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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 were used in this investigation to study the effect of cultivars and different doses of N mineral fertilization along with three isolates of N-fixing bacteria on growth, yield and chemical composition of those cultivars. Results showed that the three cultivars varied in their flowering time, fresh and dry yield and almost differed in response to mineral fertilization doses and bio-fertilization isolates. The bio-fertilization increased pea plants vegetative growth, yield and yield components. Also, the bio-fertilization increased pod and seed qualities and increased their protein content along with reducing their contents of nitrate and nitrite.

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; El-Hak *et al*., 2012). Pea is a very nutritious vegetable grown in the cool season throughout the world. It is grown as a vegetable crop for both fresh and dried seed. Pea contains high percentage of digestible protein, along with carbohydrates and vitamins. 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 in self pollinated crops 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 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 nutrients These fertilizers contain living microorganisms, which increase or promote the supply of important nutrients crucial for the overall productivity of the soil. An increasing number of farmers and agriculturists are turning to the use of bio-fertilizers as these are gentler on the soil comparing with the chemical fertilizers (Mahdi *et al*., 2010). Recent attention has been given to less 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 chemical fertilizers (Saber, 1993; El-Agory *et al*., 1996; Hanafy Ahmed *et al*., 2000). Meanwhile, nitrogen fertilization is among the most important agricultural treatments that affects the yield of unit area and is the most commonly used mineral nutrient. It is important for protein production and plays a pivotal role

in many critical functions (such as photosynthesis) in the plant and is a major component of proteins consisting of amino acids used in forming protoplasm, cell division and plant growth (El-Agory *et al*., 1996). 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 nitrogen is part of the nucleic acids DNA and RNA (Marschner, 1995).

Therefore, the present study aimed to evaluate three pea cultivars and investigate the effect of N mineral fertilization in combination with 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. Such study aimed also 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, Egypt, during the two successive fall and winter seasons of 2010/2011 and 2011/2012, respectively to evaluate the effect of three strains of N-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 N fertilizer on growth and chemical constituents on three Pea (*Pisum sativum* L.) cultivars; Master B, Palmoral and Jaguar. Soil analysis was carried out and the averages of the obtained data are shown in Table 1.

Pea seeds were sown on November 5th, 2010/2011 and November 8th, 2011/2012, respectively on one side of line ridge (400 cm long and 60 cm wide). Seeds were drilled at spacing averaged 10 cm apart. Each experimental plot consisted of 4 ridges, so the area of each plot was 9.6 m^2. Nitrogen was applied in the form of ammonium sulfate (20.6% N) at levels 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 rate of 50 kg K_2O/fed. The different amounts of fertilizers were added at two equivalent doses two and six weeks after seed sowing. The bio-fertilizers were applied to pea plants after four weeks from sowing the seeds. The study included 30 treatments. All treatments as follow were (10 treatments) carried out with three varieties of pea:

- Control (recommended doses of N% (40 kg N/fed (about 200 kg of ammonium sulfate (20.6% N) 500 g for each plot) without any inoculation of bio-fertilizers
- Strain 1+0% of N mineral fertilizer
- Strain 1+25% of N mineral fertilizer (10 kg/fed (125 g for each plot))
- Strain 1+50% of N mineral fertilizer (20 kg/fed (250 g for each plot))
- Strain 2+0% of N mineral fertilizer
- Strain 2+25% of N mineral fertilizer
- Strain 2+50% of N mineral fertilizer
- Strain 3+0% of N mineral fertilizer
- Strain 3+25% of N mineral fertilizer
- Strain 3+50% of N mineral fertilizer

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 was recorded: plant height and number of branches.

Table 1: Physical and chemical analyses of the experimental soil

Soil constituent	Values
Texture grade	Clay loam
Sand (%)	7.15
Silt (%)	54.25
Clay (%)	38.60
pH (1: 2.5 soil suspension)	8.16
EC (dS m^{-1}, 1:5 soil water extract)	1.17
$CaCO_3$ (%)	3.20
Organic matter (%)	1.40
Available N (ppm)	45.18
P (ppm)	11.37
K (ppm)	78.16

EC: Electrical conductivity

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

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

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 of the green and dry pods were harvested after planting for all treatments of the three cultivar are shown in Table 2.

Green pod yield and its components: At harvest time, reproductive characteristics were recorded by using number and weight of green pod taken from each plot, hence, average number of green pods/plant, green pods yield/plant and total yield of green pods (t/fed.) were calculated.

Physical and chemical fruit quality: In order to record the physical pod characters, random samples of 20 pods were taken from every plot and the following data were recorded: Average pod length, pod diameter and pod weight, number of seeds/pod, weight of 1000 dry seeds and dry seeds yield/fed.

Chemical composition of dry seeds: Each of N, P and K were determined in the dry seeds of 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 Lilleland (1946).

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

For the 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).

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 NPK was used in combination with the bacterial bio-fertilization. On the other hand, plants bio-fertilized with isolate 1 without any mineral fertilization gave the lowest mean values in almost all of the studied characteristics. Table 1 is showing the chemical and physical properties of the soil used in this study and Table 2 is showing the harvesting time of the three used cultivars in the two successive seasons.

Plant height (cm): Data in Table 3 showed that the highest plants were observed in Jaguar (89.2 and 90.3 cm) followed by Palmoral (81.9 and 82.4 cm) and Master B which gave the shortest plants (69.9 and 71.1 cm) in the first and second seasons, respectively with significant differences among them. The treatment with the bacterial isolate 1 gave the highest plants (81.7 and 82.8 cm) and 50% NPK in combination with isolate 3 gave the highest values of plant height (86.8 and 88.6 cm). Interestingly, the triple interactions among cultivars, bacterial Isolates and NPK fertilizer resulted in the highest plants when Master B was fertilized with 50% of NPK in combination with isolate 2 (97.2 and 99.6 cm) in the first and second seasons, respectively (Table 3).

Flowering time (days): This character means the days from planting the pea seeds till the appearance of the first flower on plants. Master B was the earliest cultivar to begin flowering (33.8 and 45.0 days) followed by Palmoral (46.4 and 47.4 days) and finally by Jaguar (57.0 and 47.9 days). Treatment with isolate 3 helped pea plants to flower earlier (46.2 and 47.1 days) comparing to the other Isolates. The chemical fertilization with NPK only made plants flower later (46.7 and 47.3 days) than the bio-fertilization. Bio-fertilization alone helped plants flower earlier and Master B plants bio-fertilized without any mineral fertilizers were the earliest plants to flower (32.1 and 33.6 days) in the first and second seasons, respectively (Table 4).

No. of branches/plant: Jaguar gave the highest values of No. of branches/plant (4.3 and 4.3) followed by Palmoral (3.3 and 3.3) and Master B (2.5 and 2.4) as shown in Table 5. Bio-fertilizing the plants with isolate 3 increased the number of branches per plant in the first season (3.2) and Isolates 1 and 2 increased it in the second season (3.4 for both). The 50% dose of NPK in combination with isolate 3 increased No. of branches/plant in the two seasons (4.0 and 3.9). Moreover, the significant highest mean values of this character were obtained when Master B was fertilized with 50% NPK in combination with isolate 2 in the two seasons (5.4 and 5.2), respectively (Table 5).

No. of pods/plant: Jaguar also showed the highest values of No. of pods/plant in the two seasons (13.0 and 14.7) as shown in Table 6. Fertilizing the plants with isolate 2 also enhanced the plants to give higher No. of pods/plant (14.3) in the first season but in the second season all isolates almost

Table 3: Effect of bio and/or mineral fertilization on plant height (cm) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	69.8	60.1	73.3	77.2	91.2	82.3	94.3	97.2	82.4	76.3	85.0	90.7	69.9	81.7	81.10
Jaguar	69.2	61.3	74.4	78.4	91.9	75.2	92.2	96.3	82.0	74.1	84.2	88.8	89.2	80.7	70.20
Palmoral	69.4	58.8	71.8	75.5	91.7	73.1	92.2	93.0	81.9	70.0	83.1	84.2	81.9	78.7	83.40
$LSD_{0.05}$	A = 3.15		B = 2.61		AB = 2.03		C = 0.47		AC = 0.36		BC = 0.63		ABC = 3.89		86.80
2011/2012															
Master B	68.4	62.0	76.0	79.1	90.7	83.9	95.8	99.6	80.9	77.9	86.4	92.8	71.1	82.8	80.80
Jaguar	68.6	63.4	73.8	81.0	91.3	77.4	93.4	98.3	80.2	76.0	85.4	90.7	90.3	81.6	72.10
Palmoral	68.2	61.3	73.2	77.8	91.1	74.3	93.6	94.6	80.4	72.5	81.9	83.1	82.4	79.3	84.40
$LSD_{0.05}$	A = 3.26		B = 2.82		AB = 2.14		C = 0.56		AC = 0.38		BC = 0.82		ABC = 3.92		88.60

Table 4: Effect of bio- and/or mineral fertilization on flowering time (days after planting) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	34.7	32.1	33.1	33.5	58.1	54.9	56.5	56.8	47.2	45.9	46.4	46.6	33.8	45.5	46.7
Jaguar	34.8	32.7	33.8	33.9	58.1	55.5	56.8	57.2	47.2	44.9	45.2	45.9	57.0	45.5	44.8
Palmoral	34.8	33.4	34.4	34.4	58.1	56.9	57.4	57.5	47.2	46.6	46.7	46.7	46.4	46.2	45.6
$LSD_{0.05}$	A = 0.03		B = 0.04		AB = 0.05		C = 0.03		AC = 0.04		BC = 0.04		ABC = 0.07		45.8
2011/2012															
Master B	35.5	33.6	34.6	35.1	58.6	56.6	57.7	57.9	47.8	47.2	47.6	47.7	45.0	46.7	47.3
Jaguar	35.5	33.9	35.1	35.3	58.6	56.8	57.8	58.0	47.8	46.0	46.6	46.8	47.9	46.5	46.1
Palmoral	35.5	34.7	35.4	35.4	58.6	58.0	58.3	58.3	47.8	47.6	47.9	48.0	47.4	47.1	46.7
$LSD_{0.05}$	A = 0.03		B = 0.03		AB = 0.04		C = 0.03		AC = 0.04		BC = 0.05		ABC = 0.08		46.9

Table 5: Effect of bio- and/or mineral fertilization on No. of branches/plant of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	2.3	1.9	2.6	3.4	4.2	3.8	4.5	5.4	3.3	3.0	3.7	4.0	2.5	2.5	3.3
Jaguar	2.4	1.9	2.7	3.6	4.2	3.7	4.5	5.1	3.3	2.8	3.5	3.8	4.3	2.5	2.8
Palmoral	2.4	1.8	2.6	2.9	4.1	3.6	4.0	4.4	3.2	2.8	3.3	3.4	3.3	3.2	3.5
$LSD_{0.05}$	A = 0.20		B = 0.20		AB = 0.10		C = 0.13		AC = 0.14		BC = 0.14		ABC =	0.21	4.0
2011/2012															
Master B	3.2	1.9	2.5	3.2	4.0	3.9	4.4	5.2	3.2	3.1	3.5	3.9	2.4	3.4	3.2
Jaguar	2.2	2.0	2.6	3.4	4.0	3.8	4.3	5.0	3.2	3.0	3.6	3.7	4.3	3.4	2.9
Palmoral	2.3	1.8	2.4	2.8	4.0	3.7	4.2	4.6	3.1	2.8	3.2	3.3	3.3	3.2	3.4
$LSD_{0.05}$	A = 0.24		B = 0.22		AB = 0.11		C = 0.14		AC = 0.14		BC = 0.16		ABC = 0.23		3.9

Table 6: Effect of bio and/or mineral fertilization on No. of pods/plant of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	12.8	9.3	10.4	11.2	15.6	10.6	15.0	15.6	14.5	9.9	12.6	14.6	12.6	11.2	14.3
Jaguar	12.9	9.8	10.5	11.4	15.6	10.7	14.8	15.5	14.6	9.5	12.6	13.6	13.0	14.3	9.9
Palmoral	12.8	8.9	11.8	12.9	15.7	10.9	15.6	15.7	14.6	9.1	13.5	14.2	12.6	12.8	13.0
$LSD_{0.05}$	A = 0.27		B = 0.27		AB = 0.30		C = 0.27		AC = 0.36		BC = 0.36		ABC = 0.56		13.9
2011/2012															
Master B	13.1	11.1	11.3	11.9	15.8	11.8	15.2	15.9	14.5	10.7	13.2	14.9	11.9	13.3	14.5
Jaguar	13.2	10.9	11.3	11.7	15.8	11.4	15.5	15.7	14.3	10.4	13.7	14.3	14.7	13.2	10.7
Palmoral	13.2	9.6	12.3	13.2	15.8	11.1	15.3	16.5	14.6	9.6	13.7	14.5	13.2	13.3	13.5
$LSD_{0.05}$	A = 0.28		B = 0.27		AB = 0.29		C = 0.27		AC = 0.35		BC = 0.37		ABC = 0.59		14.3

behaved the same. The mean value of the fertilizer concentrations was the highest when the NPK fertilizer was used alone (14.3 and 14.5) in the two seasons. Regarding the triple interaction, Palmoral fertilized with 50% of NPK in combination with isolate 2 gave the significant highest mean values of this character (15.7 and 16.5) in the first and second seasons, respectively (Table 6).

Fresh weight of green pods/plant (g): Table 7 showed that Jaguar gave the significant highest mean values of fresh weight of green pods/plant in the two seasons (78.1 and 92.8 g). Bio-fertilizing pea plants with isolate 2 gave the highest values in the first season (87.0 g) and was similar to isolate 1 in the second season (79.8 and 82.1 g), respectively (Table 7). Furthermore, fertilizing pea plants with 50% NPK in combination with the bio-fertilization gave the highest mean values of fresh green pod weights/plant in the two seasons (88.0 and 93.7 g) comparing to other fertilizers combinations. On the other hand, Master B fertilized with 50% NPK+isolate 2 gave the significant highest mean values of this character (109.8 and 117.7 g) in the first and second seasons, respectively (Table 7).

Fresh green pod yield/fed (t): Jaguar gave the significant higher mean values of fresh yield/fed of green pods in the two seasons (5.2 and 6.2 t/fed) followed by Palmoral (4.7 and 5.0 t/fed) and then by Master B which gave the lowest values (4.8 and 4.5 t/fed). Bio-fertilizing pea plants with isolate 2 resulted in the higher mean values of this character (5.8 t/fed) in the first season and almost similar to isolate 1 in the second season (5.3 and 5.5 t/fed), respectively. The combination of 50% of NPK and bio-fertilizations gave higher mean values of fresh green pod yield/fed (5.9 and 6.2 t/fed) than the other combinations. The triple combination among cultivars, mineral and bio-fertilizations showed that Master B fertilized with 50% NPK and isolate 2 gave the highest mean values (7.3 and 7.9 t/fed) in the first and second seasons, respectively comparing to the other combinations (Table 8).

No. of seed/pod: Master B cultivar showed the highest No. of seeds/pod (8.7 and 9.1) in the two seasons (Table 9). Isolates 1 and 2 gave almost the same mean values of this character in the two seasons. The dose of 50% NPK+Bio-fertilizers gave the highest mean values in the two seasons (9.0 and 9.7). Furthermore, Jaguar fertilized with 50% NPK in combination with isolate 1 showed the significant highest mean values in the seasons (10.4 and 10.8), respectively (Table 9).

Dry weight of 1000 seeds (g): The three pea cultivars differed significantly in this characteristic as Jaguar showed the highest mean values (190.1 and 212.0 g) comparing to 181.9 and 205 g for Palmoral and 177.6 and 192.4 g for Master B in the first and second seasons, respectively (Table 10). The bio-fertilization alone decreased the seeds weight (130 g for Palmoral with isolate 1 in the first season for example) while the combination of 50% of NPK plus the bio-fertilization gave the highest mean values (253.8 g for Master B with isolate 2 in the second season) and the mineral fertilization alone gave average mean values for all cultivars in the two growing seasons (Table 10).

Dry seed yield/fed (t): Jaguar gave the highest mean value of dry seed yield/fed (1.25 and 1.25 t) followed by Palmoral (1.02 and 1.07 t) and Master B showed the significant lowest mean

Table 7: Effect of bio- and/or mineral fertilization on fresh green pod weight/plant (g) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	65.5	40.9	64.6	73.3	95.2	57.7	98.9	109.8	86.5	48.4	76.5	93.6	71.4	61.0	80.0
Jaguar	64.8	45.7	67.2	78.4	94.8	55.8	93.3	104.2	79.6	45.4	72.8	84.4	78.1	87.0	46.9
Palmoral	65.1	34.9	61.5	69.7	94.9	52.3	88.8	98.9	79.5	40.7	69.8	79.5	69.3	70.9	77.0
$LSD_{0.05}$	A = 1.85		B = 1.85		AB = 2.07		C = 1.76		AC = 2.35		BC = 2.35		ABC = 3.62		88.0
2011/2012															
Master B	69.4	49.9	72.1	79.8	99.3	69.4	105.1	117.7	83.5	54.3	84.9	99.6	67.4	82.1	84.7
Jaguar	76.0	54.6	74.2	83.2	99.6	61.6	100.2	108.3	82.9	51.4	76.6	89.4	92.8	79.8	53.5
Palmoral	69.7	39.4	66.7	74.1	99.0	55.3	92.2	105.4	82.8	45.6	74.1	85.3	75.9	74.1	82.9
$LSD_{0.05}$	A = 1.76		B = 1.76		AB = 2.07		C = 1.78		AC = 2.34		BC = 2.34		ABC = 3.88		93.7

Table 8: Effect of bio- and/or mineral fertilization on fresh green pod yield/fed (ton) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	4.4	2.7	4.3	4.9	6.3	4.0	6.7	7.3	5.3	3.2	5.1	6.2	4.8	4.1	5.3
Jaguar	4.3	3.0	4.8	5.2	6.3	3.7	6.2	6.9	5.3	3.0	4.9	5.2	5.2	5.8	3.1
Palmoral	4.3	2.3	4.1	4.7	6.3	3.5	5.9	6.5	5.3	2.7	4.7	4.7	4.7	4.8	5.1
$LSD_{0.05}$	A = 0.11		B = 0.11		AB = 0.13		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.18		5.9
2011/2012															
Master B	4.6	3.3	4.8	5.3	6.6	4.6	7.0	7.9	5.6	3.6	5.6	6.6	4.5	5.5	5.6
Jaguar	4.7	3.6	5.0	5.6	6.6	4.1	6.7	7.2	5.6	3.4	5.1	6.1	6.2	5.3	3.6
Palmoral	4.7	2.6	4.5	4.9	6.6	3.7	6.2	7.0	5.5	3.0	4.9	5.6	5.0	4.9	5.5
$LSD_{0.05}$	A = 0.12		B = 0.12		AB = 0.14		C = 0.10		AC = 0.12		BC = 0.12		ABC = 0.19		6.2

Table 9: Effect of bio and/or mineral fertilization on No. seeds/pod of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	5.8	6.7	9.5	10.1	7.7	6.5	8.1	9.3	7.5	6.0	7.9	8.6	8.7	8.1	7.9
Jaguar	5.8	7.0	9.7	10.4	7.7	6.2	8.2	8.9	7.4	5.8	7.7	8.3	7.7	8.0	6.2
Palmoral	5.8	6.5	9.2	9.8	7.7	5.9	7.8	8.3	7.5	5.4	7.5	7.7	7.3	7.6	8.4
$LSD_{0.05}$	A = 0.35		B = 0.35		AB = 0.39		C = 0.12		AC = 0.12		BC = 0.12		ABC = 0.18		9.0
2011/2012															
Master B	9.0	7.2	8.9	10.7	8.2	6.9	8.5	9.7	9.7	6.5	8.5	9.4	9.1	8.4	8.3
Jaguar	9.0	7.4	10.2	10.8	8.2	6.6	8.6	9.6	9.7	6.2	8.4	9.1	8.2	8.5	6.6
Palmoral	8.9	6.9	9.6	10.2	8.1	6.3	8.3	9.2	9.7	5.8	8.2	8.4	7.8	8.2	8.8
$LSD_{0.05}$	A = 0.38		B = 0.38		AB = 0.41		C = 0.13		AC = 0.13		BC = 0.13		ABC = 0.19		9.7

Table 10: Effect of bio- and/or mineral fertilization on 1000 seeds dry weight (g) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	167.8	145.1	184.8	189.0	184.9	167.8	215.9	232.7	179.7	165.8	201.7	225.9	177.6	172.0	177.9
Jaguar	170.4	153.8	188.7	201.9	184.5	154.7	205.0	223.7	179.8	150.8	195.2	214.1	190.1	192.7	149.9
Palmoral	170.6	130.9	179.7	181.1	184.6	145.0	197.9	216.0	179.5	135.7	189.0	201.9	181.9	184.9	195.3
$LSD_{0.05}$	A = 1.87		B = 1.87		AB = 2.01		C = 1.68		AC = 2.23		BC = 2.23		ABC = 3.45		209.6
2011/2012															
Master B	190.7	164.0	203.7	208.1	205.2	189.0	238.0	253.8	199.2	185.6	225.3	248.8	192.4	209.3	198.3
Jaguar	191.5	175.0	207.1	222.7	203.9	174.0	224.1	241.0	200.1	169.9	214.8	233.1	212.0	204.7	169.4
Palmoral	191.6	148.8	199.9	205.7	202.5	163.8	215.7	232.2	200.6	155.0	207.7	219.2	205.0	195.2	215.1
$LSD_{0.05}$	A = 1.89		B = 1.89		AB = 2.22		C = 1.79		AC = 2.24		BC = 2.24		ABC = 3.56		229.4

value (0.74 and 0.80 t) in the first and second seasons, respectively (Table 11). Furthermore, plants treated with isolate 1 gave the highest mean value of dry seed yield (1.05 t/fed in the first season and 1.09 t/fed in the second season). Also, plants treated with the combination of bio-fertilizers+50% NPK gave the highest mean value (1.20 and 1.25 t/fed) comparing to the bio-fertilizers alone without NPK which gave the lowest one (0.78 and 0.76 t/fed) in the first and second seasons, respectively. Regarding the combination of the three factors, plants of Master B treated with isolate 2+50% NPK gave the highest mean values of total dry seed yield/fed (1.49 and 1.54 t) while Palmoral plants treated with isolate 1 without NPK fertilizer gave the lowest values (0.42 and 0.50 t/fed) in the first and second seasons, respectively with significant differences between them (Table 11).

Total crude protein content in dry seeds (%): Data in Table 12 showed that the mean values of total crude protein content of dry seeds slightly differed in the three cultivars and Jaguar showed the highest content (22.9 and 25.1%) followed by Palmoral (22.7 and 24.6%) and then Master B (22.3 and 25.1%) in the first and second seasons, respectively. Moreover, bio-fertilizing the three cultivars increased their protein content (23.0 and 25.2%) for isolate 2 (22.7 and 25.1%) for isolate 3 and then (22.3 and 24.3%) for isolate 1 in the first and second seasons, respectively. The combination between the isolates and 50% of NPK enhanced the plants to increase their seeds content of protein (25.2 and 27.3%) comparing to plants only bio-fertilized (18.4 and 21.0%) in the first and second seasons, respectively. The highest content of protein in the dry seeds (28.2%) was obtained when plants of Jaguar fertilized with isolate 2+50% of NPK in the second season (Table 12).

Nitrate (NO_3^{-1}) and Nitrite (NO_2^{-1}) contents (mg kg^{-1} dry weight): The low content of nitrate and nitrite in edible plants is recommended and the present results showed that the three pea cultivars had almost similar content of these substances. These contents were increased by fertilizing the plants with the NPK mineral fertilization alone (189 mg kg^{-1} dry weight nitrate for Palmoral in the second season as the highest content) and were decreased about 50% by fertilizing the plants with the bio-fertilizers ((for example from 147.9 mg kg^{-1} dry weight nitrate (with the mineral fertilizers) to 98.0 mg kg^{-1} dry weight nitrate (with the bio-fertilization) for Palmoral in the first season)). Also, the same trend was observed in the nitrite content as the bio-fertilization decreased its content in the seeds of Palmoral (7.3 to 3.7 mg kg^{-1} dry weight in the first season). The seed content of both nitrate and nitrite was increased by increasing the NPK doses (Table 13 and 14).

NPK contents in dry seeds (%): Table 15-17 are showing the NPK contents in pea dry seeds of the three cultivars bio-or mineral fertilized. Data showed that the three cultivars slightly differed in their contents of these three elements and the contents were increased by the mineral fertilization and decreased by the bio-fertilization. When the combination of 50% NPK plus isolate 2 was used to fertilize Palmoral plants the N content was 4.90% comparing to 3.90% with the bio-fertilization alone and 4.31% with the 100% NPK in the second season as an example (Table 15). Also, when the combination of 50% NPK and isolate 1 was used to fertilize Jaguar plants; this resulted in 0.42 mg kg^{-1} P in dry seeds comparing to 0.37 mg kg^{-1} with the bio-fertilizing with isolate 1 alone and 0.40 mg kg^{-1} when 100% of NPK was used alone (Table 16). Regarding the K content, data in Table 16 showed almost the same results as plants of Master B

Table 11: Effect of bio- and/or mineral fertilization on total dry seed yield/fed (ton) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	0.80	0.45	0.77	0.91	1.24	1.01	1.28	1.49	1.07	0.86	1.16	1.33	0.74	0.74	1.04
Jaguar	0.81	0.50	0.82	0.98	1.23	1.02	1.22	1.42	1.05	0.72	0.99	1.24	1.25	1.05	0.78
Palmoral	0.82	0.42	0.71	0.86	1.25	0.83	1.08	1.34	1.06	0.61	0.93	1.17	1.02	1.02	1.00
$LSD_{0.05}$	A = 0.10		B = 0.10		AB = 0.11		C = 0.08		AC = 0.11		BC = 0.11		ABC = 0.17		1.20
2011/2012															
Master B	0.90	0.51	0.81	1.00	1.30	1.10	1.40	1.54	1.13	0.91	1.21	1.40	0.80	1.09	1.09
Jaguar	0.90	0.60	0.90	1.05	1.30	1.00	1.30	1.50	1.10	0.80	1.10	1.30	1.25	1.05	0.76
Palmoral	0.90	0.50	0.80	0.92	1.30	0.90	1.14	1.40	1.11	0.70	1.00	1.22	1.07	0.98	1.05
$LSD_{0.05}$	A = 0.10		B = 0.10		AB = 0.13		C = 0.10		AC = 0.11		BC = 0.12		ABC = 0.17		1.25

Table 12: Effect of bio- and/or mineral fertilization on total crude protein content (%) in dry seeds of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
	100%				100%				100%				Mean	Mean	Mean
Fertilizers	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	NPK	0.0	25.0	50.0	of (A)	of (B)	of (C)
2010/2011															
Master B	22.9	18.6	23.4	25.3	23.8	17.5	23.3	24.0	23.3	17.1	23.8	24.6	22.3	22.3	23.3
Jaguar	22.9	19.7	23.8	26.0	23.8	18.3	24.2	26.1	23.3	18.6	24.0	25.3	22.9	23.0	18.4
Palmoral	22.9	17.0	22.0	23.6	23.8	19.9	24.4	26.3	23.2	19.4	24.4	26.1	22.7	22.7	23.6
$LSD_{0.05}$	A = 0.11		B = 0.11		AB = 0.13		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.18		25.2
2011/2012															
Master B	25.9	21.7	25.2	27.4	25.2	21.6	25.7	27.8	25.3	18.5	25.4	26.5	25.1	24.3	25.5
Jaguar	26.0	22.9	26.8	28.0	25.4	23.6	26.3	28.2	25.3	19.8	25.8	27.4	25.1	25.2	21.0
Palmoral	26.0	19.2	25.0	26.6	25.2	18.5	25.4	26.5	25.4	21.5	26.3	28.0	24.6	25.1	25.8
$LSD_{0.05}$	A = 0.13		B = 0.13		AB = 0.14		C = 0.10		AC = 0.13		BC = 0.14		ABC = 0.20		27.3

Table 13: Effect of bio- and/or mineral fertilization on nitrate content (%) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	158	110	132	158	179	114	152	169	169	111	144	157	152.9	140.0	170.5
Jaguar	163	121	140	168	182	125	160	184	170	119	155	173	154.9	161.9	119.2
Palmoral	162	98	119	161	180.7	141	167	189	171	134	162	182	147.9	153.9	114.7
$LSD_{0.05}$	A = 0.10		B = 0.10		AB = 0.11		C = 0.08		AC = 0.11		BC = 0.11		ABC = 0.17		170.0
2011/2012															
Master B	164	112	136	165	187	116	156	177	175	113	147	167	145.3	151.3	177.0
Jaguar	169	124	145	177	189	127	164	188	176	121	158	177	166.9	159.6	121.6
Palmoral	168	101	124	169	188	144	175	192	177	136	165	186	158.2	159.6	152.2
$LSD_{0.05}$	A = 0.11		B = 0.11		AB = 0.12		C = 0.09		AC = 0.12		BC = 0.11		ABC = 0.18		176.4

Table 14: Effect of bio- and/or mineral fertilization on nitrite content (%) of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	6.7	4.2	5.4	6.5	7.3	5.1	5.6	6.1	7.1	5.0	5.5	6.1	6.0	5.7	7.0
Jaguar	6.6	4.5	5.6	6.6	7.3	5.4	5.8	6.8	7.1	5.2	5.7	6.7	6.2	6.3	4.9
Palmoral	6.6	3.7	5.2	6.4	7.3	5.5	5.9	7.0	7.1	5.5	5.8	6.9	5.8	6.1	5.6
$LSD_{0.05}$	A = 0.11		B = 0.11		AB = 0.13		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.18		6.6
2011/2012															
Master B	7.0	4.5	5.7	6.6	7.6	5.3	5.9	6.4	7.4	5.2	5.8	6.4	6.0	6.1	7.3
Jaguar	7.0	4.9	6.0	6.9	7.7	5.6	6.0	7.1	7.4	5.4	5.9	6.9	6.5	6.4	5.1
Palmoral	6.9	3.9	5.5	6.6	7.6	5.7	6.2	7.3	7.4	5.7	6.1	7.2	6.4	6.3	5.9
$LSD_{0.05}$	A = 0.14		B = 0.14		AB = 0.14		C = 0.11		AC = 0.13		BC = 01.3		ABC = 0.20		6.8

Table 15: Effect of bio- and/or mineral fertilization on N % of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	3.64	2.76	3.55	3.74	3.85	2.92	3.78	4.15	3.74	2.75	3.73	4.33	3.59	3.42	3.73
Jaguar	3.65	2.96	3.69	3.84	3.73	3.14	3.91	4.22	3.73	2.96	3.81	4.14	3.84	3.79	2.96
Palmoral	3.67	2.55	3.44	3.55	3.82	3.36	4.18	4.33	7.73	3.22	3.98	4.28	3.47	3.70	3.78
$LSD_{0.05}$	A = 0.42		B = 0.42		AB = 0.47		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.19		4.07
2011/2012															
Master B	4.14	3.24	4.1	4.30	4.40	3.44	4.34	4.60	4.30	3.30	4.24	4.51	3.9	4.1	4.2
Jaguar	4.20	3.50	4.2	4.40	4.33	3.63	4.43	4.71	4.23	3.44	4.31	4.63	4.3	4.2	3.5
Palmoral	4.20	3.10	3.9	4.03	4.31	3.90	4.70	4.90	4.24	3.73	4.41	4.70	4.2	4.2	4.3
$LSD_{0.05}$	A = 0.46		B = 0.46		AB = 0.53		C = 0.11		AC = 0.14		BC = 0.14		ABC = 0.21		4.5

Table 16: Effect of bio- and/or mineral fertilization on P % of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	0.33	0.29	0.32	0.33	0.28	0.23	0.26	0.28	0.25	0.20	0.24	0.25	0.32	0.32	0.29
Jaguar	0.33	0.30	0.33	0.33	0.28	0.25	0.28	0.29	0.25	0.22	0.25	0.27	0.27	0.27	0.25
Palmoral	0.33	0.29	0.32	0.33	0.28	0.26	0.29	0.31	0.25	0.24	0.26	0.27	0.25	0.25	0.28
$LSD_{0.05}$	A = 0.42		B = 0.42		AB = 0.47		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.19		0.29
2011/2012															
Master B	0.4	0.37	0.38	0.40	0.37	0.32	0.34	0.36	0.3	0.28	0.31	0.32	0.39	0.35	0.36
Jaguar	0.4	0.37	0.39	0.42	0.36	0.34	0.36	0.40	0.3	0.30	0.31	0.32	0.36	0.35	0.33
Palmoral	0.4	0.35	0.38	0.40	0.36	0.35	0.37	0.40	0.3	0.30	0.32	0.34	0.31	0.35	0.35
$LSD_{0.05}$	A = 0.14		B = 0.14		AB = 0.14		C = 0.11		AC = 0.13		BC = 01.3		ABC = 0.20		0.40

Table 17: Effect of bio- and/or mineral fertilization on K % of three cultivars of peas in two successive winter seasons of 2010/2011 and 2011/2012

		Isolate 1+NPK (%)				Isolate 2+NPK (%)				Isolate 3+NPK (%)					
Fertilizers	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	100% NPK	0.0	25.0	50.0	Mean of (A)	Mean of (B)	Mean of (C)
2010/2011															
Master B	2.22	1.52	1.77	2.10	2.47	2.02	2.43	2.58	2.33	1.95	2.18	2.41	2.27	1.92	2.35
Jaguar	2.24	1.75	1.96	2.28	2.49	1.91	2.35	2.48	2.34	1.72	1.97	2.36	2.09	2.30	1.73
Palmoral	2.21	1.40	1.64	1.95	2.50	1.73	2.23	2.42	2.35	1.75	1.85	2.27	2.00	2.11	2.04
$LSD_{0.05}$	A = 0.42		B = 0.42		AB = 0.47		C = 0.09		AC = 0.12		BC = 0.12		ABC = 0.19		2.32
2011/2012															
Master B	2.30	1.70	1.84	2.22	2.6	2.11	2.52	2.70	2.41	2.03	2.30	2.51	2.02	2.3	2.43
Jaguar	2.31	1.90	2.03	2.40	2.6	2.00	2.44	2.60	2.42	1.83	2.10	2.44	2.40	2.3	1.84
Palmoral	2.30	1.52	1.80	2.08	2.6	1.81	2.32	2.52	2.43	1.70	1.93	2.34	2.20	2.1	2.13
$LSD_{0.05}$	A = 0.14		B = 0.14		AB = 0.14		C = 0.11		AC = 0.13		BC = 01.3		ABC = 0.20		2.41

fertilized with the combination of 50% NPK plus isolate 2 gave 2.70% of K content comparing to 2.11% when the plants were only bio-fertilized with isolate 2 and 2.60% when the plants were only mineral fertilized with 100% of NPK (Table 17).

DISCUSSION

Pea is a very nutritious vegetable grown in the cool season throughout the world. It is grown as a vegetable crop for both fresh and dried seed. Pea contains high percentage of digestible protein, along with carbohydrates and vitamins. That's why we chose here to evaluate novel pea cultivars newly grown under the Egyptian winter growing conditions. Master B is a commonly known cultivar and is a very early maturing cultivar suitable for mechanic harvesting and Jaguar and Palmoral cultivars are recently imported British cultivars which are highly yielding sweet peas cultivars. The later ones are very promising pea cultivars under the Egyptian growing conditions. Our results confirmed all these characteristics of these cultivars and confirmed our previous results (Gheeth *et al*., 2012).

It is crucial to mention here that all the bio-fertilization isolates used here are produced in our university (The centre of Bio-fertilization, Minia University, Egypt). This centre produces many bio-fertilization products for different crops including the non legumes and legumes crops. The centre is also doing a good research on genetic modifying the bacteria of these products and got very beneficial mutants which are broadly distributed to the Egyptian farmers. The present isolates used in this study are three promising products of this centre.

The present bio-fertilization isolates treatment 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 are organic and safe in their content of nitrate and nitrite. That's because bio-fertilizers are important components of integrated nutrients management. Bio-fertilizers are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus etc.) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi *et al*., 2008).

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 and protect plants from pests and diseases which also enhances growth and increases yield (El-Yazeid *et al*., 2007). The 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).

Using the combination of bio-fertilizers and half dose of 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 carrot and tomato, on sugar beet (Stajner *et al*., 1997), on cauliflower (Bambal *et al*., 1998), on faba bean (Wyszkowska, 1999) and on cabbage (Sharma, 2002). 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 and Bhargava (1994) on *Brassica napus* and Verma *et al*. (1997) on cabbage, Verma *et al*. (2000) on pea and Panwar *et al*. (2000) on radish indicated that both inoculation with Azotobacter and application of N increased seed yield.

Moreover, 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; 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 fixation, dissolving phosphorus and production of organic acids in the soil which consequently lowering soil pH and increase the availability of most of the plants needed elements (Hanafy Ahmed *et al*., 2000). In addition, the beneficial effects of biofertilizers 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 present results.

CONCLUSION

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 these cultivars potentials of growth and total fresh yield, protein content and dry seed production and also decreased the seeds contents of nitrate and nitrite. The best results were obtained when the 50% of the recommended NPK dose was used along with the bio-fertilization isolates. 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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