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Effects of *Azotobacter* and Nitrogen Chemical Fertilizer on Yield and Yield Components of Wheat (*Triticum aestivum* L.)

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Abstract: The effect of seed inoculation by *Aazotobacter* and different levels of nitrogen fertilizer on growth and yield of sunflower (Azargol cultivar) was studied in experiment farm of Islamic Azad University, Par Abad Mogha Branch during 2010-2011 growing seasons. The experiment treatments were arranged in factorial based on a complete randomized block design with three replications. Four nitrogen fertilizer levels of 25, 50, 75 and 100% N recommended with two levels of *Azotobacter*: with and without *Azotobacter* (control) were assigned in a factorial combination. Results showed that plant height, grains number per ear and biological yield were significantly higher in inoculated plants than in non-inoculated plants. Plant height, ear length, grains number per ear, biological yield and grain yield were increased with increasing N level above 75% N recommended in non-inoculated plants, whereas no significant difference was observed between 50 and 100% N recommended. The positive effect of *Azotobacter* inoculation was decreased with increasing N levels. According to the results of this experiment, application of *Azotobacter* in present of 50% N recommended had an appropriate performance and could increase grain yield to an acceptable level, so it could be considered as a suitable substitute for chemical nitrogen fertilizer in organic agricultural systems.

Key words: *Azotobacter* · Grain yield · Nitrogen fertilizer · Wheat

INTRODUCTION

Every increasing population of the world demands the increase in food production which intern depends upon the improved agricultural practices. More recently, a real challenge faces the workers in the agricultural research field to stop using the high rates of agro-chemicals which negatively affect human health and environment [1, 2]. Some of these problems can be tackled by using bio-fertilizers, which are natural, beneficial and ecologically friendly. The bio-fertilizers provide nutrients to the plants and maintain soil structure. Nitrogen is a major limiting nutrient for crop production. It can be applied through chemicals or biological resources, but chemical nitrogen fertilizers are expensive. Nitrogen is a fundamentally important element in biologically mediated production and nutrient cycling processes. N_2 containing constituents of organic molecules often confer bioactivity to these molecules. Free living prokaryotes with the ability to fix atmospheric di-nitrogen (diazotrophs) are ubiquitous in the soil. But our knowledge of their ecological importance and their diversity remains incomplete [3]. *Azotobacter* is a free living N_2 fixing bacterium. The mechanisms by which Azotobacter promote plant growth are not fully understood [4]. But, several mechanisms have been suggested by which Azotobacter can promote plant growth, including phytohormone production, enhancing stress resistance, N2 fixation, stimulation of nutrient uptake and biocontrol of pathogenic microorganisms [5, 6], increasing the supply or availability of primary nutrients to the host plant [7], the synthesis of antibiotics, enzymes and fungicidal compounds [8-10]. The experimental results obtained by Vermani *et al*. [11] showed that although *Azotobacter* in general are nitrogen fixers, addition of nitrogen in the medium decreases the lag phase and generation time and thus fermentation time. When nitrogen is supplied in the $NaNO_3$ form, up to 0.5 $g.l^{-1}$ concentration, there was an increase in growth, but further increases in concentration did not altered the growth pattern.

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There is little information available on effects of Azotobacter on wheat under field conditions in north of Iran. Hence, the objectives of this study were to determine the effect seed inoculation (priming) with Azotobacter on grain yield of wheat in condition of Pars Abad Moghan in Iran.

MATERIALS AND METHODS

The experiment was initiated in Research Farm of College of Agriculture, Islamic Azad University, Pars Abad Moghan Branch during 2010-2011 growing seasons. Pars Abad Moghan is classified among the temperate climatic regions in the country with average rainfall of 389.5 mm per year. The height of the experimental site from sea level was 50m. The mean annual temperature was 15°C while the mean maximum and minimum temperatures were 31.4 and 1.4 °C, respectively. The soil physical and chemical characteristic of the experimental area is presented in Table 1.

The experimental treatments were arranged as factorial on the basis of a Randomized Complete Block Design with three replications. Treatments were included four levels of nitrogen chemical fertilizer (urea) consisting of N_1 = 25% (50 N kg.ha^{-1}), N_2 = 50% (100 N kg.ha^{-1}), N_3 = 75% (150 N kg.ha^{-1}) and N_4 = 100% N recommended (200 N kg.ha^{-1}) and two levels of *Azotobacter* (without and with inoculation by *Azotobacter chorococum* with population 10^8 number per each ml) on winter wheat *(Ttiticum aestivum* L. cv. pishtaz).

Seed bed preparation was done in early autumn. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third at tillering stage and the remain before stem elongation stage. Inoculated seeds and non-inoculated seeds were sown in experimental plots of 4 × 8 m in dimensions. The cultivation rows were 20 cm apart in each plot (at 300 plants m^2 density). Weeds were removed by hand and plots were irrigated as required through the growing season.

In the harvest stage, the ten middle rows were used for sampling and measured parameters such as: plant height, number of grains per ear, 1000 grain weight, grains yield, biological yield and harvest index were assessed. Grain yield in each plot measured with 14% humidity.

Statistical Analysis: Using SAS [12] data were subjected to analysis of variance. Mean comparison was conducted using the Duncan's Multiple Range Test (DMRT) at 5% level of probability [13].

RESULTS AND DISCUSSION

Results from the present study indicated that plant height, grains number per ear and biological yield have been affected by inoculation with *Azotobacter*. In the other word, *Azotobacter* could proper part of nitrogen for feed plants in the rhizosphere. N fertilizer could affect significantly on traits such as: plant height, ear length, grains number per ear, grain yield and biological yield.

Table 1: Soil physical and chemical properties of experimental area

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture	PH	E.C (dS/m)	Organic Carbon (%)	Total N (%)	Available P (ppm)	Available K (ppm)
0-30	40.0	30.0	30.0	Clay loam	7.8	3.4	0.49	0.05	6.7	220
Optimum				loam	6.5-7.5	2.0<	>1.0	1.0>	10-15	200-300

Table 2: Analysis variance of measured parameters

		MS						
S.O.V	d.f	Plant height	Ear length	Grains number /ear	1000-grain weight	Grain Yield	Biological Yield	Harvest Index
Rep	2	6.994^{ns}	0.757^{ns}	150.136^{ns}	1.901^{ns}	0.302^{ns}	0.116^{ns}	15.161^{ns}
A	1	111.513*	3.511^{ns}	320.622*	0.346^{ns}	0.322^{ns}	4.638*	3.640^{ns}
N	3	293.301**	8.300*	376.784*	6.553^{ns}	0.895**	8.452**	0.871^{ns}
A*N	3	8.718^{ns}	0.102^{ns}	28.241^{ns}	4.116^{ns}	0.043^{ns}	0.519^{ns}	0.283^{ns}
Error	14	22.928	1.985	153.960	3.971	0.114	0.877	11.807
C.V (%)		4.7	11.2	10.8	5.4	12.7	11.6	9.6

*:Significant at 0.05 level, **: Significant at 0.01 level, ^{ns}: Non significant difference.

Table 3: Mean comparisons of the main effects

Treatment	Plant height (cm)	Ear length (cm)	Grains number / ear	1000-grain weight (g)	Grain Yield (t. ha^{-1})	Biological Yield (t. ha^{-1})	Harvest Index (%)
A levels							
A_0	88.8^b	10.2^a	38.5^b	41.2^a	6.143^a	16.785^b	36.5^a
A_1	91.9^a	11.1^a	41.3^a	41.4^a	6.418^a	17.896^a	35.8^a
N levels							
N_1 (25%)	84.4^c	9.2^b	38.3^b	40.1^c	5.695^c	15.447^c	36.8^a
N_2 (50%)	88.6^b	10.5^{ab}	40.1^{ab}	41.2^{ab}	6.162^{bc}	16.870^{bc}	36.5^a
N_3 (75%)	92.7^{ab}	11.6^a	41.3^{ab}	42.1^b	6.517^{ab}	18.239^{ab}	35.7^a
N_4 (100%)	95.8^a	12.2^a	42.9^a	41.9^a	6.749^a	18.806^a	35.8^a

For a given means within each column of each section followed by the same letter are not significantly different ($p<0.05$)

Table 4: Mean comparisons of the interaction effect

Treatment	Plant height (cm)	Ear length (cm)	Grains number /ear	1000-grain weight (g)	Grain Yield (t. ha^{-1})	Biological Yield (t. ha^{-1})	Harvest Index (%)
A_0N_1	81.8^c	8.4^b	37.2^c	39.5^b	5.420^c	14.448^d	37.5^a
A_1N_1	87.1^{bc}	9.6^{ab}	39.3^b	40.8^{ab}	5.971^{bc}	16.447^{bcd}	36.3^a
A_0N_2	86.9^{bc}	10.2^{ab}	39.2^b	41.1^{ab}	6.005^{bc}	16.162^{cd}	37.1^a
A_1N_2	90.3^{ab}	10.8^{ab}	40.9^{ab}	41.2^{ab}	6.318^{ab}	17.577^{abc}	35.9^a
A_0N_3	91.5^{ab}	11.1^{ab}	40.2^{ab}	43.1^a	6.458^{ab}	17.880^{abc}	36.1^a
A_1N_3	93.8^a	12.2^a	42.3^a	41.3^{ab}	6.575^{ab}	18.599^{ab}	35.3^a
A_0N_4	95.1^a	11.8^a	43.1^a	41.2^{ab}	6.687^{ab}	18.649^{ab}	35.8^a
A_1N_4	96.5^a	12.7^a	42.9^a	42.4^{ab}	6.810^a	18.965^a	35.9^a

For a given means within each column of each section followed by the same letter are not significantly different ($p<0.05$)

The results of analysis of variance and the comparison of the means of main effects of treatments on different crop traits are presented in Tables 2 and 3, respectively.

Plant Height: The analysis of variance (Table 2) showed significant effect of nitrogen fertilizer and *Azotobacter* on plant height. It seems that nitrogen plays an important role in enhancement of plant height. The application of chemical nitrogen fertilizer and *Azotobacter* had significant effect to increase the height. In general, the maximum plant height (91.9 cm) was obtained to seed inoculation with *Azotobacter*, while the least value (88.8 cm) was recorded at without inoculation. Similar results have been reported by Kader *et al*. [14]. They reported that inoculation of plants with *Azotobacter* could result in significant changes in various growth parameters, such as plant height. Means of comparisons for nitrogen levels indicated the maximum (95.8 cm) plant height was recorded for N4 and minimum value (84.4 cm) was recorded for N1 treatment (Table 3).

Ear length: Data regarding the effect of Azotobacter and nitrogen fertilizer on the ear length are given in table 2. The response of ear length to Azotobacter was not significant but response of it to nitrogen fertilizer was significant. Maximum (11.1 cm) ear length was recorded with *Azotobacter* inoculation and minimum it was recorded at control treatment (10.2 cm). Means comparisons indicated that maximum ear length (12.2 cm) was observed for 100% N recommended and minimum value (9.2 cm) was observed for 25% N recommended.

Number of Grains per Ear: Number of grains per ear was significantly affected by *Azotobacter* and nitrogen levels, but no in their interaction effect (Table 2). Maximum number of grains per ear was recorded to inoculation with *Azotobacter* (41.3) and minimum it was recorded at control treatment (38.5). *Azotobacter* plants had about 8% more number of grains per ear in comparison with non-*Azotobacter* plants. It means that *Azotobacter* plays an important role in wheat generative growth and therefore to make a significant increase in the number of grains per ear. Kader *et al*. [14] reported that *Azotobacter* increase the available nitrogen in the soil which could enhance the grain number in plant. Our results concur partly with observations made by Golami *et al*. [15] who reported that the grains number increased with seed priming with

Azotobacter in maize. Increase in grains per ear with inoculation might be due to the positive response of wheat at inoculation with *Azotobacter*. These results are also in agreement with De Freitas [16] who concluded that grain number per ear in wheat was highest at inoculation with *Azotobacter*. Means comparison indicated that the maximum (42.9) number of grains per ear was recorded for N4 and minimum value (38.3) was recorded for N1 treatment. Number of grains per ear plays an important role to determining grain yield.

Grain Yield: Grain yield is the main target of crop production. Results from the present study are indicated that grain yield have been affected by the nitrogen fertilizer. The maximum grain yield (6.749 t. ha^{-1}) was observed in 100% N recommended and the minimum grain yield (5.695 t. ha^{-1}) in 25% N recommended. But *Azotobacter* had no significant effect on grain yield. Maybe, the reasons of no success at inoculation with *Azotobacter* to increasing of grain yield, interaction effects and eating between meals with native races of *Azotobacter* in soil, soil PH and no to exist enough time for arrived to highest efficiency of *Azotobacter* activity. But *Azotobacter* plants had higher grain yield (6.418 t. ha^{-1}) in compared of non-*Azotobacter* plants (6.143 t. ha^{-1}). A similar trend in yield differences across seed priming with *Azotobacter* have been reported by Dobbelaere *et al*. [17] and Cakmakı [18]. They have been reported that PGPR can increase yield. Kloepper and Beauchamp [19] have been shown that wheat yield increased up to 30% in seed priming with PGPR. Mirzakhani *et al*. [20] reported that *Azotobacter* increased the available nitrogen in the soil which could enhance the grain yield in safflower.

Biological Yield: *Azotobacter* and nitrogen fertilizer had significantly effects on biological yield, but interaction of theirs had no effect on the biological yield. *Azotobacter* could with activity itself, cause to increasing N nutrient in around root plants and addition absorb by roots. Biological yield increased with seed inoculation with *Azotobacter*, as the maximum (17.896 t. ha^{-1}) and the minimum (16.785 t. ha^{-1}) biological yield obtained from seed inoculation with *Azotobacter* and without priming, respectively (Table 3). Zaidi and Khan [21] have suggested that seed priming with PGPR increased biological yield. The increase in biological yield with *Azotobacter* inoculation indicates the favorable response of wheat to seed priming with *Azotobacter*. Similar observations were also made by Golami er al. [15] in corn. Perveen *et al*. [22]; Wani *et al*. [23] have been reported increase in biological yield due to inoculation with PGPR. At all the levels of nitrogen fertilizer, the *Azotobacter* plants had higher biological yield. In fact, at 25% level of N fertilizer, the *Azotobacter* plants had 13% higher biological yield compared to non-*Azotobacter* plants, while at 50, 75 and 100% N recommended, *Azotobacter* association resulted in higher biological yield of 9, 4 and 2%, respectively. The biological yield in A_0N_4 (12.401 t. ha^{-1}) had no significant effect with A_1N_2 (11.672 t. ha^{-1}). The results of using biological fertilizer treatment (inoculation by *Azotobacter*) with 50% nitrogen recommended was not significantly different from the high rate of chemical nitrogen application (A_0N_4 treatment). According to the results of this experiment, application of *Azotobacter* in present of 50% N recommended had an appropriate performance and could increase biological yield to an acceptable level, so it could be considered as a suitable substitute for chemical nitrogen fertilizer in organic agricultural systems.

CONCLUSIONS

The Bio-fertilizers are considered as the most favorable natural compounds to enhance the microorganism activities in the soil. The highest privilege of application of these fertilizers in Iran is providing with organic matter in desperately needed arid and semi-arid soils. Also providing with the nutrients in accordance to natural abilities of plant uptake potential, enhancing and improving the soil biodiversity, developing the biological activities, increasing the environmental hygiene, conversation and supporting the natural and non-renewable resources are among the most important reasons to increase the utilization of biological fertilizers.

The results from the present study indicated that plant height, grains number per ear and biological yield have been affected significantly by inoculation with *Azotobacter*, because this biofertilizer can enhance absorb of nitrogen by plant. According to calculated, inoculation wheat grains in planting date with *Azotobacter* to cause increase yield about 6 percentage and, also reduce using of about 50 percentage chemical nitrogen.

REFERENCES

1. El-Kholy, M.A., S. El-Ashry and A.M. Gomaa, 2005. Biofertilization of maize crop and its impact on yield and grains nutrient content under low rates of mineral fertilizers. Journal of Applied Sciences Research, 2: 117-121.

2. Kader; M.A., A.A. Mamun, S.M.A. Hossain and M.K. Hasna, 2000. Effects of azotobacter application on the growth and yield of transplant rice and nutrient status of post-harvest soil. Pakistan Journal of Biological Sciences, 3: 1144-1147.
3. Brown M, 1976. Role of *Azotobacter paspali* in association with *Paspalum notatum*. J. Appl. Bacteriol., 40: 341-348.
4. Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255: 571-586.
5. Rodriguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotecnology Advances, 17: 319-339.
6. Sindhu, S.S., S.K. Gupta and K.P. Dadrawal, 1999. Antogonistic effect of *pseudomonas spp.* on phatogenic fungi and inhancement of growth of green gram (*Vigna radiate* L.). Biology and Fertility of Soils, 29: 62-68.
7. Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung and M.H. Wong, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125: 155-166.
8. Ahmad, F., I. Ahmad and M.S. Khan, 2006. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Icrobial. Res., 36: 1-9.
9. Bharathi, R., R. Vivekananthan, S. Harish, A. Ramanathan and R. Samiyappan, 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protec., 23: 835-843.
10. Jeun, Y.C., K.S. Park., C.H. Kim, W.D. Fowler and J.W. Kloepper, 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. Biol. Contorl, 29: 34-42.
11. Vermani, M.V., S.M. Kelkar and M.Y. Kamat, 1997. Studies in Polysaccharide Production and Growth of *Azotobacter vinelandii* MTCC 2459, a Plant Rhizosphere Isolate. Letters in Applied Microbiology, 24: 379-383.
12. SAS Institute Inc, 1988. SAS/STAT User's Guide. Version 6, fourth ed. Statistical Analysis Institute Inc. Cary. NC.
13. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn. Mc Graw Hill. New York. USA., ISBN-13: 978-0070609259.
14. Kader, M.A., M.H. Mian and M.S. Hoque, 2002. Effects of *Azotobacter* inoculant on the yield and nitrogen uptake by wheat. Journal of Biological Science, 4: 259-261.
15. Gholami, A., S. Shahsavani and S. Nezarat, 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Proceedings of World Academy of Science. Engineering and Technology, 37: 2070-3740.
16. De Freitas, J.R., 2000. Yield and N assimilation of winter wheat (*Triticum aestivum* L., var Norstar) inoculated with rhizobacteria. Pedobiologia, 44: 97-104.
17. Dobbelaere, S., J. Vanderleyden, Y. Yaacov and L. Okon, 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical Rev. Plant Sci., 22: 107-149.
18. Cakmakı, R., 2005. Bitki geliflimini teflvik eden rizobakterilerin tarımda kullanımı. Ataturk Univ. Ziraat Fakutesi Dergisi, 36: 97.
19. Kloepper, J.W. and C.J. Beauchamp, 1992. A review of issues related to measuring of plant roots by bacteria. Can. J. Microbiol., 38: 1219-1232.
20. Mirzakhani, M., M.R. Ardakani, A. Aeene Band, F. Rejali and A.H. Shirani Rad, 2009. Response of Spring Safflower to Co-Inoculation with *Azotobacter chroococum* and *Glomus intraradices* Under Different Levels of Nitrogen and Phosphorus. American Journal of Agricultural and Biological Sciences, 3: 255-261.
21. Zaidi, A. and M.S. Khan, 2005. Interactive effect of rhizospheric microorganisms on growth, yield and nutrient uptake of wheat. J. Plant Nutr., 28: 2079-2092.
22. Perveen, S., M.S. Khan and A. Zaidi, 2002. Effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiatus* L.). Ind. J. Agric. Sci., 72: 421-423.
23. Wani, P.A., M.S. Khan and A. Zaidi, 2007. Synergistic effects of the inoculation with nitrogen fixing and phosphate-solubilizing rhizobacteria on the performance of field grown chickpea. J. Plant Nutr. Soil Sci., 170: 283-287.