J. Agric. Sci. Mansoura Univ., 34 (4): 3495 -3518, 2009

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

Farag, Amal M.*; Sahar S, Taha.*; Hanaa F. Ahmed and A.A. Ragab*****

*** Vegetable Department, Faculty of Agriculture, Cairo University**

**** Plant Physiology Division, Faculty of Agriculture, Cairo University**

***** Soil, Water and Environment, Research Institute, A.R.C, Giza, Egypt**

ABSTRACT

Two field experiments were carried out on spinach (*Spinasia 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 cerculans* 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 pervious 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 stter-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 concentrationwere 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 (*Spinasia oleracae* 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 micro organisms 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 in 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 dissoloving and potassium release bacteria promote the growth of plants either directly through N_2-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 rgizobacteria 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 rihizopheric bacteria isolates were tested. The beneflcal 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 4m^2. (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 mgl^{-1} of 8- hydroxyzuinoline 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% $NaCO_3$ indicated the production of 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-Britto 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 un-inoculated 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 ($meql^{-1}$), Cl^- 11.71($meql^{-1}$), SO_4 14.92 ($meql^{-1}$), Ca^{++} 9.53($meql^{-1}$), Mg^{++}2.57($meql^{-1}$), Na^+ 22.93 ($meql^{-1}$). 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 rat 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 + ¼ dose of chemical fertilizers (NPK) + PGPR.
- ¼ dose of poultry manure + ½ dose of chemical fertilizers (NPK) + PGPR.
- ¾ dose of chemical fertilizers (NPK) + PGPR.
- ½ dose of chemical fertilizers (NPK) + PGPR.
- ¼ dose of chemical fertilizers (NPK) + PGPR.
- ¾ dose of chemical fertilizers (NPK) + PGPR + spraying setter-2.
- ½ dose of chemical fertilizers (NPK) + PGPR + spraying setter-2.
- ¼ 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 apparotus of Parnos – Wagner as described by Van Schouwenburg and Walinga (1978). Phosphorus was estimated colorometically by using chlorostannous reduced molybdophosphoric blue color method according to Chapman and Parker (1961). Potassium was determined using the flame photometer. NO_3 – 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.cerculans* appeared negative reaction on the tests of siderophore and P- solublizers. 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.

Bacterial Strains	Relative reaction			
	Azotobacter chroococcum **(AZ4)**	***Bacillus megaterium*** **(BM3)**	***Basillus cerculans*** **(BCI)**	***Bradyrhizobium japonicum*** **USDA 100 sp4**
Siderophores	++	-	-	+++
Cyanogens (HCN)	+	-	+	-
IAA	+++	+	++	+++
P-solubilizers	++	+++	-	-

(-) 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 $^1/_4$ poultry fertilizer + ½ (NPK) recommended chemical fertilizer + PGPR as compared to chemical fertilizer alone. On the other hand, applying ½ (NPK) + PGPR+ spraying with setter – 2 and ½ poultry fertilizer + PGPR + spraying with setter-2 as well as poultry fertilizer, ½ (NPK) chemical fertilizer + PGPR, ½ poultry + ½ NPK + spraying setter-2 and PGPR sightly increased plant length as compared with chemical fertilizer (NPK), Meanwhile, adding ½ poultry + ½ NPK, ½ poultry + PGPR, ½ poultry + $^1/_4$ chemical + PGPR, $^1/_4$ NPK + PGPR + spraying setter-2 resulted in highly decreased comparing with NPK. The lowest values were obtained with using ¾ NPK + PGPR and $^1/_4$ NPK + PGPR. Length of plants receiving ¾ NPK + PGPR + spraying setter-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 + $\frac{1}{4}$ chemical (NPK) + PGPR and ½ chemical (NPK) + PGPR + spraying setter-2.

Length of plants receiving poultry fertilizer or ½ chemical (NPK) + PGPR were nearly equal to these 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 $\frac{1}{4}$ chemical (NPK) + 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 $\frac{1}{4}$ 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 + $\frac{1}{4}$ 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 fertilize by PGPR. The lowest values were obtained from plants received ¾ NPK + PGPR or $\frac{1}{4}$ 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 + ½ poultry + PGPR, ¾ chemical NPK + PGPR, ½ chemical NPK + PGPR or ¼ 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 ½ poultry + ¼ chemical + PGPR resulted in higher values than that obtained when plants grown in soil supplemented with ¼ poultry + ½ NPK + PGPR. Leaves dry matter percentage of plants supplemented with ½ NPK (chemical dose) + PGPR+ spraying setter-2 were higher than ones supplemented with ¾ 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 ¼ 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 ½ poultry + PGPR + spraying setter-2 followed by ½ poultry + ½ 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 ½ poultry + ½ chemical NPK or ½ chemical NPK + PGPR + spraying setter-2. Also, plants receiving ¼ poultry + ½ chemical + PGPR gave higher total yield than plants receiving ½ poultry + ¼ chemical + PGPR.

In the second season, the highest total yield was obtained with application of ½ poultry + ½ chemical + spraying setter-2 followed by ½ poultry + PGPR + spraying setter-2 or ½ poultry + ½ chemical, then poultry fertilizer. On the other hand, plants grown on soil supplemented with ½ poultry + ¼ chemical + PGPR gave higher yield than ones supplemented with ¼ poultry + ½ chemical + PGPR.

In both seasons, application of PGPRT alone cause lower total yield than application poultry or chemical fertilization. Also, ½ chemical + PGPR + spraying setter-2 resulted in higher values than ¾ chemical +PGPR without spraying. Moreover, the results indicated that spraying setter-2 caused the 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.

Growth characters	Plant length (cm)		Plant weight (gm)		Leaves number/per plant		Leaves dry matter %		Yield (ton/fed.)	
Season / Treatments	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
NPK	33.0	30.5	34.0	23.5	9.6	10.1	9.00	9.02	9.173	7.074
PGPR	33.8	26.3	23	22.3	8.8	9.7	8.27	8.17	6.718	6.811
Poultry manure	34.2	30.3	23.8	27.3	8.8	10.1	9.21	9.10	8.995	9.216
½ poultry + ½ NPK	32.1	31.2	24.2	27.0	8.9	9.2	8.10	8.40	8.531	9.275
½ poultry + ½ NPK + Setter-2	33.7	31.0	28.3	30.3	9.3	10.9	9.61	9.58	9.141	10.693
½ poultry + PGPR	32.0	29.1	29.5	31.7	9.0	9.2	8.59	8.50	7.987	7.622
½ poultry + PGPR + setter-2	34.3	32.5	36.5	33.7	10.1	10.7	9.23	8.96	11.595	9.297
½ poultry +¼NPK + PGPR	32.9	31.0	30.3	31.2	9.0	10.6	8.75	8.72	7.099	8.119
¼ poultry + ½ NPK + PGPR	35.4	26.3	33.3	23.3	9.2	9.8	8.63	8.58	7.543	7.078
¾ NPK + PGPR	30.4	28.4	22.7	18.0	9.1	9.3	8.48	8.29	6.953	5.611
½ NPK + PGPR	34.0	30.3	29.8	16.8	9.8	9.0	8.53	8.51	7.005	6.505
¼ NPK + PGPR	30.4	26.1	27.1	16.3	8.5	8.8	8.04	8.20	5.737	4.291
¾ NPK + PGPR + setter-2	33.1	28.9	30.2	22.0	9.7	9.4	8.73	8.67	7.082	5.697
½ NPK + PGPR + setter-2	34.5	31.0	35.2	20.0	9.5	9.9	8.61	8.61	8.612	7.167
¼NPK + PGPR + setter-2	32.8	28.2	27.3	16.8	9.6	9.3	8.21	8.30	6.878	4.939
L.S.D 0.05	2.19	2.43	2.79	5.65	0.84	0.69	1.068	0.599	2.267	1.545

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 N^{15} sulphate and composts significantly increased spinach yield compared with single application of N^{15} 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 affected on spinach plants, which caused increasing the prameters 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 verity their potential for growth enhancement in lettuce. A total of 77 fluorescent *Pseudomonads*, 23 *Bacillus* and 11 other *rihizospheric* 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 cased significant increase in plant height, fresh and dry weight per plant of lettuce or peaplants 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). Microelements, 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 simulative 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

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

On the other hand, application of ¾ , ½ or ¼ 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 ½ poultry + ½ chemical or ½ poultry + PGPR gave lower values of nitrogen percentage of leaves than ½ poultry + ½ chemical + spraying setter-2 or ½ 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 ¾ NPK + PGPR + spraying setter-2 followed by ½ NPK + PGPR + spraying setter-2 gave the highest values in both seasons.

On the other hands, spinach plants ¾, ½ or ¼ 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 ½ poultry + PGPR had higher phosphorus% than that obtained by using ½ poultry + ½ 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 ½ poultry + PGPR or ½ poultry + ¼ chemical + PGPR gave the highest values in the first season. Whereas plants grown in soil supplied with ¾, ½ or ¼ 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 ½ poultry + PGPR gave the higher potassium% as compared with ½ poultry + ½ chemical or NPK (recommended dose). Also, plants received ¾, ½ or ¼ + PGPR + spraying setter-2 gave the higher values of potassium percentage than NPK (recommended dose) as well as ¾, ½ or ½ 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.

Chemical composition	N %		P %		K %	
Season / Treatments	2005	2006	2005	2006	2005	2006
NPK	3.44	3.39	0.236	0.238	2.94	2.98
PGPR	3.32	3.26	0.134	0.129	2.70	2.72
Poultry manure	4.24	4.32	0.112	0.115	2.90	2.92
½ poultry + ½ NPK	3.39	3.15	0.123	0.119	2.85	2.81
½ poultry + ½ NPK + Setter-2	3.36	3.19	0.144	0.138	2.93	2.89
½ poultry + PGPR	4.13	4.00	0.320	0.300	3.20	3.00
½ poultry + PGPR + setter-2	4.47	4.28	0.329	0.351	2.82	2.84
½ poultry + ¼ NPK + PGPR	3.71	3.79	0.247	0.262	3.17	3.00
¼ poultry + ½ NPK + PGPR	3.62	3.58	0.250	0.255	2.91	2.89
¾ NPK + PGPR	4.18	4.11	0.329	0.312	2.91	2.91
½ NPK + PGPR	3.96	4.00	0.322	0.288	2.93	2.94
¼ NPK + PGPR	3.97	4.10	0.256	0.269	2.83	2.86
¾ NPK + PGPR + setter-2	4.25	4.22	0.428	0.410	3.07	3.10
½ NPK + PGPR + setter-2	4.07	4.18	0.368	0.365	3.07	3.15
¼ NPK + PGPR + setter-2	4.15	4.16	0.300	0.291	3.07	3.11
LSD 0.05	0.492	0.384	0.0237	0.0539	0.155	0.259

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

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 concenteration 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

Chemical composition	Total sugars		Total soluble phenols		Total free amino acid		Nitrate		Total chlorophyll	
Season / Treatments	2005	2006	2005	2006	2005	2006	2005	2006	2004	2005
NPK	84.8	91.1	44.3	45.6	91.7	107.5	401.0	415.8	40.8	40.5
PGPR	170.6	171.0	27.1	26.9	123.4	146.5	200.1	205.3	37.9	38.5
Poultry manure	122.5	125.1	42.3	40.9	426.8	399.2	230.1	221.6	39.6	39.1
½ poultry + ½ NPK	129.4	129.8	51.0	52.7	206.2	194.1	362.4	346.7	40.1	40.2
½ poultry + ½ NPK + Setter-2	196.3	201.5	35.3	36.1	225.3	239.5	334.2	328.4	40.3	40.5
½ poultry + PGPR	118.8	127.2	39.5	38.8	275.6	281.4	290.5	293.1	39.5	39.8
½ poultry + PGPR + setter-2	194.9	201.5	25.4	27.6	325.3	327.2	275.8	280.4	41.1	40.9
½ poultry +¼ NPK + PGPR	183.4	182.5	39.9	38.7	204.0	209.0	329.3	311.5	39.3	39.2
¼ poultry + ½ NPK + PGPR	187.0	185.7	38.9	39.8	175.9	180.5	300.6	319.20	42.1	42.0
¾ NPK + PGPR	98.8	105.3	48.7	49.0	124.1	130.6	381.9	386.1	40.8	40.8
½ NPK + PGPR	135.4	140.2	44.3	42.9	143.8	151.9	363.5	359.2	40.2	39.9
¼ NPK + PGPR	89.3	105.0	36.8	33.2	133.2	147.0	335.7	341.0	38.8	38.8
¾ NPK + PGPR + setter-2	124.9	136.1	47.2	47.6	293.2	299.4	350.6	362.9	41.9	41.6
½ NPK + PGPR + setter-2	146.3	141.0	37.1	36.9	310.8	321.5	332.4	339.0	40.9	40.7
¼ NPK + PGPR + setter-2	110.8	120.0	33.7	36.4	215.1	208.9	300.2	298.5	38.5	38.2
L.S.D 0.05	9.51	9.51	4.14	4.37	34.21	24.94	27.5	30.1	ns	ns

In the second season, the effect of ½ poultry + PGPR was nearly similar to that of ½ 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 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 indicat 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 indicat 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

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 indicat 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 + ½ chemical NPK + PGPR followed by those treated with ¾ 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, bradyrhzobia 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 rhizospher area, as average of the two season.

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

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.

REFERENCES

Abdel-Hafez, A.H.(1966). Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolves. Ph.D. thesis Fac. Agric., Ain shams Univ., Egypt ,pp.29-30.

Abd-Elmoniem, E.M.; El-Shinawy, M.Z; Abou-Hadid, A.F and Helmy, Y.I.(2001). Response of lettuce plant to feeding with unconventional sources under hydroponic system. Acta Horticulturae, 559(2): 549-554.

Abotaleb, H.H; Mona A.M. Soleman and Mahrous, N.M. (2002). The enhancement of wheat productivity by inoculation with associative diazotroph and foliar application of methanol under different N-fertilizer level. Assiut J. Agric. Sci., 33:113-127.

Adediran, J.A; Mnkeni, P. N.S and Mafu, N.C .(2004).Changes in chemical properties and temperature during the composting of tobacco waste with other organic materials, and effects of resulting composts on lettuce (*Lactuca sativa* L.) and spinach (*Spinacea oleracea* L.). Biological Agriculture & Horticulture, 22(2)101-119.

Ae, N.; Matsumoto, S.; Koyama, Y.and Iijima, K.(2006) . The potential role of PEON (Phosphate buffer-Extractable Organic Nitrogen) in the soil for plant nutrition and its implication for organic farming. Food and Fertilizer Technology , 17:3- 7.

Alexander, D. and Zubeter, D.A. (1991). Use of chrome azural S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils, 12:39-45.

Al-Kahal, A.A.; A.A. Ragab ; Saied, S.A. and Omar, S.A. (2003). Use of plant growth promoting rhizobacteria for controlling faba bean disease caused by *Fusarium oxysporum*. in; Eleventh Conference of Microbiology; Cairo; Egypt, Oct.12-14.

Amer, S. S. and El-Assiouty, F. M. M. (2004). Pea seeds a production as affected by foliar application with citrine and nofatrain. J. Agric. Sci. Mansoura. Univ., 29: 3531-3544.

Antoun, H.C.; Beauchamp, J.; N. Houssard; Chabat, R. and Lonland, M. (1998). Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes. Effect on radishes (*Rophanous sativus,* L). Plant and Soil, 204:762-767.

Babik, I.and Kowalczy, k, W.(2004).The use of organic fertilizer obtained from composting of plant materials in vegetables cultivation.. Nowosci Warzywnicze. Instytut Warzywnictwa , 39: 49-54.

Baker, A.W. and Schippers, M. (1987). Microbial cyanid production in the rhizosphrer in relation to potato yield reduction and *Pseudomonas spp.* Mediated plant growth- stimulation. Soil Biol. Biochem., 19:451-457.

Bashan, Y. and Holguin, G. (1998). Proposal for the division of plant growth promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth promoting bacteria) and PGPB. Soil Biol Biochem, 30:1225-1228.

Beiero.L. D.Perrig; O.Masciarelli; C.pennaj; Cassan, F. and luna, V. (2007).Photo hormone production by three strains (Bradyrizobiun japonicum) and possible phsiologcal and technological. Mplications. Appl. Microbial.Biotechnol.74:874-880.

Black,C.A.(1982). Methods of soil analysis.Part2 .Chemical and microbiological Properties. Second Edition. Amer .Soc. of Agron. Madison, Wisconsin, U.S.A.

Cataldo, D. A.; Harnon, M.; Schrader, L. E. and Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commune in Soil Science and Plant Analysis, 6 (1): 71-80.

Chapman, H. D. and Parker, F. (1961). Methods of analysis for soil, plant and water. Journal of Plant Nutrition , 22 (1): 121-128.

Chishaki, N.; Hara, K.and Horiguchi, T.(2000). Availability of nutrients in animal waste composts for the growth of crops. Bulletin of the Faculty of Agriculture, 50, 41-48.

De-Britto Alvarez, M.A.; Gagne, S. and Antoun, H. (1995). Effect of compost on rhizophrer microflora of the tomato and on the incidence of plant growth- promoting rhizobacteria. Appl. Environ. Microbiol., 61:194-199.

Dubois, M.; Smith, F.; Gilles, K. A.; Hamilton J. K. and Rebers, P. A. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28 (3): 350-356.

Elfstrand, S.; Bath, B.and Martensson, A. (2007). Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Applied Soil Ecology, 36(1): 70-82.

El-Quesni, F. E. M. and Radwan, S.M. (1993). Effect of top gad and micro nutrients on growth, symbiotic nitrogen fixation and chemical composition of faba bean plants. Bull. Fac. of Agric., Univ. of Cairo, 44: 315-322.

Freitas, S.S; Melo, A.' M.Tand Donzeli, V.P.(2003) Growth enhancement of lettuce by rhizobacteria. Revista Brasileira de Ciencia do Solo , 27 (1): 61-70.

Ferreira,C.M.and Hungria, M. (2002). Recovery of soybean inoculant strains from uncroped soils in Brazil. Field Crops Research, 79: 139-152.

Garate A and Bonilla I. (2000). Nutricion mineraly produccion vegetal. In: Azcon-Bieto j, Talon, E. (eds) Fundamentos de Fisiologia Vegetal. McGraw-Hill Interam, Madrid, pp. 113-130

Gent, M. P (2005). Effect of genotype, fertilization and season on free amino acid in leaves of salad green of plant nutration 28 (7) : 1103-1116.

Goldstein, A.H. (1986). Baterial solubilization of mineral phosphates: historical prespective and future prospect. Amer. J. Altern. Agric., 1:51-57.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedres for Agric. Res. 2nd. Ed. John Wiley & Sons. Bup. pp139-153.

Green wood, D. J. and Hunt, J. (1986). Effect of nitrogen fertilizer on the nitrate contents of field vegetables grown in Britain. J. Sci. Food Agric., 37:373-383.

Hammermeister, A.M; Astatkie, T and Jeliazkova, E.A. (2006). Nutrient supply from organic amendments applied to unvegetated soil, lettuce and orchard grass. Canadian Journal of Soil Science, 86(1): 22-31.

Hanafy-Ahmed, A.H. (1996). Physiological studied on tipburn and nitrate accumulation in lettuce plants. J. Agric., Sci. Mansoura Univ., 21: 3971-3994.

Hanafy-Ahmed, A.H., Kheir, N. F., Abdel-Latif, E.A. and Amin, M.A. (1995). Effect of NPK fertilizers and foliar application of faba bean and wheat. Egypt. J. Appl. Sci., 10: 652-676.

Hegazi, N.A. and Neimela, S. (1976). A note on the estimation of Azotobacter densities by membrane filters technique. J. Appl. Bacteriol., 41:311-313.

Ismail, R. H.A. (2002). Physiological sutios on biofertilization in pea plants under calcareous soil condition. Ph. D. Thesis, Fac. Agric., Cairo Univ., Egypt.

Jakse, M. and Mihelic, R. (1999) .The influence of organic and mineral fertilisation on vegetable growth and N availability in soil: preliminary results. Acta Horticulturae, 506: 69-75.

Jakse, M. and Mihelic, R. (2001). Comparison of fertilisation with organic or mineral fertilisers in a three year vegetable crop rotation. Zbornik Biotehniske Fakultete Univerze v Ljubljan, 77 (2):179-190.

Kang Bong Kyoon. (2004). Effects of microbial liquid manure on growth and yield of leaf lettuce. Journal of the Korean Society for Horticultural Science. , 45: 3, 115-118.

Katoh, M.; Hayashi, Y.and Morikuni, H. (2008). Effect of combined applications of ^{15}N-labeled ammonium sulfate and cattle manure composts on nitrogen supply potential of soil to spinach . Japanese Society of Soil Science and Plant Nutrition, 79 (3): 283-289.

Kloepper, j ; Lifshitz. R.and Zablotowicz, R. (1989). Free-living bacteria inocula for enhancing crop productivity. Trends Biotechnol.,7:39- 44.

Kodashima, R.; Takahashi, M.and Hiraka, M. (2006).Effect of continuous application of compost made from cattle waste and sawdust on the growth and nitrogen uptake of spinach (*Spinacia oleracea* L.). Horticultural Research , 5 (4): 389-395.

Kutuk, C.; Topcuoglu, B. and Demir, K.(1999). Effects of different organic matters applied to soil on yield and some quality factors and mineral contents in spinach plant. Ziraat Fakultesi Dergisi, 12(1) 31-36.

Li Huihe; Wang ZhengYin; Zhang Hao and Li BaoZhen.(2003).Effects of organic manures on the nutritional quality of foliage vegetables in soilless culture. Journal of Southwest Agricultural University, 25(1) 66-69.

Linder, R. C. (1944). Rapid analytical methods for some of the more common inorganic constituents of plant tissues. Plant Physiol., 19 (1): 76-79.

Maftoun, M.; Moshiri, F.; Karimian, N. and Ronaghi, A.M .(2004).Effects of two organic wastes in combination with phosphorus on growth and chemical composition of spinach and soil properties. Journal of Plant Nutrition, 27(9): 1635-1651.

Marschner, H. (1995). Mineral nutrition of higher plants. Academic press Inc. San Diego, CAq2101, pp. 889.

Maruo, N.; Furukawa, Y.; Taira, K.and Asano, T.(2002) .Transition of soil nitrogen with high levels of compost application under greenhouse culture. Bulletin of the Nara Prefectural Agricultural Experiment Station, 33, 35-37.

Matsumoto, S.; Ae, N.and Yamagata, M.((1999). Nitrogen uptake response of vegetable crops to organic materials. Soil Science and Plant Nutrition, 45(2): 269-278.

Maynard, A. A. (1991). Intensive vegetable production using composted animal manures. Bulletin - Connecticut Agricultural Experiment Station, 894, 13 pp.

Medeiros, D.C.; Freitas, K.C.; Veras, F. de S.; Anjos, R. S.B. and Borges, R.D. (2008). Quality of lettuce seedlings depending on substrates with and without biofertilizer addition. Horticultura Brasileira. Sociedade de Olericultura do Brasil , 26(2): 186-189.

Mills, H. A.; Barker, A. V. and Naynard, D. N. (1976). Effects of nitrapyrin on nitrate accumulation in spinach. J. Amer. Hort. Sci., 101:202-204.

Moore, S. and Stein, W. H. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 211: 907-913.

Morra, L.; Bilotto, M.and Tonini, A.(2003).Organic fertilization of lettuce in tunnels. Colture Protette , 32(2): 83-90.

Nguyen Van Hiep and Preston, T.R. (2006) Effect of cattle manure and biodigester effluent levels on growth and composition of water spinach . Livestock Research for Rural Development, 18: 4, 48.
no.9, 2nd Ed., pp.539-624.

Nofal, M.A., Eleiwa, N.M.E., Mohamed, A.F. and Ibrahim, E.M. (1991). Effect of ascorbic and citric acids applied as seed soaking and/or foliar spray on growth and micronutrient composition of lettuce plant. Zagazig J. Agric. Res., 18:1033-1038.

Page,A.L.;.Meller, R.H. and Keeneyeds, D.R. (1982). "Methods of Soil Analysis" Part2. Chemical and microbiological properties. Agronomy monograph

Pavlou, G.C. ; Ehaliotis, C.D. and Kavvadias, V.A . (2007). Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. Scientia Horticulturae , 111(4): 319-325.

Peyvast, G.; Olfati, J.and Kharazi, P.R.(2008). Effect of organic fertilizers on nitrate accumulation by vegetables. Environment and Biotechnology, 49(1): 58-62

Piper, C.S. (1950). Soil and Plant Analysis.1st Ed. Interscience Publishers, N.Y.p.48

Pondy, A.;. Ekalabya,S. and Lokman, P. (1998). Influence of bacterial inoculation on maize in upland forming system of the sikkim Himalaya. Soil Biol. Biochem., 30:379-384.

Porto, M.L.; Alves, J. da. C.; Souza, A.P; Araujo, R. da C.and Arruda, J.A . (2008) Nitrate production and accumulation in lettuce as affected by mineral Nitrogen supply and organic fertilization. Horticultura Brasileira. Sociedade de Olericultura do Brasil, 26 (2): 227-230.

Porto, V. C.N; Negreiros, M.Z.; Bezerra Neto, F. and Nogueira, I. C.C . (1999). Organic matter sources and doses on lettuce yield. Escola Superior de Agricultura de Mossoro, 1. 12: 1/2, 7-11.

Premuzic, Z.; Garate, A.and Bonilla, I. (2002). Production of lettuce under different fertilisation treatments, yield and quality. Acta Horticulturae, 571, 65-72.

Rabia Ashraf; Faiza Shahid and Ali, T.A. (2007). Association of fungi, bacteria and actinomycetes with different composts. Pakistan Journal of Botany, 39(6): 2141-2151.

Ragab, A.A. and Rashed, M.H. (2003). Enhancement of nutrients uptake and metabolism efficiency of strohum *(Sorghum bicolor, L.)* plants by biofertilizers under water stress. Zagazig J. Agric. Res., 30 (1):147-166.

Ragab, A.A.; Abotaleb, H.H. and Nadia M. A. Ghalab. (2006). Response of Lupine plants to *inoculation with Bradyrhizobium sp.* (lupinus) combined with plant growth promoting Rhizobateria (PGPR) under newly reclaimed soil condition. J. Agric. Sci. Mansora Univ., 31(7): 4613-4622.

Rodrigues, E.Tand Casali, V. W.D.(1999). Yield and nutrient concentration in lettuce in relation to organic and mineral fertilizer application. Horticultura Brasileira, 17(2):125-128.

Santos, R. H.S. ; Silva, F. da and Casali, V. W.D. (2001) . Residual effect of organic compost on lettuce growth and yield. Pesquisa Agropecuaria Brasileira, 36(11):1395-1398.

Sarg,S.M.(2005).Effect of foliar application of vitamin B, and C on growth, yield and quality of potato. J. Agric. Sci. Mansoura Univ., 30: 4089-4095.

Sottero, A.N. ; Freitas, S. dos S.; Melo, A. M.T. and Trani, P.E.(2006) Rhizobacteria and lettuce: root colonization, plant growth promotion and biological control Revista Brasileira de Ciencia do Solo, 30 (2): 225-234.

Souza, P.A. ; Negreiros, M.Z. ; Menezes, J.Band Bezerra Neto, F.(2005). Chemical evaluations of lettuce leaves grown under the residual effect of soil fertilized with organic compost. Horticultura Brasileira, 23 (3): 754-757.

Suchorska-Orowska, J.(1996). The use of unconventional fertilizers for vegetable crop fertilization in a three-year crop rotation . Zeszyty Naukowe Akademii Rolniczej w Szczecinie, 63: 201-210.

Swain. T. and Hillis, W. F. (1959). The quantitative analysis of phenolic constituent. J. Sci., Food Agric., 10: 63-69.

Talaat, N. B. (1995). Physiological studies on reducing the accumulation of nitrate in some vegetable plants. M.Sc. Thesis, Agric. Bot. Dep., Fac. of Agric., Cairo Univ.

Tanahashi, T. and Yano, H .(2004) .The method of presuming the effect of nitrogen based on the nitrogen content of a poultry manure compost. Japanese Journal of Soil Science and Plant Nutrition, 75(2):257-260.

Thompson, H. C. and Kelly, W. C. (1983). Vegetable crop. McGraw Hill Book Company, Inc. New York, USA, (pp. 642-644).

Urashima, Y. and Hori, K.(2003). The development of an efficient method of inoculating PGPR onto spinach seed. Japanese Journal of Soil Science and Plant Nutrition, 74(2):163-168.

Urashima, Y.; Suga, Y.and Hori, K.(2005). Growth promotion of spinach by fluorescent *Pseudomonas* strains under application of organic materials. Soil Science and Plant Nutrition , 51(6):841-847.

Van-Schouwenburg, J. C. H. and Walinga, I. (1978). Methods of analysis of plant material. Agric. Univ., Wagejnen – Netherlands.

Vincent, J.M. (1970). A Manual for the practical Study of the Root-nodule Bacteria. IB p.15 prentic Hall international, Ltd., New Jersey, USA.

Wahdan, H.M. and Mansour, A. A. (2002). Effect of gibberellic acid and micronutrients on head yield of artichoke. J. Agric. Sci. Mansoura Univ., 27 (12): 8603-8609.

Yadava, U.L. (1986). A rapid and non-dastructure method to determine chlorophyll in intact leaves. Hort. Sci., 21: 1449-1450.

Yamazaki, H.and Roppongi, K.(1998). The effect of organic matters application for leaf vegetable yield and quality. Bulletin of the Saitama Horticultural Experiment Station, 21: 7-20

Yobo, KS. ; Laing, M. D. and Hunter, C.H. (2004) Effect of commercially available rhizobacteria strains on growth and production of lettuce, tomato and pepper. South African Journal of Plant and Soil , 21(4):230-235.

Zahra, M.K. (1969). Studies on silicate bacteria. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt, pp.44, 71-73,101-111.

Zhao, X.; Carey, E.E. ; Young, J.E.; Wang, W.Q. and Iwamoto, T. (2007).Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. Hort . Science, 42(1):71-76.

Zhou Yan and Luo AnCheng (2004). Effect of organic manure on the quality of vegetables in plastic-sheet-covered sheds. Acta Agriculturae Zhejiangensis , 16(4):210-212.

استجابة نباتات السبانخ لسماد مخلفات الدواجن والتلقيح بالبكتريا المنشطة للنمو (PGPR) والرش بالمنشط الحيوى

امل محمد فراج*، سحر سميح طه* ، هناء فتوح احمد** و عاطف عبد العزيز رجب***
* قسم الخضر كلية الزراعة جامعة القاهرة
** فرع فسيولوجى النبات قسم النبات الزراعى كلية الزراعة جامعة القاهرة
***معهد بحوث الاراضى والمياة -مركز البحوث الزراعية

أجريت تجربتان حقليتان حيث زرعت نباتات السبانخ وسمدت بسماد الدواجن بمعدل 4 طن / فدان والأسمدة الكيماوية الموصى بها وهى 250 كجم سلفات أمونيوم و 200 كجم سوبر فوسفات الكالسيوم الأحادى و 75 كجم سلفات بوتاسيوم واستعملت بمعدلات مختلفة (معدل كامل، 2/1 المعدل ، 4/1 المعدل لكل من السمادين الكيماوى والعضوى) وكذلك تقييم بعض السلالات البكترية من حيث انتاجها لبعض مركبات الفوتوهرمون والسيدروفور وقدرتها على اذابة الفوسفور الغير ميسر واستخدامها كبكتريا مشجعة للنمو.

بذور السبانخ بعضها لم يلقح والبعض الأخر لقح بـ PGPR وهو يشمل بكتريا مثبتة للنتروجين مثل *Azotobacter chrooccoum* و بكتريا مذيبة للفوسفور (*Bacillus megaterium*) وبكتريا ميسرة للبوتاسيوم(*Bacillus cerculans*) (*Bradyrhizobium japonicum.*) , رشت بعض النباتات بالماء والبعض الآخر بمنشط ورقى Stter-2 يحتوى فى تركيبه على حامض سكوربيك وحامض ستريك ونتروجين ونحاس وكالسيوم وبورون ومنجنز بعد 15 يوم وبعد 25 يوم من الزراعة. عوملت نباتات السبانخ بالمعاملات السابقة مفردة أو متجمعة.

أوضحت النتائج أن استعمال 2/1 كمية سماد الدواجن + PGPR + رش setter-2 أعطت أعلى قيمة من وزن النبات فى الموسمين وطول النبات فى الموسم الثانى وكمية المحصول للفدان وعدد الأوراق للنبات فى الموسم الأول مقارنة بمعدل التسميد الكيماوى الموصى به. ومن ناحية أخرى ادى استعمال 2/1 سماد الدواجن + 2/1 السماد الكيماوى + الرش بـ Setter-2 الى زيادة فى عدد الأوراق للنبات والمحصول الكلى للفدان فى الموسم الثانى والنسبة المئوية للمادة الجافة للأوراق فى الموسمين.

أما محتوى النبات من المكونات الكيماوية فقد أدى استعمال 2/1 كمية سماد الدواجن + PGPR + الرش Setter-2 الى الحصول على أعلى قيم للنسبة المئوية نتروجين والسكريات الكلية فى الموسمين.بينما سجلت أعلى قيم للنسبة المئوية للفوسفور فى الموسمين والبوتاسيوم فى الموسم الثانى باستعمال NPK 4/3 + PGPR + الرش Setter-2 ، وكذلك أعلى نسبة مئوية للبوتاسيوم باستعمال 2/1 سماد الدواجن + PGPR فى الموسم الاول .

تم الحصول على اعلى تركيزمن الفينولات الذائبة الكلية والأحماض الامينية الحرة الكلية تم عليها باستعمال 2/1 كمية سماد الدواجن + 2/1 الكمية الموصى بها NPK والكمية الكاملة من سماد الدواجن على التوالى فى الموسمين. تم الحصول على أعلى تركيز للنترات فى النبات باستعمال كمية السماد الكيماوى الموصى بها. بينما النباتات المعاملة PGPR يليه المعاملة بسماد الدواجن أعطت أقل القيم فى الموسمين. لا يوجد اختلاف معنوى بين المعاملات فى تركيز النباتات من الكلورفيل الكلى فى الموسمين.