

EFFECT OF MINERAL AND BIOFERTILIZATION ON NODULATION, NODULE ACTIVITY AND YIELD IN SOYBEAN (*Glycine max*, L.).

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ABSTRACT: *Two field experiments were carried out in the Experimental Farm, Faculty of Agriculture, Minufiya University, Shebin El-Kom to study the effect of nine mineral fertilization treatments (0, 30 kg N, 60 kg N, 15 kg P₂O₅, 30 kg P₂O₅, 24 kg K₂O, 48 kg K₂O, 30 kg N + 15 kg P₂O₅ + 24 kg K₂O and 60 kg N + 30 kg P₂O₅ + 48 kg K₂O/ fed) and eight biofertilization treatments (0, Rhi., Pho., Pot., Rhi.+ Pho., Rhi. + Pot., Pho + Pot. and Rhi.+ Pho.+ Pot.) on nodulation and productivity of soybean (Giza 111 cultivar) during 2006 and 2007 seasons. The obtained results could be summarized as follows:*

- 1- Mineral fertilization with 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed enhanced nodulation and microbial activation as expressed in dry weight of nodules/plant and activities of nitrogenase and dehydrogenase enzymes as well as yield and its components (number of pods/plant, number of seeds /pod, 100-seed weight, seeds weight/pod, seed yield/plant and seed, straw and biological yields/fed) compared with the other mineral fertilization treatments. However, unfertilized plants produced the highest values of number of nodules/plant in both seasons.*
- 2- Seed inoculation with the tested biofertilizers reflected a marked effect on nodulation and microbial activation as well as yield and its components in favour of triple inoculation with Rhi. + Pho. + Pot. followed by dual inoculation with Rhi. + Pho. in most characters with the exception of number of nodules/plant which recorded the highest values by dual inoculation with Rhi. + Pho. in the two seasons.*
- 3- The interactions between the tested mineral and biofertilizers were found to be significant for most traits studied herein. Unfertilized N, P and K plants when inoculated with Rhi. + Pho. recorded the highest number of nodules/plant. However, the application of 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed combined with Rhi. + Pho. + Pot. was the most effective treatment for increasing the dry weight of nodules, nitrogenase and dehydrogenase activities, number of pods, seed yield/plant and seed and straw yields/fed compared to the other tested treatments.*
- 4- Significant positive correlation coefficients were detected between nodulation and microbial activation as well as yield and its components indicating that the productivity of soybean could be increased by improving the nodulation and microbial activation, particularly dehydrogenase activity which showed more stronger association with the seed yield/fed than with any other nodulation or enzyme activity attribute.*

Key Words: *Mineral fertilizers, biofertilizers, nodulation, enzymes activities, yield, soybean.*

INTRODUCTION

Soybean (*Glycine max*, L.) belongs to the family Fabaceae, has been introduced to Egyptian agriculture which could help in reducing the food gap owing to high seed quality. Protein and oil in seeds reach 40 % protein and 20 % of oil. Indicating, it is high nutritive value for both human and livestock. In addition, soybean plants improve soil fertility through nitrogen fixation by symbiotic bacteria (*Bradyrhizobium japonicum*). Symbiotically, soybean fixes 125-150 kg N/ha (Chandel *et al.*, 1989) and add about 30-40 kg N/ha for succeeding crop (Saxena and Chandel, 1992).

Productivity of soybean could be sustained with balanced nutrition. Therefore, mineral fertilizers applications with N (Abd El- Fattah, 2001), P (Mehasen *et al.*, 2002) and K (Darwish, 2003) played great roles in the physiological and metabolic processes in the soybean plants. The amounts of mineral fertilizers have been doubled during the last period and hence caused environmental pollution, harmed the soil beneficial bacteria and introduced numerous environmental troubles (Lampkin, 1990) which led to a public health risk (Top *et al.*, 2002).

Recently, using biological fertilizers became very important to reduce the environmental pollution as result of heavy use of mineral fertilizers that increases production cost (Hussein and El- Melegy, 2005). Inoculation of seeds or soil with N₂-fixing bacteria could compensate about 40% of plant requirements of mineral nitrogen. Biofertilizers are the most advanced biotechnology which can increase the output, necessary to support developing organic agriculture, non pollution agriculture, improved fertility, produced toxic metabolites inhibitory to many pathogenic fungi, improved nodulation (Groppa *et al.*, 1998 and Hamissa *et al.*, 2000) and yield (Gomaa, 1996; Badr El-Din and Moawad, 1998 and Okba, 2009).

Therefore, the present study was planned to find out the response of soybean (Giza 111 cv.) to varying levels of macro mineral fertilizers, i.e. N, P and K and some biofertilizers, i.e. Rhizobacterien (as N₂ fixing bacteria), Phosphorien (as P dissolving bacteria) and Potassiumage (as K mobilizing microorganisms). Nodulation and microbial activation as well as yield and its components were, also, investigated.

MATERIALS AND METHODS

Two field experiments were carried out in the Experimental Farm, Faculty of Agriculture, Minufiya University, Shebin El-Kom to study the effect of mineral and biofertilization on nodulation and microbial activation as well as yield and its components of soybean (Giza 111 cultivar) during 2006 and 2007 seasons. The experiment included seventy two treatments which were the combination of nine mineral fertilizers and eight biofertilizers as follows:

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A- Mineral fertilizers

- (1) 0 (without fertilization) (2) 30 kg N/fed (3) 60 kg N/fed
- (4) 15 kg P₂O₅ / fed (5) 30 kg P₂O₅ /fed (6) 24 kg K₂O /fed
- (7) 48 kg K₂O / fed (8) 30 kg N + 15 kg P₂O₅ + 24 kg K₂O /fed
- (9) 60 kg N + 30 kg P₂O₅ + 48 kg K₂O /fed

B- Biofertilizers

- (1) 0 (Uninoculation)
- (2) Rhizobacterien (Rhi.): as N₂-fixing included bacteria of *Bradyrhizobium japonicum* and *Azotobacter chroococcum*.
- (3) Phosphorien (Pho.): as phosphate dissolving included bacteria of *Bacillus megaterium var. phosphaticum*.
- (4) Potassiumage (Pot.): Potassiumage (as potassium mobilizing) included bacteria of *Bacillus circulans*, *pseudomonas sp* and actinomycetes and fungi of *Aspergillus sp* and *Mucor sp*.
- (5) Rhi. + Pho. (6) Rhi. + Pot.
- (7) Pho. + Pot. (8) Rhi. + Pho. + Pot.

Mineral fertilizers were soil fully applied in the forms of urea (46.5% N), ordinary superphosphate (15 % P₂O₅) and potassium sulphate (48 % K₂O) before the first irrigation in both seasons. Soybean seeds were inoculated with the tested biofertilizer treatments at a rate of 30 g /kg seeds for each treatment by using Arabic gum solution as an adhesive agent before sowing. The experimental design was a split plot with four replications. The mineral fertilizers were arranged at random in the main plots, whereas the biofertilizers were assigned at random in the sub-plots. The area of each experimental plot was 16.8 m², including seven ridges, four meters long and 60 cm apart. Seeds were sown on April 28th and May 5th in first and second seasons, respectively in hills 20 cm apart on the two sides of the ridge. Twenty days after sowing, plants were thinned to two plants /hill, i.e. 140000 plants/fed. The preceding crops were Egyptian clover and wheat in first and second seasons, respectively. Soil mechanical and chemical analyses of the experimental site are presented in Table (1)

Table (1): Soil Mechanical and chemical properties of the top soil depth (0-30 cm) during 2006 and 2007 seasons.

Properties Seasons	Mechanical					Chemical					
	Fine sand %	Coarse sand %	Silt %	Clay %	Texture class	pH	E.C. dS/m	O.M. %	Available macronutrients (ppm)		
									N	P	K
2006	15.2	6.5	41.5	36.8	Clay loam	7.9	0.82	1.7	29.4	7.53	300.5
2007	16.5	6.0	39.0	38.5	Clay loam	7.5	0.50	2.0	33.8	8.75	348.3

Characters studied

Nodulation and microbial activity:

At 70 days after sowing, five plants were uprooted by mattock at random in each experimental plot. The roots were dipped in water to remove the soil then washed with distilled water. The following data were recorded on the roots and rhizosphere soil per each plant.

- 1- Number of nodules /plant.
- 2- Dry weight of nodules /plant (g.).
- 3- Nitrogenase activity $\mu\text{mole C}_2\text{H}_4/\text{g D.Wt nodules/hr}$: It was measured in the root samples, chromatography by acetylene reduction technique as described by Hardy *et al.* (1973) and Somasegaran and Hoben (1985).
- 4- Dehydrogenase activity ($\mu\text{g TPF/ g dry soil /24 hours}$): It was measured in rhizosphere soil, spectrophotometry for triphenyl formazan (T.P.F) produced from the reduction of 2,3,5 triphenyl tetrazolium chloride (T.T.C) using acetone for extraction according to Thalmann (1967).

Yield and yield components:

At harvest, ten guarded plants were taken to determine individual plant characters. The seed, straw and biological yields of two ridges were determined and calculated in ton/fed.

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|-----------------------------|----------------------------------|
| 1- Number of pods / plant. | 2- Number of seeds / pod. |
| 3- 100-seed weight (g.). | 4- Seeds weight / pod (g.). |
| 5- Seed yield / plant (g.). | 6- Seed yield / fed (ton). |
| 7- Straw yield / fed (ton). | 8- Biological yield / fed (ton). |
| 9- Harvest index (%) | |

Correlation coefficients

Simple correlation coefficients were calculated between each of nodulation attributes (number and dry weight of nodules/plant) and microbial activity (nitrogenase and dehydrogenase activities) on one hand and yield and its components on the other.

The data were statistically analyzed according to the method described by Snedecor and Cochran (1967). Duncan's multiple range test (Duncan, 1955) was used to compare between the treatment means.

RESULTS AND DISCUSSION

A- Effect of mineral fertilizers

A. 1- Nodulation and microbial activity:

The data of number and dry weight of nodules per plant as well as activities of nitrogenase and dehydrogenase enzymes at 70 days after sowing as influenced by mineral fertilizers in the two growing seasons are shown in Table (2).

Results recorded in Table (2) show that the application of the different NPK fertilizers resulted in reducing number of nodules/ soybean plant

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compared to unfertilized plants in the two seasons. The data, also, show that the highest average of the number of nodules was recorded by the application of 15 kg P₂O₅ followed by 24 kg K₂O in the two seasons. In this respect, Hamissa *et al.* (2000) suggested that nodulated legumes dependent on symbiotic nitrogen for growth may require more phosphorus than those dependent on combined nitrogen. Moreover, the data obtained herein show that the lowest rates of N, P and K either separately or their combination took the next rank in a descending order. In this respect, other researcheres found that number of nodules/soybean plant was increased by the application of 20 kg P₂O₅/ha (Sherif *et al.*, 1993), but decreased by the application of 45 kg N/fed (El- Essawi *et al.*, 1993) and 48 kg K₂O/fed (El- Saady, 2004) compared to untreated plants with mineral fertilizers.

Data in the same Table indicate that the values of dry weight of nodules/plant and activity of nitrogenase enzyme tend to be significantly highest in plants treated with 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed treatment but without significant differences with 15 kg P₂O₅/fed in both seasons. However, when plants were fertilized with the highest levels of the three tested elements, i.e. treatment of 60 kg N + 30 kg P₂O₅ + 48 kg K₂O they recorded the lowest averages of the two traits in both seasons. The positive effect of 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed treatment on nitrogenase activity might be attributed to the role of these nutrients in increasing the number and dry weight of nodules /plant and consequently led to more nitrogen fixation. In this respect, similar results were obtained by other researcheres who found that nodules dry weight/plant and nitrogenase activity were increased by the application of N (El- Howeity *et al.*, 2009) in faba bean plants and P (Mehasen *et al.*, 2002 and Mehasen and El- Ghozoli, 2003) in soybean plants compared to unfertilized plants. However, Hamissa *et al.* (2000) suggested that phosphorus play a vital role in reactions involving energy transfer and more specifically, ATP in nitrogenase activity.

It is evident, also, that all mineral fertilizers caused a remarkable increase in dehydrogenase activity as compared with unfertilized plants (Table 2). The greatest dehydrogenase activity was obtained by the application of 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed in the two seasons. On the contrary, the obtained data show that the application of 60 kg N + 30 kg P₂O₅ + 48 kg K₂O/fed recorded poor activity of dehydrogenase enzyme compared to that of the other mineral fertilizers. In this connection, Burns (1982) indicated that dehydrogenase enzyme is not only responsible for oxidation – reduction processes, but also involved in the recycling of elements in soil for the benefit of both macro and microflora. These results are in accordance with those obtained by Abd El Maksoud *et al.* (1995) who found that the high application of P and/or N was inhibitory factor for the activity of microorganisms. In this connection, El- Howeity *et al.* (2009) noticed that dehydrogenase activity in the soil rhizosphere was significantly increased

with increasing nitrogen level up to 40 kg N/fed compared to unfertilized faba bean plants.

Table (2): Effect of mineral fertilizers application on nodulation and microbial activation at 70 DAS during 2006 and 2007 seasons.

Mineral fertilizers (kg/fed)	Nodules /plant		Nitrogenase □μmole C ₂ H ₄ / g D.Wt nodules / hr)	Dehydrogenase (μg TPF/ g dry soil /24 hours)
	Number	Dry weight (g.)		
2006 season				
0	105.19 a	0.489 b	38.16 b	44.74 g
30 N	75.83 b	0.474 b	31.27 c	59.11 bc
60 N	36.96 d	0.241 c	27.48 d	54.68 de
15 P ₂ O ₅	96.77 a	0.574 a	41.20 ab	61.10 ab
30 P ₂ O ₅	59.90 c	0.473 b	34.00 c	57.55 cd
24 K ₂ O	95.56 a	0.548 a	41.05 ab	56.63 cde
48 K ₂ O	67.45 bc	0.452 b	32.90 c	53.58 e
30 N+15 P ₂ O ₅ +24 K ₂ O	69.32 bc	0.582 a	42.01 a	63.76 a
60 N+30 P ₂ O ₅ +48 K ₂ O	32.23 d	0.193 d	20.92 e	50.29 f
2007 season				
0	98.04 a	0.450 c	35.33 cd	47.70 f
30 N	67.01 d	0.390 d	28.15 f	62.24 c
60 N	36.20 g	0.234 e	23.09 g	56.82 de
15 P ₂ O ₅	89.07 b	0.514 ab	38.67 ab	64.97 b
30 P ₂ O ₅	61.73 ef	0.383 d	30.80 ef	60.98 c
24 K ₂ O	82.94 c	0.494 b	36.79 bc	58.42 d
48 K ₂ O	59.77 f	0.387 d	32.47 de	56.54 de
30 N+15 P ₂ O ₅ +24 K ₂ O	66.43 de	0.521 a	40.30 a	68.85 a
60 N+30 P ₂ O ₅ +48 K ₂ O	28.41 h	0.161 f	17.65 h	54.87 e

A. 2- Yield and its components:

Results in Table (3) reveal that number of pods/plant was significantly increased by the application of all tested mineral fertilizers in both seasons as compared with the unfertilized plants in favour of 30 kg N + 15 kg P₂O₅ + 24 kg K₂O followed by 15 kg P₂O₅ /fed in the two seasons. These increases occurred herein by the such treatments may be due to the increase in the number and dry weight of nodules as well as nitrogenase and dehydrogenase activities as shown in Table (2). These results are in agreement with those obtained by other investigators who found that number

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of pods of soybean plant was increased by the application of N (Mehasen and Saeed, 2005), P (Abdel- Mohsen *et al.*, 2002) and K (Darwish, 2003) as well as in combination at levels of 20, 60 and 40 kg N, P₂O₅ and K₂O/ha, respectively (Saxena *et al.*, 2001) compared with the unfertilized plants.

The data in Table (3) demonstrate that the number of seeds/pod, 100-seed weight and seeds weight/pod were significantly enhanced by the application of all tested mineral fertilizers in comparison to the control treatment in both seasons. Moreover, it could be noticed that the application of the lower NPK levels followed by the lower NP levels seemed to be the most effective treatments in increasing the values of those traits in the two seasons. Therefore, it can be suggested that the translocation of photoassimilates from the vegetative plant tissues (source) to the seeds (sink) was much affected by plant nutrition status which led to the promotion of cell division, build up of storage sink capacity and hence increased the translocation of metabolites to sink tissues (Mengel and Kirkby, 1987). These findings are in harmony with those obtained by Mohamed (2000) who mentioned that adding some mineral fertilizers as a mixture consisted of ammonium nitrate + superphosphate + potassium sulphate/fed had a promoting effect in increasing number of seeds/pod, 100-seed weight and seeds weight/pod of broad bean compared to unfertilized plants.

The data in Table (3) show that significant increases in seed yield per plant and fed were obtained by the application of all tested mineral fertilizers more than the control treatment in the two growing seasons. The highest seed yield per plant and fed were recorded when the plants received 30 kg N + 15 kg P₂O₅ + 24 kg K₂O followed by 15 kg P₂O₅ and 30 kg N treatments in the two growing seasons. From these results, it can be noticed that, the application of 15 kg P₂O₅ /fed or 30 kg N/fed seemed to be satisfactory in producing almost the same seed yield/fed which obtained by adding 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed. Therefore, it can be suggested that the treatment of 15 kg P₂O₅ /fed or 30 kg N/fed are considered to be economic treatments for soybean yield because it save about 30 kg N or 15 kg P₂O₅ beside 24 kg K₂O/fed under the present experimental condition. It is evident from Table (1) the soil of the experimental site was very rich in its content from available K (324.4) and moderately rich in its content from available P (8.14) as an average of the two seasons. These contents could be seemed to explain the failure of K addition or the increase of P level to 30 Kg P₂O₅/fed to add further significant increase in seed yield/fed. Similar results were reported by other investigators who found that seed yield per plant and unit area were increased when plants were fertilized with N (Mehasen and Saeed, 2005), P (Abdel- Mohsen *et al.*, 2002 and Mehasen *et al.*, 2002) and K (Darwish, 2003) for soybean as well as NPK (Ahmed *et al.*, 2003) for some legume crops such as faba bean, chickpea and lupine compared to unfertilized plants.

Table 3

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It is evident that the application of 30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed gave the highest significant values of straw and biological yields/fed compared to the other tested mineral fertilizer followed by 30 kg N/fed in a descending order (Table 3). Adding such treatments were the most effective treatments in enhancing straw and biological yields/fed which may be owing to its effect on enhancing plant growth and consequently was reflected finally in producing more dry matter production. These findings are in harmony with those of other investigators who found that straw and biological yields/unit area were increased by the application of N (Ahmed *et al.*, 2002), P (Sherif *et al.*, 1993) and K (Hassanein *et al.*, 1996).

The data in Table (3) show, also, that application of 15 kg P₂O₅ /fed gave the highest significant values of harvest index, but without significant difference with 30 kg P₂O₅/fed in both seasons. On the contrary, the lowest value was obtained by unfertilized plants. On possible explanation, more amount of dry matter was retranslocated from the vegetative plant organs (stem, leaves and other non seed tissues) to the major sink (seed) was enhanced by phosphorus. These results are in agreement with those obtained by Chaturvedi and Chandel (2005) who found that increasing fertility level up to 20, 60 and 40 kg N, P₂O₅ and K₂O /ha, respectively caused a significant increase in the harvest index compared to untreated soybean plants which recorded the lowest one.

B- Effect of biofertilizers

B. 1- Nodulation and microbial activity:

Data in Table (4) indicate that the number and the dry weight of nodules /plant significantly responded to the tested biofertilizers. It is evident that the two traits were increased by seed inoculation with Rhi. either separately or mixed with any tested biofertilizers in favour of inoculation with Rhi. + Pho. for number nodules and Rhi. + Pho. + Pot. for dry weight of nodules which produced the highest values in both seasons. Therefore, there is a vital importance to inoculation soybean seed with Rhizobacterien which contains effective *Bradyrhizobium japonicum* strains. In this respect, Okba (2009) concluded that inoculating soybean seeds with a mixture of *Bradyrhizobium japonicum* as N₂ fixing bacteria, *Bacillus megaterium* as P dissolving bacteria and *Bacillus circulans* as K mobilizing bacteria produced higher number and heavier dry weight of nodules/plant than the uninoculated plants.

The results in Table (4) reveal, also, that seed inoculation with the tested biofertilizers either separately or mixed significantly increased the activities of nitrogenase and dehydrogenase enzymes compared to the uninoculated plants. Seed inoculation with Rhi. + Pho. + Pot. followed by Rhi. + Pho. produced higher significant values than the other tested biofertilizers in the two seasons. However, uninoculated seeds produced the lowest values of the two traits in the both seasons. The favourable effect of biofertilizers on

the activity of nitrogenase enzyme might refer to a noticeable increase in the amount of organic acids devoted to fuel nitrogenase enzyme and hence its activity was increased. Moreover, El- Shinnawi *et al.* (1997) stated that dehydrogenase enzyme catalyze the electron transfer reaction and yielding the energy required for the anabolic processes of various microorganisms inhabiting the soil. Through such reactions, the energy source or electron donor is converted to a simpler form that could be utilized by other organisms (plants and microbes). These results are in agreement with those obtained by El- Howeity *et al.* (2009) who demonstrated that faba bean seeds inoculation with *Rhizobium leguminosarum* alone and in combination with *Bacillus polymyxa* or *Azospirillum brasilense* or *Bacillus megaterium* caused an increase in nitrogenase and dehydrogenase activities compared to uninoculated plants.

Table (4): Effect of biofertilizers application on nodulation and microbial activation (at 70 DAS) during 2006 and 2007 seasons.

Biofertilizers	Nodules /plant		Nitrogenase □ μmole C ₂ H ₄ / g D.Wt nodules/ hr)	Dehydrogenase (μg TPF/ g dry soil /24 hours)
	Number	Dry weight (g.)		
2006 season				
0	0.83 e	0.018 e	0.78 e	43.01 f
Rhi	80.31 c	0.567 b	43.07 ab	57.23 c
Pho	54.63 d	0.332 d	32.90 d	59.97 b
Pot	55.28 d	0.424 c	31.88 d	50.62 e
Rhi+ Pho	123.21 a	0.638 a	43.65 a	62.09 b
Rhi+ Pot	113.31 b	0.597 b	41.09 b	53.50 d
Pho+ Pot	52.08 d	0.363 d	37.19 c	53.04 de
Rhi+Pho+Pot	88.54 c	0.642 a	44.10 a	66.26 a
2007 season				
0	0.88 e	0.022 g	0.58 e	46.96 e
Rhi	92.28 b	0.449 c	41.12 a	59.87 c
Pho	47.44 d	0.290 f	29.98 cd	60.82 bc
Pot	46.46 d	0.325 e	28.37 d	54.95 d
Rhi+ Pho	104.18 a	0.604 a	42.09 a	63.44 b
Rhi+ Pot	87.19 c	0.472 b	36.12 b	57.68 cd
Pho+ Pot	51.39 d	0.373 d	30.86 c	58.66 c
Rhi+Pho+Pot	94.27 b	0.606 a	42.65 a	69.97 a

B. 2- Yield and its components:

Data in Table (5) show significant differences in the number of pods/plant were noted due to biofertilizers inoculation. The highest number of pods per plant was recorded for triple seed inoculated with Rhi. + Pho. + Pot. followed by the dual inoculation with Rhi. + Pho. and single inoculation with Rhi. and Pho. in a descending order. On the other hand, the uninoculated plants produced the lowest number of pods/ plant in the both growing seasons. This superiority may be attributed to a greater amount of assimilates which contributed to dry matter accumulation and this in turn increased the number of pods/plants as a result of the inoculation with *Rhizobium* and *Bacillus megaterium* together which increased nutrients uptake over the single inoculation or uninoculated plants (Ewais, 2006). In this respect, similar results were obtained by other researcheres who found that number of pods/soybean plant was increased by inoculation with *Bradyrhizobium japonicum* (Salih and Nawar, 2003), phosphorien (Abdel- Mohsen *et al.*, 2002) and mixed *Bradyrhizobium japonicum* and *Bacillus megaterium* (Govindan and Thirumurugan, 2005) compared to uninoculated plants. From Table (5) it is clear that the inoculation of all tested biofertilizers either separately or mixed tended to significant increase the number of seeds/pod, 100- seed weight and seeds weight /pod compared to uninoculated treatment. The data show that the highest values of these traits were recorded by seed inoculation with Rhi. + Pho. + Pot. in both seasons. Moreover, dual inoculation with Rhi. + Pho. took the second rank in this respect. However, the uninoculated plants with any tested biofertilizers produced the lowest values in two seasons. In this respect, other researcheres reported the importance of some bacterial inoculation such as *Bradyrhizobium japonicum* (Jat *et al.*, 1998) and *Bacillus megaterium* (Mehasen and El- Ghozli, 2003) as well as mixed biofertilizers, i.e. nitrobien (*Bradyrhizobium japonicum*), phosphorien (*Bacillus megaterium*) and potassien (*Bacillus circulans*) as reported by Okba (2009) for enhancing number of seeds/pod, seed index and/or seeds weight /pod compared to uninoculated soybean plants.

The data, also, show that seed yield per plant and fed were significantly increased by inoculation with all tested biofertilizers either separately or mixed as compared with uninoculated plants in the two growing seasons (Table 5). Moreover, it is worthy to mention that the triple inoculation with Rhi. + Pho. + Pot. caused a greater increase in seed yield than that obtained by using the rest biofertilizers followed by dual inoculation with Rhi. + Pho. as a second rank in the two seasons. The increase in seed yield obtained herein is well agrees with the increases in the number and dry weight of nodules, enzymes activity (Table 4) and yield components (Table 5).

Table 5

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These results are in agreement with those obtained by other researchers who mentioned that seed yield per plant and unit area of soybean were enhanced with inoculation with nitrogen fixing bacteria (*Bradyrhizobium japonicum*) as reported by Mehasen *et al.* (2002), phosphate dissolving bacteria (*Bacillus megaterium*) as reported by Mehasen and El- Ghozoli (2003) and with mixed biofertilizers, i.e. nitroben (*Bradyrhizobium japonicum*), phosphorien (*Bacillus megaterium*) and potassien (*Bacillus circulans*) as detected by Okba (2009).

Straw and biological yields/fed were significantly and positively responded to inoculation with biofertilizers in both seasons as shown in Table (5). The data indicate that triple inoculation with Rhi. + Pho. + Pot. treatment produced the highest significant values followed by dual inoculation with Rhi. + Pho. in both seasons. As an average of the two seasons, the increase in straw and biological yields/fed due to seed inoculation with Rhi. + Pho. + Pot. amounted to 26.12 and 29.45 % more than the uninoculated plants, respectively. This superiority might be attributed to their effect in different roles for increasing (i) nitrogen fixation, (ii) phosphate dissolving and potassium mobilizing as well as more availability of some essential micronutrients and/or (iii) production of some growth regulators like IAA, GA₃ and cytokinins which stimulated vegetative growth and resulted in increase the dry matter production. These findings are in harmony with those of other researchers who found that straw and biological yields/unit area were increased by seed inoculation with *Bradyrhizobium japonicum* (El- Haddad *et al.*, 1998) and mixed nitrogen fixation bacteria and phosphate dissolving organisms (Ahmed *et al.* (2002) compared to uninoculated soybean plants.

The highest values of harvest index were obtained by the inoculation with Rhi. + Pho. + Pot. and Rhi. + Pot. in the first and second seasons, respectively. This result may be due to the effect of biofertilizers on improving retranslocation of dry matter from the vegetative plant organs (source) to the fruiting parts (sink). This finding seems to be in confirmation with that obtained by Okba (2009) who concluded that inoculation of soybean seeds with mixture of three biofertilizers, i.e. nitroben, phosphorien and potassien increased harvest index compared to uninoculated plants.

C- Effect of the interaction

The interactions between the mineral fertilizers and biofertilizers were found to be significant for nodulation and microbial activation (number and dry weight of nodules/plant as well as the activities of nitrogenase and dehydrogenase enzymes) and yield and some its components (number of pods, seed yield/plant and seed and straw yields/fed) in the two seasons. As an average of the two seasons, the data of nodulation and microbial activation are illustrate in Fig (1) and yield and some its components in Fig (2).

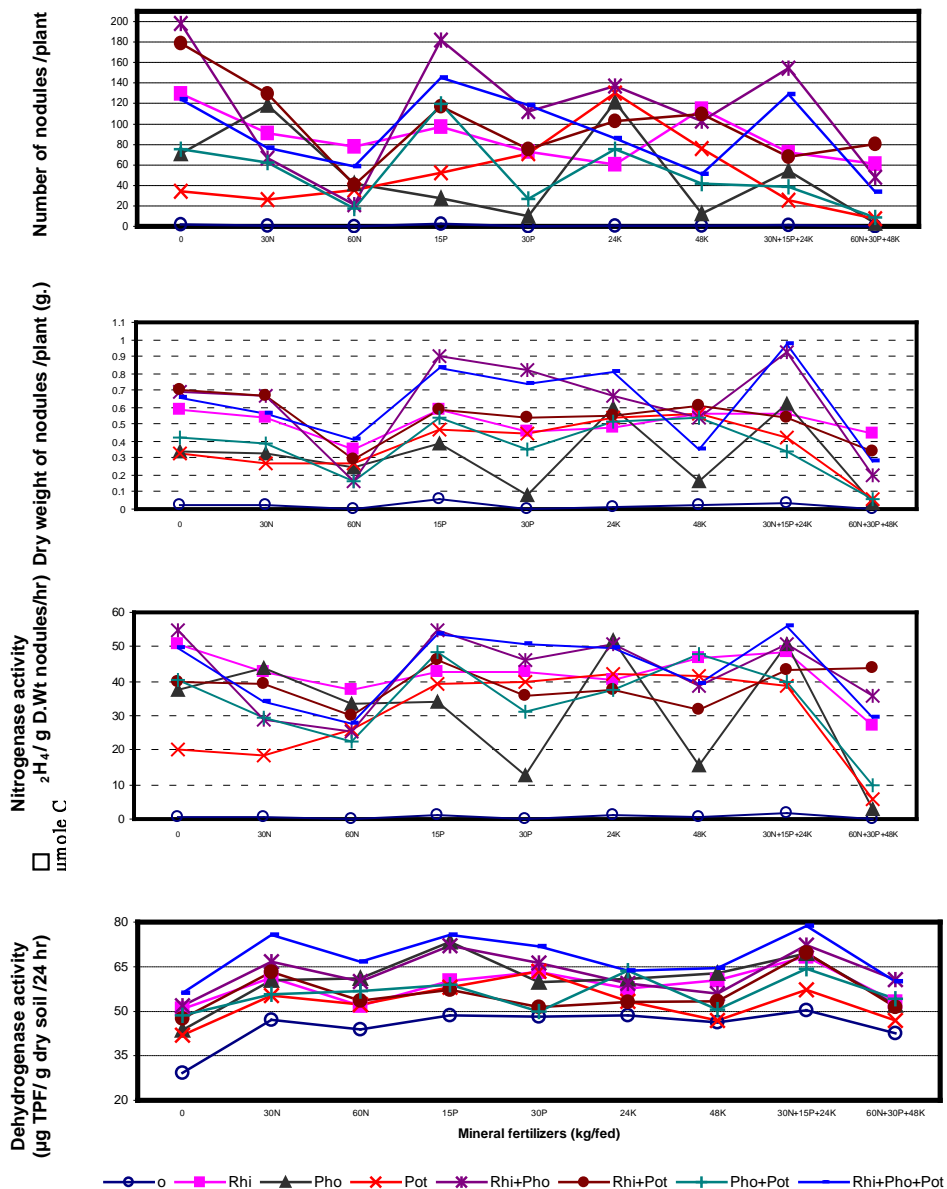


Fig (1): Effect of the interaction between mineral fertilizers and biofertilizers on nodulation and microbial activities as an average of the two seasons.

Effect of mineral and biofertilization on nodulation, nodule

Data of the interaction as illustrated in Fig (1) reveal that the unfertilized plants and inoculated with Rhi. + Pho. had the highest number of nodules/plant, while the highest values of dry weight of nodules/plant and nitrogenase and dehydrogenase activities were obtained from plants fertilized with 30 kg N + 15 kg P₂O₅ + 24 kg K₂O and seed inoculated with Rhi. + Pho. + Pot. However, the lowest values of number and dry weight of nodules/plant and nitrogenase activity were obtained generally by plants which were uninoculated and either unfertilized or fertilized with mineral fertilizers. On the other hand, the lowest value of dehydrogenase activity was obtained by untreated plants with either mineral or biofertilization. It is evident from Fig (1) that the addition of high levels of mineral fertilizers especially 60 kg N was followed by in a noticeable decrease in the number of nodules/plant and also their dry weights. This was also observed in nitrogenase and dehydrogenase activities but with different magnitudes for the different biofertilization treatments. Number and dry weight of nodules/plant were maximized when soybean seeds were treated with the combination of the three biofertilizers under study. This was reflected in the activity of nitrogenase and dehydrogenase. These results clearly indicate that the over use of nitrogen might have negative effects in one or more steps of *Rhizobium* stimulation and/or invasion in the root hair and its curling (Ghazal *et al.*, 1990) . Hemoglobin synthesis was also reported to decrease due to the over use of N. The release of bacteria from the infection thread was, also, reported to fail due to this high N addition. In this respect, similar results were obtained by Abd El- Fattah (2001) who found that the application of low rates of nitrogen (15 or 30 kg N/fed) and bacterial inoculation (*Bradyrhizobium japonicum* and *Azospirillum*) produced the highest values of number and dry weight of nodules/soybean plant compared to other levels of nitrogen (0, 45 and 60 kg N/fed). Moreover, El- Howeity *et al.* (2009) mentioned that the highest values of microbial activation were obtained by seed inoculation with *Rhizobium leguminosarum* and *Bacillus megaterium* combined with 20 kg N/fed for nitrogenase activity and with 40 kg N/fed for dehydrogenase activity compared to untreated faba bean plants.

Data of the interaction effect between the tested mineral and biofertilizers were illustrated in Fig (2) for number of pods, seed yield/plant and seed and straw yields/fed. Fig (2) confirms the results observed in Fig (1) where the highest averages of the abovementioned traits were recorded due to the combination of the lower NPK levels (30 kg N + 15 kg P₂O₅ + 24 kg K₂O/fed) with biofertilization with the three biofertilizers under study (Rhi. + Pho. + Pot.). From these results, it could be concluded that the application of the previous combined treatment may be recommended for promoting the plant growth characteristics which led to an encouragement the seed formation owing to increasing the plant capacity in building metabolites, and this in turn increased in the yield and its components.

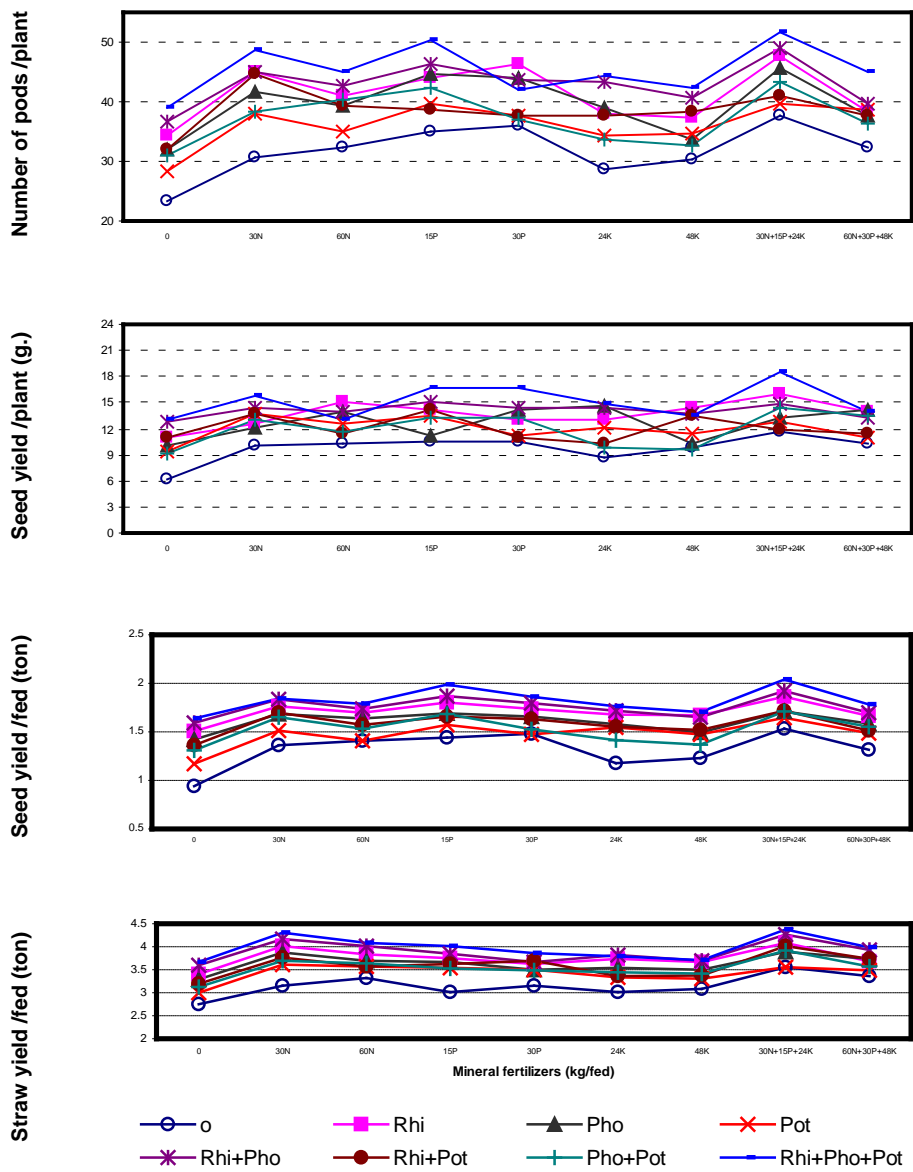


Fig (2): Effect of the interaction between mineral fertilizers and biofertilizers on yield and some its components as an average of the two seasons.

Effect of mineral and biofertilization on nodulation, nodule

However, the untreated plants with neither mineral and/nor biofertilizers had the lowest values of the all such traits. These findings are in harmony with those obtained by Mohamed (2000) who indicated that application of mixed mineral fertilizers (ammonium nitrate + calcium superphosphate + potassium sulphate) combined with mixed biofertilizers included some bacteria (*Rhizobium*, *Azotobacter* and *Azospirillum*) increased number of pods and seed yield per plant compared to untreated broad bean plants. Moreover, Ahmed *et al.* (2002) found that seed and straw yields/fed were increased with the application of chemical fertilization (60 kg N/fed and 30 Kg P₂O₅/fed) combined with biofertilization with some microorganisms (phosphate dissolving organisms and nitrogen fixing bacteria) compared to untreated soybean plants.

D- Correlation studies

Simple correlation coefficients between nodulation attributes, i.e. number and dry weight of nodules as well as microbial activation, i.e. nitrogenase and dehydrogenase activities on one hand, and yield and its components on the other, over all tested mineral and biofertilizers during 2006 and 2007 seasons are shown in Table (6). The data clear that positive and significant correlation coefficients were obtained between the number of nodules/plant and each of number of pods/plant, 100-seed weight, seeds weight/pod, seed yield/plant, seed yield/fed, straw yield/fed, biological yield/fed and harvest index in the two seasons as well as number of seeds/ pod in second one only. In addition, dry weight of nodules/plant showed a high significant positive correlation between each two of all characters studied of yield and its components, but its correlation coefficients with harvest index did not reach the 1% level of significance in the second season. Moreover, microbial activation characters (nitrogenase and dehydrogenase activities) were strongly correlated with each of yield and its components characters. The correlation coefficients were highly significant for the aforementioned traits with exception of that between harvest index and each of nitrogenase activity and dehydrogenase activity in the second season. Comparing the values of correlation coefficients between the soybean seed yield/fed on one hand and the nodulation attributes on the other, it is quite evident that stronger association could be observed between the dry weight of nodules/plant and seed yield/fed. Also, stronger association could be detected between dehydrogenase activity and seed yield/fed than between nitrogenase activity and seed yield/fed. These results clearly indicate that rhizosphere activation, as expressed in the activity of dehydrogenase was more effective on the variation of seed yield fed than nitrogenase activity. It seems evident that enhancement of microbial activity through mineral and/or biofertilization was the corner stone for the further steps of nodulation and thereafter N fixation as expressed in nitrogenase activation. Finally, it can be concluded that the productivity of soybean could be increased by improving the number and dry

weight of nodules per plant as well as microbial activation in each of nodules and soil rhizosphere.

Table (6): Simple correlation coefficients between nodulation and microbial activation on one hand and yield and its components of soybean on the other, over all tested mineral and biofertilizers during 2006 and 2007 seasons.

Characters	Number of nodules/plant	Dry weight of nodules/plant	Nitrogenase activity	Dehydrogenase activity
2006 season				
Number of pods/plant	0.332 **	0.476 **	0.506 **	0.788 **
Number of seeds/ pod	0.229 ^{NS}	0.363 **	0.490 **	0.564 **
100-seed weight	0.244 *	0.431 **	0.496 **	0.562 **
Seeds weight/pod	0.266 *	0.413 **	0.450 **	0.544 **
Seed yield/plant	0.399 **	0.506 **	0.494 **	0.738 **
Seed yield/fed	0.410 **	0.569 **	0.554 **	0.814 **
Straw yield/fed	0.294 *	0.478 **	0.508 **	0.747 **
Biological yield/fed	0.360 **	0.539 **	0.550 **	0.806 **
Harvest index	0.405 **	0.492 **	0.458 **	0.659 **
2007 season				
Number of pods/plant	0.397 **	0.549 **	0.495 **	0.816 **
Number of seeds/ pod	0.408 **	0.577 **	0.531 **	0.813 **
100-seed weight	0.377 **	0.513 **	0.514 **	0.692 **
Seeds weight/pod	0.296 *	0.465 **	0.439 **	0.738 **
Seed yield/plant	0.496 **	0.626 **	0.641 **	0.827 **
Seed yield/fed	0.503 **	0.608 **	0.605 **	0.802 **
Straw yield/fed	0.380 *	0.500 **	0.504 **	0.734 **
Biological yield/fed	0.432 **	0.551 **	0.553 **	0.779 **
Harvest index	0.285 *	0.274 *	0.267 *	0.261 *

*, ** Significant and highly significant at 5 and 1% probability, respectively.

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تأثير التسميد المعدني و الحيوي على تكوين العقد الجذرية ونشاطها والمحصول في فول الصويا

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الملخص العربي

أجريت تجربتان حقليتان بمزرعة كلية الزراعة جامعة المنوفية بشبين الكوم خلال موسمي الزراعة ٢٠٠٦ ، ٢٠٠٧ بهدف دراسة تأثير تسع معاملات من التسميد المعدني (صفر ، ٣٠ كجم ن ، ٦٠ كجم ن ، ١٥ كجم فو،أه ، ٣٠ كجم فو،أه ، ٢٤ كجم بو،أ ، ٤٨ كجم بو،أ ، ٣٠ كجم ن + ١٥ كجم فو،أه + ٢٤ كجم بو،أ ، ٦٠ كجم ن + ٣٠ كجم فو،أه + ٤٨ كجم بو،أ / فدان) وثمانى معاملات من التسميد الحيوي (صفر ، ريزوباكترين ، فوسفورين ، بوتاسيوماج ، ريزوباكترين + فوسفورين ، بوتاسيوماج ، فوسفورين + بوتاسيوماج ، ريزوباكترين + فوسفورين + بوتاسيوماج) على تكوين العقد الجذرية ونشاطها والمحصول فى فول الصويا (صنف جيزة ١١١). ويمكن إيجاز أهم النتائج المتحصل عليها على النحو التالي :

- ١- أعطى التسميد المعدني بمعدل ٣٠ كجم ن + ١٥ كجم فو،أه + ٢٤ كجم بو،أ / فدان أعلى القيم لمعظم صفات العقد الجذرية والنشاط الميكروبي (الوزن الجاف للعقد الجذرية للنبات ونشاط انزيمى النيتروجينيز و الديهيدروجينيز) والمحصول ومكوناته (عدد القرون على النبات ، عدد البذور فى القرن ، وزن ١٠٠ بذرة ، وزن بذور القرن ، محصول البذور للنبات ، محصول البذور والقش و البيولوجي للفدان) وذلك مقارنة ببقية معدلات التسميد الأخرى وكذلك النباتات غير المسمدة. فى حين أعطت النباتات غير المسمدة أعلى القيم لعدد العقد الجذرية للنبات وذلك خلال موسمي الزراعة.
- ٢- أدى تلقيح البذور بأى من الأسمدة الحيوية المختبرة إلى تحسين صفات العقد الجذرية والنشاط الميكروبي ، المحصول ومكوناته وخاصة معاملة التلقيح الثلاثي (ريزوباكترين +

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فوسفورين + بوتاسيوماج) تبعتها معاملة التلقيح المزدوج (ريزوباكترين + فوسفورين) لمعظم الصفات تحت الدراسة فيما عدا عدد العقد الجذرية للنبات حيث أعطت معاملة التلقيح المزدوج (ريزوباكترين + فوسفورين) أعلى القيم وذلك خلال موسمي الزراعة.

٣- تشير نتائج التفاعل إلى تأثير معظم الصفات تحت الدراسة تأثيراً معنوياً بالتفاعل بين عاملي الدراسة خلال موسمي الزراعة حيث أعطت النباتات التي لم تسمد معدنياً وتم تلقيح بذورها بالأسمدة الحيوية (ريزوباكترين + فوسفورين) أعلى قيم لعدد العقد الجذرية للنبات، في حين أدى استخدام معدل التسميد المعدني ٣٠ كجم ن + ١٥ كجم فوسفور + ٢٤ كجم بوتاسيوم/أ/فدان مع التلقيح بمخلوط الأسمدة الحيوية الثلاثة إلى زيادة الوزن الجاف للعقد الجذرية للنبات، نشاط انزيمي النيتروجيني و الديهيدروجيني، وكذلك إلى زيادة في عدد القرون/نبات، محصول البذور/نبات، محصول البذور والقش للقدان مقارنة ببقية المعاملات الأخرى المختبرة. في حين سجلت النباتات التي لم تعامل بأي من تلك الأسمدة المعدنية أو الحيوية أقل القيم لمعظم الصفات تحت الدراسة.

٤- أظهرت نتائج الارتباط وجود علاقة معنوية وموجبة بين عدد ووزن العقد الجذرية والنشاط الميكروبي (نشاط انزيمي النيتروجيني والديهيدروجيني) من جانب وبين المحصول ومكوناته (عدد القرون على النبات، عدد البذور في القرن، وزن ١٠٠ بذرة، وزن بذور القرن، محصول البذور للنبات، محصول البذور والقش و البيولوجي للقدان، دليل الحصاد) من جانب آخر مما يشير إلى إمكانية زيادة إنتاجية محصول فول الصويا عن طريق زيادة عدد ووزن العقد الجذرية وكذلك النشاط الميكروبي وخصوصاً نشاط انزيم الديهيدروجيني والذي سجل ارتباطاً عالياً بمحصول البذور/فدان يفوق الارتباط بين المحصول ونشاط انزيم النيتروجيني بما يوضح أن تنشيط عمل الميكروبات في ريزوسفير التربة له دور كبير ويمثل حجر الزاوية لبقية خطوات تكوين العقد الجذرية ومن ثم تثبيت الآزوت الجوي.

Table (3): Effect of mineral fertilizers application on yield and its components of soybean plant during 2006 and 2007 seasons.

Characters Mineral fertilizers (kg/fed)	No. of pods/plant	No. of seeds/ pod	100-seed weight (g.)	Seeds weight/ pod (g.)	Seed yield/plant (g.)	Seed yield/fed (ton)	Straw yield/fed (ton)	Biologi cal yield/fe d (ton)	Harvest index (%)
2006 season									
0	30.68 f	2.39 c	13.93 c	0.344 b	10.32 e	1.33 g	3.20 f	4.53 f	29.36 d
30 N	40.56 ab	2.73 b	15.49 ab	0.419 a	13.14 bc	1.64 bc	3.71 b	5.34 b	30.71 bc
60 N	38.75 bcd	2.70 b	15.70 ab	0.410 a	12.76 bcd	1.57 de	3.65 bc	5.22 cd	30.08 cd
15 P ₂ O ₅	40.74 ab	2.71 b	15.82 ab	0.412 a	13.56 ab	1.69 b	3.55 cd	5.24 c	32.25 a
30 P ₂ O ₅	39.97 abc	2.66 b	15.48 ab	0.409 a	13.05 bc	1.61 cd	3.50 de	5.12 d	31.45 ab
24 K ₂ O	35.91 de	2.63 b	15.65 ab	0.408 a	12.22 cd	1.53 ef	3.43 e	4.96 e	30.85 bc
48 K ₂ O	35.25 e	2.62 b	15.43 ab	0.400 a	11.98 d	1.48 f	3.40 e	4.89 e	30.27 cd
30 N+15 P ₂ O ₅ +24 K ₂ O	42.28 a	2.87 a	15.97 a	0.438 a	14.17 a	1.75 a	3.82 a	5.57 a	31.42 ab
60 N+30 P ₂ O ₅ +48 K ₂ O	36.87 cde	2.69 b	14.79 bc	0.405 a	12.66 bcd	1.54 e	3.62 bc	5.16 cd	29.84 cd
2007 season									
0	33.58 f	2.26 f	13.18 f	0.355 e	11.15 f	1.40 e	3.31 f	4.71 e	29.72 c
30 N	42.42 bc	2.76 b	15.36 c	0.428 a	13.60 c	1.70 abc	3.94 b	5.64 b	30.14 bc
60 N	40.10 cd	2.63 cd	15.48 bc	0.404 c	12.93 d	1.63 cd	3.78 c	5.41 c	30.13 bc
15 P ₂ O ₅	44.56 ab	2.68 bc	15.68 b	0.417 b	14.49 b	1.74 ab	3.70 cd	5.44 c	32.00 a
30 P ₂ O ₅	41.20 cd	2.59 de	15.22 cd	0.402 c	13.37 c	1.68 bc	3.62 de	5.29 c	31.76 ab
24 K ₂ O	38.74 de	2.58 de	15.31 cd	0.398 c	12.53 de	1.58 d	3.58 e	5.15 d	30.68 abc
48 K ₂ O	37.19 e	2.54 e	15.08 de	0.390 d	12.46 e	1.55 d	3.53 e	5.08 d	30.51 bc
30 N+15 P ₂ O ₅ +24 K ₂ O	46.63 a	2.90 a	16.11 a	0.434 a	15.20 a	1.78 a	4.09 a	5.87 a	30.32 bc
60 N+30 P ₂ O ₅ +48 K ₂ O	39.66 d	2.61 cde	14.93 e	0.401 c	12.77 de	1.61 cd	3.75 c	5.36 c	30.04 bc

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Table (5): Effect of biofertilizers application on yield and its components of soybean plant during 2006 and 2007 seasons.

Characters Biofertilizers	No. of pods/plant	No. of seeds/pod	100-seed weight (g.)	Seeds weight/pod (g.)	Seed yield/plant (g.)	Seed yield/fed (ton)	Straw yield/fed (ton)	Biological yield/fed (ton)	Harvest index (%)
2006 season									
0	29.72 f	2.38 c	13.81 b	0.363 c	9.76 d	1.28 f	3.12 f	4.40 f	29.09 d
Rhi	40.03 bc	2.74 ab	15.26 a	0.410 ab	13.67 b	1.68 c	3.60 c	5.28 c	31.82 a
Pho	38.58 cd	2.68 ab	15.35 a	0.405 ab	12.66 c	1.55 d	3.55 d	5.10 d	30.39 b
Pot	35.45 e	2.67 ab	15.55 a	0.406 ab	11.90 c	1.46 e	3.47 e	4.94 e	29.55 cd
Rhi+ Pho	42.29 ab	2.76 ab	15.77 a	0.415 ab	14.14 b	1.75 b	3.73 b	5.48 b	31.93 a
Rhi+ Pot	37.31 de	2.66 ab	15.73 a	0.409 ab	12.02 c	1.52 d	3.53 d	5.05 d	30.10 bc
Pho+ Pot	36.40 de	2.64 b	15.70 a	0.396 b	11.96 c	1.47 e	3.52 de	4.99 e	29.46 cd
Rhi+Pho+Pot	43.32 a	2.80 a	15.96 a	0.434 a	15.11 a	1.85 a	3.83 a	5.67 a	32.63 a
2007 season									
0	33.81 f	2.22 e	13.62 e	0.356 f	10.25 g	1.37 f	3.19 f	4.56 f	30.04 b
Rhi	42.64 bc	2.70 b	15.29 c	0.388 d	14.05 c	1.74 b	3.91 b	5.65 b	30.80 ab
Pho	40.94 cd	2.62 bc	15.15 c	0.411 c	13.52 d	1.66 c	3.71 c	5.37 c	30.91 ab
Pot	36.95 e	2.52 d	14.88 d	0.417 c	12.25 f	1.49 e	3.40 e	4.89 e	30.47 ab
Rhi+ Pho	43.81 b	2.84 a	16.00 b	0.440 b	14.77 b	1.77 ab	4.04 a	5.81 a	30.46 ab
Rhi+ Pot	39.85 d	2.57 cd	15.08 cd	0.381 de	12.68 e	1.64 c	3.66 cd	5.30 c	30.94 a
Pho+ Pot	38.12 e	2.55 cd	14.85 d	0.377 e	12.63 e	1.58 d	3.55 d	5.13 d	30.80 ab
Rhi+Pho+Pot	47.48 a	2.92 a	16.32 a	0.455 a	15.18 a	1.80 a	4.13 a	5.93 a	30.36 ab