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BIO-FERTILIZATION AND ITS IMPACT ON QUALITY AND PRODUCTIVITY OF SOME NEW PEA (*PISUM SATIVUM* L.) CULTIVARS

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ABSTRACT

Three different pea cultivars Master B، Jaguar and Palmoral were used in this investigation to study the effect of cultivars and different doses of N mineral fertilization along with three isolates of N_2-fixing bacteria (Rhizobium) on growth, yield and chemical composition of those cultivars. Results showed that the three cultivars varied in their flowering time and yield production and almost behaved different under mineral and bio-fertilization. The bio-fertilization increased the plants vegetative growth, yield and yield components. Furthermore, the bio-fertilization increased the quality of seeds and their protein content, but reduced their contents of nitrate and nitrite in comparison with those fertilized by N mineral fertilization only.

Key words: Peas, New Cultivars Evaluation, Mineral and Bio-Fertilizations, Organic Production.

INTRODUCTION

Field pea (*Pisum sativum* L.) derives from the Middle East and was first cultivated roughly 10,000 years ago (Mithen, 2003; Gad El-Hak *et al*., 2012). Pea is a very nutritious vegetable grown in the cool season in different regions of the world. It is grown as a vegetable crop for both fresh and dried seeds. Pea contains high percentage of digestible protein, along with carbohydrates and vitamins (Davies *et al*., 1985 and Akhilesh and Singh, 2013). A wide range of genetic variability is available in pea, providing a good scope for improvement in yield and associated characters of pea through selection. Selection among cultivars and lines for different purposes is very crucial nowadays (Gheeth *et al*., 2012).

It has been recognized that fertilizers are effective means to increase the yield of vegetable crops and to reduce the shortage in food supplies

especially in countries with population of high density. Bio-fertilizers are microorganisms that help plants to grow by increasing the quantity of available nutrients. Since these fertilizers contain living microorganisms it increases or promotes the supply of important nutrients crucial for the overall productivity of the soil. Recent attention has been given to reduce soil pollution practices in modern agriculture. One of the ways to reduce soil pollution is the use of bio-fertilizers which have been recommended by several investigators to substitute partially chemical fertilizers (Saber, 1993, El-Agory *et al*., 1996 ; Hanafy *et al*., 2000).

Among the most important agricultural treatments that affect crops yield of unit area nitrogen fertilization. Nitrogen is the most commonly used mineral nutrient, which is important for protein production. It plays a pivotal role in many critical functions such as photosynthesis in the plant and is a major component of proteins containing amino acids used in forming protoplasm, cell division and plant growth. Nitrogen is necessary for enzymatic reactions in plants since all plant enzymes are proteins. It is a necessary component of several vitamins, e.g., thiamine, niacin and riboflavin and is a part of the nucleic acids; i.e. DNA and RNA (Marschner, 1995).

Therefore, the present study aimed to evaluate three pea cultivars and investigate the effect of N mineral fertilization and/or three Isolates of bio-fertilizers (Minia Azotein) on pea plant growth, chemical composition and green pod yield and its components as well as dry seed yield and seeds quality. Moreover, the study aimed to decrease the pollution resulting from using mineral fertilizers by the application of bio fertilizers and encourage the organic production of pea in Egypt.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of Mallawy Agricultural Research Station, Minia Governorate, Egypt, during the two successive winter seasons of 2010/2011 and 2011/2012, respectively to evaluate the effect of three strains of N_2-fixing bacteria as a bio-fertilizer (Minia Azotein; was friendly obtained from Minia University Bio-fertilization Center, Minia, Egypt) in addition to different levels of mineral N fertilizer on growth and chemical constituents of three Pea (*Pisum sativum* L.) cultivars; namely Master B, Palmoral and Jaguar**.** Soil analysis was carried out according to Wilde *et al*. (1985) and the averages of the obtained data are shown in Table 1.

Table 1: Physical and chemical analyses of the experimental soil (average two seasons).

Soil constituents	Value
Texture grade	Clay loam
Sand	7.15%
Silt	54.25%
Clay	38.60%
pH (1: 2.5 soil suspension)	8.16
E.C. (dsm^{-1}, 1:5 soil water extract)	1.17
$CaCO_3$	3.20%
Organic matter	1.40%
Available N	45.18 ppm
Available P	11.37 ppm
Available K	78.16 ppm

E.C.: Electrical Conductivity.

Pea seeds were sown on November 5th, in 2010 and November 8th, in 2011, respectively on one side of line ridge (4m long and 60cm wide). Seeds were drilled at spacing averaged 10 cm apart. Each experimental plot consisted of 4 ridges, so the area of each plot was $9.6m^2$. Mineral nitrogen was applied in the form of ammonium sulfate (20.6% N) at four levels (0.0, 25, 50 and 100%) with or without bio-fertilizers. Phosphorus in the form of calcium super phosphate (15.5% P_2O_5) at 31 kg/fed. and potassium in the form of potassium sulfate (48% K_2O) at the rate of 50 kg K_2O/fed. The different amounts of fertilizers were added at two equivalent doses after two and six weeks from seed sowing. The bio-fertilizers were applied to pea plants after three weeks from sowing. These treatments were arranged in split plot design with three replicates, pea cultivars were assigned at random in the main plots, while sub plots were devoted to levels of mineral N fertilizer and N_2-fixing bacteria.The study included 30 treatments as follows:

Recommended doses of mineral N was (40 kg N/fed (about 200 kg of ammonium sulfate (20.6% N) 500g for each plot) without any inoculation of bio-fertilizers.

Statistical design

Each experiment consisted of two factors (pea cultivars, bio-fertilizer isolates, and/or the added doses of mineral N fertilizers) in a split design, where cultivars were in the main plots, the combination of isolates and the mineral nitrogen doses were in the sub plots.

Data Recorded:

Vegetative Growth Characters: A random sample of five plants from each experimental plot was taken at full blooming stage; i.e., after 60 days from sowing seeds. The following data were recorded: Plant height and number of branches / plant.

Flowering Behavior: Number of days from sowing till the appearance of the first flower was determined in representative samples of three plants which were labeled at each plot for this purpose.

Harvesting Time: Averages days taken by the green and dry pods from plantation time to ripen and to be ready for harvesting were recorded for all treatments of the three cultivars are shown in Table 2.

Table 2: Harvesting time (days) of green pods and dry seeds of the three pea cultivars

Days to harvest				
		Fresh yield		
Season	**Cultivar**	**First harvest**	**Second harvest**	**Dry yield+ (2-5day)**
First season	**Master B**	87	100	135
	Palmoral	115	127	163
	Jaguar	101	115	148
Second season	**Master B**	88	102	142
	Palmoral	118	129	172
	Jaguar	104	117	157

Green Pod Yield and Its Components: At harvest time, reproductive characteristics were recorded by using green pods taken from each plant and/or plot, hence, the average number of green pods/plant, green pods yield/plant (g) and total yield of green pods (ton/fed.) were recorded.

Physical and Chemical Fruit Quality: In order to record the physical pod characters, random samples of 20 pods were taken from each plot and the following data were recorded: average pod length (cm) and pod weight (g), number of seeds/pod, weight of 1000 dry seeds(g), and dry seed yield/fed.(Ton).

Chemical Composition of Dry Seeds

NPK determination: N, P and K were determined in the dry seeds of all cultivars treatments the total nitrogen was determined in the digested dry matter of seeds using the micro-kjeldahl method according to Pregl (1945). Phosphorus was determined by using the spectrophotometer method as

described by John (1970). Potassium was determined by using the flame photometer method as described by Brown and Lilliland (1946).

Crude protein (N% × 6.25) it was determined according to AOAC (2005). All determinations were performed in triplicates and the means were recorded.

Extraction and determination of nitrite and nitrate: the nitrite and nitrate were extracted from finally powdered meal of all samples by 1% K_2SO_4 solution and determined spectrophotometrically as described by Saad (1991).

Statistical Analysis:

All recorded data were subjected to the statistical analysis of variance procedures and treatment means were compared using the L.S.D as described by Gomez and Gomez (1984). The statistical analysis was done using the computer program MSTAT-C software version 4.0.

RESULTS

Generally, the three evaluated pea cultivars varied in their field performance, horticultural characteristics, yield and chemical prosperities. The three bacterial Isolates used in this study also varied in their effect on pea plants growth, yield and chemical composition. Furthermore, the best data values were obtained when 50% of N was used in combination with the bacterial bio-fertilization. On the other hand, plants bio-fertilized with Isolate 1 without any mineral N fertilization gave the lowest mean values in almost all of the studied characteristics.

Growth Characters

Effect of cultivars

Data presented in Table 3 show that the highest plants were observed by Palmoral cultivar (88.7 and 90.1 cm) followed by Jaguar cultivar (81.9 and 82.7 cm) while, Master B cultivar gave the shortest plants (70.1 and 71.9 cm) in the first and second seasons, respectively, with significant differences among the three cultivars. Regarding number of branches per plant, it is clear from the same data that Palmoral cultivar gave the highest values (4.32 and 4.32) followed by Jaguar cultivar (3.37 and 3.32), while, Master B recorded the lowest values in this respect (2.56 and 2.48) in the first and second seasons, respectively with significant differences among them. As for flowering time, this character means the number of days elapsed from sowing seeds till the appearance of the first flower on pea plant, it is obvious from the data in Table 3 that *cv*. Master B was the

Table 3: Effect of bio- and/or mineral N fertilization on growth characters of three pea cultivars, during 2010/2011 and 2011/2012 seasons

Characters / Treatments	Growth characters / plant					
	Plant height (cm)		Number of branches		Flowering time (day)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
			Pea cultivars			
Master B	70.1	71.9	2.56	2.48	33.6	34.9
Palmoral	88.7	90.1	4.32	4.32	56.8	57.7
Jaguar	81.9	82.7	3.37	3.32	46.2	47.3
L.S.D at 5%	**2.41**	**1.14**	**0.25**	**0.09**	**0.09**	**0.11**
			bio- and mineral N fertilization			
100% mineral N	81.1	80.0	3.25	3.16	46.7	47.3
Isolate 1+ 0% N	72.9	74.6	2.90	2.97	44.3	45.8
Isolate 1+ 25% N	84.2	86.0	3.60	3.50	45.3	46.6
Isolate 1+ 50% N	88.3	90.4	4.24	4.10	45.6	46.9
Isolate 2+ 0% N	70.2	72.2	2.87	2.93	44.4	45.6
Isolate 2+ 25% N	83.6	85.2	3.55	3.42	45.3	46.4
Isolate 2+ 50% N	87.8	90.0	4.17	4.02	45.7	46.7
Isolate 3+ 0% N	67.3	69.3	2.73	2.80	45.6	46.8
Isolate 3+ 25% N	82.3	82.8	3.27	3.23	46.2	47.2
Isolate 3+ 50% N	84.2	85.1	3.58	3.57	46.1	47.2
L.S.D at 5%	**0.72**	**0.87**	**0.15**	**0.08**	**0.12**	**0.12**

earliest cultivar to begin flowering (33.6 and 34.9 days) followed by *cv.* Jaguar (46.2 and 47.3 days) and finally by cv. Palmoral (56.8 and 57.7 days) in the first and second seasons, respectively.

Effect of bio and mineral nitrogen fertilization

Illustrated data in Table 3 indicate that inoculation of the soil with bacterial isolate 1 plus fertilization with 50% mineral nitrogen recorded the tallest plants (88.3 and 90.4 cm), followed by treatment which included the inoculation with isolate 2 plus 50% nitrogen fertilizer without significant differences between them during the two seasons of study, while the shortest plants were obtained from plants which treated with bacterial isolate 3 and without mineral nitrogen fertilization which gave 67.3 and 69.3 cm in the first and second seasons, respectively. As for number of branches per plant it is clear from the same data that the combination between isolate 1 or isolate 2 plus 50% mineral nitrogen increased significantly number of

branches per plant and recorded 4.24 and 4.10 and 4.17 and 4.02, respectively, while the lowest values in this respect were recorded by the combination between isolate 3 without addition mineral nitrogen fertilization (2.73 and 2.80) in the first and second seasons, respectively.

Concerning flowering time, it is obvious from the data in Table 3 that the combination between isolates 1 and 2 without addition mineral nitrogen fertilization helped pea plants to flower earlier in the first season without significant difference, while the combination between isolate 2 and without mineral nitrogen fertilization helped pea plants to flower earlier in the second season (45.6 days). On the other side, fertilization of pea plants with mineral nitrogen at 100% of recommended dose led to delaying flowering of plant (46.7 and 47.3 days) in the first and second seasons, respectively.

Effect of the interaction between pea cultivars, bio and mineral nitrogen fertilization:

Results in Table 4 illustrate that the interaction between inoculation of pea plants of Palmoral cultivar with isolate 1 plus fertilization with 50% mineral nitrogen increased significantly plant height (97.2 and 99.5 cm) and number of branches per plant (5.23 and 5.36) followed by inoculation seeds of Palmoral cultivar with isolate 2 plus fertilization with 50% mineral nitrogen (96.2 and 98.3 cm) for plant height and (4.95 and 5.13) for number of branches per plant in the first and second seasons, respectively, without significant differences between them. On the other side, the lowest values in this respect were recorded by the interaction between inoculation seeds of Master B cultivar with isolate 3 and without mineral nitrogen fertilization which gave 58.8 and 61.3 cm for plant height and (1.83 and 1.76) for number of branches per plant in the first and second seasons, respectively.

As for flowering time, the same results in Table 4 indicate that the interaction between inoculation plants of Master B cultivar with isolate 1 and without mineral nitrogen fertilization recorded the lowest values of flowering time (32.1 and 33.6 days) in the first and second seasons, respectively, while the highest values in this respect were recorded by the interaction between Palmoral cultivar and fertilization of 100% mineral nitrogen which recorded 58.1 and 58.6 days in the first and second seasons, respectively.

Yield and Its Components

Effect of cultivars

It is obvious from the data presented in Table 5 that Palmoral cultivar recorded the highest values of yield and its components expressed as number of pods per plant average pod weight, number of seeds per pod,

Table 4: Effect of interaction between bio- and/or mineral N fertilization on growth characters of three pea cultivars during 2010/2011 and 2011/2012 seasons.

Characters / Treatments		Growth characters / plant					
		Plant height (cm)		Number of branches		Flowering time (day)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Master B	100% mineral N	69.8	68.4	2.23	2.34	34.6	35.5
	Isolate 1+ 0% N	60.1	62.0	1.89	1.85	32.1	33.6
	Isolate 1+ 25% N	73.3	76.0	2.45	2.59	33.1	34.5
	Isolate 1+ 50% N	77.2	79.1	3.18	3.39	33.5	35.1
	Isolate 2+ 0% N	61.3	63.4	1.97	1.92	32.7	33.9
	Isolate 2+ 25% N	74.4	76.8	2.57	2.68	33.8	35.1
	Isolate 2+ 50% N	78.4	81.0	3.41	3.55	33.8	35.3
	Isolate 3+ 0% N	58.8	61.3	1.83	1.76	33.4	34.7
	Isolate 3+ 25% N	71.8	73.2	2.36	2.55	34.3	35.3
	Isolate 3+ 50% N	75.5	77.8	2.83	2.94	34.4	35.4
Palmoral	100% mineral N	91.2	90.7	4.03	4.15	58.1	58.6
	Isolate 1+ 0% N	82.3	83.9	3.91	3.82	54.9	56.6
	Isolate 1+ 25% N	94.3	95.8	4.46	4.54	56.4	57.7
	Isolate 1+ 50% N	97.2	99.5	5.23	5.36	56.8	57.9
	Isolate 2+ 0% N	75.2	77.4	3.78	3.73	55.5	56.7
	Isolate 2+ 25% N	92.2	93.4	4.33	4.46	56.7	57.5
	Isolate 2+ 50% N	96.2	98.3	4.95	5.13	57.2	58.0
	Isolate 3+ 0% N	73.1	74.2	3.73	3.64	56.9	58.0
	Isolate 3+ 25% N	92.2	93.5	4.15	3.96	57.4	58.2
	Isolate 3+ 50% N	93.0	94.6	4.58	4.41	57.5	58.3
Jaguar	100% mineral N	82.4	80.9	3.22	3.26	47.2	47.7
	Isolate 1+ 0% N	76.3	77.9	3.11	3.03	45.9	47.2
	Isolate 1+ 25% N	85.0	86.4	3.58	3.66	46.4	47.6
	Isolate 1+ 50% N	90.7	92.8	3.87	3.96	46.6	47.7
	Isolate 2+ 0% N	74.1	76.0	3.02	2.95	44.9	46.0
	Isolate 2+ 25% N	84.2	85.4	3.35	3.49	45.2	46.6
	Isolate 2+ 50% N	88.8	90.7	3.68	3.82	45.9	46.8
	Isolate 3+ 0% N	70.0	72.5	2.83	2.79	46.6	47.6
	Isolate 3+ 25% N	83.1	81.9	3.18	3.29	46.7	47.9
	Isolate 3+ 50% N	84.2	83.1	3.28	3.38	46.7	48.0
L.S.D at 5%		**1.15**	**1.40**	**0.12**	**0.24**	**0.2**	**0.2**

weight of 1000 seeds, yield per plant and green pods yield per feddan as well as dry seed yield per feddan . On the other hand, the lowest values in this respect were recorded by Master B cultivar in both seasons of study.

Effect of bio and mineral nitrogen fertilization:

Illustrated data in Table 5 indicate that inoculation pea plants with bacterial isolate 3 plus fertilization with 50% mineral nitrogen of the recommended dose recorded the highest number of pods per plant, while inoculation pea plants with the same bacterial isolate and without mineral nitrogen fertilization recorded the lowest values in this respect in the two seasons of growth. On the other side, inoculation pea plants with bacterial isolate 1 plus fertilization with 50% mineral nitrogen recorded the highest values of average pod weight (6.64 and 6.92 g), number of seeds per pod (9.35 and 9.91), weight of 1000 seeds (215.8 and 236.8 g), pods yield per plant (92.29 and 98.90 g) and green yield per feddan (6.15 and 6.59 ton) as well as dry seed yield per feddan (1.247 and 1.303 ton) in the first and second seasons, respectively, while the lowest values in this respect were recorded when inoculated pea plants with bacterial isolate 3 and without mineral nitrogen fertilization, these results are true in both seasons of study.

Effect of the interaction between pea cultivars, bio and mineral nitrogen fertilization

Data in Table 6 illustrate that the interaction between inoculation of Palmoral cultivar plants with isolate 1 plus fertilization with 50% mineral nitrogen increased significantly yield and its components of pea plants as compared to other treatments, and recorded (15.70 and 15.92) for number of pods per plant, 7.01 and 7.39 g for average pod weight, 9.32 and 9.72 for number of seeds per pod, 232.7 and 253.7 g for weight of 1000 seeds, 109.7 and 117.7 g for yield per plant and 7.32 and 7.85 ton for green yield per feddan as well as 1.493 and 1.543 ton for dry seed yield per feddan in the first and second seasons, respectively. On the other side, the interaction between inoculation of Jaguar cultivar seeds with bacterial isolate 3 and without mineral nitrogen fertilization recorded the lowest values for number of pods per plant (9.11 and 9.57) and number of seeds per pod (5.37 and 5.76) in the first and second seasons, respectively, while the interaction treatment between inoculation plants of Master B cultivar seeds with bacterial isolate 3 and without mineral nitrogen fertilization recorded the lowest values of average pod weight, weight of 1000 seeds, yield per plant and green yield per feddan as well as dry seed yield per feddan, these results are true in both seasons of study.

Table 6: Effect of interaction between bio- and/or mineral N fertilization on yield and its components of three pea cultivars during 2010/2011 and 2011/2012 seasons

Characters / Treatments		Yield and its components													
		No. of Pods /plant		Average pod weight (g)		No. of Seeds /pod		Weight of 1000 seeds (g)		Yield/ plant (g)		Green yield /fed. (ton)		Dry seed yield/fed. (ton)	
		1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*
Master B	100% mineral N	12.83	13.05	5.10	5.31	8.49	9.00	170.7	190.6	65.5	66.1	4.37	4.63	0.803	0.863
	Isolate 1+0% N	9.27	11.05	4.40	4.51	6.73	7.15	145.1	163.9	40.9	49.1	2.73	3.32	0.453	0.513
	Isolate 1+25%N	10.44	11.27	6.18	6.39	9.48	9.88	184.7	203.7	64.6	72.1	4.31	4.81	0.763	0.813
	Isolate 1+50%N	11.25	11.93	6.51	6.69	10.2	10.7	188.9	208.0	73.4	79.8	4.89	5.32	0.913	0.973
	Isolate 2+0% N	9.79	10.89	4.66	5.00	6.96	7.39	153.7	174.9	45.7	54.6	3.05	3.64	0.503	0.563
	Isolate 2+25%N	10.49	11.28	6.39	6.57	9.67	10.2	188.7	207.0	67.1	74.2	4.48	4.95	0.823	0.883
	Isolate 2+50%N	11.37	11.67	6.89	7.12	10.5	10.8	201.9	222.6	78.4	83.2	5.23	5.55	0.983	1.053
	Isolate 3+0% N	8.90	9.61	3.91	4.09	6.53	6.93	130.9	148.7	34.9	39.4	2.33	2.63	0.423	0.463
	Isolate 3+25%N	11.82	12.27	5.19	5.43	9.18	9.59	179.7	199.8	61.4	66.7	4.07	4.45	0.713	0.773
	Isolate 3+50%N	12.87	13.21	5.41	5.60	9.78	10.2	187.1	205.6	69.7	74.1	4.65	4.94	0.863	0.923
Palmoral	100% mineral N	15.57	15.77	6.11	6.29	7.68	8.17	184.8	205.1	95.2	99.3	6.35	6.62	1.243	1.293
	Isolate 1+0% N	10.62	11.77	5.71	5.89	6.51	6.89	167.8	188.9	57.3	69.4	4.05	4.63	1.013	1.093
	Isolate 1+25%N	14.99	15.22	6.59	6.90	8.06	8.45	215.9	237.9	98.9	105.1	6.59	7.01	1.283	1.353
	Isolate 1+50%N	15.65	15.92	7.01	7.39	9.32	9.72	232.7	253.7	109.7	117.7	7.32	7.85	1.493	1.543
	Isolate 2+0% N	10.73	11.37	5.19	5.38	6.18	6.57	154.7	173.9	55.7	61.6	3.72	4.11	0.953	1.003
	Isolate 2+25%N	14.81	15.47	6.29	6.47	8.20	8.60	204.9	224.0	93.2	100.1	6.22	6.68	1.223	1.263
	Isolate 2+50%N	15.50	15.73	6.72	6.88	8.92	9.60	223.7	240.9	104.2	108.2	6.95	7.22	1.423	1.473
	Isolate 3+0% N	10.93	11.07	4.78	4.99	5.89	6.32	144.9	163.7	52.3	55.3	3.49	3.69	0.833	0.893
	Isolate 3+25%N	15.56	15.33	5.70	6.01	7.79	8.29	197.8	215.6	88.7	92.2	5.92	6.15	1.083	1.143
	Isolate 3+50%N	15.70	16.51	6.29	6.38	8.28	9.22	215.9	232.1	98.8	105.4	6.93	7.03	1.343	1.383
Jaguar	100% mineral N	14.49	14.46	5.50	5.78	7.49	7.90	179.7	199.1	79.7	83.6	5.32	5.58	1.073	1.133
	Isolate 1+0% N	9.88	10.68	4.89	5.08	6.02	6.49	165.7	185.5	48.4	54.2	3.23	3.62	0.863	0.913
	Isolate 1+25%N	12.57	13.18	6.09	6.34	7.86	8.48	201.7	225.2	76.6	83.6	5.11	5.58	1.163	1.213
	Isolate 1+50%N	14.63	14.85	6.40	6.68	8.57	9.37	225.9	248.7	93.7	99.2	6.25	6.62	1.333	1.393
	Isolate 2+0% N	9.52	10.41	4.76	4.93	5.79	7.52	150.7	169.8	45.4	51.4	3.03	3.43	0.723	0.773
	Isolate 2+25%N	12.64	13.67	5.77	6.04	7.75	8.41	195.1	214.7	73.0	76.6	4.87	5.11	0.933	1.053
	Isolate 2+50%N	13.64	14.14	6.18	6.41	8.28	9.06	214.0	233.0	80.4	90.7	6.03	6.04	1.243	1.293
	Isolate 3+0% N	9.11	9.57	4.48	4.61	5.37	5.76	135.7	154.9	40.9	44.2	2.73	2.95	0.613	0.653
	Isolate 3+25%N	13.51	13.75	5.19	5.36	7.48	8.23	188.9	207.6	70.1	73.7	4.68	4.92	0.933	0.983
	Isolate 3+50%N	14.18	14.54	5.61	5.79	7.70	8.37	201.8	219.2	79.6	84.2	5.31	5.62	1.173	1.223
	L.S.D at 5%	**0.58**	**0.60**	**0.04**	**0.05**	**0.52**	**0.51**	**5.0**	**5.2**	**5.73**	**3.62**	**0.16**	**0.51**	**0.054**	**0.051**

Chemical Constituents of Seeds

Effect of cultivars

It is obvious from the data presented in Table 7 that Palmoral cultivar recorded the highest values of total nitrogen percentage (3.78 and 4.28), potassium (2.26 and 2.35%) and crud protein (23.63 and 26.75%) in the first and second seasons, respectively, while Master B cultivar recorded the highest values of phosphorus (0.317 and 0.385%) and gave the lowest values of nitrate (135 and 141 mg/kg) and nitrite (5.48 and 5.78 mg/kg) in the first and second seasons, respectively.

Effect of bio and mineral nitrogen fertilization

Illustrated data in Table 7 indicate that inoculation pea plants with bacterial isolate 2 and fertilization with 50% mineral nitrogen recorded the highest values of total nitrogen (4.07 and 4.57%), potassium (2.36 and 2.46%) and protein (25.43 and 28.56%) in the first and second seasons, respectively, followed by inoculation with bacterial isolate 3 and fertilization with 50% mineral nitrogen without significant differences between them. On the other side, inoculation pea plants with bacterial isolate 1 without mineral nitrogen fertilization recorded the lowest values of nitrate (111 and 113 mg/kg) and nitrite (4.79 and 5.02mg/kg) in the first and second seasons, respectively.

Effect of the interaction between pea cultivars, bio and mineral nitrogen fertilization

Data in Table 8 illustrate that the interaction between inoculation pea plants of Palmoral cultivar with bacterial isolate 3 plus fertilization with 50% mineral nitrogen increased significantly chemical constituents of pea seeds as compared to other treatments, it recorded 4.33 and 4.85 % for total nitrogen, 0.306 and 0.428 % for phosphorus and 27.08 and 29.24% for protein, while seed inoculation of the same cultivar with bacterial isolate 1 and fertilization with 50% mineral nitrogen gave the highest values of potassium percentage (2.58 and 2.65%) in the first and second seasons, respectively. On the other side, inoculation plants of Master B cultivar with bacterial isolate 3 without mineral nitrogen fertilization recorded the lowest values of nitrate (98 and 101 mg/kg) and nitrite content (3.65 and 3.93 mg/kg) in the first and second seasons, respectively.

DISCUSSION

Pea is a very nutritious vegetable grown in the cool season and it grown as a vegetable crop for both fresh and dried seed. It contains high percentage

Table 8: Effect of interaction between bio- and/or mineral N fertilization on chemical constituents of 2010/2011 and 2011/2012 seasons cultivars during three pea

Characters / Treatments		Chemical constituents of seeds											
		N %		P %		K %		Protein %		Nitrate (mg/kg)		Nitrite (mg/kg)	
		1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*	1st s*	2nd s*
Master B	100% mineral N	3.64	4.14	0.332	0.402	2.22	2.30	22.77	25.87	158	164	6.68	6.98
	Isolate 1+ 0% N	2.76	3.24	0.288	0.369	1.52	1.65	17.27	22.25	110	112	4.24	4.54
	Isolate 1+ 25% N	3.55	4.06	0.314	0.383	1.77	1.84	22.21	25.37	132	136	5.41	5.73
	Isolate 1+ 50% N	3.74	4.26	0.332	0.404	2.10	2.22	23.40	26.62	158	165	6.45	6.63
	Isolate 2+ 0% N	2.96	3.45	0.303	0.372	1.75	1.90	18.52	21.56	121	124	4.54	4.91
	Isolate 2+ 25% N	3.69	4.20	0.328	0.389	1.96	2.03	23.08	26.25	140	145	5.64	6.02
	Isolate 2+ 50% N	3.84	4.37	0.332	0.421	2.25	2.37	24.02	27.31	168	180	6.56	6.91
	Isolate 3+ 0% N	2.55	3.06	0.293	0.345	1.40	1.53	15.98	19.13	98	101	3.65	3.93
	Isolate 3+ 25% N	3.44	3.88	0.318	0.375	1.64	1.76	21.52	24.25	119	124	5.16	5.47
	Isolate 3+ 50% N	3.55	4.03	0.327	0.392	1.95	2.08	22.19	25.18	151	159	6.42	6.64
Palmoral	100% mineral N	3.85	4.37	0.282	0.365	2.47	2.55	24.08	27.31	179	187	7.26	7.56
	Isolate 1+ 0% N	2.92	3.44	0.231	0.323	2.02	2.11	18.27	21.50	114	116	5.11	5.31
	Isolate 1+ 25% N	3.78	4.34	0.363	0.342	2.43	2.52	23.65	27.12	152	156	5.62	5.85
	Isolate 1+ 50% N	4.15	4.55	0.275	0.369	2.58	2.65	25.92	28.42	169	177	6.14	6.39
	Isolate 2+ 0% N	3.14	3.63	0.247	0.336	1.91	2.00	19.65	22.68	125	127	5.36	6.56
	Isolate 2+ 25% N	3.91	4.43	0.280	0.359	2.35	2.44	24.46	26.77	160	164	5.80	6.03
	Isolate 2+ 50% N	4.22	4.71	0.288	0.385	2.48	2.57	26.40	28.67	184	188	6.82	7.07
	Isolate 3+ 0% N	3.36	3.85	0.256	0.350	1.73	1.81	21.02	24.06	141	144	5.54	5.74
	Isolate 3+ 25% N	4.18	4.66	0.286	0.370	2.23	2.32	26.15	28.27	167	178	5.94	6.17
	Isolate 3+ 50% N	4.33	4.85	0.306	0.428	2.42	2.52	27.08	29.24	189	192	7.04	7.29
Jaguar	100% mineral N	3.71	4.25	0.251	0.302	2.33	2.41	23.18	26.54	169	175	7.07	7.37
	Isolate 1+ 0% N	2.75	3.26	0.200	0.275	1.95	2.03	17.21	20.39	111	113	5.01	5.21
	Isolate 1+ 25% N	3.73	4.24	0.235	0.309	2.18	2.26	23.33	26.48	144	147	5.54	5.77
	Isolate 1+ 50% N	3.99	4.51	0.250	0.348	2.41	2.41	24.96	28.19	157	161	6.11	6.36
	Isolate 2+ 0% N	2.96	3.44	0.220	0.293	1.72	1.83	18.52	21.50	119	121	5.24	5.44
	Isolate 2+ 25% N	3.81	4.31	0.246	0.310	1.97	2.07	23.83	26.93	155	158	5.66	5.89
	Isolate 2+ 50% N	4.14	4.63	0.265	0.320	2.36	2.44	25.90	28.93	173	177	6.67	6.92
	Isolate 3+ 0% N	3.22	3.73	0.239	0.306	1.57	1.66	20.15	23.30	134	136	5.46	5.66
	Isolate 3+ 25% N	3.89	4.41	0.255	0.322	1.85	1.93	24.33	27.36	162	165	5.84	6.07
	Isolate 3+ 50% N	4.28	4.70	0.272	0.335	2.27	2.34	26.77	29.36	182	186	6.93	7.18
L.S.D at 5%		**0.054**	**0.054**	**0.051**	**0.054**	**0.27**	**.27**	**0.53**	**0.72**	**5.1**	**0.52**	**0.29**	**0.36**

of digestible protein, along with carbohydrates and vitamins. That's why we chose this crop here to evaluate novel pea cultivars newly grown under the Egyptian winter growing conditions. Master B is a commonly known cultivar and it is a very early maturing cultivar suitable for mechanic harvesting, and Jaguar and Palmoral cultivars are recently imported British cultivars which are highly yielding peas cultivars. The later ones are sweeter than Master B and are very promising pea cultivars to be grown under the Egyptian conditions. The obtained results in this work confirmed all these characteristics of these cultivars and confirmed the previous results.

It is crucial to mention here that all the bio-fertilization isolates used here are produced in Minia university (The centre of Bio-fertilization, Minia University, Egypt.). The isolates used in this work are three promising products of this centre.

The bio-fertilization isolates treatments increased all horticultural characteristics, fresh pod yield and dry seed yield. Furthermore, they increased the protein content and decreased the nitrate and nitrite contents in dry seeds. So, the obtained seeds from bio-fertilized treatments are organic and safe in their content of nitrate and nitrite. That's because bio-fertilizers are important components of integrated nutrients management. They play a key role in productivity and sustainability of soil and also protect the environment (El-Kalla *et al*. 1997; Sharma and Nanadeo, 1999; Hewedy, 1999). They are cost effective, eco-friendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system (Abraham and Lal, 2002; Anany, 2002; Estefanous *et al.,* 2003). Moreover, beneficial microorganisms in bio-fertilizers accelerate and improve plant growth along with protecting plants from pests and diseases which also enhances growth and increases yield (Abou El-yazeid *et al*., 2007). Moreover, soil microorganisms have a great role in sustainable development of agriculture and this has been reviewed by Lee and Pankhurst (1992) and Wani *et al*. (1995).

Obtained results showed that, using the combination of bio-fertilizers and half dose of mineral nitrogen gave the best results. Many authors showed that the favourable effect of *Azotobacter* and mineral nitrogen fertilizer on growth, chemical composition of leaves, and yield was reported on sugar beet (Stajner *et al*., 1997), on cauliflower (Bambal *et al*., 1998), and on faba bean (Wyszkowska, 1999). Also, Dakhly *et al*. (2004) on squash obtained the highest total yield and the best N uptake by using 45 kg nitrogen and chicken manure in addition to inoculation with *Azotobacter*. Prabhjeet *et al*. (1994) on *Brassica napus* and Verma *et al*. (1997) on cabbage, Verma *et al*. (2000) on pea and Panwar *et al*. (2000) on radish claimed that both inoculation with *Azotobacter* and application of N increased seed yield.

Other scientists claim that bio-fertilizers increased the concentration of simple organic molecules such as sugars, free amino acids and total soluble phenols which played a role in regulation of plant osmosis and consequently better plant growth and yield (Panwar *et al*. (2000) and Verma *et al*. (2000). In other words, bacteria in bio-fertilizers can produce different compounds such as organic acids, plant growth promoters (auxins, gibberellins and cytokinins) as well as nitrogen fixing, dissolving phosphorus and producing organic acids in the soil which consequently lowers soil pH and increases the availability of most of the plants needed elements (Hanafy *et al*., 2000). In addition, the beneficial effects of bio-fertilizers such as production of organic acids (lowering soil pH) and production of plant growth regulators may contribute to a better plant growth and yield through enhancing nutrient uptake (Ibrahim and Abdel-Aziz, 1977). On the other hand, it is well known that bio-fertilizers can lower the amount of added chemical nitrogen fertilizer to the soil and consequently mitigation of pollution (Ibrahim and Abdel-Aziz (1977). All these statements were clear and confirmed by the obtained results.

Conclusively, the three promising pea cultivars showed very desirable characteristics *e.g.*, short time to flowering, high yield of fresh fruits and dry seeds. The bio-fertilization increased the used cultivars potentials of growth, protein content and dry seed production and also decreased the seeds content of nitrate and nitrite.

The best results were obtained when the 50% of the recommended mineral nitrogen dose was used along with the bio-fertilization isolates as compared to the other treatments and the control treatment. These results encourage Egyptian pea farmers to produce the organic pea as the demand for organic pea is increasing in the Egyptian markets and also for exportation along with enhancing sustainable agricultural system in Egypt.

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التسميد الحيوي وأثره على جودة وإنتاجية بعض أصناف البسلة الحديثة

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تم استخدام ثلاثة أصناف من البسلة (صنف قديم وهو Master B وصنفان حديثان هما Jaguar و Palmoral فى هذا البحث وذلك لدراسة تأثير الأصناف وكذلك تأثير التسميد بجرعات مختلفة من السماد الكيماوى النيتروجينى (N) مع التسميد الحيوى بثلاثة عزلات مختلفة من بكتريا الريزوبيوم التى تم إنتاجها بوحدة المخصبات الزراعية بكلية الزراعة بجامعة المنيا على النمو والإنتاجية وكذلك التركيب الكيماوى لثمار وبذور هذه الأصناف الثلاثة. وأوضحت النتائج أن الأصناف الثلاثة اختلفوا فى عدد الأيام اللازمة لبداية الإزهار (فكان الصنف Master M هو الأكثر تبكيراً فى الإزهار والإثمار) وكذلك الإنتاجية من القرون الطازجة أو البذور الجافة وكان سلوكها متبايناً تجاه نوع وكمية السماد الآزوتى أو الحيوى أو التوليفة بينهما (فتفوق الصنفان الحديثان على الصنف القديم فى معظم الصفات التى تم دراستها). وزادت التوليفة من التسميد الحيوى مع نصف الكمية الموصى بها من التسميد الكيماوى وأعطت معظم النتائج المرجوة من النمو الجيد والمحصول العالى من القرون والبذور وكذلك محتوى البذور العالى من البروتين.

ومن الناحية الأخرى قلل التسميد الحيوى معنوياً من محتوى البذور من النترات والنيتريت بالمقارنة بتلك المأخوذة من النباتات التى تم تسميدها بالسماد الكيماوى فقط.

التوصية: نستنتج من النتائج السابقة بأنه يمكن إستبدال التسميد النيتروجينى المعدنى بمعدل ٥٠% مع تلقيح بذور البسلة صنف Palmoral بالعزلات ١، ٢ من بكتريا الريزوبيوم بديلا عن التسميد النيتروجينى المعدنى بمعدل ١٠٠%والذى كان كافيا للحصول على أعلى معدل للنمو الخضرى ، والمحصول ومكوناته وكذلك المحتوى الكيماوى لبذور البسلة.

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