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Response of Yield and Yield Components of Safflower (*Carthamus tinctorius* L.) To Seed Inoculation with *Azotobacter* and *azospirillum* and Different Nitrogen Levels under Dry Land Conditions

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Abstract: In order to study the effect of bio-fertilizer on yield and yield components of safflower under dry land conditions, a factorial experiment was conducted based on randomized completed block design with three replications in Ilam, Iran in 2008-2009. The factors consisted of three levels of nitrogen fertilizer (0, 30 and conventional consumption (60) kg/ha) and bio-fertilizer (non-inoculation, *Azotobacter* and *Azospirillum*). The results showed that nitrogen rates had significant effects on yield and yield components. Significant increase was observed in all characters with applying bio-fertilizers and increasing nitrogen from zero to 30 kg/ha, but not 30 to 60 kg/ha and no significant differences between 30 and 60 kg/ha was observed in most studied traits. Applying *Azotobacter* and *Azospirillum* increased seed yeield and yield components by 35 and 21%, respectively compared with control. There were significant interaction between nitrogen levels and bio-fertilizers regarding yield, yield components and seed oil and protein contents. The highest seed yield was obtained by applying 30 or 60 kg/ha with *Azotobacter*. Also, the highest seed oil and protein contents was obtained by applying 30 or 60 kg/ha nitrogen, *Azotobacter* and *Azospirillum*. There were no significant differences between conventional consumption of nitrogen fertilizer (i.e. 60 kg/ha) and 30 kg/ha. According to results of present study, it can be concluded that farmers can obtain the same safflower yield if they apply half of conventional consumption of nitrogen with bio-fertilizers. In this way, decreasing nitrogen fertilizer can be reduced the environment pollution and developed sustainable agriculture.

Key words: Safflower (*Carthamus tinctorius* L.) · Nitrogen fertilizer · Seed yield and yield components · Inoculation

INTRODUCTION

Intensive use of chemical fertilizers and other chemicals has produced environmental problems and increased production costs. The recent economic crisis and environmental problems has raised interest in environmental friendly sustainable agricultural practices, which can reduce input costs [1]. N2-fixing play an important role for plant nutrition by increasing N uptake by the plants and playing a significant role as plant growth promoting rhizobacteria (PGPR) in the biofertilization of crops. Plant growth-promoting rhizohacteria (PGPR) are able exit a beneficial upon plant growth. Nitrogen fixation and P solubilization [2] production of antibiotic [3] and increased rood dry weight are the principal mechanism for the PGPR. A number of different bacteria promote plant growth, including *Azotobacter sp. Azospirillum sp. Pseudomones sp. Bacillus sp. Acetobacter sp* [4]. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increased plant growth and yield. The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: - the ability to produce phytohormons,. - asymbiotic N2 fixation,. - against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and also- solubilisation of mineral phosphates and other nutrients [5]. In Behl *et al*. [6] indicated that *Azotobacter* and micorhiyza increased seed yield, seed numbers, 1000-seed weight and biological yield of wheat. Zahir *et al*. [7] reported 19.8 % increase in seed yield of maize due to dual inoculating seed with *Azotobacter* and Pseudomonas. Tilak *et al*. [8] reported improving seed yield of maize due

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to duel inoculation of *Azotobacter* and *Azospirillum*. Boddy and Dbereinezer [9] also reported that yield and nitrogen content were increased due to inoculation of wheat with *Azospirillum*. Inoculation with *Azotobacter* sp. *Enterobacter* sp. or *Klebsiella* sp. increased number of root hairs, dry matter concentration, N-uptake or yields [1]. Other reasons are related to producing amino acids, carbohydrates, organic acids and growth simulating materials [10]. With due attention to irregular chemical fertilizers consumption and to safflower importance as new oil crop and lack of supported and comprehensive information about growth reaction of this plant to bio-fertilizer, this study was conducted in order to evaluating bio-fertilizer on yield and yield components of safflower in nitrogen levels under dry land conditions.

MATERIALS AND METHODS

In order to investigating and studying bacterial bio-fertilizer application on agronomic traits of safflower (Sina), a factorial experiment was conducted based on randomized complete block design with three replications in Ilam, Iran during 2008/2009 growing season. Soil physical and chemical properties of experimental site are shown in Table 1. The treatments used consisted of three levels of bio-fertilizer(non-inoculation, *Azotobacter* and *Azospirillum*) and three level of nitrogen fertilizers at a rate of 0, 30 and 60 kg/ha

Each plot consisted of six lines with 4 meter length, 30 cm row spacing and 10 cm plant spacing. Seeds were moistured with 2% sugar water and inoculated with 7 gram inoculation including 10^7 alive and active bacteria before planting. In order to determining agronomic traits (i.e. heads per plant, seeds per head and 1000-seed weight) 10 plants were randomly selected in each plot. In order to determine seed yield, all lines of each plot except marginal ones and 50 cm from beginning and end of the lines were harvested at maturity stage and appropriate seed moisture. Seed oil and protein contents were determined using Nuclear Magnetic Resonance Spectrophotometer (NMR) and Micro Kejeldahl digestion, respectively. Statically analysis was conducted using MSTAT-c software. Mean comparison was also conducted with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Seed Yield: Analyzing seed yield variance showed that there was a significant difference between nitrogen fertilizer levels (Table2). Seed yield was increased with applying nitrogen fertilizer (Table 3). However, there was no significant difference between 30 and 60 kg/ha. Bio-fertilizers had significant effect on seed yield. Application of bio-fertilizers increased seed yield in comparison with the control treatment. *Azotobacter* and *Azospirillum* improved seed yield by 35 and 21%, respectively, in comparison with non- inoculation treatment. These results are agreement with those obtained by Singh *et al.* [10] who believed to maximize production of different wheat cultivars which inoculated with *Azotobacter* under normal conditions. Data also showed that by using bio-fertilizer inoculation methods, seed yield was improved but also consumption of nitrogen chemical fertilizer reduces remarkably. Such increases in seed yield may be due to increasing plant accessibility to nutrients using dual use chemical fertilizers and more their absorption by plant and as a result improving growth and photosynthesis by increasing leaf area per plant. Nanda *et al.* [11] reported that grain maize inoculation with *Azospirillum* and *Azotobacter* significantly increased forage yield of

Table 1: Soil physical and chemical properties of experimental area

Soil texture	Available P (mg kg-1)	Available K(mg kg-1)	Total N(%)	Organic Carbon (%)	E.C(dS/m)	pH
Silty loam	4.8	305	0.09	1.01	0.62	7.1

Table 2: Analysis variance of measured parameters

		MS					
S.O.V	d.f	Seed yield	Heads per plant	Seeds per head	1000-seed weight	Oil content	Protein content
Replication	2	127570.3	56.7	21.7	49.3	22.2	4.4
N	2	1286514.8**	190.03**	386.3**	181.4**	27.2**	8.6**
Az	2	424692.5**	96.03**	245.7**	198.3**	13.1**	3.5**
N*AZ	4	66175.9**	16.2**	16.7*	26.3*	5.2*	0.87*
Error	16	13170	2.3	4.3	8.2	1.5	0.26
CV(%)		11.2	11.6	9.5	10.5	4.7	3.2

*: Significant at 0.05 level, **: Significant at 0.01 level

Table 3: Mean comparisons of the main effects

Treatment	Seed yield (kg/ha)	Heads per plant	Seeds per head	1000-seed weight	Oil content (%)	Protein content (%)
Nitrogen						
N_1	583.3^{b}	7.7^{b}	14.2^{c}	22.5^{c}	24.7^{b}	14.8^{b}
N_2	1216.6^{a}	15.4^{a}	25.2^{b}	31.4^{a}	27.5^{a}	16.4^{a}
N_3	1257.7^{a}	16^{a}	29.8^{a}	28.1^{b}	27.9^{a}	16.6^{a}
Az						
Az_1	801.1^{c}	9.4^{b}	15.7^{b}	23^{c}	25.3^{b}	15.2^{b}
Az_2	1235.5^{a}	15.7^{a}	25.3^{a}	32.3^{a}	27.5^{a}	16.4^{a}
Az_3	1021.1^{b}	14^{a}	24.2^{a}	26.7^{b}	27.3^{a}	16.2^{a}

Mean which have at least once common letter are nit significant different at the 5%level using (DMRT)

N_1, N_2 and N_3= 0, 30 and 60 kg/ha, respectively

Az_1, Az_2 and Az_3= No- inoculation, *Azotobacter and Azospirillum* , respectively.

Table 4: Mean comparisons of the interaction effects

Treatment	Seed yield (kg/ha)	Heads per plant	Seeds per head	1000-seed weight (g)	Oil content (%)	Protein content (%)
N_1Az_1	516.6^{e}	7^{d}	11.3^{d}	21.6^{c}	21.6^{b}	13.8_{b}
N_1Az_2	623.3^{e}	8.3^{cd}	16^{cd}	26.3^{b}	26.3^{a}	15.4_{b}
N_1Az_3	610^{e}	8^{cd}	15.3^{cd}	26.3^{b}	26.3^{a}	15.3_{b}
N_2Az_1	896.6^{d}	10^{bc}	18^{c}	26.6^{b}	26.6^{a}	15.3_{b}
N_2Az_2	1516.6^{a}	18.3^{a}	29.3^{b}	28^{a}	28^{a}	17.1_{a}
N_2Az_3	1186.6^{bc}	16.3^{b}	25.6^{b}	28^{a}	28^{a}	16.8_{a}
N_3Az_1	990^{cd}	11.3^{b}	18^{c}	27.8^{a}	27.8^{a}	16.6_{a}
N_3Az_2	1566.6^{a}	19^{a}	30^{a}	27.9^{a}	27.9^{a}	16.7_{a}
N_3Az_3	1266.6^{b}	17.6^{a}	29^{ab}	28.2^{a}	28.2^{a}	16.6_{a}

Mean which have at least once common letter are nit significant different at the 5%level using (DMRT)

N_1, N_2 and N_3= 0, 3O and 60 kg/ha, respectively

Az_1, Az_2 and Az_3= No- inoculation, *Azotobacter and Azospirillum* , respectively

the plant. They also reported that inoculation with PGPR strains significantly promoted growth of seedling safflower. In general, inoculation resulted in early seedling growth and expansion. Similar results was recorded by Dobbelaere *et al.* [12].

Heads per Plant: Heads per plant was significantly influenced by nitrogen fertilizer and bio-fertilizer treatment (Table 2). Data indicated that heads per plant was increased with increasing nitrogen fertilizer. This may be attributed to improving water absorption and plants nourishing due to nitrogen. However, there were no significant differences between 30 kg/ha and 60 kg/ha. The lowest number of heads per plant was observed with non-inoculation treatment ,while bacteria treatments increased heads per plant. Effect of bio-fertilizers on heads per plant and flowering levels were positive in this experiment. In other word, using nitrogen fertilizer at appropriate levels provide better nutrient uptake and plant photosynthesis through improving bio-fertilizers activity which results in better flowering and heading. Also, positive effect of using bio-fertilizer may be attributed to increase water and nutrient uptake due to development and expansion of roots and also to biological nitrogen fixation by bio-fertilizers. The highest and the lowest number of heads per plant was obtained with applying 30 or 60 kg/ha with *Azotobacter* treatments and non-nitrogen and non-inoculation treatment, respectively (Table 4). Lower nitrogen levels accompanied by bacteria and higher nitrogen levels accompanied by coexistent bacteria were set at same group. This indicated that these bacteria are active in rhizospher and can minimize the need of applying nitrogen fertilizer. This is probably resulted from synthesize of bacteria simulating plant growth promoting rhizobacteri and also nitrogen fixation by these bacteria. This current investigation confirms the earlier works. It revealed that under conditions, seed treatment with PGPR improved seed germination, seedling vigor, seedling emergence and seedling stand over the control. corresponding enhancement of seed germination parameters by PGPR has been reported by Raju *et al.* [13], Niranjan *et al.* [14] and Niranjan *et al.* [15].

Seeds per Head: Seed numbers per head was influenced by nitrogen fertilizer and bio-fertilizer treatments and their interaction (Table 2). This trait was increased by increasing nitrogen level. The lowest seeds per head belonged to non -inoculation treatment. Bio-fertilizers application increased seeds per head; both kinds of bio-fertilizers had same effects on this trait. Also seeds per head was increased due to the interaction between nitrogen fertilizer and both bio-fertilizers (*Azotobacter* and *Azospirillum*) and significant and positive effects was recorded. The highest seeds per head was produced by using 30 and 60 kg/ha in combination with *Azotobacter* and *Azospirillum* (Table 4). In general *Azotobacter* followed by nitrogen fertilizer increased seed yield and yield components by positive influence on macro elements absorption such as N, P and K [16], micro elements such as Zn and Fe [17], improving water distributing in plant, developing nitrate reductase activity and finally increased the plant hormones which play an important role in plant growth.

1000-seed Weight: 1000-seed weight showed that an increase by multiplying nitrogen fertilizer amount, but between 30 and 60 kg/ha wasn't observe a significant difference. 1000-seed weight also increased due to inoculating seed with studied bacteria in comparison with non- inoculation. Bio-fertilizers improved photosynthesis maybe by increasing water and nutrients absorption leading to produce more assimilate and improving plant growth and thus, 1000-seed weight increased compared with non-inoculation treatment. The lowest 1000-seed weight was produced at bio-fertilizer non-inoculation and non-utilizing nitrogen fertilizer treatments. Data in Table 4 indicated that the highest 1000-seed weight was recorded by applying 30 and 60 kg/ha nitrogen followed by *Azotobacter* and *Azospirillum*, however no significant differnecs between 30 and 60 kg/ha treatments or by *Azotobacter* were observed. Applying 30 kg/ha N, provided better nourishment condition to activity and reproducing *Azotobacter* and *Azospirillum*, because these bacteria need this element to grow, develop and fix nitrogen. Bio-fertilizer treatments provided more suitable condition to improving bioactivities of soil and increased 1000-seed weight through absorbing nutrients by root compared with control treatment. This result was agreement by Yasari and Patwardhan [18]who indicated that application of *Azotobacter* and *Azospirillum* increased canola 1000-seed weight. Idris [19] confirmed positive effect of *Azotobacter* on 1000-seed weigh.

Oil and Protein Content: The analysis of variance indicated that there are significant difference between nitrogen fertilizer, bio-fertilizer and their interaction effects on oil and protein contents (Table2). The lowest oil and protein contents was recorded with non-utilizing nitrogen fertilizer treatment. Oil and protein contents were increased by using nitrogen fertilizer, however no statistical significant differences was observed between 30 and 60 kg/ha. The highest oil and protein contents due to bio-fertilizers followed by nitrogen fertilizer. Shehata and El-Khawas [20] reported a significant increase in oil content of sunflower with applying bio-fertilizer. Nitrogen fertilizer ×bio-fertilizer interaction indicated that increasing nitrogen and bio-fertilizers increased oil and protein contents (Table 4).

CONCLUSION

In general, using bio-fertilizers and manage integrated nourishment quantitatively and qualitatively is one of the efficient ways to improve plants production and environment would have a better condition if chemical fertilizers consumption reduce. Recent studies indicated that using bio- fertilizers also improving soil physiological structure and also increase organic matters content and nitrogen available to coexistent plant. Of course, before it is recommended to massive production and widely application it is necessary to implement and replicate this experiment in different regions.

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