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The effect of seed inoculation with growth stimulus bacteria and nitrogen fertilizer on the yield and yield components of mung bean in the Ivan Gharb city

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ABSTRACT: In order to effect of nitrogen fertilizer and seed inoculation with growth stimulus bacteria on the yield and yield components of mung bean, a factorial experiment was conducted in a randomized complete block design with three replications in the research farm located 15 km from the city of West Ivan in the 40 km northwest of Ilam In the 2012. Factors included the levels of nitrogen fertilizer at four levels of urea source (Zero, 25, 50 and 75 kg urea per acre) and seed inoculation with growth stimulus bacteria at four levels included (Non-inoculated of the seed as control, seed inoculation with Pseudomonas, Putida strain 186, Azotobacter strain 5 and Azospirillum lipoferum strain). The results showed that the nitrogen fertilizer and seed inoculation with bacteria had significant effect on the number of pods per plant, number of seeds per plant and biological yield. In addition to the effect of nitrogen and growth bacteria, the effect of treatments was significant on grain yield and harvest index. The highest grain yield was obtained with the consumption of 50 kg urea per hectare and seed inoculation with Azospirillum (3182 kg per ha). It seems that seed yield can be increased when seed inoculation with growth bacteria the Azospirillum in the lower urea values. Also in the experiment, all traits except thousand seed weight had positive and significant correlations with grain yield.

***Keywords*:** mung bean, growth stimulus bacteria, yield

INTRODUCTION

Legumes affect soil fertility due to atmospheric nitrogen stabilizer bacteria in the root. Every year a large amount of nitrogen is added to the soil after harvesting the crops. Some other parts of legume plants such as leave, stem, flower, immature pods, gland plus germinated seeds can be used as human and animal food and green manure to strengthen and improve the physical condition of the soil. Mung bean is the most common plants that are cultivated after wheat harvest in the most parts of the country and it is harvested before autumn planting. It is superior on other plants in the second cultivation due to the short-term development, the fixation of atmospheric nitrogen, the soil strengthen and prevent erosion. Also, in the some areas the plant provided that can be economically justified, it can be applied as green manure in crop rotations to achieve sustainable agriculture (Majnoun Hosseini, 2008). Efforts to increase production per unit area and excessive use and unbalanced of chemical fertilizers have had negative environmental implications in addition to increasing production costs and low yields. Long-term studies show that excessive use of chemical fertilizers reduces the crop yield due to the soil acidification, reducing biological activities, reducing soil physical properties and lack of micronutrients (Adrian et al, 2004). So that inappropriate use of chemical fertilizers, especially during the last four decades, in addition to the financial and economic losses by farmers, has led to environmental pollution, food, groundwater and surface water, loss of trace elements such as zinc, iron, Cu and manganese and finally, threaten the health of consumers.
To absorb the soil mineral nutrients and increase the absorption efficiency in the plants, it can be exploited from different methods such as the use of fertilizer sources, organic materials and plants inoculation with growth

stimulation bacteria PGPR. These bacteria increase nutrient uptake and plant growth through the effect on morphology and physiology of inoculated plants root (Mahdavi pour et al, 2009). Mung bean is able to stabilize atmospheric nitrogen as absorbable; it does through certain strains of Rhizobium which live in its root nodules form symbiotic (Osouli and Taleshi, 2011). The plant due to the short period of development, nitrogen fixation capacity of air, land improve and prevent soil erosion is superior to other plants in second culture. Also, in the some areas the plant provided that can be economically justified, it can be applied as green manure in crop rotations to achieve sustainable agriculture (Majnoun Hosseini, 2008).

Providing appropriate soil fertility using a balanced fertilizer and supply required nutrients for plant is one of the important aspects of crop management to achieve maximum yield and optimum quality of crops and to minimize their deleterious effect on the environment. Crop need to have numerous nutrients for optimal growth and development. Some elements, such as nitrogen, phosphorus and potassium are required by plants in relatively large amounts and plant growth and production will decline in case of lack of these elements in the soil (Saidi, 2007).

Nitrogen fertilizers are important in plant nutrition. Access to nitrogen for the crops is important limiting factors for agricultural productions. The importance of adequate nitrogen nutrition and diminish the soil nitrogen reserves due to nitrogen is exposure to loss than other nutrients and its recycling rate is about 33%, has aroused the farmers to excessive use of nitrogenous fertilizers (Ghorban Ali et al, 2006).

Soil quality can be evaluated based on improvement of quantitative and qualitative indicators of environmental community. For this reason, use of biofertilizers is considered as most effective management practices to maintain desirable soil quality (Fatma et al, 2008). Microorganisms used in biological fertilizers are either free-living or have symbiotic relationship with plants. These microorganisms directly or indirectly participate in the plant nutrition.

Sadeghipour and Monem (2010) stated that there were significant difference between the different usages of nitrogen in terms the mung bean protein percentage at 1% level. So that, grain protein percentage increased with nitrogen consumption increase. The highest protein percentage was obtained from the use 120 kg nitrogen per hectare and its minimum was lack of nitrogen.

Cheragi et al (2011) stated that nitrogen fertilizer has had significant effect on grain yield, biological yield, number of pods per plant, seeds per pod, thousand seed weight and harvest index of the mung bean. Results of Singh et al (2003) showed that there is a synergistic effect between biological, manure and chemicals fertilizers. They reported that increased levels of nitrogen as chemical fertilizers, improved millet grain yield and water use through significant improvements in growth characteristics. Seed inoculation with growth bacteria significantly increased the number of pods per plant (11%) and the mean of number of seeds per plant (14%) of soybeans than lack inoculation with bacteria. Also inoculation with bacteria significantly increased grain weight (5%) than lack inoculation with bacteria (Kazemi et al, 2005). Narula et al (2005) in the effect of biofertilizers along with chemical fertilizers on wheat yield reported that in the seed inoculation with Azotobacter combination with values of 90 and 120 kg N ha, dry matter and NPK absorption increased by plants compared to control. The survival of the Azotobacter bacteria in the Azotobacter treatment plus 90 kg N ha respectively was more than Azotobacter plus 120 kg N ha. Sayadi et al (2012) stated that mung bean seed inoculation with Azospirillum bacteria had a significant effect on the chlorophyll index, root dry weight, number of branches, roots, pods, number of seeds per plant, hundred grain weight, biological yield and pod length and width. Thus, the researchers stated that mungbean plant showed positive responses to the pretreatment with Azospirillium bacteria.

Therefore, this study was conducted to improve the quality yield of the mung bean to determine the most appropriate race of growth bacteria and as possible to reduce or mitigate the nitrogenous fertilizers by replacing it with biological bacteria to be examined the effect of nitrogen and seed inoculation with bacteria to increase plant growth (PGPR) on yield and some agronomic characteristics of the mungbean.

MATERIALS AND METHODS

This experiment was conducted in the research farm located 15 km from the city of West Ivan In the 2012. West Ivan is located in the 40 km northwest of Ilam province. Its height from sea level is 1303 m; the longitude 46 ° and 21 min and longitude 33° and 46 min. Precipitation average of the zone have been reported 645 mm per year. This area is part of the temperate regions. To determine the soil properties before running the test, sampling were taken from a depth of 0 to 30 cm the soil and its properties were examined. According to the results, the experimental land soil was loam. Analysis of soil samples are shown in Table 1.

Table 1. Physical and chemical properties of soil tested

Silt (%)	Sand (%)	Clay (%)	PH	K (ppm)	P (ppm)	(%)N	(cm) Depth
46	27	27	7.39	200	9.5	0.04	0-30

The factorial experiment was conducted in a randomized complete block design with three replications. Factors included the levels of nitrogen fertilizer at four levels of urea source (Zero, 25, 50 and 75 kg urea per acre) and seed inoculation with growth stimulus bacteria at four levels included (Non-inoculated of the seed as control, seed inoculation with Pseudomonas, Putida strain 186, Azotobacter strain 5 and Azospirillum lipoferum strain). Each unit consisted of 5 rows, distance 50 cm and a length of 3 m. Plants spacing within the row was considered 15 cm. A row plant was considered between two plots. Distance between two replications was calculated1 m. sowing seed on the line was done at a depth of 3-4 cm. land preparation, including plowing, disk leveler that was done before planting. Irrigation was performed based on environmental conditions, crop and farmers needs approximately every 10 days.

For mixing and seed inoculation with bio-fertilizer (growth stimulating bacteria), initially, seeds were spread on clean plastic, then amount of inoculation fluid (One liter per 35 kg seed) were slowly sprinkled on seeds and then seeds were inoculated with stirring, then inoculated seeds hanging in the shade and they were prepared for cultivating after drying (Akbari et al, 2009).

The traits included the number of pods per plant, seed weight, and number of seeds per plant, grain yield and biological yield. Harvest index was calculated by dividing the yield on biological function. The obtained data were analyzed using SAS statistical software and Excel was used to plot the graphs.

RESULTS AND DISCUSSION

The results of variance analyses (Table 2) showed that the effect of nitrogen and growth stimulating bacteria were significant on all traits except grain weight. Also, the interaction between nitrogen fertilizers in the bacteria was significant on grain yield and harvest index.

Table 2. Summary of variance analysis the effect of nitrogen and bacteria on yield and its components

MS						DF	
Harvest index	Biological yield	Grain yield	Number of seeds per plant	Seed weight	Number of pods per plant		Change sources
13.33	95467	5333	596.28*	293**	4.47	2	Repetition
47.11*	1655759**	835507**	3475.88**	36.8ns	168.6**	3	Nitrogen
48.98*	1849496**	936554**	2785.78**	16.2ns	46.33**	3	Bacteria
32.08*	62571	109370**	87.71	72.8	9.70	9	B × N
12.28	97124	22843	144.65	36.2	11.48	30	E
7.84	5.54	5.99	5.82	13.01	12.11	-	CV %

*, ** And ns respectively significant at 5%, 1% level and no significant

Table 3. Correlation between evaluated traits in the mung bean

Grain yield	Biological yield	Seed weight	Number of seeds per plant	Number of pods per plant	
				0.62**	Number of seeds per plant
			0.30*	0.28*	Seed weight
		0.18ns	0.71**	0.49**	Biological yield
	0.78**	0.19ns	0.83**	0.61**	Grain yield
0.79**	0.24ns	0.0ns	0.61**	0.48**	Harvest index

*, ** And ns respectively significant at 5%, 1% level and no significant

Table 4. Average comparison the effect of nitrogen fertilizer on traits

Harvest index (%)	Biological yield (kg)	Grain yield (kg)	Number of seeds per plant	Number of pods per plant	nitrogen fertilizer (kg/ha)
38.71 c	5076.9 c	1795 c	181.76 c	22.6 c	0
39.4 c	5674.8 b	1932 b	200.9 b	26.2 bc	25
42.3 b	6013 a	2216 a	222.2 a	29 b	50
47.6 a	5897 a	2483 a	220 a	32.7 a	75

Duncan's multiple range tests was used for means comparison. The difference between the mean have at least one common letter in each row statistically is not significant

Table 5. Mean comparison the effect of growth stimulating bacteria on traits

Harvest index (%)	Biological yield (kg)	Grain yield (kg)	Number of seeds per plant	Number of pods per plant	growth stimulating bacteria
44.2 a	5827 a	2374 a	214 a	29.1 a	Azotobacter
39.6 b	5869 a	2182 b	216.1 a	29.5 a	Azospirillum
43.5 a	5802.6 a	2446 a	213.2 a	29.3 a	Pseudomonas
38.71 c	5009.8 b	1795 c	181.20 b	24.6 b	Control

Duncan's multiple range tests was used for means comparison. The difference between the mean have at least one common letter in each row statistically is not significant.

The effect of nitrogen and bacteria on the number of pods per plant

Results showed that nitrogen fertilizer and growth bacteria had significant effect on the number of pods per mung bean plant, but the effect of experimental treatments was not significant on the traits (Table 2). The highest number of pods per plant was obtained the value of 32.7 psc, consumption of 75 kg N ha that increased 34% than lack of nitrogen fertilizer application (Table 4). Poor najaf (2006) stated that the nitrogen fertilizer has significantly increased on the number of pods per peas. A clear symptom of nitrogen deficiency in crops is reducing the number of flowers and pods. Number of pods is one of the ingredients grain yield. In the present experiment, the positive and significant correlation was observed between these traits and yield (Table 3).

Results showed that the seed inoculation with three types of bacteria had positive effect on increasing number of pods per plant. So that, with the Azospirillum bacteria, the number of pods per mung bean plant were obtained number 29.5 numbers that increased 17% than lack of inoculation (Table 5). Sayyadi et al, (2011) stated that the Azospirillum significantly has increased the number of pods of the mung bean. Since the number of pods per plant is one of the factors associated with performance, so, anything that will increase yield, also affects on this factor. Shokuhfar et al, (2008) reported increase the number of pods per soybean affected seed inoculation with bacteria.

Effect of nitrogen and bacteria on grain weight

The results showed that none of the main effects and interaction between treatments had no significant effects on mung bean seed weight (Table 2).

Effect of nitrogen and bacteria on the number of seed per plant

Results showed that nitrogen fertilizer and growth bacteria had significant effect on the number of seeds per mung bean plant, but the interaction of experimental treatments was not significant on the traits (Table 2). Significant differences were not observed between 50 and 75 kg per ha in terms the number of seed per plant, so that, the highest number of seeds per plant were obtained 222.2 number with the consumption of 50 kg N ha that revealed 20% increase compared to the lack of nitrogen fertilizer (Table 4). Poor najaf (2006) stated that the nitrogen fertilizer had significant effect on the number of seed per peas. Cheraghi et al, (2011) stated that the effect of nitrogen on pod and number of seed was positive and carbon metabolism can have a greater impact on the number and yield of grain.

Results showed that in the seed inoculation with three types of bacteria, there were no significant difference between three bacteria in terms the effect on the number of seed per plant. So that, with the Azospirillum bacteria, the number of seed per plant was obtained 216.1 numbers that increased 18% than lack of inoculation (Table 5). Poor najaf (2006) stated that the growth bacteria had significant effect on the number of seed per peas. Sayyadi et al, (2011) stated that the Azospirillum significantly has increased the number of seed in the mung bean. Shokuhfar et al, (2008) reported that seed number per soybean pod increased affected by seed inoculation with bacteria. Number of grain specifies tank capacity of the plant. More seeds per pod make to stored more photosynthetic materials and increased performance (Cheraghi et al, 2011).

In the present experiment, it was observed significant and positive correlation between the number of seeds per plant and grain yield (r = 0.83 **) (Table 3).

Effect of nitrogen and bacteria on grain yield

Results showed that in addition to the effect of nitrogen and growth bacteria, the experimental treatment had significant effect on the mung bean yield (Table 2). Results showed that at all levels of nitrogen, the lowest yield was obtained in the non-inoculated seed with growth bacterial (control). The highest grain yield was obtained with the consumption of 50 kg N ha and inoculated seed with Azospirillum amount of 3182 kg ha that it was 77% more than control. However, the lowest yield in the treatment was related to the lack of seed inoculation and nitrogen with 1.795 kg per ha (Figure1). Poor najaf (2006) stated that the growth bacteria and nitrogen fertilizer had

significant effect on the peas yield and with increasing nitrogen use and seed inoculation, grain yield has increased. The main reason for this increase may be a positive effect on nitrogen in increase stem weight and leaves, leaf surface expansion and preventing from abortion of flowers and pods.

Shiri Azar et al, (2012) stated that bacteria have a significant effect on grain yield. Gholi Nejad et al, (2009) stated that investment of assimilates in higher levels of nitrogen has increased in leaf and stem segments and as a result concentrated material increases in the grain and consequently increases the yield.

In the present study, it was observed a positive and significant correlation between grain yield and number of pods per plant and seed number per plant (Table 3).

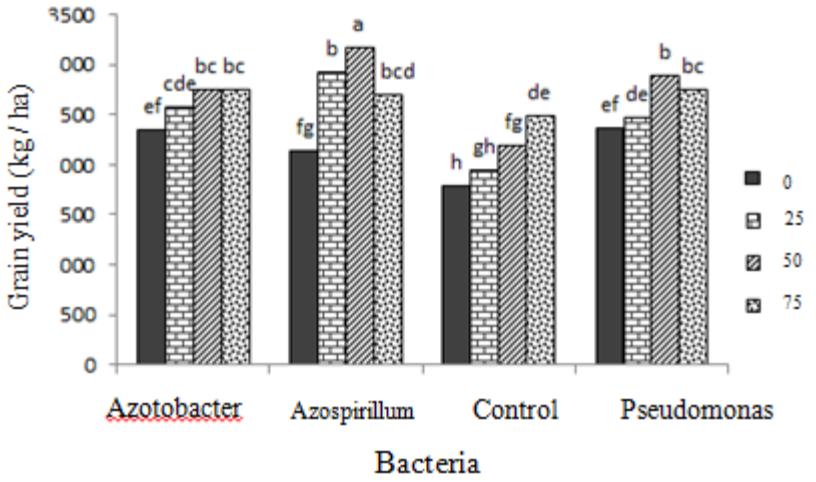

Figure 1. Compare the average of nitrogen manure composition (kg/ha) and growth bacteria on the grain yield

Effect of nitrogen and bacteria on the biological performance

Results showed that nitrogen fertilizer and growth bacteria had significant effect on the biological yield of the mung bean but the interaction of experimental treatments was not significant on the traits (Table 2). Though, there was no significant difference between 50 and 75 kg ha fertilizer use in their effects on biological function, but the highest biological yield with the use of 50 kg N ha was obtained amount of 6013 kg per hectare that increased 16% compared to the lack of nitrogen fertilizer (Table 4).

Ghorbanli et al, (2006) in their reviews about effect of nitrogen on the dry weight of rice plants showed that nitrogen increase caused to increased total plant dry weight. In their experiments correlation between the nitrogen content and net exchange of the carbon dioxide in different rice genotypes confirms that nitrogen increase is essential for dry matter production. Gholi Nejad et al, (2009) stated that nitrogen deficiency due to reduced leaf size and durability; probably reduce the amount of received light, light use efficiency, crop photosynthetic and then began to decrease biomass. In this experiment, there is a significant and positive correlation between biological and grain yield (Table 3).

Results showed that there was no significant difference between bacteria in terms the effect on the biological yield, so that, the biological function in the seed inoculation with Azospirillum was obtained the amount of 5869 kg per hectare that increased 16% compared to the lack of inoculation (Table 5).

The researchers stated that inoculation of the mung bean seed with Azospirillum has increased the biological yield of the plant (Rousta et al, 2009). Khalil zadeh et al, (2012) reported the mung bean dry weight increase using the biologic fertilizers. Taherkhani et al, (2007) stated that bean shoots at different levels of fertilizer; the highest rate was related to inoculation with growth bacteria and had significant difference with non-inoculated.

Effect of nitrogen and bacteria on the HI

Results showed that in addition to the effect of nitrogen and growth bacteria, the experimental treatment had significant effect on the mung bean harvest index (Table 2). Results showed that at all levels of nitrogen use except 75 kg per ha, the lowest harvest index was obtained in the non-inoculated seed with growth bacterial (control). The highest HI was obtained with the consumption of 25 kg N ha and inoculated seed with Azospirillum amount of 51.57 kg ha. However, the lowest harvest index was obtained in the lack of nitrogen use and non-inoculated seed

amount of 38.71 % (Figure 2). Poor Najaf (2006) stated that the growth bacterial and nitrogen fertilizer had significant effect on the peas HI.

In the present study it was found that harvest index rather than is correlated with biological yield (r = 0.24ns), it is correlated with grain yield (r = 0.79 **) (Table 30. Therefore, increase or decrease the yield has more influence on the character.

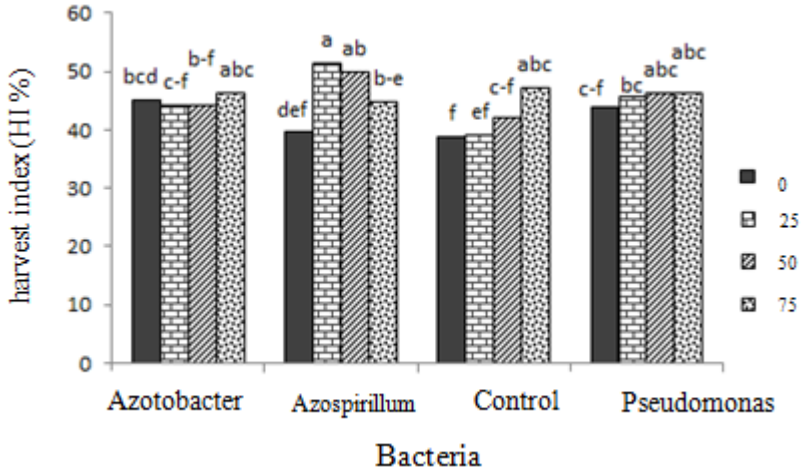

Figure 2. Mean comparison of the nitrogen fertilizer composition (kg per hectare) and growth bacteria on the HI

CONCLUSION

Results showed that nitrogen fertilizer and seed inoculation with bacteria had significant effect on the number of pods per plant, number of seeds per plant and biological yield. In addition to the effect of nitrogen and growth bacteria, the experimental treatment had significant effect on the grain yield and harvest index. The highest grain yield was obtained with the consumption of 50 kg urea ha and inoculated seed with Azospirillum amount of 3182 kg ha. Seems to grain yield in seed inoculation with Azospirillum growth bacteria can be increased in smaller amounts of urea. Also in this experiment, except for seed weight, all traits had positive and significant correlation with grain yield.

It is recommended this test can be used at least two consecutive years and using other mung bean repeated varieties and different growth bacteria to determine the best way.

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