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 substitute the expensive plants with nitrogen and phosphorus during the growth that, the use of bio-fertilizers is a cheap means for supplying plants with nitrogen and phosphorus during the 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 P2O5/ fed. (66.7 or 33% of the recommended dose of P2O5) as well as seed inoculation with 300 g Azotobacter inoculum in the presence of the full dose of P2O5 (22.5 kg P2O5/ 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 P2O5 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 P2O5 + 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 chroococcum; Phosphorien; 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 chroococcum 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 P2O5/ fed (as a control treatment)
- **T2** – 20 kg N/ fed + 22.5 P2O5 kg/ fed + 300 g/ fed. *Azotobacter* inoculum
- **T3** – 10 kg N/ fed + 22.5 P2O5 kg/ fed + 300 g/ fed. *Azotobacter* inoculum
- **T4** – 40 kg N/ fed + 15.0 P2O5 kg/ fed + 300 g/ fed. Phosphorein inoculum
- **T5** – 40 kg N/ fed + 7.5 P2O5 kg/ fed + 300 g/ fed. Phosphorein inoculum
- **T6** – 20 kg N/ fed + 15.0 P2O5 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 chroccocum* and *Bacillus megatherium* peat based inocula.** High efficient strains of *Azotobacter chroccocum* (a free living nitrogen fixing bacteria) and *Bacillus megatherium* 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 were determined calorimetrically as described in A.O.A.C. (1970), Murphy and Riely (1962) and Brown and Lilleland (1946), respectively, as follows (Abo Sedera, 1981):

- A) *Azotobacter chroccocum* and *Bacillus megatherium* peat based inocula
- B) Without N and P mineral fertilizers + 300 g/ fed. *Azotobacter* + 300 g/ fed phosphorein inocula

**Table I. Mechanical and chemical analysis of soil**

<table>
<thead>
<tr>
<th>Texture class</th>
<th>A- Physical Analysis</th>
<th>B- Chemical analysis</th>
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<tbody>
<tr>
<td>Clay loam</td>
<td>Coarse sand % 8.4</td>
<td>Available N ppm 82.4</td>
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<tr>
<td></td>
<td>Fine sand % 12.7</td>
<td>Available P ppm 6.6</td>
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<tr>
<td></td>
<td>Silt % 18.5</td>
<td>Available K ppm 214.1</td>
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<tr>
<td></td>
<td>Clay % 60.4</td>
<td>E.C. (m mhos/cm) 1.42</td>
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<tr>
<td></td>
<td>Ca-Cr % 3.1</td>
<td>pH 8.1</td>
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<td></td>
<td>Organic matter % 2.8</td>
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**3. Chemical composition of leaves.** a) Photosynthetic pigments i.e., chlorophyll a, b and carotenoids 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 Rely (1962) and Brown and Lileland (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 blooming stage, the number of male plants (M) and female plants (F) were recorded to determine the sex ratio as follows (Abo Seder, 1981):

**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).

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).

\[
\text{MDG} = \frac{(G_1 \times N_1) + (G_2 \times N_2) + \ldots \ldots + G_n \times N_n}{G_1 + G_2 + \ldots \ldots + G_n}
\]

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 chroococum 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 chroococum) 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 chroococum 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, Estefanous et al. (2003) on pigeon pea mentioned that seed inoculation with PDB increased PDB density in the plant rhizosphere.

2. Growth parameters and yield. The effect of both bio - (i.e. Azotobacter chroococum & 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 + P2O5 at 15 or 7.5 kg/ fed (i.e., T4 & T5) followed by T2 (20 kg N/ fed + 22.5 kg P2O5 / 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 P2O5 / 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 chroococum & 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 P2O5 / fed (Treatments 4 & 5) or 300 g/ fed Azotobacter inoculum plus 20 kg N/ fed + 22.5 P2O5 / 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 carotenoides content in the plant leaves was achieved by inoculation with Azotobacter.
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 phosphoraein plus 40 kg N/ fed or 7.5 kg P2O5/ fed or 300 g/ fed Azotobacter inoculum plus 20 kg N + 22.5 kg P2O5 / fed, respectively compared with the control and other treatments. This may be due to the enhancing effect of inoculating the plants with Azotobacter.

5A. Sex ratio. Data illustrated in Table VI show that the treatments T4 (40 kg N + 15.0 kg P2O5 + 300 g phosphoraein), T5 (40 kg N + 7.5 kg P2O5 + 300 g phosphoraein) and T2 (20 kg N + 22.5 kg P2O5 + 300 g Azotobacter) 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. This may be due to the enhancing effect of inoculation on plant growth and subsequently mineral uptake.

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 P2O5 with 300 g phosphoraein (T4, T5) followed by 20 Kg N plus 22.5 Kg P2O5 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. Referring 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.
The highest seed yield and seed index were recorded in treatments inoculated with 300 (g) phosphorin in combination with 40 kg (N) fed + 15.0 or 7.5 kg P₂O₅. The increase in both seeds yield and seed index due to inoculation with both phosphorin and Azotobacter chroococum 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 (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 insignificant response. The best results for seed quality characteristics were obtained in Treatments 4 and 5 (300 g phosphorin with 40 kg N plus 15.0 or 7.5 kg P₂O₅) followed by treatment 2 (300 g Azotobacter plus 20 kg N + 22.5 P₂O₅) 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 P₂O₅/fed showed the best results for germination percentage and germination rate of sow 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 P₂O₅ dose, or with 300 g Azotobacter in the presence of 100% P (full recommended P₂O₅ 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 cost and subsequently environmental pollution load.

REFERENCES


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