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Effects of Bio and Chemical Nitrogen Fertilizer on Grain and Oil Yield of Sunflower (*Helianthus annuus* L.) under Different Rates of Plant Density

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ABSTRACT

*Considering the environmental problems arising from chemical fertilizers usage, many attentions has been drawn to the application of biological fertilizers in agriculture. In order to study the effects of chemical nitrogen fertilization and biofertilizer inoculation on grain yield, yield components and oil yield of sunflower (*Helianthus annuus *L.) under different rates of plant density, a Factorial experiment based on Randomized Complete Block design was conducted in three replications. Experimental factors were: (i). Four levels of chemical nitrogen fertilizer (0, 100, 150 and 200 kg N ha^{-1}), (ii). Two levels of biofertilizer (with and without inoculation) containing of *Azotobacter *sp. and *Azospirillum *sp. and (iii). Three levels of plant density (8, 10 and 12 plant m^{-2}). The highest plant height, stem and head diameter, number of grains per head, 1000-grains weight, grain yield, oil yield and biological yield were obtained from the highest level of nitrogen fertilizer (200 kg N ha^{-1}) and biofertilizer inoculation. Application of 150 and 200 kg N ha^{-1} showed no significant difference in these traits. Furthermore, the highest rate of plant density (12 plant m^{-2}) showed the maximum values of grain yield, oil yield and biological yield that was statistically in par with 10 plant m^{-2} density. The results pointed out that medium plant density (about 10 plant m^{-2}) with moderate N rate (about 150 kg N ha^{-1}) can be beneficial to improve growth, development and total yield of inoculated sunflower.*

Keywords: Biofertilizer, Chemical nitrogen, Density, Grain yield, Oil yield, Sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops, because it offers advantages in crop rotation systems, such as high adaptation capability, suitability to mechanization and low labor needs. Sunflower is the third most widely grown oilseed in the world after groundnut and soybean. Moreover, its oil has excellent nutritional properties and has a relatively high concentration of linoleic acid [5, 10,19].

Agronomic practices such as plant density are known to affect crop environment, which influence the yield and yield components [2, 4, 8, 12]. Stand density affects plant architecture and alters growth and development pattern. Optimum population levels should be maintained to exploit maximum natural resources, such as nutrient, sunlight, soil moisture and to ensure satisfactory yield. On the other hand, in order to increase grain yield, density must be kept at proper plant population [9, 17, 18]. Barros et al. (2004) found that the grain yield in sunflower increased to the maximum with increasing plant density and remained constant at even higher plant density under favorable environmental conditions. Under less favorable conditions however, the grain yield started to decline at very high plant density.

Nitrogen (N) deficiency is frequently a major limiting factor for crops production in over the world [6, 13, 15]. The most important role of nitrogen in the plant is its presence in the structure of protein and nucleic acids, which are the most important building and information substances of every cell. In addition, nitrogen is also found in chlorophyll that enables the plant to transfer of energy from sunlight by photosynthesis [4, 7, 11, 19]. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Moreover, it influences cell size, leaf area and photosynthetic activity [7, 15, 16]. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crops.

Increasing and extending the role of biofertilizers can reduce the need for chemical fertilizers and decrease adverse environmental effects. They can play a significant role in fixing atmospheric nitrogen and production of plant growth promoting substances. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization has great importance in alleviating environmental pollution and deterioration of nature [1, 10, 14, 20]. Existence of microbial communities like *Azotobacter* and *Azospirillum* in the soil or rhizosphere promotes the growth of the plant through the cycling and availability of nutrients, increasing the health of the roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients [21].

Determination of the growth and yield response of sunflower crop to N fertilization and plant density is very important to maximize yield and economic profitability of sunflower. Moreover, it seems that there is little investigation on combined effects of organic and inorganic nitrogen fertilization in some crops such as sunflower. Therefore, the objective of this study was to evaluate the effects of chemical nitrogen and biofertilizer on grain and oil yield of sunflower under different rates of plant density.

MATERIALS AND METHODS

The study was carried out at the Research Farm of Tabriz University (Latitude 38˚05´ N, Longitude 46˚17´E and Altitude 1360 m above sea level). The climate is characterized by mean annual precipitation of about 250 mm and mean annual temperature of 10˚C with annual maximum and minimum temperature of 16.6˚C and 4.2˚C, respectively. The soil type was clay loam with EC about 0.68 ds m^{-1}, pH about 7.9 and organic matter about 0.6%.

The effects of four levels of chemical nitrogen fertilizer (0, 100, 150 and 200 kg N/ha), two levels of biofertilizer (with and without inoculation) and three levels of plant density (8, 10 and

12 plant/m^2) on grain yield, yield components and oil yield of sunflower (*Helianthus annuus* L.) were studied by a Factorial experiment based on Randomized Complete Block design with three replications. Chemical nitrogen fertilizer (used in urea form) in each level, divided into two equal parts; the first part of the N was broadcasted by hand and incorporated immediately in planting time, second part used in 6-8 leaves (v_6-v_8) stage. Nitroxin used as a biological fertilizer (containing of *Azotobacter* sp. and *Azospirillum* sp.) in the amount of one liter per 30 kg of seed. The field was prepared well before sowing by plowing twice with tractor followed planking to make a fine seed bed. Seeds of sunflower cv. Hysun-33 were hand planted in four rows plots, 7 m long with spacing of 0.5 m between rows. Plots were separated by 1.5 m and blocks by 2.5 m unplanted distances. Two seeds were sown per hill. The field was immediately irrigated after planting to ensure uniform germination. After germination, the plants were thinned to one seedling per hill at 3-4 leaves stage. Weeds were controlled over the growth period with hand hoeing. All other agronomic operations except those under study were kept normal and uniform for all treatments.

The plants were harvested at maturity and traits such as plant height, stem diameter, head diameter, number of grains per head and 1000-grains weight were recorded on 10 randomly selected plants in each plot. Grain and biological yield were obtained by harvesting the middle two rows of each plot. To determine the seed oil, ground seed samples were assayed using an Inframatic 8620 instrument (Perten Instruments, Inc, IL) that functions based on infrared spectrometry. Analysis of variance was carried out using SAS computer software packages. The comparison of means was investigated using Duncan's Multiple Range Test (DMRT) at 0.05% probability.

RESULTS AND DISCUSSION

The effects of N fertilization, biofertilizer inoculation and plant density on grain yield, yield components and oil yield of sunflower were presented in Tables 1. The results obtained from the variance analysis of data indicated that nitrogen fertilization and plant density had significant effects on all of studied traits. Moreover, inoculation with biofertilizer affected significantly studied traits except stem diameter and 1000-grains weight.

Plant height (P H): The plant height was significantly affected by nitrogen application, biofertilizer inoculation and plant density (Table 1). The highest plant height were observed in the maximum rate of nitrogen application (200 kg N/ha) that not differed significantly with 150 kg N/ha. The minimum plant height recorded in control. Application of 200 kg N/ha increased plant height by 14.50%, compared to control (Table 1). Moreover, inoculated plants had more height than non-inoculated plants. Inoculation with biofertilizer increased plant height about 5.70% than non-inoculated plants. These results are in line with the findings of other researchers [4, 10, 16, 17]. Increasing of plant density significantly increased the plant height (Table 1). The highest rate of plant density (12 plant/m^2) increased plant height by 9.91% compared to the lowest level of plant density (8 plant/m^2). Taller plants at higher densities may be due to inter plant competition for light and aerial resources as reported by Barros et al. (2004) that probably related to hormonal changes in plant tissues.

Table1. Effects of chemical nitrogen fertilization, biofertilizer inoculation and plant density on yield and it's components in sunflower (*Helianthus annuus* L.)

Treatments	P H (cm)	S D (mm)	H D (cm)	N G (per head)	1000-G W (g)	G Y (kg/ha)	O Y (kg/ha)	B Y (kg/ha)
Nitrogen rates (kg/ha)								
N_0	164.61 **c**	24.18 **c**	16.07 **c**	619.73 **c**	41.15 **c**	2696.55 **c**	1125.80 **c**	8094.23 **c**
N_{100}	179.04 **b**	27.51 **b**	19.38 **b**	667.26 **b**	48.73 **b**	3164.17 **b**	1296.36 **b**	8837.09 **b**
N_{150}	188.87 **a**	29.63 **a**	21.73 **a**	693.48 **a**	50.86 **ab**	3442.62 **a**	1361.21 **a**	9521.35 **a**
N_{200}	192.53 **a**	30.47 **a**	22.69 **a**	707.32 **a**	53.11 **a**	3537.49 **a**	1374.66 **a**	9753.62 **a**
Biofertilizer								
B_{non}	175.94 **b**	27.51 **a**	18.86 **b**	651.68 **b**	47.92 **a**	3047.83 **b**	1215.60 **b**	8665.94 **b**
B_{with}	186.58 **a**	28.37 **a**	21.07 **a**	692.21 **a**	49.01 **a**	3372.57 **a**	1363.41 **a**	9437.21 **a**
Density (plant/m^2)								
D_8	171.41 **c**	29.89 **a**	21.97 **a**	695.54 **a**	50.32 **a**	2908.15 **b**	1160.42 **b**	8097.41 **b**
D_{10}	182.12 **b**	27.78 **b**	19.72 **b**	673.82 **b**	47.98 **ab**	3319.97 **a**	1334.61 **a**	9392.58 **a**
D_{12}	190.25 **a**	26.16 **c**	18.21 **c**	646.47 **c**	47.09 **b**	3402.49 **a**	1373.48 **a**	9664.73 **a**
Mean	**181.26**	**27.94**	**19.96**	**671.94**	**48.46**	**3210.20**	**1289.50**	**9051.57**
Nitrogen (N)	**	**	**	**	**	**	**	**
Biofertilizer (B)	*	ns	*	**	ns	*	**	**
Density (D)	**	*	**	**	*	**	**	**
N × B	ns	ns	ns	ns	ns	ns	ns	ns
N × D	ns	ns	ns	ns	ns	ns	ns	ns
B × D	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	**7.86**	**6.33**	**8.54**	**7.12**	**9.28**	**8.67**	**10.01**	**9.46**

*- **P H:** Plant Height, **S D:** Stem Diameter, **H D:** Head Diameter, **N G:** Number of Grains, **1000-G W:** 1000 Grains Weight, **G Y:** Grain Yield, **O Y:** Oil Yield, **B Y:** Biological Yield- Mean values followed by same letters in each column and treatment showed no significant difference by DMRT (P = 0.05). - *, ** and ns showed significant differences at 0.05, 0.01 probability levels and no significant, respectively.*

Stem diameter (S D) and Head diameter (H D): Stem and head diameter increased with increasing of nitrogen application rate. Increasing of nitrogen fertilizer from 0 to 200 kg N/ha increased the stem and head diameter by 20.64 and 29.17%, respectively. The lowest and the highest values of these traits recorded in 0 and 200 kg N/ha, respectively (Table 1). Killi (2004) and Soleimanzadeh et al. (2010) reported same results in sunflower. Inoculation with biofertilizer showed no significant effects on stem diameter but, had statistically significant effects on head diameter. The plants that treated with biofertilizer showed about 10.48% more head diameter than non-inoculated plants (Table 1). Moreover, the maximum values of stem and head diameter recorded in the lowest rate of plant density. The highest plant density (12 plant/m^2) decreased stem and head diameter about 12.47 and 17.11% respectively, compared to 8 plant/m^2 density (Table 1). These results demonstrated that increasing plant density resulted in a decrease in stem and head diameter, probably caused by competition for available space, as has been observed previously [2, 5, 11].

Number of grains per head (N G): Variance analysis of data indicated that nitrogen fertilization, biofertilizer inoculation and plant density had statistically significant effects on number of grains per head (Table 1). The highest number of grains per head recorded in 200 kg N/ha application that, showed no significant difference with 150 kg N/ha. The least number of this trait obtained from control. Application of 200 kg N/ha increased the number of grains per head about 12.38% compared to control in each plant (Table 1). Similar trends were reported by

Elfadl et al. (2009) and Namvar and Seyed Sharifi (2011). Biofertilizer inoculation had statistically more number of grains per head than non-inoculated plants. Inoculation with biofertilizer increased this trait by 5.85% compared to plants that no treated with inoculum (Table 1). Vessey (2003) reported that *Azotobacter* and *Azospirillum* increase the available nitrogen in the soil which could enhance the grain number. Furthermore, the highest number of grains per head observed in the minimum rate of plant density (8 plant/m^2). This rate of density showed about 7.06% more number of grains per head than the 12 plant/m^2 density (Table 1). More number of grains per head got an advantage of larger head size [5, 9].

1000-grains weight: 1000-grains weight was affected significantly by nitrogen fertilizer and plant density while, inoculation with biofertilizer showed no significant effects on this trait (Table 1). Increasing of nitrogen application rate increased significantly the weight of 1000-grains in sunflower. The highest 1000-grains weight recorded in the application of 200 kg N/ha while the lowest values of this trait obtained from the no fertilized plants. Application of 200 kg N/ha increased the 1000-grains weight by 22.51% compared to control (Table 1). Moreover, the maximum 1000-grains weight recorded in the lowest level of plant density (8 plant/m^2). The density of 12 plant/m^2 decreased 1000-grains weight about 6.41% compared to the 8 plant/m^2 (Table 1). Increasing 1000-grains weight was attributed to bold grains as a result of more nutrition at low densities. These results are in accordance with the works of Dordas and Sioulas (2008), Massignam et al. (2009) and Dong et al. (2012).

Grain yield (G Y) and oil yield (O Y): Data presented in table 1 showed that all of studied experimental factors (Nitrogen fertilizer, biofertilizer inoculation and plant density) had significant effects on grain and oil yield of sunflower. Grain and oil yield continuously increased with increasing of N application however, these traits of sunflower increased until 150 kg N/ha and further increase in N rate resulted in no significant grain yield increase. The highest rate of nitrogen fertilizer (200 kg N/ha) showed the greatest grain and oil yield, however this rate of nitrogen fertilizer was statistically in par with 150 kg N/ha (Table 1). Application of 200 kg N/ha increased grain and oil yield per unit of area by 23.77 and 18.11% respectively, compared to the least application of nitrogen fertilizer (control). Scheiner et al. (2002) investigated the nitrogen requirements of sunflower and found that nitrogen fertilization increased the seed yield by 17%. Moreover, inoculated plants indicated more grain and oil yield rather than non-inoculated plants. Biofertilizer inoculation increased grain and oil yield about 9.62 and 10.84% than control, respectively (Table 1). Similar results reported about the effects of nitrogen fertilizer [7, 12, 15, 19] and biofertilizer [1, 10, 14, 20] on grain and oil yield of different crop plants. The increase in the yield components, grain and oil yield in the inoculated plants could be attributable to the exudation of plant growth regulators (PGRs), such as auxins and gibberellin and cytokinin by *Azotobacter* and *Azospirillum* [14, 21]. Jalilian et al. (2012) showed that the grain yield of sunflower improved when sunflower plants were grown with a combination of chemical N and biofertilizer. Furthermore, the highest level of plant density (12 plant/m^2) showed the maximum grain and oil yield that not differed significantly with the density of 10 plant/m^2. The maximum rate of density increased grain and oil yield by 14.53 and 15.51% respectively, compared to 8 plant/m^2 density (Table 1). Previous studies were justified these results in various crops [2, 3, 6, 12, 18].

Biological yield (B Y): Biological yield of sunflower also showed the same trend as grain and oil yield. As shown in Table 1, the highest biomass obtained from the application of 200 kg N/ha, however there was no significant difference among the application of 150 and 200 kg N/ha in biological yield. Usage of 200 kg N/ha increased the biological yield by 17.02%, compared to control. Massignam et al. (2009) noted that decrease in biomass production with decreasing N supply was associated with decreases in both radiation interception and radiation use efficiency (RUE). Nitrogen is known to be an essential nutrient for plant growth and development that involved in vital plant function such as photosynthesis, DNA synthesis, protein formation and respiration [4, 8, 9, 16, 19]. The growth parameters such as LAI, biomass, and leaf photosynthesis significantly decreased due to unsatisfactory N availability [15, 16]. Results obtained from this study indicated that usage of N fertilization had positive effects on sunflower yield and its attributes. Adding N increases the production of total dry matter in plants [17, 20] which can increase the potential of plant to produce more plant height, stem and head diameter, grains number and weight that ultimately resulted in high grain and biological yield. Nitrogen fertilization increases the total dry matter for number of reasons: (i). Nitrogen can increase the LAI in plants [4, 7, 16]. More LAI increases the interception of solar radiation by plants that resulted in the more accumulation in plants [16]. (ii). Nitrogen can increase the photosynthesis rate in plants. Increasing photosynthetic rate with N fertilization can be attributed to increasing amount of chlorophyll pigments, since N is one of the main components of chlorophyll [15, 19]. In contrast, supplementation of adequate nitrogen for crops can increase their growth and development. In this condition, plants are able to produce more yield components that result in more grain yield.

Moreover, sunflower plants that treated with biofertilizer had the more biomass than plants that no treated with this inoculum. Inoculation with biofertilizer increased the biological yield about 8.17% compared to control (Table 1). The observed benefits on sunflower by biofertilizer inoculation seem to be due to the supply of N to the crop [1, 14]. Moreover, growth promoting substances (phytohormones) produced by these organisms. Phytohormones as secondary metabolites are known to play a key role in plant growth regulation. They promote seed germination, root elongation and stimulation of leaf expansion. In addition, great root development and proliferation of plants in response to biofertilizer activities i.e. *Azotobacter* and *Azospirillum* enhance water and nutrient uptake [1, 20, 21].

Furthermore, the highest rate of plant density (12 plant/m^2) had the maximum magnitude of biological yield. This rate of plant density was statistically in par with the 10 plant/m^2 density in biomass production. The highest level of plant density increased biological yield about 16.22% than the least level of plant density (Table 1). Dry matter production of crops depends on the amount of intercepting solar radiation and its conversion to chemical energy. When plant density is too high, it encourage inter plants competition for resources. Then crop net photosynthesis process will be affected due to less light penetration in the crop canopy as well as increase in the competition for available nutrient which will affect grain and biological yield. On the other hand, application of optimum plant density helps for the proper utilization of solar radiation [5, 9, 18]. Seyed Sharifi and Raei (2011) stated that leaf area index (LAI) and leaves architecture are two main characteristics that define light interception in the canopy. Plant population modifies the canopy structure and influence light interception, dry matter production and crop yield.

CONCLUSION

In summary, results obtained from this study clearly indicated that: chemical nitrogen application, biofertilizer inoculation and plant density had significant effects on grain yield, yield components and oil yield of sunflower (*Helianthus annuus* L.) except the effects of biofertilizer inoculation on stem diameter and 1000-grains weight. The highest values of plant height, stem and head diameter, number of grains per head, grain yield, oil yield and biological yield were obtained from the highest level of nitrogen fertilizer (200 kg N ha^{-1}) and biofertilizer inoculation. Application of 150 kg N ha^{-1} was statistically in par with 200 kg N ha^{-1} in all of these traits. Furthermore, the highest rate of plant density (12 plant m^{-2}) showed the maximum values of grain yield, oil yield and biological yield that not differed significantly with 10 plant m^{-2} density. The results pointed out that medium plant density (about 10 plant m^{-2}) with moderate N rate (about 150 kg N ha^{-1}) can be beneficial to improve growth, development and total yield of inoculated sunflower.

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