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EFFECT OF DIFFERENT BIOFERTILIZER TYPES AND NITROGEN FERTILIZER LEVELS ON GROWTH, YIELD AND CHEMICAL CONTENTS OF PEA PLANTS (*PISUM SATIVUM* L.)

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ABSTRACT

The current study was carried out to investigate the effects of seed inoculation with five different biofertilizer types (Azotobacter, Azospirillum, Rhizobium, a mixture of the previous types of the biofertilizers and uninoculated control) and four different fertilization levels of mineral nitrogen (0, 30, 60 and 90 Kg N fed^{-1}.) as well as their interactions on the vegetative growth characters, flowering traits , green pods yield and its components and some chemical contents of pea plants (*Pisum sativum L.*) cv. "Victory Freezer ".Two field experiments were carried out during the winter seasons of 2003 and 2004 at EL-Mahmodia region, Behera Governorate, Egypt. A factorial experiment in a randomized complete blocks design was used, with three replications. The obtained results indicated that inoculation of pea seeds with any utilized biofertilizer type significantly, increased all studied vegetative growth characters and yield components (plant height, number of leaves, number of branches, plant fresh weight , plant dry weigh , green pods yield fed^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, and shelling %).Also leaves contents of N, K and chlorophyll were increased compared to the uninoculated control, in both seasons. The mixed biofertilizer was the most effective one on all studied vegetative growth characters and yield components. The application of mineral nitrogen at the rates of 30, 60 and 90 Kg N fed^{-1} to the growing pea plants, significantly, increased

plant height, number of leaves and branches plant^{-1}, plant fresh weight, plant dry weight, yield of green pods fed^{-1}, number of green pods plant^{-1}, number of seed pod^{-1} . Also leaves N, K and chlorophyll contents were increased over the control treatment, in both seasons. The highest two nitrogen levels (60 and 90 Kg N fed-1.) were remarkable and associated with the highest mean values in this concern. The interaction effects of biofertilizer types and nitrogen levels on most studied characters were found significant in both seasons. The combined treatment of mixed biofertilizer and 60 or 90 Kg N fed^{-1} together, were considered the best treatment combinations, as they gave the highest mean values of most studied characters.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the major winter crops grown in Egypt for local consumption and export. Pea seeds, fresh or dry, had a high nutritional values due to their high contents of carbohydrates, proteins, vitamins and minerals (Smart, 1990).

Nitrogen nutrition is one of the paramount factors which influence growth and yield potential of many different vegetable crops. Suitable application of nitrogen to the growing pea plants was extensively studied by many investigators to attain favorable enhancing effects on growth, yield and quality. However, the excessive use of nitrogen fertilizers increased the total costs of crop production, created pollution of agro-ecosystem and increased deterioration of soil fertility (Marschner, 1995). Therefore, it became essential for us to evolve and adopt a strategy of supplementing or substituting the inorganic nitrogen with organic sources, especially those of microbial origin.

Nitrogen fixing microorganisms are either symbiotic of the genous Rhizobium; which associate with plant and form nodules, or non-symbiotic as bacteria of the genera Azotobacter, Azospirillum and Klebsiella; that generate ammonia for their own use and provide the plant with nitrogen as an exchange for carbon and protected habitat (Marschner, 1995).

Accordingly, the present study was conducted to investigate the influence of seed inoculation with different biofertilizer types and various nitrogen fertilizer levels as well as their different interactions on vegetative growth, flowering traits, green pods yield and its components, and chemical composition of pea plants.

METERIALS AND METHODS

This study was carried out during the two winter seasons of 2003 and 2004 at El-Mahmodia region, Behera Governorate, A.R.E.

Preceding the initiation of each experiment, soil samples of 25 cm depth, from each experimental site, were collected and analyzed for some important physical and chemical features, according to the standard and published procedures, reported by Page *et al*. (1982). The results of these analyses are shown in Table (1).

There were 20 treatment combinations consisting of 4 nitrogen levels (0, 30, 60 and 90 kg N fed^{-1}) and 5 biofertilizer treatments (uninoculated control**,** *Azotobacter chroococcum* **,** *Azospirillum lipoferium* , *Rhizobium Leguminsarum* and a Mixed biofertilizer containing equal parts of the single aforementioned biofertilizers). Bacterial population of the inoculants used was1.2 x 108 cell/cm^3 obtained from the Biofertilizer Unit, Faculty of Agriculture, Ain Shams University.The biofertilizer were supplied by the Biofertilizer Unit, Faculty of Agriculture, Ain Shams University, Cairo.

The seeds were surface sterilized with 1% sodium hypochlorite for 20 min, then rinsed with water several times. The surface of disinfected seeds was coated by soaking seeds in a liquid culture medium of each organism for 15 minutes using 10% arabic gum as Adhesive. For combined inoculations, the liquid cultures of the three organisms were mixed in equal proportions and the seeds were then dipped in it**(**Fernandez and Miller, 1986**).** Uninoculated seeds (control) were soaked in distilled water for 15. minutes also.

Table (1). Physical and chemical properties of the experimental sites during 2003 and 2004 seasons.

Parameters	Seasons	
	2003	2004
Physical properties		
Clay%	44.00	44.00
Silt%	40.00	40.00
Sand%	16.00	16.00
Soil texture	Silty clay	Silty clay
Chemical properties		
PH	8.3	7.6
EC(dsm^{-1}) soil paste	2.4	2.6
$CaCO_3$%	2.00	2.1
Macro-elements (ppm)		
Total N	85.00	90.00
Total P	29.00	27.00
Total K	316.16	416.00

The equivalent amount of each mineral N rate, in the form of ammonium sulphate (20.5% N), was side banded at two equal portions, after 3 and 5 weeks from seed sowing. All treatments received 200 kg of calcium superphosphate (15.5 % P_2O_5) and 50 kg potassium sulphate (48% K_2O) fed^{-1}. Superphosphate was applied pre-sowing date, whereas, potassium sulphate was added in two equal portions at 3 and 5 weeks after sowing date.

A factorial experiment in a randomized complete blocks design of twenty treatments combinations replicated three times was conducted each season. The experimental unit consisted of 5 rows, 4 meters long and 0.6 meter wide. The adjacent experimental units were separated by a guard row. Seeds of pea (*Pisum sativum L.*) Victory Freezer cultivar were inoculated and directly sowed on one side of the row at 15 cm apart on October 30, 2003 and October 26, 2004. Ten

random chosen plants from each plot, at 60 days from seed sowing were used to measure the following :

1-Vegetative growth characters: Plant height, Number of leaves and branches plant-1, Plant fresh weight, Plant dry weight.

2-Flowering traits: Earliness of flowering(the number of days from seed sowing till 25 % full blooming of pea plants) and Fruit set percentage(calculated as A/Bx100; where, A denotes number of setted pods plant^{-1} throughout the entire season and B denotes number of flowers plant-1 throughout the entire flowering period).

3-Green pods yield and its components: Number of green pods plant^{-1}, number of seeds pod^{-1}, total green pods yield feddan^{-1} and Shelling ratio calculated as:

$$\frac{\text{weight of green seeds per plot}}{\text{weight of green pods per plot}} \times 100$$

4- Leaves chemical contents : Total chlorophyll content (in SPAD unit) using digital chlorophyll meter (model minolta chlorophyll meter SPAD-502);Nitrogen content, according to Chapman and Pratt (1961);Phosphorus content according to the procedure of John (1970) and Potassium content as described by Jackson(1973).

All obtained data were statistically analyzed using SAS software program (1996). Comparisons among the means of different treatments were achieved using the New Least Significant Difference procedure at P=0.05 level, as illustrated by Al-Rawy and Khalf-Allah (1980).

RESULTS AND DISCUSSION

The main effects of nitrogen and biofertilizers and the interaction on vegetative growth:

The main effects of the biofertilizers on the various studied vegetative growth characters of pea plants, in both seasons of 2003and 2004, are illustrated in Table (2). Inoculation of pea seeds either with a single biofertilizer of Azotobacter, Azospirillum and Rhizobium or the mixture of them resulted in significant increments on plant height,

Table (2).The main effects of biofertilizer types and nitrogen fertilizer rates on the vegetative growth characters of pea plants

Treatments	Plant height (cm)		Number of leaves plant $^{-1}$		Number of branches plant $^{-1}$		Plant fresh weight (gm)		Plant dry weight (gm)	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
Biofertilizer type										
Control	36.53 C	38.45 C*	9.62 C	9.39 D	1.48 C	1.32 C	28.49 D	27.92 C	3.99 C	4.48 C
Azotobacter	41.26 B	43.36 B	10.76 B	10.49 C	1.74 B	1.45 C	33.30 C	31.05 B	4.49 B	5.03 B
Azospirillum	42.13 B	43.70 AB	10.87 B	10.54 C	1.75 B	1.53 B	33.48 C	30.38 B	4.48 B	5.06 B
Rhizobium	39.64 B	43.86 AB	10.17 B	10.90 B	1.75 B	1.72 A	35.49 B	30.94 B	4.79 B	5.24 A
Mixed	50.78 A	46.05 A	12.32 A	11.86 A	2.10 A	1.72 A	39.35 A	31.18 A	5.27A	6.03 A
N rate (Kg N fed^{-1})										
0	35.00 C	43.7 C	8.26 C	8.76 C	1.48 C	1.14 C	22.06 C	25.24 C	2.91 C	3.46 C
30	40.58 B	43.08 B	10.68 B	10.19 B	1.63 A	1.48 B	33.32 B	30.79 B	4.60 B	5.11 B
60	46.26 A	46.79 A	12.34 A	11.77 A	1.95 A	1.85A	40.08 A	33.43 A	5.41 A	6.00 A
90	46.42 A	47.78 A	12.51 A	11.85 A	1.99 A	1.82 A	41.12 A	33.44 A	5.50 A	6.11 A

during the winter season of 2003 and 2004

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

number of leaves, number of branches, plant fresh weight and plant dry weight, compared to the uninoculated control in both seasons. Additionally, the mixed biofertilizer was found superior and associated with the highest mean values of the vegetative growth characters comparing with the other single biofertilizers. These enhancing effects of the different biofertilizers could be due to the efficiency of the different bacterial strains, on N_2-fixation,dissolving immobilized P and producing appropriate amounts of phytohormones necessary for activating plant growth parameters. Jagnow *et al.* (1991) and Noel *et al.*(1996) demonstrated that the non-symbiotic N_2-fixing bacteria of the genera Azotobacter and Azospirillum produced adequate amounts of IAA and cytokinins which increase the surface area per unit of root length and enhanced root branching with an eventual increase in the uptake of nutrients from the soil and finally accelerated plant growth. The current results generally, agreemed with those results reported by many investigators such as Abd EL-Fattah and Arisha (2000) and Shiboob (2000), on common bean ; Abd EL-Naby (1998) and Adam (2002), on broad bean and EL-Mansi *et al.*(2000) and Ishaq (2002) on pea. Ismail (2002) reported that the inoculation of pea seeds with Rhizobium was responsible for significant increments on plant height, number of leaves and branches, fresh and dry weight plant^{-1} and dry matter %, compared to the uninoculated plants.

Table 2, clearly, showed that the main effects of nitrogen fertilizer rates on plant height, number of leaves, number of branches, plant fresh weight and plant dry weight reflected significant differences in both seasons.

The recorded results (Table 2), also clarified that the application of 30, 60 and 90 Kg N fed^{-1} to growing plants, significantly, increased plant height, number of leaves, number of branches, plant fresh weight and plant dry weight over the control treatment, in both seasons. Also, whether the nitrogen applied level was at either 60 or 90 Kg N fed^{-1}, all the studied vegetative growth characters showed the highest values. Additionally, the differences between these two nitrogen levels appeared insignificant on all previously mentioned characters. Such favorable effects of nitrogen application on vegetative growth could be expected, since nitrogen is known as an essential plant nutrient and plays a major role in nucleic acids and protein synthesis, cell division

and elongation and protoplasm formation (Marschner, 1995). Similar results were reported by several researchers such as EL-Neklawy *et al*. (1985), Vigai *et al.* (1990) and Ishaq (2002) on pea; EL-Oksh *et al*. (1991), Arisha and Bardisi (1999) and Merghany (1999) on common bean; Abd El-Mouty (2000) on cowpea and Abd El-Naby (1998) on broad bean. Ismail (2002) reported that vegetative growth characters of pea plants ,significantly ,increased by increasing nitrogen fertilizer up to 90 Kg N fed^{-1}. Also ,Shiboob (2000) indicated that increasing nitrogen level from 0 to 40 Kg N fed^{-1}.,significantly, enhanced the vegetative growth characters of common bean plants as expressed by plant height , shoot fresh weight , number of leaves, leaf area and leaf dry matter percentage .

Concerning the effects of interactions between biofertilizer types and nitrogen fertilizer levels on the vegetative growth characters of pea plants, the obtained results of the two seasons (Tables 3 & 4) revealed that inoculating pea seeds with the mixed biofertilizer combined with 90 Kg N $fed^{-1}$to the growing plants was pronounced and aided in attainment of the best vegetative growth as expressed by plant height, number of leaves $plant^{-1}$, plant fresh weight and plant dry weight. The desirable effects of the aforementioned treatment combination could be expected on the assumption that the promoting effects of this particular biofertilizer type and the used level of nitrogen, probably coupled together and, in turn, encouraged the vegetative growth to go more forward. The present results appeared to be in close agreements with those reported by many investigators, e.g. Abd EL-Mouty (2000) on cowpea and Ishaq (2002) on pea who reported that inoculation of pea seeds with the biofertilizer Halex-2 (mixture of azotobacter, azospirillium and klebsiella) and application of 40 Kg N fed^{-1}, attained the best results on plant height, fresh and dry weight $plant^{-1}$ and dry matter content of leaves and stems.

Table (3).Interaction effects of biofertilizer types and nitrogen fertilizer rates on the vegetative growth characters of pea plants, during the winter season of 2003.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Plant height (cm)					
0	30.48 f*	35.55 e	35.55 e	35.03 e	38.40 d e
30	35.80 e	37.86 d e	37.86 d e	38.40 d e	53.00 a
60	39.19 d	45.97 b c	47.15 b	42.18 c d	56.85 a
90	40.64 d	45.66 b c	47.97 c d	42.96 c	54.89 a
No. Leaves plant^{-1}					
0	6.41 I	8.67 g h	8.16 h	8.81 f g	9.25 f
30	8.43 g h	10.26 e	10.82 e	10.82 e	13.07 a b
60	11.74 d	11.67 d	12.43 b c	12.43 b c	13.46 a
90	11.91 c d	12.43 b c	12.09 b-d	12.64 b	13.50 a
No. branches plant^{-1}					
0	1.15	1.52	1.41	1.52	1.83
30	1.41	1.69	1.52	1.59	1.93
60	1.59	1.90	1.95	2.00	2.31
90	1.79	1.86	2.08	1.89	2.35
Plant fresh weight (gm)					
0	16.47 I	20.47 k	22.60 j k	23.19 j	27.61 I
30	23.67 j	33.83 g h	32.24 h	34.60 f-h	42.25 a-c
60	35.74 e-g	37.81 d e	40.53 c d	43.07 a b	43.27 a b
90	38.10 d e	41.11 b c	40.01 c d	41.10 b c	44.27 a
Plant dry weight (gm)					
0	2.47 I	2.78 h	2.86 h	3.12 g h	3.33 g
30	3.19 g h	4.65 f	4.64 f	4.71 e f	5.80 a b
60	5.13 d e	5.23 c d	5.16 d	5.68 b c	5.85 a b
90	5.17 d e	5.31 b-d	5.26 b-d	5.67 b c	6.09 a

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

Table (4). Interaction effects of biofertilizer types and nitrogen fertilizer rates on the vegetative growth characters of pea plants during the winter season of 2004.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Plant height (cm)					
0	29.25 h*	34.59 g	34.30 g	35.47 g	39.88 f
30	33.63 g	44.54 d e	44.61 d e	44.80 c-e	47.80 a b
60	44.96 d e	46.37 a-d	47.62 a b	47.40 a-c	47.60 a b
90	45.98 b-d	47.96 a b	48.26 a b	47.78 a b	48.91 a
No. Leaves plant^{-1}					
0	7.09 j	8.88 h	9.08 g h	9.43 g	9.52 g
30	8.11 I	10.35 f	10.37 f	10.87 e	12.39 a
60	11.10 d e	11.66 b c	11.27 c-e	11.61 c	12.75 a
90	11.28 c-d	11.71 b c	11.43 c d	11.70 b c	12.78 a
No. branches plant^{-1}					
0	1.00	1.00	1.10	1.37	1.26
30	1.28	1.40	1.47	1.73	1.93
60	1.45	1.65	1.89	1.92	2.15
90	1.43	1.76	1.96	1.87	1.97
Plant fresh weight (gm)					
0	21.90 h	24.27 g	25.26 g	24.83 f	26.97 f g
30	28.15 f	30.05 e	30.26 d e	31.27 d e	34.20 a b
60	30.80 e	35.58 b c	32.38 c d	34.05 a b	34.34 a b
90	30.83 e	34.30 a b	33.64 b	33.63 b	34.82 a
Plant dry weight (gm)					
0	3.09 h	3.34 h	3.35 h	3.55 h	4.00 g
30	3.33 h	5.18 e f	5.02 f	5.41 d-f	6.60 a b
60	5.76 b-e	5.81 b-d	5.81 b-d	5.91 b-d	6.73 a
90	5.76 b-e	5.80 b-d	6.06 b-d	6.11 b c	6.80 a

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

The main effects of nitrogen and biofertilizers and the interaction on flowering traits:

Data of the studied flowering traits, listed in Table (5) show that the inoculation of pea seeds with the mixed biofertilizers or with Rhizobium alone caused a significant delay of flowering in both seasons. However, inoculating pea seeds with any biofertilizer type was significantly associated with higher mean value of fruit set than the uninoculated control. These results could be attributed to the

effects of the symbiotic and non- symbiotic nitrogen fixing bacteria on the morphology and physiology of the root system which promoted the vegetative growth to continue for a longer time and hence delayed flowering. Similar findings were obtained by Sorial and EL-Khateeb (1992) and Barakat and Gabr (1998) on tomato; Gabr *et al.* (2001),on sweet pepper and Ishaq (2002), on pea who reported that seed inoculation with the biofertilizer Halex-2 caused significant increases on the number of days from seed sowing to flowering.

Table (5). Main effects of biofertilizer types and nitrogen fertilizer rates on flowering traits of pea plants during the winter seasons of 2003 and 2004.

Treatment	Season 2003		Season 2004	
	Earliness of flowering (days)	Fruit set (%)	Earliness of flowering (days)	Fruit set (%)
Biofertilizer type				
Control	56.75 C*	91.81 B	54.25 B	91.68 C
Azotobacter	57.75 B	93.98 A	55.25 B	92.79 B
Azospirillium	57.00 C	94.86 A	55.17 A B	93.38 B
Rhizobium	58.08 A B	95.54 A	55.83 A	94.39 A
Mixed	58.50 A	95.06 A	56.33 A	94.89 A
N rat (kg N Fed^{-1})				
0	56.80 B	90.66 C	49.80 C	89.38 C
30	57.07 B	94.21 B	53.27 B	93.08 B
60	58.20 A	95.65 A	58.87 A	95.31 A
90	58.27 A	96.48 A	59.53 A	95.54 A

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

Concerning the effects of nitrogen levels on the studied flowering traits, the results of the two growing seasons (Table 5) indicated that nitrogen fertilization with 60 and 90 Kg N fed^{-1}. , significantly, delayed flowering and increased fruit set percentage. Such results might be attributed to the stimulation effects of nitrogen on the vegetative growth characters that, consequently, resulted in delaying the flowering and increasing fruit set percentage. Similar results were obtained by Bakry *et al.* (1984), on pea; Edrees (2001) on lettuce and Gabr *et al.* (2001), on sweet pepper who showed that the increased applied levels of nitrogen from 40 to 80 Kg N fed^{-1}, significantly

delayed the flowering, whereas, the application of 120 Kg N fed^{-1} increased significantly fruit set percentage.

The effects of interaction between biofertilizer types and N fertilizer levels on earliness of flowering and fruit set were found significant in both seasons (Table 6).

Table (6).The interaction effects of biofertilizer types and nitrogen fertilizer rates on the vegetative growth characters of pea plants during the winter season of 2004.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Season 2003 Earliness of flowering (days)					
0	55.66 f*	56.67 e	56.00 e	57.67 c d	57.67 c d
30	56.33 e f	57.67 c d	56.33 e	57.33 d	58.67 a b
60	57.33 d	58.33 a-c	57.67 c d	58.67 a b	58.67 a b
90	57.67 c d	58.33 a-c	58.00 b c	58.67 a b	59.00 a
Fruit set (%)					
0	88.33 f	88.82 f	91.38 e	93.18 d	91.57 e
30	91.49 e	94.26 c d	94.57 c d	95.21 b-d	95.52 a-c
60	93.17 d	96.04 a-c	96.45 a b	96.82 a b	95.79 a-c
90	94.26 c d	96.81 a b	96.04 a b	96.95 a b	97.35 a
Season 2004 Earliness of flowering (days)					
0	46.67 f	50.67 e	50.33 d e	50.67 d e	50.67 d e
30	52.00 c-e	53.67 c	52.33 c d	53.67 c	59.67 a b
60	58.67 a b	57.67 b	58.67 a b	59.33 a b	60.00 a
90	59.67 a	59.00 a b	59.33 a b	59.67 a	60.00 a
Fruit set (%)					
0	87.58 g	89.89 e f	89.15 f	90.18 e f	90.10 e f
30	90.87 e	92.50 d	93.37 c d	94.00 c	96.65 a
60	94.33 b c	94.14 b c	95.54 a b	96.48 a b	96.05 a
90	94.93 b c	94.65 b c	95.47 a b	96.90 a	96.77 a

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

The results of the two growing seasons showed that inoculation of pea seeds with the mixed biofertilizer accompanied with nitrogen fertilization at the rate of 60 or 90 Kg N fed^{-1}, significantly , delayed flowering and increased fruit set . Similar findings were obtained by Barakat and Gabr (1998) on tomato; Abd

EL-Fattah and Sorial (2000), on summer squash and Gabr *et al.* (2001),on sweet pepper.

The main effects of nitrogen and biofertilizers and the interaction on green pods yield and its components:

The recorded results of the two growing seasons(Table 7) showed that the yield of green pods fed^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, and shelling % of the inoculated plants with any biofertilizer types were comparatively higher than those of the uninoculated ones and the mixed biofertilizer treatment was the most pronounced in this concern. These results might be expected on the basis that the different studied bacterial inoculants, probably, favoured one or more of the following beneficial influences; N_2 fixation, acquisition of P and various micro-nutrients due to production of chelators and organic acids, producing adequate amounts of indole acetic acid, cytokinins and abscisic acid which affect growth, morphology and physiology of roots and secretion of fungistic substances responsible for the inhibition of some pathogens like Alternaria and Fusarium (Martin *et al.*,1989 and Jagnow *et al.*,1991). The present results are in line with those of Dejensen *et al.*(2002) and EL-Bassiony (2003) on beans; Gabr *et al*. (2001) on sweet pepper and Ismail (2002), on pea. Shiboob (2000) indicated significant positive effects on green pods yield and its components of bean due to the inoculation of seeds with different biofertilizer types.

The effects of nitrogen levels on the studied green pods yield and its components are illustrated in Table 7. The results of the two growing seasons, clarified that increasing the application of nitrogen from 30 to 90 Kg N fed^{-1}, caused significant increase in yield of green pods fed^{-1}, number of green pods plant^{-1}, number of seeds pod^{-1} over the control treatment. The higher two nitrogen doses (60 and 90 Kg N fed^{-1}.) were remarkable in this concern, but the difference between them did not appear to be significant. However, shelling % was not significantly responded to nitrogen application. The obtained increments of green pods yield fed^{-1} as a result of nitrogen application might be directly attributed to the increased pods number plant^{-1} and number of seeds pod^{-1}. The enhancing effects of nitrogen may be related to the role of nitrogen in activating the vegetative growth which was reflected as significant increases on the different studied

Treatments	Green pods yield fed^{-1} (ton)		No. green pods plant^{-1}		No. seeds pods $^{-1}$		Shelling (%)	
	2003	2004	2003	2004	2003	2004	2003	2004
Biofertilizer type								
Control	2.32* D	2.54 D	24.63 D	25.75 D	7.74 C	8.17 C	42.84 B	43.71 B
Azotobacter	3.32 C	3.16 C	27.17 C	28.69 C	8.28 B C	8.61 B C	44.83 A	46.07 A B
Azospirillum	3.69 B C	3.50 B C	28.61 B	30.24 B	8.64 B	8.81 A B	44.06 A B	44.88 A B
Rhizobium	3.80 A B	3.74 A B	28.76 AB	30.67 A B	9.06 A	9.15 A	44.86 A	45.66 A
Mixed	4.40 A	4.04 A	29.71 A	31.08 A	9.11 A	9.18 A	44.14 A	47.81 A
Nitrogen fertilizer rate (kg N fed^{-1})								
0	2.30 C	2.06 C	23.30 C	23.78 C	7.73 B	7.46 B	43.82 A	45.01 A
30	3.64 B	3.54 B	27.86 B	29.76 B	8.71 A	8.67 B	44.48 A	45.96 A
60	4.09 A	4.05 A	29.79 A	31.73 A	8.98 A	9.46 A	44.14 A	46.27 A
90	3.84 A B	3.92 A B	29.64 A	31.96 A	8.85 A	9.51 A	44.35 A	45.25 A

Table (7). Main effects of biofertilizer types and nitrogen fertilizer rates on the green pods yield and its components of pea plants during the winter season of 2003 and 2004

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

growth parameters (Tables 3 and 4). It is also possible that the sufficient quantity and perhaps the efficient absorption of nitrogen coupled together promoted the production of more photosynthates required for pea seed production. The present results agreed to a great extent with those reported by Arisha and Bardisi (1999), who reported that the number and yield of green pods plant^{-1} as well as the total yield fed^{-1} were significantly increased with increasing the applied nitrogen up to 80 Kg N fed^{-1}. Ismail (2002) indicated that the addition of 90 Kg N fed^{-1}., to the growing pea plants, was sufficient for the plants to express their best performance on green pods yield and its components.

The interaction effects of biofertilizer types and N fertilizer rates on green pods yield and its components, in the two seasons of 2003 and 2004 are presented in Tables (8 & 9). the results of the two growing seasons indicated that green pods yield fed^{-1}, number of pods plant^{-1}, number of seeds pod^{-1} were significantly increased through the inoculation of seeds with different biofertilizers and different N levels, relative to the control treatment. The combined treatment of mixed biofertilizer and 30 or 60 Kg N fed^{-1}., gave the highest mean values of green pods fed^{-1} in both seasons. These results might be explained on the basis that the promoting effects of biofertilizer and nitrogen together on growth of pea plants were reflected on the increased of green pods yield and its components. Many investigators, working on different vegetable crops, emphasized the beneficial effects of the interaction between inoculation with biofertilizers and mineral nitrogen application on yield and its components as El-Araby *et al.* (2003) on peas; Abd EL-Mouty (2000) on cowpea; Shiboob (2000) on common bean; Elkhatib *et al*.(2004) on onion.

Table (8).Interaction effects of biofertilizer types and nitrogen fertilizer rates on the green pods yield and its components of pea plants, during the winter season of 2003.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Green pods yield fed^{-1} (ton)					
0	1.22 L	2.60 j	2.60 j	2.52 j	2.55 j
30	1.91 K	3.37 h I	3.62 f-h	4.31 c d	4.97 a
60	3.09 I	3.83 e-g	4.41 b-d	4.33 b-d	4.77 a b
90	3.09 I	3.50 g-I	4.09 c-e	4.04 d-f	4.52 a-c
No. pods plant^{-1}					
0	20.03 g	23.75 f	24.10 f	24.29 e f	24.35 e
30	23.32 f	28.58 c-e	28.72 b-e	30.05 a-d	31.39 a b
60	27.73 d e	29.25 b-d	30.13 a-d	30.40 a b	31.46 a b
90	27.46 e	28.49 d e	30.30 a-c	30.30 a-c	31.63 a
No. seeds pod^{-1}					
0	7.24 h	7.75 f g	7.45 h	7.97 f g	8.26 e f
30	7.59 g h	8.13 e f	8.89 c	9.52 a	9.42 a
60	8.10 e f	8.83 c d	8.99 b c	9.56 a	9.42 a
90	8.06 e f	8.42 d e	9.24 a-c	9.22 a-c	9.33 a b
Shelling (%)					
0	42.67 e-g	45.03 a-d	42.62 e-g	45.12 a-e	42.66 e-g
30	46.05 a-d	47.25 f g	41.23 a b	43.60 d-g	44.26 c-e
60	41.16 I-g	43.40 d-g	47.71 a	43.93 d-f	44.51 b-e
90	41.48 d-g	43.67 d-f	44.66 b-c	46.78 a-c	45.11 a-e

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

Table (9).Interaction effects of biofertilizer types and nitrogen fertilizer rates on the green pods yield and its components of pea plants during the winter season of 2004.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Green pods yield fed^{-1} (ton)					
0	1.24 I	1.59 h I	2.12 h I	2.56 g	2.80 g
30	1.54 h I	3.35 f	3.94 b-e	4.27 a-d	4.61 a
60	3.72 e	3.92 c-e	4.04 b-e	4.12 b-e	4.44 a b
90	3.62 e f	3.80 d e	3.89 d e	3.99 d e	4.34 a-c
No. pods plant^{-1}					
0	20.76 k	23.00 j	24.52 h	24.90 h	25.7 g
30	23.98 i	28.82 f	30.81 d	32.06 b c	33.14 a
60	28.35 f	31.37 c d	32.83 a b	33.00 a	33.09 a
90	29.92 e	31.58 c d	32.80 a b	32.71 a b	32.81 a b
No. seeds pod^{-1}					
0	6.47 h	7.10 g	7.62 e f	8.05 c-e	8.04 d e
30	7.57 f g	8.37 c d	8.38 c	9.57 a b	9.47 a b
60	9.18 b	9.37 a b	9.78 a	9.40 a b	9.60 a b
90	9.27 b	9.60 a b	9.48 a b	9.57 a b	9.62 a b
Shelling (%)					
0	42.89	45.86	44.01	43.63	48.69
30	44.76	47.06	46.48	45.24	46.25
60	77.19	47.34	46.20	46.37	47.27
90	42.96	44.03	42.84	47.37	49.04

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

The main effects of nitrogen and biofertilizers and the interaction on chemical contents of leaves:

Data presented in Table 10 show that inoculation of pea seeds with the different biofertilizer types increased significantly N, K and Chlorophyll contents of leaves as compared with the uninoculated treatment in both seasons. However, P content in leaves was not significantly differed. The mixed biofertilizer was more effective in this concern than the other three biofertilizers. The enhancing effects of biofertilizers on the chemical contents of leaves can be related to the hormonal exudates of the non-symbiotic bacteria which modify root growth, morphology and physiology resulting in more acquisition of nutrients by the growing plants(Jagnow *et al.*, 1991). Similar trends

were reported by Choudhary *et al*. (1984) and Solieman *et al*.(2003)on pea; and Gabr *et al.* (2001), on sweet pepper.

Regarding the effects of nitrogen levels on the various studied chemical contents of leaves of pea plants (Table10) the recorded results clarified some significant increments on leaves N, K and chlorophyll contents due to increasing N fertilizer from 30 to 90 Kg N fed^{-1}., compared with the control treatment. However, P content was not significantly affected. The application of 60 Kg N fed^{-1} seemed to be sufficient and pronounced in this concern. The improving effects of nitrogen fertilizer on leaves and seeds chemical contents might be related to the vital role of nitrogen, for the formation of chlorophyll pigments and stimulation of photosynthesis process (Marschner,1995). Similar trends were reported by several researchers such as Merghany (1999), Shiboob (2000) and Nassar *et al.* (2001) on common bean; Abd EL-Mouty (2000), on cowpea; Solieman *et al*. (2003) and Ishaq (2002), on pea.

Concerning the interaction effects of biofertilizer types and nitrogen fertilizer rates on chemical contents of leaves of pea plants (Tables11 and 12), the results revealed that the highest mean values for nitrogen, potassium and chlorophyll contents of leaves were obtained from the plants that were previously inoculated with the mixed biofertilizer and given either 60 or 90 Kg N fed^{-1}. Similar trends were reported by Merghany (1999), on snap bean and Ismail (2002) on pea.

Table (10). Main effects of biofertilizer types and nitrogen fertilizer rates on the chemical contents of pea plants ,during the winter season of 2003 and 2004

Treatments	The Chemical contents of leaves							
	N (%)		P (%)		K (%)		Chlorophyll content (SPAD)**	
	2003	2004	2003	2004	2003	2004	2003	2004
Biofertilizer type								
Control	3.81 C*	3.89 C*	0.26 A	0.27 A	0.79 C	0.79 B	49.96 B	49.84 C
Azotobacter	4.08 B	4.10 B	0.26 A	0.28 A	0.82 B	0.82 A	55.25 A	54.96 B
Azospirillum	4.14 B	4.12 B	0.27 A	0.28 A	0.82 B	0.82 A	54.93 A	54.49 B
Rhizobium	4.26 A	4.19 A B	0.26 A	0.29 A	0.83 B	0.82 A	56.22 A	55.59 A B
Mixed	4.26 A	4.21 A	0.28 A	0.29 A	0.86A	0.83 A	56.15 A	56.67 A
N rate (Kg N fed^{-1})								
0	3.58 C	3.70 C	0.25 A	0.28 A	0.76 C	0.77 C	45.66 C	45.61 C
30	4.09 B	4.11 B	0.26 A	0.29 A	0.82 B	0.81 B	55.39 B	55.23 B
60	4.37 A	4.27 A	0.26 A	0.28 A	0.86 A	0.84 A	58.34 A	57.89 A
90	4.40 A	4.31 A	0.27 A	0.29 A	0.86 A	0.85 A	58.62 A	58.50 A

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

**SPAD= 10 mg chlorophyll g^{-1} fresh weight.

Table(11). Interaction effects of biofertilizer types and nitrogen fertilizer rates on the chemical contents of leaves of pea plants, during the winter season of 2003.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Leaf N content (%)					
0	3.11 g*	3.44 f	3.60 f	3.90 e	3.85 e
30	3.50 f	4.17 d	4.21 c d	4.31 a-d	4.28 b-d
60	4.29 b-d	4.34 a-d	4.36 a-c	4.49 a b	4.45 a b
90	4.28 b-d	4.37 a-c	4.38 a-c	4.43 a b	4.48 a
Leaf P content (%)					
0	0.23	0.25	0.24	0.26	0.26
30	0.27	0.24	0.28	0.27	0.29
60	0.27	0.23	0.27	0.27	0.29
90	0.28	0.30	0.27	0.27	0.27
Leaf K content (%)					
0	0.72 f	0.77 e f	0.77 e f	0.77 d-f	0.78 c d
30	0.75 f	0.84 a-d	0.82 b-e	0.83 b-e	0.85 a b
60	0.85 a b	0.85 a b	0.84 a-b	0.87 a b	0.91 a
90	0.85 a b	0.85 a b	0.85 a b	0.87 a b	0.89 a
Leaf chlorophyll content (SPAD)**					
0	39.02 g	46.36 f	45.87 f	48.07 e f	48.90 e
30	47.63 e f	56.33 c d	57.21 c d	58.10 a-c	57.71 a-d
60	57.61 b-d	59.06 a b	57.58 b-d	59.49 a b	57.98 a-c
90	55.57 d	59.27 a b	59.06 a b	59.21 a b	59.99 a

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

**SPAD= 10 mg chlorophyll g^{-1} fresh weight.

Table(12). Interaction effects of biofertilizer types and nitrogen fertilizer rates on the chemical contents of leaves of pea plants,during the winter season of 2004.

Biofertlizer / N rate (kg N Fed^{-1})	Control	Azotobacter	Azospirillium	Rhizobium	Mixed
Leaf N content (%)					
0	3.19 f*	3.81 d e	3.70 e	3.86 d	3.93 d
30	4.70e	4.13 c	4.25 a-c	4.26 a-c	4.19 b c
60	4.29 a b	4.17 b c	4.26 a-c	4.29 a b	4.36 a
90	4.36 a b	4.29 a b	4.27 a-c	4.34 a	4.37 a
Leaf P content (%)					
0	0.28	0.28	0.28	0.29	0.30
30	0.29	0.26	0.30	0.30	0.30
60	0.28	0.26	0.29	0.29	0.29
90	0.29	0.30	0.29	0.29	0.29
Leaf K content (%)					
0	0.74 c	0.77 c	0.79 b c	0.77 c	0.77 c
30	0.67 c	0.84 a b	0.82 a b	0.82 a b	0.82 a b
60	0.84 a b	0.84 a b	0.83 a b	0.84 a b	0.85 a
90	0.84 a b	0.84 a	0.84 a	0.85 a b	0.86 a
Leaf chlorophyll content (SPAD)**					
0	39.19 g	46.77 f	46.86 f	47.60 f	47.65 f
30	45.85 f	57.05 c-d	56.55 e	57.61 b-e	59.10 a-d
60	57.96 c-e	57.02 c-e	57.39 c-e	57.97 b-e	59.72 a
90	56.96 d e	59.01 a-d	57.17 c-e	59.16 a-c	60.21 a

*Values having a common alphabetical letter (s), do not significantly differ, using the revised L.S.D. test at P=0.05

**SPAD= 10 mg chlorophyll g^{-1} fresh weight.

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الملخص العربى

تأثير أنواع مختلفة من الأسمدة الحيوية و معدلات التسميد النتروجينى على النمو والمحصول والمحتوى الكيماوى للبسلة

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أجريت هذه الدراسة بهدف اختبارتأثيرات تلقيح بذور البسلة بأربعة أنواع مختلفة من الأسمدة الحيوية، ثلاثة منهم منفردة من أجناس Azotobacter، Azospirillum، Rhizobium ، سماد حيوي مختلط من الأجناس الثلاثة السابقة بالإضافة إلى الكنترو ل الغير الملقح . وأربعة مستويات من التسميد النيتروجينى المعدنى (صفر، ٣٠ ، ٦٠، ٩٠ كجم ن للفدان) والتداخلات بينهم وذلك على صفات النمو الخضري، الإزهار، و المحصول الكلى للقرون الخضراء و مكوناته و كذلك بعض المكونات الكيماوية لأوراق البسلة صنف فكتورى فريزر.

أجريت تجربتان حقليتان خلال موسمي الشتاء ٢٠٠٣و ٢٠٠٤م في منطقة المحمودية بمحافظة البحيرة – مصر. نفذت التجارب باستخدام تجربة عامليه في تصميم القطاعات العشوائية الكاملة بثلاث مكررات.

يمكن تلخيص النتائج المتحصل عليها على النحو التالى :

1 - أدى تلقيح بذور البسلة بأي سماد حيوي إلى زيادة معنوية فى ارتفاع النبات، عدد الأوراق، عدد الفروع، الوزن الطازج و الجاف وكذلك أعطى زيادة معنوية فى المحصول الكلى من القرون الخضراء و عدد القرون/ نبات و عدد البذور فى القرن و نسبة التصافي للنبا ت وذلك أدى أيضا إلى زيادة معنوية فى محتوى الأوراق من النيتروجين و البوتاسيوم والكلوروفيل مقارنة بالبذور الغير ملقحة فى كل من الموسمين و قد تفوق السماد الحيوى الخليط فى كل الصفات السابقة .

2 - أدت إضافة السماد النيتروجين المعدنى بمعدل ٣٠، ٦٠ و ٩٠ كجم ن للفدان ، لنباتات البسلة ، إلى زيادة معنوية فى ارتفاع النبات،عدد الأوراق ،عدد الفروع ، الوزن الطازج و الجاف للاوراق وأعطى أيضا زيادة معنوية فى المحصول الكلى من القرون الخضراء للفدان و عدد القرون/ نبات و عدد البذور فى القرن و نسبة التصافي وكذلك أدى إلى زيادة معنوية فى محتوى الأوراق من النيتروجين و الفوسفور و الكلوروفيل مقارنة بالكنترول فى الموسمين هذا وقد ارتبطت أعلى القيم المسجلة لهذه الصفات بإضافة السماد النيتروجينى المعدني بمعدلات٦٠ ، ٩٠ كجم ن للفدان ولم يكن أن الفرق بينهما معنويا".

3 - أظهرت نتائج موسمي الدراسة وجود تأثيرات معنوية للتداخل بين التسميد الحيوى و النيتروجينى المعدنى فى معظم صفات النمو الخضرى والمحصول التى تم دراستها و كانت أفضل النتائج عند تلقيح بذور البسلة بالسماد الحيوى المختلط مع تسميد النباتات بالنتروجين المعدنى بمعدل 60 أو 90 كجم ن للفدان .