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Response of Yield and Yield Components of Maize (*Zea mays* L.) to Different Biofertilizers and Chemical Fertilizers

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Abstract: In order to study the effect of different biofertilizers and chemical fertilizers on corn yield and yield components, a field experiment was conducted at the research farm of Islamic Azad University, Karaj Branch, Iran. Treatments included azotobacter]with (a_1) and without (a_0) [mycorrhiza] with (m_1) and without (m_0) [nitrogen] 0 kg/ha (n_0), 100 kg/ha (n_1) and 200 kg/ha (n_2) [and phosphorus] 0 kg/ha (P_0), 50 kg/ha (P_1) and 100 kg/ha (P_2) [arranged as factorial in the form of a randomized complete block design with three replications. Results indicated that mycorrhizal symbiosis increased nutrients uptake by the plant. Azotobacter improved the measured traits significantly as the result of biological nitrogen fixation and exudation of plant growth promoters. The interactions of azotobacter × mycorrhiza and mycorrhiza × 50 kg/ha phosphorus had the most increasing effect on the measured traits. The interaction between azotobacter × mycorrhiza increased dry matter yield by 10.26%, grain yield by 15.78% and forage yield by 4.9% compared with control. Although the individual application of all four factors significantly affected traits; their interactions were more effective. Overall, results of this experiment indicated that applying azotobacter and mycorrhiza reduced the need for phosphorus (50 kg/ha) and nitrogen (100 kg/ha) fertilizers and improved quality and quantity of yield.

Key words: Azotobacter · Mycorrhiza · Nitrogen · Phosphorus · Maize

INTRODUCTION

In order to obtain more agricultural production, either more lands should be cultivated, which is not applicable in most cases, or higher yield must be produced in the currently cultivated lands. For this purpose, large amount of chemical fertilizers and pesticides are being used, but the problem is that they increase the costs of production and damage human and environmental health. Moreover, using chemical pesticides to control pests and diseases threaten beneficial insects and microorganisms which have many vital roles in the environment. These problems make it required to study the alternative and natural ways of increasing yield production. One ways is using inoculants of microorganisms which provide many nutrients to plants; azotobacter and mycorrhiza are the two examples of these microorganisms. Mycorrhizal symbiosis increases absorption of some elements such as phosphorus, nitrogen and micronutrients, improves water uptake, produces phytohormones, reduces damages caused by the environmental stresses, improves quality of soil aggregate, decreases root damages at transplanting and affects some other microorganisms in soil [1-4]. These effects are mainly due to mycorrhiza's mycelia which are connected to the plant roots and increase their absorptive surface. On the other hand, mycorrhiza produces some enzymes which affect soil phosphates [5, 6].

Azotobacter is a beneficial free living (non symbiosis) nitrogen fixing bacteria which is reported to fix 20-60 kg/ha nitrogen in soil annually. Azotobacter was the first and is the most common biofertilizer for some plants such as maize, wheat, sorghum and rice which produces some plant growth promoting metabolites, enzymes and hormones (auxin, cytokinin and gibberelin) in addition to fixing air nitrogen [7]. This bacterium also produces growth inhibitors which in low doses (10 ppm) eliminate some plant pathogens [8]. By producing different amino

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acids, vitamins and hormones, azotobacter improves solubility and availability of phosphorus, iron and zinc to plants [9, 10]. Finally, the objective of this experiment was to evaluate the effects of some biofertilizers, chemical fertilizers and their interactions on corn yield production in order to reduce chemical fertilizers application.

MATERIALS AND METHODS

The experiment was conducted in 2009 at the Research Farm of Islamic Azad University, Karaj Branch, Iran. The field area was 2500 m^2 and under fallow for one year before the experiment. Physical and chemical properties of field's soil are listed in Table 1. The experiment was conducted in factorial arrangement in the form of randomized complete block design with three replications.

Four factors were studied in this experiment: mycorrhiza (*Glomus intradices*), azotobacter (*Azotobacter chroococum*), nitrogen and phosphorus fertilizers. Mycorrhiza was in two levels, with and without, based on 1 kg/ha (with clay career) which was weighted for each treatment and inoculated with seeds right before sowing. Azotobacter was also in two levels, with and without, based on 2 Li/ha which was weighted for each treatment and inoculated with seeds right before sowing. Phosphorus fertilizer was in three levels (0, 50 and 100 kg/ha P, applied as triple-super phosphate) which was weighted for each plot and all applied before planting. Finally, nitrogen was in three levels (0, 100 and 200 kg/ha N, applied as urea) which was weighted for each plot and splited into two parts; one part was applied before planting and the other was applied at 4-6 leaves stage.

The field was prepared using moldboard, two disks and leveler at early spring. According to prepared experiment map, each plot contained five rows (each 5 m long and 65 cm wide) and corn seeds were planted with 20 cm of spacing. An interspace of 75 cm between plots and 2 m between replication were left uncultivated to prevent treatments interference. When the field was prepared, corn was seeded on May 6^{th} and field was irrigated weekly. No chemical pesticide was used because no pest or disease was observed and weeds were controlled mechanically during corn growth period.

Traits were measured at two stages. Some of them such as fresh forage yield were measured at dough stage. To do this, the weight of 10 plants in each plot was used to calculate forage production in hectare. At next stage, which was at physiological maturity, 20 plants were harvested in each plot, their ears were detached by the cob end; ear length and ear diameter were measured using a caliper. Number of rows per ear, kernels per row and kernels per ear were counted and averaged over the 20 harvested plants in each plot. To measure the 1000 kernels weight, five samples each containing 100 kernels were randomly taken from the whole 20 plants in each plot and then weighted. Measured weights of five samples were added together and multiplied by two to reach the weigh of 1000 kernels. Obtained data were subjected to standard humidity equation (14%). To measure the total grain yield, grain weight of 20 plants which were harvested from 2.4 m^2 was used. These data were also subjected to standard humidity equation at 14% and finally changed to one hectare. At last, the whole harvested biomass of 20 harvested plants was divided into different plant parts and oven dried at 70°C for 2 days and after that, weighted. Harvest index (HI) was also calculated using the dry weights of all 20 plants and their grains weights.

RESULTS AND DISCUSSION

Analysis of variances (Table 2) showed the significant effect of azotobacter, mycorrhiza, nitrogen and phosphorus on the number of kernels per row and rows per ear, 1000 kernels weight, grain yield, dry matter, forage yield and harvest index. Mean comparison for the effect of azotobacter on yield and yield components showed that azotobacter significantly affected traits (Table 3) and increased the number of kernels per row (5.97%), 1000 kernels weight (0.57%), grain yield (7.19%), dry matter yield (5.5%), forage yield (5.65%) and harvest index (1.9%) compared with control, but had no effect on number of rows per ear. Azotobacter fixes air nitrogen and produces plant growth promoters which increase plant growth and the number of hair roots. This condition increases absorptive surface of plant roots and plants take up more water and nutrients [11].

Table 1: Physico-chemical properties of the field soil

Depth	Texture	$EC_{dS/m}$	pH	T.N.V%	O.C%	Total N%	P_{ppm}	K_{ppm}
0-30 cm	Loamy Silt	2.42	7.88	12.42	0.46	0.05	6	220

Table 2: Analysis of variances for the measured traits

		Mean Squares (MS)						
SOV	df	Rows per ear	Kernels per row	1000 kernels weight	Forage yield	Grain yield	Biomass yield	Harvest Index
Rep	2	0.004^{ns}	0.0356^{ns}	0.03^{ns}	7127719.6^{ns}	75056.3^{ns}	18590.3^{ns}	1.53^{ns}
A	1	0.001^{ns}	11.89**	53.63*	50506972.1**	10838108.1**	13778632.1*	0.48*
M	1	0.001^{ns}	11.88**	71.86*	12304912.6**	8840146.6**	7520938.8*	41.56*
N	2	0.022^{ns}	137.55**	40.87*	40056296.6**	10371580.4**	88950.5**	2.61*
P	2	0.005^{ns}	148.69**	63.95*	47778.4**	9042872.6**	3926630.4**	22.07**
A×M	1	0.015^{ns}	2.83*	9.57*	68700.2**	119467.2**	15317.6*	19.42*
M×N	2	0.002^{ns}	2.81*	6.42^{ns}	32041.2^{ns}	196294.8^{ns}	57688.4*	1.64^{ns}
M×P	2	0.006^{ns}	57.40*	23.45*	907236.8*	2600531.8^{ns}	1114745.6*	22.97^{ns}
A×N	2	0.002^{ns}	15.63*	0.02*	10090121.4*	1003200.1^{ns}	3806204.1*	1.75^{ns}
A×P	2	0.006^{ns}	1.95^{ns}	1.04^{ns}	150446.9^{ns}	110396.1^{ns}	49043.9^{ns}	8.27^{ns}
N×P	2	0.004^{ns}	3.82^{ns}	3.34^{ns}	480614.0^{ns}	86817.7^{ns}	374223.2^{ns}	5.61^{ns}
A×M×N	2	0.002^{ns}	2.24^{ns}	3.54^{ns}	710971.0^{ns}	30332.0^{ns}	79894.3^{ns}	1.67^{ns}
A×M×P	2	0.011^{ns}	5.00^{ns}	2.95^{ns}	485517.6^{ns}	167808.5^{ns}	34193.3^{ns}	0.60^{ns}
A×N×P	2	0.025^{ns}	6.62^{ns}	0.81^{ns}	357007.8^{ns}	261589.3^{ns}	37443.6^{ns}	3.99^{ns}
M×N×P	2	0.002^{ns}	1.23^{ns}	0.96^{ns}	406351.4^{ns}	94535.8^{ns}	31859.5^{ns}	2.06^{ns}
A×M×N×P	2	0.002^{ns}	4.38^{ns}	1.82^{ns}	277741.8^{ns}	134361.6^{ns}	15766.2^{ns}	6.15^{ns}

A, azotobacter; M, mycorrhiza; N, nitrogen; P, phosphorus; NS, nonsignificant; **, significant at 0.01; *, significant at 0.05

Table 3: Effect of azotobacter, mycorrhiza, nitrogen and phosphorus and their two-fold interactions on measured traits

Treatments	Rows per ear	Kernels per row	1000 kernels weight (g)	Grain yield (kg/ha)	Dry matter yield (kg/ha)	Forage yield (kg/ha)	Harvest Index (%)
A_0	18.21a	27.10b	173.67b	6504b	12078b	40399b	53.55b
A_1	18.22a	28.72a	174.32a	7008a	12778a	42799a	54.99a
M_0	18.21a	26.61b	173.60b	6434b	12110b	40762b	53.17b
M_1	18.21a	29.23a	174.40a	7007a	12747a	42415a	55.52a
N_0	18.20a	26.23c	173.55b	6322b	11821c	39998b	53.47b
N_1	18.20a	28.41b	174.16a	6902a	12601b	41524a	54.77a
N_2	18.20a	29.11a	174.31a	7045a	12862a	41755a	54.47a
P_0	18.22a	21.38b	173.42b	6305b	11960b	40747b	52.72b
P_1	18.21a	28.56a	174.24a	6899a	12598a	54121a	54.77a
P_2	18.21a	28.80a	174.31a	7064a	12727a	54130a	55.50a
A_1M_1	18.23a	29.86a	174.8a	7266a	13060a	42160a	55.63a
A_1M_0	18.22a	28.61b	174.3a	6889b	12500b	41250b	55.11a
A_0M_1	18.22a	27.69b	174.2a	6750b	12440b	40880b	54.26a
A_0M_0	18.21a	25.61c	173.3b	6119c	11720c	40090c	52.39b
A_1N_0	18.22a	27.49b	173.9b	6614b	12480b	41320bc	53.99ab
A_1N_1	18.23a	29.41a	174.6a	7218a	13050a	41990ab	55.32a
A_1N_2	18.22a	29.29a	174.5a	7193a	13800a	42090a	55.19ab
A_0N_0	18.20a	24.98c	173.1c	6030c	11160c	38660d	53.21b
A_0N_1	18.22a	27.42b	173.8b	6611b	12400b	41060c	53.31b
A_0N_2	18.22a	28.93a	174.1a	6872ab	12670a	41420abc	55.23ab
A_1P_0	18.22a	27.23c	173.7ab	6485b	12290b	41510a	52.76c
A_1P_1	18.23a	29.39a	174.7a	7240a	12970a	41970a	55.82a
A_1P_2	18.22a	29.56a	174.6ab	7299a	13070a	41920a	55.84a
A_0P_0	18.20a	25.56d	173.2b	6126c	11630c	39980b	52.69c
A_0P_1	18.22a	27.73c	173.9ab	6558b	12220b	40460b	53.66bc
A_0P_2	18.21a	28.06b	173.9ab	6829b	12380b	40690b	55.16a
M_1N_0	18.22a	27.39bc	173.9ab	6595b	12270c	40410c	54.74b

Table 3: Continued

Treatments	Rows per ear	Kernels per row	1000 kernels weight (g)	Grain yield (kg/ha)	Dry matter yield (kg/ha)	Forage yield (kg/ha)	Harvest Index (%)
M_1N_1	18.22a	29.93bc	174.7a	7246a	12930ab	41830ab	56.04ab
M_1N_2	18.21a	30.37a	174.7a	7391a	13190a	42010a	56.11a
M_0N_0	18.22a	25.07d	173.1b	6048c	11530d	39570d	52.45c
M_0N_1	18.21a	26.89c	173.7ab	6558b	12120c	41220b	54.08b
M_0N_2	18.23a	27.85b	173.9ab	6698b	12530bc	41500ab	54.88ab
M_1P_0	18.22a	28.36b	174.1a	6838a	12450b	41200a	54.95b
M_1P_1	18.21a	29.81a	174.8a	7265a	12900a	41580a	56.36a
M_1P_2	18.23a	29.54a	174.4a	7129a	12800a	41460a	55.69a
M_0P_0	18.21a	24.42d	172.8b	5772c	11420c	40220b	52.71c
M_0P_1	18.21a	27.32c	173.9a	6533b	12290b	40850a	53.97b
M_0P_2	18.22a	28.07b	174.1a	6599a	12710a	41250a	55.06a

A, azotobacter] with (a_1) and without (a_0) [M, mycorrhiza] with (m_1) and without (m_0) [N, nitrogen] 0 kg/ha (n_0), 100 kg/ha (n_1) and 200 kg/ha (n)[P, phosphorus] 0 kg/ha (P_0), 50 kg/ha (P_1) and 100 kg/ha (P_2). [Means in a column followed by the same letter are not significantly different at $P \leq 0.01$

Azotobacter enhances plant growth and yield through above mentioned mechanisms. Mean comparison of the effect of mycorrhiza also showed the significant effect on all measured traits, except for the number of rows per ear (Table 2). In mycorrhizal symbiosis, the fungus mycelia connect to plant roots; helping plants to absorb more phosphorus and other nutrients. In fact, mycorrhiza has improved the number of kernels per row, 1000 kernels weigh, grain yield, forage yield and harvest index through the improvement of plant nutrition, nitrogen fixation, plants pollination, photosynthesis and leading carbohydrates toward reproductive organs. Similar results were obtained by Colomb *et al.* [12].

Different application rates of nitrogen fertilizer application significantly affected the number of kernels per row, 1000 kernels weight, grain and forage yield, dry mater yield and harvest index, but hat no effect on the number of rows per ear (Table 2). Mean comparison (Table 3) showed that higher N application rate increased the number of kernels per row (9.9%), 1000 kernels weight (0.46%), grain yield (10.27%), dry matter yield (8.1%), forage yield (4.2%) and harvest index (2.23%). No significant difference was observed between 100 and 200 kg/ha N. Nitrogen is a key element in corn yield production. Plants which absorb sufficient amount of nitrogen produce more photosynthetic produced; resulting in higher yield. In this experiment, the absorption of sufficient nitrogen at the grain filling stage increased grains weight. Researchers found that nitrogen increased leaf area index (LAI) which ended in higher growth rate and yield production [13].

Phosphorus application rates significantly affected on number of rows per ear, 1000 kernels weight, grain yield, dry matter yield, forage yield and harvest index (Table 2), but the effect was not significant on number of rows per ear. Mean comparison (Table 3) showed that higher P application rates enhanced the number of kernels per row (8.4%), 1000 kernels weight (0.52%), grain yield (10.75%), dry matter yield (6%), fresh forage yield (2.35%) and harvest index (4%). The effect of 50 and 100 kg/ha P application rates was not significantly different. Phosphorus is an important element for corn growth and affects plant metabolism and yield production [14]. Taking up sufficient P increases plants leaf area and photosynthesis and finally, growth and yield. Abdali [14] represented that higher P content in soil would enhance plant yield production.

The interaction between azotobacter × mycorrhiza significantly affected all measured traits except for the number of rows per ear (Table 2) and mean comparison (Table 3) showed higher effect of azotobacter and mycorrhiza co-application, compared with their individual application. The interaction between azotobacter × mycorrhiza enhanced the number of kernels per row (14.2%), 1000 kernels weight (0.86%), grain yield (10.26%), fresh forage yield (4.9%) and harvest index (5.7%) compared with content. It seems that the two microorganisms' symbiosis increases their activity. Azotobacter has symbiotic relations with other microorganisms such as azospirillum and researches have indicated that this symbiosis increases dry matter in maize and sorghum [15]. Azotobacter fixes air nitrogen which helps plant shoot and root to grow more and produce more hair roots. This condition is more favorable for mycorrhiza mycelia as they can connect to young roots better than older ones. More connection between plant roots and mycorrhiza mycelia would improve nutrients obsorption, plant growth and yield. Moreover, azotobacter has antagonistic relations with some harmful microbes in soil and controls growth of alternaria, fusarium and helminthosporium by the exudation of antibiotics [16]. Beneficial metabolites synthesis, plant growth promoters exudation and air nitrogen fixation are other azotobacter functions [17]. Mycorrhizal symbiosis also improves plants growth through different mechanisms such as better uptake of water and nutrients, controlling stomata, root hydrolic conductivity, photosynthesis and increasing plants resistance to pathogens [18, 19].

Analysis of variances showed the significant effect of interaction between azotobacter × nitrogen on most measured traits except for the number of rows per ear and harvest index (Table 2). According to mean comparison (Table 3), there was no significant difference between azotobacter × 100 kg/ha N and azotobacter × 200 kg/ha N, so application of 100 kg/ha N along with azotobacter is advisable because lower chemical fertilizer is applied. Azotobacter × 100 kg/ha N increased the number of kernels per row (14.7%), 1000 kernels weight (0.9%), grain yield (16.3%), dry matter yield (14.5%) and fresh forage yield (8.1%) compared with control (Table 3). Azotobacter has probably increased plant shoot growth, photosynthesis and yield by the production of plant growth promoters, siderophores and enhancement of root development and root absorptive surface [11, 20].

The interaction between mycorrhiza × phosphorus significantly affected the number of kernels per row, 1000 kernels weight, grain yield, dry matter yield, forage yield and harvest index (Table 2). Mean comparison (Table 3) indicated no significant difference between 100 kg/ha P and 100 kg/ha P × mycorrhiza; representing that when soil contains more available phosphate than plant needs, mycorrhizal symbiosis does not have any benefits to plants and the fungus acts like a parasite [14, 21]. Mycorrhiza × 50 kg/ha P enhanced the number of kernels per row (18%), 1000 kernels weight (1.5%), forage yield (20%), dry matter yield (11.5%) and harvest index (4.6%) compared with control and was the most effective treatment.

All other interactions had no significant effect on measured traits.

CONCLUSION

The main objective of biofertilizers application is to reduce the need for chemical fertilizers. Results of this experiment represents that applying lower rates of phosphorus and nitrogen (50 and 100 kg/ha, respectively) along with azotobacter and mycorrhiza is advisable. The effect of the two biofertilizers' co-application was considerable on dry matter and grain yield because of synergistic relations between the two microorganisms.

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