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Effect of Different Levels of Nitrogenous Fertilizer in Combination with Phosphate Bio-fertilizer on Growth Characteristics and Yield in Maize Cultivar of Single Cross 704

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ABSTRACT

In order to study the effect of different levels of nitrogenous fertilizer in combination with phosphate bio-fertilizer on grain yield of maize single cross 704 test, an experiment in split-plot in randomized complete block design with three replications was conducted in Islamic Azad University Khorasgan Isfahan Branch in 2011-2012. Treatments consisted of four levels of nitrogenous fertilizer including 50, 100, 150 and 250 kilogram per hectare as the main plot and three levels of fertilized phosphate fertilizer type 2 including 0, 100 and 200 grams per hectare as sub-plots. Maize cultivar of single cross 704 was used in this experiment. Results obtained from analysis of variance of data showed that different levels of nitrogenous fertilizer in combination with phosphate bio-fertilizer had a significant effect on grain yield. However, this had not significant effect on seed protein percentage, days to tasseling, days to flowering, days to physiologic maturity and yield of number of ears per plant. The results obtained from comparison of means considering different levels of nitrogenous fertilizer showed that the highest grain yield was obtained when using 150 kilogram per hectare and 200 kilogram per hectare nitrogenous fertilizer. Moreover, the results obtained from comparison of means considering different levels of phosphate bio-fertilizer showed that 200 gram per hectare phosphate bio-fertilizer had the highest effect on grain yield (6571.30). Comparison of the interactions between nitrogenous fertilizer and bio-fertilizer showed that the highest grain yield was obtained when 200 kg per hectare nitrogenous fertilizer in combination with 200 kilogram per hectare bio-fertilizer was used. In contrast, the lowest grain yield (4460.60 gram per meter) was obtained when 100 kilogram per hectare nitrogenous fertilizer in combination with 0 kilogram per hectare bio-fertilizer was used.

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INTRODUCTION

Sustainable agriculture as a farming system includes such approaches that decrease dependency of farmers to several agricultural inputs. This decreases environmental degradation and may lead to intergenerational balance. Environmental problems resulting from application of chemical fertilizers, energy as well as costs of production and consumption of such resources is the most important issues in the world nowadays (Ghalavand *et al*, 2006). Management of soil fertility through application of bio-fertilizers is one of the vital components of sustainable agriculture systems (Orhan *et al*, 2006). Although bio-fertilizers were less used over the past few decades for various reasons, they are widely used in sustainable development of agriculture as an essential element nowadays. This is due to various problems caused by excessive application of chemical fertilizers (Alexandr Atus, 2003).

Bio-fertilizers are either solid, semi-solid or liquid materials containing live microorganisms or their products. They actively and biologically supply nitrogen, phosphorus, sulfur and other nutrients especially micronutrients in soil. When consumed, the microorganisms are colonized around root of the plant or within the plant. Then, they increase supply of nutrients. As a result, they increase growth of the host plants (Amoo Aghayi and Mostajeran, 2007). Currently, bio-fertilizers are discussed as an alternative for chemical fertilizers. They increase soil fertility in sustainable agriculture. Cereals are important sources of human food. They need chemical fertilizers more than other plants (Zaheer *et al*, 1999).

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Organic phosphorus fertilizer with the brand name of fertilized bio-fertilizer type 2 contains two types of phosphate solubilizing bacteria such as strains of *Pseudomonas putida* and *Bacillus lentus*. These respectively use two mechanisms to produce organic acids and acid phosphatase to make phosphorus compounds soluble. As a result, the soluble phosphorus can be absorbed by the plant (Melbooyi, 2007). These bacteria can tolerate a pH between 5 and 11 and salinity up to 3.5%. Such circumstances make it possible to apply this fertilizer in a wide range of different areas (Malbooyi, 2007). Regarding importance of phosphate bio-fertilizer type-2, it can be stated that using 100g of this fertilizer is as efficient as using 100 kilograms phosphorus chemical fertilizer. This fertilizer also increases LAD (leaf area duration), which may lead to increased use of solar energy. As a result, higher rate of photosynthesis might be obtained (Melbooyi, 2007). Nitrogen is the most important element in soil fertility. Chemical fertilizers are mostly used to supply this nutrient for soil. Agricultural soils lose a significant amount of nitrogen through leaching annually. Then, the amount of nitrogen available to plants sharply reduces (Bashen Velvani, 1990).

Nitrogen is a critical and vital element for the plant. It is also found in proteins, nucleic acids and chlorophyll. It is more susceptible to be lost compared to other nutrients. The amount recovered is less than half the amount used (Boswell *et al.*, 1985).

MATERIALS AND METHODS

This research was conducted in Islamic Azad University Khorasgan Isfahan Branch located in 32° and 40 minutes north latitude and 51 degrees 48 minutes eastern longitude with height of 1555 m. This region has a dry climate based on Koppen classification. Long-term average annual rainfall and temperatures with dry summers and very hot temperature are respectively as 120 mm and 16 °C in this region. The experiment was conducted as a split plot design in three replications. The experimental treatments consisted of four levels of nitrogenous fertilizer (50,100,150,200 kg per hectare) as the main factor and three levels of bio-fertilizer fertilized phosphate type 2 (0,100,200 g ha) as the subplot. The main plot had 12 meters length and 3 meters width. The main plot was specified by labor manually with 4 meters length and 3 meters width. Between each replication, a stream was set for farm irrigation. After preparing the field, it was fertilized with two-thirds of urea from the determined amount. The amount of fertilizer for each treatment is given in Table 1. The seeds were treated with fertilized bio-fertilizer type-2 based on treatments determined for the experiment. Then, they were planted in the field. The amount of fertilized bio-fertilizer type-2 is given in table (2).

The planting operation included as follows: the seeds were planted in a row with 75 cm distance. The space between the rows was considered as 15 cm apart in a row. The planting depth was considered as 5 cm. In this experiment, traits such as seed protein percentage, grain yield, number of ears per plant, days to tasseling, days to flowering, days to physiologic maturity were observed.

Measuring the traits:

Seed protein percentage:

The extraction method was performed using wet digestion. Then, prepared extract was poured down Kjeldahl nitrogen measurement balloon. The total nitrogen in the sample was extracted with dovarda alvin index and magnesium oxide (by boric acid index). Then, measured values were converted to protein percentage after calculation based on protein coefficients (IAOAC, 1983).

Grain Yield:

At the time of complete maturity, the ears were isolated from plants (14 plants from each plot based on the number of density of plants per 2 square meter). After the ears were dried, the seeds were removed from the cob. Then, whole grains of each plot were weighed separately to determine grain yield.

Number of ear per plant:

The number of ear was counted and recorded based on density of 14 plant per 2 square meter per plot.

Days to tasseling:

The number of days from planting date to 50% development of tassels was counted and recorded in the field in the farm.

Days to flowering:

Number of days between planting and 50% development of male inflorescences was counted and recorded in the field.

Days to physiologic maturity:

Number of days between planting and physiologic maturity of Mays

The results obtained from each measured trait were analyzed using MS-TATC software. The results obtained from comparison of both simple and interactive effects were analyzed with Duncan test. The charts relevant to comparison of means were designed in Excel software.

RESULTS AND DISCUSSION

Seed protein percentage:

Based on the analysis of variance, the effect of none of the treatments was significant on seed protein percentage (Table 1). Seed protein percentage was slightly the same when different levels of nitrogenous fertilizer were used. It showed no significant difference and differed slightly within a range from 4.963 to 5.6 within a group. Moreover, the results obtained from comparison of means of phosphorus bio-fertilizer showed that the highest seed protein percentage (equal to 5.48) was obtained in 200 grams per hectare bio-fertilizer treatment while the lowest one (4.9) was obtained in zero kg per hectare bio-fertilizer treatment. This was not statistically significant (Table 2). Comparison of means of interactions of nitrogenous fertilizer on bio-fertilizer also showed that highest seed protein percentage (5.83) was obtained in 150 kg per hectare nitrogenous fertilizer with 200 gram per hectare bio-fertilizer while the lowest one (4.40) was obtained in 100 kg per hectare nitrogenous fertilizer with 100 gram per hectare bio-fertilizer. This was not significantly different. Mehrvarz *et al* (2008) conducted a similar study on barely and showed that application of phosphate solubilizing microorganisms and bacteria combined with appropriate amounts of chemical fertilizer promote plant growth and improves vegetative growth. Thus, grain yield and seed protein percentage significantly increase. Ibochi *et al* (2007) also showed that application of chemical fertilizer with bio-fertilizers and organic fertilizers increase seed protein percentage, dry matter accumulation and leaf area in maize. However, the effect of nitrogenous fertilizer and bio-fertilizer on this trait was not examined in this study. While an increasing trend was shown between these two variables, this was not significant. This was due to selection of inappropriate levels of nitrogen fertilizer and bio-fertilizer. This may be also due to increased amount of absorbable phosphorus in the soil and soil salinity.

Grain Yield:

Analysis of variance of obtained data showed that simple effect of nitrogen fertilizer, bio-fertilizer as well as the interaction between bio-fertilizer and nitrogenous fertilizer on grain yield was significant in 0.01 level of significance. Comparison between the means also showed that the highest grain yield was obtained in 150 and 200 kg per hectare of nitrogenous fertilizer while other two levels showed no significant difference. The latter were categorized in one group. In addition, the comparison between different levels of bio-fertilizer showed that 200 grams per hectare bio-fertilizer had the greatest effect on grain yield, which was equal to 6571.30 grams per square meter. The comparison between means of interactions between bio-fertilizers and nitrogenous fertilizers showed that the highest grain yield was obtained in 200 kg per hectare nitrogenous fertilizer and 200 gram per hectare bio fertilizer while the lowest one (4460.60 gram per square meter) was obtained in 100 kg per hectare nitrogenous fertilizer with zero grams per hectare bio-fertilizer. The effects of nitrogenous fertilizer, bio-fertilizer and the interaction between nitrogenous fertilizer and bio-fertilizer on grain yield were statistically significant in 0.01 level of significance. Comparison of means was also statistically significant. This shows that high levels of nitrogenous fertilizer and bio-fertilizer increase transfer of dry material from stalks of maize. These findings are in line with those obtained by Mashram and Shend (1993), Martinez Toledo *et al* (1998), Tilak *et al* (1992) and Nieto and Frankenberger (2000). Maleki Narg Mussa *et al* (2012) reported in their studies that interaction between chemical, biological, and nitrogenous and phosphorus fertilizers on following traits is significant in both 0.05 and 0.01 level of significance: number of ears per plant, number of rows per ear, number of kernels per row, canned yield, thousand grain weight. They stated quantitative and qualitative yield in plants inoculated with bacteria such as *Azospirillum* increases due to production of stimulative materials by these bacteria.

Days to tasseling:

Based on results obtained from analysis of variance, the effect of none of the experimental treatments was significant on days to tasseling (Table 1-4). Different levels of nitrogenous fertilizer showed that number of days to tasseling using various levels of nitrogenous fertilizers showed no significant difference. Then, all were in a group, which ranged from 5 days to 5.55 days. Based on results obtained from comparison of means of phosphate bio-fertilizer, maximum number of days to tasseling (5.41) was observed in 200 kg per hectare treatment while the minimum number of days to tasseling (5) was observed in 100 kg per hectare treatment without bio-fertilizer. This was not significantly different (Table 2-4). Based on results obtained from comparison of means of interactions between nitrogenous fertilizer and bio-fertilizer, the maximum days to tasseling was obtained in 150 kg per hectare nitrogenous fertilizer with 200 gram per hectare bio-fertilizer per

hectare while the lowest on the minimum number of days to tasseling (5) was obtained in other levels, which was not significantly different.

Days to flowering:

Based on results obtained from analysis of variance, the effect of none of experimental treatments on days to flowering was significant. Based on results obtained from comparison of means, the maximum number of days to flowering was observed in 50 kg per hectare nitrogenous fertilizer and zero kg bio-fertilizer while the minimum number of days to flowering was obtained in 200 kg per hectare nitrogenous fertilizer and 200 gram per hectare bio-fertilizer. This was not significantly different. Based on results obtained from comparison of means, the interactions between nitrogenous fertilizer and bio-fertilizer, maximum number of days to flowering was observed in 50 kg per hectare nitrogenous fertilizer with zero grams per hectare bio fertilizer. This showed no significant difference with other treatments and all were in a group.

Table 1: results of analysis of variance of effect of experimental treatments on phenological traits

Source of change	Mean of squares	Degree of freedom	Days to tasseling	Days to flowering	Days to physiologic maturity	Number of ear per plant	Grain yield	Seed protein percentage	Soil pH	Leaf phosphorus content
Repeat	2		0/69	9/75	1/36	0.21	/139 32226	0/53	/038 0	0/002
Nitrogen (A)	3		0/69	8/22	3/29	0.21	/7** 33148 40	1/48	/011 0	0/0017*
Error (A)	6		0/69	6/53	3/1	0.87	/97 15518 4	1/9	/014 0	0/0004
Phosphate bio-fertilizer (B)	2		0/69	3/08	0/444	0.39	** 14689 261	1/19	0/01	0/0004
A*B	6		0/69	1/97	0/407	0.09	/7** 20900 93	0/23	/013 0	0/0007
Error B	16		0/69	3/17	0/375	0.41	/51 13138 8	0/92	/007 0	0/0004
Coefficient of variation	2		24/3	29/66	5/89	14.67	6/81	18/76	/004 1	6/34

Table 2: comparison of means of effects of experimental treatments on phenological traits

treatment	Seed protein percentage	Number of ear per plant	Days to tasseling	Days to flowering	Grain yield (kg/ha)	Days to physiologic maturity
Nitrogenous fertilizer (kg/ha)						
50 (A1)	4/74	4/49	5/00	7/22	^b 4748/80	56/9
100 (A2)	6/65	4/31	5/00	6/22	^b 4851/10	11/00
150 (A3)	5/6	4/47	5/55	5/55	^a 5836/60	10/56
200 (A4)	5/26	4/16	5/00	5/00	^a 5860/40	10/44
Bio-fertilizer (g/ha)						
0 (B1)	8/43	4/15	5/00	6/33	^c 4460/60	10/5
100 (B2)	8/49	43.4	5/55	6/25	^b 4940/70	16/10
200 (B3)	8/45	4/49	5/41	41 5/	^a 6571/30	10/5

Means who have at least one common letter in each row and for every component have no significant difference based on Duncan test at 0.05 level of significance.

Number of days to physiologic maturity:

No significant difference was observed between various levels of nitrogenous fertilizer, bio-fertilizers and their interactions with number of days to physiologic maturity. Moreover, according to results obtained from comparison of means, the interaction between phosphate bio-fertilizer and nitrogenous fertilizer, maximum number of days to maturity (11.33) was observed in 100 kg per hectare nitrogenous fertilizer and 100 gram per hectare bio-fertilizer while the minimum number of days to maturity (5) was observed in 200 kg per hectare nitrogenous fertilizer with three levels of bio fertilizers. This was not significantly different. The time of emergence of tassels and male inflorescences and physiological maturity are considered as the main phenological stages of growth in maize. Grain yield is significantly influenced by phenology of maize hybrids (Stevens *et al*, 1986). However, none of these traits was influenced by nitrogenous fertilizer and bio-fertilizers in

this study. Although a decreasing trend was observed, it was not significant. This may be due to selection of inappropriate levels of nitrogen fertilizer and bio-fertilizer. It may also be due to increased amount of absorbable phosphorus in the soil and soil salinity.

Conclusion:

Indiscriminate application of chemical fertilizers to obtain high grain yield and compensate food shortage led to an increase in the cost of manufacture and degradation of soil and water resources. Then, specialists attempted to use ecologically healthy and sustainable cropping systems (Tilak *et al*, 1992).

The results obtained from this experiment showed that application of chemical fertilizer with bio fertilizers could have positive effects on yield and yield components of maize. Grain yield of maize was assessed as positive in this experiment. Given that a one-year experiment cannot yield reliable results, no recommendation can be given based on this experiment. However, based on results obtained from this experiment, it seems that application of bacterial compounds as bio-fertilizer can effectively improve and enhance the product. By widely applying this measure in our country, this can be used as an alternative to chemical fertilizers, which not only increase production, but also enhance security of arable soil and reduce environmental pollution. Further studies should be conducted on this issue, so that it can be concluded that application of bio-fertilizer can increase grain yield as chemical fertilizer does. Then, it is worthy to replace chemical fertilizers with bio-fertilizer or use them in combination with each other. This not only increase grain yield, but also ensure environmental health by less using chemical fertilizers.

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