 F 0.56 P.B 1.27 P.F 0.79 B.F N.S. P.B.F 1.57
	P_1	11.42^{g}	14.51^{ef}	15.44^{ef}	20.03^{c}	15.35^{b}	13.43^{d}	17.27^{b}	12.97^{e}	17.73^{c}	
	P_2	14.46^{f}	14.78^{ef}	17.20^{d}	24.41^{a}	17.46^{a}	15.83^{c}	19.59^{a}	14.62^{d}	20.80^{a}	
	P_3	15.01^{ef}	16.05^{de}	16.05^{de}	22.62^{b}	17.71^{a}	15.58^{c}	19.33^{a}	15.53^{d}	19.38^{b}	
	Mean	12.67	14.12	15.06	20.34	15.56	13.87	17.23	13.40	17.71	

F_0 = without foliar application F_1= with foliar application P_0=without P fertilizer P_1= 15 kg P_2O_5/fed P_2= 22.5 kg P_2O_5/fed P_3= 30 kg P_2O_5 /fed

Concerning the effect of foliar application of micronutrients under different levels of P fertilizer, the data in Table 3 showed that N and K uptake were significantly affected. Also, the data reveal that P_3 with foliar applied achieved the highest values of macronutrient uptake. This may be due to the effect of micronutrients which can play an important role in the availability of phosphorus in the soil. Imtiaz *et al*. (2003) stated that adequate nutrition of plant with micronutrients depends on many factors. Among them is the ability of soil to supply these nutrients, rate of nutrients absorption by the plants, distribution of nutrients to function sites and nutrients mobility within the plant. In this respect, El-Fouly and Fawzi (1996) noticed that micronutrients application encourages the growth of roots, which in turn take up higher contents of N, P and K and finally being reflected on the yield.

Regarding the effect of interaction between P biofertilizer and different levels of mineral phosphorus fertilizer, the results in Table 3 show that there was a significant enhancement in the uptake of N, P and K in comparison to plants untreated with biofertilizer. It is also reported that, P_2+ P biofertilizer gave the highest values compared with the other levels of phosphorus fertilizer. This may be explained as the application of biofertilizers could make such nutrients more available for plants. While, the combined treatment of P-biofertilizer and foliar spray of micronutrients insignificantly increases the uptake of N, P and K. Phosphate solubilizing microorganisms increased available phosphorus and nitrogen in the soil which increases their concentration by plant, also increased the grain protein. Likewise, phosphate solubilizing bacteria have the capability to solubilize the residual or fixed soil P and increase the availability of P in the soil (Singh *et al*., 2008) producing the growth promoting substances (Selvakumar *et al*., 2012) and thereby increase the overall P-use efficiency of the crops. Moreover, bio-phosphate bacteria efficiency increased by application of biological phosphorus fertilizer combined with chemical phosphorus fertilizer. Mahfouze and Sharaf - Eldin (2007) reported that phosphorus solvent bacteria have the ability to produce organic acids that would increase solubility of phosphorus available for plant. It can be noted that plants treated with P biofertilizer and foliar application of micronutrients were more effective than plants without them, they were recording 38%, 35% and 32% for N, P and K uptake, respectively.

Regarding the trible interaction, the data in Table 3 illustrated that N and P uptake showed non-significant response to applied mineral P + P bio + foliar micronutrients, while K uptake significantly responsed. The data showed that maximum values of N, P, K uptake under P_2 + B_1 + foliar were obtained than those in treatments which received other levels of phosphorus mixed with biofertilizer and foliar application or that achieved by the single use of inorganic P fertilizer or either of P bio-fertilizer and foliar micronutrient. In general, application of P_2+B_1+foliar exhibited the best treatments in enhancing the uptake of the studied macronutrients by seeds as it achieved 58%, 71% and 63% increases in N, P and K uptake by seeds, respectively comparing with P_3 (30 kg P_2O_5) without bio-fertilizer or micronutrients spraying. It is interesting to mention that the high increased uptake could be explained as a result of the remarkable increase in the seeds weight of yield achieved by this treatment. This increase is mainly attributed to the effect of microorganisms which can play a very significant role in making available nutrients elements for plants. It is essential to bring about some microbial transformation of both inorganic and organic compounds in the soil to make available of these elements to soybean plant. These results are in agreement with those found by Cabrera (2006) who reported that the application of biofertilizers enabled plants to extract a higher amounts of N, P, K, Ca^{+2} and Mg^{+2} and increased yield.

Micronutrients

Data in Table 4 show that the positive effect of P application on Fe, Zn and Mn uptake and graded the rate of P application up to P_3 achieved the highest values over that of P_0. Application of bio-fertilizer or micronutrients spraying individually caused significant superiority in the uptake of micronutrients to that without any of them. This may be due to improved physical and chemical properties of the soil and increased the available nutrients to plant. Foliar application is very fast method for providing required elements in plant because nutrients are taken up quickly as compared to that through plant roots (El-Haggan, 2014).

The double interaction between each of those three factors and another presented in Table 4 clarifies that the combination of bio-fertilizer with P at the rate P_2 was the best and significantly encouraged the uptake of micronutrients (El-Gizawy and Mehasen, 2009). While the effect of combined treatment between P application and foliar spraying with micronutrients had insignificant effect on the uptake of Mn only. Also, the interaction between P-biofertilizer and foliar micronutrients caused significant increase in the uptake of Fe and Mn. The increase percent for plants treated with P biofertilizer and foliar application of micronutrients were more effective than plants without P biofertilizer and foliar application of micronutrients recording 22%, 42% and 104% for Fe, Zn and Mn uptake, respectively.

Regarding the effect of the three factors together on uptake of micronutrients, the results reveal that Fe and Mn significantly responded while, Zn was insignificant. As well as the results mentioned that P_3 + P- biofertilizer + foliar spraying of micronutrients gave the highest values compared to the other levels of P application due to promoting root growth which in turn enhancing nutrients and water uptake from the soil. The relative increase was 28, 82 and 195% (for Fe, Zn and Mn, respectively) comparing with P_3 (30 kg P_2O_5) without bio-fertilizer or micronutrients spraying (Zaghloul *et al*., 2015). In this regard, zinc absorption reduced by phosphorus application in the soil, but biological phosphorus fertilizers due to having phosphate solubilizing bacteria had no interference on other elements uptake (Solimpour *et al.*, 2010).

TABLE 4. The effect of all treatments used on micronutrients uptake in seeds of soybean plants.

Micronutrients (gm/fed)	P levels	Without Bio-P-Fert.(B_0)		With Bio-P-Fert.(B_1)		P mean	Mean of P.F		Mean of P.B		L.S.D at 5 %
		F_0	F_1	F_0	F_1		F_0	F_1	B_0	B_1	
Fe	P_0	114.59^{m}	114.91^{m}	119.69^{lm}	135.92^{jk}	121.28^{c}	117.14	125.41	114.75^{h}	127.81^{g}	P 4.22 B 4.79 F 2.86 P.B 9.58 P.F N.S B.F 4.05 P.B.F 8.09
	P_1	128.20^{k}	138.03^{ij}	158.57^{f}	184.30^{c}	152.28^{b}	143.39	161.17	133.12^{f}	171.44^{c}	
	P_2	149.66^{h}	150.25^{gh}	182.06^{c}	218.52^{a}	173.12^{a}	165.86	184.39	149.96^{e}	200.29^{a}	
	P_3	158.27^{fg}	166.09^{ef}	173.88^{de}	194.95^{b}	175.30^{a}	166.08	180.52	162.18^{d}	184.42^{b}	
	Mean	137.68	142.32	158.55	183.42	155.50	148.12	162.87	140.0	171.0	
Zn	P_0	35.2	40.12	43.12	52.65	42.78^{c}	39.17	46.39	37.67^{e}	47.88^{cd}	P 3.66 B 2.74 F 2.21 P.B 5.48 P.F N.S B.F N.S P.B.F N.S
	P_1	40.74	44.51	53.59	72.72	52.89^{b}	47.17	58.62	42.62^{de}	63.16^{b}	
	P_2	44.33	48.15	64.47	86.63	59.70^{a}	54.35	67.39	46.19^{d}	75.55^{a}	
	P_3	47.50	57.65	56.90	76.75	60.87^{a}	52.20	67.20	52.58^{c}	66.83^{b}	
	Mean	41.94	47.61	54.52	72.19	54.06	48.22	59.90	44.77	63.36	
Mn	P_0	8.10^{j}	12.99^{hi}	14.30^{gh}	28.27^{d}	15.92^{c}	11.20^{e}	20.63^{cd}	10.55^{g}	21.29^{d}	P 2.69 B 1.65 F 1.10 P.B 3.29 P.F 3.12 B.F 0.55 P.B.F 3.11
	P_1	11.00^{ij}	16.48^{g}	25.01^{e}	31.80^{c}	21.07^{b}	18.00^{d}	24.14^{b}	13.74^{f}	28.40^{c}	
	P_2	13.83^{ghi}	19.31^{fg}	30.63^{cd}	44.91^{a}	26.16^{a}	22.23^{bc}	32.11^{a}	16.57^{ef}	37.7^{a}	
	P_3	15.22^{gh}	21.82^{f}	28.71^{cd}	38.89^{b}	27.17^{a}	21.97^{bc}	30.35^{a}	18.58^{e}	33.80^{b}	
	Mean	12.04	17.65	24.66	35.97	22.58	18.35	24.31	14.86	30.30	

F_0 = without foliar application F_1= with foliar application P_0= without P fertilizer P_1= 15 kg P_2O_5 /fed P_2= 22.5 kg P_2O_5/fed P_3= 30 kg P_2O_5 /fed

Yields of protein and oil

Data presented in Table 5 reveal that raising the rate of P application up to P_3 significantly increased the contents of protein and oil in comparison with untreated plant. In addition, application of bio-fertilizer or spraying micronutrients individually caused significant response in the content of protein and oil over to that without any of them (El-Haggan, 2014). Concerning the combined effect between different levels of P application and both of P solubilizing bacteria and foliar spraying of micronutrients, the results in Table 5 reveal that protein and oil contents were significantly affected by using foliar treatment and P_3 recorded the highest values. Amara (1998) found also that spraying with micronutrients resulted in a large increase in protein content of broad bean. Hemati (2005) reported that wheat protein increased by application of micronutrients such as Zn, Mn and Fe. In addition, the results mentioned that P_2+ P biofertilizer gave the highest values of protein and oil contents compared with the other levels of phosphorus fertilizer. In this connection Saber *et al.* (1989) reported that application of bio-fertilizer increased oil and protein contents as well as nutrient elements in soybean seeds. It can be noted that plants treated with P biofertilizer and foliar application of micronutrients were more effective than plants without P biofertilizer and foliar application of micronutrients recording 36 and 28 % for protein and oil contents, respectively.

Furthermore, the effect of three factors on content of protein and oil was insignificant, but, the treatment of $P_2B_1F_1$ was the best one and achieved relative increase 58 and 43% for protein and oil contents compared with P_3 (30 kg P_2O_5) without bio-fertilizer or micronutrients spraying.The increased protein mainly due to the increase in N content means that biofertilizers can provide plants with essential nutrients elements required for oil and protein formation. These results are in line with those obtained by El-Kholy and Gomaa (2000). The application of inorganic P fertilizer and PBF with foliar micronutrient might have assured P availability and its subsequent utilization for formation of carbon skeletons and for the synthesis of amino acids and ATP that, in turn, might have led to an enhanced synthesis of protein during soybean seed development (Trwari and Pal, 2005). However, P_2+ PBF + foliar application of micronutrients was found to be the best interaction between inorganic P levels and bio-fertilizers treatment with micronutrients. In general, microorganism can play a very significant role in making available phosphorus to plants. It is essential to bring about some microbial transformations of both inorganic and organic compounds in soil to make available of this element to plant.

TABLE 5.The effect of all treatments on protein and oil yields contents in seeds of soybean plants.

Yield	P levels	Without Bio-P-Fert.(B_0)		With Bio-P-Fert.(B_1)		P mean	Mean of P.F		Mean of P.B		L.S.D at 5%
		F_0	F_1	F_0	F_1		F_0	F_1	B_0	B_1	
Protein (kg / fed)	P_0	236.07	263.08	252.01	367.24	279.60^{c}	244.04^{e}	315.16^{c}	249.58^{f}	309.62^{e}	P 6.03 B 4.80 F 4.81 P.B 9.61 P.F 9.63 B.F 6.81 P.B.F N.S
	P_1	274.79	291.95	331.69	435.70	333.53^{b}	303.24^{d}	363.83^{b}	283.37^{c}	383.70^{c}	
	P_2	318.57	309.68	413.60	518.9	386.62^{a}	366.09^{b}	414.29^{a}	314.13^{e}	466.25^{a}	
	P_3	327.46	330.98	396.60	491.43	390.19^{a}	362.03^{b}	411.21^{a}	329.22^{d}	444.02^{b}	
	Mean	289.22	298.92	348.48	453.32	347.49	318.85	376.12	294.08	400.90	
Oil (kg/fed)	P_0	62.02	85.82	93.90	113.05	73.70^{c}	87.96^{e}	99.43^{d}	83.92^{f}	103.47^{e}	P 2.60 B 2.30 F 4.81 P.B 4.59 P.F 3.27 B.F 2.31 P.B.F N.S
	P_1	102.33	112.50	115.02	150.73	120.14^{b}	108.67^{c}	131.62^{b}	107.41^{e}	132.88^{c}	
	P_2	115.11	121.46	151.20	185.63	143.27^{a}	133.16^{b}	153.55^{a}	118.29^{d}	168.42^{a}	
	P_3	129.94	130.45	134.88	177.80	143.35^{a}	132.41^{b}	154.13^{a}	130.20^{c}	156.34^{b}	
	Mean	102.35	112.56	123.75	156.80	120.12	115.55	134.68	110.0	140.29	

F_0 = without foliar application F_1= with foliar application P_0= without P fertilize P_1= 15 kg P_2O_5 /fed P_2= 22.5kg P_2O_5/fed P_3=30 kg P_2O_5 /fed

Conclusion

In the light of the obtained results, it could be concluded that a combined application of 22.5 kg P_2O_5/fad (P_2) + P biofertilizer+ foliar micronutrients was generally significant and provided the best condition for achieving maximum yield and its quality attributes. In general, environmental pollution could be reduced by decreasing consumption of chemical fertilizer. Overall, application of P biofertilizer with mineral phosphorus fertilizer and foliar micronutrients in addition to yield increasing could be a strategy to achieve sustainable agriculture. Therefore, the integrated fertilization including mineral (macro- and micro-nutrients) and bio-fertilizers can be considered an important strategy for sustainable agriculture.

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تقييم استخدام التسميد الحيوى والمعدنى للفوسفور مع الرش بالعناصر الصغرى على انتاجية وجودة محصول فول الصويا

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اقيمت تجربتان حقليتان خلال الموسم الصيفى 2013 و2014 فى محطة بحوث الجميزة بمحافظة الغربية وذلك لدراسة استخدام معدلات مختلفة من التسميد الفوسفاتى (بدون اضافة فوسفور- اضافة 15 كجم فوسفور /فدان - 22.5 كجم/فدان – 30كجم/فدان) سواء بمفردها او الإضافة مع وجود فسفور حيوى واستخدام الرش بالعناصر الصغرى وتأثير ذلك على محصول فول الصويا ومكوناته وجودته.

وقد كانت النتائج المتحصل عليها كما يلى :

- أدى استخدام التسميد المعدنى أو الحيوى للفوسفورأو الرش بالعناصر الصغرى سواء بصورة منفردة او مرتبطة الى حدوث زيادة معنوية لمحصول فول الصويا ومكوناته
- كذلك أظهرت النتائج ان أفضل معاملة كانت بأستخدام التسميد الفوسفاتى بمعدل 75٪ من الموصى به مع التسميد الحيوى والرش بالعناصر الصغرى حيث سجلت أعلى قيم لمحصول فول الصويا ومكوناته.
- لوحظ من النتائج المتحصل عليها ان استخدام التسميد المعدنى أو الحيوى للفوسفورأو الرش بالعناصر الصغرى سواء بصورة منفردة او مرتبطة أدى الى حدوث زيادة معنوية فى محتوى العناصر الكبرى والصغرى وكذلك محتوى فول الصويا من البروتين والزيت.
- وجد أن أستخدام التسميد الفوسفاتى بمعدل 75٪ مرتبطا مع الفوسفات الحيوى الى حدوث زيادة معنوية فى محتوى العناصر الكبرى والصغرى وكذلك محتوى فول الصويا من البروتين والزيت بينما ادى استخدام معدلات الفوسفور مع الرش بالعناصر الصغرى الى زيادة محتوى النيتروجين- البوتاسيوم - المنجنيز وكذلك البروتين والزيت .
- كذلك فقد أظهرت النتائج أن أستخدام التسميد الفوسفاتى بمعدل 75٪ مرتبطا مع الفوسفات الحيوى والرش بالعناصر الصغرى احدث زيادة معنوية فى امتصاص البوتاسيوم والحديد والمنجنبز.
- ولذلك فانه من الواضح ان استخدام التسميد الحيوى من الممكن ان يحل محل جزء من التسميد المعدنى فى انتاج محصول فول الصويا مما يؤدى الى تقليل التلوث البيئى الناجم عن استخدام الاسمدة المعدنية.