

# Effects of dual inoculation of Mycorrhizal Arbuscular Fungi and Rhizobium Bacteria on Yield and Potassium Content of Corn Grains and Green Bean Under Intercropping

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**ABSTRACT:** In order to study the effect of dual inoculation of Mycorrhizal Arbuscular fungi and Rhizobium Bacteria on the Yield and Potassium content of grains of corn and Green Bean under intercropping, experiment was carried out in the research field of Faculty of Agriculture, of Azna PNU in the growing season of 2013 using in factorial to randomized complete block design and with three replication. The results showed that cropping systems on yield and potassium content of grains of Green Bean were significant at 1% level as well as potassium content of grains of were significant at 5% level. that inoculation by bacteria had a significant effect on yield of bean, yield of corn, number of grains per pod plant and number total of grains per ear ( $P < 0.01$ ). There is significant difference between the arbuscular mycorrhizal fungus treatment effects on number total of grains per ear and harvest index bean ( $p < 0.01$ ) and yield of corn and number of grains per pod plant ( $p < 0.05$ ). Significantly greater yield of seed bean (2592.3 kg.h) end Potassium content of grains of corn (10.55 mg/gr d.wt) were obtained from inoculation with Bacteria in mono cropping. Also the maximum Potassium content of grains of bean (15.75 mg/gr d.wt) was obtained from intercropping system with arbuscular mycorrhizal fungus. The separate inoculation of seeds with fungi and bacteria increased 25 and 11 percent yield of corn rate in terms of the non-use of fungus and bacteria, respectively.

**Keywords:** Green Bean, Corn, Intercropping, Potassium, Rhizobium Bacteria, Mycorrhizal Arbuscular Fungi.

## INTRODUCTION

The use of fertilizers, including chemical fertilizers nitrogen and phosphorus and potassium to enhance soil fertility and crop productivity has often negatively affected the complex system of the biogeochemical cycles (Steinshamn, 2004). Fertilizer use has caused leaching and run-off of nutrients, especially nitrogen (N) and phosphorus (P), leading to environmental degradation (Gyaneshwar, 2002). Microbial inoculation and intercropping are promising components for integrated solutions to agro-environmental problems because inoculation possesses the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Adesemoye, 2008). In nature, many plants establish a symbiosis with soil microorganisms, including arbuscular mycorrhizal (AM) fungior and N<sub>2</sub>-fixing bacteria like Rhizobium in legume (Leguminosae) plants. Both symbionts are known to improve plant growth under several environmental conditions. In fact, it is common that Rhizobium and AM fungi in dual symbiosis enhance growth and yield of many legumes (Franzini, 2009). Intercropping is the simultaneous growing of two or more crops in the same field. Farmers practice different cropping systems to increase productivity and sustainability (Hauggard Nielson, 2001). Intercropping systems, among the most important cropping

systems, are widely distributed in the world because of its high overall productivity, good pest and disease control, ecological services, and economic profitability (Malezieux , 2009). Intercropping of cereal and legume crops helps maintain and improve soil fertility. Legumes fix atmospheric nitrogen, which may be utilized by the host plant or may be excreted from the nodules into the soil and be used by other plants growing nearby (Andrew, 1979). Also legume–rhizobium symbiotic system is the most important biological nitrogen fixation system in nature (Peoples , 1995), providing about 65% biosphere’s available nitrogen (Lodwig , 2003). Rhizobium and mycorrhizal also produce plant growth regulators (auxins, gibberellins, cytokinins, abscisic acid and ethylene) (Paul, 2007). Rhizobial and mycorrhizal inoculants are now widely used in various parts of the world being inexpensive, environment-friendly and easy to use and have no side effects in most of the cases. They play an important role in improving crop yields (Paul, 2007). In this context, the present work aims to evaluate was the behavior of mycorrhizal -rhizobia symbiosis on yield, yield components, Harvest index and Potassium contents the Green Beans and corn intercropping system.

## MATERIALS AND METHODS

### **Site description**

The field experiments were conducted in 2013 at the Research Farm of the Faculty of Agriculture, Azna PNU, Iran (latitude 38.050 N, longitude 46.170 E, Altitude 1360 m above sea level). The climate is characterized by mean annual precipitation of 245.75 mm, mean annual temperature of 10°C, mean annual maximum temperature of 16.6 °C and mean annual minimum temperature of 4.2°C and a sandy- loam soil texture.

### **Experimental design**

The experimental design was a factorial to randomized complete block with three replications. The experimental factors include cropping systems such as mono cropping of corn, mono cropping of green beans, intercropping, Arbuscular Mycorrhizal fungus (use and non-use) and Rhizobium bacteria (use and non-use). The biological fertilizer of mycorrhiza was produced from a trap culture of clover (*Trifolium alexandrium*), which contained soil, plant roots and fragments of *Glomus mosseae* (obtained from Turan Biotech Co). And Mesorhizobium Bacteria was obtained from the Mehr Asia Biotechnology Company (MABCo.). For mycorrhizal treatments the amount of 15 g of fungal inoculum was placed in 3 cm below each seed. Each plots consisted a length of 6 meters for all treatments and 7 rows with spaced 50 cm as well as spacing of seeds were considered 20 cm for corn and bean. Practices including irrigation, thinning and weeding were kept normal and uniform for all the treatments.

### **Data collection**

At harvest time, for measuring the yield and yield components of the two species, in each plot six plants of corn and bean accidentally were selected after removing marginal effects and traits were measured. Also, Harvest index (HI) was calculated by the following equation.

$$HI = \frac{\text{Economical yield}}{\text{Biological yield}}$$

### **Potassium contents**

For Potassium (K+) contents determination, samples (0.5 g) of grains were ashed in a furnace for 6 h at 500 °C. The ash was dissolved in chloride acid. This solution was diluted with distilled water and filtered on Whatman paper. The K+ contents were determined by flame emission photometry (Ghoulam, 2002).

### **Data analysis**

Statistical analysis of the data was performed with SAS software. The significance of difference between treatments was determined using the least significant difference (LSD) multiple range test at the 5% probability level.

## RESULTS AND DISCUSSION

### **Bean Seed Yield**

The results analysis of variance showed that the effect of culture system, using Rhizobium bacteria, Rhizobium bacteria and fungus mycorrhizal interactions and interactions between systems were statistically significant in 1% level and the interaction of culture and fungus mycorrhizal was statistically significant in 5% level. But bean yield is not good under the effect of mycorrhizal fungi and Rhizobium bacteria and interaction between culture systems (Tab 1). The results showed that in intercropping system compared to mono crop was reduced yield by 26 Percent So that

treatment of mono culture bean with an average of 1958.34 Kg.Ha and intercropping treatment with an average of 1440.18 Kg.Ha are the highest and lowest yield, respectively (Tab 2).

Also use of Rhizobium bacteria significantly increased the yield so that Rhizobium bacteria treatment with 1748.57 Kg.Ha is the most and avoiding the use of bacteria treated with 1550.95 Kg.Ha is the lowest (Tab 2). Safapoor (2010) stated that use mycorrhizal fungi in different varieties of beans make reduced yield. In the study it was reported that soybean seed yield in both years was more mixed in pure culture to mixed culture (Undie et al, 2012). Also results showed interaction between use of Rhizobium bacteria and mycorrhizal fungi that The highest and lowest yield with 2102.5 Kg.Ha and 1295.70 Kg.Ha averages are make from use of Rhizobium bacteria treatment and non-use treatment (Tab 2). The results of interaction between cultivation system, Rhizobium bacteria and mycorrhizal fungi show that the most yields is from pure cultivation of beans with use of Rhizobium bacteria treatment with 2592.3 Kg.Ha as well as the lowest yields is from pure cultivation of beans with 1475.1 Kg.Ha (Tab 3).

### ***Bean Harvest Index***

The results of variance analysis showed that cultivation system and use of bacteria as well as interaction between cultivation system and bacteria, interaction between cultivation system, fungus and bacteria is not show statistically significant impact on the bean harvest index (Tab 1). The mixed cropping and manure fertilizer on growing corn and beans was also reported that the harvest index was not affected by the type of culture (Najafi , 2013). This can be due from coordinate changes of biological yield and grain yield per unit area (Ghanbari et al, 2006). The effects Comparison of mycorrhizal fungi show that use of mycorrhizal fungi treatment with 49.83 percent is the most bean harvest index and non-use of it with 55.41 percent is the lowest bean harvest index (Tab 2). Rajabzadeh Motlagh (2012) Reported that increased harvest index inoculated with mycorrhiza can be so justified that Mycorrhiza fungus with effects on sharing dry matter and increased grain yield make high rate of harvest index beans. The results of mean comparison culture system and mycorrhiza fungus show that the highest and the lowest bean harvest index are 55.74 percent and 36.7 percent, respectively as well as it was for intercropping treatments with mycorrhizal fungi and intercropping treatments with non mycorrhizal fungi (Tab 2).

### ***The number of seeds per pod beans***

To improve the performance of bean seeds per pod should be noted, because these factors are highly correlated with crop yield (Zafarani , 1991). In the analysis of variance seeds per pod beans was significant under the effect of bacteria on in 1% level and the effect of mycorrhizal fungi as well as the interaction of cultivation system and bacteria in 5% (Tab 1). On the other hand it has been reported that the number of seeds per pod in the mixed and pure cultivation are significantly different, although grown in pure culture is more mixed (Marzban et al, 2012). With the use of Rhizobium bacteria was observed that seeds per pod also increased so that the maximum and minimum number of seed per pod was 5.65 and 4.07 in use of bacteria treatment and nonuse of bacteria treatment (Tab 2).

Research has shown that use of seed that bacteria inoculation can be used in the economic production and savings product will cause nitrogen fertilizers. As well as bacterial inoculation with promoting against soil borne diseases and lack of adverse effects of the influx of environmental is an important step in the direction of sustainable agriculture (Rajabzadeh Motlagh, 2012). Results showed that highest and lowest number of seeds per pod with 5.27 and 4.47 are for nonuse of mycorrhizal fungi and use of mycorrhizal fungi, respectively (Tab 2). As well as result show that with use of bacteria the number of seeds per pod was more (Tab 2).

### ***Bean Seeds Potassium***

In the analysis of variance potassium content of seeds under the influence of cultivation, interaction cultivation system and rhizobium bacteria was be significant in 1% level as well as under influenced by the interaction cultivation system, Rhizobium bacteria and mycorrhizal fungi was significant at 5% level (Tab 1). In general, potassium absorption (Tab 2) in the mixed cropping (12.66 mg/gr d. wt) was being more than mono cultivation (10.98 mg/gr d. wt). This applies in mixed cropping systems can supplement the effects of components in search of nutrients in the soil profile because of the difference in the depth of root development, absorption elements that are not available In the pure culture or because of differences over the course the components of mixed cropping (Marzban, 2012). Also mean comparison showed that the highest and lowest potassium content of grains are made from mixed cropping with mycorrhiza fungi treatment and pure cultivation (Tab 3). Mycorrhiza fungi with an extensive network and increasing the speed of root uptake efficiency of plants to absorb water and nutrients, especially of phosphorus, potassium, zinc and copper have increased and improved their growth pick (Smith and Read, 2008). Also in this study, it seems that the use of mixed cropping with mycorrhizal fungi enhance the development of deep, side and increase the density of the bean roots that this absorption of nutrients, particularly potassium has increased in comparison with pure cultivation.

**Corn Grain Yield**

Total number seed s affected by use of bacteria and mycorrhizal fungi as well as was be significant in 1% level, on the other hand interaction of mycorrhizal fungi and bacteria were significant in 5% level. While farming systems was not a significant effect on the total number seed (Tab 1). In Table mean comparison of effects bacteria consume an average of 502.5 made the most grains as well as nonuse of bacteria an average of 404.77 made the lowest grains (Tab 2). Based on the mean comparison result the effects of combination of bacteria and mycorrhizal fungi made the most grain (536.13) from the combined treatment of bacterial and mycorrhizal fungi as well as the lowest (303.25) was be for nonuse of them (Tab 2). Phosphorus and nitrogen are important factors in aggregation and the formation of seed corn. So it seems that mycorrhiza and Rhizobium bacteria dissolved phosphate and has more access Phosphorus for plant. The plant access to a greater volume of soil can absorb more water and food as a result of the increased the number of grains (Smith and Read, 2008; Etesami et al, 2009).

**Corn Harvest Index**

The results of analysis variance showed that the bacterium Rhizobium is affected by harvest index and it was significant in 5% level (Tab 1). Mean comparison showed that the application of Rhizobium bacteria increased harvest index so that the highest and the lowest harvest index with average 29.91 and 23.85 was found bacteria use and nonuse of bacteria treatment (Tab 1). Andrei et al (2001) have announced that Rhizobium bacteria through the elongation roots and increase the root system in cereals increase root contact with the soil and absorption of nutrients which increases the production of dry matter in vegetative phase and specific to reproductive organs as a result of increased harvest index.

Table 1. Mean-square characteristics of corn and bean

Sources of change	df	Grain yield Bean	Grain yield Corn	Number of grains per pod plant	Number total of grains per ear	Harvest Index Bean	Harvest Index Corn	k content of grains Bean	k content of grains Corn
Error	2	1706.08 <sup>ns</sup>	444869.11 <sup>ns</sup>	0.0012 <sup>ns</sup>	14183.87 <sup>ns</sup>	67.93 <sup>ns</sup>	46.63 <sup>ns</sup>	5.12 <sup>ns</sup>	1.35 <sup>ns</sup>
Cropping system	2	1610928.3 <sup>**</sup>	25271.74 <sup>ns</sup>	0.033 <sup>ns</sup>	16419.58 <sup>ns</sup>	6.66 <sup>ns</sup>	25.98 <sup>ns</sup>	16.83 <sup>**</sup>	10.35 <sup>*</sup>
Bacteria	1	527918.3 <sup>**</sup>	14920888.01 <sup>**</sup>	14.88 <sup>**</sup>	63422.46 <sup>**</sup>	73.32 <sup>ns</sup>	90.58 <sup>*</sup>	4.59 <sup>ns</sup>	8.44 <sup>*</sup>
Fungus	1	3.26 <sup>ns</sup>	4584903.64 <sup>*</sup>	4.08 <sup>*</sup>	101497.52 <sup>**</sup>	411.1 <sup>**</sup>	23.54 <sup>ns</sup>	1.815 <sup>ns</sup>	2.44 <sup>ns</sup>
Cropping system x Bacteria	x 2	700.92 <sup>ns</sup>	751933.17 <sup>ns</sup>	3.15 <sup>*</sup>	15288.87 <sup>ns</sup>	5.08 <sup>ns</sup>	4.42 <sup>ns</sup>	71.41 <sup>**</sup>	16.60 <sup>**</sup>
Cropping system x Fungus	x 2	137637.76 <sup>*</sup>	78141.96 <sup>ns</sup>	2.34 <sup>ns</sup>	4405.81 <sup>ns</sup>	695.84 <sup>**</sup>	13.32 <sup>ns</sup>	1.98 <sup>ns</sup>	0.07 <sup>ns</sup>
Fungus x Bacteria	1	1558917.4 <sup>**</sup>	185663.33 <sup>ns</sup>	0.0937 <sup>ns</sup>	31955.75 <sup>*</sup>	575.55 <sup>**</sup>	26.17 <sup>ns</sup>	3.15 <sup>ns</sup>	22.55 <sup>**</sup>
Cropping system x Fungus x Bacteria	2	620913.17 <sup>**</sup>	6097.85 <sup>ns</sup>	0.0037 <sup>ns</sup>	662.71 <sup>ns</sup>	0.04 <sup>ns</sup>	54.97 <sup>ns</sup>	12.61 <sup>*</sup>	8.09 <sup>*</sup>
Error	12	15852.92	529679.61	0.55	6670.17	39.97	15.35	1.99	1.45
Coefficient of Variation		7.4	10.43	15.36	17.09	12.94	12.29	11.94	14.01

ns= non-significant, \* significant in 5%, \*\* significant in 1%

Table 2. Mean comparison some characteristics of corn and bean

Treatment	Grain yield Bean (kg.ha-1)	Grain yield Corn (kg.ha-1)	Number of grains per pod	Number total of grains per ear	Harvest Index Bean (%)	Harvest Index Corn (%)	k content of grains Bean (mg . g DW <sup>-1</sup> )	k content of grains Corn (mg . g DW <sup>-1</sup> )
<b>Cropping system</b>								
Mono cropping	1958.34 a	6940.22 a	4.8 a	430.02 a	45.6 a	32.90 a	10.98 b	7.95 b
Intercropping	1440.18 b	7005.1 a	4.9 a	482.23 a	46.22 a	30.82 a	12.66 a	9.26 a
<b>Bacteria</b>								
Non use of Bacteria	1550.95 b	6184.1 b	4.07 a	404.07 b	47.44 a	29.91 b	12.26 a	8.01 a
Use of Bacteria	1847.58 a	7761.1 a	5.65 a	502.85 a	43.94 a	33.82 a	11.38 a	9.2 a
<b>Fungus</b>								
Non Use of Fungus	1698.88 a	6535.5 b	5.27 b	391.15 b	41.55 b	30.78 a	12.1 a	8.92 a
Use of Fungus	1699.66 a	7409.7 a	4.45 a	521.21 a	49.83 a	32.85 a	11.55 a	8.28 a
<b>Cropping system x Bacteria</b>								
Mono Cropping	1815.43 b	5975.7 b	4.4 bc	353.38 b	46.45 a	30.53 ab	9.8 c	6.52 b
Mono Croppingx Bacteria	2101.25 a	7905.6 a	5.25 ab	506.67 a	43.88 a	35.27 a	12.27 b	9.37 a
Intercropping	1286.47 d	6393.6 b	3.7 c	456.17 a	48.43 a	29.30 b	14.85 a	9.50 a
Bacteriax Intercropping	1593.90 c	7616.5 a	6.5 a	508.5 a	44.01 a	32.33 ab	10.5 c	9.02 a
<b>Cropping system x Fungus</b>								
Mono Cropping	2033.68 a	6560.1 b	5.55 a	378.53 c	46.41 b	31.16 a	10.97 b	7.57 b
Mono Croppingx Fungus	1823.83 a	7320.2 ab	4.1 b	481.5 ab	43.95 bc	34.63 a	11 b	8.32 ab
Intercropping	1516.3 b	6510.6 ab	5 ab	403.75 bc	36.7 c	30.57 a	13.22 a	9 ab
Fungus x Intercropping	1364.07 b	7499.2 a	4.8 b	506.92 a	55.74 a	31.06 a	12.8 ab	9.52 a
<b>Fungus xBacteria</b>								
Non Use ( Fungus, Bacteria)	1295.7 d	5835 c	4.5 b	303.25 b	38.4 b	29.98 b	12.17 a	6.72 b
Fungus	1806.2 b	6533.2 bc	3.6 c	506.29 a	56.48 a	29.85 b	12.35 a	9.30 a
Bacteria	2102.05 a	7236.1 b	6 a	479.04 a	46.7 b	31.75 ab	12.02 a	9.58 a
Bacteriax Fungus	1593.10 c	8286.1 a	5.3 ab	536.13 a	43.19 b	35.85 a	10.75 a	8.55 a

Mean that have at least a share word don't significant difference in LSD test (5% level)

Table 3. Compared the effects of three characteristics of corn

Treatment	Grain yield Bean (kg.ha-1)	Grain yield Corn (kg.ha-1)	Number of grains per pod	Number total of grains per ear	Harvest Index Bean (%)	Harvest Index Corn (%)	k content of grains Bean (mg . g DW <sup>-1</sup> )	k content of grains Corn (mg . g DW <sup>-1</sup> )
Mono cropping	1475.1 c	5666.7 e	5.2 a-c	270.5 c	42.76 b-d	31.36 b	8.6 d	4.6 c
Mono Croppingx Fungus	2155.8 b	6282.7 c-e	3.6 d	436.25 ab	50.15 b	29.69 b	10.8 cd	8.45 ab
Mono Croppingx Bacteria	2592.3 a	7453.7 a-c	5.9 ab	486.58 a	50.06 b	30.97 b	13.35 ab	10.55 a
Mono Cropping x Fungusx Bacteria	1610.2 c	8357.7 a	4.6 cd	526.57 a	37.69 d	39.57 a	11.2 bc	8.2 b
Intercropping	1116.3 d	6003.3 de	3.9 cd	336 bc	34.05 d	28.60 b	13.9 a	8.85 ab
Fungus x Intercropping	1456.6 c	6783.8 c-e	3.6 d	576.33 a	62.81 a	30.12 b	15.75 a	10.15 ab
Bacteriax Intercropping	1611.8 c	7018.5 bc	6.1 a	471.5 ab	39.43 cd	32.54 b	10.7 cd	9.15 ab
Intercropping x Fungusx Bacteria	1576 c	8214.6 ab	6 a	545.5 a	48.68 bc	32.12 b	10.3 c	8.9 ab

Mean that have at least a share word don't significant difference in LSD test (5% level)

## CONCLUSION

It can be stated that the share of each two plants other than the composition of the mixture increases, the plant will prevail and on the contrary. Also morphology and structure of the components are effective of such mixtures are dominant and recessive (Undie, 2012). And as the results of this experiment showed that a mixture of green beans with corn will be the dominant species. Also results of this experiment show that in the most cases the inoculation of Rhizobium bacteria and fungus mycorrhiza together or separately no effect on both qualitative and quantitative traits of plants. The positive effects of these microorganisms are probably due to nutrient availability and growth hormone. In total, according to the results can be expected using mixed cropping and organic fertilizers Improved nitrogen fixation and absorption of other nutrients and ultimately improve yield stability and rabies.

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