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The effect of application using nitragin and nitroxin biofertilizers on reduce the use of nitrogen chemical fertilizer in sunflower cultivation (*Helianthus annuus* L.)

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Abstract

For the aim of sustainable agriculture and reduce the use of chemical fertilizers, the experiment carried out by using of nitroxin and nitragin biofertilizers and chemical fertilizer of urea on yield and yield components of sunflower. This experiment was conducted in a farm located at 8 km distance of south west of Khoy city, northwest of Iran, in 2016. The experiments carried out using factorial design based on Randomized Complete Block design with three replications and two factors. The factors were as follows: The first factor consumption method of Nitragin biofertilizer in three levels (Control, seed inoculation and consumption along with irrigation) and Second factor nitrogen fertilizer in four levels (Control, Nitroxin biofertilizer with 100 kg chemical fertilizer of urea per hectare, Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare, 200 kg chemical fertilizer of urea per hectare).The results showed that consumption method of Nitragin biofertilizer on steam diameter, head diameter, Number of seed per head, 1000-seed weight and seed yield was significant. The maximum seed yield at rate of 3172.7 kg.ha^{-1} was obtained from experimental treatments of Nitragin consumption along with irrigation. The effect of nitrogen fertilizer on plant height, steam diameter, head diameter, Number of seed per head, 100-seed weight, seed yield and seed oil percentage were significant. The maximum seed yield 3277.6 and 3206.4 kg.ha^{-1} was obtained from experimental treatments 200 kg chemical fertilizer of urea per hectare and Nitroxin biofertilizer together urea at rate of 150 kg.ha^{-1} respectively. According to the results of this experiment, using Nitroxin biofertilizer could reduce 25% urea chemical fertilizer consumption.

Key words: Biofertilizer, Seed oil percentage, Sunflower, yield

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseeds that contribute considerably to edible oil in the world with an intermediate water requirement and adapted to a wide range of climatic conditions (Skoric *et al.*, 2008). Fertilization needs to be used rationally in order to avoid a negative ecological impact and undesirable effects on the sustainability of agricultural production systems. Excessive application of fertilizers also affects the farmer's economy (Zubillaga *et al.*, 2002). Nitrogen is one of the elements that the plant needs in all its periods of growth. Nitrogen fertilizers play an important role in increasing the yield of crops through the development of vegetative parts and the production of carbohydrate by increasing photosynthesis. Although the use of chemical fertilizer improves the quality and quantity of crops, but farmers use it more than the recommended amount in order to maximum yield (Zang *et al.*, 2007). This factor causes environmental problems such as water pollution, soil degradation and degradation of Biological balance in soil environment (Malro *et al.*, 2008). Excessive use of chemical fertilizers in recent years has caused problems in soils (Bashan *et al.*, 2004). The use of bio-fertilizers in agro-ecosystems with the aim of eliminating or significantly reducing the use of chemical inputs is one of the important components of sustainable agriculture (Sharma, 2002). Biological fertilizers are made up of bacteria and also useful fungi, each of which is produced for a specific purpose (such as nitrogen Fixation and release of phosphate, potassium and iron from insoluble compounds). These bacteria are usually located around the root and help the plant absorb the elements (Han and Lee, 2006). But the use of bio-fertilizers alone cannot meet all the nutritional requirements of the

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plant and often ensure the sustainability of production in agricultural systems as a supplement to chemical fertilizers (Shaharuna et al., 2006). Therefore, researchers recommend the use of nitrogen fertilizers along with biofertilizers (zadeh *et al.*, 2013). Akbari *et al.*, (2009) showed that with increased nitrogen consumption, seed and biological yield of sunflower increased. Shaukat *et al.*, (2006) reported that the highest percentage of sunflower oil was obtained in seed inoculation with *Azotobacte*r bacteria and *Pseudomonas*. Fagus and Arsac (1991) stated that the application of growth enhancing bacteria in sunflower compared with control treatment causing the increase seed yield. Experimental results on sunflower showed that all treatments of inoculated seeds (*Azotobacter* and *Azospirillum*) had 1000 seed weight, seed number and head diameter more than non-inoculated treatments (Akbari *et al.,* 2009). Application of biofertilizer including enhancing growth bacteria improved yield and qualitative traits of sunflowers compared to control Treatment (non-inoculation), which increased seed yield and seed oil content (Shehata and Khawas, 2003). Yassari *et al.*, (2008) reported that the combined application of nitrogen chemical and bio fertilizers increased the yield and yield components of rapeseed compared to control treatment (Nitroxin-treated plants and non-use of nitrogen fertilizers). This study was carried out to evaluate the effects of different sources of nitrogen fertilizers on yield and yield components of sunflower in Khoy, Iran.

Material and Methods

This experiment was conducted in a farm located at 8 km distance of south west of Khoy city, northwest of Iran, in 2016 growing seasons. Height of this region is 1157 meters above sea level and longitude and latitude are 44°, 55´ E, and 38°, 37´ N. Soil texture was loam with pH 7.63 and 1.08% organic matter (Table 1).

Table 1: Results of soil analysis of experiment place

(pH)	K (ppm)	P (ppm)	N (ppm)	OC (ppm)	Sand (%)	Clay (%)	Silt (%)	Soil Texture	EC (ds/m)
7.63	215.8	7.8	0.108	1.08	25	26	49	loam	0.82

The experiment carried out using factorial design based on Randomized Complete Block design with three replications and two factors. The factors were as follows: The first factor was consumption method of Nitragin biofertilizer in three levels (Control, seed inoculation and consumption along with irrigation) and Second factor was nitrogen fertilizer in four levels (Control, Nitroxin biofertilizer with 100 kg chemical fertilizer of urea per hectare, Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare, 200 kg chemical fertilizer of urea per hectare). Cultivation operations were conducted on May 15th, 2016. The oily sunflower variety was Iro-Flour. With regard to the soil test results, 100 kg super phosphate fertilizer per hectare and 150 kg of potassium sulphate fertilizer per hectare were spread in the farm and mixed with soil and different amounts chemical fertilizer of urea was added to experimental plot based on experimental treatments in two stages before cultivation and the time of flowering. Nitragin biofertilizer contains different bacteria including *Azotobacter* spp., *Azospirillum* spp. and *Pseudomonas* spp. at a concentration of 10^8 colony forming units (CFU) mL^{-1} and Nitroxin biofertilizer contains two bacteria, including *Azotobacter* spp., *Azospirillum* spp. at a concentration of 10^8 CFU mL^{-1}. The mentioned biofertilizers were manufactured by Mehr Asia Biotechnology Company in Iran.For bacterial inoculation, the seeds were soaked in biofertilizer treatments for 15 minute and then were planted and in Nitragin biofertilizer consumption along with irrigation, the first 5 liters per hectare of Nitragin biofertilizer in 10 liters of water (according to the manufacturer) was dissolved and then was used in experimental plot based on experimental treatments along with the second irrigation. Each experimental plot included 5 cultivation rows with 4 meters in length and 60cm distances in row and distance

between plans in each row was 20 cm. the seeds were planted at 5 cm depth through dry soil with density of 8.3 plants per m^2. Irrigation was carried out at intervals every 10 days and weeds were removed manually during the growing season. In this study, plant height, steam diameter, head diameter, Number of seed per head, 100-seed weight, seed yield and seed oil percentage were measured. Two side rows and half of meter of the beginning and end of the middle row were eliminated for sampling due to marginal effects. To determine plant height, steam diameter, head diameter, Number of seed per head, 8 plants from each experimental plot were randomly chosen and their average was recorded as the mentioned traits. Yield seed was calculated at a level equal to 2 m2after drying and reaching the seeds moisture to 13 - 14 percent. To determine the percent of seed oil, the Soxhlet Extraction Machine was used and then data were analyzed by MSTATC soft ware and means compared with Duncan's Multiple Range Test at 5% probability level.

Results and Discussion

Plant height

The effect of nitrogen fertilizer on plant height was significant at 1% probability level (Table 2). Increasing nitrogen consumption increased sunflower plant height, so that the highest plant height was 153.2 and 151.7 cm, respectively, in 200 kg chemical fertilizer of urea per hectare and Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare and the lowest plant height was 140.6 cm in control or non-fertilized treatment (Table 3). The use of nitrogen chemical fertilizer with Nitroxin biofertilizer by expanding root area and increasing the absorption of water and nutrients from soil stimulates vegetative growth of plant and increases the period of vegetation and increases the amount of Assimilates by increasing the leaf area and photosynthetic surface of the plant. And thus, through the division and enlargement of the cells, increases plant heights (Yusef *et al.,* 2014). According to Burd *et al.,* (2000), growth-enhancing bacteria can increase plant height and production capability by synthesizing phytocchromes and increasing nutrient availability. Zahir *et al.,* (2000) reported a 5.8% increase in maize plant height by inoculation with *Azotobacter*. Kader *et al.,* (2002) evaluated the seed inoculation with *Azotobacter* at different levels of nitrogen fertilizer for the final height of the wheat plant with significant and positive outcome.

Stem diameter

The effect of consumption method of nitragin biofertilizer on stem diameter was significant at 5% probability level (Table 2). The maximum stem diameter was 22.27 mm in biofertilizer treatment with irrigation and the lowest stem diameter was 20.68 mm in control treatment (Table 3). Selosse *et al.,* (2004) showed that biofertilizers containing nitrogen fixation bacteria help the plant absorb more elements, resulting in an increase in plant growth. The stem diameter trait was exposed at the 1% probability level to nitrogen fertilizer application (Table 2). The highest stem diameter was 22.39 and 22.11 mm, respectively, in 200 kg chemical fertilizer of urea per hectare and Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare and the lowest stem diameter was 20.3 mm which belonged to control or non-fertilized treatment (Table 3). As soil nutrients increase, plant utilization of nutrients will increase and plant growth will increase, which will increase the diameter of the stem. Babaei *et al.,* (2009) in their study expressed the increase in stem diameter with increasing nitrogen fertilizer in sunflower plant.

Head diameter

The head diameter at 1% level was influenced by consumption method of Nitragin biofertilizer (Table 2). The use of Nitragin biofertilizer along with irrigation with 18.4 cm and control treatment or non-consumption of Nitragin with 16.9 cm had the highest and lowest diameter of the head, respectively (Table 3).The microorganisms found in biofertilizers that have been established in the root environment have a positive effect on plant growth, including head diameter.These microorganisms, through the synthesis of materials such as auxin, can increase cell growth and proliferation, and consequently increase the head diameter. According to a survey conducted in sunflower, the use of biofertilizers increases the head diameter(Yousef and Youdi, 2014). The effect of different levels of nitrogen on head diameter was significant at 1% probability level

Table 2: Variance analysis effects of consumption method of Nitragin biofertilizer and nitrogen fertilizer on different traits of sunflower

S.O.V	Df	Means of Squares						
		Plant height	Stem diameter	Head diameter	Number of seeds per head	the weight of 1000 seeds	Seed yield	Oil percent
Replication	2	139.36	43.93	11.71	15059.3	0.05	385231.9	2.84
consumption method of nitragin biofertilizer	2	47.58	8.60*	11.17**	21167.6*	9.59*	568528.7**	2.49
Nitrogen fertilizer	3	300.46**	7.73**	20.06**	94335.8**	44.27**	1178623.1**	8.71*
Method of application×Nitrogen fertilizer	6	26.55	2.70	0.34	4024.5	0.43	81379.02	3.25
Error	22	63.95	1.84	0.322	6368.24	2.46	90100.80	2.20
CV (%)		5.41	6.29	3.22	10.60	2.66	10.11	3.77

***, ** = Significant at 5% and 1%, respectively**

Table 3. Comparison of mean effects of consumption method of Nitragin biofertilizer and nitrogen fertilizer on different traits of sunflower (Dissimilar letters in each column indicate significant differences at the 5% level)

Experimental factors		Plant height (cm)	Stem diameter (mm)	Head diameter (cm)	Number of seeds per head	1000 seed Weight (g)	seed yield (kg.ha^{-1})	Seed oil percentage (%)
Consumption method of nitragin biofertilizer	Control(no fertilizer)	145.79	20.68 b	16.70 c	709.3 b	58.06 b	2724.9 b	38.9
	seed inoculation	148.15	21.67 ab	17.57 b	757 ab	58.97 ab	3012.4 a	39.41
	Consumption along with irrigation	149.75	22.37 a	18.63 a	793.1 a	59.84 a	3172.7 a	39.80
Nitrogen fertilizer	Control(no fertilizer)	140.6 b	20.3 b	15.56 d	632.4 c	56.63 b	2482.3 c	38.6 b
	Nitroxin biofertilizer with 100 kg.ha^{-1} chemical fertilizer of urea	146.1 ab	21.5 ab	17.64 c	711.4 b	57.49 b	2912.7 b	40.1 a
	Nitroxin biofertilizer with 150 kg.ha^{-1}chemical fertilizer of urea	151.7 a	22.11 a	18.32 b	803.4 a	60.72 a	3206.4 a	40.3 a
	200 kg.ha^{-1} chemical fertilizer of urea	153.2 a	22.39 a	19.02 a	865.3 a	60.98 a	3277.6 a	38.5 b

(Table 2). As the nitrogen level increased, the head diameter increased. The head diameter has been the highest in the treatment of 200 kg chemical fertilizer of urea per hectare with 19.02 cm and has been the lowest in control or nitrogen non-use treatment with 15.56 cm (Table 3).

Number of seeds per head

The effect of consumption method of Nitragin biofertilizer on number of seeds per head was significant at 5% probability level (Table 2). Nitragin biofertilizer along with irrigation had the greatest effect on increasing the number of seeds per head and the lowest number of seeds per head was observed in the control treatment or non-consumption of Nitragin, which decreased about 12% (Table 3). This indicates the optimal use of Nitragin by irrigation from the point of view of providing bio-nitrogen for the better growth of the sunflower plant. Bio-fertilizers play an important role by enhancing nitrogen uptake and increasing the efficiency of this element in the process of photosynthesis and production of green areas, resulting in increased growth and flowering. Further, bio-fertilizers provide different nutrients as solution to plant through production of solvent secretions and soil pH reduction (Kapoor *et al.,* 2003). The effect of nitrogen fertilizer application on seed number per head was significant at 1% probability level (Table 2). The highest number of seeds per head with 865.3 and 803.4 numbers was observed in 200 kg chemical fertilizer of urea per hectare and Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare and the lowest number of seeds per head was obtained at 632.4 numbers which belonged to control or non-fertilized treatment (Table 3). As the nitrogen chemical fertilizer increased from zero to 200 kilograms per hectare, the number of seeds per head increased. Various studies have shown the increase of yield components as a result of application of nitrogen fertilizers (Farah *et al.,* 2011; Eidizadeh *et al.,* 2011). It is believed that nitrogen increases the photosynthetic efficiency per unit area due to increasing the Leaf area duration, thereby maintaining the flow of nutrients to the flower and fruit increases the number of seeds per head and yield in the plants (Cheema *et al.,* 2001). Ramra *et al.,* (2007) stated that the use of chemical and biological fertilizers increases the physiology and metabolism activities of sesame seeds and causes more accumulation of dry material in the plant and the number of seeds per capsule.

1000 seed weight

Consumption method of Nitragin biofertilizer was significant on 1000 seed weight at 5% probability level (Table 2).The highest 1000 seed weight (59.84 g) belonged to Nitragin biofertilizer along with irrigation and the lowest 1000 seed weight (58.06 g) belonged to control treatment (Table 3). Nitragin can have a positive effect on 1000 seed weight by exacerbating the photosynthesis activity and increasing the nutrients inside the plant. Also, increasing the weight of 1000 seeds after the application of Nitroxin biofertilizer can be attributed to the effect of bacteria on nitrogen fixation and better development of the root system and hence better absorption of nutrients, especially nitrogen (Yousef and Youdi, 2014).

The effect of nitrogen fertilizer application on 1000 seed weight was significant at 1% probability level (Table 2). Considering the comparison of mean, it is observed that with increasing nitrogen fertilizer, the weight of 1000 seeds increases. The highest 1000 seed weight with 60.98 and 60.72 g was observed in 200 kg chemical fertilizer of urea per hectare and Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare and the lowest 1000 seed weight with 56.63 g belonged to control or non-fertilized treatment (Table 3). Babaei *et a.,* (2009) also reported that the increase in nitrogen up to 200 kg/ha increased the 1000 seeds weight of sunflower seeds. Nitrogen element increases the weight of seeds due to its important role in plant metabolism processes by participating in plant metabolism and increasing the amount of dry matter accumulation in plant organs, especially seeds. Improving seeds weight can be attributed to enhancing the photosynthetic efficiency of the plant and ultimately improving plant growth by adding nitrogen to the soil.

Seed yield

The effect of consumption method of Nitragin biofertilizer on seed yield was significant at 1% probability level (Table 2). The use of Nitragin biofertilizer along with irrigation with 3172.7 $kg.ha^{-1}$ and control treatment or non-consumption of Nitragin with 2724.9 $kg.ha^{-1}$ had the highest and

lowest seed yield, respectively and use of Nitragin biofertilizer along with irrigation increased 16.4% seed yield of sunflower (Table 3). In the experiment by Moghimi and Yusefi (2013), Nitroxin bio-fertilizer with irrigation on safflower had more efficiency than use of seed treatment on seed yield. The effect of biofertilizer on growth parameters, yield and yield components of sunflower were studied and it was determined that its application improved qualitative and quantitative traits as compared to control (no fertilizer application) (Shata *et al.,* 2007). The effect of nitrogen fertilizer was significant at 1% probability level on seed yield (Table 2). The comparison of the mean showed that the highest seed yield (3277.62 $kg.ha^{-1}$) was related to 200 kg/ha urea fertilizer application, which was not significantly different from Nitroxin bio-fertilizer treatment plus 150 kg/ha urea fertilizer, and the lowest seed yield was 2482.3 $kg.ha^{-1}$ related to control treatment (Table 3). Roesti *et al.,* (2006) attributed the increase in seed yield by growth-promoting bacteria with nitrogen fertilizer to the positive role of bacteria in regulation and production of hormones that enhance growth and development of plant roots, helping for improvement in yield by providing optimal conditions for further absorption. Other researchers also consider the increase in yield in integrated systems due to the compatibility of the most available nitrogen in soil with soil requirements (Nasrollah Zadeh , 2017). So that at early growth which nutrient requirements is low, their inorganic nitrogen content is lower than that of chemical fertilizers, but in reproductive growth stages due to the continuation of the mineralization process, absorption continues for a longer time.

Seed oil percentage

The results of analysis of variance indicate the significance of effect of nitrogen fertilizer application on sunflower seed oil content (Table 2). The highest percentage of seed oil belonged to Nitroxin biofertilizer with 150 kg chemical fertilizer of urea per hectare with 40.3% and the least amount of this trait with 38.5% related to treatment of 200 kg chemical fertilizer of urea per hectare (Table 3). Increasing nitrogen consumption in this experiment did not only increase the percentage of seed oil but also reduced its percentage. As the results show, there is an inverse relationship between the amount of nitrogen consumed and the percentage of sunflower seed oil, so that the amount of seed oil decreases with increasing nitrogen fertilizer application. In the experiment of Yousef and Youdi, (2014) with increasing chemical fertilizer to a certain extent, the percentage of oil increased but then decreased. With the increase in nitrogen consumption, the formation of nitrogen-containing protein precursor's increases and protein formation increases in providing photosynthetic materials, resulting in a decrease in the amount of materials needed to convert to oil (Taherkhani and Golchin, 2006). Fathi *et al.,* (2002) also reported on rapeseed that the percentage of seed oil decreased in very high levels of nitrogen (250 kg/ha) and increased by the percentage of protein. Kumar *et al.,* (2009) stated that application of biological fertilizers with low percentage of chemical fertilizers on sesame plant could significantly increase seed oil content.

Conclusion

The maximum seed yield was obtained by consuming Nitragin bio-fertilizer along with irrigation. Further, the use of Nitroxin fertilizer with 150 kg/ha of urea fertilizer produced the equivalent yield of 200 kg chemical fertilizer of urea per hectare, which could save 25% of the consumption of urea chemical fertilizer. This can have an important role in preventing the high consumption of nitrogen chemical fertilizers.

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