INTERACTION EFFECT BETWEEN PHOSPHATE DISSOLVING MICRO-ORGANISMS AND BORON ON SQUASH (*Cucurbita pepo* L.) GROWTH, ENDOGENOUS PHYTO-HORMONES AND FRUIT YIELD

Journal

J Biol Chem Environ Sci, 2006, Vol. 1(4): 751-774
www.acepsag.org

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ABSTRACT

Combination between plant growth promoting rhizobacteria; *Paenibacillus polymyxa* and two phosphate dissolvers; *Bacillus megaterium* and arbiscular mycorrhizal fungi AM (*Glomus mosseae*) were applied to the rock-phosphate amended soil in two field experiments during 2005 and 2006 seasons. Interaction effects between those biofertilizers application and foliar spraying with boron on some microbial activities, growth characteristics as well as fruit yield and quality of squash were studied. Significant positive effects were obtained of dehydrogenase, phosphatase, and N_2-ase activities as well as nitrogen forms, available phosphorous and soluble potassium in rhizosphere of squash after inoculation with *P. polymyxa*. Also, dual inoculation of *P. polymyxa* with either AM or *B. megaterium* gave maximum values of all microbial activities and available N, P and K in squash rhizosphere in both seasons especially when foliar spraying with boron was applied. Meanwhile, application of both dual inoculants either alone or with boron spraying led to considerable improvement in growth characteristics, photosynthetic pigments as well as biochemical composition of squash plants when compared with untreated ones. Also, dual inoculation with *P. polymyxa* and AM increased gibberellins and auxins content in squash leaves while, co-inoculation with *P. polymyxa* and *B. megaterium* increased cytockinins content either in the presence or absence of boron spraying. However, boron has positive effect on endogenous phytohormones particularly in the inoculated plants with biofertilizers. Similar positive trend was observed in sex ratio; early fruit yield and total yield with good quality of fruits especially when dual inoculation combined with boron foliar spraying were applied. Obtained results confirm the positive influence of co-inoculation with plant growth promoting rhizhobacteria as an active tool to improve squash fruit yield and quality as well, also, support the role of boron for enhancing biofertilization performance.

Key words: Biofertilizers, mycorrhizae, *P. polymyxa*, *B. megaterium*, boron, squash, chlorophyll, endogenous hormones, sex ratio, early yield, fruit quality

INTRODUCTION

Nowadays, biofertilizers considered the most advanced biotechnology can increase the output, improve the quality and it is responsible for developing organic, green and non-pollution 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 improve the physical properties of soils; enrich air aeration, water and nutrients retention capacity. Biofertilizers provide the cultivated plants 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 well pesticides application, based on yield (Shehata and El- Khawas, 2003). 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 nutrients use or increase nutrients availability can provide sustainable solutions for present and future agricultural practices (Rai, 2006). An alternative approach for using of phosphate-solubilizers as a microbial inoculants is the use 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 (Olsson *et al*., 1996). Some bacteria such as *Paenibacillus polymyxa* (previously *Bacillus* *polymyxa* Ash *et al*., 1994) 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 (Mansfeld-Giese *et al*., 2002). A promising trend for increasing the efficiency of biofertilizers was studied by using different mixture of nitrogen fixing bacteria, phosphate solubilizers and potassium solubilizer. Wu *et al*. (2005) 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 biofertilizers 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 without considering micro-elements 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 (Blevins and Lukaszewski, 1994). 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. (Goldbach *et al*., 1990).

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.

MATERIAL 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 interaction effect between 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.). Mechanical and chemical analyses of the experimental soil are presented in Table (A). Mechanical and chemical analyses were estimated according to Jackson (1973) and Black *et al* (1982), respectively.

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

Bacterial inocula

Paenibacillus polymyxa and *B. megaterium* var. *phosphaticum* (pure local strains) were obtained from Biofertilizers 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.

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 (Gerdemann and Nicolson, 1963) 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). The amount of boric acid was used at a rate of 200 L/fed by spraying onto the plants at the 30th and 60th day from planting.

Determination of phosphate solubilizers efficiency

Paenibacillus polymyxa and *B. megaterium* var. *phosphaticum* were tested for their efficiency in phosphate solubilizing on Pikovskaya (1948) medium where rock phosphate was replaced instead of tricalcium 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:

$$\mathbf{SE} = \frac{\textbf{Solubilization diameter} \times \mathbf{100}}{\textbf{Growth diameter}}$$

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

Strains	**Solubilization efficiency**	
	Tricalcium-P	Rock-P
Paenibacillus polymyxa	188.33	175.42
Bacillus megaterium	228.57	241.05

Experimental design

The experiment consisted of eight treatments of biofertilizers and boron foliar application arranged in a split plot 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 rock phosphate (26.4% P_2O_5) while, uninoculated treatments were fertilized with calcium superphosphate (15.5% P_2O_5) at the same dose. Inoculated and uninoculated seeds of squash were directly sown on the 20th and 23rd of January for 2005 and 2006 seasons, respectively. All plots were fertilized with potassium sulphate (48% K_2O) at the rate of 48 kg K_2O/fed. Inoculated treatments were supplemented with a half dose of inorganic N-fertilizer (30 kg N/fed) as ammonium sulphate (20.5%N) while uninoculated treatments received full dose of inorganic nitrogen (60 kg N/fed). Potassium and nitrogen fertilizers were applied in two equal doses, at the first irrigation (3 weeks from sowing) and at the beginning of flowering.

Determinations

Microbial activities of the plants rhizhosphere after 30 and 60 days from sowing were conducted. The samples were analyzed for dehydrogenase activity according to the method described by Casida *et al.* (1964) while nitrogenase activity was estimated according to Hardy *et al.* (1973). Phosphatase activity was determined by the method given by Drobnikova (1961). Mycorrhizal infection was microscopically estimated on a sample of fresh root as described by Giovannetti and Mosse (1980) after clearing and staining (Phillips and Hayman, 1970). After 50 days from planting, rhizosphere samples were analyzed for NH_4-N and NO_3-N according to Bremner and Keeny (1965), available phosphorus according to (A.P.H.A, 1992) and available potassium according to Chapmen and Pratt (1961).

Growth parameters and chemical constituents

At the middle of fruit picking stage (50 days from sowing) four plants were chosen at random from each plot to measure stem length (cm), stem diameter (cm), number of leaves/plant, dry weight of stem and leaves (g/plant) and leaf area(cm^2/plant) using the disk method according to Koller (1972). Photosynthetic pigments were calorimetrically determined as described in A.O.A.C. (1990). Total sugars and total carbohydrates were determined according to Thomas and Dutcher (1924) and Dubois *et al*. (1956), respectively. Total nitrogen, phosphorous, potassium, magnesium and boron were determined according Chapmen and Pratt (1961) while, crude protein was determined according A.O.A.C. (1990). Endogenous phytohormones were quantitatively determined in squash leaves during 2006 season according Koshioka *et al*. (1983) for auxin, gibberellic acid and abscisic acid while, cytokinins were determined according to Nicander *et al*. (1993).

Yield 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 (g/plant), total fruits/plant and total yield (kg/plant) were recorded. Total soluble solids was measured in the juice of squash fruit by using a hand refractometer while, both vitamin C content and titratable acidity were determined according A.O.A.C. (1990).

Data obtained in this study were subjected to statistical analysis according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Phosphate-solubilization

Solubilization efficiency of tricalcium phosphate and rock phosphate by *P. polymyxa* and *B. megaterium* was shown in Table (B). Both strains were able to solubilize of 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*. (2000) and Muthukumar and Udaiyan (2006) found that *P. polymyxa* play 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 due to *P. polymyxa* that play an important role as plant growth promoting rhizobacteria

via P-solubilization (Muthukumar and Udaiyan, 2006) or N_2-fixation (El-Howeity *et al.*, 2003). This might led to accumulate available nutrients and stimulate the microorganisms in rhizosphere soil. 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 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 (Vazquez *et al.*, 2000). 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 (Vivas *et al.*, 2003).

Data in Table (1) also show that relatively high of 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 (Hauka, 2000 and El-Komy, 2005). On the other hand, data also show that mycorrhizal root infection percentage was high in inoculated plants with 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 (Vazquez *et al*., 2000).

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 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*. (2002) who found that available-P content was increased when the plants were inoculated with mycorrhiza and amended with rock phosphate. Han *et al*. (2006) also reported that co-inoculation with Bacillus and phosphate solubilizer in the presence of rock-P increased the availability of phosphorus and potassium 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 enzymes 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*. (2002).

Growth parameters

The growth parameters of squash plants as stem length, diameter and dry weight, leaves number, area and dry weight per plant were significantly increased by all biofertilizers application (Table 3). 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 (2006) 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 (2002) and Giri and Mukerji (2004) 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 has a positive effect on all growth parameters for all treatments especially in the presence of biofertilizers. This may be due to enhancement of boron to sugar synthesis and translocation from plant leaves which in turn may activate the photosynthesis and led to an increase in plant growth (Blevins and Lukaszewski, 1994). Also, Goldbach *et al*. (1990) reported that boron has a role in activation of cell division and cell elongation, which might be attributed to an increase in leaf area.

Photosynthetic pigments and chemical constituents

Results in Tables (4 & 5) 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 carotenoids contents (Table 4) as well as chemical constituents of squash plants (Table 5). It is evident from the obtained data that plants inoculated with AM or *B. megaterium* combined with *P. polymyxa* gave the highest photosynthesis pigments and nutrients content either with or without boron application. In this respect, Abou-Aly and Gomaa (2002) stated that mixed biofertilizers increased both nutrients content and leaf chlorophyll concentration than control. Also, Han *et al*. (2006) 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 (1995) found that boron increased chlorophyll and nutrients 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 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 photosynthesis characters (Tables 3&4) may explain the increase in total carbohydrates in plants (Table 5).

Endogenous phytohormones

Endogenous phytohormones of squash plants as affected by the interaction between biofertilizers and boron are shown in Table (6). 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 (Brian, 2004; Timmusk *et al*., 2005 and Rai, 2006). 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 acid. Generally, application of boron in the presence of biofertilizers improved endogenous phytohormones of squash plants.

Yield and yield components

Data illustrated in Table (7) 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 (2005) 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 significantly increased in response to biofertilizer application compared to control. Also, boron has positive effect on the same parameters.

It may be stated that under such this work conditions, the stimulatory effect of 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 5) as well as endogenous phytohormones (Table 6) as previously resulted and discussed in this work.

Concerning the fruit quality, data presented in Table (8) 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 (2006) reported that applied of rock-phosphate with P-solubilizers especially *B. megaterium* increased sugarcane yield and also, improved the juice quality.

Table (8). Some chemical fruit quality of squash as affected by biofertilizers and boron application in two seasons.

Treatments	Vitamin C mg/100 g fresh weight		Total soluble solids (%)		Titratable acidity (%)	
	S1	S2	S1	S2	S1	S2
Without boron addition						
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						
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**

Generally, the present study is strongly admit the use of phosphate solubilizing microorganisms as an active tool to improve microbial activities and nutrients 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

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.

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.

A.P.H.A, American Public Health Association (1992). Standard methods for the examination of water and wastewater. Washington, D.C., USA.

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.

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.

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.

Bremner, J.M. and D.R. Keeny (1965). Steam distillation method for determination of ammonium, nitrate and nitrite. Annals Chem. Acta, 32: 485-495.

Brian, B.M.G. (2004). Ecology of *Bacillus* and *Paenibacillus* spp. in agricultural system. Phytopathology, 94(11):1252-1258.

Casida, L.E.; D.A. Klein and T. Santoro (1964). Soil dehydrogenase activity. Soil Sci., 98: 371-378.

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, p.309.

Drobnikova, V. (1961). Factors influencing the determination of phosphatase in soil. Folia. Microbiol., 6, 260.

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

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.

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.

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.

Fathy, E.L.E. (1995). Physiological studies on tomatos. Ph.D Thesis, Fac. of Agric., Mansoura Univ., Egypt.

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.

Giovannetti, M. and B. Mosse (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizae infection in roots. New Phytol. 84:489.

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.

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.

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.

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.

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.

Jackson, M.L. (1973). Soil Chemical Analysis. Prentice-Hall of India, Private New Delhi.

Koller, H.R.C. (1972). Leaf area-leaf weight relationship in soybean canopy. Crop Sci., 12:216-220.

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.

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.

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.

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.

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.

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.

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.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiologiya, 17:362-370.

Rai, M.K. (2006). Handbook of microbial biofertilizers. Food Products Press, an imprint of The Haworth Press, Inc, Binghamton, New York.

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.

Snedecor, G.W. and W.G. Cochran (1980). Statistical method 7th Ed., Iowa state University Press, Ames Iowa, USA.

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.

Thomas, W. and R.A. Dutcher (1924). The colorimetric determination of carbohydrates methods. J. Amr. Chem. Soc., 46:1662-1669.

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.

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.

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.

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.

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.

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[illegible] (*G. mosseae*) [illegible] *P. polymyxa* [illegible] (*B. megaterium*) [illegible].

[illegible] .*P. polymyxa* [illegible] *B. megaterium* [illegible] *P. polymyxa* [illegible].

[illegible] *P. polymyxa* [illegible] *B. megaterium* [illegible] *P. polymyxa* [illegible].