African Journal of Biotechnology Vol. 11(27), pp. 7028-7037, 3 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3194
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of biofertilizers on grain yield and protein content of two soybean (*Glycine max* L.) cultivars

Iraj Zarei[1], Yousef Sohrabi[1]*, Gholam Reza Heidari[1], Ali Jalilian[2] and Khosro Mohammadi[3]

[1]Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran.
[2]Kermanshah Agriculture and Natural Resources Center, Kermanshah, Iran.
[3]Department of Agronomy, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

Accepted 18 January, 2012

Nutrient management is one of the most important factors in successful cultivation of plants. Biofertilizers can affect the quality and quantity of crop. In order to study the effects of biofertilizers on grain yield and protein content of two soybean (*Glycine max L.*) cultivars, an experiment was conducted using a factorial arrangement based on randomized complete block design with four replications, at the Mahidasht Research Station of Kermanshah in 2010. The factors were soybean cultivar (Williams and Line no. 17) and fertilizer application (b_1= N + P, b_2= *Bradyrhizobium japonicum* + P, b_3= N + *Bacillus* and *Pseudomonas* + 50% of P, b_4= *B. japonicum* + *Bacillus* and *Pseudomonas* + 50% of P, b_5= *B. japonicum* + 50% of N + *Bacillus* and *Pseudomonas* + 50% of P). Results show that Line no. 17 with 2911.2 kg/ha had higher seed yield than Williams with 2711 kg/ha. Also, fertilizer levels of b_3 with 3058.2 and b_2 with 2643.8 kg/ha produced the highest and the lowest seed yield, respectively. Plants treated with fertilizer levels of b_1, b_2 and b_5 in comparison with other fertilizer levels significantly produced lower thousand seed weight. In Line no. 17 fertilizer level of b_3 with 2.88 produced the highest seed per pod. Results show that fertilizer levels had a significant effect on the number of pod per plant and treatments containing biological fertilizers in terms of the number of pods per plant were equal or superior to chemical fertilizer. It was also observed that fertilizer levels of b_1, b_3 and b_5, produced the highest protein percentage. It therefore seems that biofertilizers can be considered as a replacement for part of chemical fertilizers in soybean production.

Key words: *Bacillus*, *Bradyrhizobium japonicum*, phosphate solubilizing, protein, *Pseudomonas*.

INTRODUCTION

Nutritional management is an important factor in success of planting crops. Soybean in the case of protein and oil is known as a world's most important crop (Raei et al., 2008). Throughout history, legumes have been used for the supply of food, fodder, fuel and traditional medicine (Howieson et al., 2008). Protein of soybean seed contains amino acids required for human nutrition and livestock (Raei et al., 2008). For optimum plant growth, nutrients must be balanced and should be sufficient for plant, or in other words the soil must have nutrients that are needed for plants (Ayoola, 2010); however, most of these resources are in the unavailable form and each year only a little part of them are released through biological activity and chemical processes (Chen, 2006). Hence, in order to increase crop yield per unit area, largely chemical fertilizers are used. The result of these activities in recent years has been the crisis of environmental pollution, especially water and soil pollution that threatens human society.

Sustainable agriculture based on using biological fertilizers is an effective solution for overcoming these problems (Darzi et al., 2006; Ekin et al., 2009). Biological fertilizers can affect on yield and quality of product. Biological fertilizers containing useful enzymes and microorganisms that can increase plant growth and quality of crops, and reduce the cost of fertilizer and pesticide application (Chen, 2006). Phosphate-solubilizing microorganisms produce various organic acids such as oxalate, lactate, acetate, glycolate, gluconate, tartrate,

*Corresponding author. E-mail: y.sohrabi@uok.ac.ir. Tel: +989141421300. Fax: +988716620553.

Table 1. Some physical and chemical characteristics of soil.

Texture	O.C (%)	Ec (Ds/m)	pH	N_{tot} (%)	P	K	Fe	Mn	Zn
					(mg/kg)				
Clay silty	0.91	0.71	7.1	0.1	10	410	7	9.7	0.75

citrate and succinate. Plants of the legume family establish symbiotic relationship by rhizobial bacteria and through this symbiosis can fix nitrogen by rhizobial bacteria and provide all or part of their required nitrogen in this way (Gan and Peoples, 1997). Kuntal et al. (2007) during the research on medicinal plant, *Stevia rebaudiana* Bert, showed that application of phosphate solubilizing bacteria, improved biological function and absorption of nutrient elements in this plant. Shaharoona et al. (2007) also reported that phosphate-solubilizing bacteria would increase wheat yield. In addition, Jat and Ahlawat (2006) by using of phosphate solubilizing bacteria and one strain of rhizobial bacteria on the pea plant, stated that biological yield, grain yield and grain protein level significantly increased compared with control treatment. Olivera et al. (2002) declared that the effect of combined inoculation of bean by phosphate solubilizing bacteria and *Bradyrhizobium japonicum* bacteria were positive on dry weight.

Moreover, Zhang et al. (2002) reported that *B. japonicum* bacteria increased number of pods per plant, number of seeds per plant, hundred seed weight, grain protein, total protein and development of plant leaves in two soybean cultivars. Kazemi et al. (2005) also stated that soybean seed inoculation by rhizobial bacteria significantly increased the number of pods per plant, number of seeds per plant, thousand grain weights and finally the yield of soybean. Yadeghari et al. (2003) in research to examine the effects of inoculation of four strains of *B. japonicum* on yield and yield components of soybean showed that line no.11 was superior to Williams cultivar. This superiority was attributed to more vegetative period of line no. 11 and better symbiosis by *B. japonicum*. Asadi Rahmani et al. (2000) reported that during seed filling stage of soybean in treatments inoculated by *B. japonicum* bacteria, more photo-assimilate transport to grain due to higher photosynthesis level and this factor can increase the seed size and seed weight. Stefan et al. (2010) stated that inoculation of soybean by *Bacillus pumilus* significantly increased plant height, leaf number, leaf area, grain protein and nodulation. While Rosas et al. (2002) reported that combined inoculation of soybean by symbiotic bacteria of soybean and phosphate solubilizing bacteria improved dry weight of soybean.

Considering the importance of soybean in production of oil, its nutritional importance and status of biological fertilizers in sustainable agriculture, the study of yield and yield components of soybean, possible changes of oil and protein percentage affected by biological fertilizer is therefore essential to improve yield and quality of product, effort to provide food and health security and also decrease use of chemical inputs with adverse effects on environmental health. In most researches carried out, little attention has been paid to combined effect of phosphate solubilizing bacteria and *B. japonicum* bacteria. Moreover, doing such an experiment in a different climatic condition may have different results.

MATERIALS AND METHODS

The experiment was conducted at the Station of Agriculture and Natural Resources of Mahidasht, Iran with latitude 34° 16′ 12″ N and longitude 46° 50′ 01″ E and elevation of 1380 m above the sea level during the 2010 growing season. The experiment was conducted as a factorial arrangement based on randomized complete block design with four replications. The experimental treatments consisted of two soybean cultivars (Williams and line no. 17) and five different levels of fertilizer as follows: b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50 percent P; b_4: *Bradyrhizobium japonicum* + *Bacillus* + *Pseudomonas* + 50 percent P; b_5: *Bradyrhizobium japonicum* + 50 percent N + *Bacillus* + *Pseudomonas* + 50 percent P.

Soil samples were collected prior to the experiment (Table 1). Considering fertilizer requirement and results of soil analysis, 150 kg/ha triple super phosphate and 300 kg/ha urea was used for fertilizer treatment. Urea fertilizer was used during the three-stage, 30 kg, simultaneously with the planting (starter) in all experimental plots and 270 kg was applied as an equal in four-leaf stage and early reproductive stage. Planting soybean was done in early June. Biological fertilizers fully were mixed with seeds in the shadow and dried for 10 min (inoculated seeds were planted before two hours). Inoculated seeds were planted in rows with 3 cm depth. To ensure the success of biological fertilizers, once again in two to four leaf stages, biological fertilizers were consumed in plots by irrigation.

Measurement of growth parameters

In this study, plant height, number of stem node, number of lateral branches, the height of the first fertile node of the soil, plant dry weight, number of seed per plant, number of pod per plant, number of seed per pod, seed thousand weight, biological yield, economic yield, harvest index, protein percentage and protein yield were measured. For measuring traits of plant height, number of stem node, number of lateral branches, plant dry weight, number of seed per plant, number of pod per plant, number of seed per pod and biological yield, 10 plants from each plot were harvested and studied. To measure plant dry weight, 10 plants from each plot were put in oven at 75°C for 48 h. Two lines in each plot were considered for measuring seed yield (economic yield). Harvest index was calculated by using this formula:

$$\text{Harvest index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

Table 2. Analysis of variance of number of lateral branches, number of stem node, plant height, height of first fertile node from the soil, number of pod per plant, number of seed per pod and number of seed per plant in response to various Cultivar and fertilizer in soybean plant.

Source of variation	Df.	Number of lateral branches	Number of stem node	Plant height	Height of first fertile node from the soil	Number of pod per plant	Number of seed per pod	Number of seed per plant
Block	3	0.090^{ns}	3.698**	79.46**	0.043^{ns}	26.58^{ns}	0.00428^{ns}	168.9^{ns}
Cultivar	1	0.090^{ns}	4.160**	0.09^{ns}	0.009^{ns}	8.93^{ns}	0.00062^{ns}	54.8^{ns}
Fertilizer	4	0.968**	1.723**	162.90**	0.067^{ns}	53.82**	0.04560**	723.3**
Cultivar × Fertilizer	4	0.204^{ns}	0.580^{ns}	10.40^{ns}	0.433^{ns}	8.96^{ns}	0.04436*	24.7^{ns}
Error	27	0.124	0.405	13.9	0.225	8.5	0.00326	67.5
CV	-	11.1	3.1	3.5	9.1	7.5	2.1	7.8

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; Df: Degree of freedom; CV: Coefficient of variation. ns, Non significant.

Protein was measured by Kjeldahl method (Peach and Tracey, 1956) and by multiplying seed yield in protein percentage, protein yield was determined.

Statistical analysis

The data were analyzed using the SAS software package. Comparisons of all means were done at the 5% probability level based on Duncan's method. Moreover, for significant interaction, slicing was used. Graphs were generated using Excel software.

RESULTS AND DISCUSSION

Number of lateral branches

Results show that fertilizer levels of b_2 with 3.65 and b_1 with 2.80 had the highest and lowest number of lateral branches, respectively (Table 3). Increase in the number of lateral branches could be caused by increase in plant growth that was the result of improved nutrient absorption of phosphorus and nitrogen. Nitrogen as part of the protein compounds, enzymes, effective compounds in energy transfer, takes part in structure of DNA, present in the structure of chlorophyll and has a direct impact on vegetative growth (Assiouty and Sedera, 2005). It seems that a gradual release of nitrogen by nitrogen stabilizer bacteria caused the number of lateral branches in the fertilizer levels of b_2 and b_4 to increase. Priority of b_2 and b_4 could be due to balancing the absorption of nutrients (supply of continuous and stable mineral elements especially nitrogen to plants) in the root environment and beneficial effect of bacteria in these treatments on the enzymes and hormones, which results in plant growth.

However, in level of fertilizer b_5, despite having nitrogen stabilizer bacteria, the number of lateral branches was less than those of b_2 and b_4. It seems that consuming of 50% nitrogen fertilizer in this fertilizer levels led to decrease in the activity of nitrogen fixation in nodules. Sundara et al. (2002) during their research in sugarcane plant reported that application of phosphate solubilizing bacteria (*Bacillus megaterium*) increased the number of stems per plant.

Number of stem node

Line no. 17 with 20.67 nodes, had more node than Williams cultivar with 20.02. Among the studied fertilizer levels, b_3 with 20.74 and b_2 with 19.81 had the highest and lowest stem node, respectively (Table 2). Between fertilizer levels of b_2 and b_4, and also b_1, b_3 and b_5, no significant difference was observed (Table 3). Priorities of b_1, b_3 and b_5 in terms of number of stem node can be induced from developed root system, improved water supply and adequate plant nutrients. Lower number of stem node in b_2 and b_4 may be due to the fact that in these two fertilizer levels, supplied nitrogen by nitrogen stabilizer bacteria has been gradually given to the plant. In fertilizer levels of b_2 and b_4 that had used *B. japonicum* bacteria, stem node was less than other fertilizer treatments. Increasing lateral branches per plant in fertilizer levels containing *B. japonicum* may have caused the stem node in plants under these treatments to decrease.

Plant height

Fertilizer levels of b_3 with 112.21 and b_4 with 101.6 had the highest and lowest plant height, respectively (Table 3). As shown in Table 3, in

Table 3. Effect of variety and fertilizer levels on soybean traits.

Treatment	Number of lateral branches	Number of stem node	Plant height (cm)	The height of first fertile node from the soil (cm)
Cultivar				
a_1	3.12[a]	20.02[b]	107.98[a]	5.17[a]
a_2	3.21[a]	20.67[a]	107.88[a]	5.20[a]
Fertilizer				
b_1	2.80[b]	20.74[a]	110.82[a]	5.31[a]
b_2	3.65[a]	19.81[b]	104.84[b]	5.12[a]
b_3	2.96[b]	20.59[a]	112.21[a]	5.07[a]
b_4	3.40[a]	19.87[b]	101.60[b]	5.19[a]
b_5	3.02[b]	20.72[a]	110.19[a]	5.22[a]

Means with one common letter have no significant difference ($P \leq 0.05$). a_1: Williams cultivar, a_2: line no. 17; b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

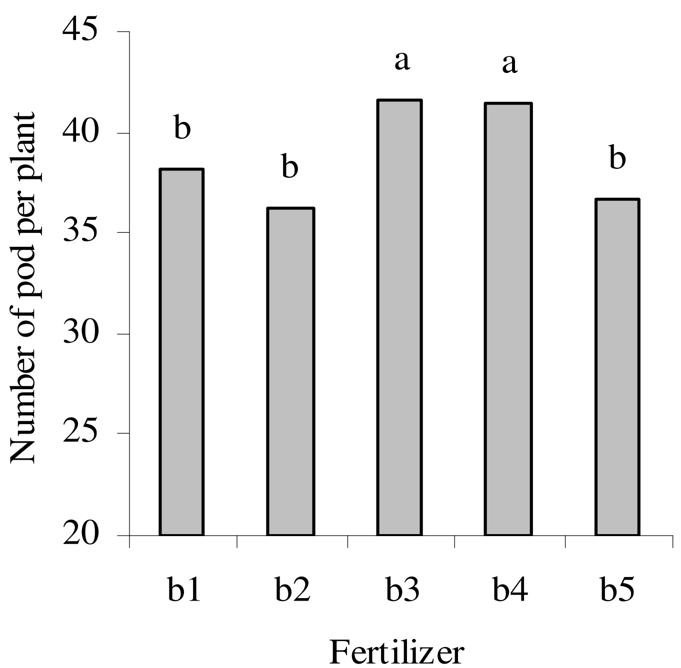

Figure 1. Effect of fertilizer levels on number of pod per plant. Means with one common letter have no significant difference ($P \leq 0.05$). b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

fertilizer levels of b_2 and b_4 that used *B. japonicum* bacteria, plant height was less than other fertilizer treatments. Increasing lateral branches per plant in fertilizer levels containing *B. japonicum* may have caused the plant height in plants under these treatments to decrease. As can be observed in this table, fertilizer levels of b_1, b_3 and b_5 had a similar effect on plant height, this is probably due to sufficient supply of required nutrients to the plant, which finally caused the photosynthesis and soybean growth to be improved. These results are in agreement with the results of Darzi et al. (2006) that they performed their study on the fennel plant. Dileep Kumar et al. (2001) also reported that combined inoculation of pea seeds with rhizobial and phosphate solubilizing bacteria increased plant height.

The height of first fertile node from the soil

Results show that the effects of cultivar, fertilizer treatments and their interaction were not significant on the height of first fertile node from the soil.

Number of pod per plant

The results obtained showed that fertilizer levels had a significant effect on number of pod per plant. Fertilizer levels of b_3 with 41.6 and b_2 with 36.16 had the highest and lowest number of pod per plant, respectively (Figure 1). Treatments containing biological fertilizers in terms of number of pods per plant were equal or superior to chemical fertilizer. Fertilizer levels of b_3 and b_4 had the higher number of pods per plant than chemical fertilizer. This result probably was because of balancing uptake of nutrients in root environment, the beneficial effects of these bacteria on enzymes and hormones and their effects on plant growth. Mahfouz and Sharaf-Eldin (2007) reported that phosphorous solvent bacteria have the ability to produce organic acids that would increase solubility of phosphorus available for plants. Continuous and stable supply of mineral elements especially P to the plants, can increase growth and flowering rate. Phosphorus along with nitrogen, improves reproductive growth

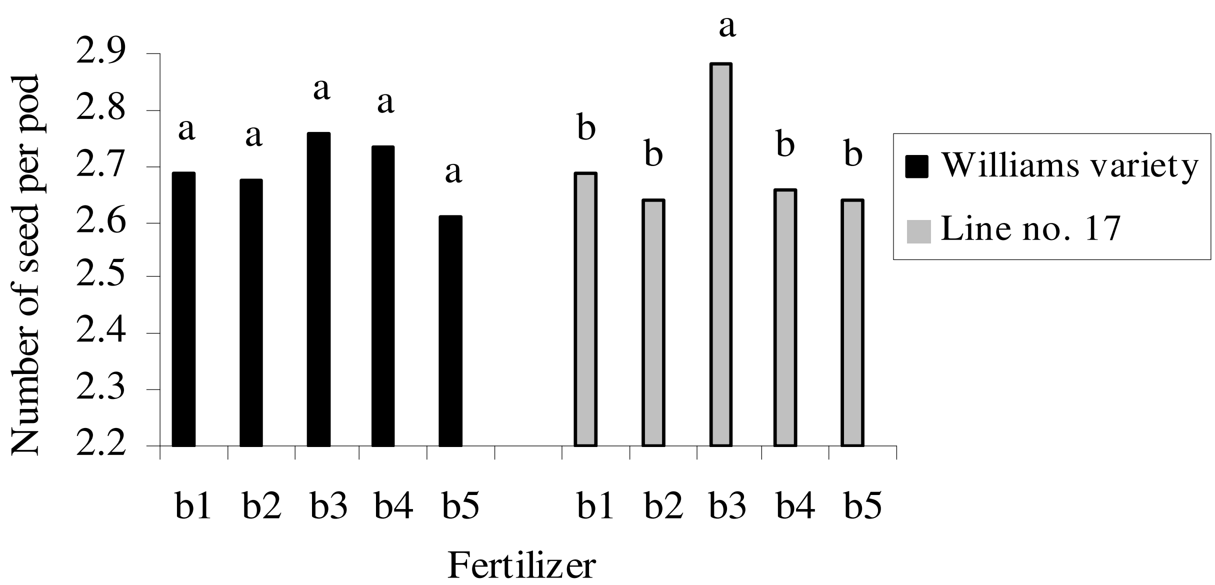

Figure 2. Effect of fertilizer levels on number of seed per pod of two soybean cultivars. Means with one common letter have no significant difference ($P \leq 0.05$). b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

Table 4. Effect of fertilizer levels on number of seed per plant, seed thousand weight and plant dry weight.

Fertilizer	Number of seed per plant	Seed thousand Weight (g)	Plant dry weight (g)
b_1	102.49^{b}	134.77^{b}	33.91^{bc}
b_2	96.00^{b}	128.70^{b}	32.16^{c}
b_3	117.25^{a}	145.98^{a}	37.54^{a}
b_4	111.85^{a}	148.89^{a}	36.93^{ab}
b_5	96.19^{b}	133.56^{b}	31.71^{c}

Means with one common letter have no significant difference ($P \leq 0.05$). b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

and fruit produce in the plant. The b_5 consumption of nitrogen fertilizer (up to 50 percent of plant requirement), may have led to reduction of nitrogen fixation by *B. japonicum* and finally the number of pod per plant, compared to chemical fertilizer. Gan and Peoples (1997) during their research on soybean, however, observed that there was no difference in the number of pods per plant and grain yield between plants inoculated by *B. japonicum* and plants treated by chemical fertilizer.

Number of seed per pod

Results show that the studied cultivars had different responses to fertilizer levels. In line no. 17, fertilizer levels of b_3 with 2.88 and b_5 with 2.63 had the highest and lowest seed per pod, respectively. Also in line no. 17, only b_3 showed significant differences with other fertilizer levels (Figure 2). It seems that different genetics characteristics results in plants showing different responses to fertilizer levels. Priority of b_3, in Line no. 17 may have been because of hormonal effects of phosphate solubilizing bacteria and continuous and stable supply of P to the plants during growth and flowering periods. Nabila et al. (2007) observed that application of *Azospirillum* on wheat had significant effect on number of grain per spikelet.

Number of seed per plant

The highest and lowest number of seed per plant belonged to fertilizer levels of b_3 with 117.25 and b_2 with 96.00, respectively. The b_3 and b_4 in terms of number of seed per plant were significantly different from b_1, b_2 and b_5 (Table 4). This priority of b_3 and b_4 may be due to the more pods per plant, which may have been provided from adequate phosphorus and nitrogen needed for plants. P is an essential element for cell division, root development and seed formation (Gizawy and Mehasen, 2009). Similarly, Zhang et al. (2002) reported that *B. japonicum* bacteria application increased the number of seed per plant of two soybean cultivars.

Seed thousand weight

Results show that fertilizer levels of b_4 with 148.89 and b_2 with 148.70 g had the highest and lowest seed thousand weight, respectively. Plants treated with fertilizer levels of b_1, b_2 and b_5 in comparison with other fertilizer levels significantly produced lower seed thousand weight (Table 4). Better developed root systems and better absorption of nutrient elements in fertilizer levels of b_3 and b_4, may increase seed thousand weight. On the other hand, improvement of photosynthesis by these bacteria may increase seed thousand weight 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 (Saleh Rastin, 2005). Priority of seed thousand yield in b_3 and b_4 possibly is attributed to enzymatic and hormonal effects of phosphate solubilizing bacteria.

On the other hand, the lower yield in b_2 may be due to the absence of phosphate solubilizing bacteria. In $b_{5,}$ consumption of nitrogen fertilizer (up to 50% of plant requirement) may have led to the reduction of nitrogen fixation by *B. japonicum* and seed thousand weight as well. Kazemi et al. (2005) reported that soybean seed inoculation with rhizobial bacteria significantly increased seed thousand weight. Zhang (2002) reported that inoculation with *B. japonicum* bacteria increased 100 seed weight of two soybean cultivars. Asadi Rahmani et al. (2000) also observed that in the grain filling stage of soybean due to higher levels of photosynthesis in treatments inoculated with *B. japonicum* bacteria, more phosphate is transported to the grain and this factor could increase the size and weight of seed.

Plant dry weight

Result show that among fertilizer levels, b_3 with 37.54 and b_5 with 31.71 g had the highest and lowest plant dry weight, respectively. Fertilizer levels of b_3 and b_4 compared to other fertilizer levels had higher plant dry weight (Table 4). To justify these results, it could be suggested that phosphate solubilizing bacteria in these fertilizer levels had a positive impact on plant growth and increased plant dry weight. This can be caused by stimulating secretion of growth hormones which is produced by this bacteria and their effect on plant growth. In $b_{5,}$ consumption of nitrogen fertilizer (up to 50% of plant requirement), may lead to reduction of nitrogen fixation by *B. japonicum* and finally plant dry weight compared to chemical fertilizer. Kandil et al. (2004) reported that the use of biological fertilizers in sugar beet, significantly increased plant dry weight. Raeipour and Aliasgharzadeh, (2004) also stated that *Bradyrhizobium* bacteria has positive effect on shoot dry weight, and interaction of phosphate solubilizing bacteria and *B. japonicum* was significant on shoot dry weight. Hernandez et al. (1995) reported that effect of *Pseudomonas fluorescens* bacteria was positive on the increasing weight of plant maize.

Seed yield

Results show that Line no. 17 with 2911.2 kg/ha had higher seed yield than Williams with 2711 kg/ha. Between fertilizer levels, b_3 with 3058.2 and b_2 with 2643.8 kg/ha produced higher and lower seed yield respectively (Table 5). Between plants treated with fertilizer levels of b_3 and b_4, no significant differences were observed, but these plants significantly produced higher seed yield compared to plants under fertilizer treatment of b_1, b_2 and b_5 (Figure 3). Fertilizer levels of b_3 and b_4 significantly produced higher seed yield compared to plants under fertilizer treatment of b_1 (chemical fertilizer). It seems that nitrogen stabilization and phosphate solubilizing bacteria, by increasing yield component such as number of pods per plant, seeds per pod, seed number and seed thousand weight, increased seed yield. For high yield, plant should have proper balance between vegetative and reproductive growth, and developmental stages of seeds completely. This status can be created when we have balanced the necessary elements for vegetative growth (nitrogen), with the necessary elements for reproductive growth (P) (Bashan et al., 1992). Bacteria used in these treatments (b_3 and b_4), maybe increase seed yield by providing macro and micro nutrients for plant growth, production of stimulate material, development of root system and anti-pathogenic effects (Jat and Ahlawat, 2006).

It is reported that soybean inoculated by *Bradyrhizobium* bacteria and phosphate solubilizing bacteria increased the seed yield (Singh, 1994; Jat and Ahlawat, 2006). Phosphate solubilizing bacteria led to increased absorption of other elements by increasing the ability to access phosphorus and thereby can increase crop yield (Mahfouz and Sharaf-Eldin, 2007). Priority of fertilizer level of b_4 than fertilizer level of b_2 was probably because phosphate-solubilizing bacteria had positive effect on activities of nitrogen stabilizer bacteria due to provision of phosphorus and other nutrients. In fertilizer level of b_5, consumption of nitrogen fertilizer (up to 50% of plant requirement) may have led to reduction of nitrogen fixation by *B. japonicum* and thus, seed yield was reduced.

Table 5. Analysis of variance of seed thousand weight, plant dry weight, seed yield, biological yield, harvest index, protein percentage and Protein yield in response to various Cultivar and fertilizer in soybean plant.

Source of variation	Df.	Seed thousand Weight	Plant dry weight	Seed yield	Biological yield	Harvest index	Protein percentage	Protein yield
Block	3	467.1**	84.05**	62917^{ns}	2310557**	24.080^{ns}	12.961^{ns}	3819.1^{ns}
Cultivar	1	45.3^{ns}	4.91^{ns}	401001*	2134408**	206.025**	8.546^{ns}	75836.2^{ns}
Fertilizer	4	596.4**	57.44**	309321**	1120716**	17.304^{ns}	148.475**	196601.9**
Cultivar × Fertilizer	4	165.4^{ns}	20.11^{ns}	98406^{ns}	456005^{ns}	16.931^{ns}	19.379^{ns}	11238.4^{ns}
Error	27	86.7	10.18	66232	244231	15.419	7.569	19286.3
CV	-	6.7	9.3	9.2	6.1	11.3	7.7	13.7

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; Df: degree of freedom; CV: coefficient of variation. ns, Non significant.

Biological yield

Results show that Williams cultivar with 8376.7 had higher biological yield than Line no. 17 with 7914.7 kg/ha. Soybean treated with fertilizer levels of b_3 with average of 8502.8 and b_5 with average of 7709.3 kg/ha had the highest and lowest biological yield, respectively. Plants treated with fertilizer levels of b_2 and b_5 significantly had less biological yield (Figure 4). Biological yield represents the total biomass of plant organs and effective absorption of nutrient elements.

Considering that nitrogen is available in the structure of proteins, nucleic acids, chlorophyll, enzymes and vitamins, so enough nitrogen in the plant, provide plant better growth. In treatment of b_1, adequate nitrogen supply increased vegetative growth and thus biological yield was increased. The b_2 treatment inoculated with nitrogen stabilizers bacteria may be unable to fix nitrogen for complete need of plant. In treatment of b_5, it is likely that consumption of nitrogen fertilizer (50% needed by plant) reduced nitrogen fixation by *B. japonicum* and consequently biological yield was reduced compared to chemical fertilizer. In fertilizer levels containing phosphate solubilizing bacteria (b_3 and b_4), due to the solubility of phosphate and production of plant hormones that affect nutrient uptake and photosynthesis processes, can increase growth, development of plant root systems and biological yield (Assiouty and Sedera, 2005). Gharib et al. (2008) reported that developed root systems increase water and nutrient uptake and consequently, increased photosynthesis, and this caused the production of photosynthetic material and biological yield to increase.

Harvest index

As seen from Table 6, Line no. 17 with average of 36.89% had higher harvest index than Williams cultivar with 32.35%. Priority of Line no. 17 than cultivar of Williams can be attributed to the fact that cultivar of Williams had lower seed yield and higher biological yield than Line no. 17, according to the equation related to harvest index (Equation 1); thus Williams cultivar had been a lower harvest index. Shirastava et al. (2001) and Narne et al. (2002) stated that in soybean, harvest index has highly correlated with grain yield.

Protein percentage and protein yield

Results reveal that fertilizer levels of b_1, b_3 and b_5 produced the highest protein percentage (Figure 5). Priority of levels of fertilizer of b_1, b_3 and b_5, may be that in these treatments, sufficient nitrogen for protein synthesis and the production was put at the disposal of plant. However, plants treated with fertilizer levels of b_2 and b_4 significantly produced lower protein percentage in grain. This may be due to the low efficiency of nitrogen stabilizer nodes in the late growth period of soybean. Priority of fertilizer level of b_4 than b_2, was probably because of presence of phosphate solubilizing bacteria (in fertilizer level of b_4) that caused the gradual and balanced supply of phosphorus, part of the energy needed for nitrogen fixation (by stabilizer bacteria) provided. Plants treated with fertilizer levels of b_1, b_4 and b_5, significantly produced lower seed protein yield compared with plants treated with fertilizer level of

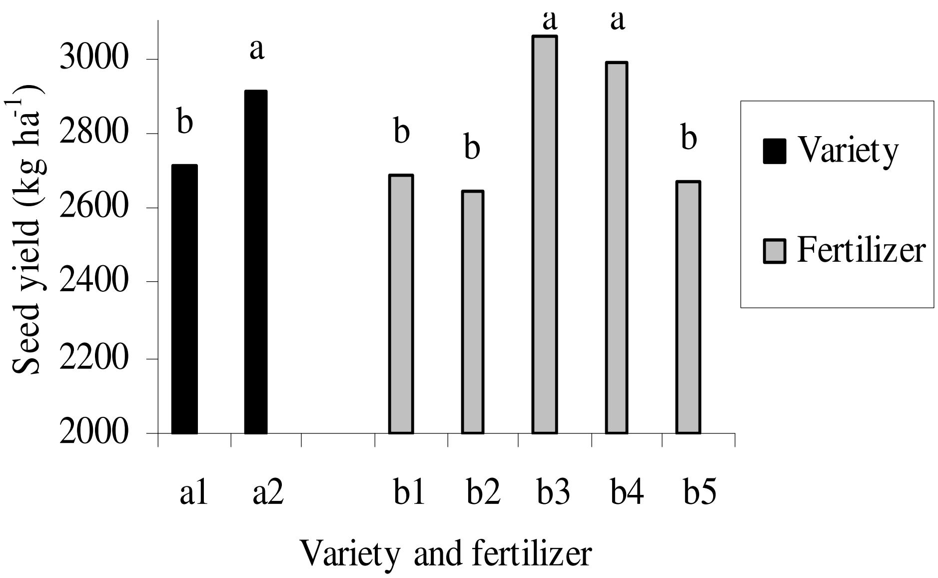

Figure 3. Effect of cultivar and fertilizer level on seed yield. Means with one common letter have no significant difference ($P \leq 0.05$). a_1: Williams cultivar, a_2: line no. 17; b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

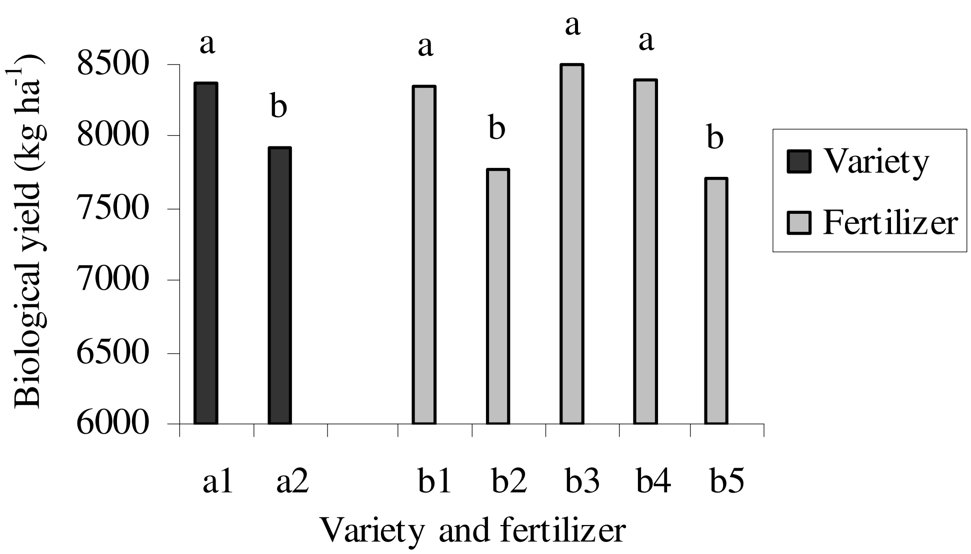

Figure 4. Effect of cultivar and fertilizer level on biological yield. Means with one common letter have no significant difference ($P \leq 0.05$). a_1: Williams cultivar, a_2: line no. 17; b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

Table 6. Effect of cultivar on harvest index.

Treatment	Cultivar	
	Williams	**Line no. 17**
Harvest index (%)	32.35^{b}	36.89^{a}

Means with one common letter have no significant difference ($P \leq 0.05$).

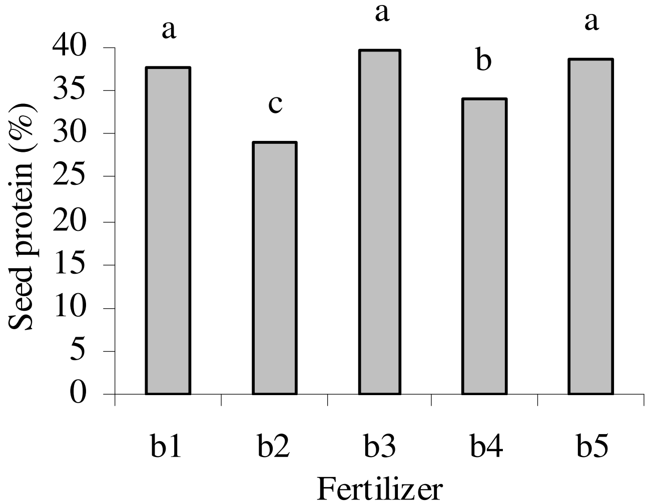

Figure 5. Effect of different levels of fertilizer on protein percentage. Means with one common letter have no significant difference ($P \leq 0.05$). b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

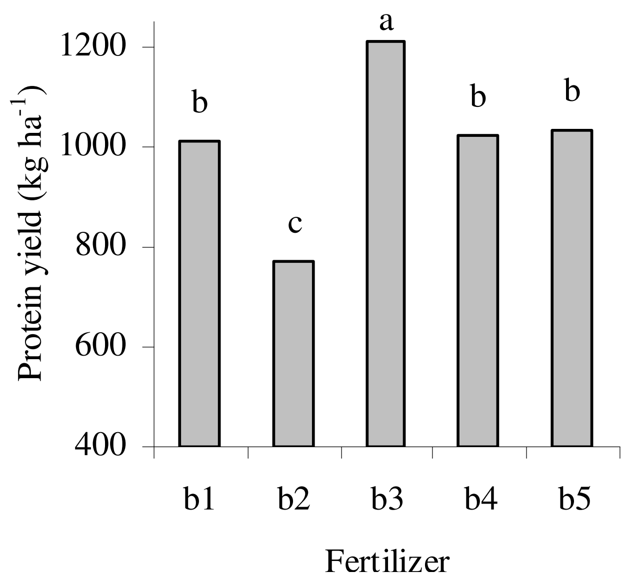

Figure 6. Effect of different levels of fertilizer on protein yield. Means with one common letter have no significant difference ($P \leq 0.05$). b_1: N + P; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* + *Pseudomonas* + 50% P; b_4: *B. japonicum* + *Bacillus* + *Pseudomonas* + 50% P; b_5: *B. japonicum* + 50% N + *Bacillus* + *Pseudomonas* + 50% P.

b_3 (Figure 6).

Considering that seed protein yield, by multiplying the seed yield and protein percentage can be derived, hence the plants treated with fertilizer level of b_3, due to having higher yield and protein percentage produced higher protein yield. On the other hand, plants treated with fertilizer level of b_2, compared with other treatments, produced lower seed yield and protein percentage, therefore their protein yield was lowest. Stephen et al. (2010) stated that soybean inoculated with *Bacillus pumilus* had higher seed protein content. Rahmani et al. (2008) reported that nitrogen is the most important element in protein synthesis and its increase in optimum conditions increases the amount of protein. In addition, Shehata and Khawas (2003) showed that application of biological fertilizer on sunflower increased seed protein.

Conclusion

So far, the application of phosphate-solubilizing bacteria in most examined traits was better than chemical fertilizer. Moreover, the inoculation with *B. japonicum* in most examined traits did not have significant difference with chemical fertilizer. So the impact of phosphate-solubilizing on examined traits was more than inoculation with *B. japonicum*. Overall, the results obtained in this experiment showed that Line No. 17 had a more quantitative and qualitative performance than cultivar of Williams. Between examined fertilizer levels, treatment of b_4, including *B. japonicum*, phosphate-solubilizing and 50% super phosphate triple, provided the best conditions for achieving maximum grain yield and oil yield in soybean.

ACKNOWLEDGEMENTS

We thank the employees of the Agricultural Research Center, Kermanshah, and also Mr. Yousef dezfuli and Mr. Ali Coraei for their assistance in this study.

REFERENCES

Asadi Rahmani H, Saleh Rastin N, Sajadi A (2000). Investigate the possibility of predicting the need of inoculation of soybeen, based on the determine number of *Bradyrhizobium japonicum* bacteria and index of availability of soil nitrogen. Soil Water J. 12(7): 21-32.

Assiouty FM, Abo-Sedera SA (2005). Effect of bio and chemical fertilizers on seed production and quality of spinach (*Spinacia oleracea* L.). Int. J. Agric. Biol. 6: 947-952.

Ayoola OT (2010). Yield performance of crops and soil chemical chsnges under fertilizer treatments in a mixed cropping system. Afr. J. Biotechnol. 9(26): 4018-4021.

Bashan Y, Alcaraz L, Toledo G (1992). Responses of soybean and cowpea root membranes to inoculation with *Azospirillum brasilense*. Symbiosis, 13: 217-228.

Chen J (2006). The combined use of chemical and organic fertilizer and/ or biofertilizer for crop growth and soil fertility. Taipei Food Fertilizer Technol. Bull. 17: 1-9.

Darzi MT, Ghalavand A, Rejali F, Sefydkan F (2006). Study of application of biological fertilizers on the yield and yield components of fennel herbs. J. Med. Arom. Plants Res. 22(4): 276-292.

Dileep Kumar SB, Berggren I, Martensson AM (2001). Potential for improving pea production by co- inoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant Soil, 229(1): 25-34.

Ekin Z, Oguz F, Erman M, Ogun E (2009). The effect of *Bacillus* sp.

OSU-142 inoculation at various levels of nitrogen fertilization on growth, tuber distribution and yield of potato (*Solanum tuberosum* L.). Afr. J. Biotechnol. 8(18): 4418-4424.

Gan Y, Peoples MB (1997). The effect of N fertilizer strategy on N2 fixation, growth and yield of vegetable soybean. Field Crops Res. 51: 221-229.

Gharib FA, Moussa LA, Massoud ON (2008). Effect of compost and Bio-fertilizers on growth, yield and essential oil of sweet marjoram (*Majorana hortensis*) plant. Int. J. Agric. Biol. 10: 381-387.

Gizawy NKB, Mehasen SAS (2009). Response of Faba bean to bio, mineral phosphorus fertilizers and foliar application with zinc. World Appl. Sci. J. 6(10): 1359-1365.

Hernandez AN, Hernandez A, Heydrich M (1995). Selection of rhizobacteria for use in maize cultivation. Cultivos Tropicales, 6: 5- 8.

Howieson JG, Yates RJ, Foster K, Real D, Besier RB (2008). Prospects for the future use of legumes. In: Dilworth, MJ, James EK, Sprent JI, Newton WE. (Eds.). Nitrogen-Fixing Leguminous Symbioses. Springer, Dordrecht, The Netherlands, pp. 363-387.

Jat RS, Ahlawat IPS (2006). Direct and residual effect of vermicompost, biofertilizers and phosphorus on soil nutrient dynamics and productivity of chickpea-fodder maize sequence. J Sustain Agr. 28(1): 41-54.

Kandil A, Badawi MA, El-Mursy SA, Abdou UMA (2004). Effect of planting dates, nitrogen levels and biofertilization treatments on growth attributes of suger beet (*Beta vulgaris* L.). Scientific Journal of King Faisal University (Basic Appl. Sci.), 5(2): 227-236.

Kazemi Kazemi S, Ghaleshi S, Ghanbari A, Kianoush GE (2005). Effects of planting date and seed inoculation by the bacteria on the yield and yield components of two soybean varieties. Agri. Sci. Nat. Resour. 12(4): 20-26.

Kuntal D, Dang R, Shivananda TN, Sekeroglu N (2007). Comparative efficiency of bio and chemical fertilizers on nutrient contents and biomass yield in medicinal plant *Stevia rebaudiana* Bert. J. Med. Plants Res. 1: 35-39.

Mahfouz SA, Sharaf-Eldin MA (2007). Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). Int. Agrophys, 21: 361-366.

Nabila M, Zaki MS, Karima M, EL-Din G (2007). Growth and yield of some wheat cultivars irrigated with saline water in newly cultivated land as affected by biofertilization. J. Appl. Sci. Res. 3:1121-1126.

Narne C, Aher RP, Dahat DV, Aher AR (2002). Selection of protein rich genotypes in soybean. Crop Res. His. 24(1): 106-112.

Olivera M, Iribarne C, Lluch C (2002). Effect of phosphorus on nodulation and N fixation by bean (*Phaseolus vulgaris*). Proceedings of the 15th International Meeting on Microbial Phosphate Solubilization. Salamanca University, 16-19 July, Salamanca, Spain.

Peach K, Tracey MV (1956). Modern methods of plant analysis. Vol 1, Peach K, Tracey MV (ed), Springer-Verlag, Berlin, pp. 468-502.

Raeipour L, Aliasgharzadeh N (2004). Interaction of phosphate solubilizing bacteria and *Bradyrhizobium japonicum* on yield and absorption of some nutrients in soybean, J. Agric. Know. 4(15): 141-156.

Raei E, Sedghi M, SayedSharifi R (2008). Effect of *Bradyrhizobium* inoculation, application of nitrogen and weeding on growth and seed filling rate in soybean. J. Agric. Tech. 12(43): 81-91.

Rahmani N, Valadbighi SA, Daneshian J, Bidgholi VM (2008). Effect of different levels of drought stress and nitrogen on oil yield in evergreen herb (*Calendula officinalis* L.). J. Res. Arom. Plant Iran. 24(1): 101-108.

Rosas S, Rovera M, Andres J, Correa N (2002). Effect of phosphorous solubilizing bacteria on the rhizobia- legume symbiosis. Proceedings of the 15th International Meeting on Microbial phosphate Solubilization. Salamanca University, 16-19 July, Salamanca, Spain.

Saleh Rastin N (2005). Sustainable management from the perspective of soil biology. Proceedings of the necessity of manufacturing bio-fertilizers in the country, pp. 261-276.

Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007). Effectiveness of various *Pseudomonas* Sp and Burkholderia improving growth and yield of wheat (*Triticum aestivam* L.). J. Microbiol. Biotechnol. 17(8): 1300-1307.

Shehata MM, EL-Khawas SA (2003). Effect of two biofertilizers on growth parameters, yield characters, nitrogenous components, nucleic acids content, minerals, oil content, protein profiles and DNA banding pattern of sunflower yield. Pak. J. Biol. Sci. 6: 1257-1268.

Shirastava MK, Shukla RS, Jain PK (2001). Path coefficient analysis in diverse genotype of soybean (*Glycine max* L.). Adv. Plant Sci. 4: 47-51.

Singh HP (1994). Response to inoculation with *Bradyrhizobium*, vesicular-arbuscular mycorrhiza and phosphate solubilizing microbes on soybean in a mollisol. Indian J. Microbiol. 34: 27-31.

Stefan M, Dunca S, Olteanu Z, Oprica L, Ungureanu E, Hritcu L, Mihasan M, Cojocaru D (2010). Soybean (*Glycine max* L.) inoculation with *Bacillus pumilus* RS3 promotes plant growth and increases seed protein yield: Relevance for environmentally-friendly agricultural applications. Carpath J. Earth Environ. 5(1): 131-138.

Sundara B, Natarajan V, Hari K (2002). Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugar cane and sugar yields. Field Crop Res. 77: 43-49.

Yadeghari M, Akbari GE, Daneshyan JF (2003). Effects of inoculation of four strains of bacteria (*Bradyrhizobium japonicum*) on yield and yield components of soybean in Karaj climate. Iran J. Field Crop. Res. 1(1): 93-107.

Zhang H, Charles TC, Driscoll B, Prithiviraj T, Smith DL (2002). Low temperature-tolerant *Bradyrhizobium Japonicum* strains allowing improved soybean yield in short-season. Agron. J. 94: 870-875.