



Effect of seed inoculation with *Azotobacter* and *Azospirillum* and different nitrogen levels on yield and yield components of canola (*Brassica napus* L.)

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Abstract

In order to investigate the effect of different levels of nitrogen and bio-fertilizers on yield of canola plants in Qazvin, Iran, a split-plot design was conducted on the base of randomized complete block design with four replications. Treatments included 3 levels of nitrogen (0, 75 and 150 kg ha⁻¹) and bio-fertilizers on four levels (no inoculation, *Azotobacter*, *Azospirillum* and a combination of *Azotobacter* and *Azospirillum*). Results showed that nitrogen treatment had a significant effect on yield and yield components. Significant increase was observed in all characters with applying bio-fertilizers. Interaction of effect of nitrogen and bio-fertilizers treatments on seed yield showed when no nitrogen fertilizer was used, the highest seed yield was obtained from the combined use of bio-fertilizers. But with application of 150 N (kg ha⁻¹), the highest seed yield was obtained when no bio-fertilizers were applied. The oil yield was also influenced by experimental treatments so that the highest oil yield was obtained from 75 and 150 kg ha⁻¹ N treatments. The combined use of bio-fertilizers also was statistically superior to other treatments. According to findings of the present study, it can be concluded that farmers can obtain the same canola yield if they apply half the conventional allocation of nitrogen with bio-fertilizers.

Keywords: *Brassica napus* L.; *Azotobacter*; *Azospirillum*; nitrogen

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Introduction

Canola requires a relatively high supply of nitrogen fertilizer as the content of this nutrient in seeds and plant tissues is greater than

in most grain crops. This is why canola is considered a nitrogen demanding crop (El-Habbasha and Abd El-Salam, 2010). Nitrogen fertilizers on the other hand, pose a health hazard and microbial population problem in soil besides the high cost of their application

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(Mahfouz and Sharaf-Eldin, 2007; Hasaneen et al., 2009).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield. The action mechanisms of PGPRs can be divided into direct and indirect ones. Direct mechanisms include N_2 fixation, soil mineral solubilization, production of plant-growth-promoting substances (auxins, cytokinins or gibberellins) and reduction of ethylene levels, among others. Indirect mechanisms include favoring colonization by other beneficial soil microorganisms, such as mycorrhizal fungi, and repressing the growth of plant pathogenic microorganisms (Lugtenberg et al., 2009; Marulanda et al., 2010; Gholami et al., 2009). Seed inoculation and the foliar spray of bio-fertilizers (products containing PGPR) have been used for boosting plant growth and reducing the negative effects of stress conditions (Saleem, et al. 2007).

El-Habbasha and Abd El-Salam (2010) illustrated that increasing nitrogen fertilization significantly decreased the oil content in canola seeds. The excessive use of nitrogen fertilizer has generated several environmental problems. Some of these problems can be tackled by use of bio-fertilizers, which are naturally beneficial and ecologically friendly. Bio-fertilizers provide nutrients to the plants and maintain soil structure. It has been revealed that the effect of nitrogen fixation induced by nitrogen fixers is not only significant for legumes, but also non-legumes. Moreover, some microorganisms like *Azotobacter* have multiple functions for plant growth which may derive both from their nitrogen fixation and stimulating effect on root development. Soil microorganisms, viz. *Azotobacter* and *Azospirillum* as N_2 -fixing bacteria could be a beneficial source to enhance plant growth and produce considerable amounts of biologically active substances that can promote growth of reproductive organs and increase the plants' productivity (Yasari et al., 2009). Yasari et al. (2008) reported that the application of *Azotobacter* and *Azospirillum* helped increase the oil content of canola seeds. *Azotobacter* along with other N_2 -fixing bacteria like rhizobium play an important role in yield-

attributed characters owing to the production of siderophores which regulate the availability of nutrients to the crop (Boiero et al., 2007). The present research was carried out to evaluate the effects of different nitrogen levels and bio-fertilizers on yield and yield components of canola plants.

Materials and Methods

This experiment was carried out in order to investigate the effects of different levels of nitrogen and bio-fertilizers on yield of canola plants in Qazvin province, Iran. The experimental design was split-plot based on completely randomized block design in four replications in Ismael Abad Research Station (36 N, 16 E, and 1285 m above the sea level) during 2009-2010. Mean precipitation per year was 310-320 mm and mean temperature per year was 13.9 °C. The experimental soil had silty-loam texture with pH 7.9 - 8 which was slightly alkaline and EC of $1 - 1.39^3 \times 10 \text{ ds m}^{-1}$. Each replication of this design consisted of 12 treatments and a total of 48 plots were analyzed. The factors examined were four levels of nitrogen (0, 75 and 150 kg ha^{-1}) equal to the application of 0, 150 and 300 kg ha^{-1} of urea, as main plot, respectively. Also bio-fertilizers in 4 levels (no inoculation, *Azotobacter*, *Azospirillum* and combined inoculation of *Azotobacter* + *Azospirillum*) comprised the sub-plot factors.

The area of each main plot was 6 x 12 m and that of sub-plot was 6 x 2.4 m. There was a 6 m distance between two adjacent main plots in order to prevent nitrogen fertilizer from moving to the next plot. There was a 60 cm distance between two adjacent sub-plots as well. There were 12 plots in each block and the treatments were allocated randomly to each plot. Seeds were moisturized with 10% sugar water and inoculated with 30 gram inoculation including 10^7 living and active bacteria before planting. Based on the soil physico-chemical test, the total amount of recommended doses of P and K was considered as 150 kg ha^{-1} and 100 kg ha^{-1} , respectively. Nitrogen fertilizer was added in three splits: 1/3 at cultivation time as basal fertilization, 1/3 at stem initiation stage and the remaining 1/3 was applied at bud initiation before flowering stage in accordance with the

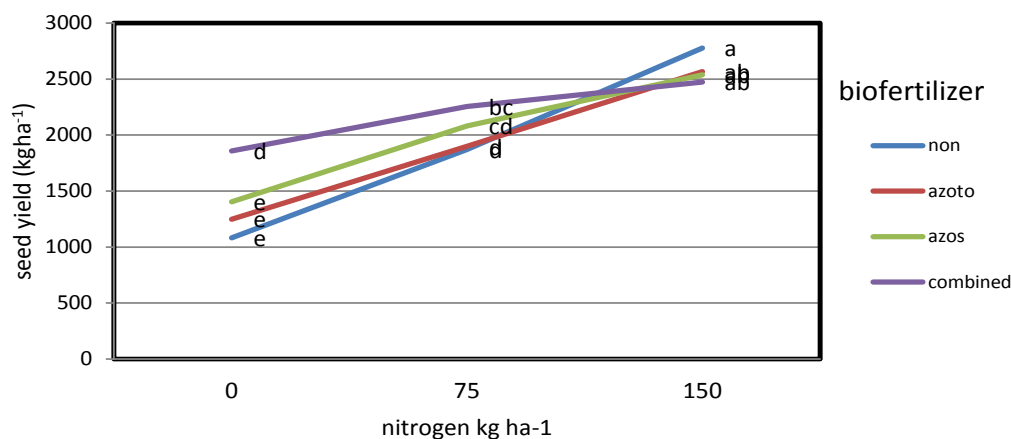


Fig. I. Interaction of nitrogen and biological fertilizer treatment in seed yield

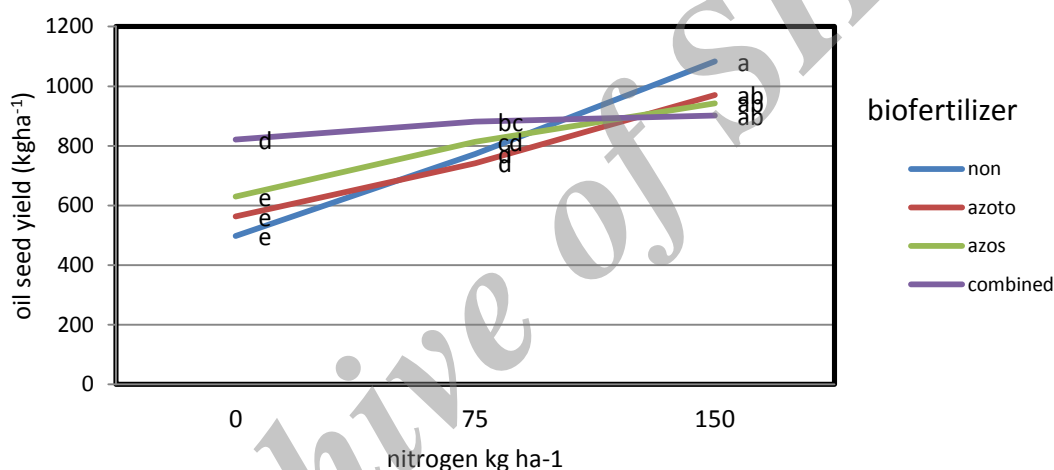


Fig. II. Interaction nitrogen and biological fertilizer treatment in the seed oil yield

treatment variables. Traits under study, included seed number per silique, number of siliques per plant, 1000 seed weight, biological yield, seed yield and oil yield. Statistical analysis was conducted using MSTAT-c software. Mean comparison was also conducted with Duncan's Multiple Range Test.

Results

Seed yield

N fertilizer had a significant effect on canola seed yield. Increasing N fertilizer rate from

0 to 75 kg ha⁻¹ and from 75 to 150 kg ha⁻¹ increased the seed yield.

Results obtained from analysis of variance indicated that there are significant differences between the effects of nitrogen fertilizer, bio-fertilizer and their interaction effects on seed yield (Table 2). The lowest seed yield was observed in the treatment with no application of nitrogen fertilizer and this increased when nitrogen fertilizer was applied. The highest seed yield belonged to bio-fertilizers followed by nitrogen fertilizer. Using bio-fertilizer inoculation methods, not only improved seed yield improved but also reduced consumption of nitrogen chemical fertilizer remarkably.

Table 1

Soil physical and chemical properties of experimental area

| PH | E.C(dS/m) | Organic Carbon (%) | Total N (%) | Available K (mg kg ⁻¹) | Available P (mg kg ⁻¹) | Soil texture |
|-------|-----------|--------------------|-------------|------------------------------------|------------------------------------|--------------|
| 7.9-8 | 1-1.39 | 0.5-0.8 | 0.05-0.08 | 215-308.5 | 7-11.2 | Loam |

Table 2

Analysis of variation for the studied traits

| S.O.V | D.f | Biological yield | Oil seed yield | seed yield | 1000 seed weight | number of silques per plant | Seed number per silque |
|---------------------------|-----|------------------------|----------------|---------------|---------------------|-----------------------------|------------------------|
| replication | 3 | 1048.989 | 42796.352 | 90034.211 | 0.406 | 159.019 | 15.63 |
| nitrogen | 2 | 719171.686** | 479993.535** | 5668170.081** | 4.876** | 4884.021** | 410.083** |
| Error | 6 | 36171.949 | 10682.989 | 75810.555 | 0.148 | 56.722 | 4.042 |
| bio fertilizer | 3 | 13743.255** | 26423.887* | 368.219787* | 0.271 ^{ns} | 11.339 ^{ns} | 3.047** |
| Nitrogen × bio-fertilizer | 6 | 1281.918 ^{ns} | 45043.674** | 723.210488** | 0.009 ^{ns} | 11.396 ^{ns} | 0.333 ^{ns} |
| Error | 27 | 2802.508 | 9063.097 | 50318.278 | 0.007 | 31.506 | 0.645 |
| CV | | 6.18% | 11.88% | 11.19% | 4.43% | 7.39% | 3.86% |

**Significant at 0.01 level; *Significant at 0.05 level; ns: Non significant

1000 seed weight

Nitrogen and bio-fertilizers had significant effect on 1000 seed weight at 1% level. The highest 1000 seed weight was obtained from 150 kg ha⁻¹N treatment. The trait of 1000 seed weight also increased due to inoculating of the seeds with the bacteria under study in comparison with the case of no inoculation. Bio-fertilizers may improve photosynthesis by increasing water and nutrients absorption leading to produce more assimilate and improve plant growth and thus, 1000 seed weight increased in comparison with no inoculation treatment.

Seed number per silques

Seed number per silques was influenced by nitrogen fertilizer and biofertilizer treatments (Table 2). This trait increased by increasing nitrogen level. The lowest seed number per silques belonged to no inoculation treatment. Biofertilizers increased seed number per silques; both kinds of biofertilizers had the same effects on this trait.

Number of silques per plant

Nitrogen and bio-fertilizers had a significant effect on silques per plant at 1% level. The trait of number of silques per plant also increased after inoculation of the seeds with bacteria under study. Application of 150 kg ha⁻¹ nitrogen and inoculation treatments resulted in the highest number of silques per plant (Table 2).

Biologic yield

The analysis of variance showed that the biologic yield was also influenced by the experimental treatments. The highest biologic yield was obtained from 75 and 150 kg ha⁻¹ N treatments. The combined use of bio-fertilizers also was statistically superior to other treatments (Table 2).

Seed and oil yield

The obtained results showed that the interaction of effects of nitrogen and bio-fertilizer was significant for the seed and oil yield. Maximum seed yield (2777 kg ha⁻¹) and maximum oil yield (974.5 kg ha⁻¹) were obtained from 150

Table 3
Means for studied traits in canola under seed inoculated

| Nitrogen (kg ha ⁻¹) | Biological Fertilizer | Biological yield (kg ha ⁻¹) | Oil Seed Yield (kg ha ⁻¹) | Seed Yield (kg ha ⁻¹) | 1000 Seed Weight (g) | Number of Silique per plant | Seed Number per Silique |
|------------------------------------|---------------------------------|---|--|--------------------------------------|----------------------------|-----------------------------------|-------------------------------|
| 0 | | 620.1b | 628.1c | 1399 c | 1.361c | 58.28c | 15.72c |
| 75 | | 922.6a | 802.4b | 2026 b | 1.964b | 76.28b | 20.84b |
| 150 | | 1029a | 974.5a | 2588 a | 2.464a | 93.22a | 25.84 a |
| | Non inoculation | 808.2b | 784.2b | 1911 b | 1.867a | 75.13a | 20.08 b |
| | Azotobacter | 862.1a | 758.8b | 1905 b | 1.862a | 75.08a | 21a |
| | Azospirillum | 884a | 795.5ab | 2008 ab | 1.902a | 76.5a | 20.88a |
| | Azotobacter and Azospirillum | 874.2a | 868.2a | 2195 a | 1.851a | 77a | 21.25a |

Values followed by the same letters are not significantly different at 5% level.

kg ha⁻¹N nitrogen with no inoculation treatment (Table 3).

The findings suggested that with the combined application of *Azotobacter* and *Azospirillum*, seed oil yield was almost constant at all levels of nitrogen fertilizer. Combined application of bio-fertilizers with 75 kg nitrogen fertilizer resulted in a relatively good performance (Fig. II).

The mean comparison showed that increasing the level of nitrogen resulted in increased canola seed yield and yield components. Increased number of seeds per silique (25.84), number of silques per plant (93.22), 1000 seed weight (2464 kg ha⁻¹), seed yield (2588 kg ha⁻¹), biologic yield (1029 kg ha⁻¹) and oil seed yield (974.5 kg ha⁻¹) were obtained with the application of maximum level of nitrogen (150 kg ha⁻¹). Minimum value of the above mentioned characteristics, i.e., the minimum number of seeds per silique (15.72), the minimum total number of silques in plant (58.28 silques), minimum 1000 seed weight (1.361 g), minimum biologic yield (620.1 kg ha⁻¹), minimum seed yield (1399 kg ha⁻¹) and seed oil yield (628.1 kg ha⁻¹) resulted with no nitrogen treatment control plots.

Discussion

N fertilizer in this study had a significant effect on canola seed yield. Another study on the effect of N fertilization on growth and yield components showed increase in canola seed yield (Ahmadi and Bahrani, 2009). Comparison of N-requirement of canola and cereals including wheat crop has also been investigated and the results showed that genotypes of canola required higher level of N fertilizers to maintain their seed yield (Balint and Rengel, 2008). Sakari et al., (2012) reported a significant increase in seed yield between 100 and 150 kg ha⁻¹ (Table 2).

Hamidi et al. (2007) reported that 1000 seed weight increased by multiple inoculations compared to single inoculation. The increase in dry seed weight were derived mainly from increase in 100 seed weight and number of seeds per ear. This finding was supported by Yasari and Patwardhan (2007) who reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield (21.17%), pod per plant (16.05%), number of branches (11.78%) and weight of 1000 grain (2.92%). These results are also in harmony with those obtained by Yasari et al. (2008, 2009).

Different genera such as *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconoacetobacter*, *Pseudomonas*, and *Serratia* have been used as PGPR (Jaleel et al., 2009). From among these different PGPR, genera such as *Azotobacter*, *Azospirillum*, *Psuedomonas* and *Bacillus* are widely used as biofertilizers in the field of

agriculture. Performance of biofertilizers could be explained by the fixation of sufficient atmospheric nitrogen, production of plant growth promoters, decreasing the ethylene production in plants and solubilization of minerals such as phosphorus (Karthikeyan et al., 2008 a, b). Thus, the effect of biofertilizers in this experiment can be justified, at least partly, to the ability of PGPR to fixing nitrogen.

The ability of many rhizobacteria to produce plant hormones or hormone-like substances has often been evoked to explain how PGPR can promote plant growth (Jaleel et al., 2007). The application of both biofertilizers via any of the various applications methods (seed inoculation and foliar application) significantly alleviated the growth-inhibiting effects of waterlogging stress. The increase in grain yield confirms this effect. The number of siliques per plant was responsible for the significantly greater grain yield over the waterlogged control. The positive correlation between the number of siliques per plant and the grain yield ($r = 0.972^{**}$) also confirms this influence. The positive effects of PGPR inoculation on plant growth were confirmed by various studies (Ibiene et al., 2012). Vijayan et al. (2007) demonstrated the beneficial effect of foliar application of *Azotobacter chroococcum* on mulberry under salinity stress condition.

Some characteristics of canola such as plant height, number of branches per plant, number of pods per plant, seed yield and oil content are positively correlated with soil N level (Ahmadi and Bahrani, 2009). Biofertilizer microorganisms are more suitable for high crop yield, protection from different pathogens and pesticides. They also help in maintaining soil health by decomposition of dead and decaying matters in the soils (Verma et al., 2010). For example, cultures of *Azotobacter* used as inoculants were reported as producers of gibberellic acid, indole 3 acetic acid and cytokinins which promote germination and plant growth (Keyeo et al., 2011). Auxins produced by rhizobacteria can influence plants growth, including root development which improve uptake of essential nutrients thus increasing plant growth (Vikram, 2007).

Megawer and Mahfouz (2010) reported that inoculation of canola seeds by either *Azotobacter*, *Azospirillum* or the mixed inoculum and adding half recommended dose of nitrogen showed superiority and high productivity with saving half of the mineral nitrogen recommended dose. Ahmadi and Bahrani (2009) showed that nitrogen fertilizer affected the oil content negatively and decreased it by 3.3% in canola.

The result of the present study will help to determine the input fertilizer need suitable to have a greater and sustained yield.

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