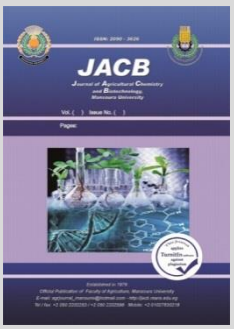
Journal of Agricultural Chemistry and Biotechnology

Journal homepage: www.jacb.mans.edu.eg
Available online at: www.jacb.journals.ekb.eg

Complementary Effect of Biofertilizer with Mineral Nitrogen Fertilizer on Canola Plant Productivity

Selim, M. A. E.* and Sabrien A. Omar

Microbiology Department, Faculty of Agriculture, Mansoura University, Egypt



ABSTRACT

Biofertilizers are recently used on a large scale to reduce chemical fertilizers and their pollutant effect on soils and water. In this work, a pot experiment was carried out to evaluate the effect of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Klebsiella oxytoca* with different levels of nitrogenous fertilizer on the growth and yield of canola (*Brassica napus* L.) and microbial population in the soil during the winter season, 2018. Results reveal that used bacterial species as biofertilizers have a great effect on morphological and yield traits of canola plants, where the plants treated with *Azotobacter*, *Azopirillum* and *Klebsiella* were characterized by increases in plant heights, plant weights as well as numbers of branches and pods. Also, *Azotobacter* and *Azospirillum* increased nitrogen, whereas *Klebsiella oxytoca* increased phosphorus in plants and seeds. The results showed that using triple inoculum (*Az. chroococcum*, *Azosp. lipoferum* and *K. oxytoca*) with 75% of nitrogenous fertilizer (33.75 kg N/feddan) achieved the highest seed yield per plant (24.35 g/plant), while oil percent in seeds was 46.86%, which was not the highest but very close to oil percentage in plants treated with the recommended dose of nitrogen, which was 47.47%. On the other side, using bacterial inoculum stimulate microbial population and increased total bacteria, diazotrophs (*Azotobacter* and *Azospirillum*) and phosphate dissolving bacteria counts in the rhizosphere of canola plants.

Keywords: Canola (*Brassica napus* L.), biofertilization, *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Klebsiella oxytoca*

INTRODUCTION

Canola (*Brassica napus* L.) is one of the main oil crops in many countries especially in Canada, the EU and the USA. Canola oil production comes in the third position after soybean and palm, and in the second position in total world area for oil crops. Canola produces approximately 14.7% of total vegetable edible oil in the world (Yasari *et al*., 2008). It contains 40 - 45% oil and 36 - 40% protein.

Canola oil has a high content of omega 3 and vitamin E, and low content of erucic acid and glucosinolates. Thus, it recognized as functional food by medicine (Brown *et al*., 2008). Canola oil is used widely as cooking and salad oil, and in making margarine. Also, it is used in lubricants and hydraulic fluids, lamp oils and soap making (Malhi & Gill, 2007; Megawer & Mahfouz, 2010; Naderifar & Daneshian, 2012; Sharifi, 2012).

Egypt has been faced with the problem arising from the shortage in the local production of edible oils as compared to the rate of their consumption. The wide gap between the production and consumption of edible oils reached 90%, which created a need for importation. Finding a new source of edible oil has been a necessity.

Cultivation of canola in Egypt is an opportunity to decrease the local deficiency in the production of vegetable edible oils, especially due to its ability to tolerate harsh environmental conditions such as salinity and drought frequently prevailing in newly reclaimed soil as well as being successfully grown during the winter season in Egypt. (Weiss, 1983; Kandil, 1984; Sharaan, 1986; Ghallab & Sharaan, 2002; Sharaan *et al*., 2002).

In Egypt, efforts are being made to increase the areas under cover oilseed crops as much of the edible oil is important. It is well known that the chemical N-P-K fertilizer help in the healthy growth of crops like canola (Yasari & Patwardhan, 2007; Yasari, *et al*., 2009). On the other hand, the continuous and intensive use of chemical fertilizers may lead to environmental pollution especially to groundwater, and the cost of production increased. So, the need to find alternatives was crucial to reduce the use of chemical fertilizers. Bio-fertilizers are the most advanced biotechnology enhanced environmental sustainability, lower-cost production and good crop yields (Sharma *et al*., 2007; Hakeem *et al*., 2016).

Biofertilizers are the organic preparations containing microorganism(s), which are added to soil as an alternative to chemical fertilizers. biofertilizers include nitrogen fixers, phosphate solubilizing bacteria, plant growth-promoting rhizobacteria, etc… (Shekh, 2006). A number of different bacteria promote plant growth including *Azotobacter* spp., *Azospirillum* spp., *Pseudemonas* spp., *Bacillus* spp. were used in the inoculation of canola plants. The biofertilizer had a significant and positive effect on plant height and yield of canola as well as the reduction in the quantity of chemical fertilizers used (Gupta & Samnotra 2004, Turan *et al*., 2006; Mohamed *et al*., 2017). The present study was undertaken to find the integrated effects of N-P-K and Bio-fertilizer on the growth and yield of canola, as well as some soil microorganisms in the rhizospheric region.

* **Corresponding author.**
E-mail address: maalawady@mans.edu.eg
DOI: 10.21608/jacb.2020.123549

MATERIALS AND METHODS

Bacteria used as biofertilizers

Azotobacter chroococcum MF135558, *Azospirillum lipoferum* and *Klebsiella oxytoca* MF13559 were kindly obtained from the Microbiology Dept. Faculty of Agriculture, Mansoura University, Egypt. Both *Azotobacter chroococcum* MF135558 and *Azospirillum lipoferum* were used as nitrogen-fixing bacteria, while *Klebsiella oxytoca* MF13559 was used for its high capability of dissolving phosphorus (Hauka *et al.*, 2018).

Preparation of bacterial Inocula

Bacterial strains were grown on the specific liquid media to reach sufficient bacterial growth for using as a biofertilizer. *Azotobacter chroococcum* MF135558 was grown on modified Ashby's medium (Abd El-Malek and Ishac, 1968) at 30ºC for 7 days, *Azospirillum lipoferum* was grown on Döbereiner' medium (Döbereiner, 1978) at 30ºC for 7 days and *Klebsiella oxytoca* MF13559 was grown on nutrient broth medium at 30ºC for 3 days.

Bacterial cultures were centrifuged at 3000 rpm for 30 min at 20ºC, then the sediment was resuspended in 50 ml 0.8% KCl (w/v) solution and added to tap water. The inoculum was adjusted to 10^7 cell/ml. Bacterial inoculum was mixed with soil in pots before seed sowing at rate of 10 ml of inoculum per 10 kg soil. Ten ml of bacterial inoculum were added to each plant in the rhizospheric region in two doses; the first one after 15 days and the second one after 30 days of seed germination.

Pot experiment

A pot experiment was carried out during winter season 2017/2018 at the Experimental Farm, Fac. Agri., Mansoura Univ., Egypt. To each pot, 30 kg of clay loamy soil was added, and the experiment was designed as a complete randomized block with 13 treatments and three replicates. Phosphorus and potassium fertilizers were added with soil preparation. Nitrogenous fertilizer was added in three equal doses (before sowing, after thinning and at floral buds initiation). Plants received the recommended dose of phosphorus (30 Kg P_2O_5 /fed) as calcium superphosphate (15% P_2O_5) and the recommended dose of potassium (24 kg K2O/fed) as potassium sulphate (48% K_2O) for all treatments, while nitrogen was applied as ammonium nitrate (33.5%N) in four levels as follow; without nitrogen fertilizer, recommended dose (45 kg N/fed), 75% of the recommended dose (33.75 kg N/fed) and 50% of the recommended dose (22.5 kg N/fed). The canola seeds was kindly obtained from Station of Agricultural Research Center, Giza, Egypt. Nine seeds of canola were added to each pot at sowing, then thinned to three plants per pot after two weeks of germination. The design of the experiment is shown in Table (1), and the physical, chemical and microbiological properties of used soil are shown in Table (2).

Table 1. Design of pot experiment

Treatment no.	Treatment
T1	45 kg N/fed (Recommended dose of N)
T2	33.75 kg N/fed (75% of N recommended dose)
T3	22.5 kg N/fed (50% of N recommended dose)
T4	*Azotobacter* + 75%N
T5	*Azotobacter* + *Klebsiella* + 75%N
T6	*Azospirillum* + 75%N
T7	*Azospirillum* + *Klebsiella* + 75%N
T8	*Azotobacter* + *Azospirillum* + *Klebsiella* + 75%N
T9	*Azotobacter* + 50%N
T10	*Azotobacter* + *Klebsiella* + 50%N
T11	*Azospirillum* + 50%N
T12	*Azospirillum* + *Klebsiella* + 50%N
T13	*Azotobacter* + *Azospirillum* + *Klebsiella* + 50%N

Table 2. Physical, chemical and microbiological analysis of used soil

Physical properties		Chemical properties			
Texture analysis		Soluble cations (meq/L)		Soluble anions (meq/L)	
Sand	27.7%	K^+	0.11	CO_3^{--}	ND
Silt	38.8%	Na^+	1.58	HCO_3^-	0.55
Clay	33.5%	Ca^{++}	1.14	Cl^-	0.18
Soil texture	Clay loamy	Mg^{++}	0.92	SO_4^{--}	2.94
pH	7.73	OM (%)	2.15	Available NPK (mg/kg)	
EC	1.4	$CaCO_3$ (%)	5.53	N	155.19
WH (%)	5			P	8.09
				K	438.03
Microbial counts					
Total bacterial counts x10^5	16.5				
Azotobacter spp. x10^2	0.014				
Azospirillum spp. x10^2	0.026				
Phosphate dissolvers x10^3	3.65				

Plant morphological and yield traits

Samples of canola plants were collected two times, the first sample was taken 40 days after sowing (DAS) and the second sample was taken at the harvesting time (180 days after sowing). Three plants were collected from each treatment to measure both plant traits and chemical analysis. Plant traits which measured in samples collected 40 DAS includes; plant heights (cm), plant dry weights (g) and number of leaves per plant, while morphological and yield traits which measured in harvesting samples were; plant heights (cm), number of branches, number of pods, seed yield of a plant (g) and oil percentage in seeds (%).

Chemical analysis

N, P, and K (mg/g) were determined in plant samples and seeds. To prepare plant samples for N, P, and K determination, samples of plants and seeds were dried at 80ºC until constant weight in the oven, then ground well. 0.1 g of the sample was digested according to the method of (McGill & Figueiredo, 1993).

Nitrogen was measured by the Kjeldahl method according to Jackson (1962), while phosphorus was measured colorimetrically by the method of Boltz and Mellon (1948) and potassium was measured by atomic absorption spectroscopy (Jackson, 1973).

Oil percentage

Oil was determined in canola seeds (%) by using a soxhlet apparatus according to A.O.A.C. (1995).

Bacterial counts

Soil samples were obtained from the rhizosphere of canola roots; after 40 and 70 days of sowing to determine total bacteria, *Azotobacter* spp., *Azospirillum* spp. and phosphate dissolving bacterial counts. Total bacteria counts were counted on nutrient agar medium (Oxoid), while phosphate-dissolving bacteria were counted on the Pikovskaya medium (Pikovskaya, 1948) using the pour-plate method. *Azotobacter* spp. were counted on Ashby's medium (Abd-El-Malek and Ishac, 1968), while *Aspsirillum* spp. were counted on Döbereiner's medium (Döbereiner, 1978), using the most probable numbers (MPN) technique.

Statistical analysis

Statistical analysis of obtained data was carried out using COSTAT (2005) software of variance analysis (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Morphological traits of canola plants 40 Days after sowing

The effect of chemical and bio-fertilizer on canola plant growth 40 DAS is presented in Table (3). Data revealed that chemical and bio-fertilizers significantly improved plant growth traits such as plant heights, plant dry weights, and number of leaves. Data show that using single or mixed bacterial inoculum as a biofertilizer significantly increased plant morphological traits, after 40 DAS. There were no significant differences in the heights of plants treated with *Az. chroococcum* + 75% of mineral nitrogen, plants treated with mixture of *Az. chroococcum* and *K. oxytoca* + 75 % of recommended nitrogen dose, and plants treated with the nitrogen recommended dose. The highest value of plant heights was 34.5 cm, recorded by plants treated with the recommended dose of N, followed by 33.6 cm, which was recorded by plants treated *Az. chrocooccum* + *K. oxytoca* + 75% mineral nitrogen.

Inoculation with *Az. chrocooccum* + *K. oxytoca* + 75% N, resulted in significant increases in plant dry weights compared with all other treatments, which reaches 8.2 g/plant, while plant treated with the recommended dose of nitrogen gives 6.65 g/plant. Also treatment with *Az. chroococcum* + 75% N and *Azosp. lipoferum* + *K. oxytoca* + 75% N. give good plant dry weights. Also application with *Az. chroococcum* + *Azosp. lipoferum* + *K. oxytoca* + 75% N gives the highest numbers of leaves (14.3), compared with (13.3 and 12.7) which are achieved with plants applied with *Az. chroococcum* + *K. oxytoca* + 75% N and the recommended dose of nitrogen respectively, but the differences are not significant.

Morphological traits of canola plants at harvesting stage

Data in Table (4) show the effect of chemical and bio-fertilizers on yield traits of canola plants at the harvesting stage. Data show that bacterial inoculation increase plant heights and numbers of branches and pods in canola plants. Addition of *Az. chroococcum* and *K. oxytoca* gave the highest plant heights with saving 25% of N fertilizers. The highest plant height (120 cm) was recorded in plants treated with the full dose of N and plants treated with *Az. chroococcum* + *K. oxytoca* + *Asozp. lipoferum* + 75% N. There were significant increases in branches formation with the addition of *Az. chroococcum* and *K. oxytoca*, although different N levels affected branches numbers also. The highest numbers of branches recorded by plants received the full dose of nitrogen, which is 14.33 branches/plant, followed by 14 and 13.67 in plants treated with *Az. chroococcum* + *K. oxytoca* + 75% N and *Az. chroococcum* + *K. oxytoca* + *Asozp. Lipoferum* + 75% N, respectively, although there were no significant differences between the three treatments. Same trend happened with numbers of pods per plant, the highest numbers of pods/plant recorded were 230.3, 228.3 and 227 with treatments; *Az. chroococcum* + *K. oxytoca* + *Asozp. lipoferum* + 75% N, full dose of nitrogen (recommended dose) and *Az. chroococcum* + *K. oxytoca* + 75% N, with no significant differences.

Table 3. Morphological traits of canola plants treated with mineral nitrogen and bio-fertilizers at 40 DAS

Treatments	Plant height (cm)	Plant dry weight (g)	Number of leaves
100% N (Recommended dose)	34.50a	6.65bc	12.7a-c
75% N	28.50cd	5.83b-d	11.3c-e
50% N	22.67f	4.99de	7.7g
Az. + 75% N	32.83ab	6.07b-d	9.7ef
Az. + *K.* + 75% N	33.00ab	7.00ab	13.3ab
Azosp. + 75% N	29.13cd	5.33c-e	12.7a-c
Azosp. + *K.* + 75% N	30.43bc	7.03ab	11.7b-d
Az. + *Azosp.* + *K.* + 75% N	33.60a	8.20a	14.3a
Az. + 50% N	28.33cd	5.79b-d	10.3d-f
Az. + *K.* + 50% N	29.33cd	6.05b-d	10.3d-f
Azosp. + 50% N	25.20ef	4.30e	7.7g
Azosp. + *K.* + 50% N	27.17de	6.35bc	8.7fg
Az. + *Azosp.* + *K.* + 50% N	28.33cd	6.84b	10.7de

Different letters following the data within each column mean significant difference at *P < 0.05*.

Az. = Azotobacter chroococcum*; *Azosp. = Azospirillum lipoferum* and *K. = Klebsiella oxytoca

Table 4. Yield traits of canola plants treated with mineral nitrogen and bio-fertilizers at harvesting time

Treatments	Plant height (cm)	Number of branches /plant	Number of pods /plant
100% N (Recommended dose)	120.0a	14.33a	228.3a
75% N	106.3c	10.33c-e	195.7b
50% N	83.8f	8.33ef	133.7d
Az. + 75% N	116.0ab	12.33a-c	201.3b
Az. + *K.* + 75% N	118.3a	14.00a	227.0a
Azosp. + 75% N	117.0ab	9.67d-f	189.0b
Azosp. + *K.* + 75% N	111.0bc	11.33b-d	197.0b
Az. + *Azosp.* + *K.* + 75% N	120.0a	13.67ab	230.3a
Az. + 50% N	96.8d	9.33d-f	152.7cd
Az. + *K.* + 50% N	98.3d	9.67d-f	160.3c
Azosp. + 50% N	86.0ef	7.67f	142.3cd
Azosp. + *K.* + 50% N	90.0e	8.67ef	152.0cd
Az. + *Azosp.* + *K.* + 50% N	97.7d	9.67d-f	162.0c

Different letters following the data within each column mean significant difference at *P < 0.05*.

Soil microorganisms play an important role in the enhancement of plant growth especially, their role in

nitrogen-fixing and phosphate dissolving. Results obtained in Tables (3 & 4) show that using bacterial inoculation raised morphological and yield traits of canola plants. Also, data reveal that using mixed inoculum of diazotrophs and phosphate dissolving bacteria increased plants morphological and yield traits, our results are in agreement with Abd-Elgawad (2009), which found that mixed inoculum of *Azotobacter chroococcum* and *B. megaterium* gave the highest numbers of branches and pods in canola plant. Data also reveal that using the inoculum of *Az. chroococcum* gave better results than inoculum of *Azosp. lipoferum*, which differs with El-Howeity and Asfour (2012), who revealed that using *Azospirillum brasilense* is more effective on canola plants than *Azotobacter chroococcum.* Also, El-Sawah, *et al.* (2018) revealed that the used bacteria strains in our research; *Azotobacter chroococcum* MF135559 and *Klebsiella oxytoca* MF135559 are high producers for indole acetic acid (IAA), which is considered one of the most common plant growth factors. This could explain their positive impact on canola plant growth and yield traits.

N, P and K concentrations in canola plants, 40 DAS and at harvesting stage

Data in Table (5) show the effect of chemical and bio-fertilizer on N, P and K concentrations (mg/g) in canola plants after 40 DAS and at harvesting time. Data show that nutrients concentrations increased with the age of plants, which almost doubled at harvesting compared with plants at 40 DAS. Also, data show that nitrogen fertilizer levels significantly impact on nutrients (N, P and K) concentrations during growing stages of canola plants, while the effect of biofertilizers varied depending on the kind of bacterial inoculum used. Data reveal that using *Az. chroococcum* and *K. oxytoca* resulted in significant increases in N concentrations in canola plants. The highest levels of nitrogen concentration were recorded in plants applied with the recommended dose of nitrogen (45kg N/fed), which recorded 26.30 mg/g after 40 days of sowing and 51.2 mg/g at harvesting time. Also data reveal that there were an increases in K concentrations in plants treated with *K. oxytoca* compared with plants without treatment of *K. oxytoca*. Highest P concentrations achieved by application with *Az. chrococcum* + *Azosp. lipoferum* + *K. oxytoca* + 75% N, which recorded 2.75 mg/g after 40 days of sowing and 2.79 mg/g at harvesting timw. Also the treatment: *Az. chrococcum* + *Azosp. lipoferum* + *K. oxytoca* + 75% N. recorded the highest K concentrations in canola plants (12.15 mg/g) after 40 days of sowing, while the treatment: *Az. chrococcum* + *K. oxytoca* + 75% N. recorded the highest K concentrations in canola plants (22.17 mg/g) at harvesting time.

Table 5. Concentrations of N, P, and K (mg/g) in canola plants treated with mineral nitrogen and bio-fertilizers at harvesting time

Treatments	40 DAS			Harvesting		
	N	P	K	N	P	K
100% N (Recommended dose)	26.30a	1.91e	8.33gh	51.20a	2.61b-d	18.27cd
75% N	20.31e	1.56f	10.58c	43.73c	2.59cd	15.46h
50% N	15.13j	1.39fg	9.13e	32.80f	2.27g	15.03i
Az. + 75% N	22.25d	2.29bc	8.89e-g	47.07b	2.36-g	18.01de
Az. + *K.* + 75% N	24.43c	2.66a	11.38b	47.80b	2.74ab	22.17a
Azosp. + 75% N	20.34e	2.02de	10.33cd	42.93c	2.43ef	15.76h
Azosp. + *K.* + 75% N	20.63e	2.38b	8.88e-g	42.63c	2.48d-f	19.94b
Az. + *Azosp.* + *K.* + 75% N	25.35b	2.75a	12.15a	48.43b	2.79a	21.89a
Az. + 50% N	18.79f	1.87e	8.95ef	38.80e	2.26g	15.47h
Az. + *K.* + 50% N	18.03gh	2.11cd	8.16h	37.70e	2.66a-c	17.77e
Azosp. + 50% N	17.50h	1.30g	8.47f-h	33.47f	2.38fg	16.58g
Azosp. + *K.* + 50% N	16.33i	2.10d	8.95ef	38.10e	2.54c-e	17.12f
Az. + *Azosp.* + *K.* + 50% N	18.16g	2.04de	9.96d	40.67d	2.69a-c	18.34c

Different letters following the data within each column mean significant difference at *P < 0.05*.

Many studies linked between biofertilizers and nutrients uptake by plants. Free-living diazotrophs such as *Azotobacter* and *Azospirillum* play an important role in providing nitrogen to the plants. *Azotobacter* species fixes about 40-200 kg N/ha, while *Azospirillum* species fixes about 20-40 kg N/ha, which can provide good amounts of nitrogen to plants (Mazid *et al.,* 2011 and Brusamarello-Santos *et al.,* 2017). Soluble P in soils is usually very low (Beever and Burns 1981), since 75-90% of added P in soil rapidly fixed in soils because it is precipitated by metal-cation complex (Sharma *et al.,* 2013), so phosphate dissolving bacteria takes its importance from its role in providing phosphate in soluble form to plants by releasing mineral dissolving compounds such as organic acids, protons, hydroxyl ions, siderophores and CO_2 (Rodriguez & Fraga, 1999 and Sharma *et al.,* 2013). The role of soil microorganisms in nitrogen and phosphorus availability to plants could explain the increases in N content in canola plants treated with *Az. chroococcum* and *Azosp. lipoferum* and the increases in P content in canola plants treated with *K. oxytoca*.

Seeds yield, oil percentage (%) and NPK concentrations in canola seeds

In line with previous results, Data in Fig. (1) Reveal that seeds yield (g/plant) of canola plants and oil percentage (%) in seeds were significantly improved with raising nitrogen levels and with the application of bacterial inoculation. The highest seeds yield (24.35 and 24.1 g/plant) achieved by plants treated with *Az. chroococcum* + *K. oxytoca* + *Azosp. lipoferum* + 75% N and plants treated with *Az. chroococcum* + *K. oxytoca* + 75% N respectively, which there were no significant differences between the two treatments. Also, data prove that using *Az. chroococcum* and *K. oxytoca* could save 25% of nitrogenous fertilizers given that plants fertilized with the recommended dose of nitrogen achieved just 21.87 g/plant.

On the other hand, plants fertilized with the recommended dose of nitrogen recorded the highest oil percentage in canola seeds, which recorded (47.47 %), while there were significant slight decreases in oil percentage in plants treated with *Az. chroococcum* + *K. oxytoca* + *Azosp. lipoferum* + 75% N and *Az. chroococcum* + *K. oxytoca* + 75% N, which recorded 46.86 and 46.85 % oil, respectively.

Our results show that bacterial inoculation increased seeds yield and % oil in canola seeds, this may be explained by their effect on plant growth discussed before as they can produce phytohormones (IAA and GA) which improve plant growth (El-Sawah *et al.,* 2018 and El-Howeity & Asfour, 2012), and also their role in providing nutrients to plants during growth stages, which reflects on the good growth of canola plants. Different species of *Azotobacter* could increase yield up to 50% in some crops such as rice, maize, sugarcane and some vegetables (Mazid *et al.,* 2011). Also, data show that *Az. chroococcum* and *K. oxytoca* have more effect than *Azosp. lipoferum*, these results disagree with El-Howeity and Asfour (2012), who revealed that *Asopirillum brasilense* gives plant seeds yield over than *Azotobacter chroococcum.*

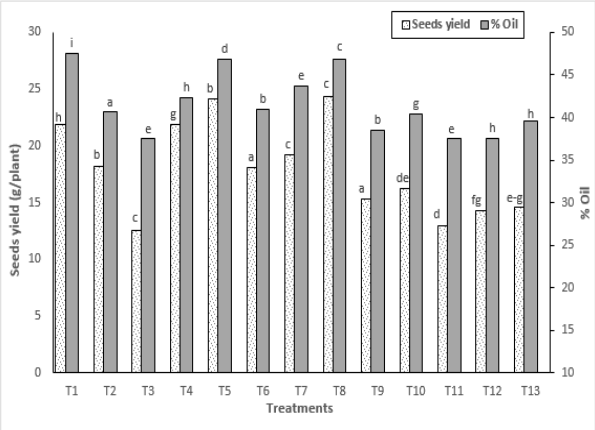


Fig .1. Seeds yield and oil percentage in seeds of canola plants treated with mineral nitrogen and bio-fertilizer

Different letters following the data within graph mean significant difference at *P < 0.05*.

T1 100% N; T2 75% N; T3 50% N; T4 *Az.* + 75% N; T5 *Az.* + *K.* + 75% N; T6 *Azosp.* + 75% N; T7 *Azosp.* +*K.* + 75% N; T8 *Az.* + *Azosp.* + *K.* + 75% N; T9 *Az.* + 50% N; T10 *Az.* + *K.* + 50% N; T11 *Azosp.* + 50% N; T12 *Azosp.* + *K.* + 50%N; T13 *Az.* + *Azosp.* + *K.* + 50%N

The contents of N, P and K in canola seeds were significantly affected with chemical and bio-fertilizer as shown in Table (6). Data show that N, P and K concentrations in canola seeds was significantly increased with raising N levels. Also, data reveal that the application of *Az. chroococcum* increased N contents, while the application of *K. oxytocca* increased P contents in canola seeds. The highest N concentrations obtained in seeds of plants treated with *Az. chroococcum* + *K. oxytoca* + *Azosp. lipoferum* + 75% N which recorded 79.13, followed by 76.57 and 75.83 mg/g in treatments of *Az. chroococcum* + 75% N and *Az. chroococcum* + *K. oxytoca* + 75% N, respectively. Otherwise, the highest P concentrations in seeds were 6.5 and 6.47 mg/g in plants treated with *Az. chroococcum* + *K. oxytoca* + 75% N and *Az. chroococcum* + *K. oxytoca* + *Azosp. lipoferum* + 75% N respectively, as they were much more than other treatments. The highest K contents in canola seeds were 9.53 and 9.1 mg/g, recorded by plants fertilized with *Az. chroococcum* + *K. oxytoca* + *Azosp. lipoferum* + 75% N and *Az. chroococcum* + *K. oxytoca* + 75% N, respectively.

Table 6. Concentrations of N, P and K (mg/g) in canola seeds treated with mineral nitrogen and bio-fertilizers

Treatments	N	P	K
100% N (Recommended dose)	71.20d	5.57b	8.75c
75% N	62.37f	4.99e	6.73g
50% N	56.43h	4.53fg	6.03h
Az. + 75% N	75.83b	5.09de	7.84e
Az. + *K.* + 75% N	76.57b	6.50a	9.10b
Azosp. + 75% N	70.47d	4.60f	8.27d
Azosp. + *K.* + 75% N	73.80c	5.59b	7.71e
Az. + *Azosp.* + *K.* + 75% N	79.13a	6.47a	9.53a
Az. + 50% N	62.77f	4.37gh	7.07f
Az. + *K.* + 50% N	65.63e	4.90e	8.17d
Azosp. + 50% N	56.97h	4.21h	6.90fg
Azosp. + *K.* + 50% N	59.40g	5.21cd	7.03f
Az. + *Azosp.* + *K.* + 50% N	67.50e	5.38bc	7.67e

Different letters following the data within each column mean significant difference at *P < 0.05*.

Bacterial counts in the rhizosphere of canola plants

Data in Tables (7&8) show the effect of mineral nitrogen and bio-fertilization on the counts of total bacteria, phosphate dissolving bacteria, *Azotobacter* spp. and *Azospirillum* spp. (c.f.u/g dry soil) in the rhizospheric region of canola plants. Data show that there was a noticeable effect of plant age and type of fertilization on the bacterial counts in the soil. Bacterial counts 70 DAS recorded high increases compared with bacterial counts 40 DAS. Data in Table (7) show that raising nitrogen doses and the addition of biofertilizers caused an increases in total bacterial counts in the rhizosphere of canola plants. On the other hand, the counts of phosphate dissolving bacteria were affected with the type of biofertilizer added as the plants were treated with *K. oxytoca* recorded noticeable increases in phosphate dissolving bacteria, compared with plants without treatment with *K. oxytoca*.

Table 7. Effect of mineral nitrogen and bio-fertilizers on total bacterial counts (10^7 cfu g^{-1}) and phosphate dissolving bacteria counts (10^4 cfu g^{-1}) in the rhizosphere of canola plants after 40 and 70 DAS

Treatments	Total bacteria		Phosphate dissolving bacteria	
	40 days	70 days	40 days	70 days
100% N (Recommended dose)	17.75	147.5	7.85	75.5
75% N	16.8	143.5	8	70
50% N	16.15	149	7.35	90.5
Az. + 75% N	19.3	161.5	9.55	81
Az. + *K.* + 75% N	18.85	183	21.1	242
Azosp. + 75% N	17.5	222	6.25	96.5
Azosp. + *K.* + 75% N	19.7	230.5	20.95	213
Az. + *Azosp.* + *K.* + 75% N	19.9	221	21	202.5
Az. + 50% N	16.55	211.5	5.05	72
Az. + *K.* + 50% N	15.1	217	19.05	211.5
Azosp. + 50% N	15.4	180.5	8.15	80
Azosp. + *K.* + 50% N	16.15	196	19.2	220.5
Az. + *Azosp.* + *K.* + 50% N	19.25	212.5	18.85	228.5

Data in Table (8) show that high counts of *Azotobacter* sp. were recorded in the rhizosphere of canola

plants treated with *Az. chroococcum*, the highest counts of *Azotobacter* spp. (253.540×10^5 cfu g^{-1}) was recoded in plants fertilized with *Az. chroococcum* + *Azosp. lipoferum* + *K. oxytoca* + 50% N, 70 DAS, followed by (193.094×10^5 cfu g^{-1}) in plants fertilized with *Az. chroococcum* + *K. oxytoca* + 50% N. Also *Azospirillum* spp. recorded high counts in canola plants treated with *Azosp. lipoferum.* The highest counts of *Azospirillum* sp. recorded in plants fertilized with *Azosp. lipoferum* + 75% N, which recorded 81.576×10^5 cfu g^{-1} soil, followed by plants fertilized with *Azosp. lipoferum* + *K. oxytoca* + 50% N, which recorded 48.681×10^5 cfu g^{-1} soil.

Table 8. Effect of mineral nitrogen and bio-fertilizers on *Azotobacter* spp. and *Azospirillum* spp. counts (10^4 cfu g^{-1}) in canola plants rhizosphere after 40 and 70 DAS

Treatments	*Azotobacter* spp.		*Azospirillum* spp.	
	40 days	70 days	40 days	70 days
100% N (Recommended dose)	0.201	18.656	0.047	0.331
75% N	0.232	4.720	0.031	0.175
50% N	0.202	4.041	0.039	0.350
Az. + 75% N	4.738	56.544	0.018	0.292
Az. + *K.* + 75% N	4.560	162.792	0.027	0.289
Azosp. + 75% N	0.235	3.369	0.452	81.576
Azosp. + *K.* + 75% N	0.276	4.004	2.346	19.576
Az. + *Azosp.* + *K.* + 75% N	3.735	160.992	1.826	12.522
Az. + 50% N	3.737	124.320	0.037	0.178
Az. + *K.* + 50% N	4.712	193.094	0.071	0.211
Azosp. + 50% N	0.234	4.246	0.452	5.060
Azosp. + *K.* + 50% N	0.232	4.057	0.596	48.681
Az. + *Azosp.* + *K.* + 50% N	5.318	253.540	2.908	25.354

Our results showed a relationship between bacterial counts in soil and canola plants' growth and yield traits. The high counts of *Azotobacter*, *Azospirillum* and *K. oxytoca* in the soil of canola plants treated with biofertilizers may explain the high values of plant morphological traits, seeds yield, oil contents, and nitrogen concentrations in plants and seeds, this is probably due to the ability of these bacteria to excrete plant growth phytohormones. On the other hand, we recorded a high N concentrations by plants that recorded high counts of nitrogen-fixing bacteria; *Azotobacter* and *Azospirillum*, which could be explained by the additional nitrogen supplies by these bacteria for plants as their high nitrogen fixation capacity. Also, high counts of phosphate dissolving bacteria in soil resulted in high P concentrations by canola plants due to the ability of *K. oxytoca* to release phosphorus in dissolved form to plants.

REFERENCES

A.O.A.C., (1995). Official methods of analysis of the association of official analytical chemists.15th Ed. Published by the association of official analytical chemists. INC. Suite 400,200 Wilson Baulevared – Arligton.Virgina 2221 USA, 69 – 90.

Abd El-Gawad, A., Hendawey, M. H., & Farag, H. I. A. (2009). Interaction between biofertilization and canola genotypes in relation to some biochemical constituents under Siwa Oasis conditions. *Research Journal of Agriculture and Biological sciences*, *5*(1), 82-96.

Abd-El-Malek, Y., & Ishac, Y. Z. (1968). Evaluation of methods used in counting azotobacters. *Journal of Applied Bacteriology*, *31*(3), 267-275.

Beever, R. E., & Burns, D. J. W. (1981). Phosphorus uptake, storage and utilization by fungi. In *Advances in botanical research* (Vol. 8, pp. 127-219). Academic Press.

Boltz, D. F., & Mellon, M. G. (1948). Spectrophotometric determination of phosphorus as molybdiphosphoric acid. *Analytical Chemistry*, *20*(8), 749-751.

Brown, J., Davis, J. B., Lauver, M., & Wysocki, D. (2008). USCA Canola Growers' Manual. *Oregon. P*, *71*.

Brusamarello, L. C., Gilard, F., Brule, L., Quillere, I., Gourion, B., Ratet, P., ... & Hirel, B. (2017). Metabolic profiling of two maize (*Zea mays* L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria *Herbaspirillum seropedicae* and *Azospirillum brasilense*. *PloS one*, *12*(3), e0174576.

Döbereiner, J. (1978). Influence of environmental factors on the occurrence of *Spirillum lipoferum* in soils and roots. *Ecological Bulletins*, 343-352.

El-Howeity, M. A., & Asfour, M. M. (2012). Response of some varieties of canola plant (*Brassica napus* L.) cultivated in a newly reclaimed desert to plant growth promoting rhizobacteria and mineral nitrogen fertilizer. *Annals of Agricultural Sciences*, *57*(2), 129-136.

El-Sawah, A. M., Hauka, F. I. A., & Afify, A. H. (2018). Dual inoculation with *Azotobacter chroococcum* MF135558 and Klebsiella oxytoca MF135559 enhance the growth and yield of wheat plant and reduce N-fertilizers usage. *Journal of Food and Dairy Sciences*, *2018*, 67-76.

Ghallab, K. H., & Sharaan, A. N. (2002). Selection in canola (*Brassica napus* L.) germplasm under conditions of newly reclaimed land. II. Salt tolerant selections. *Egypt. J. Plant Breed*, *6*(2), 15-30.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & Sons.

Gupta, A. K., & Samnotra, R. K. (2004). Effect of biofertilizers and nitrogen on growth, quality and yield of cabbage (*Brassica oleracea* var. *capitata* L.) cv Golden Acre. *Environment and Ecology*, *22*(3; SUPP), 551-553.

Hakeem, K. R., Akhtar, M. S., & Abdullah, S. N. A. (Eds.). (2016). *Plant, soil and microbes*. Springer.

Hauka, F., Afify, A., & El-Sawah, A. (2017). Efficiency Evaluation of some Rhizobacteria Isolated from Egyptian Soils, In vitro as Biofertilizers. *Journal of Agricultural Chemistry and Biotechnology*, *8*(9), 231-235.

Jackson, M. L. (1962). Soil chemical analysis, constable and Co. *Ltd. London*, *497*.

Jackson, M. L. (1973). Soil chemical analysis Prentice Hall of India Ltd. *New Delhi*, 219-221.

Kandil, A. A. (1984). Preliminary study on the effect of NPK fertilization on oil seed rape (*Brassica napus* L.) in Egypt. In *6. Congres International sur le Colza, Paris (France), 17-19 May 1983*. GCIRC.

Malhi, S. S., & Gill, K. S. (2007). Interactive effects of N and S fertilizers on canola yield and seed quality on S-deficient Gray Luvisol soils in northeastern Saskatchewan. *Canadian Journal of Plant Science*, *87*(2), 211-222.

Mazid, M., Khan, T. A., & Mohammad, F. (2011). Cytokinins, A classical multifaceted hormone in plant system. *Journal of Stress Physiology & Biochemistry*, *7*(4).

McGill, W. B., & Figueiredo, C. T. (1993). Total nitrogen. *Soil sampling and methods of analysis*, 201-211.

Megawer, E. A., & Mahfouz, S. A. (2010). Response of canola (*Brassica napus* L.) to biofertilizers under Egyptian conditions in newly reclaimed soil. *International Journal of Agriculture Sciences*, *2*(1), 12-17.

Mohamed, S. M., Mohamed, H. M., Shahata, H. M., & Ahmed, H. M (2017). Growth, Yield and Yield Components of Canola Crop (*Brassica napus*) in El-Kharga Oasis New Valley as affected by bio, Nitrogen and Phosphorus Fertilization. *Assiut Journal of Agricultural Sciences, 48, 319-330*

Naderifar, M., & Daneshian, J. (2012). Effect of different nitrogen and biofertilizers effect on growth and yield of *Brassica napus* L. *International Journal of Agriculture and Crop Sciences (IJACS)*, *4*(8), 478-482.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, *17*, 362-370.

Rodríguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology advances*, *17*(4-5), 319-339.

Sharaan, A. N. (1986). Variation in character expression in rapeseed (*Brassica napus* L.) cultivars in relation to environmental changes. *Bull. Fac. of Agric. Univ. of Cairo*, *37*(1), 35-48.

Sharaan, A. N., Ghallab, K. H., & Yousif, K. M. (2002). Performance and water relations of some rapeseed genotypes grown in sandy loam soils under irrigation regimes. *Annals of Agric. Sc., Moshtohor*, *40*(2), 751-767.

Sharifi, R. S. (2012). Study of yield, yield attribute and dry matter accumulation of canola (*Brassica napus* L.) cultivars in relation to sulfur fertilizer. *International Journal of Agriculture and Crop Sciences (IJACS)*, *4*(7), 409-415.

Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M., & Sharma, R. (2007). Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. *Journal of Herbal Medicine and Toxicology*, *1*(1), 61-63.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, *2*(1), 587.

Shekh, B. A. (2006). Biotechnology and biofertilization: Key to sustainable agriculture. *Scientific Issue,(1) Das, K., R. Dang, TN*.

Turan, M., Ataoğlu, N., & Şahın, F. (2006). Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *Journal of Sustainable Agriculture*, *28*(3), 99-108.

Weiss, E. A. (1983). *Oilseed crops*. Longman Group, 660.

Yasari, E., & Patwardhan, A. M. (2007). Effects of (*Azotobacter* and *Azosprillium*) inoculants and chemical fertilizers on growth and productivity of canola (Brassica napus L.). *Asian Journal of Plant Sciences*, *6(1), 77-82*.

Yasari, E., Azadgoleh, A. E., Pirdashti, H., & Mozafari, S. (2008). *Azotobacter* and Azospirillum inoculants as biofertilizers in canola (*Brassica napus* L.) cultivation. *Asian Journal of Plant Sciences, 7(5), 490-494*.

Yasari, E., Azadgoleh, M. E., Mozafari, S., & Alashti, M. R. (2009). Enhancement of growth and nutrient uptake of rapeseed (*Brassica napus* L.) by applying mineral nutrients and biofertilizers. *Pakistan Journal of Biological Sciences*, *12*(2), 127-133.

التأثير المكمل للسماد الحيوى مع التسميد النيتروجينى المعدنى على إنتاجية نبات الكانولا

محمد عبدالله العوضى سليم و صابرين أحمد عمر

قسم الميكروبيولوجيا الزراعية، كلية الزراعة، جامعة المنصورة، مصر

تستخدم الأسمدة الحيوية مؤخرا بصورة كبيرة للتقليل من استخدام الأسمدة الكيماوية وأثارها الملوثة على التربة والماء. فى هذا العمل تم زراعة تجربة أصص خلال صيف 2018 لدراسة تأثير الأنواع الميكروبية الأزوتوباكتر كروكوكام والأزوسبيريللم ليبو فيرام والكليبسييلا أوكسيتوكا مع مستويات مختلفة من السماد النيتروجينى على نمو وإنتاجية نبات الكانولا، وكذلك تأثيرهم على المجتمع الميكروبى فى رايزوسفير نباتات الكانولا. وقد أوضحت النتائج أن الأنواع الميكروبية المستخدمة كلقاح حيوى كان لها تأثير كبير على الصفات المورفولوجية والمحصولية لنبات الكانولا، حيث تميزت النباتات المعاملة بالأزوتوباكتر والأزوسبيريللم والكليبسييلا بزيادات ملحوظة فى أطوال وأوزان النباتات وعدد التفرعات والقرون فى النبات الواحد. كما كان لميكروبى الأزوتوباكتر والأزوسبيريللم تأثيرا ملحوظا فى زيادة مستوى عنصر النيتروجين، بينما لميكروب الكليبسييلا تأثيرا ملحوظا فى زيادة مستويات عنصر الفوسفور فى النباتات والبذور. ووفقا لنتائجنا فقد حققت المعاملة باللقاح الحيوى الثلاثى (الأزوتوباكتر والأزوسبيريللم والكليبسييلا) مع 75% من السماد النيتروجينى (33.75 كجم نيتروجين / فدان) أعلى إنتاج فى محصول البذور حيث حققت (24.35 جرام بذور / نبات) فى حين كانت نسبة الزيت فى بذور الكانولا مع هذه المعاملة هى 46.86%، والتى لم تكن النسبة الأعلى إلا أنها كانت قريبة جدا من أعلى نسبة للزيت والتى سجلت 47.47% فى بذور النباتات المسمدة بالكمية الموصى بها من التسميد النيتروجينى. من ناحية أخرى فقد أدى استخدام اللقاح الحيوى فى تنشيط المجتمع الميكروبى فى التربة وتسبب فى زيادة العدد الكلى للبكتيريا فى رايزوسفير نباتات الكانولا وأعداد البكتيريات المثبتة لنيتروجين الهواء الجوى (الأزوتوباكتر والأزوسبيريللم) والبكتيريا المذيبة للفوسفات المعدنية فى التربة.