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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 Condition

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Abstract: In order to study the effect of bio-fertilizer on yield and yield components of safflower under dry land condition, 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 observed in all characters with applying bio-fertilizers and increasing nitrogen from zero to 30 kg/ha but not 30 to 60 kg/ha. The latter two treatments were in same statistical class in most characters. Applying *Azotobacter* and *Azospirillum* leaded to increase 35 and 21% in yield and yield components, respectively compared with control. There were significant interaction between nitrogen levels and bio-fertilizers regarding yield, yield components and seed oil and protein content. The highest amount of yield obtained from applying 30 or 60 kg/ha with *Azotobacter*. Also, the highest amount of seed oil and protein content obtained from 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 help to reduce environment pollution and develop 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 may be important 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 increase 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] experiment integrated use of *Azotobacter* and micorhiyza caused to increasing seed yield, seed number, 1000-seed weight and bio- yield of wheat. Zahir *et al.* [7] reported 19.8 % increase in seed yield of maize due to dual

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inoculating seed with *Azotobacter* and *Pseudomonas*. Tilak *et al.* [8] reported improving seed yield of maize due to duel inoculation of *Azotobacter* and *Azospirillum*. Boddy and Dbereinezer [9] also reported that yield and nitrogen content 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 oily plant and lack of supported and comprehensive information about growth reaction of this plant to bio-fertilizer, this study, thus, 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 completed block design with three replications in Ilam, Iran in 2008-2009 growing season. Soil physical and chemical properties of experimental area are shown in Table 1. The factors consisted of three levels of fertilizer (0, 30 and 60 kg/ha) and bio-fertilizer (non-inoculation, *Azotobacter* and *Azospirillum*).

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 measuring 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 measure 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 content were measured 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 (Table 2). Seed yield 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. In due attention to table results, comparing mean bio-fertilizers application caused to increasing seed yield in compared with control treatment. *Azotobacter* and *Azospirillum* improved seed yield 35 and 21%, respectively, in compared with non- inoculation treatment. These results are agreed with Singh *et al.* [10] who believed to maximize production of different wheat cultivars which inoculated with *Azotobacter* under normal conditions. Results obtained from this study showed that with using bio-fertilizer inoculation methods, not only seed yield can be improved but also consumption of nitrogen chemical fertilizer reduces remarkably. Seed yield improving factors

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

causes a tinted rated treatment include 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 leave area. Nanda *et al*. [11] reported that grain maize inoculation with *Azospirillum* and *Azotobacter* significantly increased forage yield of the plant. In this experiment, it was observed 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 as well (Table 2). Heads per plant increased with increasing nitrogen fertilizer. This can 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 observed in non-inoculation treatment and bacteria treatments caused to increasing 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 can 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 by *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 indicates 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 in other plants [13, 14, 15].

Seed Per Head: Seed number per head influenced by nitrogen fertilizer and bio-fertilizer treatments and their interaction as well (Table 2). This trait increased by increasing Nitrogen level. The lowest seeds per head belonged to non -inoculation treatment. Bio-fertilizers caused to increasing seeds per head; both kinds of bio-fertilizers had same effects on this trait. Also interaction of this traits indicated, seeds per head increase due to multiplying nitrogen fertilizer, both bio-fertilizers (*Azotobacter* and *Azospirillum*) have significant and positive effects on this trait. The highest seeds per head produced in 30 and 60 kg/ha with *Azotobacter* and *Azospirillum* (Table 4). In general *Azotobacter* followed by nitrogen fertilizer can increase seed yield and yields 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 Redoctaz activity and finally producing plant hormones which have 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 compared with non- inoculation. Bio-fertilizers improved photosynthesis maybe by increasing water and nutrients absorption leading to produce more assimilate and improve plant growth and thus, 1000-seed weight increased in compared with non-inoculation treatment. This trait interaction showed that lowest 1000-seed weight produced at bio-fertilizer non-inoculation and non-utilizing nitrogen fertilizer treatments. As showed in Table 4, highest 1000-seed weight observed at 30 and 60 kg/ha nitrogen fertilizer followed by *Azotobacter* and *Azospirillum*, both 30 and 60 kg/ha treatments followed by *Azotobacter* were statically on a same statistical class. Applying 30 kg/ha chemical fertilizer nitrogen, 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 caused to increasing 1000-seed weight through absorbing nutrients by root in compared with control treatment. This result was agreement by Yasari and Patwardhan [18] indicated that application of *Azotobacter* and *Azospirillum* increased canola weight of 1000-seed. Idris [19] confirmed positive effect of *Azotobacter* on 1000-seed weigh.

Oil and Protein Content: Results obtained from analysis variance indicated that there are significant difference between nitrogen fertilizer, bio-fertilizer and their interaction effects on oil and protein content (Table 2). The lowest oil and protein content was observed in non-utilizing nitrogen fertilizer treatment. Oil and protein content increased with using nitrogen fertilizer, but statistical significant differences weren't observed between 30 and 60 kg/ha. The highest oil and protein content belong 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 increases oil and protein content, as shown in 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.

REFERENCES

1. Salantur, A., A. Ozturk, S. Akten, F. Sahin and F. Donmez, 2005. Effect of inoculation with non-indigenous and indigenous rhizobacteria of Erzurum (Turkey) origin on growth and yield of spring barley. Plant and Soil., 275: 147-156.
2. Zaidi, A. and S. Mohammad, 2006. Co-inoculation effects of phosphate solubilizing micro-organisms and glomus fasciculatum on green grambradyrhizobium symbiosis. Agricultural Science, 30: 223-230.

3. Zahir, A., Z.M. Arshad and W.F. Frankenberger, 2004. Plant growth promoting rhizobacteria: Advances in Agronomy., 81: 97-168.
4. Turan, M., N. Ataoglu and F. Sahin, 2006. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. Sustainable Agricultural., 28: 99-108.
5. Gholami, A., S. Shahsavani and S. Nezarat, 2009. The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. World Academy of Science, Engineering and Technol., 49: 19-24.
6. Behl, R.K., H. Sharma, V. Kumar and K.P. Singh, 2003. Effect of duel inoculation of VA micorrhyza and *Azotobacter* chroococcum on above flag leaf characters in wheat. Aechives of Agronomy and Soil Science., 49(1): 25-31.
7. Zahir, A.Z., M. Arshad and A. Khalid, 1998. Improving maize yield by inoculation with plant growth promoting rhizobacteria. Soil Sci. J. Pak., 15: 7-11.
8. Tilak, K.V.B., C.S. Singh, V.K. Roy and N.S. Rao, 1982. *Azospirillum* brasilense and *Azotobacter* chroococcum inoculum: effect on yield of maize and sorghum. Soil Bio and Biochem., 14: 417-418.
9. Boddey, R.M. and J. Dobereinezr, 1998. Nitrogen fixation associated with grasses and cereals. Plant and Soil., 102: 53-65.
10. Singh, R., R.K. Behl, K.P. Singh, P. Jain and N. Narula, 2004. Performance and gene effects for wheat yield under inoculation of arbuscular mycorrhiza fungi and *Azotobacter chroococcum*. Haryana Agricultural University. Hisar, India. Plant Soil Environ., 50: 409-415.
11. Nanda, S.S., K.C. Swain, S.C. Panda, A.K. Mohanty and M.A. Alim, 1995. Effect of nitrogen and biofertilizers in fodder rainfed upland conditions of Orisa. Current Agricultural Res., 8: 45-47.
12. Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labendera-Gonzalez, J. Caballero-Mellado, F. Aguirre, Y. Kapulnik, S. Brener, S. Burdman, D. Kadouri, S. Sarig and Y. Okon, 2001. Response of Agronomically important crops to inoculation with Azospirillum. Aust. J. Plant. Physiol., 28: 871-879.
13. Raju, N.S., S.R. Niranjana, G.R. Janardhana, H.S. Prakash, H.S. Shetty and S.B. Mathur, 1999. Improvement of seed quality and field emergence of Fusarium moniliforme infected sorghum seeds using biological agents. J. Sci. Food. Agric., 79: 206-212.
14. Niranjan, S.R., N.P. Shetty and H.S. Shetty, 2004. Seed bio-priming with Pseudomonas fluorescens isolates enhances growth of pearl millet plants and induces resistance against downy mildew. J. Pest. Manage., 50: 41-48.
15. Niranjan, S.R., S.A. Deepak, P. Basavaraju, H.S. Shetty, M.S. Reddy and J.W. Kloepper, 2003. Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet. Crop Protection., 22: 579-588.
16. Narula, N., V. Kumar, R. Behl, A. Deubel, A. Gransee and W. Merbach, 2000. Effect of p-solubilizing (*Azotobacter chroococcum)* on N,P,K uptake in p-responsive wheat genotypes grown under greenhouse condition. J. Plant Nutrition and Soil Sci., 163: 393-398.
17. Wani, S.P., S. Chandrapalaiah, M.A. Zambre and K.K. Lee, 1988. Association between nitrogen-fixing bacteria and pearlmillet plants, responses mechanisms and resistance. Plant and Soil, 110: 284-302.
18. Yasari, E. and A.M. Patwardhan, 2007. Effects of Aztobacter and Azospirillium inoculations and chemical fertilizers on growth and productivity of Canola. Asian. J. Plant. Sci., 6: 77-82.
19. Idris, M., 2003. Effect of integrated use of Mineral, organic N and *Azotobacter* on the yield, yield components and N-nutrition of wheat (*Triticum Aestivum*). Pakistan J. Biological Sci., 6: 539-543.
20. Shehata, M.M. and S.A. EL-Khawas, 2003. Effect of two biofertilizers on growth parameters, yield characters, nitrogenous components, nucleic acids content, minerals, oil content, protein profiles and DNA banding pattern of sunflower yield. Pakistan J. Biological Sci., 6: 1257-1268.