Australian Journal of Crop Science

AJCS

AJCS 5(13):1776-1782 (2011) ISSN:1835-2707

Assessment of growth and yield components following the application of different biological fertilizers on soybean (*Glycine max* L.) cultivation

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Abstract

In the present study, the effect of five biological fertilizers [b_1 (control), b_2, b_3, b_4 and b_5] on the yield and quality properties of the seeds of two well-established in Iran soybean genotypes (*Glycine max* L. cv. Wlliams and Line No. 17) was examined, in order to evaluate their use as an alternative to chemical fertilizers. An experiment was conducted using a factorial arrangement based on a complete block randomized design with four replications. The results showed that by increasing Growing Degree Days (GDD), the Leaf Area Index (LAI) of cv. Williams was relatively higher than that of Line no. 17, whereas Line no. 17 had significantly more chlorophyll (a, b and total) than that of cv. Williams. The highest and lowest weight measurements of 1000 seeds were recorded when fertilizers b_4 and b_2 were applied. Overall, Line no. 17 had a higher total yield of seeds than that of cv. Williams. The application of fertilizer b_1, b_3 and b_5, resulted in the highest seed protein content. In addition, the highest and lowest measurements of seed oil content for cv. Williams, were recorded when fertilizers b_2 and b_3 were applied. Seeds of Line no. 17 had (a) higher phosphorus and zinc content than of cv. Williams, whereas cv. Williams had (a) higher iron and manganese content than of Line no. 17, regardless of the applied fertilizer. K content of seeds had the highest and lowest mean values when b3 and b5 fertilizers were applied, respectively. In conclusion, the implementation of biological fertilizers (especially b3 and b4) could be a useful means for minimizing the chemical inputs during soybean cultivation, allowing at the same time for high yield and high quality products.

Keywords: *Bacillus* sp., Biofertlizers, *Bradyrhizobium japonicum*, Chlorophyll content, Leaf area index, Protein and oil content, *Pseudomonas* sp.

Abbreviations: GDD (Growing Degree Days), LAI (Leaf Area Index), b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum*+ *Bacillus* sp.+ *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum* + 50% N + *Bacillus* sp. + *Pseudomonas* sp.+ 50% P.

Introduction

Nutritional management is of major importance for (a) efficient crop production. The application of biological fertilizers can affect not only the yield but also the quality of the final product. Soybean might easily be considered one of the world's most important crops, regarding its protein and oil content of seeds (Raei et al., 2008). Throughout history, legumes have been traditionally used as (a) food source, fodder, also valued for medicinal purposes and more recently for bio-fuel production (Howieson et al., 2008). Proteins of soybean seeds are composed of essential amino acids, important for human and livestock nutrition (Raei et al., 2008). For optimum plant growth, nutrients must be in sufficient quantities within soil, in order to allow for unhindered plant development. However, it is rather the case that nutrient content within soil is either poor or in unavailable forms for plants, whereas only scarcely are nutrients replenished annually through biological activity and chemical processes (Chen, 2006). In order to ensure high crop yield per unit, a large amount of synthetic chemical fertilizers is used on a yearly basis quite often throughout the world as a common cultivation practice. Sustainable agriculture, with its use of biological fertilizers instead of chemical ones, bears high importance in overcoming the problems that have arisen from environmental pollution (Darzi et al., 2006). Biological fertilizers contain various useful enzymes and microorganisms which increase both plant growth and product quality, and reduce the cost of both fertilizer and pesticide application (Chen, 2006). The use of biological fertilizers in Iran includes stabilizers of soybean nitrogen fixation bacteria (*Bradyrhizobium japonicum*) and phosphate fertilizers (Fertile 2, Zeis Fanavar Co, Iran). Fertile 2 contains two types of phosphate solubilizing bacteria, such as *Bacillus lentus* (strain P5) and the *Pseudomonas* sp. (strain P13). Phosphate solubilizing microorganisms produce various organic acids such as Aksalat, lactate, succinate, acetate, glycolat, gluconate, tartrate, citrate and succinate. Legume plants have established a symbiotic relationship with rhizobial bacteria through which they can fix aerial nitrogen and cover their nitrogen needs (Gan and Peoples, 1997). Kuntal et al. (2007) studied the medicinal plant *Stevia rebaudiana* (Bert.) and reported that the application of phosphate solubilizing bacteria improved biological activity and absorption of nutrient elements. Shaharoona et al. (2007) also reported that phosphate solubilizing bacteria increased the yield of wheat. Jat and Ahlawat (2006) examined the effect of phosphate solubilizing bacteria and one strain of rhizobial bacteria on pea plants, and reported that biomass yield, grain yield and grain protein content were significantly increased compared with the control treatment. Some studies showed that inoculation of chilli-pepper plants (*Capsicum annuum* L.) can induce plant growth by increasing growth characteristics such as the total number of fruit, fruit-weight and total yield in plants grown under field conditions.

Olivera et al. (2002) proposed that the combined inoculation of bean plants by phosphate solubilizing bacteria and *Bradyrhizobium japonicum* had a positive effect on the weight of 1000 seeds. The application of *Bradyrhizobium japonicum* on soybean plants increased the number of pods and seeds per plant, the weight of 1000 seeds, the protein content of seeds and the development of plant leaves (Zhang et al., 2002;Kazemi et al., 2005). Similarly, Yadeghari et al. (2003) examined the effect of inoculation of soybean with four strains of *Bradyrhizobium japonicum* on total yield and various yield components and reported that Line no.11 was superior to cv. Williams, mainly due to the larger vegetative period of Line no. 11 and the better symbiosis with *Bradyrhizobium japonicum*. The seed size and seed weight of soybean increased when plants were inoculated with *Bradyrhizobium japonicum* bacteria at seed filling stage due to a more efficient photosynthetic activity level and a higher transport of photo-assimilates to grains (Rahmani et al., 2000). The water stress often has a negative effect on the nodulation and the seed yield in legumes but this effect could be minimized through N management. It was suggested that the combined action of water stress and inoculation with and without *Mesorhizobium cicer* at various rates of N fertilizers could affect the biochemical property of nodules, the biomass partitioning among plant parts (shoot, roots and nodules), and the seed yield in chickpea. Moreover, inoculation of soybean with *Bacillus pumilus* increased significantly plant height, leaf number, leaf area, nodulation and protein content of seeds (Stefan et al., 2010), whereas *Bradyrhizobium japonicum* application resulted in significantly higher biomass production, phosphorous, potassium and nitrogen content of shoots and fresh and dry weight of root nodules (Raeipour and Aliasgharzadeh, 2007). Rosas et al. (2002) reported that combined inoculation of soybean with symbiotic bacteria of soybean and phosphate solubilizing bacteria improved dry weight of soybean, whereas in pea plants it resulted in higher shoot length, root length and dry weight (Dileep Kumar et al., 2001). Considering the importance of soybean for oil production, its nutritional value and the increasing trend of using biological fertilizers in sustainable agriculture, we examined the effect of the implementation of biological fertilizers on the yield and various yield components of soybean plants, as well as their effects on seed quality features such as the oil and protein content. The aim of the present study was to evaluate the use of this alternative form of fertilizers as a means towards increasing yield and quality of soybean while decreasing the chemical inputs during cultivation and thus minimizing their adversary effects on the environment.

Results and discussion

Analysis of variance showed no significant interaction between the two factors (data not shown). Therefore, no second order interactions were examined, only main effects .

Leaf Area Index

The results of the present study showed that LAI index increased gradually from 0 to a peak level of 1200 GDD, irrespectively of cultivar or fertilizer (Table 2, Fig 1 and 2). However, cv. Williams had a larger LAI than the one in Line no. 17 at 900 and 1200 GDD. LAI was highest and lowest at 1200 GDD for fertilizers b_3 and b_2 respectively and differed significantly from all the other treatments. Therefore, the absence of *Bradyrhizobium japonicum* combined with the positive nutritional effects of phosphate solubilizing bacteria of *Bacillus* and *Pseudomonas* sp, seems to be more beneficial to the total leaf area than any of the other fertilizing combinations applied. In other studies, Stefan et al. (2010) reported that inoculation of soybean seeds resulted in significant increase of both the number and the total area of leaves. Kandil et al. (2004) reported that the application of biological fertilizers on sugar beet plants significantly increased the Leaf Area Index, whereas Zhang et al. (2002) reported similar results for two soybean cultivars inoculated with *Bradyrhizobium japonicum* bacteria.

Chlorophyll content

Line no. 17 had significantly more chlorophyll a, chlorophyll b and total chlorophyll content than of cv. Williams (Fig 3.). The use of b_2, b_3 and b_4 combinations resulted in higher chlorophyll content (a, b and total) than the other two combinations, without significant differences between them (Fig 4.). Therefore, both genotype and fertilizer composition seem to play an important role on chlorophyll content of leaves, probably due to differences in assimilation rate that affect chlorophyll content. Qrbanly et al. (2006) reported a positive correlation between the nitrogen and the chlorophyll content of leaves, mainly due to the presence of nitrogen in the structure of chlorophyll molecules. In addition, Chandrasekar et al. (2005) and Swedrzynska (2000) observed an increase in chlorophyll content of white millet and wheat leaves respectively, after inoculation by *Azosperilium* bacteria, probably due to nitrogen fixation which increases nitrogen content of vegetative tissues.

Weight of 1000 seeds- Total yield of seeds

The application of b_3 and b_4 fertilizers resulted in both higher weight of 1000 seeds and total yield of seeds than the remaining combinations, regardless of the genotype, probably due to the improved development of root system, combined with the higher assimilation rate of nutrients (Fig 5. and 6). Regarding the two genotypes, cv. Williams had a higher total yield of seeds than Line no. 17, regardless of the applied fertilizer (data not shown). The effect of seed inoculation on the number of pods per plant, the number of seeds per pod and the weight of 1000 seeds, consequently produced a higher total yield of seeds. Rahmani et al. (2000) reported that improved plant nutrition during the seed filling stage resulted in sufficient allocation of photoassimilates in seeds and therefore in higher seed weight. Similarly, Zhang et al. (2002) reported that seed inoculation of two soybean cultivars with *Bradyrhizobium japonicum* bacteria led to higher weight of 1000 seeds. Jat and Ahlawat (2006) has proposed that the increase of total yield of seeds is due to the stimulation of growth production, the improvement in macro and micro nutrient uptake, as well as the antipathogenic effects. In addition, the combination of *Bradyrhizobium* and phosphate solubilizing bacteria improves phosphate uptake and nitrogen stabilizing bacteria activity, resulting in a higher total yield of seeds (Singh, 1994; Jat and Ahlawat, 2006; Mahfouz and Sharaf-Eldin, 2007).

Seed protein content and total protein yield

Seed inoculation with fertilizers containing nitrogen (b_1, b_3, b_5) resulted in a higher protein content and total protein yield (data not shown) than that recorded in combinations where

Table 1. Physical and chemical characteristics of soil samples.

Texture	O.C. (%)	E.C. (dS/m)	pH	N total % w/w	P	K	Fe	Mn	Zn
					mg Kg^{-1}				
Clay silty	0.91	0.71	7.1	0.1	10	410	7	9.7	0.75

O.C.: Organic Components, E.C.: Electrical Conductivity

Table 2. The effect of genotype and fertilizer composition on P, K, Fe, Mn and Zn concentrations of soybean seeds (expressed in mg/100g).

Treatment	P concentration (mg/100g)	K concentration (mg/100g)	Fe concentration (mg/100g)	Mn concentration (mg/100g)	Zn concentration (mg/100g)
Genotype*1					
a_1	438.1b	2611.0a	12.01a	3.28a	6.54b
a_2	626.2a	2649.4a	8.74b	2.42b	8.46a
Fertilizer composition*2					
b_1	396.1e	1997.7c	9.67b	2.48b	6.76c
b_2	603.6b	2821.7b	10.68a	3.26a	9.20a
b_3	667.6a	3374.0a	10.37a	3.12a	7.44b
b_4	539.5c	3207.5a	10.85a	3.23a	7.41b
b_5	453.7d	1750.1d	10.31a	2.16c	6.67c

Means within the same column, followed by different Latin letters are statistically different at $p \geq 0.05$ according to Duncan Multiple Range Test (DMRT). *1a_1: cv. Williams, a_2: line no. 17. *2b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum*+ *Bacillus* sp.+ *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum* + 50% N + *Bacillus* sp. + *Pseudomonas* sp.+ 50% P.

nitrogen was not present (Fig 7). Nitrogen is essential for protein synthesis, therefore improving nitrogen availability for plants, accounts for a higher assimilation rate for the specific element and a higher rate protein synthesis within plant tissues that are allocated in seeds (Rahmani et al., 2008). Similarly, Shehata and Khawas (2003) reported that applying biological fertilizers on sunflower plants increased protein content of seeds, especially when applied during the seed filling stage (Lhuillier-Soundele et al., 1999).

Seed oil content and total oil yield

The inoculation of seeds with biological fertilizers affected the oil content of seeds only in the case of cv. Williams, where the use of b_2 fertilizer resulted in the highest seed oil content, suggesting that this parameter is dependent on both the fertilizer composition and the genotype (Fig 8). In contrast, total oil yield of seeds was affected by fertilizer composition in the case of Line no. 17 and not in the case of cv. Williams, mainly due to the higher total yield of seeds of Line no. 17 compared to cv. Williams (Fig 9). Similarly, El-Kramany et al. (2007) reported that oil yield of peanuts was affected by fertilizer composition, whereas Shehata and Khawas (2003) reported that both seed oil content and total oil yield were affected by the use of biological fertilizers.

P and K content of seeds

Phosphorus content of seeds was significantly higher for Line no. 17 than cv. Williams, whereas regarding fertilizer composition, combination b_2 b_3 and b_4 resulted in the highest content of phosphorus and potassium within seeds respectively (Table 2). The fertilizers that contained any kind of bacteria (b_2-b_5) improved mineral uptake of plants, as opposed to chemical fertilizers (b_1). Phosphate solubilizing bacteria produce organic acids that dissolve phosphorus from soil minerals, rendering this element available for plants (Rashid et al., 2004; Chen et al., 2006). In addition, these bacteria are related with plant hormones production that affect roots development and therefore plant uptake potential, as well as with proton release within soil solution that increases potassium exchange capacity (Raeipour and Aliasgharzadeh, 2007).

Fe, Mn and Zn content of seeds

The present study demonstrated that cv. Williams had a higher content in Fe and Mn than Line no. 17, whereas, in the case of Zn (Table 2), it indicated converse results. As to fertilizer composition, following application of b4 and b2 combinations Fe content of seeds had higher recordings, still with minor differences between them. Mn content was higher when b_2, b_3 and b_4 combination were applied, whereas b_2 combination had a beneficial effect also on Zn content of seeds. A similar effect of fertilizer composition on mineral content of seeds was reported by El-Kramany et al. (2007) and Khan (2005).

Material and methods

Plant material and experiment

The experiment was conducted at the Station of Agriculture and Natural Resources of Mahidashat, Iran (longitude: 46° 50' E, latitude: 24° 16' N, elevation: 1380 m above sea level) during the 2009 growing season in field conditions. The treatments consisted of two soybean (*Glycine max* L.) cultivars (cv. Williams and cv. Line no. 17) and five fertilizers with the following composition:
b_1: N + P* (Control)
b_2: *Bradyrhizobium japonicum* + P
b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P
b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P
b_5: *Bradyrhizobium japonicum* + 50% N + *Bacillus* sp. + *Pseudomonas* sp. + 50 percent P.
*[N= 300 Kg ha^{-1} of urea (46-0-0)]
[P = 150 Kg ha^{-1} of triple super phosphate (0-46-0)]

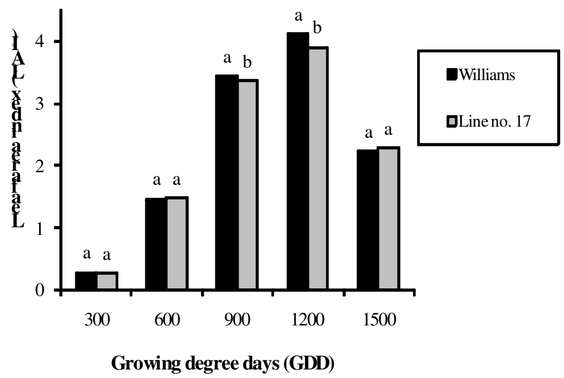

Fig 1. The effect of genotype on leaf area index in relation to Growing Degree Days (GDD). Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT).

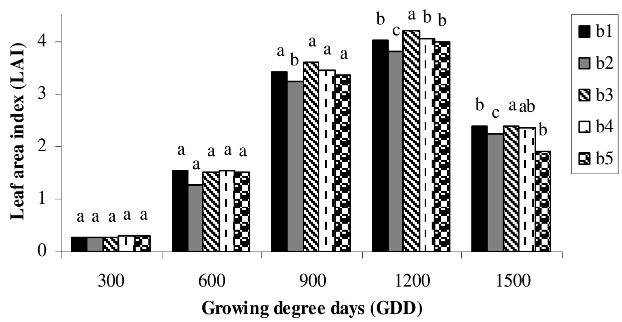

Fig 2. The effect of fertilizer composition on Leaf Area Index (LAI) of two soybean cultivar in relation to Growing Degree Days (GDD). Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum*+ *Bacillus* sp.+ *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum* + 50% N + *Bacillus* sp. + *Pseudomonas* sp.+ 50% P.

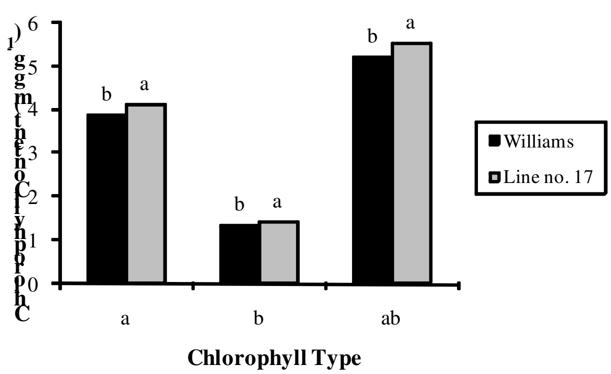

Fig 3. The effect of genotype on chlorophyll a, b and total chlorophyll content of leaves of soybean plants, regardless of fertilizer composition. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT).

Measurements

30 samples of soil were taken prior to the experiment and the results of their analysis are presented in Table 1. Considering nutritional plant requirements and the results of soil analysis, a base dressing of 150 Kg ha^{-1} of triple super phosphate (0-46-0) and 300 Kg ha^{-1} of urea was added. Throughout cultivation, planting was promptly followed up by urea addition in three equivalent doses of 30 Kg ha^{-1}30; the four-leaf stage and early reproductive stage were also enhanced by urea addition. Seeds were sown on June 5th, whereas 2 hours prior to seeding, seeds were inoculated with the biological fertilizers. Seed inoculation was carried out in shading conditions and followed by air-drying for 10 minutes before sowing. Inoculated seeds were planted in rows at a depth of 3 cm. The distance between the rows was 50 cm, whereas the plants within each row were 8 cm apart. Plant density was 25 plants per m^2. Inoculation with biological fertilizers was repeated at the 2-4 leaf stage by adding the fertilizers into the irrigation water. During cultivation, Leaf Area Index was recorded at 300, 600, 900, 1200 and 1500 Growing Degree Days (GDD), with Leaf Area Meter (LI- COR LI 3100, Linkoln, Nebraska, USA) according to the following formula (Karimi and Siddique, 1991):

$GDD= \Sigma \{[(T_{max} + T_{min})/2]-T_b\}$

Where:

T_{max}= Maximum daily temperature and up to the maximum of 30 ^oC.

T_{min}= Minimum daily temperature and up to the the maximum of 10 ^oC.

T_b= Basement temperature for plant that is 10 ^oC for soybean.

In order for leaf chlorophyll content (a, b and total chlorophyll) evaluation to be made, samples of young leaves at the R5 stage (onset of grain filling) were collected, placed in plastic bugs and kept under freezing conditions (4-5° C) until analysis. Chlorophyll content was determined according to a modified Arnon (1949) method. Zero point five (0.5) g of fresh leaf samples (ten samples for each treatment=100 samples in total) were homogenized with 5 ml of 80% acetone, then centrifuged at 13.000 rpm for 15 minutes at 4° C. The supernatant was removed and acetone was added to a final volume of 10 ml. The extracted pigments were evaluated by spectophotometry with the implement of a Shimadzu Spectrophotometer Model UV 180 (Shimadzu, Kyoto, Japan) at 645 and 663 nm wavelengths. The amount of chlorophyll (Chlorophyll a, b and total) present in the acetone extract was calculated in terms of mg chlorophyll per g of tissue by the use of the following equations:

chlorophyll a= $12.7(A663) - 2.69 (A645) \times V/1000 \times W$

chlorophyll b= $22.9 (A645) – 4.69 (A663) \times V/1000 \times W$

total chlorophyll= $20.2 (A645) + 8.02 (A663) \times V/1000 \times W$,

where:

A= Absorbance at specific wavelength

V= Final volume of chlorophyll extract in 80% acetone

W= Fresh weight of tissue extracted.

Plants were hand-harvested in mid-October; yield and quality features of seeds were recorded (weight of 1000 seeds, total yield, protein content, total protein yield, oil content, total oil yield and P, K, Fe, Mn and Zn content). Seed protein content was determined (ten samples for each treatment=100 samples in total) along the lines of the Kjeldhal method (Peach and Tracey, 1956). For mineral composition, samples of 50 g of seeds from each plot were selected randomly, washed with distilled water and oven dried at a constant weight at 70° C

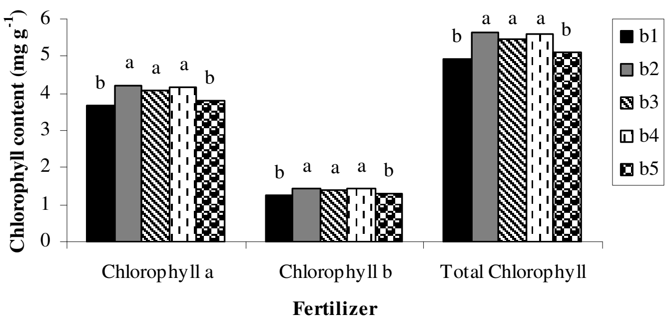

Fig 4. The effect of fertilizer composition on chlorophyll a, b and total chlorophyll content of leaves of soybean plants, regardless of cultivar. Different letters over the columns, when present, indicate statistical differences at at p≤0.05 according to Duncan Multiple Range Test (DMRT). b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum* + 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.

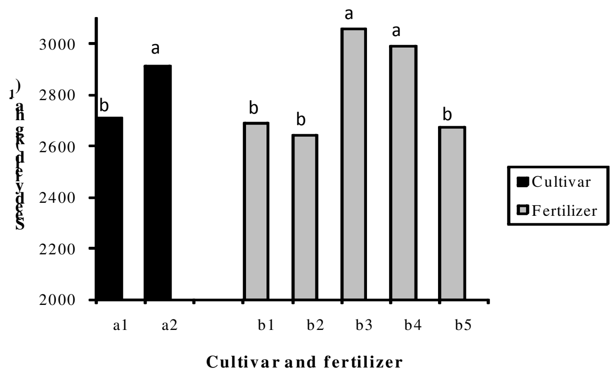

Fig 6. The effect of fertilizer composition and genotype on total seed yield of two soybean cultivars. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). a_1: cv. Williams; a_2: Line no. 17. b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.;
b_5: *Bradyrhizobium japonicum* + 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.

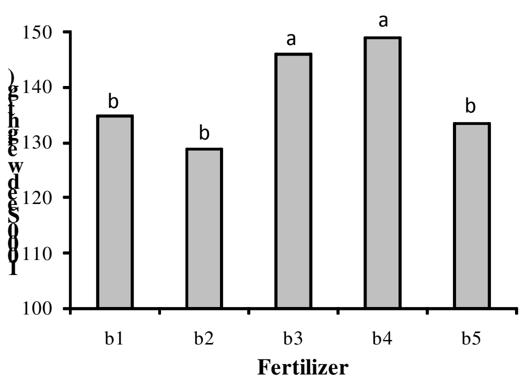

Fig 5. The effect of fertilizer composition on the weight of 1000 seeds of soybean plants regardless of cultivar. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum*+ 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.

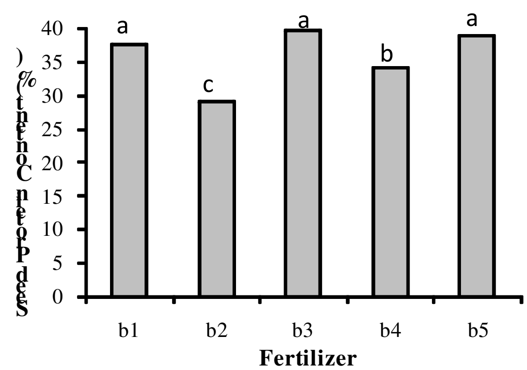

Fig 7. The effect of fertilizer composition on seed protein content [expressed as % percentage (w/w)], regardless of cultivar. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P., b_5: *Bradyrhizobium japonicum*+ 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.

or 48 hours. To determine the mineral content, samples (ten samples for each treatment=100 samples in total) were powdered in an electric mill (Teif Azmoon Pars M1415, Tehran, Iran), passed through a 40-mesh sieve, subjected to dry ashing in a muffle furnace at 550° C for 5 h, and used to extract K, Fe, Mn and Zn by means of 1 N HCl. The amount of P present in the samples was evaluated by spectrophotometer using a Shimadzu Spectophotometer Model UV 180 (Shimadzu, Kyoto, Japan) at 470 nm wavelength, according to Emami (1996). The concentration of Fe, Mn and Zn in the aqueous extracts was determined by atomic absorption spectrophotometer and K content by flame photometry (Teif Azmoon Pars P7c, Tehran, Iran) (Chapman, and Pratt, 1961). Seed oil content (ten samples from each block, n=40) was determined with the use of a Soxhlet apparatus.

Statistical analysis

The experiment was a factorial design laid out as a randomized complete block. Each block consisted of 5 fertilizer levels and 2 cultivars (i.e. 10 treatments and four replications, that is, 40 plots in total). Statistical analysis was conducted with the SAS (SAS Institute Inc., Cary, NC, USA) and Microsoft Office Excel (Microsoft Inc., USA). Data were evaluated by analysis of variance (ANOVA), whereas means

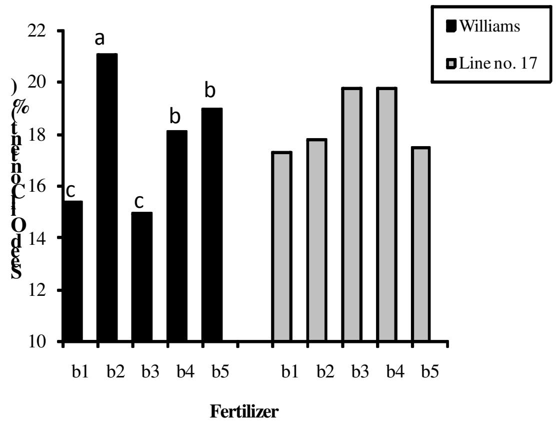

Fig 8. The effect of fertilizer composition on seed oil content [expressed as % percentage (w/w)] of two soybean genotypes. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). a_1: cv. Williams; a_2: Line no. 17. b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_5: *Bradyrhizobium japonicum* + 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.

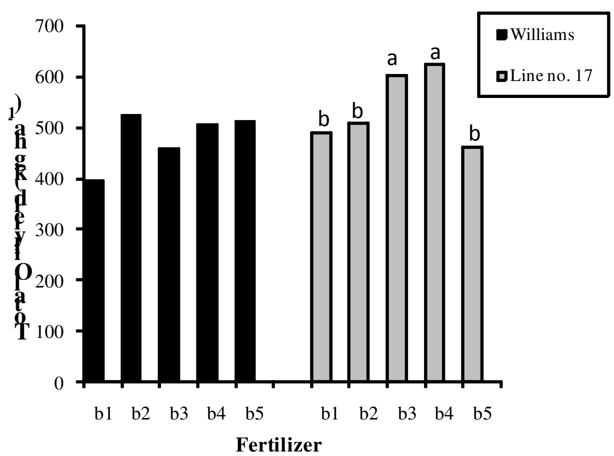

Fig 9. The effect of fertilizer composition on seed total oil yield of two soybean genotypes. Different letters over the columns, when present, indicate statistical differences at p≤0.05 according to Duncan Multiple Range Test (DMRT). b_1: N + P.; b_2: *Bradyrhizobium japonicum* + P.; b_3: N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P.; b_4: *Bradyrhizobium japonicum* + *Bacillus* sp. + *Pseudomonas* sp. + 50% P; b_5: *Bradyrhizobium japonicum*+ 50%N + *Bacillus* sp. + *Pseudomonas* sp. + 50% P were compared by the Duncan Multiple Range Test (p ≤0.05).

Conclusions

In conclusion, the application of fertilizers containing phosphate solubilizing (b_3, b_4 and b_5) and/or nitrogen fixation bacteria (b_2) resulted in an improvement of the yield components and quality features of soybean seeds, except for the case of seed protein content where the presence of nitrogen in fertilizer composition is of major importance. Fertilizers containing only nitrogen fixation bacteria (b_2) seem to be more beneficial than any other chemical or biological fertilizers, when seed oil and Zn content is considered. Therefore, fertilizer composition significantly affected yield components (especially b_3 and b_4 fertilizers) and quality features of soybean seeds, whereas differences between genotypes were observed only in the case of total seed yield and mineral composition of seeds. Overall, bio-fertilizers could be used as efficient substitutes of chemical fertilizers, and, more importantly environment-friendly ones not compromising yield and quality features.

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