Enhancement Growth, Yield Production and Quality of Kale Plants by Using Plant Growth Promoting Bacteria

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Abstract: The experiments were carried out in pots in greenhouse conditions on Kale plants (*Brassica oleracea var. sabellica*) during seasons of 2016 and 2017. The objective of this study was to investigate the effect of plant growth promoting bacteria (PGPB) (*Serratia marcescens*, *Pseudomonas poae*, *Plantibacter flavus* and *Bacillus amyloliquefaciens* subsp. *Plantarum*) inoculation to roots and medium on kale plants growth and productivity. The combined analysis data in both seasons showed that the highest vegetative growth was obtained from strain 303 such as plant height (17.17 cm), Leaf number (13.83) and leaf area (105.03 cm2) in comparison to untreated plants. PGPB significantly increased the nutritional compositions in kale leaves compared to the control plants. Inoculation of strain 303 gave the highest recorded values in Chlorophyll a (Chl. a 15.08 mg/100 g FW), Chlorophyll b (Chl. b 9.58 mg/100 g FW), ascorbic acid (13.52 mg/100 g FW), Phenols (2.26 mg/g FW) and Rosmarenic acid (5.89 mg/g FW). The highest yield of kale plants was obtained from strain 303 with registered number 102.13 g/plant compared to control plants (84.53 g/plant). Bacterial (PGPB strains) inoculation significantly affected kale leaves nutrient elements contents than untreated plants. Strain 303 recorded the highest value in nitrogen contents (N, 1.67% DW), Phosphorus (P, 0.27% DW), potassium (K, 2.49% DW), magnesium (Mg, 0.70% DW), Iron (Fe, 82.12 ppm DW) and Zinc (Zn, 27 ppm DW) in comparison to the control. The inoculation methods didn`t show clear trend, however root inoculation exhibit significant differences in the traits majority. The study is highly useful as initial work to introduce a new member of vegetable crops which is rich in their nutritional value and can be a benefit to cultivate in Egypt for commercial purposes.

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1. Introduction

Kale (*Brassica oleracea var. sabellica*) belongs to *Brassicaceae* family is mainly consumed for its leaves and cultivated commonly in North America, Central and Northern Europe (Thomson et al., 2007 and Schmidt et al., 2010). Macronutrients in the growing media such as nitrogen, phosphorus, and potassium are essential for plant growth and development. Further, Biofertilizers such as plant growth promoting bacteria (PGPB) are essential substitutes to avoid the environmental and soil pollution caused by overuse of chemical fertilizers (Lugtenberg and Kamilova, 2009). Furthermore, inoculation of PGPB to the seeds and medium can promote plant growth due to increasing the availability of nutrients, plants protection from diseases, inducing of plant hormones, decreasing of ethylene levels in plants and plants tolerant to the environmental stresses (Glick 1995, Glick et al. 1999, Compant et al., 2010 and Glick, 2010). Finally, Plant growth promoting rhizobacteria can competethe chemical fertilizers as a biofeltilizers application to improve plant growth and productivity, as well as alleviate the environmental pollution (Han and Lee 2005 and Munteanu et al., 2007).

Seeds inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens* caused significantly increase the leaves photosynthetic pigments (Mohamed and Gomaa, 2012 and Abeer et al., 2015). The highest chlorophyll contents were obtained from inoculation with Bacillus strains (S4 and S7) compared to untreated plants (Stefan et al., 2013). Mung bean Seeds was inoculated with Pseudomonas strain GRP3 significantly increased chlorophyll contents and reduced the leaves chlorosis compared with untreated plants (Sharma et al., 2003).

The phytochemicals contents such as ascorbic acid, anthocyanins, flavonoids and lycopene were increased with elevated phosphorus in to tomato plants (Dorais et al., 2008). In strawberry, arbuscular mycorrhizae fungi and rhizosphere of microorganisms can modulate and release phosphorus contents (Malusa et al., 2006). The flavonoids were increased significantly by the inoculation with PGPB relatively to non-inoculated plants (Del Amor and Porras 2009). The most of phosphorus was in two forms, either organic or inorganic and most of used phosphorus as chemical fertilizer is in complicated picture

unavailable to plant roots (Khan et al., 2007 and Glick 2012). Also, PGPR such as *Azospirillum* , *Klebsiella*, *Burkholderia* and *Bacillus* inoculationenhanced biological nitrogen fixation, phosphate solubilization and synthesis of phytohormone, which in turn decreased the chemical fertilization (Glick 2012).

Plant Growth Promoting Rhizobacteria (PGPR) strains inoculation encouraged root and plant growth and increased the vegetative growth production in Mung bean (Glick 2012 and Stefan et al., 2013). PGPR strains such as PGPR1, PGPR2 and PGPR4 were inoculated to mung bean seeds produced high significant pod yield than uninculated seeds, PGPR were capable of affecting the growth and yield of numerous plant species such as citrus, sweet cherry, apple, apricot, raspberry, high bush blueberry and mulberry (Bashan et al., 2004, Aslantas et al., 2007; Karlıdagˇ et al., 2007 and Esitken et al., 2010). Thus, total accumulated ‘‘Extra’’ fruit yield was increased by 45.8 and 58.3% in inoculated and non-inoculated plants, respectively, compared with the control plants.

PGPB strains can increse the bioavailability of iron in the soil, which will facilitate the uptakeby plants and alleviate stresses in case of growning under stresse condition such as heavy metal (Saravanakumar and Samiyappan 2007 and Jalili et al., 2009). Further, rhizobacterial isolates enhanced significantly the contents of nitrogen and phosphorus in the soil, shoot, and kernel. Furthermore, inoculation strawberry plants with PGPB strains increased P, Fe and Zn contents (Dey et al., 2004 and Esitken et al., 2010). Dursun et al (2010) studied the effects of PGPR (*Bacillus and Pseudomonas*) on plant mineral contents, and they found that the bacterial applications increased mineral contents in tomato and cucumber fruit as compared to control treatment. All bacterial applications showed improving N, P, Mg, Ca, Na, K, Cu, Mn, Fe and Zn contents in the fruits.

The objectives of this study were to obviate the environmental pollution associated with the excessive use of chemical fertilizers through the application of PGPB as an alternative to these harmfull chemicals. Chemical fertilizers one of sources of human and environmental pollution, need crud material cost a lot of money, depletes several sources such as oil and gass for their production. PGPB are able to release the macro and micro nutrients from soil in the root rhizosphere zone and made it easy to uptake by plants. In addition to determining effects of the bacterial strains of strain AP-4 (*Serratia marcescens*), strain AP-19 (*Pseudomonas poae*), strain AP-21 (*Plantibacter flavus*) and strain AP-303 (*Bacillus amyloliquefaciens* subsp. *Plantarum*) on kale plants growth and yield grown in greenhouse conditions.

2. Material and Methods

Plant material and growth conditions

The present study was carried out during the two successive seasons of 2016 and 2017 on Kale (*Brassica oleracea var. sabellica*) cv Dwarf Blue Curled Vates from cruciferae family. The seeds of kale plants were purchased from Burpee Garden Products Company, Pennsylvania USA. The experiments were conducted in a greenhouse at Massachusetts University, Amherst, USA under the control of environmental condition, the day length was 16/8 hrs, and the temperature of growth condition was 22˚C / 18˚C using randomized blocks complete design with three replications. Control and treated plants were grown randomly into plastic pots (6 inches standard) filled with the commercial growing media (Sunshine Lc1, RSi) obtained from Sungro Horticulture company, Canada. The seeds were sown in plastic tray cells at the nursery first, then transplanting after two weeks to the plastic pots number 6.

Bacterial strains and their preparation

The experiments contain three methods of using four strains of (PGPB). The PGPB strains utilized in this study were strain AP-4 (*Serratia marcescens*), strain AP-19 (*Pseudomonas poae*), strain AP-21 (*Plantibacter flavus*), and strain AP-303 (*Bacillus amyloliquefaciens* subsp. *Plantarum*). All bacteria strains were obtained from the culture collections from the Department of Entomology and Plant Pathology, Auburn University, Auburn, AL. Bacterial strains were maintained in nutrient broth (NB) (Sigma Aldrich, St. Louis, MO) with 30 % glycerol at -80 ^{o}C for long-term storage. Pre-cultures were prepared in 2 ml NB at 32 ^{o}C for 24 hours with shaking. The prepared cultures were then scaled up to 50ml and incubated in NB at 32 ^{o}C for 16 hours with shaking. The obtained cultures were then centrifuged at 5,000 G for 5 min. The bacterial pellet was then dissolved in distilled water and adjusted to 10^{9}- 10^{10} CFU/ml for seed treatment and 10^{8}-10^{9} CFU/ml for root and medium treatment. CFU = Colony forming units = Bacteria cell numbers per gram substrate.

Treatments

Three methods of PGPB were separately applied as follow: **1**) Control plants were subjected to water only. **2**) The seeds were soaked (incubated) in PGPB for one hour then sowed in pots. **3**) Seedlings roots two weeks old were incubated in PGPB culture for one hour then transplanted to the pots. **4**) 4 ml of PGPB were added to each pot.

Plant Harvest

The leaves of kale and collard plants were harvested after 60 days from transplanting date for the first harvest and subsequently by 30 days for the second harvest. The freshly harvested leaves from each plant were immediately weighed to determine

fresh weights followed by drying of some selected samples under 60 ºC at 72 hours at oven for further investigations.

Data were recorded and measured as the following:

A – Physical characteristics

Data of physical characteristics were taken as the following: Plant height (cm), Leaf number/plant was counted. The leaf area (cm^2) was calculated by mean of leaves number four, five and six from the growing tip according to Shaik and Murthy (2001) which measured by Li-300 leaf area meter produced by Li-Cor, Pinclivania).

B - Yield

The yield of kale was harvested and weighted for their two cutting times. The sums of two harvests of Kale yield were calculated per plant in gram.

C – Chemical characteristics

a – Chemical characteristics in fresh leaves

The leaves number four, five and six from the apical tip of plant were harvested for the chemical composition estimation in fresh tissue such as chlorophyll a, chlorophyll b, carotenoids, ascorbic acid, rosmarenic acid and total phenols. Chlorophyll a (chl. a), chlorophyll b (chl. b), and carotenoids (mg/100g fw) were determined spectrophotometrically according to Hipkins and Baker (1986).

Ascorbic acid (mg/100g fw) was determined spectrophotometrically according to Kapur et al., (2012). The sample was ground in liquid nitrogen, 1.2 g of sample was mixed with 6 ml of 5% metaphosphoric acid-10% acetic acid solution in a 15 ml plastic falcon tube. The mixture was shacked gently and incubated at room temperature for 30 minutes then filtrated using Watman pepper number 1. Subsequent, 115 µl of 3% bromine water was added to 2 ml of the filtrated sample followed by 65 µl of 10% thiourea and 500 µl of 2,4- DNPH. Finally, after the incubation at 37˚C for 3 hours, 2.5 ml of 85% sulfuric acid was added and the absorbance was measured at 521 nm (Spectrophotometer UV 1800, 120V, Shemadzu corporation). Phenolic levels (mg/g fw) in the leaf tissue were determined according to Chandler and Dodds (1983) and Helaly et al., (2015). The level of rosmarinic acid (mg/g fw) in kale leaves was determined using the modified UV assay of López-Arnaldos et al. (1995 and Helaly et al., (2015).

b - Chemical characteristization in dry leaves

Kale leaves were dried at 60 ˚C for 72 hours, then ground into fine powder for sub sequential analysis**.** Total flavonoid content (mg/g dw) was determined using aluminium chloride (AlCl3) method according to Rohman et al., (2010). 50 mg of plant tissue fine powder was mixed with 10 ml 80% methanol in 15 ml falcon tube. The mixture was shacked at room temperature for 1 hour 2ml of the extracted solution were centrifuged at 4000 rpm for 15 minutes. 0.8 ml of the supernatant was mixed with an equal volume from each of distilled water and 10% AlCl3. After incubation for 5 minutes at room temperature, 4 ml of distilled water was added and the absorbance measured at 415 nm.

Total nitrogen (%) was determined by transferring 200 mg of leaf fine powder into 50 ml kjeldahl digestion flask with 1.5g of potassium sulfate and 0.13 g of copper sulfate followed by 4 ml of sulfuric acid. After the sample completely digested and cooled, 46 ml of distilled water was added. The samples were analyzed by flow injection analysis using a spectrophotometer, Lachat Instruments, Milwaukee, WI according to Wendt, (2000).

Determination of phosphorus (P %), Potassium (K %), magnesium (%), iron (Fe ppm) and zinc (Zn ppm) were done according to Weil, (2014). 0.5 g of plant sample was placed into porcelain crucible with a lid and ignited in a muffle furnace at 500˚C for 8 hours. The ash was dissolved in 15 ml of 10% hydrochloric acid and filtrated into 25 ml glass vials for elements analysis. The samples were analyzed using Instrument upgraded to G8006A, MP. AES series spectrophotometer.

Statistical analysis

The analysis of variance was performed to determine the effect of treatment with bacteria strains (A), inoculation methods (B) and A X B interaction were subjected to statistical analysis for variance by using split plot design as mentioned by Gomez and Gomez (1984). Means treatments were compared by Least Significant Difference (L.S.D) at the level of 5% of probability in the two seasons of the experiment.

3. Results

The effect of plant growth promoting bacteria (PGPB) on kale plants characteristics in the combined analysis in the two seasons of 2016 and 2017 was tabulated and explained as the following:

1 - Effect of PGPB on kale vegetative growth

The data presented in Table (1) show the effect of the different PGPB strains, inoculation methods and their interaction on kale plant height, leaf number/plant and leaf area during the two seasons of 2016 and 2017. The obtained results showed that inoculation kale with strain Ap -303 had the highest significant differences on kale plant height (17.17 cm) and leaf area (105.03cm2) against the untreated plants (11.17 and 78.60 cm^2) in roots and medium inoculation respectively. On contrast, there is no significant change in kale leaf number under treatment with PGPB in both seasons. The inoculation of PGPB in medium gave the highest plant height with recorded value 15.93 cm in comparison with root inoculation (13.20 cm). On the other side, no significant effect between PGPB strains and inoculation methods in all

vegetative growth parameters.

2 - Effect of PGPB on kale leaf pigments contents

Bacterial inoculation significantly affected Chl.a and Chl.b while no significance was noticed in carotenoids (Table 2). Strain Ap – 303 exhibited the highest value of Chl.a (15.08 mg/100g FW) and Chl.b (9.58 mg/100g FW), while the control plants exhibit the least value of Chl.a (10.58) and Chl.b (5.93 mg/100g FW). The results showed that the highest significant of Chl.a (13.92 mg/100g FW) and Chl.b (8.89 mg/100g FW) was obtained from medium inoculation, while the best carotenoids value (6.32 mg/100g FW) was obtained from root inoculation. Regarding the interaction between bacterial strains and inoculation methods on pigments content, the obtained resultswere showed significant differences in the Chl.b and carotenoids. This increase was found ininoculated kale plants with bacterial strain Ap-303 on medium with register value 10.34 mg/100g FW in Chl.b and strain Ap - 19 on roots with register value 6.75 mg/100g FW in carotenoids.

Table (1): Effect of plant growth promoting bacteria (PGPB) on vegetative growth of kale plants in combined analysis in the two seasons of 2016 and 2017.

Traits	Plant height Cm			Leaf number/plant			Leaf area cm^2		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	10.67	11.67	11.17	11.67	11.33	11.50	82.53	74.67	78.60
Strain AP- 4	13.33	17.00	15.17	12.00	12.67	12.33	95.80	90.80	93.30
Strain AP-19	14.67	17.00	15.83	12.33	14.00	13.17	100.73	88.00	94.37
Strain AP-21	13.00	14.00	13.50	12.33	14.33	13.33	92.17	90.17	91.17
Strain Ap-303	14.33	20.00	17.17	13.33	14.33	13.83	101.13	108.93	105.03
Mean	13.20	15.93		12.33	13.33		94.47	90.51	
LSD at 5%	Treatments (A)		3.96	Treatments (A)		N.S	Treatments (A)		14.11
	* Inoc. methods (B)		2.51	* Inoc. methods (B)		N.S	* Inoc. methods (B)		N.S
	Interaction A X B		NS	Interaction A X B		N.S	Interaction A X B		N.S
*Inoc. methods = Inoculation methods									

Table (2): Effect of plant growth promoting bacteria (PGPB) on chlorophyll a, b and carotenoids of kale leaves in combined analysis in the two seasons of 2016 and 2017.

Traits	Chlorophyll a mg/100g FW			Chlorophyll b mg/100g FW			Carotenoids mg/100g FW		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	10.54	10.63	10.58	6.29	5.57	5.93	6.40	5.65	6.02
Strain AP- 4	11.33	14.34	12.84	5.94	10.32	8.13	6.22	5.66	5.94
Strain AP-19	12.71	14.75	13.73	6.66	9.22	7.94	6.75	5.69	6.22
Strain AP-21	12.25	13.66	12.96	6.42	8.99	7.71	6.73	5.66	6.19
Strain Ap-303	13.91	16.24	15.08	8.82	10.34	9.58	5.51	5.96	5.73
Mean	12.15	13.92		6.82	8.89		6.32	5.72	
LSD at 5%	Treatments (A)		2.15	Treatments (A)		1.11	Treatments (A)		N.S
	* Inoc. methods (B)		1.36	* Inoc. methods (B)		0.70	* Inoc. methods (B)		0.34
	Interaction A X B		N.S	Interaction A X B		1.56	Interaction A X B		0.76
*Inoc. methods = Inoculation methods									

3 - Effect of PGPB on kale nutritional value in fresh leaves

Bacterial treatment with strain Ap-303 showed significant increase in ascorbic acid (13.52 mg/100 g FW), phenols (2.26 mg/g FW) and rosmarenic acid (5.89 mg/g FW) contents in kale leaves in comparison to untreated plants (Table 3). Untreated plants exhibited the least value of ascorbic acid (9.34 mg/100 g FW), phenols (1.66 mg/g FW) and rosmarenic acid (4.11 mg/g FW) contents of kale leaves. In particular, inoculation methods and the interaction between PGPB strains and inoculation methods didn`t showed any significant differences.

4 - Effect of PGPB on kale fresh weight (Yield), dry weight and flavonoids

Results in Table (4) show the effect of inoculation with PGPB strains, inoculation methods

and their interaction on the Fresh weight (Yield g/plant), Dry weight % and flavonoids (mg/g DW) contents of kale leaves in combined analysis in the two seasons. The obtained results highlighted the effect of inoculated kale plant with PGPB strain AP-303 with a significant increment in fresh weight (Yield 102.13 g/plant) compaired to untreated plants (Yield 84.53 g/plant). Dry weight % and flavonoids (mg/g DW) contents have no significance among all PGPB strains and control plants. The inoculation methods of PGPB show only significant differences in dry matter % (17.53 %) with inoculation medium compared to the control plants (16.09%). The interaction between inoculation with PGPB strains and inoculation methods on fresh weight (Yield g/plant), dry matter % and flavonoids contents in leaves showed no significant differences were observed. The present study clearly highlighted that the inoculation with PGPB strains has promoted significant plant growth yield and t quality of kale plants, but growth responses were strain-specific.

Table (3): Effect of plant growth promoting bacteria (PGPB) on ascorbic acid, total phenols and rosmarinic acid of kale leaves in combined analysis in the two seasons of 2016 and 2017.

Traits	**Ascorbic acid (mg/100g FW)**			**Phenols (mg/g FW)**			**Rosmarinic acid (mg/g FW)**		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	8.23	10.44	9.34	1.72	1.60	1.66	4.05	4.17	4.11
Strain AP- 4	12.31	11.54	11.93	1.93	2.08	2.01	4.75	4.41	4.58
Strain AP-19	13.45	12.55	13.00	2.02	2.30	2.16	5.28	5.16	5.22
Strain AP-21	12.48	12.41	12.45	2.00	2.29	2.15	4.90	4.77	4.84
Strain Ap-303	14.31	12.73	13.52	2.12	2.40	2.26	6.06	5.72	5.89
Mean	12.16	11.94		1.96	2.14		5.01	4.85	
LSD at 5%	Treatments (A)		2.38	Treatments (A)		0.38	Treatments (A)		0.6
	* Inoc. methods (B)		N.S	* Inoc. methods (B)		N.S	* Inoc. methods (B)		N.S
	Interaction A X B		N.S	Interaction A X B		N.S	Interaction A X B		N.S
*Inoc. methods = Inoculation methods									

Table (4): Effect of plant growth promoting bacteria (PGPB) on fresh yield, dry matter and flavonoids of kale leaves in combined analysis in the two seasons of 2016 and 2017.

Traits	**Yield (g/plant)**			**Dry matter (%)**			**Flavonoids (mg/g DW)**		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	86.10	82.96	84.53	14.94	16.09	15.52	25.62	20.49	23.06
Strain AP- 4	87.03	87.59	87.31	15.94	17.14	16.54	26.66	28.08	27.37
Strain AP-19	99.05	95.42	97.24	16.51	17.67	17.09	27.91	30.45	29.18
Strain AP-21	88.92	86.26	87.59	16.31	16.85	16.58	25.75	26.79	26.27
Strain Ap-303	105.27	99.00	102.13	16.98	19.90	18.44	35.95	39.95	37.95
Mean	93.27	90.25		16.13	17.53		28.38	29.15	
LSD at 5%	Treatments (A)		10.51	Treatments (A)		N.S	Treatments (A)		N.S
	* Inoc. methods (B)		N.S	* Inoc. methods (B)		1.34	* Inoc. methods (B)		N.S
	Interaction A X B		N.S	Interaction A X B		N.S	Interaction A X B		N.S
*Inoc. methods = Inoculation methods									

5 - Effect of PGPB on kale macro and microelements contents in dry leaves

Bacterial inoculation significantly affected kale leaves nutrient elements content (Table 5). Available NPK in kale leaves was significantly affected by PGPB strains inoculation compared with the control. The highest N (1.67%), P (0.27%) and K (2.49%) contents were obtained from strain Ap -303 compared to the control (NPK 1.18%, 0.22% and 1.62% respectively). The inoculation methods exhibited no significant differences in nitrogen content, while the P (0.27%) and K (2.03%) contents were significantly increased from roots inoculation. The interaction between inoculation with PGPB strains and

inoculation methods on NPK % contents in leaves exhibit no significant differences was reached.

The presented data in Table (6) show that the effect of PGPB strains, inoculation methods and their interaction on Mg (%), iron (ppm) and Zinc (ppm) contents in kale leaves dry matter. Treatments with PGPB strain Ap-303 significantly increased the contents of Mg (0.70% DW), iron (82.12 ppm DW) and zinc (27 ppm DW) in kale leaves than untreated plants (0.45 % DW, 50.43 ppm DW and 24.15 ppm DW) respectively. Regarding the effect of inoculation methods, the root inoculation exhibits the highest significant differences in Mg (0.70 % DW), Fe (70.30 ppm DW) and Zn (28.80 ppm DW) than medium inoculation. In addition, there no statistical differences in Mg, Fe and Zn contents between PGPB strains treatments and inoculation methods.

Table (5): Effect of plant growth promoting bacteria (PGPB) on nitrogen, phosphorus and potassium of kale leaves in combined analysis in the two seasons of 2016 and 2017.

Traits	Nitrogen %			Phosphorus %			Potassium %		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	1.09	1.26	1.18	0.24	0.20	0.22	1.69	1.55	1.62
Strain AP- 4	1.41	1.49	1.45	0.29	0.22	0.25	2.11	1.79	1.95
Strain AP-19	1.54	1.42	1.48	0.28	0.25	0.26	1.81	1.82	1.82
Strain AP-21	1.48	1.58	1.53	0.25	0.23	0.24	1.73	1.71	1.72
Strain Ap-303	1.74	1.61	1.67	0.28	0.27	0.27	2.83	2.15	2.49
Mean	1.45	1.47		0.27	0.23		2.03	1.80	
LSD at 5%	Treatments (A)		0.22	Treatments (A)		0.03	Treatments (A)		0.35
	* Inoc. methods (B)		N.S	* Inoc. methods (B)		0.02	* Inoc. methods (B)		0.22
	Interaction A X B		N.S	Interaction A X B		N.S	Interaction A X B		N.S
*Inoc. methods = Inoculation methods									

Table (6): Effect of plant growth promoting bacteria (PGPB) on iron, magnesium, and zinc of kale leaves in combined analysis in the two seasons of 2016 and 2017.

Traits	Magnesium %			Iron (ppm)			Zinc (ppm)		
Inoculation methods	Inoculation methods		Mean	Inoculation methods		Mean	Inoculation methods		Mean
Treatments	Roots	Medium		Roots	Medium		Roots	Medium	
Control	0.59	0.30	0.45	58.07	42.80	50.43	29.10	19.20	24.15
Strain AP- 4	0.66	0.38	0.52	69.70	55.20	62.45	31.00	25.30	28.15
Strain AP-19	0.72	0.50	0.61	76.73	57.90	67.32	29.80	19.50	24.65
Strain AP-21	0.63	0.46	0.55	68.67	61.00	64.83	24.23	25.70	24.97
Strain Ap-303	0.89	0.51	0.70	78.33	85.90	82.12	29.87	24.13	27.00
Mean	0.70	0.43		70.30	60.56		28.80	22.77	
LSD at 5%	Treatments (A)		13.83	Treatments (A)		0.09	Treatments (A)		N.S
	* Inoc. methods (B)		8.75	* Inoc. methods (B)		0.06	* Inoc. methods (B)		3.35
	Interaction A X B		N.S	Interaction A X B		N.S	Interaction A X B		N.S
*Inoc. methods = Inoculation methods									

Discussions

Plant growth-promoting bacteria (PGPB) are one of important modern technique to obviate negative effects of used chemical fertilization. The PGPB strains increased plant growth according to plant height and leaf area of kale plants. These result was agreement with finding with Bashan et al., 2004; Bashan et al., 2005, Zahir et al., 2003. Plant growth promoting rhizobacteria might enhance plant growth and productivity by synthesizing phytohormones (auxin, cytokinin and gibberellin), increasing the local availability of nutrients, facilitating the uptake of nutrients and decreasing heavy metal toxicity in the plants (Burd et al., 2000, Gholami et al., 2009 and Erturk et al., 2012). Not only this, plant growth regulator (phytohormone) was response to seeds or roots inoculation with various PGPB to improvement plant growth and development. (Zahir et al., 2004).

The present experiment revealed that root and medium inoculation with all bacteria strains resulted in

an increased plant height and leaf area and similar increases in these characters were observed in various crops which inoculated with PGPB (*Pseudomonas, Azospirillum and Azotobacter*) strains (Siddiqui and Shaukat 2002, Shaukat, et al. 2006 and Gholami et al. 2009). Thus, various PGPRs having the ability to produce the IAA, cytokinine and other plant hormones which play an important role in plant growth and yield. It has been reported that the bacterial strain Bacilluswas capable of producing IAA, cytokinine, nitrogen and phosphate solubilizing capacity in cauliflower plants (Shaukat et al., 2006, Esitken et al. 2010 and Ekinic et al., 2014). Similarly, seed inoculation with PGPR increased growth parameters such as (plant height, stem width, root length, internode length) of cabbage plants (Ekinci et al., 2014), tomato (Ibiene et al. 2012 and Garcia et al., 2003 Walia et al. 2013). Nezarat and Gholami, 2009 showed that leaf and shoot dry weight, and leaf area was increased by bacterial inoculation (Nezarat and Gholami 2009). Increasing in plant growth from bacterial inoculation may be due to the increasing in nutrients uptake which enhance plant growth hormones stemuli, increasing chlorophyll formation and organic acids. (Ekinci et al., 2014).

In this study, the application of PGPB strains enhanced plant growth, secondary metabolites, and N, P, K, Mg, Fe and Zn in kale leaves. The macro and micro elements are very important key to play a role in enhancing plant growth and pigment content. These results due to the played role by PGPB strains by increasing the solubility of the nutrients in the soil to plant and that effect on pigments formation. These results showed agreement with finding by Sharma et al., 2003, Mohamed and Gomaa 2012, Stefan et al., 2013 and Abeer et al., (2015). Nitrogen, Mg application increased leaf growth, leaf area and plant growth which affected photosynthesis and chlorophyll formation and chlorophyll content are approximately proportional to leaf nitrogen content (Terry and Ulrich 1974 and Bojovic and Markovic 2009). The role of potassium in plant growth and development is major influence on leaf photosynthesis may be due to control gas exchange. Seeds inoculation with PGPB may be increased NPK and phytohormones which promote the photosynthesis processes followed by high chlorophyll formation in plants (Lamrani et al., 1996, Duli et al., 2001 and Onanuga et al., 2012).

Kale yield was significantly increased in case of root inoculation method with Plant growth promoting bacteria. It was shown previously that the application of bacteria strongly stimulated yield and quality parameters in barley and sugar beet (Cakmakci et al., 2001), apricot (Esitken et al., 2002), and raspberry (Orhan et al., 2006). The effect of application with microbial strains in increasing crop yield and nitrogen fixation has been previously reported (Jia et al., 2004 and Erturk et al., 2012). The effect of PGPB on plant growth and yield in Maiz and Mung bean were significantly increased (Gholami et al., 2009 and Singh et al., 2015). In this study inoculation with Bacillus strain Ap-303 affected plant height, leaf area, and pigments contents, resulted in overall plant growth may be due to higher process of photosynthesis. A higher leaf area index revealed a higher biomass as more leaf fresh weight and yield (Heuvelin et al., 2005). Malusa and Vassilev (2014) proposed that a biofertilizer is the formulated product containing one or more microorganisms that enhance plant nutrient status such as growth and yield by release the macro and micronutrients from soil and more availability to plants. PGPB show significant improvement on the growth and yield of crops in response to microbial inoculation and the application had positive effects on the agricultural yield and crop quality (Lugtenberg and Kamilova 2009). Through my view of experiment results admitted I could say that, PGPB strains application on kale plants gave the highest yield and their quality due to the highest vegetative growth, highest pigment contents and the highest N, P, K, Mg, Fe and Zn contents in kale leaves and all that associated with direct bacteria inoculation with roots instead of medium inoculation.

In the present study, the highest yield quality in kale leaves such as ascorbic acid, phenolic acid, and flavoinoids was obtained from PGPB strains compared with the control. This result has consistence wtih Erturk et al., 2012 on strawberry which reported that ascorbic acid contents increased from 47.41 mg 100 g^{-} 1 (control) to 53.88 mg 100 g^{-}1 (bacteria strain RC05). Ordookhani et al., 2013 worked on tomato plants stated that the inoculation with PGPB increased Vitamin C, TSS, pH, P, K, Ca compared to controls. PGPB enhanced plants phenolics contents when challenged to several stresses and increased the strengthen of plant cell wall (Loganathan et al., 2014). In this study, the application of PGPB strains increased the phytochemical levels in kale plants. The enhanced accumulation of phenolic and flavonoids compounds may be related to an increased activity of phenylalanine ammonia lyase (PAL) and peroxidases (PO) enzymes which cause an increase in the available phenolic free pool (Jayaraman et al., 2011). The higher concentrations of phenolics in kale leaves can be explained by the role of application PGPB strains Strains which enhance the acetate shikimate biosynthesis pathway to produced high content of phenolics and flavonoids (Sousa et al., 2008). Application PGPR strains mostly associated with plant rhizosphere, are found to be useful to plant growth, productivity and their quality (Esitken et al., 2010). Root inoculation was the most profitable for kale leaf

productivity and quality, due to the direct inoculation with root than medium inoculation may be not cover the root rhizosphere zone.

PGPB used as biofertilizer which becoming an important technique for organic farming and plays crucial role for the agriculture economic and production over the world. Biofertilizers are known as products that contain living microorganisms; when inoculated to seeds, plant surfaces, or soil, they colonize the rhizosphere or inter inside plant, and promote plant growth by increasing the release of availability of macro and microelements to treated plants (Vessey 2003). The high availability of N, P, and K could enhance soil fertility, improve antagonistic isolates bio-control effects, and extend microorganisms survival rates in soil (Yang et al., 2011 and Mohamed et al., 2017). Similar result was found on cabbage exhibited that PGPR inoculation with *Bacillus* M3 OSU- 142 elevated N, P, Ca, Fe, and Zn contents of cabbage leaves (Turan et al., 2014). Auxins elevated by bacteria can promote root growth, resulting in an increased uptake of essential nutrients (Vikram, 2007). The processes of plant development are controlled by internal signals that depend on the adequate supply of mineral nutrients from soil to roots (Turan et al., 2014). PGPB application is one of the most source for environment friendly fertilizer in agriculture field and can be promote plant growth indirectly by reducing plant pathogens infection, or directly by accelerate the release and uptake of nutrients from the soil, by impacting phytohormone assimilation such as giberallin, auxin and/or cytokinin by enzymatic change to lower in plant ethylene synthasis (Bashan and de-Bashan, 2010 and Ekinic et al., 2014).

Conclusions

The plant growth promoting bacteria increased vegetative growth and yield productivity and quality in kale plants in respect to untreated plants (control). Strain Ap - 303 ((*Bacillus amyloliquefaciens* subsp. *Plantarum*) was recorded the highest vegetative growth, yield and their nutritional compositions followed by strain AP-19 (*Pseudomonas poae*). The inoculation methods did not give clear trend, however the most traits had positive response to the root inoculation. The highest significant result was obtained from strain 303 and the interaction between strain 303 and roots inoculation.

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References

1. Abeer, H., E.F. Abdallah, A.A. Alqarawi, A. Al-Huqail, S.R.M. Alshalawi, S. Wirth and E. Dilfuza (2015): Impact of plant growth promoting Bacillus subtilis on growth and physiological parameters of Bassia indica (Indian Bassia) grown under salt stress. Pak. J. Bot., 47 (5): 1735-1741.
2. Aslantas, R., Cakmakci, R., Sahin, F., (2007): Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Sci. Hortic. 111, 371–377.
3. Bashan, Y. and LE de-Bashan. (2005): Bacteria Plant Growth-Promotion. In: Hillel D, Encyclopedia of Soils in the Environment. vol. 1. Oxford, UK: Elsevier, 103–115.
4. Bashan, Y. and L.E. de-Bashan (2010): How the Plant Growth-Promoting Bacterium Azospirillum Promotes Plant Growth-A Critical Assessment. Advances in Agronomy 108: 77-136.
5. Bashan, Y.G. Holguin and L.E. de-Bashan (2004): Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can J Microbiol. 50: 521-577.
6. Bojovic, B. and A. Markovic (2009): Correlation between nitrogen and chlorophyll content in wheat (Triticum astivum L.). Kragujevac J. Sci. (31) 69-74.
7. Burd, G.I., D.G. Dixon and B.R. Glick (2000): Plant growth promoting rhizobacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol., vol.33, pp.237-245.
8. Cakmakci R., F. Kantar, and F. Sahin, (2001): Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. Journal of Plant Nutrition Soil Science 164: 527–531.
9. Chandler, S.F. and J.H. Dodds. (1983): The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of Solanum laciniatum. Plant Cell Rpt. 2:105-108.
10. Compant, S, C. Clément and A. Sessitsch (2010): Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization,

mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42:669-678.
11. Del Amor F.M. and I. Porras (2009): Effects of plant-growth-promoting bacteria on growth and yield of pepper under limited nitrogen supply. Canadian Journal of Plant Science 89 (2): 349-358.
12. Dey, R., K.K. Pal, D.M. Bhatt and S.M. Chauhan (2004): Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. Microbiological Research, 159 (4): 371–394.
13. Dorais, M., D.L. Ehret and A.P. Papadopoulos (2008): Tomato (Solanum lycopersicum) health components: From the seed to the consumer. Phytochem. Rev. 7, 231-250. 55.
14. Duli Zhao, D.M. Oosterhuis and C.W. Bednarz (2001): Influence of potassium deficiency on photosynthesis, chlorophyll content and chloroplast ultrastructure of cotton plants. Photosynthetica, 39 (1): 103-109.
15. Dursun, A., M. Ekinci and M.F. Donmez (2010): Effect of foliar application of plant growth promoting bacterium on chemical contents, yield and growth of tomato (lycopersicon esculentum L.) and cucumber (Cucumis sativus L.). Pak. J. Bot., 42 (5): 3349-3356.
16. Ekinci M., M. Turan, E. Yildirim, A.G., R. Kotan, A. Dursun (2014): Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid hormone content of cauliflower (Brassica oleracrea L. var. botrytis) transplants. Acta Sci. Pol., Hortorum Cultus 13(6): 71-85.
17. Erturk Y., S. Ercisli and R. Cakmakci (2012): Yield and growth response of strawberry to plant growth promoting Rhizobacteria inoculation. Journal of Plant Nutrition, 35:6, 817-826.
18. Esitken, A., H. Karlidag, S. Ercisli, and F. Sahin (2002): Effects of foliar application of Bacillus subtilis Osu-142 on the yield, growth and control of shot-hole disease (Coryneum blight) of apricot. Gartenbauwissenschaft 67: 139–142.
19. Esitken A., Yildiz, H.E., S. Ercisli, M.F. Donmez, M. Turan and A. Gunes (2010): Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Scientia Horticulturae 124 (2010): 62-66.
20. Garcia L.J.A., A. Probanza, B. Ramos and F.J.G. Manero (2003): Effects of three plant growth promoting rhizobacteris on the growth of transplants of tomato and pepper in two different sterilized and nonsterilized peats. Arch. Agro. Soil Sci., 49, 119–127.
21. Gholami A., S. Shahsavani, and S. Nezarat (2009): The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize World Academy of Science, Engineering and Technology, (49): 19 – 24.
22. Glick B.R. (2012): Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation Scientica, (2012): 1 – 15.
23. Glick BR (1995) The enhancement of plant-growth by free-living bacteria. Can. J. Microbiol 41:109–117
24. Glick B.R., C.L. Patten, G. Holgin and D.M. Penrose (1999): Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, p 267.
25. Glick, B.R. (2010): Using soil bacteria to facilitate phytoremediation. Biotechnol. Adv. 28:367-374.
26. Gomez, K.A. and A.A. Gomez (1984): Statistical procedures for agricultural research (2 ed.). John wiley and sons, New York, 680p.
27. Han, H.S. and K.D. Lee (2005): Plant Growth Promoting Rhizobacteria Effect on Antioxidant Status, Photosynthesis, Mineral Uptake and Growth of Lettuce under Soil Salinity. Res. J. Agric. & Biol. Sci., 1(3), 210-215.
28. Helaly, A.A., J.P. Baek, E. Mady, M.H.Z. Eldekashy and L. Craker (2015): Phytochemical analysis of some celery accessions. Journal of Medicinally Active Plants 4, (1):1-7.
29. Heuvelin k., M.J. Bakker, A. Elings, R. Kaarsemaker and L.F.M. Marcelis (2005): Effect of Leaf Area on Tomato Yield. Acta Hort. 691: 43 – 50.
30. Hipkins, M.F. and N.R. Baker (1986): Photosynthesis energy transduction. In: M.F. Hipkins and N.R. Baker (eds.). Spectroscopy. IRL, Oxford, UK. pp.51-105.
31. Ibiene A.A., J.U. Agogbua, I.O.Okonko and G.N. Nwachi (2012): Plamt growth promoting rhizobacteria (PGPR) as biofertilizer: effect on growth of Lycopersicum esculentus. J. American Sci., 8 (2), 318-324.
32. Jalili F., K. Khavazi, E. Pazira et al., (2009): Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (Brassica napus L.) growth. Journal of Plant Physiology, 166 (6): 667–674.
33. Jayaraman, J., J. Norrie and Z. Punja. (2011): Commercial extract from the brown seaweed Ascophyllum nodosum reduces fungal diseases in greenhouse cucumber. Journal of Applied Phycology 23: 353–361.
34. Jia, Y., V. Gray, and C.J. Straker (2004): The

influence of Rhizobium and arbuscular mycorrhizal fungi on nitrogen and phosphorous accumulation by Vicia faba. Annals of Botany 94: 251–258.
35. Kapur, A., A. Hasković, A. Čopra-Janićijević, L Klepo, A. Topčagić, I. Tahirović and E. Sofić (2012): Spectrophotometric analysis of total ascorbic acid contetnt in various fruits and vegetables. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, (38): 39-42.
36. Karlidag, H., A. Esitken, M.S. Turan and F. ahin (2007): Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. Sci. Hortic. 114, 16–20.
37. Khan M.S., A. Zaidi, and P. A. Wani (2007): Role of phosphatesolubilizing microorganisms in sustainable agriculture a review. Agronomy for Sustainable Development, 27 (1): 29–43.
38. Lamrani, Z., A. Belakbir, J.M. Ruiz, L. Ragala, I. Lopez-Cantarero, and L. Romero (1996): Influence of nitrogen, phosphorus, and potassium on pigment concentration in cucumber leaves. Commun. Soil Sci. Plant Anal., 27: 1001–1012.
39. Loganathan M., R. Garg, V. Venkataravanappa, S. Saha and A.B. Rai (2014): Plant growth promoting rhizobacteria (PGPR) induces resistance against Fusarium wilt and improves lycopene content and texture in tomato. African Journal of Microbiology Research, 8 (11):1105-1111.
40. López-Arnaldos, T., M. López-Serrano, A. Ros Barcelo, A.A. Calderon, and J.M. Zapata (1995): Spectrophotometric determination of rosmarinic acid in plant cell cultures by complexation with Fe2+ ions. Freesnius J. Anal. Chem. 351:311-314.
41. Lugtenberg, B., and F. Kamilova (2009): Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63: 541-556.
42. Malusa, E., L. Sas-Paszt, W. Popinska and E. Zurawicz (2006): The effect of a substrate containing arbuscular mycorrhizal fungi and rhizosphere microorganisms (Trichoderma, Bacillus, Pseudomonas and Streptomyces) and foliar fertilization on growth response and rhizosphere pH of three strawberry cultivars. Int. J. Fruit Sci., 6, 25–41.
43. Malusa, E. and N.A. Vassilev (2014): Contribution to set a legal framework for biofertilisers. Appl. Microbial. Biotechnol. 98: 6599-6607.
44. Mohamed, H.I. and E.Z. Gomaa (2012): Effect of plant growth promoting Bacillus subtilis and Pseudomonas fluorescens on growth and pigment composition of radish plants (Raphanus sativus) under NaCl stress. Photosynthetica, 50, (2): 263-272.
45. Mohammed S. A., E. A. Salem and A. A. Helaly (2017): Impact of Mycorrhizae and Polyethylene Mulching on Growth, Yield and Seed Oil Production of Bottle Gourd (Lagenaria siceraria). Journal of Horticultural Science & Ornamental Plants 9 (1): 28-38.
46. Munteanu, N., L.D. Popa and G.C. Teliban (2007): Suitability of Phaseolus coccineus L. species for cultivation in sustainable agriculture systems. Lucrări ştiinţifice, seria Horticultură, (50): 501-504.
47. Nezarat S. and A.Gholami (2009): Screening plant growth promoting rhizobacteria for improving seed germination, transplant growth and yield of maize. Pakistan J. Biol. Sci., 12(1): 26-32.
48. Onanuga, A.O., P. Jiang and S. Adl (2012): Phosphorus, Potassium and Phytohormones Promote Chlorophyll Production Differently in Two Cotton (Gossypium hirsutum) Varieties Grown in Hydroponic Nutrient Solution. Journal of Agricultural Science, 4, (2): 157 – 166.
49. Ordookhani K., A. Moezi, K. Khavazi, F. Rejali (2013): Effect of plant growth promoting rhizobacteria and mycorhiza on tomato fruit quality. Acta Hortic. 989: 9.
50. Orhan, E., A. Esitken, S. Ercisli, M. Turan, and F. Sahin (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Scientia Horticulturae 111: 38–43.
51. Rohman A., S. Riyanto, N. Yuniarti, W.R. Saputra, R. Utami and W. Mulatsih (2010): Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (Pandanus conoideus lam) Int. Food Res. J. (17): 97–106.
52. Saravanakumar D. and R. Samiyappan (2007): ACC deaminase from Pseudomonas fluorescens mediated saline resistance in groundnut (Arachis hypogea) plants. Journal of Applied Microbiology, 102, (5): 1283-1291.
53. Schmidt, S., M. Zietz, M. Schreiner, S Rohn, L.W. Kroh and A. Krumbein (2010): Genotypic and climatic influences on the concentration and composition of flavonoids in kale (Brassica oleracea var. sabellica). Food Chemistry (119): 1293–1299.
54. Shaik, M. and V.R.K. Murthy (2001): Rapid, simple indirect method of leaf area estimation in bottle gourd (Cucurbitaceae sp.). Crop Research (Hisar). 21(2): 178-180.
55. Sharma A., B.N. Johri, A.K. Sharma, and B.R.

Glick (2003): Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (Vigna radiata L. Wilzeck). Soil Biology and Biochemistry. 35, (7): 887 – 894.
56. Shaukat, K., S. Affrasayab and S. Hasnain (2006): Growth responses of Helianthus annus to plant growth promoting rhizobacteria used as a biofertilizer. J Agric Res 1: 573–581.
57. Siddiqui I.A. and S.S. Shaukat (2002): Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens. Biol. Fertil. Soil, (36): 260–268.
58. Singh N., A. Yadav and A. Varma (2015): Effect of plant growth promoting activity of rhizobacteria on Cluster bean (Cyamopsis tetragonoloba L.) plant growth and biochemical constituents. Int. J. Curr. Microbiol. App. Sci 4 (5): 1071-1082
59. Sousa, C., D.M. Pereira, J.A. Pereira, A. Bento, M.A. Rodrigues, S. Dopico-Garcıa, P. Valentao, G. Lopes, F. Federico, R.M. Seabra and P.B. Andrade, (2008): Multivariate analysis of tronchuda cabbage (Brassica oleracea L. var. costata DC) phenolics: influence of fertilizers. J. Agric. Food Chem. 56, 2231-2239.
60. Stefan, M., Munteanu, N., V. Stoleru, M. Mihasan (2013): Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean. Romanian Biotechnological Letters 18, (2): 2013- 8132.
61. Terry, N. and A. Ulrich (1974): Effects of Magnesium Deficiency on the Photosynthesis and Respiration of Leaves of Sugar Beet. Plant Physiol. (1974) 54, 379-381.
62. Thomson, C.A., T.R. Newton, E.J. Graver, K.A. Jackson, P.M. Reid, V.L. Hartz, E.C. Cussler, and I.A. Hakim (2007): Cruciferous vegetable intake estimate. Journal of American Dietetic Association, 107:631-643.
63. Turan M., M. Ekinci, E. Yildrim, A. Gunes, K. Karagoz, R. Kotan and A. Dursun (2014): Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (Brassica oleracea) seedlings. Turk J Agric For 38: 327-333.
64. Vessey, J.K. (2003): Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255, 571–586.
65. Vikram, A., (2007): Efficacy of phosphate solubilizing bacteria isolated from vertisols on growth and yield parameters of sorghum. Res. J. Mcrobiol., (2): 550-559.
66. Waddington, S.R. (1998): Organic matter management: From science to practice. Soil Fertil., 62, 24–25. .
67. Walia A., P. Metha and A. Chauhan (2013): Effect of Bacillus subtilis strain CKT1 as inoculum on growth of tomato transplant under net house conditions. Proc. Nail. Acad. Sci. India, Sect. B, Biol. Sci., 84 (1): 145–155.
68. Weil, S. (2014): Calcium and potassium accumulation in lettuce under different nitrogen regimes. M. Sc. Plant and soil science, University of Massachusetts, Amherst, USA, 234 pages, http://scholarworks.umass.edu/masters -theses -2.
69. Wendt, K. (2000): The pathway of calcium movement to the xylem. Journal of Experimental potany, 52 (358): 891 – 899.
70. Yang, X., L. Chen, X. Yong and Q. Shen (2011): Formulations can affect rhizosphere colonization and biocontrol efficiency of Trichoderma harzianum SQR-T037 againts Fusartium wilt of cucumbers. NBiol. Fertil. Soils, (47): 239-248.
71. Zahir, Z.A., M. Arshad and W.T. Frankenberger (2003): Plant growth promoting bacteria: applications and perspectives in agriculture. Adv Agron;81:97-168.
72. Zahir, A. Z., M. Arshad, and W. T. Frankenberger (2004): Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. Advances in Agronomy 81: 97–168.

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