RESPONSES OF SPINACH PLANTS TO POULTRY MANURE, INOCULATION WITH PLANT GROWTH – PROMOTING RHIZOBACTERIA (PGPR) AND SPRAYING BIO-STIMULANT

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ABSTRACT

Two field experiments were carried out on spinach (Spinacia oleracea L.) plants cv. Baladi to study the effects of 4 tones / fed. of poultry manure or recommended chemical fertilizers [250 kg/fed. ammonium sulphate (20.5%N)+ 200 kg/fed. calcium super phosphate (15.5%P2O5)+ 75kg /fed. potassium sulphate (84.5%K2O)] at the rates of full dose, ½ dose or 1/4 dose for each. Seeds were uninoculated or inoculated with plant growth – promoting rhizobacteria (PGPR) which included Azotobacter chroococcum, phosphate dissolving bacteria Bacillus megaterium, potassium release bacteria Bacillus circulans and Bradyrhizobium japonicum. Moreover, plants were sprayed with water or foliar biostimulant (Setter-2) which containing ascorbic, citric acids N, Cu, Ca, B and Mn twice after 15 and 25 days of planting on plant growth and yield. Spinach plants received the previous treatments in combination or single as well as evaluate phytohormone biosynthesis, cyanogens (HCN) and siderophores production and phosphate solubilization in some bacterial strains to be used as a plant growth promoting rhizobacteria (PGPR). The results indicated that using ½ dose of poultry manure + PGPR + spraying with setter-2 led to the highest plant weight in both seasons and plant length in the second season as well as leaves number/plant and total yield/fed. in the first season. Meanwhile, ½ dose of poultry manure + ½ dose of chemical fertilizer + spraying with setter-2 resulted in the highest leaves number / plant and total yield/fed. in the second season as well as dry matter percentage of leaves in both seasons.

Concerning chemical components in spinach planting, applying ½ dose of poultry manure + PGPR + spraying with setter-2 caused the highest N% and total sugars concentration in the leaves in both seasons. The highest P and K % were obtained with supplying ¾ NPK + PGPR + spraying setter-2 in both seasons and second season, respectively. ½ dose of poultry manure + PGPR caused the highest K% in the first season. On the other hand, the highest total soluble phenol and total free amino acids recorded due to use ½ dose of poultry manure + ½ dose of recommended NPK and full dose of poultry manure respectively, in both seasons. Nitrate concentration were the highest in plants received recommended chemical fertilizers while plants received PGPR followed by poultry manure caused the lowest values in both seasons. Chlorophyll concentration in leaves not significantly affected by the type of fertilization in both seasons. There were differences between rhizobacteria strains in its ability to production phytohormone biosynthesis, cyanogens (HCN) and siderophores production and phosphate solubilization.
INTRODUCTION

Spinach (Spinacia oleracea L.) is one of the popular vegetable crops in Egypt. Its leaves are cooked for human feeding. Crop with such promising potentialities for local markets, would necessitate much research in order to improve its production. The nutritional requirements of any crop play a major role in its improvement possibilities. The importance of nitrogen, phosphorus and potassium for spinach plant growth and metabolism has been investigated. Excessive application of chemical fertilizers, to enhance growth rates and yield of crops, is a common agricultural practice in developing countries. This extreme fertilizer application often leads to the accumulation of high levels of nitrates in plant tissue (Greenwood and Hunt, 1986).

Organic farming has become popular due to consumer concern with human health and the environment. However, the defined features of organic manure remain vague. Stimulated growth in some crops, irrespective of lower amounts of inorganic nitrogen (N), after organic matter was applied has been observed (Ae et al., 2006). Available N was 70% in the poultry manure treatment (Hammermeister et al., 2006).

Growth rates of lettuce or spinach plants in organic plots were equal to or higher than those of plants grown under mineral fertilization (Morra et al., 2003). Addition the microorganisms to different compost can help to optimize compost quality standards (Rabia et al., 2007).

Soil in which the proliferation of microorganisms is induced by the presence of plant roots is termed the "rhizosphere" (Garate and Bonilla, 2000). Bacteria growing in the rhizosphere are called "rhizobacteria". Rhizobacteria that possess some direct mechanism or capacity to promote plant growth are referred to as plant growth promoting rhizobacteria "PGPR". (Kloepper et al., 1989; Antoun et al., 1998 and Boiero et al., 2007). Those that promote plant growth by some indirect mechanism are biocontrol plant growth promoting bacteria referred to as (Bashan and Holgum, 1998).

Direct promotion of growth occurs when PGPR provide compounds that effect plant metabolism or when they facilitate acquisition by plants of a nonavailable nutrient from the soil. In PGPR, the most important direct plant growth promoting mechanism besides biological nitrogen fixation is synthesis of phytohormones or plant growth – regulating compounds. Nitrogen fixing bacteria, phosphate dissolving and potassium release bacteria promote the growth of plants either directly through N2-fixation, supply of nutrients, synthesis of phytohormones (Ferreira and Hungria, 2002 and Ragab and Rashad, 2003) and solubilization of minerals, or indirectly as bio-control agent by inhibiting the growth of pathogens (Antoun et al., 1998 and Al-kahal et al., 2003). The bio-control effect of those microorganisms is due to the secretion of secondary metabolites such as antibiotics and HCN. The biofertilizer offers a way to use the chemical fertilizer safely if both types are mixed together in the soil, and it is not harmful to other soil microflora (Mills et al., 1976). Plant growth – promoting rhizobacteria PGPR can increase the productivity of lettuce (Sottero, et al., 2006). Rhizobacteria (PRPG) as plant growth boosters can be an option for increased productivity in several crops.
including lettuce. A total of 77 fluorescent *pseudomonads*, 23 *Bacillus* and other rhizospheric bacteria isolates were tested. The beneficial effect of rhizobacteria (PRPG) was superior causing an improved plant growth (Freitas, et al., 2003). The high (PGPR) population was maintained in the soil with use of organic material (Urashima and Hori, 2003).

On the other hand, Spinach leaf concentration of nitrogen, phosphorus and potassium were raised by 10 to 20% with organic compared to inorganic fertilization and lowered nitrate (Gent, 2005). Also, organic manure improved soluble sugars and amino acids content (Li et al., 2003). Organic and conventional fertilization affected on lettuce phenolics compounds (Zhao et al., 2007) and chlorophyll content (Abd-Elmoniem et al., 2001).

Foliar biostimulants contained macro and micro elements in addition amino acids as well as ascorbic acid, so the effect of spraying such components as single or in a combination on vegetative growth, yield and chemical components were studied by several investigators; Talaat (1995) worked on the effect of spraying ascorbic acid on lettuce and spinach Sarg (2005) on potato plants as well as Hanafy Ahmed (1996) and Amer and El-Assiouty (2004) worked on the effect of citric acid on lettuce plant. Nofal et al. (1991) revealed that spraying ascorbic and citric acids on lettuce were significantly affected concentration of N, P and K in plant. Some vegetables similarly responded to the foliar spray with micro and macro nutrients. Artichoke plants treated with Mn, Cu and B gave the highest early yield and affected on chemical components of plants (Wahdan and Mansour, 2002).

The aimed of this study was to evaluate the interactive effects of poultry manure, chemical, bio-fertilizer and foliar biostimulants on growth, yield and chemical composition of spinach plants as well as evaluate phytohormone biosynthesis, cyanogens (HCN) and siderophores production and phosphate solubilization in some bacterial strains to be used as a plant growth promoting rhizobacteria (PGPR)

**MATERIAL AND METHODS**

The presented investigation was carried out during the two successive winter seasons of 2005 and 2006 at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza. Seeds of spinach (*Spinacia oleracea* L.) Balady cultivar were sown in soil directly in the field on October 20th in both seasons. The area of the experimental plot was 4m² (2m x2m). A complete randomized block design with three replicates was adapted.

Qualitative assessments of siderophore, hydrocyanic acid (HCN), indol acetic acid (IAA) and phosphate solubilization were determined. A bacteria forming an orange halo on chrome azural-s (CAS) agar plates or growing on TSA (10%) agar plates containing 50 mg/l of 8- hydroxyquinoline was considered as positive siderophore producers (Alexander and Zubeter, 1991). A change of color from yellow to orange – brown of filter papers impregnated with 0.5% picric acid, 2% NaCO₃ indicated the production of
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cyanide (Baker and Schippers, 1987). IAA producing bacteria were separated from organisms producing other indoles (yellow to yellow – brown pigment) by their characteristics pink to red color produced after exposure to salkowski reagent for 0.5 – 3.0 h. (De-Brito Alvarez et al., 1995). The bacterial colonies forming clarification halos on dicalcium phosphate agar plates (Goldstein, 1986) were considered as phosphate solubilizers.

Preparations of inocula:

The plant growth promoting rhizobacteria (PGPR) used in this study included some microorganisms such as Azotobacter chroococcum, Bacillus megaterium var. phosphatcum, Bacillus cerculans and Bradyrhizobium japonium. Each bacterial strains were grown and maintained each on its specific media as yeast extract mannitol media (YEM) for Bradyrhizobium (Vincent, 1970) and modified Ashby’s N-deficient medium (Hegazi and Neimela, 1976) for Azotobacter chroococcum, modified Bunt and Rovira medium (Abdel-Hafez, 1966) for B. megaterium and modified Aleks and Rou’s medium (Zahra, 1969) for B. cerculans. Microbial inocula were prepared in this study by adding 100 ml liquid culture (ca 10^9/ml) from each microorganisms to 200g vermiculite as a carrier material to be used as a plant growth promoting rhizobacteria (PGPR), with the rate of 300g mixed inocula/fed.

Seeds of spinach were uninoculated or inoculated with PGPR before planting.

Soil physical properties:

Coarse sand 6.41 %, fine sand 23.7 %, silt 30.89 %, clay 38.99%; textural class (clay loam), SP. 41.8%, pH 7.62, EC (dsm^{-1}) 1.9, CaCO_3 1.8%.

Soil chemical properties:

Total nitrogen 0.18% organic matter 1.25%, organic carbon 0.71%, HCO_3 8.40 (meq/l), Cl 11.71(meq/l), SO_4 14.92 (meq/l), Ca^{++} 9.53(meq/l), Mg^{++}2.57(meq/l), Na^+ 22.93 (meq/l). Soil physical and chemical properties were analyzed as described by Piper (1950). The experiments included the following treatments: poultry manure at the rate of 4 tons/fed. or Chemical fertilization at the recommended dose: 250 kg/fed. ammonium sulphate (20.5 % N), 200 kg/fed. calcium super phosphate (15.5% P_2O_5) and 75 kg/fed. potassium sulphate (48% K_2O) at the rate of full, 1/2, 1/4 or 3/4 dose for each.

The chemical properties of the used poultry manure was as follow:

Macronutrients: 2%N, 0.5% P and 1.4% K. Total nitrogen were determined according to the standard methods of Page et al., (1982). Total contents of phosphorus, potassium and micronutrients were assayed according to Black (1982).

Plants were spraying with water or setter-2 which contains the flowing (5000 ppm as corbic acid, 5000 ppm citric acid, 5000 ppm N, 1000 ppm Cu, 90000 ppm chelated Ca, 15000 ppm, chelated B and 1000 ppm Mn) at the rate of 500 cm setter-2/200 liter water at 15 and 25 days after planting.

The treatment were as following:
- Full dose of chemical fertilizers (NPK)
- PGPR.
- Full dose of poultry manure
- ½ dose of poultry manure + ½ dose of chemical fertilizers (NPK)
- ½ dose of poultry manure + ½ dose of chemical fertilizers + spraying setter-2.
- ½ dose of poultry manure + PGPR.
- ½ dose of poultry manure + PGPR + spraying setter-2
- ½ dose poultry manure + ⅛ dose of chemical fertilizers (NPK) + PGPR.
- ⅛ dose of poultry manure + ½ dose of chemical fertilizers (NPK) + PGPR.
- ⅛ dose of chemical fertilizers (NPK) + PGPR.
- ½ dose of chemical fertilizers (NPK) + PGPR.
- ¼ dose of chemical fertilizers (NPK) + PGPR + spraying setter-2.
- ½ dose of chemical fertilizers (NPK) + PGPR + spraying setter-2.
- ⅛ dose of chemical fertilizers (NPK) + PGPR + spraying setter-2.

Poultry manure and super phosphate were applied during the soil preparation, while N and K fertilizers were divided into two equal portions to be added at 15 and 25 days after planting.

Data were recorded in the following characters:

1- Vegetative growth characters which were estimated at 40 days after planting, ten plants from each experiments plot were chosen for measuring the following vegetative growth characters, plant length, number of leaves per plant, fresh weight of plant and dry matter percentage.

Yield: spinach plants were harvested after 40 days from planting. Yield was estimated as kg/plot and calculated as tons/fed.

Chemical composition:

Determination of N, P and K were carried out on the ground dry materials of plants which were digested using sulfuric acid, salicylic acid and hydrogen peroxide according to linder (1944). Nitrogen was determined using the micro-kejeldahl apparatus of Parnos – Wagner as described by Van Schouwenburg and Walinga (1978). Phosphorus was estimated colorometrically by using chlorostannous reduced molybdophosphoric blue color method according to Chapman and Parker (1961). Potassium was determined using the flame photometer. NO₃ – N was determined in distilled water extracts of dried tissue by the procedure of Cataldo et al. (1975) by using salicylic acid and then calculated as mg/100g fresh weight. Ethanol extracts of fresh materials were used for the determination of total sugars, total free amino acids and total soluble phenols. Total sugar were determined by using the phenol-sulphuric acid method (Dubois et al., 1956). Total free amino acids were determined by using ninhydrin reagent according to (Moore and Stein, 1954). Total soluble phenols were estimated using the Folin-ciocalteau colorimetric method (Swain and Hillis, 1959). Total chlorophyll in leaves was measured using Minolta SPDAD chlorophyll-Meter (Yadava, 1986).

All data were statistically analyzed according to Gomez and Gomez (1984).
RESULTS AND DISCUSSION

Bioassay of the bacterial strains:

Data presented in Table 1 show that Azotobacter chroococcum showed positive reaction for IAA, siderophores, HCN and phosphate-solubilizers while the strain of B. megaterium showed negative reaction on the HCN and siderophores tests. On the other hand, strain of B. cerulans appeared negative reaction on the tests of siderophore and P- solubilizers. Also, Bradyrhizobium japonicum showed positive reaction for IAA, siderophores. This results are in harmony with those obtained by Antoun et al., (1998), Ragab and Rashad (2003), Ragab et al., (2006) and Boiero et al., (2007), they found that some rhizobia, Azotobacter, and bacillus sp. produce IAA, ABA, siderophore, HCN and soluble phosphate.

Table 1: Qualitative assessment of IAA, siderophores, cyanogens (HCN) and soluble phosphate produced by different microbial strains.

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Relative reaction</th>
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<tbody>
<tr>
<td></td>
<td>Azotobacter chroococcum (AZ4)</td>
</tr>
<tr>
<td>Siderophores</td>
<td>++</td>
</tr>
<tr>
<td>Cyanogens (HCN)</td>
<td>+</td>
</tr>
<tr>
<td>IAA</td>
<td>+++</td>
</tr>
<tr>
<td>P-solubilizers</td>
<td>++</td>
</tr>
</tbody>
</table>

(-) No, (+) Low, (+++) Moderate and (+++) High reaction.

Vegetative growth and yield:

Data presented in Table 2 indicate the effect of different fertilization (organic, inorganic) and bio fertilizers as well as spraying with setter – 2 on vegetative growth characters, i.e. plant length, plant fresh weight, number of leaves per plant and dry matter percentage of leaves. 

**Plant length:** in the first season, significant differences were detected in plant length. The highest values were obtained by adding $\frac{1}{4}$ poultry fertilizer + $\frac{1}{2}$ (NPK) recommended chemical fertilizer + PGPR as compared to chemical fertilizer alone. On the other hand, applying $\frac{1}{2}$ (NPK) + PGPR + spraying with settler – 2 and $\frac{1}{2}$ poultry fertilizer + PGPR + spraying with settler-2 as well as poultry fertilizer, $\frac{1}{2}$ (NPK) chemical fertilizer + PGPR, $\frac{1}{2}$ poultry + $\frac{1}{2}$ NPK + spraying settler-2 and PGPR slightly increased plant length as compared with chemical fertilizer (NPK). Meanwhile, adding $\frac{1}{2}$ poultry + $\frac{1}{2}$ NPK, $\frac{1}{2}$ poultry + PGPR, $\frac{1}{2}$ poultry + $\frac{1}{4}$ chemical + PGPR, $\frac{1}{4}$ NPK + PGPR + spraying settler-2 resulted in highly decreased comparing with NPK. The lowest values were obtained with using $\frac{3}{4}$ NPK + PGPR and $\frac{1}{4}$ NPK + PGPR. Length of plants receiving $\frac{3}{4}$ NPK + PGPR + spraying settler-2 were nearly equal to the length of plants receiving NPK (recommended dose). Also, it was observed
that spraying setter-2 to the some treatments resulted in higher values as compared to corresponding treatments un sprayed with setter-2.

In the second season, there were significant differences between treatments. Spinach plants receiving ½ poultry fertilizers + PGPR + spraying setter-2 had the highest length compared to NPK (recommended dose) followed by ½ poultry + ½ chemical (NPK), ½ poultry + ½ chemical (NPK) + spraying setter-2, ½ poultry + ¼ chemical (NPK) + PGPR and ¼ chemical (NPK) + PGPR + spraying setter-2.

Length of plants receiving poultry fertilizer or ½ chemical (NPK) + PGPR were nearly equal to those supplied with the recommended dose of NPK. Plants fertilized with ½ poultry + PGPR, ¾ NPK + PGPR; ¾ NPK + PGPR + spraying setter-2, ¼ NPK + PGPR + spraying setter-2 were shorter than those supplied with NPK.

On the other hand, adding PGPR, ½ poultry + ½ chemical NPK + PGPR or ½ poultry + PGPR resulted in the shortest ones.

Moreover, from the results, it can be observed that fertilizing with ½ NPK + PGPR or ½ NPK + PGPR + spraying setter-2 gave the higher length of plants than ¾ NPK + PGPR or ¾ NPK + PGPR + spraying setter-2, in both seasons. However, there were no significant differences between ½ poultry + ½ chemical and ½ poultry + PGPR, in both seasons.

**Plant weight:**

Data presented in Table 2 show that there were significant differences on plant weight in both seasons. The highest plant weight was recorded with application ½ poultry + PGPR + spraying setter-2 followed by ½ NPK + PGPR + spraying setter-2 and ¼ poultry + ½ NPK + PGPR as compared with plants treated with NPK alone (recommended dose), in the first season. Meanwhile, applying ½ poultry + PGPR + spraying setter-2 followed by ½ poultry + PGPR or ½ poultry + ¼ NPK + PGPR resulted in the heaviest ones as compared with NPK (recommended dose), in the second season.

There were no significant differences between poultry fertilizer and ½ poultry fertilizer + ½ chemical fertilizer, in both seasons. Data indicated that spraying setter-2 on plants had more effective on increasing plant weight. Heavier plants were obtained by using ½ poultry + ½ chemical fertilizers + spraying setter-2 or ½ poultry + PGPR + spraying setter-2 than ½ poultry + ½ chemical or ½ poultry + PGPR + spraying setter-2, in both seasons.

Plants receiving ½ poultry + PGPR were heavier than those obtained from treating with ½ poultry + ½ chemical, in both seasons. In this respect, it might be suggest that it is possible to replace ½ recommended doses of chemical fertilizer by PGPR. The lowest values were obtained from plants received ¾ NPK + PGPR or ¼ NPK + PGPR in the first and second season, respectively.

**Number of leaves per plant:**

Data in Table 2 indicate that there were significant differences between treatments on number of leaves per plant in both seasons. In first
season, the higher number of leaves per plant was obtained by applying ½ poultry manure + PGPR + spraying setter-2 as compared with NPK (recommended dose). Meanwhile, the values obtained by adding ½ NPK + PGPR were slightly increased compared with NPK or ¾ NPK + PGPR + spraying setter-2. However, values obtained by adding poultry fertilizers or PGPR were gave the same trend.

In the second season, the highest number of leaves per plant was obtained when fertilized the soil with ½ poultry + ½ chemical NPK + spraying setter-2 then ½ poultry + ¼ chemical (NPK) + PGPR as compared to NPK (recommended dose) treatment. On the other hand, in both seasons, spraying setter-2 on plants when fertilized with ½ poultry + ½ chemical NPK + PGPR, ¾ chemical NPK + PGPR or ½ chemical + PGPR gave higher values than without spraying treatment. Also, adding, to the soil ½ poultry + PGPR gave equal or higher values than ½ poultry + ½ chemical NPK. The lowest values were obtained with fertilization ¼ NPK + PGPR.

These results are similar to those obtained by Kutuk et al., (1999) who revealed that addition of organic composts to the soil had beneficial effects on average plant weight, leaf length and yield of spinach. Also, Kodashima et al., (2006) reported that leaf length and number of spinach leaves in plants receiving compost were higher compared with those of plants treated with chemical fertilizer.

On lettuce, Porto et al., (1999) reported that the highest plant weight and leaves number per plant were obtained of plots where the organic fertilizer was supplied with the highest rate of chicken manure. On the other hand, Morra et al., (2003) found that growth rates of lettuce plant in the organic plots were equal to or higher than those of plants grown in mineral plots.

The effect of biofertilizers on the growth characters which obtained from this study was agreement with those obtained by Kang, (2004) and Medeiros et al., (2008) on the lettuce, who reported that plant length, number of leaves and plant fresh weight were the highest with application of microbial liquid manure. On the other hand, plant growth promoting rhizobacteria (PGPR) can increase the productivity of lettuce. Isolates of rhizosphere of different lettuce varieties were tested in vitro. Twelve isolated promoted growth of plants, nine isolated enhanced the number of leaves (Sottero et al., 2006).

Leaves dry matter percentage:

Data presented in Table 2 indicate that, the effect of fertilization treatments were significant on dry matter percentage of leaves in both seasons. The highest dry matter percentage of leaves were recorded with application of ½ poultry + ½ chemical (NPK) + spraying setter-2 followed by ½ poultry + PGPR + spraying setter-2 and poultry fertilizer in the first and second seasons, respectively as compared with NPK (recommended dose).

On the other hand, plants grown in the soil supplied with ½ poultry + PGPR recorded the highest values when compared with those supplied with ½ poultry + ½ chemical. Plants receiving poultry fertilizer had higher values
than plants received PGPR. Also, applying $\frac{1}{2}$ poultry + $\frac{1}{4}$ chemical + PGPR resulted in higher values than that obtained when plants grown in soil supplemented with $\frac{1}{4}$ poultry + $\frac{1}{2}$ NPK + PGPR. Leaves dry matter percentage of plants supplemented with $\frac{1}{2}$ NPK (chemical dose) + PGPR+ spraying setter-2 were higher than ones supplemented with $\frac{3}{4}$ NPK + PGPR only. In general, spraying plants with setter-2 results in higher values than those un sprayed. The lowest leaves dry matter percentage were obtained from plants receiving $\frac{1}{4}$ NPK + PGPR.

Such results are in agreement with those reported by Jakse and Mihelic (1999) who found that the yield as dry matter of spinach grown in plots received organic manure was not significantly higher compared to mineral fertilizers. Premuzic et al., (2002) found the same results on lettuce. Meanwhile, dry matter production in cabbage (Jakse and Mihelic, 2001) and in spinach (Kodashima et al., 2006) supplied with the organic compost was higher than that applied with chemical fertilizer. Results showed that addition biofertilizer caused superiority spinach production. In this regard, Nguyen and Preston (2006) on spinach recorded that there were linear responses in biomass yield to increasing levels of biodigester effluent. The response to added N, this was approximately 1.25 kg DM per 1 kg additional N from manure compared with an additional 7.5 kg DM biomass per 1kg added from biodigester effluent. Also, Mediros et al., (2008) confirmed that shoot dry mass of lettuce were depending on substrates and biofertilizers addition.

Yield:

Data recorded in Table 2 reveal significant differences between the various fertilization treatments in total yield. In the first season, the highest values were obtained with application of $\frac{1}{2}$ poultry + PGPR + spraying setter-2 followed by $\frac{1}{2}$ poultry + $\frac{1}{2}$ chemical (NPK) + spraying setter-2 as compared with those supplied with NPK alone (recommend dose). On the other hand, total yield obtained with application of poultry fertilizers caused higher yield than that obtained with application of other treatments specially $\frac{1}{2}$ poultry + $\frac{1}{2}$ chemical NPK or $\frac{1}{2}$ chemical NPK + PGPR + spraying setter-2. Also, plants receiving $\frac{1}{4}$ poultry + $\frac{1}{2}$ chemical + PGPR gave higher total yield than plants receiving $\frac{1}{2}$ poultry + $\frac{1}{4}$ chemical + PGPR.

In the second season, the highest total yield was obtained with application of $\frac{1}{2}$ poultry + $\frac{1}{2}$ chemical + spraying setter-2 followed by $\frac{1}{2}$ poultry + PGPR + spraying setter-2 or $\frac{1}{2}$ poultry + $\frac{1}{2}$ chemical, then poultry fertilizer. On the other hand, plants grown on soil supplemented with $\frac{1}{2}$ poultry + $\frac{1}{4}$ chemical + PGPR gave higher yield than ones supplemented with $\frac{1}{4}$ poultry + $\frac{1}{2}$ chemical + PGPR.

In both seasons, application of PGPR alone cause lower total yield than application poultry or chemical fertilization. Also, $\frac{1}{2}$ chemical + PGPR + spraying setter-2 resulted in higher values than $\frac{1}{4}$ chemical +PGPR without spraying. Moreover, the results indicated that spraying setter-2 caused the
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higher values of total yield as compared with corresponding plants unsprayed with setter-2.

The previous results were agree with those obtained by Rodrigues and Casali (1999), Santos et al. (2001) and Premuzic et al., (2002). Li et al., (2003) found that organic manure increased the yield of lettuce by 55-132%. Babik and Kowalczyk (2004) and Porto et al., (2008) reported that lettuce yield was significantly higher when organic fertilizer was used. Maynard (1991) reported that with poultry manure at 50 tons/acre, the yield of spinach, broccoli and pepper were equal or to greater than those obtained with inorganic fertilizer. Also, addition of half the conventional inorganic fertilizer rate to half poultry manure rate (25 tons/acre) increased the yield of all crops above the inorganic control.

Table 2: Effect of poultry manure, chemical fertilizers, biofertilizer and foliar bio-stimulant on plant length, plant weight, leaves number per plant, dry matter % and total yield of spinach plants.

<table>
<thead>
<tr>
<th>Growth characters</th>
<th>Plant length (cm)</th>
<th>Plant weight (gm)</th>
<th>Leaves number/per plant</th>
<th>Leaves dry matter %</th>
<th>Yield (ton/fed.)</th>
</tr>
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<tbody>
<tr>
<td>NPK</td>
<td>33.0</td>
<td>30.5</td>
<td>34.0</td>
<td>23.5</td>
<td>9.6</td>
</tr>
<tr>
<td>PGPR</td>
<td>33.8</td>
<td>26.3</td>
<td>23</td>
<td>22.3</td>
<td>8.8</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>34.2</td>
<td>30.3</td>
<td>23.8</td>
<td>27.3</td>
<td>8.8</td>
</tr>
<tr>
<td>½ poultry + ½ NPK</td>
<td>32.1</td>
<td>31.2</td>
<td>24.2</td>
<td>27.0</td>
<td>8.9</td>
</tr>
<tr>
<td>½ poultry + 1/3 NPK + Setter-2</td>
<td>33.7</td>
<td>31.0</td>
<td>28.3</td>
<td>30.3</td>
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<td>32.0</td>
<td>29.1</td>
<td>29.5</td>
<td>31.7</td>
<td>9.0</td>
</tr>
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<td>½ poultry + PGPR + Setter-2</td>
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<td>32.5</td>
<td>36.5</td>
<td>33.7</td>
<td>10.1</td>
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<td>½ poultry + 1/4 NPK + PGPR</td>
<td>32.9</td>
<td>31.0</td>
<td>30.3</td>
<td>31.2</td>
<td>9.0</td>
</tr>
<tr>
<td>¼ poultry + 1/3 NPK + PGPR</td>
<td>35.4</td>
<td>26.3</td>
<td>33.3</td>
<td>23.3</td>
<td>9.2</td>
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<tr>
<td>½ NPK + PGPR</td>
<td>30.4</td>
<td>28.4</td>
<td>22.7</td>
<td>18.0</td>
<td>9.1</td>
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<tr>
<td>¼ NPK + PGPR</td>
<td>34.0</td>
<td>30.3</td>
<td>29.8</td>
<td>16.8</td>
<td>9.8</td>
</tr>
<tr>
<td>½ NPK + PGPR + Setter-2</td>
<td>30.4</td>
<td>26.1</td>
<td>27.1</td>
<td>16.3</td>
<td>8.5</td>
</tr>
<tr>
<td>½ NPK + PGPR + Setter-2</td>
<td>33.1</td>
<td>28.9</td>
<td>30.2</td>
<td>22.0</td>
<td>9.7</td>
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<tr>
<td>½ NPK + PGPR + Setter-2</td>
<td>34.5</td>
<td>31.0</td>
<td>35.2</td>
<td>20.0</td>
<td>9.5</td>
</tr>
<tr>
<td>½ NPK + PGPR + Setter-2</td>
<td>32.8</td>
<td>28.2</td>
<td>27.3</td>
<td>16.8</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Also, Adediran et al., (2004), Maftoun et al., (2004), Zhou Yan and Luo (2004) and Katoh et al., (2008) who reported that the combined application of N15 sulphate and composts significantly increased spinach yield compared with single application of N15 ammonium sulphate. On the other
hand, Maruo et al. (2002) recorded that application of compost increased the yield of spinach.

From the previous results, it observed that the biofertilizers (PGPR) actively affect on spinach plants, which caused increasing the parameters of growth and yield. In this respect, Freitas et al. (2003) and Urashima and Hori (2003) confirmed that, rhizobacteria (PGPR) as plant growth boosters can be option for increased productivity in several crops, including lettuce. Four assays with rhizobacteria isolates from different origins were carried out to verify their potential for growth enhancement in lettuce. A total of 77 fluorescent Pseudomonads, 23 Bacillus and 11 other rhizospheric bacteria isolates were tested. They found that PGPR caused an improved plant growth. Based on the substrate fertility, there were differences in the behavior of the isolates.

On the other hand, Yobo et al. (2004) found that three of the Bacillus preparations significantly increased lettuce growth and yield. The growth of spinach was promoted when fluorescent Pseudomonas strains and organic material were applied (Urashima et al., 2005).

Concerning the effect of foliar biostimulant treatments, it was clear from data presented in Table 2 that spraying spinach plants with the biostimulants led to increments in all vegetative growth characters.

These results were explained by many workers, foliar biostimulants contain macro and micro elements in addition to amino acids, as well as ascorbic and citric acid, so the effect of spraying such components, as a single and in a combination, on vegetative growth, yield and chemical composition of vegetable crops: In this respect, spraying ascorbic acid significantly increased plant height as well as fresh and dry weight of both lettuce and spinach as well as potato plants (Talaat, 1995 and Sarg, 2005). Foliar spray with citric acid caused significant increase in plant height, fresh and dry weight per plant of lettuce or pea plants as recorded by Hanafy Ahmed (1996) and Amer and El-Assiouty (2004).

Some other vegetables similar responded to the foliar spray with fertilizers containing micro- and macro elements. Artichoke plants treated with Mn, Cu and B gave the highest early yield and chemical components (Wahdan and Mansour, 2002). Micronutrients, which are main components of Setter-2, play essential roles in different physiological processes, which affect directly plant growth. Moreover, Thompson and Kelly (1983) reported that Cu and Mn are essential elements and they are components of several enzymes mostly, functioning in oxidation reactions. The stimulative effect of the biostimulants was reported by Ismail (2002) on peas.

**Chemical components of leaves (Nutritive values):**

**Nitrogen:**

As regarded to nitrogen percentage in leaves, data presented in Table 3 show that there were significant differences between fertilization treatments on nitrogen percentage in leaves. Plants were supplied with ½ poultry + PGPR + spraying setter-2 or poultry in the first and second seasons, respectively gave the highest values followed by poultry or ½ poultry + PGPR + setter-2 in the first and second seasons, respectively then ¾ NPK + PGPR

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+ spraying setter-2. Applying PGPR gave the lowest values as compared with NPK.

On the other hand, application of \( \frac{3}{4} \), \( \frac{1}{2} \) or \( \frac{1}{4} \) NPK (recommended dose) with PGPR and spraying with setter-2 or un spraying gave higher values when compared with applying NPK only. Meanwhile, fertilization treatments as \( \frac{1}{2} \) poultry + \( \frac{1}{2} \) chemical or \( \frac{1}{2} \) poultry + PGPR gave lower values of nitrogen percentage of leaves than \( \frac{1}{2} \) poultry + \( \frac{1}{2} \) chemical + spraying setter-2 or \( \frac{1}{2} \) poultry + PGPR + spraying setter.

In general, spraying setter-2 on plants supplemented with different fertilization treatments caused higher values than without spraying setter-2 in both seasons.

**Phosphorus:**

Concerning phosphorus percentage in the leaves of spinach, data presented in Table 3 show that there were significant differences between fertilization treatments on phosphorus% of leaves. Plants supplied with \( \frac{3}{4} \) NPK + PGPR + spraying setter-2 followed by \( \frac{1}{2} \) NPK + PGPR + spraying setter-2 gave the highest values in both seasons.

On the other hands, spinach plants \( \frac{3}{4} \), \( \frac{1}{2} \) or \( \frac{1}{4} \) NPK + PGPR with spraying setter-2 or without spraying setter-2 resulted in the higher % than those supplied with NPK ( recommended dose ). The lowest values were obtained by using poultry fertilizer and it was lower than those obtained by applying PGPR. Plants supplied with \( \frac{1}{2} \) poultry + PGPR had higher phosphorus% than that obtained by using \( \frac{1}{2} \) poultry + \( \frac{1}{2} \) chemical.

Plants sprayed with setter-2 gave higher phosphorus% than those unspraying by setter-2.

**Potassium:**

Results presented in Table 3 indicate that fertilization treatments significantly increased potassium percentage in spinach leaves over those of PGPR which recorded the lowest values in both seasons. Plants were supplied with \( \frac{1}{2} \) poultry + PGPR or \( \frac{1}{2} \) poultry + \( \frac{1}{4} \) chemical + PGPR gave the highest values in the first season. Whereas plants grown in soil supplied with \( \frac{3}{4} \), \( \frac{1}{2} \) or \( \frac{1}{4} \) NPK + PGPR + spraying setter-2 gave the highest values of K% but without significant differences between them, in the second season.

In both seasons, plants supplied with \( \frac{1}{2} \) poultry + PGPR gave the higher potassium% as compared with \( \frac{1}{2} \) poultry + \( \frac{1}{2} \) chemical or NPK (recommended dose). Also, plants received \( \frac{3}{4} \), \( \frac{1}{2} \) or \( \frac{1}{4} \) + PGPR + spraying setter-2 gave the higher values of potassium percentage than NPK (recommended dose) as well as \( \frac{3}{4} \), \( \frac{1}{2} \) or \( \frac{1}{2} \) NPK + PGPR.

These results were agreement with those obtained by many investigators. The mineral-organic fertilizers increased the P content in spinach (Suchorska, 1996). Meanwhile, organic application resulted in higher concentration of N,P and K compared with mineral fertilizer application with lettuce (Rodrigues and Casali, 1999; Abd-Elmoniem et al., 2001 and Souza et al., 2005).

Nitrogen uptake by spinach was higher with addition organic compost than with chemical fertilizer (Kutuk et al., 1999; Matsumoto et al., 1999; Maruo et al., 2002; Maftoun et al., 2004 and Kodashima et al., 2006). Spinach
plants supplied with animals waste composts contained more K than if grown with conventional chemical fertilizers (Kutuk et al., 1999 and Chishaki et al., 2000). Meanwhile, pigeon + chicken + inorganic fertilizers treatment resulted in the highest levels of K content (Abd-Elmoniem et al., 2001). In this respect, many investigators reported that, microorganisms, which were used as biofertilizers, induced simulation effect on plant growth and production by fixing atmospheric nitrogen. They are free living, e.g., Azotobacter and Azospirillum, or live in symbiotic associations with certain higher plants, e.g., Rhizobium. In addition, Bacillus megatherium mobilize phosphate and micronutrients. Also, Bacillus circulanse release potassium. While, Azotobacter, Azotobacter and Azopirillum secret growth promoting factors, e.g., gibberellin, cytokinin like substances and auxins (Marschner, 1995).

Table 3: Effect of poultry manure, chemical fertilizers, biofertilizer and foliar bio-stimulant on N, P and K % in leaves of spinach plants.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Season</th>
<th>N %</th>
<th>P %</th>
<th>K %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPK</td>
<td>3.44</td>
<td>3.39</td>
<td>0.236</td>
<td>0.238</td>
</tr>
<tr>
<td>PGPR</td>
<td>3.32</td>
<td>3.26</td>
<td>0.134</td>
<td>0.129</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>4.24</td>
<td>4.32</td>
<td>0.112</td>
<td>0.115</td>
</tr>
<tr>
<td>½ poultry + ½ NPK</td>
<td>3.39</td>
<td>3.15</td>
<td>0.123</td>
<td>0.119</td>
</tr>
<tr>
<td>½ poultry + ½ NPK + Setter-2</td>
<td>3.36</td>
<td>3.19</td>
<td>0.144</td>
<td>0.138</td>
</tr>
<tr>
<td>½ poultry + PGPR</td>
<td>4.13</td>
<td>4.00</td>
<td>0.120</td>
<td>0.200</td>
</tr>
<tr>
<td>½ poultry + PGPR + setter-2</td>
<td>4.47</td>
<td>4.28</td>
<td>0.329</td>
<td>0.351</td>
</tr>
<tr>
<td>½ poultry + ¼ NPK + PGPR</td>
<td>3.71</td>
<td>3.79</td>
<td>0.247</td>
<td>0.262</td>
</tr>
<tr>
<td>⅓ NPK + PGPR</td>
<td>4.18</td>
<td>4.11</td>
<td>0.329</td>
<td>0.312</td>
</tr>
<tr>
<td>⅓ NPK + NPGPR</td>
<td>3.96</td>
<td>4.00</td>
<td>0.322</td>
<td>0.288</td>
</tr>
<tr>
<td>⅓ NPK + PGPR + setter-2</td>
<td>3.57</td>
<td>4.10</td>
<td>0.256</td>
<td>0.269</td>
</tr>
<tr>
<td>½ NPK + PGPR + setter-2</td>
<td>4.25</td>
<td>4.22</td>
<td>0.428</td>
<td>0.410</td>
</tr>
<tr>
<td>⅓ NPK + PGPR + setter-2</td>
<td>4.07</td>
<td>4.18</td>
<td>0.368</td>
<td>0.365</td>
</tr>
<tr>
<td>¼ NPK + PGPR + setter-2</td>
<td>4.15</td>
<td>4.16</td>
<td>0.300</td>
<td>0.291</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.492</td>
<td>0.384</td>
<td>0.0237</td>
<td>0.0539</td>
</tr>
</tbody>
</table>

Concerning the effect of foliar biostimulant treatments on nutritive values, Setter-2 caused raising of N, P and K in all plant parts and in reading of leaves chlorophyll. These results are logical since Setter-2 contains N, and microelements. Nofal et al., (1991) working on lettuce and Hanafy Ahmed et al., (1995) working on faba bean revealed that spraying such plants with ascorbic acid showed favorable effect on the content of N, P and K in different plant organs. El-Quesni and Radwas (1993) who recorded that the chlorophyll increased in the leaves.

Nutritive values:

Total sugar.data presented in Table 4 reveal clearly significant differences between fertilization treatments in total sugar concentration of leaves in both seasons. The maximum values of total sugars were obtained with supplying soil by ½ poultry + ½ chemical + spraying setter-2 or ½ poultry + PGPR + spraying setter-2 followed by ¼ poultry + ½ chemical + PGPR or
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½ poultry + ¼ chemical + PGPR as compared with NPK (recommended dose) which recorded the lowest values.

Applying PGPR in the soil caused the moderate values of total sugars in leaves. On the other hand, adding PGPR to ¾, ½ or ¼ resulted in more increasing in total sugars concentration of leaves than NPK (recommended dose), Also spraying setter-2 on plants received the pervious treatments caused highest increasing than when compared with those unsprayed plants. It was observed that plants fertilized with ½ NPK + PGPR with or without spraying setter-2 gave the higher values of total sugars concentration than fertilization with ¾ NPK + PGPR with or without spraying setter-2. Plants supplied with ½ poultry + ½ chemical NPK or ½ poultry + PGPR gave the lower values than those sprayed with setter-2.

Table 4: Effect of poultry manure, chemical fertilizers, biofertilizer and foliar bio-stimulant on total sugars, total soluble phenols, total free amino acids, nitrate and total chlorophyll (mg/100 g F.W) in leaves of spinach plants

<table>
<thead>
<tr>
<th>Season</th>
<th>Total sugars</th>
<th>Total soluble phenols</th>
<th>Total free amino acids</th>
<th>Nitrate</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPK</td>
<td>84.8</td>
<td>91.1</td>
<td>44.3</td>
<td>45.6</td>
<td>91.7</td>
</tr>
<tr>
<td>PGPR</td>
<td>170.6</td>
<td>71.0</td>
<td>27.1</td>
<td>28.9</td>
<td>123.4</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>122.5</td>
<td>77.1</td>
<td>42.3</td>
<td>40.9</td>
<td>426.8</td>
</tr>
<tr>
<td>½ poultry + ½ NPK</td>
<td>129.4</td>
<td>72.8</td>
<td>51.0</td>
<td>52.7</td>
<td>206.2</td>
</tr>
<tr>
<td>½ poultry + ½ NPK + Setter-2</td>
<td>196.3</td>
<td>201.5</td>
<td>35.3</td>
<td>36.1</td>
<td>225.3</td>
</tr>
<tr>
<td>½ poultry + PGPR</td>
<td>118.8</td>
<td>127.2</td>
<td>39.5</td>
<td>38.8</td>
<td>276.6</td>
</tr>
<tr>
<td>½ poultry + PGPR + setter-2</td>
<td>194.9</td>
<td>201.5</td>
<td>25.4</td>
<td>27.6</td>
<td>325.3</td>
</tr>
<tr>
<td>½ poultry + ¾ NPK + PGPR</td>
<td>183.4</td>
<td>182.5</td>
<td>39.9</td>
<td>38.7</td>
<td>204.0</td>
</tr>
<tr>
<td>¼ poultry + ½ NPK + PGPR</td>
<td>187.0</td>
<td>185.7</td>
<td>38.9</td>
<td>39.8</td>
<td>175.9</td>
</tr>
<tr>
<td>¾ NPK + PGPR</td>
<td>98.8</td>
<td>105.3</td>
<td>48.7</td>
<td>49.0</td>
<td>124.1</td>
</tr>
<tr>
<td>¼ NPK + PGPR</td>
<td>135.4</td>
<td>140.2</td>
<td>44.3</td>
<td>42.9</td>
<td>143.8</td>
</tr>
<tr>
<td>¾ NPK + PGPR + Setter-2</td>
<td>129.3</td>
<td>130.5</td>
<td>36.8</td>
<td>33.2</td>
<td>133.2</td>
</tr>
<tr>
<td>½ NPK + PGPR + Setter-2</td>
<td>146.3</td>
<td>141.0</td>
<td>37.1</td>
<td>36.9</td>
<td>310.8</td>
</tr>
<tr>
<td>¼ NPK + PGPR + Setter-2</td>
<td>110.8</td>
<td>120.3</td>
<td>33.7</td>
<td>36.4</td>
<td>215.1</td>
</tr>
<tr>
<td>L.S.D 0.05</td>
<td>9.51</td>
<td>9.51</td>
<td>4.14</td>
<td>4.37</td>
<td>34.21</td>
</tr>
</tbody>
</table>

In the second season, the effect of ½ poultry + PGPR was nearly similar to that of ¾ poultry + ½ chemical. Meanwhile, in the first season the effect of ½ poultry + PGPR on total sugar concentration was the lower than ½ poultry + ½ chemical.

All foliar biostimulant treatments significantly raised dry matter of leaves. The present results are in line with those reported by Ismail (2002) who found that spraying peas with a biostimulant namely Biomagic (contains

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vitamins, amino acid and macro-and microelements) increased pod contents of carbohydrates.

**Total free amino acids:** Regarding total free amino acid concentration in leaves, data presented in Table 4 indicated that the effect of fertilization on total free amino acids concentration were significant, in both seasons. Poultry fertilization resulted in the highest values followed by ½ poultry + PGPR + spraying setter-2 then ½ chemical + PGPR + setter-2 as compared with NPK (recommended dose) which recorded the lowest total free amino acids concentration in leaves.

On the other hand, plants receiving ½ poultry + PGPR had higher values than ones receiving ½ poultry + ½ chemical. Also, inoculating spinach plants with PGPR resulted in higher values than with using NPK (recommended dose).

Applying ½ poultry + PGPR in the soil where spinach plants were grown caused the higher free amino acids concentrations in leaves than those obtained when applying ½ chemical + PGPR.

Spinach plants inoculated with PGPR alone recorded higher values than those of plants fertilized with ¾ NPK + PGPR. It was observed that spraying setter-2 on plants caused the higher values of free amino acid concentrations than those obtained without spraying.

**Nitrate concentration in leaves:** As for the effect of fertilization treatments on nitrate concentration in leaves. Data in Table 4 show significant differences between treatments on nitrate concentration in both seasons. The highest values were recorded when applying NPK (recommended dose) followed by ¾ NPK + PGPR, then ½ NPK + PGPR.

Plants supplied with PGPR followed by poultry fertilization recorded the lowest values. Applying soil with ½ poultry + PGPR significantly decreased nitrate concentration in leaves as compared with plant receiving ½ poultry + ½ chemical. The effect of ½ poultry + ¼ chemical NPK + PGPR was nearly similar to that of ¼ poultry + ½ chemical NPK + PGPR.

On the other hand, using setter-2 as spraying on spinach plants resulted in decreasing nitrate concentration in plants as compared with the values recorded by the plants unspraying with setter-2.

The same trend, better quality of spinach plant was obtained with organic fertilizer than with chemical fertilizer, leaf nitrate concentration was lower and sugar concentration was higher with organic fertilizer than with chemical fertilizer (Yamazaki and Roppongi, 1998; Gent, 2005; Zhou and Luo, 2004; Kodashima et al., 2006 Pavlou et al., 2007 and Peyvast et al., 2008).

In this respect, Li et al., (2003) reported that the effect of organic manures on nitrate; soluble sugars and amino acids on lettuce varied with manure type. In general, organic manures improved such nutritional. The effect of biofertilizer on nitrate were confirmed by Premuzic et al.,(2002) who found that biostabilized compost caused the lowest nitrate content of lettuce.

**Total soluble phenols:** Concerning the total soluble phenols concentration in leaves, data presented in Table 4 indicated the effect fertilization treatments on total soluble phenols which were significant in both seasons. Plants were supplied with ½ poultry + ½ chemical followed by ¾ NPK + PGPR or ¾
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PGPR + spraying setter-2 gave the highest values as compared with NPK (recommended dose). While, the lowest values of total soluble phenols were obtained by using ½ poultry + PGPR + spraying setter-2 or PGPR in the first and second seasons, respectively.

Applying ½ poultry + PGPR caused more reducing in total soluble phenols in leaves as compared with applying ½ poultry + ½ chemical. It was observed that adding spraying setter-2 to different treatments resulted in lower values than those without spraying, in both seasons.

The results were agree with that obtained by Zhao et al., (2007) who found that organic and conventional chemical fertilization did not consistently differentially affect lettuce phenolics.

**Total chlorophyll:** Data presented in Table 4 indicate that the effect of fertilization treatments on chlorophyll concentration in leaves which were non significant in both seasons. The highest values were recorded by the plants supplied with ¼ poultry + ¼ chemical NPK + PGPR followed by those treated with ¼ NPK + PGPR + spraying setter-2. However, the lowest values were obtained with applying ¼ NPK + PGPR + spraying setter-2 followed by ½ NPK + PGPR.

These results agree with those obtained by Abd-Elmoniem et al., (2001) who found that the treatments (chicken, pigeon, inorganic fertilizer and their mixture) had no significant effect on chlorophyll content with the exception of pigeon + inorganic treatment that was significantly decreased compared to the control treatment (inorganic fertilizer).

**Microbial status:**

Soil microbial status in the two seasons was evaluated. Data presented in Table 5 show that NPK-fertilizers at the recommended dose had a negative effect on rhizospher microorganisms (RMO) (bacteria, fungi, actinomycetes). On the other hand, inoculation with mixed inocula of azotobacter, bacillus, bradyrhizobia as a group of plant growth promoting rhizobacteria alone or with 25% NPK-fertilizer gave higher value of log number of total bacteria, fungi and actinomycetes as compared to other treatments. Generally, the inoculation with (PGPR) gave higher values of (RMO) as compared to any treatment without (PGPR). These results are in accordance with other studies where inoculation with some PGPR had stimulation effect on the population of rhizospher microorganism (RMO) and increased their number by more than 50% at the end of the experiment compared with the numbers recorded before planting (Pondy et al., 1998, Abotaleb et al., 2002 and Ragab et al., 2006 ). An initial increase in abundance of bacteria and fungi was observed after direct incorporation and amendment with red clover-derived slurry and compost, but amendment with fresh red clover sustained a higher bacterial and fungal biomass until the end of the cropping season. Mulching stimulated arbuscular mycorrhizal (AM) fungi at the end of the cropping season. The treatments with fresh red clover, direct incorporation and mulch, tended to differ in their microbial community composition from the treatments with processed red clover. The protease, acid phosphatase and arylsulphatase activities were highest in the direct incorporation treatment, whereas enzyme activity in treatments with
processed red clover was never higher than in the control treatments (Elfstrand et al., 2007).

It was recommended, when the poultry manure (Tanahashi and Yono, 2004), biofertilizers and foliar biostimulants were applied, and not only the amount of nitrogen but phosphate and potassium should be reduced from chemical fertilizers.

Table (5): Effect of PGPR, organic and chemical fertilizers on log number of total bacteria, fungi, and actinomycetes of spinach plants rhizosphere area, as average of the two season.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total bacteria (log number)</th>
<th>Fungi (log number)</th>
<th>Actinomycetes (log number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPK</td>
<td>3.15</td>
<td>3.72</td>
<td>3.10</td>
</tr>
<tr>
<td>PGPR</td>
<td>5.12</td>
<td>4.35</td>
<td>3.32</td>
</tr>
<tr>
<td>Poultry manure</td>
<td>5.1</td>
<td>4.62</td>
<td>3.70</td>
</tr>
<tr>
<td>½ poultry + ½ NPK</td>
<td>4.9</td>
<td>3.90</td>
<td>3.60</td>
</tr>
<tr>
<td>¼ poultry + ½ NPK + Setter-2</td>
<td>7.32</td>
<td>4.70</td>
<td>3.92</td>
</tr>
<tr>
<td>¼ poultry + PGPR</td>
<td>6.91</td>
<td>4.85</td>
<td>3.71</td>
</tr>
<tr>
<td>½ poultry + PGPR + setter-2</td>
<td>7.21</td>
<td>4.90</td>
<td>3.93</td>
</tr>
<tr>
<td>½ poultry + ¾ NPK + PGPR</td>
<td>5.32</td>
<td>3.90</td>
<td>3.09</td>
</tr>
<tr>
<td>¼ poultry + ½ NPK + PGPR</td>
<td>4.92</td>
<td>4.32</td>
<td>3.24</td>
</tr>
<tr>
<td>PGPR + 75% NPK</td>
<td>3.29</td>
<td>3.67</td>
<td>3.02</td>
</tr>
<tr>
<td>PGPR + 50% NPK</td>
<td>3.02</td>
<td>3.41</td>
<td>3.14</td>
</tr>
<tr>
<td>PGPR + 25% NPK</td>
<td>6.42</td>
<td>4.90</td>
<td>3.93</td>
</tr>
<tr>
<td>PGPR + 75% NPK + setter-2</td>
<td>3.24</td>
<td>3.22</td>
<td>3.04</td>
</tr>
<tr>
<td>PGPR + 50% NPK + setter-2</td>
<td>3.41</td>
<td>3.71</td>
<td>3.05</td>
</tr>
<tr>
<td>PGPR + 25% NPK + setter-2</td>
<td>6.34</td>
<td>4.78</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Our results constitute an important technological contribution to microorganisms selection, under this investigation, for inoculants formulations to be used with leafy plant specially spinach, showing different phytohormone profile excreted by different bacterial strains.

Finally, it could be concluded that. The best yield and quality were obtained in the present study with applying ½ poultry + PGPR + setter-2 or ½ poultry + ½ NPK + Setter-2. As a fact, the yield is the product of the physiological processes according the optimum plant growth.
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استجابة نباتات السببان لسماد مخلفات الدواجن والتلقيح بالبكتريا المنشطة للنمو (PGPR) 
أعمال محمد فرجاء، سميح طه، هناة فتحوح أحمد و عاطف عبد العزيز رجب

قسم الخضر كليّة الزراعة جامعة القاهرة

فرع سيسيولوجي النبات قسم النبات الزراعي كليّة الزراعة جامعة القاهرة

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أجريت تجربتان حديثتان حيث زرعت نباتات السببان وسمدت بسماد الدواجن بمعدل 4 طن/فدان والسماد الكيماوي الموسي به 250 كجم سلفات أمونيوم و100 كجم سوب وفسفات الكالسيوم الأحادي و 75 كجم سلفات البنفسجوم واستعملت مبيدات مختلفة (عامل كامل، معدل 1/4 المعدل لكل من السماد الكيماوي والعضوي) وكذلك تم تقيم بعض السلالات البكتيرية من حيث إنتاجها لبعض مركبات الفوتورام والسيديروفور وقدرتها على ذابة الفوسفور

الغير مسير واستخدامها كبكتريا مشجعة للنمو.

بتور السبان بعضها لم يلقح والبعض الآخر لحق ب-GPGR للتروجين مثل Bacillus chroococcum و بكتريا ميظعة الفوسفور Azotobacter japonicum (Bacillus megaterium) (Bradyrhizobium) (Bacillus cereus) (Bradyrhizobium japonicum. 

罗斯ت بعض النيبات بناءً على التربة شركي وهانس والامانج وبوه روح ومنجز بعد 15 يوم وبعد 30 يوم من الزراعة. عاملت نباتات السبان بالمعاملات السابقة مفردة أو متحركة.

أوضحت النتائج أن استعمال 2/1 كمية سماد الدواجن + PGPR + رش 2 وتصلح على أعلى قيمة من زر النبات في المواسم، ونور النبات في الموسم الثاني وكمية المحصول للذرة بعد 2 سنة، على السكان، في المزار، في الموسم الأول مقاومة معدل التنسيب الكيماوي الموسي به. ومن ناحية أخرى، استعمال 2/1 سماد الدواجن + PGPR + الرش 2 لي Getter يزيد في عدد الأوراق للنباتات والحصول الكلي للذرة في الموسم الثاني، ونسبة النمو للذرة، الجافة للأوراق في الموسم.

أما نباتات السبان من النيبات الكيماوية فقد أدى استعمال 2/1 كمية سماد الدواجن + Getter إلى الحصول على أعلى قيمة من نز النبات في المواسم، ونور النبات في الموسم الثاني وكمية المحصول للذرة بعد 2 سنة، على السكان، في المزار، في الموسم الأول مقاومة معدل التنسيب الكيماوي الموسي به. ومن ناحية أخرى، استعمال Getter يزيد في عدد الأوراق للنباتات والحصول الكلي للذرة في الموسم الثاني، ونسبة النمو للذرة، الجافة للأوراق في الموسم.

تم الحصول على أعلى تركيز من الفيتامينات الذائبة الكلية والأحماض الأمينية الحرّة الكلية تم PGPR + NPK في النيبات في المواسم، بينما سجلت أعلى قيمة للبروتين في PGPR + Getter وPGPR + NPK في النيبات في المواسم.

أعطت PGPR في PGPR + NPK سماستماد الدواجن على التوالي في المواسم. تم الحصول على أعلى تركيز للنوات في النيبات في PGPR + Getter وPGPR + NPK، بينما نباتات PGPR + Getter تعرضت لأقل نباتات PGPR + Getter. لذا، توجد اختلافات معنوية بين النباتات في تركيز النباتات من الكلور في المواسم.