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# Assessment of forage corn quality intercropping with green beans under influence of *Rhizobium* bacteria and arbuscular mycorrhizal fungus

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**Key words:** Forage Corn, Quality, Rhizobium Bacteria, Arbuscular Mycorrhizal Fungus, Intercropping

**Abstract**

To assess the quality of forage corn intercropping with green beans under the influence of Rhizobium bacteria and Arbuscular Mycorrhizal fungus, make a test in educational-research farm of agriculture faulty of Azna PNU that it was design in 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 results showed that cropping systems on crude protein, wet forage weight, dry forage weight were significant at 1% level as well as leaf to stem ratio was significant at 5% level. Between different levels of bacteria used, acid detergent fiber was impressed and was significant at 5% level. Arbuscular Mycorrhizal fungus was significant at 5% level on water soluble carbohydrate. The results showed that the use of separate and combined of Rhizobium bacteria and Arbuscular Mycorrhizal fungus increase the quality of corn in intercropping than mono cropping. Finally with increasing of plant diversity and micro-organism in soil increased the quality and quantity of forage.

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## Introduction

Forage corn is a plant that capable of producing high as compatible in most areas of the country as well as it has important role in providing forage for livestock, especially in the winter. Corn is an important forage species that for use it harvest in whole plant silage (Coors, 1995). This plant in spite of has a step in harvest as well as it has high operation in dry matter. It is easy to prepare and it is palatable forage with high quality for livestock and has a higher energy than other forages. Corn silage production needs fewer employees than other forages (Curran and Posch, 2000). Lack of nitrogen delays phonological step (Vegetative and reproductive) as well as decrease leaf appearance rate, leaf development rate and leaf area duration. With availability of nitrogen increased leaf area index, plant height, dry matter, forage quality and light use efficiency (Uhart and Andrade, 1995). Grains and Legumes intercropping are one of the most importances Cultivation and in compared with mono cropping these increase the grain operation, dry matter and forage quality. The atmospheric nitrogen fixation by legumes and transfer to corn can be one of cause increased protein in intercropping than mono cropping (Dahmardeh *et al.*, 2010). In addition, these system contrary to mono cropping system going toward principles of ecological as well as with effective use of them and sustainability increases agricultural systems especially in low-input (Lithourgidis *et al.*, 2007). Nowadays, for plant nutrition use nitrogen and phosphate fertilizers that in addition to environmental degradation they are high risks for human. On this way Rhizobium bacteria and Arbuscular Mycorrhizal fungus by providing nutrients for plants play an important role in sustainable agriculture. Rhizobium bacteria and Arbuscular Mycorrhizal fungus in root environment have advantages for plant growth that resulting in increased food intake. These bacteria synthesis various vitamins and amino acids to increase the growth and product quality and through different processes are caused systemic resistance in plants. This resistance causes that plants tolerated environmental stresses such as lack of ventilation, heavy metal pollution, salinity, drought, pests and diseases (Etesami *et al.*, 2009). The research that was done on corn, the results showed that inoculation with Arbuscular Mycorrhizal fungus significantly increased total shoot dry weight (Alizadeh *et al.*, 2011). Also one of way for increasing concentrations of crude protein in corn is intercropping of corn with beans. In this context, there is considerable evidence that intercropping of corn and legumes increase crude protein concentration in forage. In addition dry matter, hemicellulose and lactic acid corn increased in intercropping (Contreras-Govea *et al.*, 2008). The research, which is increase the diversity of agricultural ecosystem for study the effect of Rhizobium bacteria and Arbuscular Mycorrhizal fungus on quality of forage corn that was done in field conditions.

## Materials and methods

*Study Area*

This research done in educational-research farm of agriculture faulty of Azna PNU in April 2013 and it was design in factorial to randomized complete block with three replications.

*Analysis*

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). 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. In intercropping treatment planting seeds was carried as a mixture in the form of replacement. Then seeds were placed at a depth of 5 cm due depending on soil conditions and irrigation. To determine the characteristics of quality, fresh weight and dry weight selected 6 randomly of corn and bean forage and transported to the laboratory. After determining the weight of forage, plants were placed in an oven at temperatures 75 °C and dry matter was determined. After drying, the samples were crushed and milled and then passed through a

5.0 mm sieve for used in chemical analysis. Measurement of forage quality was done with NIRS device (Roberts *et al*, 2003). Statistical analysis was carried out using SAS software. The difference of the treatments was compared using the least significant difference (LSD) test at the 0.05 probability level.

**Result and discussion**

*Dry Matter Digestibly*

Improved of dry matter digestibly is the main goal of the reform program in forage corn because digestibly will be maximum and improve the efficiency of conversion of nutrients to the animals. Moreover digestibility is the most important characteristic to increase weight and milk production (Dahmardeh *et al.*, 2010). Dry matter digestibly is often representative of digestible energy (Coleman and Moore, 2003). According to the results of variance analysis between the different treatments of dry matter digestibility there was no difference (Tab1). But most digestible dry matter was obtained of intercropping treatments that combined with fungi and bacteria. The same study shows that intercropping of barley and annual legume has the highest digestible dry matter (Hail *et al.*, 2009). Finally the result show intercropping of Rhizobium bacteria and Arbuscular Mycorrhizal fungus than mono cropping has a positive synergistic effect and leads to an increase in dry matter digestibility in corn.

**Table 1.** Mean-square characteristics of forage corn.

| Leaf to Stem Ratio | Forage dry weight | Forage wet weight | Ash | CP | WSC | NDF | ADF | DMD | df | Sources of change |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.004 $^{n.s}$ | 136.19 $^{*}$ | 124.11 $^{*}$ | 0.0026 $^{n.s}$ | 1.34 $^{n.s}$ | 5.30 $^{n.s}$ | 3.64 $^{n.s}$ | 1.40 $^{n.s}$ | 4.27 $^{n.s}$ | 2 | Error |
| 0.017 $^{*}$ | 1125.95 $^{**}$ | 1123.40 $^{**}$ | 0.0001 $^{n.s}$ | 7.96 $^{**}$ | 6.02 $^{n.s}$ | 28.88 $^{n.s}$ | 3.04 $^{n.s}$ | 9.56 $^{n.s}$ | 2 | Cropping system |
| 0.000 $^{n.s}$ | 9.03 $^{n.s}$ | 9.12 $^{n.s}$ | 0.406 $^{n.s}$ | 0.91 $^{n.s}$ | 10.96 $^{n.s}$ | 22.95 $^{n.s}$ | 33.48 $^{*}$ | 1.4 $^{n.s}$ | 1 | Bacteria |
| 0.0005 $^{n.s}$ | 35.74 $^{n.s}$ | 38 $^{n.s}$ | 0.207 $^{n.s}$ | 0.15 $^{n.s}$ | 53.54 $^{*}$ | 24.74 $^{n.s}$ | 0.9 $^{n.s}$ | 3.26 $^{n.s}$ | 1 | Fungus |
| 0.0014 $^{n.s}$ | 5.93 $^{n.s}$ | 6 $^{n.s}$ | 0.56 $^{n.s}$ | 1.94 $^{n.s}$ | 89.39 $^{**}$ | 1.69 $^{n.s}$ | 34.92 $^{*}$ | 86.07 $^{n.s}$ | 2 | Cropping system × Bacteria |
| 0.00003 $^{n.s}$ | 8.33 $^{n.s}$ | 8.4 $^{n.s}$ | 0.056 $^{n.s}$ | 2.87 $^{n.s}$ | 66.4 $^{*}$ | 8.72 $^{n.s}$ | 0.008 $^{n.s}$ | 5.85 $^{n.s}$ | 2 | Cropping system × Fungus |
| 0.00003 $^{n.s}$ | 37.02 $^{n.s}$ | 42.66 $^{n.s}$ | 0.57 $^{n.s}$ | 1.29 $^{n.s}$ | 5.11 $^{n.s}$ | 13.39 $^{n.s}$ | 11.55 $^{n.s}$ | 18.11 $^{n.s}$ | 1 | Fungus ×Bacteria |
| 0.005 $^{n.s}$ | 64.26 $^{n.s}$ | 64.02 $^{n.s}$ | 0.83 $^{*}$ | 0.01 $^{n.s}$ | 9.90 $^{n.s}$ | 27.24 $^{n.s}$ | 88.35 $^{**}$ | 5.85 $^{n.s}$ | 2 | Cropping system× Fungus× Bacteria |
| 0.002 | 24.63 | 24.44 | 0.16 | 0.66 | 7.6 | 6.64 | 5.7 | 21.51 | 12 | Error |
| 24.63 | 10.61 | 10.59 | 7.32 | 7.07 | 12.01 | 5.39 | 9.54 | 8.58 | | Coefficient of Variation |

ns= non-significant, $^{*}$ significant in 5%, $^{**}$ significant in 1%.

*Acid Detergent Fiber*

Acid detergent fiber measure part of fiber that digestible is less and includes crude lignin and cellulose (Contreras-Govea *et al.*, 2009). Acid detergent fiber was influence under bacteria as well as was significant at the one percent level but the use of Arbuscular Mycorrhizal fungus and different intercropping not significant effect on this characteristic (Tab1). The results of the comparison table shows that with use of bacteria the most ADF was be 26.35% and in Lack use of bacteria was be 23.98% (Tab2). Comparison result of average effects of combination systems and bacterial show that the most ADF (27.2%) was for mono cropping of corn with bacteria (Tab2). According to the compared results in triple interaction table was observed that the most ADF (29.6%) was for corn mono cropping with use of Rhizobium bacteria and Arbuscular Mycorrhizal fungus (Tab3). Low levels of ADF in mono cropping show the high quality of forage. Due to forage quality standard table (Lithourgidis *et al.*, 2006) know that all system in this research are rated excellent that shows forage with high quality.

*Neutral Detergent Fiber*

Neutral detergent fiber is one of the important

attribute of forage (Contreras-Govea *et al.*, 2009) that indicating potential of forage consumed by livestock. When neutral detergent fiber content increase dry matter intake was be decrease therefore low percentage of NDF is desirable. Cropping systems, Rhizobium bacteria and Arbuscular Mycorrhizal fungus do not significant effect on the concentration of the cell walls of corn (Tab1). Similar results suggest a decline in forage NDF and reported (Contreras-Govea *et al.*, 2009). Also according to the standard table of forage quality can be concluded that NDF also intercropping treatments with Arbuscular Mycorrhizal fungus is in premium class that indicating the improving of forage quality thereby mixture of corn and green beans also inoculated with Arbuscular Mycorrhizal fungus. It seems that inoculation with Arbuscular Mycorrhizal fungus increased cytokinins in plants, increase in plant leaves and move food from other parts to leaves then also to delay leaf senescence. Finally the sum these factors increase the weight of the plant leaves as a result NDF rate decreased in this treatment and improves the quality of the forage.

**Table 2.** Mean comparison some characteristics of corn.

| Leaf to Stem Ratio | Forage dry weight (Ton/Ha) | Forage wet weight (Ton/Ha) | Ash (%) | CP (%) | WSC (%) | NDF (%) | ADF (%) | DMD (%) | Treatment |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cropping system | | | | | | |
| 0.2 a | 39.2 b | 40.21b | 5.60 a | 10.97 b | 23.28 a | 48.89 a | 25.52 a | 54.65 a | Mono cropping |
| 0.16 b | 53.62 a | 53.9 a | 5.59 a | 12.12 a | 22.27 a | 46.73 a | 25.81 a | 53.38 a | Intercropping |
| Bacteria | | | | | | | | | |
| 0.19 a | 46.16 a | 46.44 a | 5.46 a | 11.74 a | 22.21 a | 46.83 a | 23.98 b | 53.26 a | Non use of Bacteria |
| 0.18 a | 45.55 a | 47.67 a | 5.72 a | 11.35 a | 23.45 a | 48.79 a | 26.35 a | 54.26 a | Use of Bacteria |
| Fungus | | | | | | | | | |
| 0.19 a | 47.99 a | 48.31 a | 5.56 a | 11.63 a | 21.28 b | 48.83 a | 25.36 a | 53.65 a | Non Use of Fungus |
| 0.18 a | 45.55 a | 45.8 a | 5.63 a | 11.49 a | 24.27 a | 46.8 a | 24.94 a | 54.98 a | Use of Fungus |
| | | | Cropping system × Bacteria | | | | | | |
| 0.21 a | 39.81 a | 40.01 b | 5.62 b | 11.45 ab | 22.01 b | 47.65 ab | 22.4 b | 56.3 a | Mono Cropping |
| 0.22 a | 40.04 a | 40.33 b | 5.57 ab | 10.49 b | 25.88 a | 50.13 a | 27.2 b | 53 a | Mono Cropping× Bacteria |
| 0.17 a | 52.51 a | 52.78 a | 5.31 b | 12.03 a | 23.53 ab | 46.02 b | 25.55a | 51 a | Intercropping |
| 0.15 b | 54.73 a | 55.01 a | 5.88 a | 12.21 a | 20.67 a | 47.45 ab | 25.5 a | 55.25 a | Bacteria× Intercropping |
| | | | Cropping system × Fungus | | | | | | |
| 0.21 ab | 40.55 b | 40.88 b | 5.61 a | 10.54 b | 20.12 b | 51.51 a | 25.02 a | 54.77 a | Mono Cropping |
| 0.22 a | 39.22 b | 39.55 b | 5.58 a | 11.40 ab | 11.40 ab | 47.27 b | 24.6 a | 54.52 a | Mono Cropping× Fungus |
| 0.15 b | 55.43 a | 55.75 a | 5.51 a | 11.86 a | 11.86 a | 47.15 b | 25.7 a | 52.52 a | Intercropping |
| 0.16 ab | 51.81 a | 52.05 a | 5.68 a | 12.39 a | 12.39 a | 46.32 g | 25.35 a | 54.25 a | Fungus × Intercropping |
| | | | Fungus ×Bacteria | | | | | | |
| 0.185 a | 46.12 b | 46.36 b | 5.27 b | 12.5 a | 20.15 b | 78.60 a | 25.87 b | 54.27 a | Non Use (Fungus, Bacteria) |
| 0.197 a | 46.18 b | 46.51 b | 5.59 b | 44.43 a | 24.05 a | 45.07 b | 23.1 b | 53.2 a | Fungus |
| 0.183 a | 49.85 a | 50.26 a | 5.84 a | 11.20 a | 22.4 b | 49.06 a | 25.85 ab | 53.02 a | Bacteria |
| 0.190 b | 44.92 a | 45.08 a | 5.60 a | 11.5 a | 24.48 a | 48.52 a | 26.85 a | 55.5 a | Bacteria× Fungus |

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

*Water Soluble Carbohydrate*

Water soluble carbohydrate as digestibility is the most important components of forage quality and this characteristic represents the main source of energy in the diet (Coleman and Moore, 2003). In the table of variance analysis was significant the amount of water soluble carbohydrate under the effect of Arbuscular Mycorrhizal fungus and interaction cropping system as well as Arbuscular Mycorrhizal fungus in level of five percent, on the other hand was significant

interaction cropping system and Rhizobium bacteria in level of one percent (Tab1). In the table of mean comparison was observed that by use of Arbuscular Mycorrhizal fungus increase the amount of Water Soluble Carbohydrate (Tab2). So that maximum amount of carbohydrates by an average of 24.27% related to Arbuscular Mycorrhizal fungus treatment. In many species of on Arbuscular Mycorrhizal fungus on corn were reported that inoculation with different species of Arbuscular Mycorrhizal fungus increased Water Soluble Carbohydrate (Srimathiriya *et al.*, 2014). Also mean comparison interaction of cropping and Rhizobium bacteria show that intercropping corn and green beans reduced the amount of water soluble carbohydrate than pure corn plantation. So that the maximum water soluble carbohydrate with an average of 25.88% was related to mono cropping with Rhizobium bacteria and the lowest with mean 20.67% was related to intercropping with Rhizobium bacteria (Tab2). In the other studies reported that most of Water Soluble Carbohydrate pure corn and bio-phosphorus fertilizer was obtained for increased phosphorus absorption, reducing of disease, Improve soil structure and (Naghizade and Galavi, 2012).

**Table 3.** Compared the effects of three characteristics of corn.

| Leaf to Stem Ratio | Forage dry weight (Ton/Ha) | Forage wet weight (Ton/Ha) | Ash (%) | CP (%) | WSC (%) | NDF (%) | ADF (%) | DMD (%) | Treatment |
|---|---|---|---|---|---|---|---|---|---|
| 0.18 a | 40.83 c | 41.06 c | 5.29 a-c | 12.13 ab | 17.7 c | 48.95 ab | 25.25 b | 56.8 a | Mono cropping |
| 0.23 ab | 38.78 c | 39.13 c | 5.94 ab | 10.77 cd | 23.65 b | 46.35 bc | 19.6 c | 55.8 a | Mono Cropping× Fungus |
| 0.23 a | 40.28 c | 40.7 c | 5.93 a-c | 10.66 cd | 22.55 b | 52.07 a | 24.8 b | 52.75 a | Mono Cropping× Bacteria |
| 0.21 ab | 39.80 c | 39.96 c | 5.22 c | 10.32 d | 29.22 a | 48.2 a-c | 29.6 a | 53.25 a | Mono Cropping × Fungus× Bacteria |
| 0.18 ab | 51.44 ab | 51.66 ab | 5.29 a-c | 11.98 a-c | 22.6 b | 48.25 a-c | 24.5 b | 51.75 a | Intercropping |
| 0.16 ab | 53.58 ab | 53.09 ab | 5.36 a-c | 12.09 a-c | 24.46 ab | 43.8 c | 26.6 ab | 50.75 a | Fungus × Intercropping |
| 0.13 b | 59.42 a | 59.83 a | 5.76 a-c | 11.74 a-d | 22.3 bc | 46.06 c | 26.9 a | 53.3 a | Bacteria× Intercropping |
| 0.18 ab | 50.04 a | 50.2 a | 5.99 a | 12.69 a | 19.75 bc | 48.85 ab | 24.1 b | 57.57 a | Intercropping × Fungus× Bacteria |

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

*Crude protein*

Intercropping systems compared with corn mono cropping increased 30.88%-99.4% of crude protein (Liu *et al.*, 2005). In the analysis of variance table is influenced crude protein to cropping system and was significant in 1% level but use of Rhizobium bacteria and Arbuscular Mycorrhizal fungus there were no statistically significant effect on crude protein (Tab1). The results of mean comparison (Tab2) indicate that the amount of crude protein is more in intercropping (12.12%) that mono cropping (10.97%). Also more absorbed of photo synthetically active radiation, water and nitrogen biological fixation in beans intercropping could be the main reason for the increase in the percentage of crude protein than mono cropping. In other words the quality and quantity in intercropping is more. The researchers say that part of the nitrogen fixation in legume roots remain and other was be released and This will increase the crude protein in corn (Neumamn *et al.*, 2007). Also similar results have also been reported by others (Lithourgidis *et al.*, 2006; Bingol *et al.*, 2007; Contreras-Govea *et al.*, 2009).

*Ash percentage*

Ash content forage that they containing minerals. Minerals are required for vitamins, hormones, enzyme activity and many physiological processes that depend on the growth, health and manufacturing (Greene *et al.*, 1998). Results of variance analysis showed that cropping system, use of bacteria and use of Arbuscular Mycorrhizal fungus as well as the effects of combination did not show significant effect on ash. While the triple interaction such cropping systems, Rhizobium bacteria and Arbuscular Mycorhizal fungus show significant effect on ash In five percent level (Tab1). The results of mean comparison table show that the most ash with

averaging 5.99% is for intercropping with Rhizobium bacteria and Arbuscular Mycorrhizal fungus as well as the lowest percentage of ash with averaging 5.22%is for mono cropping with Rhizobium bacteria and Arbuscular Mycorrhizal fungus (Tab2). Intercropping compared mono cropping improves the quality of forage corn in percentage of ash (Dahmardeh *et al.*, 2010). In interpreting of ash increase in intercropping with Rhizobium bacteria and Arbuscular Mycorrhizal fungus can be noted to auxin in the roots. Then increased adventitious roots of corn and so increased absorption of nutrients from the soil.

**Table 4.** Quality standard table forage in legume-Grass (Lithourgidis *Et al.,* 2007).

| CP (%) | ADF (%) | NDF (%) | Standard |
|---|---|---|---|
| >19 | <30 | <40 | Prime |
| 17-19 | 31-35 | 40-46 | Premium |
| 13-16 | 36-40 | 47-53 | Good |
| 11-13 | 41-42 | 54-60 | Fair |
| 8-10 | 43-45 | 61-65 | Poor |
| <8 | >45 | >65 | Reject |

*Leaf to Stem Ratio*

Leaf to stem ratio can be a good indicator of forage quality and the leaves will produce better quality of forage. The survey found that only the effects of different cropping systems was significant on leaf to stem ratio in corn. This trait was not affected Rhizobium bacteria and Arbuscular Mycorrhizal fungus in test (Tab1). So that the highest and lowest of leaf to stem ratio were obtained from mono cropping and intercropping (Tab2). In a report obtained that the maximum leaf to stem ratio was found in mono cropping.

## Conclusion

The results showed that mono cropping have more DMD, NDF, Wsc, Ash, ADF and leaf to stem ratio than other system. Also intercropping of corn and beans due to rate performance and crude protein higher as well as less ADF and NDF than mono cropping has a higher forage quality and quantity. Also use of Rhizobium bacteria and Arbuscular Mycorrhizal fungus were respectively significant effect on ADF and Wsc and other factor don't effect on Rhizobium bacteria and Arbuscular Mycorrhizal fungus. However, the results showed that use of Rhizobium bacteria and Arbuscular Mycorrhizal fungus separately and combined inoculation in intercropping than mono cropping increase the quality and quantity of forage corn.

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