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Integration Between Nitrogen Fertilizer Levels and Bio-Inoculants and its Effect on Canola (*Brassica napus* L.) Plants

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Abstract: Two field trials were carried out at the Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behira Governorate, Egypt, during the two successive winter seasons 2008/2009 and 2009/2010 to study the response of canola cultivar (Serw 4) to nitrogen fertilizer levels (20, 40 and 60 kg N/faddan (faddan = 4200 m^2)) and inoculation with Nitrobein and *Azospirillum* bio-fertilizers as well as their combinations on canola plants. Seed inoculation with Nitrobein and *Azospirillum* bio-fertilizers as sole treatments showed the lowest values of the studied characters, except number of seeds/siliqua and seed oil content. Most of the studied characters increased significantly when the nitrogen levels were added in combination with the inoculants. However, the treatment Nitrobein +60 kg N/faddan showed the highest values of number of siliqua/plant, seed yield/plant, seed yield/faddan and oil yield/faddan in the combined data, with significant differences. Addition of 40 kg N/faddan to both inoculants showed no significant differences between each other on the studied characters. No significant differences resulting from increasing nitrogen levels from 40 to 60 kg N/faddan with seed inoculation Nitrobein and *Azospirillum*. The most successful treatment applying was Nitrobein + 60 kg N/faddan followed by the same level of nitrogen fertilizer with *Azospirillium* of most studied characters.

Key words: Canola · Nitrogen fertilizer · Nitrobein · *Azospirillum* · Inoculation

INTRODUCTION

Canola (*Brassica napus* L.) is one of the main oil crops in many developing countries, but in Egypt it is still uncommon as oil crop. Canola cultivation may provide an opportunity to overcome some of the local deficit of edible oil production particularly it could be successfully grown during winter season in newly reclaimed soil outside the Nile valley. Canola has a relatively high requirement of nitrogen fertilizer where the content of this nutrient in seeds and plant tissues is greater than in most grain crops, so canola is considered a nitrogen demanding crop. Increasing nitrogen fertilizer rates up to 60 kg N/fed significantly increased plant height, number of siliqua/plant, seed yield/faddan, seed yield/plant and oil yield/faddan [1, 2, 3]. Also, Sharief and Keshta, [4] reported that increasing nitrogen fertilizer levels up to 75 kg N/faddan significantly increased 1000-seed weight, seed yield/faddan, seed and oil yields/faddan. In reverse, Sahoo *et al.* [5] and El-Habbasha and Abd El-Salam [3] illustrated that increasing nitrogen fertilization significantly decreased the oil content in canola seeds. Ahmadi and Bahrani [6] showed that nitrogen fertilizer affected the oil content negatively and decreased it by 3.3% in canola.

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 natural beneficial and ecologically friendly. The 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 have multiple functions for plant growth like *Azotobacter* which may derive both from its 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 producing considerable amounts of biologically active substances that can promote growth

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of reproductive organs and increase its productivity [7-10]. A broad range of hosts is an obvious advantage for any given beneficial bacterium, eliminating the need for developing many specific crop-bacterial combinations that may confuse growers. In nature, a broad host range may help bacteria survive better. The full host range of *Azospirillum* has not been defined previously. Claims of *Azospirillum* specificity for certain cereal species are documented [11]. However, *Azospirillum* strains had no preference for crop plants or weeds, or for annual or perennial plants, and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. It appears that *Azospirillum* is a general root colonizer and is not a plant specific bacterium. Shukla *et al.* [12] reported that application of *Azotobacter* resulted significantly in higher number of seeds/siliqua, total dry mater and branches/plant in Indian mustard. Sharma *et al*. [13] reported that the oil content of Indian mustard decreased by successive increasing in nitrogen level and application of *Azotobacter*, suggesting that oil production increased significantly when nitrogen applied through *Azotobacter*. Similar results were obtained by Yasari *et al*. [9] noticed that the application of *Azotobacter* and *Azospirillum* helped increase the oil content of canola seeds.

The aim of this study was to investigate the role of nitrogen fertilizer levels and bio-inoculants on the productivity of canola plants under the newly reclaimed sandy soil in Egypt.

MATERIAL AND METHODS

Two field trials were carried out at the Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behira Governorate, Egypt, during the two successive winter seasons 2008/2009 and 2009/2010 to study the effect of nitrogen fertilizer levels and bio-inoculants on the productivity of canola in the newly reclaimed sandy soil. Soil samples were taken at depths of 30 cm for mechanical and chemical analysis as described by Chapman and Pratt [14]. Soil texture was sandy having the following characteristics, sand 91.20 and 92.33 %, silt 3.70 and 2.95 % , pH 7.40 and 7.50 , organic matter 0.34 and 0.29%, $CaCO_3$ 1.42 and 1.65 %, EC 0.33 and 0.46 mmhos/cm^2 , N, 8.11 and 7.45, P, 3.11 and 4.12 and exchangeable K, 20.60 and 23.14 mg/100g soil in 1st and 2nd seasons, respectively.

The treatments were arranged in randomized complete block design with three replicates where the nitrogen fertilizer levels (20, 40 and 60 kg N/faddan) (faddan= 4200 m^2) and bio-inoculants (*Azospirillum* and Nitrobein) were randomly distributed. The experimental unit area was 10.5 m^2 consisting of ten rows (3.5 m long and 30 cm between rows). The seeds of local variety Serw 4 were coated just before sowing with the bacteria inoculants using Arabic gum (40%) as adhesive agent and were sown at seeding rate of 3 kg/faddan on November 10th and 12th in the first and second seasons, respectively. Nitrogen fertilizer was added as ammonium sulfate (20.6 % N) in three equal doses after 15, 30 and 45 days after sowing. Phosphorus fertilizer, as calcium superphosphate (15.5% P_2O_5) at the rate of 15 kg P_2O_5/ faddan and potassium sulfate (48 % K_2O) at the rate of 24 kg K_2O /faddan were applied during seed bed preparation. Organic fertilizer was added at the rate of 20 m^3/faddan. The experimental treatments as follows:

- *Azospirillum*
- Nitrobein
- 20 kg N/faddan
- 40 kg N/faddan
- 60 kg N/faddan (control)
- *Azospirillum* + 20 kg N/ faddan
- *Azospirillum* + 40 kg N/ faddan
- *Azospirillum* + 60 kg N/ faddan
- Nitrobein + 20 kg N / faddan
- Nitrobein + 40 kg N / faddan
- Nitrobein + 60 kg N / faddan
- *Azospirillum* + Nitrobein + 20 kg N / faddan
- *Azospirillum* + Nitrobein + 40 kg N / faddan
- *Azospirillum* + Nitrobein + 60 kg N / faddan

Peat based inoculum contains *Azospirillum* at the concentration of 10^6 c.f.u./g, for Nitrobein inoculum which contains *Azotobacter* and *Azospirillum* microorganisms, peat based inoculum at concentration of 10^7 c.f.u./g was used. The preceding summer crop in two experimental seasons was groundnut (*Arachis hypogaea*). Sprinkler irrigation was applied as plants needed. Normal cultural practices of growing canola were followed by the farmers of this district. Canola plants were manually harvested on April 17th and 20th in the first and second season, respectively.

Data Recorded: Growth Parameters: At 75 and 105 days after sowing, ten plants were randomly taken from each plot to determine plant height (cm), number of leaves/plant, number of branches/plant and dry weight /plant (g).

Yield and Yield Attributes: At harvest, ten plants were taken randomly from center of the plot to measure, plant height, number of siliqua/plant, number of seeds/siliqua, 1000-seed weight (g), and seed yield/plant (g). Seed yield/faddan (kg/faddan) was estimated from harvesting the whole plot area.

Chemical Traits: Seed contents of NPK were determined as follow: N (%) was determined by the improved Kjeldahl method [15], P (%) was determined according to A.O.A.C. [16] and K (%) was determined by using Flame Photometer. Seed protein content calculated by multiplying N (%) by 5.75. Seed oil content was estimated according to A.O.A.C. [16] by using Soxhelt apparatus and petroleum ether 40-60°C as a solvent. Oil and protein yield (kg/faddan) were estimated from multiplying seed yield/faddan by seed oil content (%) and seed protein content (%), respectively.

Statistical Analysis: Data were subjected to statistical analysis of variance as described by Snedecor and Cochran [17] and the combined analysis of the two seasons results were conducted. Mean values of the recorded data were compared by using the least significant differences (LSD 5%).

RESULTS AND DISSECTION

Growth Characters: Data presented in Table 1 showed that plant height, number of leaves/plant, number of branches /plant and dry weight /plant at 75 and 105 days after sowing. Regarding to inoculation with bio-fertilizers only, no significant differences between *Azospirillum* and Nitrobein bio-fertilizers as sole treatments, except dry weight /plant for both growth samples (75 and 105 days after sowing). The abovementioned insignificant difference between both treatments clearly illustrate the benefit role of nitrogen fixers of *Azospirillum* and Nitrobein inocula for enhancing plant growth through their capacity in nitrogen fixation as well as producing considerable amounts of biologically active substances that can promote growth of reproductive organs and increase its productivity. The phytohormones are essential in diverse aspects of plant growth and development, the indole-3-acetic acid (IAA), is known to regulate diverse aspects of plant growth and development, including cell division, cell extension, and cell differentiation and play a crucial role in root initiation, apical dominance, tropisms, and senescence [18]. These results are in harmony with those obtained by Shukla *et al.* [12] reported that application of *Azotobacter* resulted significantly in total dry mater and branches/plant.

Increasing nitrogen fertilizer levels from 20 to 60 kg N/faddan did not show significant differences in different growth parameters at 75 days after sowing except dry weight /plant, while at 105 days after sowing, the studied growth characters were differed significantly by increasing nitrogen levels. The stimulant effect of nitrogen supply on plant growth has been reported by El-Kafoury *et al*. [1] and Kandil *et al*. [2]. With regard to the dual application of mineral nitrogen and bio-fertilizers, at 75 days after sowing, seed inoculation with Nitrobein + 40 kg N/faddan showed the highest values of number of branches/plant and dry weight/plant,

Table 1: Effect of nitrogen fertilizer levels, bio-fertilizers and their combination on growth characters of canola plants at 75 and 105 days after sowing (combined data of 2008-2009 and 2009-2010 seasons)

	Parameters							
	75 Days after sowing				105 Days after sowing			
Treatments	Plant height (cm)	Number of leaves/plant	Number of branches/plant	Dry weight /plant (g)	Plant height (cm)	Number of leaves/plant	Number of branches/plant	Dry weight /plant (g)
Azospirillum	101.07	9.41	5.69	27.29	115.43	10.88	10.56	44.33
Nitrobein	99.70	8.48	5.15	23.98	117.27	10.08	9.29	40.27
20 kg N/faddan	105.46	7.97	4.25	24.13	121.69	9.97	7.96	44.76
40 kg N/faddan	109.43	8.69	4.96	24.74	126.29	10.67	8.66	48.45
60 kg N/faddan (control)	109.37	9.54	5.37	26.91	127.33	11.68	9.37	49.06
Azospirillum + 20 kg N/ faddan	104.93	9.02	4.34	26.39	123.17	11.08	9.16	40.79
Azospirillum + 40 kg N/ faddan	108.39	8.10	4.57	26.01	125.20	11.52	10.03	43.67
Azospirillum + 60 kg N/ faddan	112.82	9.00	5.89	24.41	130.56	10.10	11.17	45.80
Nitrobein + 20 kg N / faddan	105.73	8.65	5.60	26.22	124.32	10.55	10.51	40.24
Nitrobein + 40 kg N / faddan	108.43	9.19	5.93	29.36	125.33	11.56	9.74	45.85
Nitrobein + 60 kg N / faddan	110.02	9.62	5.56	27.18	128.72	10.61	9.02	48.22
Azospirillum + Nitrobein + 20 kg N / faddan	106.96	8.47	4.54	26.67	128.07	11.65	10.56	42.88
Azospirillum + Nitrobein +40 kg N / faddan	103.05	8.43	4.65	27.50	128.91	11.99	11.57	49.55
Azospirillum + Nitrobein+ 60 kg N / faddan	107.95	9.35	5.57	26.92	130.42	10.65	10.86	47.38
LSD 5 %	NS	NS	NS	2.67	2.05	1.06	1.40	3.83

Table 2: Effect of nitrogen fertilizer levels, bio-fertilizers and their combination on yield and yield attributes of canola plants (combined data of 2008-20089 and 2009-2010 seasons)

Treatments	Parameters							
	Plant height (cm)	Number of siliqua/plant	Number of seeds/siliqua	1000-seed weight (g)	Seed yield /plant (g)	Seed yield / faddan (kg/faddan)	Protein yield/ faddan (Kg/faddan)	Oil yield/ faddan (kg/faddan)
Azospirillum	137.65	125.75	11.57	3.11	4.54	712.92	128.33	285.95
Nitrobein	136.45	129.29	11.37	3.21	4.71	726.48	130.76	292.28
20 kg N/faddan	135.63	133.52	9.46	3.36	4.25	740.61	136.64	296.61
40 kg N/faddan	142.74	138.15	9.93	3.37	4.67	753.32	143.13	300.34
60 kg N/faddan (control)	146.06	143.76	10.08	3.59	5.23	767.38	161.15	296.23
Azospirillum + 20 kg N/ faddan	137.42	136.61	10.37	3.36	4.77	750.64	135.11	298.46
Azospirillum + 40 kg N/ faddan	142.39	144.25	10.28	3.49	5.18	780.83	154.60	302.35
Azospirillum + 60 kg N/ faddan	146.17	147.34	9.65	3.65	5.42	786.13	165.08	306.04
Nitrobein + 20 kg N / faddan	144.15	137.55	10.84	3.30	4.97	770.63	138.74	303.73
Nitrobein + 40 kg N / faddan	143.25	143.40	10.65	3.61	5.38	784.01	156.80	305.93
Nitrobein + 60 kg N / faddan	145.42	148.74	9.96	3.69	5.47	791.01	166.14	309.57
Azospirillum + Nitrobein + 20 kg N / faddan	135.97	139.28	10.48	3.46	5.08	752.11	148.16	301.06
Azospirillum + Nitrobein +40 kg N / faddan	143.25	141.56	10.37	3.58	5.25	774.14	149.65	303.76
Azospirillum + Nitrobein +60 kg N / faddan	146.97	145.35	10.47	3.61	5.41	783.38	167.48	304.60
LSD 5 %	4.65	4.56	0.71	NS	NS	9.23	2.58	3.30

while Nitrobein +60 kg N/faddan recorded the highest values of plant height and number of leaves /plant, while at 105 days after sowing, *Azospirillum* + Nitrobein + 40 kg N/faddan recorded the highest values of number of leaves/plant, number of branches/plant and dry weight/plant with no significant differences between this treatment and *Azospirillum* + 40 kg N/faddan or Nitrobein + 40 kg N/faddan. The co-culture of *Azospirillum brasilense* and *Enterobacter cloacae* led to a more efficient N_2 fixation than did either of the bacteria alone [19, 20]. When *Azospirillum* sp. was co-inoculated with a mixture of cellulolytic fungi, its nitrogenase activity increased 22-fold [21]. These results are coincidence with Chela *et al*. [22] who reported that the use of nitrogen fertilizer in combinations with *Azospirillum* produced significantly higher green and dry matter yields than those from inoculation or fertilization alone.

Yield and Yield Attributes: Data in Table 2 showed that plant height, number of siliqua/plant, number of seeds/siliqua, 1000-seed weight, seed yield /plant, seed yield/faddan, protein and oil yield/faddan. Seed inoculation with Nitrobein and *Azospirillum* bio-fertilizers as sole treatment showed the lowest values of the studied characters, except number of seeds/siliqua compared to other treatments. No significant differences between the two inoculants, except seed and oil yield/faddan. Bacteria of the genera *Azotobacter* and *Azospirillum* are free-living, N-fixing organisms which live in close association with plants in the rhizosphere. Under appropriate conditions, these bacteria can enhance plant development and promote the yield of several agriculturally important crops in different soils and climatic regions [23]. These beneficial effects of *Azotobacter* and *Azospirillum* on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria, and to a lesser extent, biological N_2 fixation [24]. These results are in harmony with those obtained by Yasari *et al*. [9, 10]. Increasing nitrogen levels from 20 to 60 kg N/faddan significantly increased plant height, number of siliqua /plant, seed yield /faddan and protein yield /faddan. These results are in harmony with those obtained by Kandil *et al.* [2] and El-Habbasha and Abd El-Salam [3]. Most of the studied characters were increased significantly when the nitrogen levels were added in combination with the different inoculants as sole and dual application. However, addition of 60 kg N /faddan to seed inoculated with Nitrobein recorded the highest values of number of siliqua/plant, 1000-seed weight, seed yield /plant, seed yield/faddan and oil yield/faddan, whereas seed yield /faddan and oil yield /faddan was increased by 3.12 and 4.50 % compared to 60 kg N/faddan (control), respectively, this treatment followed by the same nitrogen level with the seed inoculated with *Azospirillum*, where *Azospirillum* + 60 kg N/faddan increased seed yield /faddan and oil yield /faddan increased by 2.4 and 3.3 % over the control, respectively. The treatment *Azospirillum* + Nitrobein +60 kg N / faddan records the highest value of protein yield /faddan (167.48 kg /faddan). No significant differences were observed with 40 or 60 kg N /faddan with seed inoculation either *Azospirillum* or Nitrobein bio-fertilizers. No significant differences resulting from increasing nitrogen fertilizer levels from 40 to 60 kg N/faddan with seed inoculation either *Azospirillum* or Nitrobein. Increasing nitrogen levels from 20 to 60 kg N/faddan in the presence of dual application of Nitrobein and *Azospirillum* increased significantly plant height, number of seeds/siliqua and seed yield/faddan.

Table 3: Effect of nitrogen fertilizer levels, bio-fertilizers and their combination on chemical analysis of canola plants (combined data of 2008/2009 and 2009/2010 seasons)

	Parameters				
	Nutrient seed contents (%)				
Treatments	N	P	K	Seed oil content (%)	Seed protein %
Azospirillum	3.11	4.11	2.03	40.11	17.88
Nitrobein	3.06	4.20	1.98	40.23	17.59
20 kg N/faddan	2.85	4.22	1.95	40.05	16.38
40 kg N/faddan	3.14	4.22	2.10	39.87	18.05
60 kg N/faddan (control)	3.67	4.15	2.15	38.60	21.01
Azospirillum + 20 kg N/ faddan	3.02	4.23	1.97	39.76	17.36
Azospirillum + 40 kg N/ faddan	3.25	4.25	2.09	39.18	18.69
Azospirillum + 60 kg N/ faddan	3.69	4.19	2.21	38.93	21.22
Nitrobein + 20 kg N / faddan	3.15	4.23	2.06	39.59	18.11
Nitrobein + 40 kg N / faddan	3.56	4.35	2.21	39.02	20.47
Nitrobein + 60 kg N / faddan	3.79	4.39	2.36	39.13	21.79
Azospirillum + Nitrobein + 20 kg N / faddan	3.42	4.21	2.31	40.03	19.67
Azospirillum + Nitrobein +40 kg N / faddan	3.36	4.36	2.25	39.24	19.32
Azospirillum + Nitrobein +60 kg N / faddan	3.95	4.42	2.41	38.88	22.71
LSD 5 %	0.64	NS	NS	0.56	1.14

The present results cleared that dual application of mineral nitrogen and bio-fertilizers was better than mineral nitrogen application or bio-fertilizers alone. This may be due to mineral nitrogen enhanced plant growth which increased plant metabolites which encouraged nodulation and nodule activity. Inoculation with recombinants between *Azospirillum* and *Azotobacter* strains was more efficient and significantly increased many of growth and yield parameters in plants, as well as, increased nitrogen fixation and consequently reduced the amounts of chemical fertilizers. In addition, transconjugants significantly increased the yield components and growth traits over the plants fertilized with control treatments. This means bio-fertilizers could reduce the mineral nitrogen fertilizer production costs as well as environmental pollution. Megawer and Mahfouz [25] reported that inoculation the canola seeds by either *Azotobacter, Azospirillum* or the mixed inoculum and addition half recommended dose of nitrogen showed superiority and high productivity with saving half of the mineral nitrogen recommended dose.

Chemical Traits: Data presented in Table 3 indicated that the different treatments significantly affected on the studied characters, except phosphorus and potassium seed contents. Seed inoculation with Nitrobein and *Azospirillum* bio-fertilizers as sole treatments showed no significant differences between the two inoculants and 60 kg N/faddan (control) except, seed oil and protein contents. Soil and rhizosphere bacteria can affect the mineral nutrition of plants by changing root-uptake characteristics, due to a modification of root morphology or alteration of uptake mechanisms, relative growth rate or internal composition of plants [26]. Increasing nitrogen levels from 20 to 60 kg N/faddan significantly increased nitrogen percentage and seed protein content, while seed oil content significantly decreased. The dual application of biofertilizers and nitrogen fertilizer recorded the highest values of the studied traits, whereas the treatment *Azospirillum* + Nitrobein +60 kg N / faddan surpassed in NPK seed content (%) and seed protein content, while Nitrobein inoculation records the highest seed oil content. Dual inoculations stimulated plant growth and significantly increased the concentrations of P, N, and total soluble sugars. Soil N content was increased by single inoculation with *Azotobacter* and by all dual inoculations [27]. Ahmadi and Bahrani [6] showed that nitrogen fertilizer affected the oil content negatively and decreased it by 3.3% in canola.

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