INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–6–947–952
http://www.ijab.org

Effect of Bio and Chemical Fertilizers on Seed Production and Quality of Spinach (*Spinacia oleracea* L.)

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ABSTRACT

The present investigation was carried out during two successive winter seasons (2002-2003 & 2003-2004) at Kaha Vegetable Farm, Horticulture Research Institute, ARC. It studies the effect of bio-fertilizers (*Azotobacter chroccocum &* phosphorein) singly or in combination with different rates of N and P chemical fertilizers on growth, yield, sex ratio, seeds (yield & quality) of spinach plants cv. *Dokki*. Results showed that seed inoculation with 300 g phosphorein inoculum/ fed. in the presence of 40 kg N/ fed (100% of the recommended N dose) + 15.0 or 7.5 kg/ fed (66.7 or 33% of the recommended dose of P_2O_5) as well as seed inoculation with 300 g *Azotobacter* inoculum in the presence of the full dose of P_2O_5 (22.5 kg P_2O_5/ fed.) + 50% of the full dose of N (20 kg/ fed) gave the highest favorable effect on growth, yield, sex ratio, and higher seed yield with the best quality compared with control treatment (40 kg N + 22.5 kg P_2O_5 fed.). Seeds inoculation with bio-fertilizers (*Azotobacter &* phosphorein) enriched the plant rhizosphere with such microorganisms compared with un-inoculated control. Application of 40 kg N + 15.0 kg P_2O_5 + 300 g phosphorein increased plant fresh yield by 27.2 and 42.3% and 16.3 and 10.4% in seed yield over the control in the first and second seasons, respectively.

Key Words: Biofertilization; *Azotobacter chroccocum;* Phosphorien; Spinach

INTRODUCTION

Spinach (*Spinacia oleracea,* L.) is one of the most important vegetable crops grown for its leaves. It is a source of chlorophyll, which gives it a dark green color quality and consumer acceptance. It has a high nutritional value due to its unusually high (Fe) and vitamin (A) contents. Among the methods, that have been followed for improving the growth, yield, seed production and quality of spinach are the application of nitrogenous and phosphorus fertilizers. However, un-controlled application of such fertilizers to the soil could result in some problems. Recently, it was found that, the use of bio-fertilizers is a cheap means for supplying plants with nitrogen and phosphorus during the growth schedule which could partially substitutes the expensive applied chemical fertilizers, thus leading to significant decrease in the production costs. In addition, pollution rate in the soil, water and the product could be lowered as a result of this practice.

The effect of N and P chemical fertilizers alone or in combination with *Azotobacter* and phosphorein [(PSB) or (PDB)] on the plant rhizosphere, growth yield, seed yield and quality of some crops were studied by many investigators. Badr El-Din *et al.* (1986) on soybean reported that dual inoculation with *Rhizobium japonicum* and phosphate dissolving bacteria (PDB) did not greatly increase PDB counts in the rhizosphere. Ghosh and Poi (1998) found that microbial population in the rhizosphere of some legume crops was increased as a result of combined inoculation with *Rhizobium* and PDB. Thamodharan *et al.* (2003) mentioned that PDB population in the rhizosphere was consistently high on pigeon pea.

The favorable effect of *Azotobacter* and mineral nitrogen fertilizer on growth, chemical composition of leaves, and yield was reported by Dakhly and Abdel Mageed (1997) on carrot and tomato, Stajner *et al.* (1997) on sugar beet, Bambal *et al.* (1998) on cauliflower, Wyszkowska (1999) on faba bean, and Sharma (2002) on cabbage. Also, Dakhly *et al.* (2004) on squash pointed out that the best treatment for six rate ratio, total yield and N uptake was 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 indicated that both inoculation with *Azotobacter* and application of N increased seed yield.

Positive effects of phosphorein (PSB or PDB) and chemical phosphorus fertilizer on growth, yield, seed yield and quality were found by Sharma and Nanadeo (1999), El-Kalla *et al.* (1997) on faba bean, Hewedy (1999) on tomato, Abraham and Lal (2002) on mustard, Anany (2002) on beans, and Estefanous *et al.* (2003) on okra.

The objective of this work was to study the effect of bio-fertilizers (*Azotobacter chroccocum* & phosphorein) singly or in combination with different rates of N and P chemical fertilizers on growth, yield, flowering, seed yield and quality of spinach.

MATERIALS AND METHODS

This study was conducted at the Vegetable Research Farm of Horticultural Research Institute, ARC, Kaha, Kalubia Governorate during two successive winter seasons (2002-2003 & 2003-2004). It studies the effect of some bio-fertilizers, i.e. *Azotobacter chroccocum* and phosphorein singly or in combination with different rates of N and P mineral fertilizers on growth, flowering, seed yield and quality of spinach cv. *Dokki.*

Mechanical and chemical analyses of the experimental soil are shown in Table I.

The experiment consisted of seven treatments of chemical and bio-fertilizers arranged in a complete randomized blocks design with four replicates. Seeds were sown in hills 15 cm apart on one side of the ridges 60 cm apart on October 30th and 19th in the two seasons, respectively. Plot area included 5 ridges, each of 3.5 m length and 60 cm width. The treatments were as follows:

T1 – 40 kg N/ fed + 22.5 kg P_2O_5/ fed (as a control treatment)

T2 – 20 kg N/ fed + 22.5 P_2O_5 kg/ fed + 300 g/ fed. *Azotobacter* inoculum

T3 – 10 kg N/ fed + 22.5 P_2O_5 kg/ fed+ 300 g/ fed. *Azotobacter* inoculum

T4 – 40 kg N/ fed + 15.0 P_2O_5 kg/ fed+ 300 g/ fed. Phosphorein inoculum

T5 – 40 kg N/ fed + 7.5 P_2O_5 kg/ fed+ 300 g/ fed. Phosphorein inoculum

T6 – 20 kg N/ fed + 15.0 P_2O_5 kg/ fed+ 300 g/ fed. *Azotobacter* + 300 g/ fed Phosphorein inocula

T7 – Without N and P mineral fertilizers + 300 g/ fed. *Azotobacter* + 300 g/ fed phosphorein inocula

Preparation of standard *Azotobacter chrococcum* and *Bacillus megatherium* peat based inocula. High efficient strains of *Azotobacter chrococcum* (a free living nitrogen fixing bacteria) and *Bacillus megaterium* var. *phosphaticum* (phosphate dissolvers) obtained from the culture collection of the Agricultural Microbiology Department National Research Center, Dokki Giza, Egypt were used. The selected two bacterial strains were propagated in sterilized proper nutrient broth media and incubated on a rotary shaker (180 rpm) at 28°C for 5 days. Turbidity, as bacterial growth indicator, of the cultures was adjusted calorimetrically to optical density of 1.6 at wave length of 420 nm to give 10^9 viable cells/mL fresh peat based inoculants preparation of the two bacterial strains were prepared by thoroughly mixing the cells suspension with sterilized peat moss at the ratio of 2:1 (V/W) under aseptic condition**s**. Aqueous solution of Arabic Gum (40%) was applied to the seeds as an adhesive agent before mixing with peat inoculants. Each peat inoculum was used at the rate of 300 g/ fed separately as well as combination of both and thoroughly mixed with the seeds, the coated seeds were left to air dry in shade, then the seed became ready for sowing.

Mineral fertilizers application. All plots were fertilized with potassium sulphate (K_2O, 48% fed.) at the rate of 100 kg/ fed. Mineral nitrogen fertilizer was applied as ammonium sulphate (20.5% N) and phosphorus was used as superphosphate (15.5% P_2O_5). The mineral fertilizer was applied in two equal doses, at the first irrigation (Three weeks from sowing) and at the beginning of flowering. The normal cultural practices for growing spinach plants were followed up. The following determinations were carried out:

Table I. Mechanical and chemical analysis of soil

A- Physical Analysis		B- Chemical analysis	
Coarse sand %	8.4	Available N ppm	82.4
Fine sand %	12.7	Available P ppm	6.6
Silt %	18.5	Available K ppm	214.1
Clay %	60.4	E.C. (m mhos/cm)	1.42
Ca Co_3 %	3.1	pH	8.1
Organic matter %	2.8		
Texture class	clay loam		

1. Microbiological analyses. Microbiological analyses of the plants rhizosphere after 45 days from sowing were conducted using the standard dilution methods. Soil extract yeast agar medium (Mahmoud *et al*., 1964) was used for total bacteria counts. *Azotobacter chroccocum* counts were determined on Ashby's medium (1907). Phosphate-dissolving bacteria was estimated using the medium of Bunt and Rovira (1955) modified by Louw and Webley (1959).

2. Plant growth parameters and yield. At the fresh marketable stage (70 days from sowing), four plants were chosen at random from each plot, where the following records were obtained:

a) Plant height (cm), b) Number of leaves per plant, c) Fresh and dry weight per plant (g), d) Leaf area per plant in cm^2 calculated as relation between area unit and fresh weight of leaves (Koller, 1972) using the following equation:

$$\text{Leaf area} = \frac{\text{Disk area X No. of disks X F.W. of leaves}}{\text{Fresh weight of disks}}$$

and, e) Fresh marketable yield (Ton/ fed.). At marketable stage plants of the two rows from each experimental plot were harvested and weighed then the total yield/ fed was calculated. The total yield/ fed was then calculated.

3. Chemical composition of leaves. a) Photosynthetic pigments i.e., chlorophyll a, b and carotenoides were determined calorimetrically as described in A.O.A.C. (1970), b) N, P and K were determined according to the methods used by Hesse (1971), Murphy and Riely (1962) and Brown and Lilleland (1946), respectively, c) Calcium was determined using EDTA (versinate) according to Richards (1954), and d) Iron was assayed using Atomic Absorption according to Chapman and Pratt (1961).

4. Sex ratio. At full bloming stage, the number of male plants (M) and female plants (F) were recorded to determine the sex ratio as follows (Abo Sedera, 1981):

$$\text{Sex ratio} = \frac{M}{F}$$

5. Seed-stalk height, number of branches and dry seed yield and its components. At seed maturity stage, the female plants were harvested, kept under shade to dry and the seed were removed manually. The following data were recorded: A) Main seed-stalk height (cm), B) Number of branches per plant, C) Seed yield per fed (kg), and D) Seed index (1000-seed weight) in (g).

6. Seeds quality (germination and seedling vigor characteristics) according to ISTA (1993).

a. Germination percentage was calculated due to the following equation:

$$\text{Germination\%} = \frac{\text{Total number of normal germinated seed}}{\text{Total number of sowing seeds}} \times 100$$

b. Germination rate was calculated according to Edmond and Drapala (1958).

$$MDG = \frac{(G_1 \times N_1) + (G_2 \times N_2) + \ldots\ldots Gn \times Nn}{G_1 + G_2 + \ldots\ldots\ldots\ldots Gn}$$

Where:

MDG= Mean number of days required for germination

G = Number of germinated seeds in a certain day

N = Number of days

Ten seedlings were randomly taken from each replicate to determine: 1) Seedling length (cm) was done 14 days from seed testing. 2) Fresh and dry weight of seedlings (g).

Statistics. Data obtained in this study were subjected to statistical analysis according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1. Microbiological changes. Data in Table II show that although *Azotobacter*, phosphate dissolving bacteria and total bacterial population occurred in high densities in the rhizosphere of un-inoculated spinach plants growing in the fertile clay loam soil. Inoculating spinach seeds with *Azotobacter chroccocum* and phosphate dissolving bacteria enriched the rhizosphere of spinach plants with such bacteria during the first 45 days from sowing in the two successive cultivation seasons. The highest increase in the counts of free living nitrogen fixers (*Azotobacter chroccocum*) was recorded in T7, T6, T3 and T2, and T7, T6, T2 and T3 in descending order in the first and second seasons, respectively. The increase in *Azotobacter chroccocum* counts in the above mentioned treatments could be attributed to the presence of adequate amounts of available phosphorous resulting from either super-phosphate applied or inoculation with phosphorein, as well as from the stimulative effect of the plant rhizosphere on the adjacent microorganisms. For phosphate dissolving bacteria (*Bacillus megatherium var phosphaticum*) the highest counts of such organism were recorded in T4, T5, T6, and T7, and T4, T5, T6 and T7 in descending order in the first and second season, respectively. In this regard, Badr El-Din *et al.* (1986) on soybean, Ghosh and Poi (1998) on legume crops, Estefanous *et al.* (2003) on okra yield and Thamodharan *et al.* (2003) on pigeon pea mentioned that seed inoculation with PDB increased PDB density in the plant thizosphere.

2. Growth parameters and yield. The effect of both bio - (i.e. *Azotobacter chroccocum* & phosphorein) and N and P chemical fertilizers (singly or in combination) on some growth parameters of spinach plants expressed as plant height, number of leaves, leaf area, fresh and dry weight of plant as well as total yield is shown in Table III. The results indicated that seed inoculation with phosphorein (300 g) and fertilizing the plants with nitrogen at the rate of 40 kg/ fed + P_2O_5 at 15 or 7.5 kg/ fed (i.e., T4 & T5) followed by T_2 (20 kg N/ fed + 22.5 kg P_2O_5 / fed + 300 g Azoto) induced significant increase in plant growth and yield in the two successive growth seasons, in comparison with the control treatment (received mineral fertilizer alone at the rate of 40 kg N/ fed + 22.5 kg P_2O_5 / fed) and other treatments.

Similar results were obtained by Dakhly and Abdel Mageed (1997) on other vegetable crops and Sharma (2002) on cabbage, for *Azotobacter* El-Kalla *et al.* (1997) on faba bean, Hewedy (1999) on tomato and Abo Sedera *et al.* (2005) on beans for phosphorein. The simulative effect of these microorganisms (*Azotobacter chroccocum* & Phosphorein) might be attributed to its efficiency in supplying the growing plants with biologically fixed nitrogen, dissolved immobilized phosphorus and produced phytohormones, which could stimulate nutrients absorption as well as photosynthesis process which subsequently increased plant growth and yield (Hewedy, 1999). The results recorded in Table III confirmed this conclusion. Although it is obvious also from the same table that the plants produced from seeds inoculated with 300 g/ fed Azotobacter + 300 g/ fed phosphorein inocula alone without N and P fertilization (T7) were the lowest in plant growth and yield. This could indicate that bio-fertilizers can be partially, but not completely, substitute chemical fertilizers.

3. Pigments content of spinach leaves. Results in Table IV indicate the effect of inoculation with *Azotobacter* and Phosphorein bio-fertilizers singly or in combination with different rates of the mineral fertilizers (N & P) on chlorophyll and carotenoids content. It is evident from the obtained data that the plant leaves treated with 300 g/ fed phosphorein and fertilized with 40 kg N and 15.0 or 7.5 kg P_2O_5 / fed (Treatments 4 & 5) or 300 g/ fed Azotobacter inoculum plus 20 kg N/ fed + 22.5 P_2O_5 / fed (T2) contained more chlorophyll (b) and carotenoids in the two growing seasons compared with the control treatment (T1) as well as the other treatments. On the other hand, chlorophyll (a) and total chlorophyll (a & b) contents did not show significant response to the studied treatments. In this regard, Stajner *et al.* (1997) on sugar beet and Bambal *et al.* (1998) on cauliflower reported that the highest chlorophyll and caroteniodes content in the plant leaves was achieved by inoculation with Azotobacter.

Table II. Effect of bio-and chemical fertilizers on total bacterial counts (T.C), *Azotobacter* (Azoto) and of phosphate-dissolving bacterial (PDB) counts after 45 days from sowing in 2002-2003 and 2003-2004 seasons (mean of four replicates)

Treatments	2002-2003 (season)			2003-2004 (season)		
	Tot. countx 10^8	Azoto x 10^4	PDB x 10^4	Tot. count x10^8	Azto x 10^5	PDB x 10^4
T1 - 40 N + 22.5 P_2O_5 …………………………. Control	35	18	26	33	18	25
T2 - 20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	45	250	35	44	240	36
T3 - 10 N + 22.5 P_2O_5 + 300 (g) Azoto.	40	280	30	41	200	31
T4 - 40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	52	30	400	45	31	400
T5 - 40 N + 7.5 P_2O_5 + 300 (g) Phospho.	55	35	350	50	34	350
T6 – 20 N + 15.0 P_2O_5 + 300 (g) Azoto. + 300 (g) Phospho.	58	320	300	51	370	320
T7 – Without (NP) fertilizer + 300 Azoto. + 300. Phospho	48	400	300	46	380	250

Table III. Effect of bio-and chemical fertilizers on some growth parameters and yield of spinach in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)						2003-2004 (season)					
	Plant heig-ht cm.	No. of leaves/ plant	Leaf area cm^2	F.W/ Plant (g)	D.W/ Plant (g)	Total yield Ton/fed	Plant height cm.	No. of leaves/ plant	Leaf area Cm^2	F.W/ Plant (g)	D.W/ plant (g)	Total yield Ton/ fed
T1 - 40 N + 22.5 P_2O_5 ……….…… Control	54.7	42.7	132.4	336.3	31.6	7.398	67.7	40.3	119.5	352.3	32.9	6.694
T2 - 20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	55.0	47.8	181.9	420.0	38.3	9.240	71.3	47.8	162.8	491.6	41.6	8.849
T3 - 10 N + 22.5 P_2O_5 + 300 (g) Azto.	55.0	45.7	149.8	395.0	34.0	7.898	68.0	45.7	140.2	391.1	33.6	7.430
T4 - 40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	59.7	55.5	205.5	427.7	51.3	9.409	73.3	65.0	186.7	518.9	56.5	9.859
T5 - 40 N + 7.5 P_2O_5 + 300 (g) Phospho.	57.0	53.5	185.1	423.3	41.0	9.312	73.3	52.0	173.8	494.5	52.9	9.394
T6-20 N+15.0 P_2O_5 + 300 (g) Azto. + 300 (g) Phospho.	53.7	41.3	130.9	300.0	29.7	6.900	67.3	39.5	114.1	335.9	32.3	6.382
T7–Without (NP) fertilizer+ 300 Azto.+ 300 Phospho.	50.7	30.7	98.1	183.0	15.2	5.132	57.3	34.3	102.6	201.1	21.8	4.625
L.S.D. 5%	1.5	1.7	4.4	1.1	3.6	0.161	1.7	1.4	3.7	1.9	3.6	0.158

Table IV. Effect of bio-and chemical fertilization on photosynthetic pigments contents of spinach leaves (mg/100 g fresh wt) in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)				2003-2004 (season)			
	Chlorophyll a	Chlorophyll b	Total (a+b)	Carotene	Chlorophyll a	Chlorophyll b	Total (a+b)	Carotene
T1-40 N + 22.5 P_2O_5 ……………. Control	51.5	23.3	74.8	33.5	52.0	23.2	75.2	24.0
T2-20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	53.1	25.9	79.0	35.2	53.0	25.1	78.1	25.5
T3-10 N + 22.5 P_2O_5 + 300 (g) Azoto.	52.0	23.5	75.5	34.5	52.5	23.6	76.1	25.0
T4-40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	58.0	26.5	84.5	36.6	54.2	29.8	84.1	26.5
T5-40 N + 7.5 P_2O_5 + 300 (g) Phospho.	53.6	26.4	80.0	36.4	54.1	25.5	79.6	26.3
T6-20 N+15.0 P_2O_5+300 (g) Azoto. +300 (g) Phospho.	51.5	22.8	74.3	33.0	51.0	22.9	73.9	23.6
T7–Without (NP) fertilizer+300Azoto. +300 Phospho.	45.7	21.6	67.3	29.5	45.0	21.0	66.0	21.5
L.S.D. 5%	NS	2.1	NS	1.7	NS	1.8	NS	1.7

4. Mineral elements content. Chemical composition of spinach leaves in this study in terms of N, P, K and Ca as well as Fe contents shown in Table V revealed that the highest values of these elements were obtained in T4, T5 and T2, respectively, which received 300 g/ fed phosphorein plus 40 kg N/ fed + 15.0 or 7.5 kg P_2O_5/ fed or 300 g/ fed Aztobacter inoculum plus 20 kg N + 22.5 kg P_2O_5 / fed, respectively compared with the control and other treatments. Similar results were obtained by Wyszkowska (1999) on K and Ca with Azotobacter and Estefanous *et al.* (2003) on N P K with phosphorein, respectively. This could be attributed to the enhancing effect of inoculation on plant growth and subsecuently mineral uptake.

5A. Sex ratio. Data illustrated in Table VI show that the treatments T4 {40 kg N + 15.0 P_2O_5 + 300 g phosphorein}, T5 {40 kg N +7.5 P_2O_5 + 300 g phosphorein} and T2 {20 kg N +22.5 P_2O_5 + 300 g Azotobecter} significantly decreased the number of male plants (M) but increased the number of female plants (F) and in turn decreased sex ratio (M/F) compared with the un-treated control and other treatments. In this regard, Dakhly *et al.* (2004), on squash pointed out that sex rate ratio was significantly affected with 45 kg nitrogen and chicken manure application beside inoculating the plants with Azotobacter.

5B. Seed-stalk height and number of branches. As shown in Table VI, the highest values for seed-stalk height and number of branches were obtained from applying 40 kg N plus 15.0 or 7.5 Kg P_2O_5 with 300 (g) phosphorein (T4, T5) followed by 20 Kg N plus 22.5 Kg P_2O_5 with 300 g Azotobacter (T2) compared with the control treatment (T1) and other treatments. This may be due to the enhancing effect on plant growth.

5C. Seed yield and seed index. Refereeing to the effect of bio-and chemical fertilization on seed yield and seed index, data in Table VI show that seed yield per feddan and seed index (the weight of 1000 seed), were significantly affected.

Table V. Mineral contents of spinach leaves as affected by bio-and chemical fertilizers in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)					2003-2004 (season)				
	N%	P%	K%	Ca%	Fe p.p.m	N%	P%	K%	Ca%	Fe p.p.m.
T1-40 N + 22.5 P_2O_5 ………… Control	4.17	1.32	3.72	1.37	1040.0	4.23	1.30	3.75	1.31	1160.0
T2-20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	4.26	1.35	3.75	1.39	1160.0	4.26	1.38	3.87	1.33	1296.0
T3-10 N + 22.5 P_2O_5 + 300 (g) Azto.	4.17	1.32	3.75	1.39	1120.0	4.25	1.35	3.78	1.31	1160.0
T4-40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	4.49	1.44	4.25	1.46	1393.0	4.53	1.40	4.50	1.55	1333.0
T5-40 N + 7.5 P_2O_5 + 300 (g) Phospho.	4.30	1.40	3.80	1.42	1357.0	4.48	1.39	4.40	1.41	1333.0
T6-40 N + without (P_2O_5) + 300 (g) Phospho.	3.90	1.10	3.57	1.12	682.0	3.99	1.03	3.50	1.20	773.0
T7–Without (NP) fertilizer+300 Azto. +300 Phospho.	3.80	1.04	3.41	1.11	656.0	3.87	1.01	3.10	1.12	727.0
L.S.D. 5%	0.1314	0.1564	0.1734	0.0626	15.4	0.1043	0.1577	0.1808	0.1110	16.2

Table VI. Effect of bio-and chemical fertilizers on sex ratio, seed-stalk height, No. of branches, seed yield and seed index in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)					2003-2004 (season)				
	Sex ratio	Seed-stalk height cm	No. of branches	Seed yield kg/ fed.	Seed index (g)	Sex ratio	Seed-stalk height cm.	No. of branches	Seed yield kg/ fed.	Seed index (g)
T1-40 N + 22.5 P_2O_5 …………..… Control	0.88	153.4	10.5	348.3	12.9	0.61	161.6	10.0	415.4	13.4
T2-20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	0.62	167.7	10.8	375.3	13.9	0.56	162.7	10.3	449.5	14.5
T3-10 N + 22.5 P_2O_5 + 300 (g) Azto.	0.83	156.0	10.7	364.5	13.5	0.58	162.7	10.0	437.1	14.1
T4-40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	0.43	176.5	11.8	405.0	15.0	0.47	179.3	12.0	477.4	15.4
T5-40 N + 7.5 P_2O_5 + 300 (g) Phospho.	0.58	173.0	10.9	386.1	14.3	0.52	163.3	11.0	458.8	14.8
T6-20 N+15.0 P_2O_5+300 (g) Azto. +300 (g) Phospho.	0.94	153.3	10.0	324.0	12.0	0.65	160.7	9.0	387.5	12.5
T7–Without (NP) fertilizer+300Azto. +300 Phospho.	1.09	143.9	9.2	248.4	9.2	0.95	140.0	8.3	294.5	9.5
L.S.D. 5%	0.0441	8.0	1.3	5.00	0.569	0.0438	7.8	1.8	5.90	0.572

Table VII. Effect of bio-and chemical fertilizers on seed germination and seedling vigour characteristics in 2002-2003 and 2003-2004 seasons

Treatments	2002-2003 (season)					2003-2004 (season)				
	Germin-ation %	Germi-nation rate (days)	Seedl-ing length cm.	F.W. of seedling (g)	D.W. of seedling (g)	Germin-ation %	Germi-nation rate (days)	Seedl-ing length cm.	F.W. of seedl-ing (g)	D.W. of seedling (g)
T1-40 N + 22.5 P_2O_5 ………..…… Control	72.0	7.4	16.1	0.780	0.0380	70.0	7.3	15.5	0.781	0.0376
T2-20 N + 22.5 P_2O_5 + 300 (g) Azotobacter	73.5	7.3	16.5	0.866	0.0405	73.0	7.2	16.2	0.855	0.0412
T3-10 N + 22.5 P_2O_5 + 300 (g) Azto.	72.5	7.3	16.4	0.804	0.0400	70.5	7.2	15.6	0.828	0.4020
T4-40 N + 15.0 P_2O_5 + 300 (g) Phosphorein	78.0	7.0	17.0	0.960	0.0448	79.5	7.1	17.3	0.982	0.0439
T5-40 N + 7.5 P_2O_5 + 300 (g) Phospho.	76.0	7.0	16.7	0.940	0.0439	74.5	7.1	17.0	0.899	0.0425
T6-20 N+15.0 P_2O_5+300 (g) Azto.+300 (g) Phospho.	68.5	7.6	15.7	0.754	0.0368	69.0	7.4	15.0	0.777	0.0375
T7–Without (NP) fertilizer+300 Azto.+300 Phospho.	62.0	7.7	13.0	0.664	0.0300	63.5	7.9	13.1	0.592	0.0304
L.S.D. 5%	1.3	0.45	1.6	0.16	NS	1.3	0.41	1.8	0.17	NS

The highest seed yield and seed index were recorded in treatments inoculated with 300 (g) phosphorein in combination with 40 kg (N)/ fed + 15.0 or 7.5 kg P_2O_5 (Treatments 4 & 5) followed by the treatment of 300 (g) Azotobacter plus 20 kg N + 22.5 P_2O_5 (T2) compared with the control treatment (T1) and other treatments. The increase in both seeds yield and seed index due to inoculation with both phosphorien and *Azotobacter chroccocum* bio-fertilizers could be attributed to the expected increase in the available amounts of nitrogen and phosphorous, which were continuously available to the spinach plants during their growth schedule in the two growing seasons. Similar reports were obtained by Parbhjeet *et al.* (1994) for *Brassica napus*, Verma *et al.* (1997) for cabbage, Verma *et al.* (2000) on pea, Sharma and Nanadeo (1999) for soybean, Panwar *et al.* (2000) for radish, Abraham and Lal (2002) for mustard, Anany (2002) for beans. As well as the seed yield increment may be due to low value of sex ratio, which is considered the best ratio for seed yield, in addition to the high values of branches number and seed index (Table VI).

6. Seed quality. Data presented in Table VII illustrated the effect of bio-and chemical fertilization on seed quality expressed as seed germination percentage, germination rate, seedling length, fresh and dry weight of seedlings. It is clear that, in the two growth seasons, all studied parameters were significantly improved due to the effect of the different treatments except dry weight of seedlings, which showed in-significant response. The best results for seed quality characteristics were obtained in Treatments 4 and 5 (300 g phosphorein with 40 kg N plus 15.0 or 7.5 kg P_2O_5) followed by treatment 2 (300 g Azotobacter plus 20 kg N + 22.5 P_2O_5) compared with the control treatment (T1) and other treatments.

In accordance with our results, Abo-Sedera *et al*. (2005) stated that, addition of phosphorein at a rate of 0.5 kg/ fed + phosphorus at 60 kg as P_2O_5 / fed showed the best results for germination percentage and germination rate of sown bean seeds.

It could be concluded that spinach seed inoculation with 300 g phosphorein in the presence of 100% N (full recommended nitrogen dose) plus 66.7 or 33.3% of the recommended P_2O_5 dose, or with 300 g Azotobacter in the presence of 100% P (full recommended P $_2O_5$ dose) + 50% of the recommended nitrogen dose significantly increased plant growth and seed yield with best quality. Bio-fertilizers can partially substitute chemical fertilizers, which could reduce production coast and subsequently environmental pollution load.

REFERENCES

Abo-Sedera, F.A., 1981. Effect of nitrogenous fertilizer and gibberellic acid (GA_3) on yield and quality of spinach. *M.Sc. Thesis*, University of Zagazig (Benha branch), Faculty of Agriculture Science, Moshtohor, Egypt

Abo-sedera, F.A, L.A.A. Badr, H.A. Mohamed and I.G.A. Ali, 2005. *Effect of bio-and mineral phosophorus Fertilizer on growth and dry seed yield of bean*. The 6th Arabian Conference for Horticulture, March 20–2, 2005 Ismailia Egypt

Abraham, T. and R.S. Lal, 2002. *Sustainable enhancement of yield potential of mustard (Brassica junce L. Czern. Coss) through integrated nutrient management (INM) in a legume based cropping system for the inceptisols. Cruciferae Newsletter*, 24: 99–100

Anany, T.G, 2002. Effect of some agricultural treatments on growth and dry seeds yield of beans. *Ph. D. Thesis*, University Zagazig (Benha Branch), Faculty of Agriculture Science, Moshtohor, Egypt

A.O.A.C., 1970. *Official Methods of Analysis*. The Association of Official Agricultural Chemists. Washington

Ashby, S.E., 1907. Some observations on the assimilation of atomospheric nitrogen by a free-living soil organism, *Azotobacter chroococcum* of Beijerink. *J. Agric. Sci*., 2: 431–7

Badr El-Din, S.M.S., M.A. Khalafallah and H. Moawad, 1986. *Response of soybean to dual inoculation with Rhizobium japonicum and phosphate dissolving bacteria. Zeitschrift-fur-pf tanzenerahrung-Bodenkunde*, 149: 130–5

Bambal, A.S., R.M. Verma, D.M. Panchbhai, V.K. Mahorkar and R.N. Khankhane, 1998. Effect of bio-fertilizers and nitrogen levels on growth and yield of cauliflower (*Brassica oleracea* var. botrytis). *Orissa J. Hort*., 26: 14–7

Brown, G.D. and O.L. Lilleland, 1946. Rapid determination of potassium and sodium in plant material and soil extract by flame photometry. *Proc. Amer. Soc. Hort. Sci*., 48: 341–546

Bunt, J.S. and A.D. Rovira, 1955. Microbiologica studies of some subantaretic soil. *J. Soil Sci*., 6: 119–205

Chapman, H.D. and P.F. Pratt, 1961. *Methods of Analysis for Soil, Plant and Water*, pp. 309. University of California, Division of Agriculture Science, USA Dakhly, O.F. and Y.T. Abdel Mageed, 1997. Estimation of effectiveness of *Azotobacter chroococcum* transformants on growth and yield of some vegetable crops. *Egypt. J. Gent. Cytol*., 26: 73–88

Dakhly, O.F, Y.T. Abdel Mageed and E.A. Hassan, 2004. Effect of nitrogen, organic fertilizers and new *Azotobacter* transformants on squash. *Minia J. Agric. Res. Develop*., 24: 1–30

Edmond, J.B. and W.J. Drapala, 1958. The effect of temperatures, sand and acetone on germination of okra seed. *Proc. Amer. Soc. Hort. Sci*., 71: 428–34

El-Kalla, S.E., A.K. Mostafa, A.A. Leilah and R.A. Awad, 1997. Mineral and bio-phosphatic fertilization for inter-cropped faba bean and onion. *Egypt. J. Agric. Res*., 77: 253–71

Estefanous, A.N., O.M. Sawan and A.F. Abou Hadid, 2003. Effect of inoculation with phosphate-bacteria, sawdus compost and nitrogen sources on okra yield and some properties of calcareous soil. *Acta Hort*., 608: 85–94

Ghosh, G. and S.C. Poi, 1998. Response of Rhizobium, phosphate soubilizing bacteria and mycorrhizal organism on some legume crops. *Environ. Ecol.*, 16: 607–10

Hesse, P.R., 1971. A Text Book of Soil Chemical Analysis. Jhon Murray (pupils), London

Hewedy, A.M., 1999. Influence of single and multi-bacterial fertilizer on the growth and fruit yield of tomato. *Egypt. J. Appl. Sci*., 14: 508–23

ISTA, 1993. International Rules for Seed Testing. *Seed Sci. Tech*., 21: 25–46

Koller, H.R.C., 1972. Leaf area-leaf weight relationship in soybean canopy. *Crop Sci*., 12: 180–3

Louw, H.A. and Webley, 1959. The bacteriology of the roat region of the oat plants grown under controlled pot culture conditions. *J. Appl. Bacteriol*., 12: 216–20

Mahamoud, S.A., M. Abou El-Fadl and M.Kh. El-Mofty, 1964. Studies on the rhizosphere microflora of a desert plant. *Folia Microbiogica*, 9: 1–6

Murphy, J. and J.P. Riely, 1962. A modified single solution method for determination of phosphate in natural waters. *Anal.Chem. Acta*, 97: 31–6

Panwar, A.S., J.S. Balyan and V.S. Verma, 2000. Yield and quality of radish (*Raphanus sativus*) seed as affected by fertility levels and biofertilizers. *Indian J. Agron*., 45: 822–6

Prabhjeet, S., S.C. Bhargava and P. Singh, 1994. Change in growth and yield components of *Brassica napus* in respons to *Aztobacter* inoculation at different rates of nitrogen application. *J. Agric. Sci*., 122: 241–7

Richards, L.A., 1954. Diagnosis and improvement of saline and alkaline soils. *Agriculture Handbook*, 60: 67–85

Sharma, K.N. and K.N. Namdeo, 1999. Effect of bio-fertilizer and phosphorus on growth and yield of soybean (*Glycine max* L. Merrill). *Crop Res. Hisar*, 17: 160–3

Sharma, S., 2002. Effect of *Azospirillum*, *Azotobacter* and nitrogen on growth and yield of cabbage (*Brassica oleracea* var. capitata). *Indian J. Agric. Sci*., 72: 555–7

Snedecor, G.W. and W.G. Cochran, 1980. *Statistical Method 7th Ed*., Iowa State University Press, Ames Iowa, U.S.A

Stajner, D., S. Kevresan, O. Gasic, N. Mimica-Dukic and H. Zongli, 1997. Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. *Biol. Pl*., 39: 441–5

Thamodharan, V., T. Lakshmi, G.C. Bajpai and L. Tewari, 2003. *Identification of phosphorus efficient pigeon pea genotypes based on phosphate-solubilizing bacteria in the rhizosphere,* 10: 44–5. International Chickpea Pigeonpea Newsletter

Verma, O.P., P. Sangeeta, M.S. Rathi and S. Paul, 2000. Synergistic effect of co-inoculation of *Azotobacter chrooccum* and *Rhizobium* on pea (*Pisum sativum*). *Annals Agric. Res*., 21: 418–20

Verma, T.S., P.C. Thakur and S. Ajeet, 1997. Effect of bio-fertilizers on vegetable and seed yield of cabbage. *Vegetable Sci*., 24: 1–3

Wyszkowska, J., 1999. Modification of faba bean chemical composition caused by precursors of growth regulators and soil microorganisms. III. Effect of precursors of ethylene. *Biuletyn Naukowy*, 5: 75–82

(Received 04 September 2005; Accepted 20 October 2005)