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Enhancing Growth, Productivity and Quality of Squash Plants Using Phosphate Dissolving Microorganisms (Bio phos-phor®) Combined with Boron Foliar Spray

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Abstract: This research paper deals with one of clay soils considering the benefits of using bio dissolving microorganisms of phosphor. Also these microorganisms could improve plant assimilation through releasing some sort of growth regulators. This research has been carried out through two different field experiments during 2005 and 2006 seasons. Traditional phosphate system fertilization was applied using super calcium phosphate with unincoulated squash seeds compared with using rock phosphate mixed with bio dissolving microorganism and inoculated squash seeds with *P. polymyxa* and micrrohiza *G. mosseae* or bio dissolving phosphor bacteria *B. megaterium* (Bio phos-phor®). The previous treatments combined with boron foliar spray to study their effects on morphological and physiological parameters and quality and productivity of squash, also to study the microbial activity and phosphor availability in the soil. Results showed that double inoculation with / or both inoculation with foliar spray of boron improved vegetative growth as stem length, stem thickness, leaf number, leaf area and plant dry weight compared with uninoculated plants. Plant pigments as chlorophyll A and B and carotenoids increased with bio fertilizer treatments, also the same positive trend noticed with chemical contents of plant including N, P, K, Mg, B, total protein, total soluble sugars / fresh weight and total carbohydrates / dry weight increased compared with uninoculated plants. Double inoculation with *P. polymyxa* and *B. megaterium* with foliar spray with boron led to an enhancement of internal level of growth promoters including GA, IAA and cytokinines combined with decreasing of ABA inhibitor. Double inoculation especially with micrrohiza with boron spray improved sex ratio and early production of fruits with high yield. Results indicated obvious positive and significant microbial activity in soil rhizosphere expressing by activity of dehydrogenase, phosphatase and nitrogenase enzymes, also nitrogen phases as NH_3 and NO_3, available phosphor and P_2O_5 reached to their highest values with double inoculation and boron application during the two seasons. The present research results that using rock phosphate mixed with bio dissolving microorganism and treating seeds before sowing with BDM combined with boron foliar spray significantly improved growth and productivity and quality of squash plant cultivated in clay soils.

Key words: Biofertilizers, mycorrhizae, *P. polymyxa*, *B. megaterium* var *phosphaticum* (Bio phos-phor®) boron, squash, chlorophyll, endogenous hormones, sex ratio, early yield, fruit quality

INTRODUCTION

Most of the Egyptian clay lands are poor in its content of organic matter, also it tend to alkalinity which lead to unavailability of some minerals especially phosphor. This problem became more serious with using mono calcium phosphate fertilizer. The mono-soluble phase changes in alkaline soil to the unavailable triple calcium phosphate phase which is difficult to uptake by most fast growing plant like squash.

Nowadays, biofertilizers are considered the most advanced biotechnology that can increase the output, improve the quality and they are responsible for developing organic, green and non-poluted agriculture. Also, biofertilizers contain a variety of beneficial microorganisms and enzymes which accelerate and improve plant growth and protect plants from pests and diseases. Completely fermented organic matters resulted in biofertilizers which improve the physical properties of soils, enrich air aeration, water and nutrient retention capacity. Biofertilizers provide the cultivated plants

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with the macro as well as micronutrients, required for healthy growth, therefore, improve yield and quality of agricultural crops, and reduce the overall cost of chemical fertilizers as pesticide application[1]. Clearly, there is an urgent need for sustainable agricultural practices on a global level. To overcome the ecological problems resulting from the loss of plant nutrients and to increase crop yield, microorganisms that allow more efficient nutrient use or increase nutrient availability can provide sustainable solutions for present and future agricultural practices[2]. An alternative approach for using of phosphate-solubilizers as a microbial inoculants is the using of mixed cultures or co-inoculation with other microorganisms. On the other hand, it has been postulated that some phosphate solubilizing bacteria behave as mycorrhizal helper bacteria. Similarly, bacteria and their growth or activities are affected by fungi and their exudates in rhizosphere[3]. Some bacteria such as *Paenibacillus polymyxa* (previously *Bacillus polymyxa* Ash *et al.*,[4] also provide plants with growth promoting substances. These bacteria almost exclusively associated with not only the rhizosphere of mycorrhizal plants but also the mycelium of the AM fungus[5]. A promising trend for increasing the efficiency of biofertilizers was studied using different mixtures of nitrogen fixing bacteria, phosphate solubilizers and potassium solubilizer. Wu *et al.*[6] found that application of triple inoculants not only increased nutritional assimilation of plant, but also improved soil properties. This was observed that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. In Egypt, however, there is a dire need to make availabilities of co-inoculation with biofertilizers and transfer the technology to farmers.

The Egyptian alluvial soils are thought to be deficient in microelements as a result of low percentage of organic matter, addition of NPK fertilization must consider the micro-element needs and the alkaline conditions of soil which decrease the availability of some trace elements such as Fe, Mn, Cu, B and Zn. In addition, many roles for boron in plants have been proposed, including functions in sugar transport, cell wall synthesis, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism and membrane transport. Also, boron has a role in cell division and hence helps in root elongation[37]. Boron caused a gradual hyperpolarization of the plasma membrane in root tips and stimulate proton secretion and the activity of plasma membrane NADH oxidase. Thus, boron could be directly associated with cell growth[7].

Therefore, the present investigation was undertaken to study the response of squash (*Cucurbita pepo* L.) for dual inoculants of plant growth promoting rhizobacteria with either endomycorrhizal fungi or P-solubilizing bacteria in presence of rock phosphate and their interaction effects with foliar application by boron on growth, yield and yield components of squash.

MATERIALS AND METHODS

Two field experiments were conducted at the Agricultural Research and Experimental Center, Fac. of Agric. Moshtohor, during 2005 and 2006 seasons to study the effect of foliar spraying with boron and two phosphate dissolvers; *B. megaterium* var. *phosphaticum* and endomycorrhizal fungi (*Glomus mosseae*) combined with plant growth promoting rhizhobacteria (*Paenibacillus polymyxa*) on microbial activities, growth, endogenous phytohormones and yield of squash (*Cucurbita pepo* L.) cv. El-Eskandarani. Mechanical and chemical analyses of the experimental soil estimated according to Jackson[8] and Black *et al* [9], respectively. are presented in Table (A).

Bacterial Seed Inoculation: *Paenibacillus polymyxa* and *B. megaterium* var. *phosphaticum* (pure local strains) were obtained from Biofertilizer Production Unit, Soil & Water and Environment Res. Inst., Agric., Res. Center, Giza, Egypt. The selected two bacterial strains were individually propagated in nutrient broth medium and incubated on a rotary shaker (180 rpm) at 30°C for 5 days. In dual inoculants, equal volumes of the suspensions obtained (about 10^8 cells/ml from each) were mixed with sterilized peat moss at the rate of 2:1 (V/W) under aseptic conditions. Arabic gum (16%) was applied to the seeds as an adhesive agent before mixing with peat inoculants. Each inoculum was used at a rate of 400 g/fed and thoroughly mixed with the seeds, the coated seeds were left to air-drying in shade, then the seed became ready for sowing.

Bacterial Rock Phosphate Inoculation: Three types of inoculations were used with rock phosphate to compare the effect of each and possible interaction on the performance of squash plants as affected by phosphate availability, the types were:

- Rock phosphate + *P. polymyxa*
- Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
- Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (BMP) (Bio phos-phor®).

(Bio phos-phor®) is a biologically active phosphate fertilizer was obtained from Union for Agric., Comp., (UAD), Egypt. (Bio phos-phor®) is certified by Academy of scientific Research and Technology

Table A. Mechanical and chemical analyses of the experimental soil.

Mechanical analysis			
Soil particles	Unit	Seasons	
		2005	2006
Coarse sand	%	13.15	16.41
Fine sand	%	13.24	12.36
Silt	%	15.50	16.60
Clay	%	58.11	54.63
Textural class		Clay	Clay
Chemical analysis			
Parameters	Unit	Seasons	
		2005	2006
Organic matter	%	1.67	2.06
Available N	ppm	61.4	67.3
Available P	ppm	7.8	6.9
Available K	ppm	21.5	30.7
$CaCo_3$	%	0.51	0.57
Iron	ppm	22.8	24.0
Zinc	ppm	2.27	3.78
Manganese	ppm	12.70	15.80
Copper	ppm	2.42	2.29
Boron	ppm	12.0	16.0
pH		7.94	8.08
EC	$\mu S^{\cdot}cm^{-1}$	0.71	0.73

(Egyptian patent office) under number (2004110482) and registered in Egyptian ministry of Agriculture& land reclamation under No.(4749).

Mycorrhizal Inoculation: Mycorrhizal fungus (*Glomus mosseae*) was obtained from Agric. Microbiol. Dept., Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt. Spores of the fungus were extracted by a wet-sieving and decanting technique[10] from rhizosphere soil of onion inoculated with AM fungus *Glomus mosseae* and grown for 4 months. The extracted spore suspension containing about 150-200 spores/ ml was used as a standard inoculum (20 ml/m^2) for the mycorrhizal treatments.

With respect to foliar spraying treatments, boric acid (17% boron) was used as a source of boron in a concentration of 25 ppm (0.148 g/L) and 0 ppm. The amount of boric acid was used at a rate of 200 L/fed by spraying onto the plants at the 15th and 30th day from sowing.

Determination of Phosphate Solubilizer Efficiency: *Paenibacillus polymyxa* and *B. megaterium* var. *phosphaticum* were tested for their efficiency in phosphate solubilizing on Pikovskaya[11] medium where rock phosphate was replaced instead of calcium super phosphate. The solubilization and growth diameter were measured after 5 days from incubation at 30°C. The results are expressed as solubilization efficiency (SE) where:

$$SE = \frac{\text{solubilization diameter}}{\text{growth diameter}} \times 100$$

Experimental Design: The experimental consisted of four treatments of inoculation with phosphate dissolving microorganisms (uninoculated treatment used calcium super phosphate as a meniral **P**. fertilizer (control); seed and rock phosphate inoculation by *p. polymyxa* , seed and rock phosphate inoculation by *p. polymyxa* + AM., seed and rock phosphate inoculation by *p. polymyxa* + BMP.) and two foliar boron concentration (0 and 25 ppm.) 0 ppm was used as a check treatment for B application by foliar spray using distilled water . The experiment was arranged in split design with three replicates. The main plots were assigned to boron foliar application ,while, the inoculation and uninoculation treatments were randomly distributed in the sub plots.

The plot area was 1/200 fed, 21 m^2 (3×7m) with three ridges which were 1m width and 7m length. Before cultivation, all inoculated treatments were supplied with 30 kg P_2O_5/fed as inoculate rock phosphate (26.4% total P_2O_5) using the tested phosphate dissolving microorganisms. While, the uninoculated treatments were fertilized with calcium superphosphate (15.5% available P_2O_5) at the same dose. Inoculated and uninoculated seeds of summer squash cv. EL-Eskandarani were directly sown on the 20th and 23rd of January for 2005 and 2006 seasons, respectively. All plots were fertilized with potassium sulphate (50% K_2O) at the rate of 50 kg K_2O/fed. and 60 kg N/fed as ammonium sulphate (20.5%N). Potassium and nitrogen fertilizers were applied in two equal doses, at the first irrigation (3 weeks from sowing) and at the beginning of flowering.

During the growing seasons all the horticultural procedures such as cultivation, manuring; irrigation, diserases and pest control wear practiced.

Microbial Activity Data Recorded: Microbial activities of the plant rhizhosphere after 30 and 50 days from sowing were conducted. The samples were analyzed for dehydrogenase activity according to the method described by Casida *et al*[12] while nitrogenase activity was estimated according to Hardy *et al*[13]. Phosphatase activity was determined by the method given by Drobnikova[14]. Mycorrhizal infection was microscopically estimated on a sample of fresh root as described by Giovannetti and Mosse[15] after clearing and staining (Phillips and Hayman)[16]. After 50 days from planting, rhizosphere samples were analyzed for NH_4-N and NO_3-N according to Bremner and Keeny[17], available phosphorus according to (A.P.H.A)[18] and available potassium according to Chapmen and Pratt[19].

Growth Parameters and Chemical Constituents: At the middle of fruit picking stage (50 days from sowing) eight plants were chosen at random from each plot to measure stem length, stem diameter, number of leaves/plant, dry weight of stem and leaves and leaf area using the disk method according to Koller[20]. Photosynthetic pigments were calorimetrically determined as described in A.O.A.C.[20]. Total sugars and total carbohydrates were determined according to A.O.A.C.[21]. Total nitrogen, phosphorous, potassium, magnesium and boron were determined according Chapmen and Pratt[19] while, crude protein was determined according A.O.A.C.[21]. Endogenous phytohormones were quantitatively determined in squash leaves during 2006 season according to Koshioka *et al.*[22] for auxin, gibberellic acid and abscisic acid while, cytokinins were determined according to Nicander *et al.*[23].

Flowering and Yield Components: For the sex ratio, number of male and female flowers were recorded through the growing season to determine the sex ratio (male/female flowers). Number of early fruits (number of first four pickings)/ plant, early yield, total fruits /plant and total yield were recorded. Total soluble solids was measured in the juice of squash fruit by a hand refractometer while, both vitamin C content and titratable acidity were determined according to A.O.A.C.[21].

Data obtained in this study were subjected to statistical analysis according to Snedecor and Cochran[24].

RESULTS AND DISCUSSIONS

Phosphate-solubilization: Solubilization efficiency of calcium super phosphate and rock phosphate by *P. polymyxa* and *B. megaterium* was shown in Table (B). Both strains were able to solubilize phosphate effectively from tricalcium phosphate or rock phosphate. *P. polymyxa* showed relatively higher solubilization efficiency and hence it was used as phosphate solubilizer beside its role as N_2-fixer. Subrahmaniyan *et al.*[25] and Muthukumar and Udaiyan[26] found that *P. polymyxa* plays an important role in phosphate solubilization.

Microbial Activities: Data in Table (1) show the determination of enzymatic activities in rhizosphere of squash plants. Dehydrogenase activity (DHA) represents the energy transfer, therefore, it is considered as an index of overall microbial activity in the soil. Inoculation with *P. polymyxa* increased DHA than uninoculated ones. This may be due to that *P. polymyxa* plays an important role as plant growth promoting rhizobacteria via P-solubilization (Muthukumar and Udaiyan)[26] or N_2-fixation[27]. This might led to accumulate available nutrients and stimulate the microorganisms in soil rhizosphere. Moreover, combination of mycorrhiza or *B. megaterium* with *P. polymyxa* recorded the highest DHA either with or without boron application.

Concerning phosphatase activity, using soluble P-fertilizer in uninoculated treatments decreased phosphatase activity than those treatments fertilized with insoluble-P as rock phosphate. This may due to

Table B. Solubilization efficiency (SE) of the tested bacteria

	Solubilization efficiency	
Strains	Calcium super Phosphate	Rock-P
Paenibacillus polymyxa	176.13	180.52
Bacillus megaterium	213.46	246.03

Table 1: Some microbial activities in rhizosphere of squash plants as affected by interactions between phosphate dissolving microorganisms combined with foliar boron application after 30 and 60 days from planting (during 2005 and 2006 seasons).

Biofertilization treatments	Dehydrogenase activity (µl DHA/g soil/day)				Phosphatase activity (µg inorganic phosphorus/g dry soil/day)				N_2-ase activity (n moles C_2H_4/hr/g rhizospheric soil)				Mycorrhizal root infection (%)			
	30 days		50 days		30 days		50 days		30 days		50 days		30 days		50 days	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Without Boron (0 ppm)																
Cont	23.41	28.79	26.24	28.42	11.84	9.57	18.30	19.20	8.2	11.4	6.5	11.2	5.7	9.5	10.8	9.4
SRI PP	35.34	31.75	28.79	34.86	17.31	19.53	24.05	21.13	34.6	45.7	43.1	51.4	14.2	12.1	18.6	13.8
SRI PP + AM	38.29	44.71	37.97	52.03	37.87	42.81	36.61	38.39	56.1	59.6	71.4	66.4	67.5	74.8	72.5	78.3
SRI PP + BMP	32.85	35.04	30.40	41.33	22.38	25.80	28.21	34.05	41.2	48.3	52.3	57.8	18.6	13.4	21.7	28.2
Foliar spray with Boron (25 ppm)																
Cont	27.63	30.53	28.26	32.43	13.67	12.87	16.36	19.35	12.3	9.6	9.7	10.1	7.8	10.7	8.7	11.3
SRI PP	42.06	40.36	37.74	48.34	28.11	28.01	29.94	33.40	44.6	53.7	52.8	62.9	17.4	11.3	17.8	16.4
SRI PP + AM	36.24	47.75	40.29	44.59	38.10	34.72	47.91	45.47	54.5	63.1	67.9	68.3	68.2	73.1	71.9	77.6
SRI PP + BMP	37.50	47.52	35.11	52.32	30.53	32.73	46.72	42.78	46.8	55.4	51.2	65.7	16.5	15.4	18.6	24.7
LSD at 5% (Boron x Biofertilization)	2.27	3.94	3.43	3.76	2.82	4.79	6.36	3.78	5.45	3.02	5.28	4.71	4.57	2.71	3.29	4.18

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*.
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®).

the P cycle enzyme activities are inversely related to P-availability and when P is a limiting nutrient its demand increases, resulting in an increase in phosphatase activity in the presence of P-solubilizers[28]. Also, dual inoculation especially with *P. polymyxa* and AM gave maximum values of phosphatase activity. There are positive interactions between bacteria such as *Bacillus* and mycorrhizae as a good co-inoculation system[29].

Data in Table (1) also show that the relatively high N_2-ase activity was observed in squash treatments inoculated with *P. polymyxa*. However, the highest N_2-ase activity was observed in co-inoculation treatments with *P. polymyxa* and either AM or *B. megaterium*. Many investigators demonstrated the positive effect of dual inoculation with N_2-fixer and P-solubilizer on N_2-ase activity[30,31].. On the other hand, data also show that mycorrhizal root infection percentage was high in inoculated plants with arbiscular mycorrhizal fungi (AM). Moreover, uninoculated plants have low percentage of mycorrhizal infection which indicates that the native AM are presented in the soil but in a low density. These results may be attributed to the presence of rock-P as P-fertlizer that may encourage VAM in inoculated treatments than uninoculated plants which received insoluble-P[28].

Concerning boron application, data showed that there were slightly increases in DHA, phosphatase and N2-ase activities by spraying of boron. Also, there was no effect of boron on mycorrhizal infection of squash root in the two seasons.

Available N, P and K in Squash Rhizosphere: According to the results obtained in Table (2), significant increases in available N, P and K were observed when squash plans were inoculated with *P. polymyxa* individually or with either AM or *B. megaterium* compared to uninoculated plants.

Mixed culture of *P. polymyxa* and *B. megaterium* exhibited maximum increases of ammoniacal and nitrate nitrogen content while combination of *P. polymyxa* with AM recorded the highest values of available-P and K either without or with boron application in the two seasons. This may be attributed to the high rates of mycorrhizal root infection, which observed with mycorrhizal inoculated treatments that attributed with maximum phosphatase values (Table 1). These results are in agreement with those reported by Mehasen *et al.*[32], who found that available-P content was increased when the plants were inoculated with mycorrhiza and amended with rock phosphate. Han *et al.*[33] also reported that co-inoculation with Bacillus and phosphate solubilizer in the presence of rock-P increased the availability of phosphorus and potassium

Table 2: Available N, P and K in rhizosphere of squash plants as affected by interaction between phosphate dissolving microorganisms combined with foliar boron at 50 days after sowing.

Biofertilization treatments	NH_4-N (ppm)		NO_3-N(ppm)		Available-P(ppm)		Soluble-K(ppm)	
	2005	2006	2005	2006	2005	2006	2005	2006
Without boron (0 ppm)								
Cont	66.9	72.6	78.2	81.4	44.5	52.3	38.7	42.4
SRI PP	80.8	78.4	81.6	90.7	68.2	89.1	42.3	48.6
SRI PP + AM	96.2	92.9	102.3	107.8	97.2	118.2	65.1	62.7
SRI PP + BMP	106.3	99.2	109.5	124.0	88.3	100.6	59.4	57.2
Foliar spray with boron (25 ppm)								
Cont	68.4	76.1	82.3	87.6	58.1	75.6	42.8	46.7
SRI PP	101.7	92.2	110.5	119.3	96.5	116.5	67.7	62.9
SRI PP + AM	105.8	117.3	115.6	124.5	117.6	136.4	73.5	69.8
SRI PP + BMP	112.2	121.9	123.4	131.9	112.7	121.8	68.6	72.4
LSD 5%	6.12	9.21	5.81	6.52	4.81	7.11	4.02	5.17

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*.
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®).

Table 3: Effect of phosphate dissolving microorganisms combined with foliar boron application on vegetative growth parameters of squash plant at 50 days after sowing (during 2005 and 2006 seasons)

Treatments	Stem length(cm)		Stem diameter(cm)		Stem dry weightg/ plant		No. of leaves/ plant		Leaf area / plant (cm^2)		Leaf dry weightg/ plant	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Boron treatments												
Without boron (0 ppm)	7.26	7.33	1.67	1.61	0.66	0.63	13.65	14.91	919.84	925.16	6.02	6.36
Foliar spray with boron (25 ppm)	7.63	7.42	1.84	1.75	0.86	0.785	15.16	16.51	1004.89	1043.92	6.66	7.01
Biofertilization treatments												
Cont	6.19	6.185	1.48	1.275	0.585	0.54	11.85	13.2	800.2	852.82	5.205	5.44
SRI PP	6.975	6.95	1.725	1.75	0.685	0.665	14.175	15.55	943.875	937.75	6.325	6.695
SRI PP + AM	8.575	8.425	2.05	1.91	0.945	0.865	16.85	18.125	1148.205	1123.025	7.42	7.86
SRI PP + BMP	8.055	7.96	1.785	1.795	0.84	0.77	14.75	15.975	957.205	1024.59	6.42	6.76
LSD at 5% level for Boron treatments	0.325	NS	0.112	0.096	0.124	0.112	NS	NS	53.20	64.72	0.310	0.477
LSD for Biofertilization treatments	0.632	0.679	0.205	0.157	0.185	0.164	2.28	2.31	60.55	70.20	0.686	0.872

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Table 4: Effect of interaction between phosphate dissolving microorganisms combined with foliar boron application on vegetative growth parameters of squash plant at 50 days after sowing (during 2005 and 2006 seasons)

Biofertilization treatments	Stem length(cm)		Stem diameter(cm)		Stem dry weightg/ plant		No. of leaves/ plant		Leaf area / plant (cm^2)		Leaf dry weightg/ plant	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Without boron (0 ppm)												
Cont	6.15	6.07	1.36	1.20	0.55	0.50	11.55	12.15	750.25	821.94	5.10	5.28
SRI PP	6.95	6.80	1.60	1.70	0.65	0.62	13.60	14.95	917.50	900.15	5.97	6.38
SRI PP + AM	8.85	8.35	2.00	1.82	0.74	0.73	15.70	17.30	1077.88	1005.30	7.02	7.50
SRI PP + BMP	7.11	8.12	1.75	1.74	0.73	0.69	13.75	15.25	933.76	973.28	6.01	6.28
Foliar spray with boron (25 ppm)												
Cont	6.23	6.30	1.60	1.35	0.62	0.58	12.15	14.25	850.15	883.70	5.31	5.60
SRI PP	7.00	7.10	1.85	1.80	0.72	0.71	14.75	16.15	970.25	975.35	6.68	7.01
SRI PP + AM	8.30	8.50	2.10	2.00	1.15	1.00	18.00	18.95	1218.53	1240.75	7.82	8.22
SRI PP + BMP	9.00	7.80	1.82	1.85	0.95	0.85	15.75	16.70	980.65	1075.90	6.83	7.24
LSD at 5% (Boron x Biofertilization)	0.69	0.71	0.17	0.19	0.05	0.10	0.95	1.20	56.77	62.25	0.71	0.81

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

in the soil. On the other hand, Boron application had no significant increases on available N, P and K in uninoculated plants, while significant increases were observed with all inoculated treatments. This may be due to the positive effect of interaction between inoculation and boron on soil enzyme activities that was observed in Table (1). Data also indicated that dual inoculation with *P. polymyxa* and *B. megaterium* gave maximum values of ammoniacal and nitrate nitrogen comparison with combination between *P. polymyxa* and mycorrhizal plants either without or with boron application. These observations are in harmony with those obtained by Zaghloul *et al.*[34].

Growth Parameters: The growth parameters of squash plants, i.e., stem length, diameter and dry weight, as well as leaf number, area and dry weight per plant, were significantly increased by all biofertilizer applications (Table 3 & 4). Maximum stimulatory effect of the biofertilizers was existed in plants treated with *P. polymyxa* and AM followed by dual inoculation with the two bacterial strains. In this regard, Rai[2] reported that *P. polymyxa* may possess a great variety of properties that are of interest in the development of biofertilizers including N_2-fixation, P-solubilization, production of growth promoting plant hormones especially cytokinins and production of antibiotics as well as improvement of soil porosity. So, *P. polymyxa* is a good example of bacteria, which may warrant further study due to their highly diverse and potential beneficial effects on plant growth. Also, Abou-Aly and Gomaa[35] and Giri and Mukerji[36] found that AM individually or in combination with N_2-fixer had a positive effect on plant growth. Moreover, the stimulatory effect of P-solubilizers can be attributed to the absorption of nutrients by plants especially P from the soil as previously observed in Table (2).

On the other hand, boron application had a positive effect on all growth parameters for all treatments especially in the presence of biofertilizers. This may be due to the enhancement of boron on sugar synthesis and translocation from plant leaves which in turn may activate the photosynthesis and led to an increase in plant growth[37]. Also, Goldbach *et al.*[7] reported that boron has a role in activation of cell division and cell elongation, which might be attributed to an increase in leaf area.

These results may be attributed to the effect of such bio treatments in increasing the photosynthetic pigments that intern helped in increasing the total carbohydrates and sugar level in the leaves that resulted in the vigorous growth indicated by increased in plant stem length, stem diameter, No of leaves/plant, leaf area / plant and leaf dry weight / plant as shown in Table (3) .

On the other hand, the phosphate dissolving microorganisms treatments showed an increasing in both GA3 and IAA level and a decreasing in ABA content that may have affected stem length and diameter, leaf area / plant and leaf dry weight.

Photosynthetic Pigments and Chemical Constituents: Results in Tables (5&6) show the effect of inoculation with *P. polymyxa* singly or in combination with AM or *B. megaterium* in the presence of boron application on chlorophyll and carotenoid contents as well as chemical constituents of squash plants (Table 7&8). It is evident from the obtained data that plants inoculated with AM or *B. megaterium* combined with *P. polymyxa* gave the highest photosynthetic pigments and nutrient content either with or without boron application. In this respect, Abou-Aly and Gomaa[35] stated that mixed biofertilizers increased both nutrient content and leaf chlorophyll concentration than control. Also, Han *et al.*[33] found that the integrated treatment of co-inoculation with P-solubilizers and application of rock-P significantly increased leaf photosynthesis over the control.

Furthermore, the maximum enhancement was observed in plants dually inoculated and sprayed with boron. In this respect, Fathy[38] found that boron increased chlorophyll and nutrient content in tomato plants. The enhancing effect of boron on chlorophyll content may be due to boron increased total sugar (Table 5) and the latter are essential for chlorophyll formation.

Generally, these results are considered as a good explanation to the obtained data regarding the favorable role of P-solubilizers and boron on growth parameters (Table 3). The availability of N and P for plant growth due to *P. polymyxa* and AM or *B. megaterium* led to large increase in the rate of photosynthesis which is sufficient to support plant growth. Moreover, the enhancing effect of biofertilizers and boron on growth and photosynthetic characters (Tables 5&6) may explain the increase in total carbohydrates in plants (Table 7&8).

Also from these results we can generally conclude that the application of p. polymyxa + AM. compiled with foliar boron application gave the highest photosynthetic pigment content. These results may be attributed to the increase in Mg++ and phosphor content that in tern resulted in an increase in active photosynthetic pigments (chlorophyll a and b) that may be due to the fact of Mg++ being the corner stone of the structure of chlorophyll molecule

On the other hand, the increase of K+ content may have affected the increase in carotenoid pigment due to its effect on carbohydrate build up and translocation.

Table 5: Effect of phosphate dissolving microorganisms combined with foliar boron application on photosynthetic pigments of squash plant at 50 days after sowing (during 2005 and 2006 seasons).

Treatments	Chlorophyll (a)		Chlorophyll (b)		Chlorophyll (a+b)		Carotenoids		Chlorophyll a+b / Carotenoids	
	2005	2006	2005	2006	2005	2005	2006	2005	2006	2005
					Boron treatments					
Without boron (0 ppm)	0.62	0.64	0.43	0.46	1.05	1.10	0.51	0.52	2.08	2.14
Foliar spray with boron (25 ppm)	0.77	0.78	0.55	0.54	1.32	1.32	0.59	0.61	2.21	2.15
					Biofertilization treatments					
Cont	0.56	0.57	0.4	0.43	0.96	0.98	0.42	0.49	2.25	2.06
SRI PP	0.67	0.70	0.49	0.51	1.16	1.21	0.55	0.53	2.08	2.25
SRI PP + AM	0.84	0.84	0.55	0.55	1.39	1.40	0.64	0.65	2.14	2.14
SRI PP + BMP	0.72	0.73	0.52	0.53	1.24	1.26	0.58	0.59	2.11	2.11
LSD at 5% level for Boron treatments	NS	NS	NS	NS	0.102	0.113	NS	NS	NS	NS
LSD for Biofertilization treatments	0.083	0.089	0.072	0.061	0.178	0.154	NS	NS	NS	NS

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Table 6: Effect of interaction between phosphate dissolving microorganisms combined with foliar boron application on photosynthetic pigments of squash plant at 50 days after sowing (during 2005 and 2006 seasons).

Biofertilization treatments	Chlorophyll (a)		Chlorophyll (b)		Chlorophyll (a+b)		Carotenoids		Chlorophyll a+b / Carotenoids	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
					Without boron (0 ppm)					
Cont	0.510	0.525	0.350	0.415	0.860	0.940	0.380	0.420	2.263	2.238
SRI PP	0.575	0.612	0.417	0.468	0.992	1.080	0.490	0.485	2.024	2.226
SRI PP + AM	0.790	0.800	0.512	0.515	1.302	1.315	0.630	0.615	2.067	2.138
SRI PP + BMP	0.625	0.630	0.460	0.473	1.085	1.103	0.552	0.562	1.966	1.963
					Foliar spray with boron (25 ppm)					
Cont	0.615	0.620	0.480	0.450	1.065	1.030	0.475	0.560	2.242	1.893
SRI PP	0.770	0.790	0.570	0.560	1.340	1.350	0.625	0.590	2.144	2.288
SRI PP + AM	0.890	0.895	0.590	0.595	1.480	1.490	0.665	0.690	2.225	2.159
SRI PP + BMP	0.815	0.830	0.584	0.588	1.399	1.418	0.618	0.627	2.264	2.262
LSD at 5% (Boron x Biofertilization)	0.06	0.07	0.045	0.04	0.13	0.15	0.05	0.055	0.17	0.16

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Table 7: Effect of phosphate dissolving microorganisms combined with foliar boron application on chemical and nutrients content of squash plant at 50 days after sowing (during 2005 and 2006 seasons).

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Magnesium (%)		Boron (ppm)		Crude protein (%)		Total sugars mg/g (FW)		Total carbohydrates mg/g (DW)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
								Boron treatments								
Without boron (0 ppm)	3.38	3.43	0.41	0.41	3.02	3.05	1.57	1.81	58.50	55.50	20.84	21.45	25.15	27.93	487.50	486.26
Foliar spray with boron (25 ppm)	3.83	3.81	0.45	0.44	3.20	3.33	1.75	2.05	62.00	59.00	23.56	25.20	29.32	31.63	581.0	549.65
								Biofertilization treatments								
Cont	3.19	3.15	0.36	0.38	2.98	2.98	1.43	1.68	53.00	57.00	19.13	20.19	26.09	27.74	471.4	463.51
SRI PP	3.52	3.60	0.41	0.40	3.13	3.03	1.47	1.72	59.50	55.50	21.87	23.66	26.16	27.87	511.34	485.97
SRI PP + AM	3.80	3.82	0.50	0.48	3.22	3.44	2.04	2.27	67.00	60.00	24.37	24.78	30.43	34.84	606.41	599.45
SRI PP + BMP	3.93	3.92	0.46	0.44	3.12	3.31	1.72	2.04	61.50	56.50	23.44	24.68	26.26	28.69	547.87	522.91
LSD at 5% level for Boron treatments	NS	NS	NS	NS	NS	NS	0.11	0.09	1.05	1.32	1.45	2.10	2.01	2.36	30.15	43.21
LSD for Biofertilization treatments	0.35	0.28	NS	NS	NS	NS	0.45	0.68	3.77	2.14	2.88	3.03	3.32	5.25	55.10	64.41

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Table 8: Effect of interaction between phosphate dissolving microorganisms combined with foliar boron application on chemical and nutrients content of squash plant at 50 days after sowing (during 2005 and 2006 seasons).

Biofertilization treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Magnesium (%)		Boron (ppm)		Crude protein (%)		Total sugars mg/g (FW)		Total carbohydrates mg/g (DW)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Without boron (0 ppm)																
Cont	2.70	2.75	0.322	0.353	2.80	2.69	1.30	1.46	52.0	56.0	16.88	17.19	22.58	26.18	425.12	438.70
SRI PP	3.25	3.50	0.417	0.398	3.11	3.02	1.40	1.75	57.0	53.0	20.31	21.88	25.18	27.40	482.18	450.66
SRI PP + AM	3.85	3.90	0.480	0.460	3.25	3.30	1.84	2.05	70.0	59.0	23.06	24.25	28.17	30.28	522.35	547.29
SRI PP + BMP	3.75	3.60	0.460	0.430	2.95	3.20	1.75	1.98	55.0	54.0	23.13	22.50	24.68	27.88	520.36	508.40
Foliar spray with boron (25 ppm)																
Cont	3.69	3.55	0.405	0.420	3.17	3.27	1.56	1.90	54.0	58.0	21.38	23.19	29.60	29.30	517.68	488.32
SRI PP	3.80	3.70	0.420	0.417	3.15	3.05	1.55	1.70	62.0	58.0	23.44	25.45	27.15	28.35	540.50	521.28
SRI PP + AM	3.75	3.75	0.525	0.510	3.20	3.58	2.25	2.50	64.0	61.0	25.69	25.31	32.70	39.40	690.48	651.61
SRI PP + BMP	4.11	4.25	0.470	0.450	3.30	3.42	1.70	2.11	68.0	59.0	23.75	26.86	27.85	29.50	575.38	537.42
LSD at 5% (Boron x Biofertilization)	0.4	0.51	0.03	0.04	0.4	0.3	0.2	0.35	4.50	4.60	3.75	4.15	5.7	5.4	34.5	26.07

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Table 9: Endogenous phytohormones content of squash shoot as affected by interactions between phosphate dissolving microorganisms combined with foliar boron application.

Biofertilization treatments	Promoters						Inhibitor	
	Gibberellins (GA3)		Auxins (IAA)		Cytokinins		Abscisic acid (ABA)	
	μg/g fresh weight	%(±) control	μg/g fresh weight	%(±) control	μg/g fresh weight	%(±) control	μg/g fresh weight	%(±) control
Without boron (0 ppm)								
Cont	115.70	0.00	17.30	0.00	7.22	0.00	2.07	0.00
SRI PP	195.45	+ 68.93	22.70	31.21	8.90	23.27	1.38	- 33.33
SRI PP + AM	270.60	+ 133.88	27.40	58.38	9.12	26.32	0.96	- 53.62
SRI PP + BMP	210.54	+ 81.97	24.76	43.12	9.38	29.92	1.22	- 41.06
Foliar spray with boron (25 ppm)								
Cont	198.70	+ 71.74	16.96	- 1.97	8.75	21.19	1.45	- 38.00
SRI PP	225.54	+ 94.94	19.40	12.14	10.11	40.03	0.98	- 52.66
SRI PP + AM	373.35	+ 222.69	32.18	86.01	8.17	13.16	1.10	- 46.86
SRI PP + BMP	268.75	+ 132.28	24.73	42.95	12.35	71.05	0.85	- 58.94

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum* (Bio phos-phor®)

Regarding the effect on crude protein, total sugars and total carbohydrates, p. dissolving microorganisms compiled with foliar boron application increased them and this may be also attributed to the k+ and boron increased levels, that has being reported to increase carbohydrates and sugar translocation and build up.

Endogenous Phytohormones: Endogenous phytohormones of squash plants as affected by the interaction between biofertilizers and boron are shown in Table (9). According to these results, all promoters (gibberellins, auxins and cytokinins) were improved by using biofertilizers either without or with boron application, yet; only the growing inhibitor abscisic acid was decreased. Dual inoculation with *P. polymyxa* and P-solubilizers gave the highest values of phytohormones. Application of AM in the dual inoculation recorded maximum values of gibberellins and auxins while, combination of *B. megaterium* and *P. polymyxa* gave the highest values of cytokinins content than that mixed with AM. Many investigators reported the role of plant growth promoting rhizobacteria such as *Paenibacillus polymyxa* and *B. megaterium* as well as mycorrhiza in the production of hormones such as gibberellins, auxins and cytokinins[2,39,40] .On the other hand, the growth inhibitor; abscisic acid was decreased with using phosphate-solubilizers especially AM without boron application while in the presence of boron, dual inoculation of *P. polymyxa* and *B. megaterium* recorded maximum reduction of abscisic

Table 10: Yield flowering behavior and yield components of squash plants (cv. El-Eskandarani) as affected by phosphate dissolving microorganisms combined with foliar boron application(during 2005 and 2006 seasons).

Treatments	No. of flowers/ plant						Early fruits No./ plant		Early yield g/plants		Total fruits No./plant		Total yield kg/plant	
	Male ♂		Female ♀		Sex ratio									
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
							Boron treatments							
Without boron (0 ppm)	40.26	39.27	26.12	27.50	1.66	1.49	4.08	4.26	285.60	320.45	16.88	18.85	2.62	2.59
Foliar spray with boron (25 ppm)	35.77	36.93	30.50	31.49	1.20	1.20	4.86	5.43	368.03	370.75	20.17	21.62	3.00	3.17
							Biofertilization treatments							
Cont	48.80	50.50	21.90	24.22	2.30	2.12	2.95	3.35	259.57	290.76	13.60	16.97	2.11	2.47
SRI PP	36.65	36.30	29.00	31.73	1.27	1.15	4.12	4.82	290.44	336.07	16.82	18.37	2.67	2.57
SRI PP + AM	35.72	34.70	33.70	33.06	1.06	1.05	5.45	6.21	378.74	406.52	22.20	24.10	3.26	3.52
SRI PP + BMP	30.90	30.92	28.65	28.98	1.08	1.07	5.37	5.02	378.52	349.06	21.49	21.50	3.19	2.98
LSD at 5% level for Boron treatments	3.24	2.54	2.11	3.41	0.22	0.29	NS	NS	39.80	22.30	2.78	1.40	0.300	0.302
LSD for Biofertilization treatments	7.21	8.55	3.89	5.47	0.90	0.87	1.30	1.02	79.38	47.26	3.71	2.80	0.480	0.620

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*.
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum*.(Bio phos-phor®)

Table 11: Yield flowering behavior and yield components of squash plants (cv. El-Eskandarani) as affected by interaction between phosphate dissolving microorganisms combined with foliar boron application(during 2005 and 2006 seasons).

Biofertilization treatments	No. of flowers/ plant						Early fruits No./ plant		Early yield g/plants		Total fruits No./plant		Total yield kg/plant	
	Male ♂		Female ♀		Sex ratio									
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
							Without boron (0 ppm)							
Cont	57.30	56.40	19.40	22.30	2.95	2.53	2.40	2.80	238.20	262.88	12.75	15.20	1.91	2.13
SRI PP	34.60	36.40	25.30	29.35	1.37	1.24	4.15	4.70	252.37	328.08	15.40	16.90	2.46	2.23
SRI PP + AM	36.75	33.70	33.10	31.20	1.11	1.08	5.20	5.62	361.94	390.74	20.70	22.50	3.12	3.27
SRI PP + BMP	32.40	30.60	26.70	27.17	1.21	1.13	4.60	3.95	289.89	300.12	18.68	20.80	2.99	2.76
							Foliar spray with boron (25 ppm)							
Cont	40.30	44.60	24.40	26.15	1.66	1.71	3.50	3.90	280.95	318.64	14.45	18.75	2.31	2.81
SRI PP	38.70	36.20	32.70	34.12	1.18	1.06	4.10	4.95	328.51	344.07	18.25	19.85	2.88	2.91
SRI PP + AM	34.70	35.70	34.30	34.92	1.01	1.02	5.70	6.80	395.54	422.31	23.70	25.71	3.41	3.78
SRI PP + BMP	29.40	31.25	30.60	30.80	0.96	1.01	6.15	6.10	467.15	398.00	24.30	22.20	3.40	3.21
LSD at 5% (Boron x Biofertilization)	5.57	5.61	4.60	5.01	0.10	0.13	0.80	0.91	16.14	14.27	1.57	1.97	0.15	0.09

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
SRI PP: seed and Rock phosphate + *P. polymyxa*.
SRI PP + AM: seed and Rock phosphate + *P. polymyxa* + Arbiscular mycorrhizal (AM).
SRI PP + BMP: seed and Rock phosphate + *P. polymyxa* + *Bacillus megaterium* var *phosphaticum*.(Bio phos-phor®)

Table 12: Some chemical fruit quality parameters of squash (cv. El-Eskandarani) as affected by interaction between phosphate biofertilizers and boron foliar application (during 2005 and 2006 seasons).

Biofertilization treatments	Vitamin C mg/100 g fresh weight		Total soluble solids (%)		Titratable acidity (%)	
	2005	2006	2005	2006	2005	2006
			Without boron (0 ppm)			
NPK-fertilization	18.10	18.00	4.75	4.80	0.475	0.490
P. polymyxa	18.75	18.75	5.15	5.30	0.497	0.507
P. polymyxa +AM	19.50	18.25	5.50	5.28	0.511	0.525
P. polymyxa +PSB	19.52	19.15	5.75	5.35	0.512	0.585
			Foliar spray with boron (25 ppm)			
NPK-fertilization	17.69	19.15	4.60	4.90	0.477	0.506
P. polymyxa	17.63	18.95	4.90	5.20	0.495	0.550
P. polymyxa +AM	19.62	19.85	5.85	5.46	0.520	0.550
P. polymyxa +PSB	18.97	19.25	5.30	5.41	0.531	0.570
LSD at 5%	1.25	1.30	0.30	0.40	0.02	0.04

acid. Generally, application of boron in the presence of biofertilizers improved endogenous phytohormones of squash plants.

Flowering Behavior and Yield Components: Data illustrated in Table (10&11) show that inoculation with *P. polymyxa* singly or in combination with AM or *B. megaterium* significantly decreased the number of male flowers but increased the number of female flowers and in turn decreased sex ratio compared with the untreated control or application of boron only. Maximum decrease of sex ratio was obtained in dual inoculation treatments. In this respect, El-Assiouty and Abo-Sedera[41] found that sex ratio was significantly affected by biofertilizers especially when dual inoculation was applied. Moreover, data showed that early and total fruits as well as early and total yield were significantly increased in response to biofertilizer application compared to control. Also, boron had positive effect on the same parameters.

It may be stated that under such this work conditions, the stimulatory effect of the used dual inoculation and boron treatments on squash fruit yield would be expected since the assigned treatments promoted microbial activities (Tables 1&2), growth parameters (Table 3), increased photosynthetic pigments (Table 4), increased nutrients content and total sugar and carbohydrates (Table 7&8) as well as endogenous phytohormones (Table 6) as previously resulted and discussed in this work.

Concerning the fruit quality, data presented in Table (12) show the same positive trend. Since inoculation with *P. polymyxa* individually or combined with AM or *B. megaterium* improved vitamin C, total soluble solids and titratable acidity of fresh squash fruits. Also, slightly increase in fruit quality was obtained when boron was applied. In this respect, Rai[2] reported that the application of rock-phosphate with P-solubilizers especially *B. megaterium* increased sugarcane yield and also, improved the juice quality.

Generally, the present study is strongly admit the use of phosphate solubilizing microorganisms as an active tool to improve microbial activities and nutrient content, consequently, growth performance was improved as well. In addition, dual inoculation in the presence of boron spraying increased plant growth, endogenous phytohormones and yield of squash. Also, phosphate solubilizing microorganisms play an important role in plant nutrition and allowing use of cheaper crude P sources such as rock-phosphate instead of superphosphate, while plant growth promoting microbes are an important contributor to biofertilization of agricultural crops.

REFERENCES

1. Shehata, M.M. and S.A. El- Khawas, 2003. Effect of two biofertilizers on growth parameters, yield characters, nitrogenous components, nucleic acids content, minerals, oil content, protein profiles and DNA banding pattern of sunflower (*Helianthus annus* L. cv. vedock) yield. Pakistan Journal of Biological Sciences, 6(14): 1257-1268.
2. Rai, M.K., 2006. Handbook of microbial biofertilizers. Food Products Press, an imprint of The Haworth Press, Inc, Binghamton, New York.
3. Olsson, P.A., M. Chalot; E. Baath, R.D. Finlay and B. Soderstrom, 1996. Ectomycorrhiza mycelium reduces bacterial activity in a sandy soil. FEMS Microbiology Ecology, 21: 81-86.
4. Ash, C., F.G. Priest and M.D. Collins, 1994. *Paenibacillus* gen. nov. and *Paenibacillus polymyxa* comb. Nov. In validation of the publication of new names and new combinations previously effectively published outside the IJSB, List 51. Int. J. Syst. Bacteriol., 44: 852.
5. Mansfeld-Giese, K., J. Larsen and L. Bodker, 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microbiology Ecology, 41: 133-140.
6. Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung and M. H. Wong, 2005. Effects of biofertilizer containing N_2-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125(1/2): 155-166.
7. Goldbach H.E, D. Hartmann and T. Rotzer, 1990. Boron is required for the stimulation of the ferricyanide-induced proton release by auxins in suspension-cultured cells of *Daucus carota* and *Lycopersicon esculentum*. Physiol Plant , 80: 114-118.
8. Jackson, M.L., 1973. Soil Chemical Analysis. Prentice-Hall of India, Private New Delhi.
9. Black, C.A., D.O. Evans, L.E. Ensminger, J.L White, F.E. Clark and R.C. Dinauer, 1982. *Methods of Soil Analysis*. Part 2. Chemical and microbiological properties. 2nd Ed. Soil Sci., Soc. of Am. Inch. Publ., Madison, Wisconsin, U.S.A.
10. Gerdmann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Brit Mycol. Soc., 46: 235-244.
11. Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiologiya, 17:362-370.

12. Casida, L.E., D.A. Klein and T. Santoro, 1964. Soil dehydrogenase activity. Soil Sci., 98: 371-378.
13. Hardy, R.W.F., B.C. Bums and R.D. Holsten, 1973. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem., 5: 47-81.
14. Drobnikova, V., 1961. Factors influencing the determination of phosphatase in soil. Folia. Microbiol., 6, 260.
15. Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizae infection in roots. New Phytol., 84:489.
16. Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit Mycol. Soc., 55: 158-161.
17. Bremner, J.M. and D.R. Keeny, 1965. Steam distillation method for determination of ammonium, nitrate and nitrite. Annals Chem. Acta, 32: 485-495.
18. A.P.H.A, American Public Health Association 1992. Standard methods for the examination of water and wastewater. Washington, D.C., USA.
19. Chapmen, H.D. and F.P. Pratt, 1961. Methods of analysis for soils, plants and water. Belmont: Wadsworth Publishing Company, Californian Division of Agriculture Science, 1961, pp: 309.
20. Koller, H.R.C., 1972. Leaf area-leaf weight relationship in soybean canopy. Crop Sci., 12: 216-220.
21. A.O.A.C., 1990. Official Methods of Analysis of the Association of Official Agriculture Chemists. Published by Association of Official Agriculture Chemists, 13th Ed. Washington, D.C., USA.
22. Koshioka, M., J. Harada, Takenok, M. Noma, T. Sassa, K. Ogiama, J.S. Taylor, S.B. Rood, R.L. Legge and R.P. Phris, 1983. Reversed-phase C_{18} high performance liquid chromatography of acidic and conjugated gibberellins. J. Chromatgr., 256: 101-115.
23. Nicander, B., U. Stahl, P.O. Bjorkman and E. Tillberg, 1993. Immune affinity co-purification of cytokines and analysis by high performance liquid chromatography with ultraviolet spectrum detection. Planta, 189: 312-320.
24. Snedecor, G.W. and W.G. Cochran, 1980. Statistical method 7th Ed., Iowa state University Press, Ames Iowa, USA.
25. Subrahmaniyan, K., P. Kalaiselven and N. Arulmozhi, 2000. Evaluation of different phosphorus solubilizing microorganisms in groundnut (*Arachis hypogaea* L.). Res. on Crops, 1(1): 55-57.
26. Muthukumar, T. and K. Udaiyan, 2006. Growth of nursery-grown Bamboo inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in two tropical soil types with and without fertilizer application. New Forests, 31(3): 469-485.
27. El-Howeity, M.A., M.N.A. Omar, M.M. El-shinnawi and S.A. Aboel-Naga, 2003. Colonization pattern of some diazotrophs on wheat (*Triticum aestivum*) and maize (*Zea mays*) roots in *vitro* and in *vivo* experiments. Eleventh Conference of Microbiology, Cairo, Egypt, Oct. 12-14, 2003.
28. Vazquez, M.M., S. Cesar, R. Azcon and J.M. Barea, 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology, 15: 261-372.
29. Vivas, A., A. Marulanda, J.M. Ruiz-lozano, J.M. Barea and R. Azcon, 2003. Influence of a *Bacillus sp.* on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza, 13(5): 249-256.
30. Hauka, F.I.A., M.M.A. El-Sawah and A.E.I. Selim, 1996. Role of phosphate and silicate-solubilizing bacteria in transformation of some macro-and micro-nutrients uptake by plants. Proc. 7th. Conf. Agronomy, Fac. Agric., Mansoura Univ., 9-10 Sept., 239-252.
31. El-Komy, H.M.A., 2005. Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol. Biotechnol., 43(1): 19-27.
32. Mehasen, S.A.S., R.A. Zaghloul and M.A. El-Ghozoli, 2002. Effectiveness of dual inoculation with *Bradyrhizobium* and endomycorrhizae in presence of different phosphatic fertilizer sources on growth and yield of soybean. Annals Agric. Sci., Ain Shams Univ., Cairo, 47(2): 477-500.
33. Han, H.S., Supanjani and K.D. Lee, 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ., 52(3): 130-136.
34. Zaghloul, R.A., M.A. El-Ghozoli and S.A.S. Mehasen, 2002. Effect of dual inoculation (VA-mycorrhizae and *Rhizobium*) and zinc foliar application on growth and yield of mungbean. Annals Agric. Sci., Ain Shams Univ., Cairo, 47(2): 501-525.

35. Abou-Aly, H.E. and A.O. Gomaa, 2002. Influence of combined inoculation with diazotrophs and phosphate solubilizers on growth, yield and volatile oil content of coriander plants (*Coriandrum sativum* L.). Bull. Fac. Agric., Cairo Univ., 53: 93-114.
36. Giri, B. and K. Mukerji, 2004. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiac*a and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza., 14(5): 307-312.
37. Blevins, D.G. and K.M Lukaszewski, 1994. Proposed physiologic functions of boron in plants pertinent to animal and human metabolism. Journal Series, 11: 821.
38. Fathy, E.L.E., 1995. Physiological studies on tomatos. Ph.D Thesis, Fac. of Agric., Mansoura Univ., Egypt.
39. Brian, B.M.G., 2004. Ecology of *Bacillus* and *Paenibacillus* spp. in agricultural system. Phytopathology, 94(11): 1252-1258.
40. Timmusk, S., G. Nina and E.G.H. Wagner, 2005. *Paenibacillus polymyxa* invades plant roots and forms biofilms. Applied and Environmental Microbiology, 71(11):7292-7300.
41. El-Assiouty, F.M.M. and S.A. Abo-Sedera, 2005. Effect of bio and chemical fertilizers on seed production and quality of spinach (*Spinacia oleracea* L.). International Journal of Agricultural&Biology, 7(6): 947-952.