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Interaction Between Biofertilization and Canola Genotypes in Relation to Some Biochemical Constituents under Siwa Oasis Conditions

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Abstract: In order to study the response of canola to biofrtilization using *Azotobacter chroococcum* as free living nitrogen fixing bacteria and *Bacillus megatherium* as phosphate dissolving bacteria (PDB) in new cultivated sandy soil, two field experiments were conducted under salt affected soil of Tegzerti experimental farm, Siwa Oasis, Matrouh, D.R.C. during 2005/06 and 2006/07 seasons. The traditional organic manure (sheep manure) was used as a base treatment, while two bacterial strains were used either individually or in combination together. The soil microbial parameters were determined at vegetative and harvesting stages of both seasons as total microbial counts, azotobacters and phosphate dissolving bacterial counts and soil nitrogen. The data revealed to the almost importance of engaging biofertilization with organic manure in unified bio-organic treatment. The order of strain influences on crop yield and bacterial count arranged as follows mixed treatment with both microorganisms gave the highest response followed by single treatment with *Azotobacter chroococcum* or *Bacillus megatherium* but the lowest effects were recorded in the control. The differences among genotypes were highly significant for all studied characters. The two newly bred lines 56/16 and 53/9 exhibited high mean performances for growth, yield and yield components i.e., Plant height (cm), number of branches/plant, No. of pods/plant, 1000-seed weight (g), Seed yield/plant (g) under mixed inoculation treatment with biofertelizers in the both seasons and combined data. Data show that mixed inoculation treatment increased oil content in all canola genotypes. Also, line 56/16 gave the maximum value of oil content after treatment with mixed inoculation. In this regard, the positive effect of biofertilizers (mixed inoculation) on oil quality is an expected result for its effect on improving physical and chemical properties of oil. Also, line 56/16 seemed to be the best genotypes in physical and chemical properties and oil content. Glutamic acid is the most abundant amino acid in all canola genotypes, followed by proline, leucine and lysine. The maximum value of glutamic acid was obtained from Line 53/9 with application of mixed inoculation. The highest value of proline content was recorded by Line 56/16 treated with mixed inoculation. The saturated fatty acids in all canola genotypes were caproic, lauric, myristic, palmitic and stearic. In this respect, application of mixed inoculation treatment decreased caproic, lauric, myristic in oil of line 56/16 and line 53/9. The predominant unsaturated fatty acid (oleic) was increased in oil of Pactol, line 56/16 and line 53/9 after treatment with mixed inoculation. Concerning the erucic acid content, it was decreased in oil of all canola genotypes after treatment with mixed inoculation. In addition, the decrease of erucic in oil of all canola genotypes under mixed inoculation gave also a good indication of its quality.

Key words: New reclaimed land, farmyard manure, biofertilization, *Azotobacter chroococcum, Bacillus megatherium* canola production, oil content, Physical and chemical properties, fatty acids, amino acids.

INTRODUCTION

In recent years Egypt is being faced by the problem arising from the shortage in local production of edible oil as compared the rate of their consumption. The wide gap between the production and consumption of edible oil reached to 90%, which has created a need for importation. It has been an urgent need for agricultural expansion in new locations out of Nile Valley and Delta.

Canola, family Brassicaceae, is a name applied to edible oil seed rape, which developed from two species

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Brassica napus and *B. campestris*. The two species have been widely cultivated as oil seed crops, containing about 40% oil and 23% protein[19]. Canola (*Brassica napus* L.) is one of the important oil crops all over the world. It has the third position in world oil production crops, second position in total world area for oil crops and the fifth in world international trade crops. During the last decade an intensive work has been carried out to grow rapeseed as a new oil crop in Egypt concerning with the high quality seed and oil. The development of new cultivars adapted to the local conditions with improved quality has been a major factor in the success of rapeseed[31]. Also, the recent varieties contain a low level of both erucic acid in oil and glucosinolate in meal. The erucic acid appeared to be the main factor which decreased digestibility and nutritive value of canola oil. Recently, biofertilization is the most important factor affecting the yield, yield components and biochemical constituents.

During the late 19 and early 20 centuries inorganic compounds containing nitrogen, potassium and phosphorus (NPK) were synthesized and used as fertilizers. Due to the growth in human populations fertilizers were used to increase crop production and meet the rising demands for food. Increases in the production cost, and the hazardous nature of chemical fertilizers for the environment has led to a resurgence of interest in the use of biofertilizers for enhanced environmental sustainability, lower cost production and good crop yields. Plant growth-promoting rhizobacteria enhance plant growth either by direct or indirect mechanisms[27]. Plant growth promoting rhizobacteria, PGPR (e.g. *Azotobacter chroococcum* as free living nitrogen fixing bacteria and *Bacillus megatherium* as phosphate dissolving bacteria) that have been successful in promoting the growth of crops such as canola, soybean, lentil, pea, wheat and radish have been isolated[46].

Rhizobacteria were isolated from the rhizosphere of different *Brassica* species and assayed for their ability to produce auxins in vitro. The isolates varied greatly in their potential for auxin production (ranging from 0.33 to 11.40 µg ml-1). Results showed that seed inoculation with different isolates of rhizobacteria significantly increased plant height (up to 56.5%), stem diameter (up to 11.0%), number of branches (up to 35.7%(, number of pods per plant (up to 26.7%), 1,000-grain weight (up to 33.9%), grain yield (up to 45.4%) and oil content (up to 5.6%) over the uninoculated control. It was hypothesized that these PGPR may influence the growth and yield of inoculated plants by production of auxins in the rhizosphere of inoculated plants from the L-TRP present in th root exudates, although other mechanisms of action might have also contributed[8]. The positive effects of PGPB on plant growth are always correlated with remarkable changes in root morphology, namely increased lateral root length and root hair number and length[11,12]. It is generally assumed that these developmental responses are triggered by phytohormones produced by the bacteria[12,37].

The present study aimed to investigate the response of the two newly bred lines compared with Serw4 and Pactol under different biofertilization treatments and its effect on growth characters, some chemical constituents, yield, yield components and soil microbial activities under Siwa Oasis conditions.

MATERIALS AND METHODS

Field experiments: This research work was conducted under salt affected soil of Tegzerti experimental farm, Siwa Oasis, Matrouh, D.R.C.during 2005/06 and 2006/07 seasons. The two field experiments included canola genotypes; the Egyptian variety (Serw,4), the French variety (Pactol) and two newly bred lines released through canola breeding program of Desert Research Center i.e. line 53/9 (C103/Sedo*2 C103 9C-6Su-1Su-13Sw-2Sw-0Sw) and line 56/16 (Cesor /Duplo 18C-21Su-4Sw-15Sw-1Sw-0Sw) as reported by [5,21]. Such genetic material treated by biofertilizers and were arranged in a split plot design in four replications. The four genotypes were allocated randomly in the main plots, while, biofertilizer treatments were distributed randomly in the sub-plots. Every sub-plot area was 12 m^2 (1/350 fed.; contained 4 lines with 60 cm width and 5 m length). The physical and chemical properties of soil sample taken from the experimental site to depth 0-60cm was analyzed as well as analysis of irrigation water (average over five irrigations) according to[17]. during each growing season was made (Table1). Phosphatic fertilizer as calcium superphosphat (15.5% P_2O_5) was added at a rate of 100 kg /fed. during seed bed preparation, 50 Kgm of potassium sulphate (50.0% K_2SO_4) was added at flowering stage, whereas nitrogen fertilizer was applied as ammonium sulfate (20.5% N) at rate of 24.0 kg/fed.(half of recommended dose) where 1/3 of the amount was incorporated in dry soil before sowing, 1/3 was added one month after sowing and the rest was added one week pre flowering stage. The data collected were subjected to the ordinary analysis of variance of the split plot design on individual plant mean basis outlined by[43]. Treatment means were compared using the new least significant difference (L.S.D.) test shown by[48]. at the 5% level.

The following data were recorded; Plant height (cm), Number of branches/plant, No. of pods/plant, 1000-seed weight (g), Seed yield/plant (g).

Biofertilization treatment: For N_2-fixing diazotrophs and Phosphate dissolving bacteria (PDB), different soil samples were collected from different sites of Tegzerti experimental farm at Siwa Oasis used for isolation. 15 *Azotobacter* isolates were isolated as shown in Table (2) The highest isolates for nitrogen fixation according to modified Keldahl method after[17]. isolate 9 was selected purified and identified as *Azotobacter chroococcum* according to[10]. and used as nitrogen fertilization, but for Phosphate dissolving bacteria (PDB), 7 isolates were isolated, the highest isolate for Phosphate solubilization[36]. isolate 4 was selected purified and identified as *Bacillus megatherium* according to[10].

The selected isolates (*A. chroococcum* and *B. megatherium*) were subjected to different biochemical tests for screening their activities toward production of Phytohormones by[45]. Antimicrobial substances[29]. Enzymes[32]. as shown in Table (3).

Fresh liquid culture of *A. chroococcum* and *B.megatherium* were used for soil inoculation single or in combinations at the rate of ≈ 10^8 colony forming unit (cfu)/ml.

Rhizosphere soil sample were collected at different stages of plant growth and analyzed for: total microbial counts on Bunt and Rovira medium[16]. Bunt and Rovira medium, modified by[44]. was used for counting inorganic phosphate-dissolvers, *Azotobacter* on nitrogen deficient medium[3].

Chemical analysis: Chemical analysis were applied over the two seasons for the best biofertilization treatment (mixed inoculation) comparing to control after recording field data as follows:

Oil extraction: The air-dried canola seeds were milled twice. The fine powdered samples were pressed with laboratory-type of Carver hydraulic press under 10.000Ib/in (pci) pressure for 1 hour at room temperature according to the method outlined by Ustum *et al.*[47]. The produced oils were filtrated and kept in dark bottles in the refrigerator till analysis.

Oil content (total lipids): The crude oil contents in samples were determined according to the procedure described by[1]. by extracting with n-hexane (b.p. 60-70°C) using Soxhlet apparatus.

Physical and chemical properties of canola oil: Refractive index, acid value, peroxide value and iodine value were estimated according to[1].

Determination of amino acids: Samples of canola seeds were dried ground to be used to determine content of amino acids in their hydrolysates as reported by[13]. In the same hydrolysates, samples of amino acids were injected in amino acid analyzer apparatus model (Eppendrof LC 3000). The peak area and percentage of each amino acid were calculated by computer software AXXIOM CHROMATOGRAPHY-727.

Table 1: Physical and Chemical properties of the experimental soil, sheep manure and water irrigation analyses.

Physical properties			Chemical Properties										
Coarse sand	Silt and clay	Soil texture	pH	E.C ds/m	O.M %	Soluble cations (meq/L)				Soluble anion (meq/L)			$CaCO_3$ %
						K^+	Na^+	Mg^{++}	Ca^{++}	HCO_3^-	Cl^-	SO_4	
			7.5	12.32	0.7	1.47	69.6	17.24	34.7	2.45	85.4	35.8	18.2
75.9	24.1	Sandy loam	Water irrigation analysis										
			7.3	4.01	-	0.48	21.5	9.08	8.69	10.3	20.5	8.74	
Sheep manure analysis													
Organic Carbon%		Nitrogen %	C/N		pH	P	K	Fe		Mn	Zn	Cu	
						ppm							
19.46		1.4	13.9		7.6	17	89	371		47	21	5.8	

Table 2: Screening for N_2 fixation by *Azotobacter* isolates and Phosphate solubilization by *Bacillus* isolates.

Azotobacter isolates	N_2 fixation (ppm)	*Azotobacter* isolates	N_2 fixation (ppm)
1	23	8	41
2	19	9	72
3	11	10	58
4	8	11	24
5	27	12	30
6	36	13	16
7	14	14	51
8	48	15	20
Bacillus isolates	P.solubilization Clear zone diameter(cm)	*Bacillus* isolates	P.solubilization clear zone diameter(cm)
1	0.8	5	0.3
2	0.5	6	0.7
3	1.4	7	0.4
4	2.2		

Determination of fatty acids: The fatty acids of the oil were converted to methyl esters using method according to[24]. Methyl esters of fatty acids were separated by using gas liquid chromatography (GLC).

Table 3: Biochemical activities of selected *Azotobacter chroococcum* and *Bacillus megatherium.*

Test	*A.chroococcum*	*B. megatherium*
N_2 Fixation	72	-
P.solubilization	0.9	2.2
Hormone production		
IAA	0.22	0.17
GA3	2.8	1.39
Cytokinie	23.7	12.1
Enzyme production		
Amylase	+	+
Celluolase	-	+
Phosphatase	+	+
Nitrogenase	+	-
Antimicrobial activity		
a)antibacterial activity (Inhibition zone mm)		
E.coli	29	32
S.aureus	11	15
S.typhi	36	25
C.albicans	18	37
b)antifungal activity		
F.oxysporum	27	30
A.solani	23	45
R.solani	19	35

RESULTS AND DISCUSSION

The test of homogeneity of error variance made using error mean squares of the two seasons revealed that error mean squares are homogeneous for all the studied traits. In such case combined analysis over seasons is expected. Mean squares of analysis of variance in the two seasons 2005/06 and 2006/07 for the investigated characters of canola genotypes are given in table (4) and combined in table (5). Significant mean squares due to biofertilization (F.) canola genotypes (G.) and (G.×F.) were detected for all the studied characters in the two growing seasons and combined except for; No. of branches under (G.×F.) in the both seasons and for all studied traits (F.XY., G.×Y. and G.× F. ×Y.) in the combined indicating that genotypes varied in their response to biofertilization treatments under Siwa oasis conditions.

Growth, yield and yield components: The mean performances of four varieties and/or lines of canola under experimental conditions in the two seasons and combined are presented in table (6). The differences among genotypes were highly significant for all studied characters (Table6). For plant height, Line 56/16 and Line 53/9 under mixed inoculation treatment with biofertelizers was the tallest genotype in both seasons (139.53cm. for Line 56/16 in the second season to 142.80cm. for Line 53/9 in the first season) and combined which recorded 140.60 for Line 56/16 and 141.87 for Line 53/9. This trend was also observed on number of branches/plant, Line 56/16 recorded the highest values under mixed inoculation treatment in the both seasons and combined (16.25,16.16 and 16.21, respectively) followed by *Azotobacter chroococcum* treatment which recorded 13.91, 14.03 and 13.97 for the both seasons and combined respectively.

Significant differences in the number of pods per plant were observed amongst the different biofertilizer treatments and genotypes. The number of pods per

plant increased linearly with mixed inoculation treatment followed by *Azotobacter chroococcum* treatment in the both seasons and combined for different genotypes. While Line 56/16 recorded the highest values under mix treatment in the both seasons and combined followed by Line 53/9 under mixed inoculation treatment, Line 56/16 and Line 53/9 with *Azotobacter chroococcum* treatment, respectively which had values ranging from 369.14 for Line 53/9 to 389.94 for Line 56/16 in the second season (Table, 6). These results are consistent with those reported by [35,18].

1000-seed weight was significantly increased especially with mixed inoculation treatment for all genotypes under study. Line 56/16 recorded the highest values under mix treatment followed by Line 53/9 under mixed inoculation treatment and Line 56/16 with *Azotobacter chroococcum* treatment which recorded values i.e., 4.08, 3.84 and 3.73 gm, respectivley (Table 6).

Seed yield/plant and oil yield content (%) of the newly bred lines 56/16 and 53/9 recorded the highest seed yield/plant and oil yield content under different biofertilization treatments. Also, Line 56/16 recorded the highest values followed by Line 53/9 with mixed inoculation for both traits followed *Azotobacter chroococcum* treatment for seed yield/plant and PDB for oil yield content (%). which had values ranging from 23.76 for Line 53/9 under *Azotobacter chroococcum* treatment to 25.94 for Line 56/16 under mixed inoculation treatment. Similar results were obtained by[12,41,49].

Microbiologicl determinations:

a- Total microbial counts: Initial total microbial counts in soil was 18×10^5 cfu/g dry soil. Results in Table (7) showed the change in counts which tend to increase in all treatments compared to the control. Also, second season recorded higher counts than first season where as vegetative stage better than harvesting stage in total microbial counts.

A mixed inoculation with *A. chroococcum* and *B. megaterium* produced the highest increase in the total microbial counts in vegetative stage of second season reached 84×10^5 cfu/g dry soil for Line 56/16 followed descendingly by Line53/9, Serw, 4 and Pectol which recorded 80, 79 and 73 $\times10^5$ cfu/m dry soil respectively. Similarly, Abd El-Gawad, 2008 reported that microbial inoculants increase the number and biological activities of desired microorganisms and improve the fertility in the root zone.

b- *Azotobacter* counts: The initial counts of azotobacters in soil were 6.2×10^3 cfu/g dry soil. Data recorded in Table (7) showed that the counts in vegetative stage were higher than harvesting stage in the first season. The same trend was recorded in the second season. The counts under a mixed inoculation treatment with *A. chroococcum* and *B. megaterium* showed the highest counts in vegetative stage of second season for Line 56/16 followed in descending order by Line 53/9 and Serw,4 while Pectol showed the least increment of azotobacters counts recorded 43, 36, 30 and 26 $\times10^3$ c.f.u./g dry soil . Also, A mixed inoculation treatment of *A. chroococcum* and *B. megaterium* reported highest counts compared with single treatments with *A. chroococcum or B. megatherium* which caused the least increasement all over the experimental periods. The promoting effect due to application of *A. chroococcum* not only due to the nitrogen fixation but also to the production of plant growth promoting substances, production of amino acids, organic acids, vitamins and antimicrobial substances as well, which increase soil fertility, microbial community and plant growth[42].

c - Phosphate dissolving bacterial counts (PDB counts): Data in Table (7) showed that the counts of phosphate dissolving bacteria under a mixed inoculation treatment with *A. chroococcum* and *B. megaterium* gave the highest counts during vegetative stage of second season for Line 56/16 followed by Line 53/9, Serw,4 and Pectol in descending order 10.3 , 9.9 , 8.6 and 8 $\times10^2$ cfu/g dry soil respectively. It is worthy to notice that the initial count of phosphate dissolving bacteria *B. megaterium* in soil was 3.4×10^2 cfu/g of dry soil. A mixed inoculation treatment with *A. chroococcum* and *B. megaterium* gave a synergistic effects on increasing densities of phosphate dissolving bacterial counts which increased the availability and mobility of phosphorous and other plant nutrients from soil to plant through production of organic acids these effects revealed on increase of plant growth, yield and oil yield quantitatively and qualitatively. This agree with[26,28].

d-Soil nitrogen: Data presented in Table (8) showed results of the soil total nitrogen in all treatments during vegetative and harvesting stages of two seasons. The data indicated that inoculation process increased the total nitrogen, the slight increase under phosphate dissolving bacteria inoculation may be due to the release of phosphorus which stimulate N_2 fixation by native microorganisms. A mixed application treatment of *A. chroococcum* and *B. megaterium* caused highest increase in soil total nitrogen compared with single treatment with *A. chroococcum or B. megatherium* all over the experimental periods. Thus, *A. chroococcum* enriched the soil by nitrogen fixation which increased

soil fertility. In the present investigation, a mixed inoculation treatment with *A. chroococcum* + *B. megatherium* gave the highest soil total nitrogen in vegetative stage of second season for Line 56/16 followed by Line 53/9, Serw,4 and Pectol in descending order recoded 297, 239, 217 and 206 ppm respectively. This result is compatible with the finding of[15,23].

Table 4: Mean squares of bioertilization (F.) and Canola genotypes (G.) for different studied traits under Siwa Oasis conditions in 2006/07 and 2007/08 seasons.

Season	First season					Second season				
Mean Square	F.	error	G.	G.×F.	error	F.	error	G.	G.×F.	error
d.f.	3	6	4	9	24	3	6	4	9	24
Plant height (cm.)	412.11**	6.30	1174.44**	26.44**	6.26	367.16**	7.444	1127.82**	32.36**	5.150
Branches no./plant	63.64**	0.469	66.130**	0.605	0.87	68.90**	0.443	63.32**	0.353	1.129
Pods no./plant	11149.92**	19.45	7586.43**	155.02**	27.50	11020.53**	28.96	7669.28**	247.85**	23.97
1000- Seed weight (gm)	0.157**	0.020	0.315**	0.0172**	0.114	0.1594**	0.041	0.3164**	0.038**	0.142
Seed yield/plant (gm)	25.82**	0.311	30.29**	0.668**	0.274	19.48**	0.529	27.06**	0.831*	0.309
Oil yield %	26.19**	0.135	31.38**	26.20**	0.053	26.19**	0.126	31.38**	26.20**	0.065*

and **: Denote significance at P £ 0.05 probability level.

Table 5: Mean squares of bioertilization (F.) and Canola genotypes (G.) for different studied traits under Siwa Oasis conditions as combined data. over the two years (y)

Combined									
Mean Square	Y.	F.	F.XY.	error	G.	G.×F.	G.×Y.	G.× F. ×Y.	error
d.f.	3	6	4	9	24	3	6	4	9
Plant height (cm.)	76.17**	775.00**	4.275	3.87	2301.94**	57.70**	0.316	1.104	5.706
Branches no./plant	0.204	132.43**	0.111	0.46	129.40 **	10.91*	0.054	0.047	0.700
Pods no./plant	93.56	22097.22**	73.24	124.21	15186.45**	455.12**	69.26	147.76	125.74
1000- Seed weight (gm)	3.57**	0.3126*	0.004	0.170	0.62**	0.066*	0.010	0.019	0.072
Seed yield/plant (gm)	11.52**	44.98**	0.312	0.320	57.24**	1.436*	0.113	0.163	0.291
Oil yield %	0.34**	0.028**	0.06	0.003	0.28**	0.016**	0.002	0.004	0.012

Table 6: Mean performance of 4 Canola genotypes (G.) under 3 biofertilizer treatments at Siwa in 2005/06and 2006/07 seasons and their combined data

Characters		Plant Height (cm)			No. of Branches/Plant			No. of Pods/Plant		
Genotypes	Biofertilizer treatments	1st	2nd	Combined	1st	2nd	Combined	1st	2nd	Combined
Line56/16	Mix	141.67	139.53	140.6	16.25	16.16	16.21	383.93	389.94	386.94
	Azotobacter	136.37	131.64	134	13.91	14.03	13.97	376.4	377.7	377.05
	PDB	123.17	120.38	121.77	11.46	11.95	11.71	355.35	348.77	352.06
	Control	118.57	116.12	117.34	9.71	10.21	9.96	343.94	337.33	340.64
Mean		129.94	129.95	126.92	128.43	12.83	13.09	12.96	364.91	363.44
Line 53/9	Mix	142.8	140.95	141.87	12.07	12.18	12.13	382.2	378.99	380.59
	Azotobacter	137.93	137.93	137.93	10.17	10.14	10.16	372.18	369.14	370.66

Table 6:Continue

	PDB	122.15	120.46	121.31	8.22	8.3	8.26	328.53	322	325.26
	Control	113.87	112.82	113.34	7.92	7.88	7.9	318.97	315.9	317.44
Mean		129.19	129.19	128.04	128.61	9.60	9.63	9.61	350.47	346.51
Serw-4	Mix	127.6	125.66	126.63	11.18	11.27	11.22	333.35	326.68	330.02
	Azotobacter	125.47	123.82	124.65	9.49	9.21	9.35	328.19	321.61	324.9
	PDB	100.11	105.98	103.05	5.03	6.81	5.92	299.52	297.9	298.71
	Control	112.78	111.38	112.08	5.86	5.83	5.85	275.64	271.22	273.43
Mean		120.63	116.49	116.71	116.60	7.89	8.28	8.09	309.18	304.35
Pactol	Mix	127.94	126.39	127.16	10.38	10.64	10.51	334.87	328.34	331.61
	Azotobacter	122.21	120.39	121.3	8.37	8.39	8.38	309.26	305.42	307.34
	PDB	114.39	112.78	113.59	6.33	6.37	6.35	291.93	287.88	289.9
	Control	109.29	108.15	108.72	5.46	5.69	5.58	275.97	269.65	272.81
Mean		118.46	118.46	116.93	117.69	7.64	7.77	7.71	303.01	297.82
Mean overall		124.56	123.52	122.15	122.83	9.49	9.69	9.59	331.89	328.03
LSD5%	Y.			0.87			N.S.			N.S.
	F.	2.11	1.91	1.39	0.76	0.61	0.49	4.41	12.61	6.51
	F.XY.			N.S.			N.S.			N.S.
	V.	2.51	1.20	1.24	0.68	0.67	0.42	4.42	15.12	7.01
	V.XF.	4.22	1.91	2.77	N.S.	N.S.	N.S.	8.84	25.22	13.02
	V.XY.			N.S.			N.S.			N.S.

Characters		1000 - Seed weight (gm.)			Seed yield/Plant			Oilyield %		
Genotypes	Biofertilizer treatments	1st	2nd	Combined	1st	2nd	Combined	1st	2nd	Combined
Line56/16	Mix	4.26	3.9	4.08	26.83	25.06	25.94	44.82	43.92	44.37
	Azotobacter	3.9	3.56	3.73	24.95	24.28	24.62	42.8	41.17	41.99
	PDB	3.79	3.25	3.52	24.06	23.06	23.56	43.9	42.9	43.4
	Control	3.66	3.33	3.5	23.29	22.72	23	41.8	40.71	41.26
Mean		364.17	3.90	3.51	3.71	24.78	23.78	24.28	43.33	42.18
Line 53/9	Mix	3.98	3.69	3.84	25.88	25.18	25.53	43.28	44.13	43.71
	Azotobacter	3.87	3.43	3.65	24	23.53	23.76	41.69	42.13	41.91

Table 6:Continue

	PDB	3.68	3.2	3.44	23.55	23.05	23.3	42.88	43.67	43.28
	Control	3.61	3.32	3.47	22.45	21.62	22.04	40.69	39.52	40.11
Mean		348.49	3.79	3.41	3.60	23.97	23.35	23.66	42.14	42.36
Serw-4	Mix	3.8	3.4	3.6	24.82	24.15	24.49	41.28	42.54	41.91
	Azotobacter	3.72	3.4	3.56	23.3	22.36	22.83	40.66	41.18	40.92
	PDB	3.42	3.22	3.32	25	22.25	23.63	41	41.87	41.44
	Control	3.52	2.99	3.25	21.3	20.56	20.93	39.62	40.12	39.87
Mean		306.77	3.62	3.25	3.43	23.61	22.33	22.97	40.64	41.43
Pactol	Mix	3.8	3.23	3.51	23.44	23.07	23.26	43.92	44.16	44.04
	Azotobacter	3.69	3.42	3.56	22.74	22.19	22.47	42.25	41.61	41.93
	PDB	3.6	3.3	3.45	20.22	19.96	20.09	43.6	43.22	43.41
	Control	3.55	3.25	3.4	19.07	18.43	18.75	41.85	40.95	41.4
Mean		300.42	3.66	3.30	3.48	21.37	20.91	21.14	42.91	42.49
Mean overall		329.96	3.74	3.37	3.56	23.43	22.59	23.01	42.25	42.11
LSD5%	Y.			N.S.			0.25			N.S.
	F.	0.02	0.03	0.38	0.23	0.60	0.31	0.20	0.21	0.26
	F.X.Y			N.S.			N.S.		N.S.	
	V.	0.03	0.08	0.18	0.33	0.73	0.36	0.35	0.37	0.15
	V.X.F.	0.06	0.06	0.16	0.46	0.51	0.63	0.39	0.36	0.16
	V.X.Y.			N.S.			N.S.		N.S.	

Azotobacter: *Azotobacter chroococcum*, **PDB:** *Bacillus megatherium.*, **Mix:***Azotobacter chroococcum +Bacillus megatherium*

Table 7: Effect of biofertilization and Canola genotypes on microbial determinations in rhizosphere of canola during stages of plant growth at two seasons.

Characters		Total microbial count (counts $\times 10^6$ cfu/g dry soil)				*Azotobacter* counts(counts $\times 10^3$ cfu/g dry soil)				PDB counts (counts $\times 10^2$ cfu/g dry soil)			
Genotypes	Biofertilizer treatments	First season		Second season		First season		Second season		First season			Second season
		Vegetative	Harvesting	Vegetative	Harvesting	Vegetative	Harvesting	Vegetative	Harvesting	Vegetative	Harvesting		Vegetative Harvesting
Line56/16	Mix	82	62	84	63	38	29	43	30	10.2	9.7	10.3	9.5
	Azotobacter	64	58	72	56	34	27	35	28	8.8	8.1	9.1	8.2
	PDB	59	47	65	51	21	15	24	19	9.4	8.9	9.5	8.8

Table 7:Continue

	Control	47	41	49	39	19	14	23	16	7.9	7	8.2	7.4
	Mean	63	52	67.5	52.25	28	21.25	31.25	23.25	9.075	8.43	9.23	8.48
Line 53/9	Mix	74	55	80	63	32	26	36	28	9.6	8.5	9.9	8.7
	Azotobacter	61	49	66	53	29	21	31	23	7.5	6.6	7.9	6.8
	PDB	57	45	62	51	17	13	20	14	8.4	7.9	8.7	8
	Control	44	35	48	36	16	11	18	12	7.1	6.3	7.4	6.7
	Mean	59	46	64	50.75	23.5	17.75	26.25	19.25	8.15	7.32	8.5	7.55
Serw-4	Mix	71	54	79	57	28	22	30	23	8.1	7.5	8.6	7.8
	Azotobacter	56	42	62	47	24	18	26	20	6	4.4	6.3	4.7
	PDB	51	39	57	44	16	11	19	13	7.2	6.4	7.6	6.5
	Control	40	33	43	37	13	7	17	9	5.7	4.2	5.9	4.3
		54.4	42	60.25	46.25	20.25	14.5	23	16.25	6.75	5.63	7.1	5.83
Pactol	Mix	67	50	73	53	25	19	26	21	7.8	6.9	8	7.3
	Azotobacter	52	38	59	45	18	12	21	14	5.9	4.6	6.1	4.5
	PDB	46	34	53	39	14	9	15	10	6.9	6.3	7.4	6.2
	Control	39	31	41	35	10	6	11	8	5.2	3.9	5.5	4
	Mean	51	38.25	56.5	43	16.75	11.5	18.25	13.25	6.45	5.42	6.75	5.5
LSD5%	F.	1.47	1.04	0.90	0.76	1.24	1.07	0.99	1.03	0.24	0.21	0.23	0.18
	V.	1.61	1.79	1.15	0.93	1.14	0.82	1.10	1.05	0.26	0.31	0.35	0.33
	V.XF.	2.95	2.08	1.81	1.53	2.48	2.15	1.98	2.05	0.48	0.41	0.46	0.37

Table 8: Effect of biofertilization and canola genotypes on soil total nitrogen of canola during stages of plant growth at two seasons

Characters		Total nitrogen in soil (ppm)			
Genotypes	Biofertilizer treatments	First season		Second season	
		Vegetative	Harvesting	Vegetative	Harvesting
Line56/16	Mix	257	229	297	262
	Azotobacter	195	174	211	185
	PDB	151	137	168	145
	Control	138	121	146	136

Table 8:Continue

Mean		185.25	162.25	205.5	182
Line 53/9	Mix	224	171	239	205
	Azotobacter	183	165	194	168
	PDB	143	133	157	136
	Control	131	110	139	127
Mean	170.25	144.75	182.25	159	
Serw-4	Mix	195	168	217	188
	Azotobacter	168	141	175	147
	PDB	142	128	156	139
	Control	125	103	132	119
		157.5	135	170	148.25
Pactol	Mix	184	159	206	175
	Azotobacter	149	126	166	144
	PDB	122	103	137	119
	Control	108	91	115	97
Mean		140.75	119.75	156	133.75
LSD5%	F.	2.88	2.86	2.4	3.48
	V.	2.74	3.31	2.96	3.25
	V.XF.	5.76	5.72	4.79	6.97

Biochemical determinations:

a. Effect of biofertilizer and canola genotypes on oil content (%), Physical and chemical properties of canola oil: From the data in table (9), it can be noticed that oil content ranged between 41.8% for Pactol cultivar to 43.6 for line 56/16 grown without biofertilization, and between 42.7 for Pactol cultivar to 44.6 for line 56/16 when plants treated with mixed inoculation treatment. It means that mixed inoculation treatment increased oil content in canola genotypes. Also, mixed inoculation treatment with line 56/16 gave the maximum value of oil content as compared to the control (without biofertilization). It is note worthy that, [4]. identified Line 56/16 as the highest oil seed content genotype among 28 Canola lines and /or varieties tested. Also,[6]. studied fingerprinting of 15 genotypes and described Line 56/16 as salt tolerante canola genotype. Similar results were obtained by[49]. they noticed that the application of *Azotobacter* and *Azospirillum* helped increase the oil content of canola seeds. In reverse,[20]. found that increasing nitrogen fertilization significantly decreased the oil content in canola seeds.[25]. showed that nitrogen fertilizer affected the oil content negatively and decreased it by 3.3% in oilseed rape (*Brassica napus* L.).

It was also noticed that acid value and peroxide value generally lower and iodine value was higher for canola genotypes treated with mixed inoculation treatment than those of control. The acid values of the crude oils was below 5.0 being agreeable with the acid value as recommended by Ministry of Industry, Egyptian organization for standardization and quality control. The positive effect of mixed inoculation treatment on oil quality is an expected result for its effect on improving Physical and chemical properties of oil. Also, line 56/16 seemed to be the best genotype in Physical and chemical properties and oil content under Siwa Oasis conditions. The obtained data were within the range reported by[22,9,7,38].

b- Effect of biofertilizer and canola genotypes on amino acids composition in seeds: Results in Table (10) indicated the presence of 16 amino acid including the most essential amino acids. Amino acid and type are very important to evaluate the protein. Also, glutamic acid is the most abundant amino acid in all canola genotypes, followed by proline, leucine and lysine. Data showed that, application of mixed inoculation treatment increased glutamic acid in seeds of Pactol, Line 56/16 and Line 53/9 as compared to the control. The reverse effect was true for such content in seeds of Serw4. In this regard, the maximum value of glutamic acid was obtained from Line 53/9 with application of mixed inoculation treatment. In this connection[22]. found that glutamic acid is the most abundant amino acid in all rapeseed varieties tested. Also[7]. showed that glutamic acid is the predominant amino acid in two canola cultivars (Pactol and Serw4) under saline conditions.

As to the effect of mixed inoculation treatment on proline acid in seeds of canola genotypes, data showed that the applying of mixed inoculation tended to increase the proline acid content as compared to the control. This was true for Serw4 and Line 56/16. While, Pactol and Line 53/9 took the reverse effect for such content under the same conditions. The highest value of proline content was recorded by Line 56/16 treated with mixed inoculation treatment. The obtained results indicated that the highest content of leucine acid was recorded with plants received biofertilizer as compared to the control. The last finding was true for Serw 4, Line 56/16 and Line53/9. On the contrary, Pactol cultivar gave the decreased of Leucine acid with applying of biofertilizer. The content of basic amino acid (lysine) in seeds of studied Canola genotypes was increased with mixed inoculation treatment. Also, Serw4 cultivar had a higher content of the same amino acid than other Canola genotypes tested when treated with both biofertilizers.

The present results indicate also that the amino acids. i.e., aspartic, threonine, serine, glycine, alanine, isoleucine, phenylalanine, histidine and arginine were presented in moderate quantities. Also, these amino acids appeared to be decreased or increased depending on the concerned amino acid, response being also dependent on studied genotypes interacted with biofertilization under Siwa Oasis conditions. In this respect[39]. found that the contents of glutamic and glycine in rapeseed cake were mainly controlled by maternal genetic effects. On the other hand, methionine and tyrosine are present in low quantities comparing with other amino acids in all Canola genotypes under investigation. Concerning methionine acid content, it was increased in seeds of Serw4, Line 56/16 and Line 53/9, when applied mixed inoculation treatment as compared to the control. In addition, such content in seeds of Pactol took the reverse effect under the same conditions. In this connection[7]. reported that methionine and tyrosine is presented in minute quantities in all samples of canola cultivars under Egyptian conditions.

c. Effect of biofertilizer and canola genotypes on fatty acids composition of seed oil:

The fatty acids composition of canola oil extracted from different canola genotypes grown under Siwa Oasis conditions and treated with mixed inoculation is presented in Table (11). The saturated fatty acids in all canola genotypes were caproic, lauric, myristic, palmitic and stearic.

Results indicate that the application of mixed inoculation treatment increased caproic, lauric, myristic in oil of Pactol and Serw4 as compared to the control (without biofertilizer). While, such contents in oil of line 56/16 and line 53/9 were decreased under the same conditions. In this regard, palmitic acid in oil of line 56/16 and stearic acid in oil of Serw4 recorded increment with application of biofertilization. In this respect[33]. suggested that $C1_{6:0}$ and $C_{18:0}$ concentrations of canola are controlled by different genes.[30]. found that nitrogen applied at 120 Kg /ha increased the palmitic, stearic, linoleic and linolenic contents and decreased the content of oleic acid in spring rape oil compared to the control.

Data presented in the same table show that the constituents of unsaturated fatty acids in oil were oleic, linoleic, linolenic and erucic acid. The predominant unsaturated fatty acid in all Canola genotypes is oleic acid. It was increased in oil of Pactol, line 56/16 and line 53/9 after treatment with mixed inoculation as compared to the control. In this connection, the applying of mixed inoculation treatment tended to decrease the linoleic acid in oil of line 56/16 and line 53/9. Also, Linolenic acid in oil of Pactol and line 56/16 took the same trend. In this regard[40]. identified nine compositions of fatty acids and the most represented were oleic, linoleic, linolenic and palmitic acid in both cultivars (Hybridol and Pactol). While,[34]. showed that the application of fungicides reduced side effects of nitrogen fertilizer and resulted an increase on oleic acid contents in oil seed rape.

Concerning the erucic acid content, it was decreased in oil of all canola genotypes after treatment with mixed inoculation as compared to the control. In addition, the decrease of erucic in oil of all canola genotypes under biofertilization conditions gave also a good indication of its quality. Also, the erucic acid appeared to be the main factor which decreased digestibility and nutritive value of canola oil.

Conclusion: The two newly bred lines, Line 56/16 and Line 53/9 recorded the best mean performance under different treatments comparing with the other two genotypes. While, the mixed biofertilization treatment recorded the highest values for all traits under study followed by single treatment with *Azotobacter chroococcum* and *Bacillus megatherium*. Also, applying of mixed inoculation treatment tended to increased glutamic acid, proline, basic amino acid (lysine) acid content and decrease the linoleic acid and erucic acid content in oil of line 56/16 and line 53/9.

Table 9: variation in oil content (%), Physical and chemical properties of canola oil due to interaction between biofertilizer (mixed inoculation) and canola genotypes.

Treatments		Oil content %	Physical and chemical properties			
Canola genotypes	Biofertilizer		Refractive index	Acid Value	Peroxide value	Iodine value
Line56/16	Control	43.6	1.4636	0.507	0.711	108.3
	Mix	44.6	1.4642	0.341	0.581	112.4
Line 53/9	Control	42.8	1.4635	0.557	0.686	108.0
	Mix	44.3	1.4641	0.359	0.642	111.2
Pactol	Control	41.8	1.4635	0.652	0.889	106.2
	Mix	42.7	1.4639	0.401	0.671	110.5
Serw,4	Control	42.1	1.4635	0.608	0.825	107.6
	Mix	43.0	1.4642	0.366	0.622	112.2

Table 10: Interactive effects of biofertilizer (mixed inoculation) and canola genotypes on amino acids contents (mg/g dry wt.) in canola seeds.

Treatments		Amino acids contents (mg/g dry wt)							
Canola genotypes	Biofertilizer	Aspartic	Threonine	Serine	Glutamic	Proline	Glycine	Alanine	Valine
Line 56/16	Control	9.095	9.022	7.319	18.9941	16.350	8.518	11.084	11.995
	Mix	9.643	9.568	8.110	20.7033	16.665	8.333	9.602	11.692
Line 53/9	Control	8.388	7.604	6.604	22.466	10.246	8.126	8.163	8.970
	Mix	10.086	9.782	8.599	27.922	1.417	9.743	10.499	10.736
Pactol	Control	8.649	8.675	7.189	19.116	12.003	7.561	9.081	10.372
	Mix	8.776	7.622	6.682	20.833	11.041	7.780	9.021	9.519
Serw4	Control	10.422	9.269	8.370	24.623	12.661	8.558	9.694	10.798
	Mix	11.011	10.434	8.887	22.162	16.290	8.769	10.873	12.258
Canola genotypes	Biofertilizer	Methionine	lsoleucine	Leucine	Tyrosine	Phenyl alanine	Histidine	Lysine	Arginine
Line 56/16	Control	3.116	9.471	14.458	6.679	10.247	8.002	12.458	11.384
	Mix	3.416	10.289	14.752	7.258	11.460	9.158	13.244	11.329
Line 53/9	Control	2.098	7.209	12.067	5.255	7.924	6.213	10.757	8.004
	Mix	2.113	8.681	14.461	4.118	9.467	7.691	13.311	9.567
Pactol	Control	1.952	7.774	12.233	5.433	8.260	18.935	10.826	8.332
	Mix	1.666	6.925	11.791	4.691	7.083	6.839	11.317	7.404

Table 10:Continue

Serw4	Control	1.711	8.750	13.871	6.094	9.507	7.311	12.734	9.318
	Mix	4.146	10.247	14.813	7.921	11.619	8.698	13.634	11.229

Table 11: Interactive effects of biofertilizer)mixed inoculation(and canola genotypes on fatty acids composition (%) of canola oil.

Treatments		Fatty acids composition (%)								
Canola genotypes	Biofertilizer	Caproic	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Erucic
		C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C22:1
Line 56/16	Control	4.93	8.83	9.99	6.44	2.48	25.26	23.35	15.32	3.35
	Mix	4.25	7.12	8.54	12.68	0.86	53.08	5.93	5.95	1.54
Line 53/9	Control	3.79	10.39	12.64	7.83	3.57	30.78	25.77	3.51	1.67
	Mix	3.12	5.58	5.93	7	3.29	37.67	2.29	34.81	0.25
Pactol	Control	3.28	6.58	6.2	6.75	1.92	29.81	19.23	23.11	3.06
	Mix	5.98	8.57	9.01	2.62	1.14	37.67	21.1	11.9	1.96
Serw4	Control	1.58	2.18	1.53	15.48	3.36	58.55	6.61	5.18	5.49
	Mix	9.09	13.08	12.57	9.89	4.55	24.05	11.37	10.05	5.31

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