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Enhancing Growth, Productivity and Quality of Tomato Plants Using Phosphate Solubilizing Microorganisms

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Abstract: In contribution to a more sustainable agriculture, a comparison was carried out between two plant growth promoting rhizobacteria, namely *Paenibacillus polymyxa* (PP) and *Bacillus megaterium* var. *phosphaticum* (BM) as phosphate dissolvers, in presence of rock phosphate. Biofertilizer treatments were applied individually to tomato plants (*Lycopersicon esculentum* L.) cv. Super Strain B during 2008 and 2009 spring seasons. Effects of rock phosphate and phosphate solubilizing microorganisms on some microbial activities, vegetative characteristics as well as fruit yield and quality of tomato were studied and compared with uninoculated treatment fertilized with mineral phosphate fertilizer (calcium super phosphate) as control. Significant positive effects were obtained with microbial activities (dehydrogenase and phosphatase) vegetative characteristics, photosynthetic pigments, mineral contents (N, P, K and Mg), total sugars, total carbohydrates and crude protein in leaves as well as fruit set, early, total yields and quality (T.S.S., Vit. C and acidity) of tomato in response to addition of rock phosphate with phosphate solubilizing microorganisms when compared with uninoculated treatments. The highest records of all tested parameters were recorded with rock phosphate inoculated with *Paenibacillus polymyxa* plus *Bacillus megaterium* treatment. This work shows that by inoculating phosphate solubilizing microorganisms with rock phosphate under field conditions in Egypt it is possible to obtain tomato yields comparable to those produced by using the expensive calcium super phosphate fertilizer.

Key words: Tomato**,** *Lycopersicon esculentum*, rock phosphate, phosphate solubilizing micro-organisms, fruit yield.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the important vegetable crops which contains some important minerals, vitamins, antioxidants and has high nutritional value. Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other things. According to FAO statistics (2008), tomato crop cultivated area in Egypt was 571844 feddan produced about 4204039 tones. In 2007, Egypt was ranked the fifth world producer of tomato with China in the lead (FAO statistics, 2007).

In favorite to more sustainable agriculture, the present study is aiming to get the most benefits of phosphate fertilizers in alkaline clay loam soil. This type of soil transforms the soluble calcium superphosphate to insoluble forms which are unavailable for plants. This phenomenon is quite famous by using the chemical calcium superphosphate fertilizer or with using natural rock phosphates individually in alkaline soils. This would lead farmers to add more supplementary chemical doses from phosphate fertilizers to avoid building up phosphor deficiency which affect plants by lowering their yield and quality. Furthermore, it will lead to put higher fertilization costs that interfere with sustainable agriculture concepts.

As the world population develops in this new millennium, there will be an increased dependence upon biological processes in soil to provide adequate crop nutrition for the majority of the world's farmers. Although a major increase in the use of artificial fertilizers will be necessary on a global scale, this will not be an option for large numbers of farmers due to their poverty (David *et al*., 2002). Phosphorus (P) is one of the major plant growth-limiting nutrients although it is abundant in soils in both inorganic and organic forms. Phosphate solubilizing micro-organisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to plants in a more environmentally friendly and sustainable manner.

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Although solubilization of P compounds by microbes is very common under laboratory conditions, results in the field have been highly variable. This variability has hampered the large-scale use of PSMs in agriculture. Many reasons have been suggested for this variability, but none of them have been extensively investigated. In spite of the importance of PSMs in agriculture, the detailed biochemical and molecular mechanisms of P solubilization are not known (Gyaneshwar *et al*., 2002). Phosphorus is usually supplied to the plant in many different forms some of which are manufactured, i.e., phosphoric acid and calcium super phosphate, while some others are common in nature form such as rock phosphate.

The appropriate utilization of rock phosphate (RP) as P source can contribute to sustainable agricultural intensification, particularly in developing countries endowed with RP resources, in addition to minimizing environmental pollution in countries where RP are processed industrially. The RP products are an agronomically and economically sound alternative P input to manufactured superphosphates (Zapata and Roy, 2004; Schneider *et al*., 2010). Singh and Reddy (2011) reported that inoculation with phosphate solubilizing fungus along with rock phosphate can substitute the chemical fertilizer in alkaline soil and help in improving the crop production. In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies (Wu *et al*., 2005). Biofertilizers are considered the most advanced biotechnology and can increase the output, improve the quality of crop production through providing the cultivated plants with macro as well as micronutrients, required for healthy growth therefore reduce the overall cost of chemical fertilizers (Shehata and El- Khawas, 2003). These bacteria also increase prospects of using phosphatic rocks in crop production (Khan *et al*., 2009). It is also responsible for developing organic, green and non-polluting agriculture. Microorganisms that allow more efficient nutrients use or increase nutrients availability can provide sustainable solutions for present and future agricultural practices (Rai, 2006).

Some bacteria such as *Paenibacillus polymyxa* (previously *Bacillus polymyxa* (Ash *et al*., 1994)) and *Bacillus megaterium* provide plants with growth promoting substances and play major role in phosphate solubilizing (Abou-Aly *et al*., 2006). Wu *et al*. (2005) found that application of triple inoculants not only increased nutritional assimilation of plant, but also improved soil properties. They observed that half the amount of biofertilizer applications 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 the farmers. The farmers will be interested in such technology especially for high cash crops such as tomato which usually is supplied with overdose chemical fertilizers aiming for higher crop production.

Therefore, the present investigation aimed to overcoming problems of alkaline soil concerning P fixation by providing an environmental friendly alternative for P manufactured fertilizers. This was undertaken by studying the response of tomato plants to rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* as compared to inoculation with either *Paenibacillus polymyxa* or *B. megaterium* var. *phosphaticum* in presence of rock phosphate with regard to growth, yield and fruit quality of tomato.

MATERIAL AND METHODS

In this study, the field experiment was carried out during the two growing spring seasons of 2008 and 2009, at the experimental farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effect of phosphate solubilizing microorganisms with rock phosphate on improving microbial activities, growth, productivity and quality of tomato plants (*Lycopersicon esculentum*, Mill, c.v. Super Strain B). The experimental soil was clay loam and its chemical analyses are shown in Table (A).

Table A: *The chemical properties of the experimental soil during 2008 and 2009 seasons.

Year	pH	EC dS|m	Paste soluble ions (meq\L)						Available (ppm)			Total%		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3	Cl^{-}	N	P	K	N	P	K
2008	8.1	1.25	7.1	3.8	5.8	1.4	4.2	7.0	25.9	90	634	0.25	0.19	0.65
2009	7.9	1.30	6.9	4.0	6.6	1.6	3.8	3.9	23.8	60	475	0.22	0.16	0.48

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Inocula Preparation:

Bacillus megaterium var. *phosphaticum* and *Paenibacillus polymyxa* (pure local strains) were kindly provided by the unit of Bio-fertilizers, Fac. Agric. Ain Shams Univ., Cairo, Egypt. *Bacillus megaterium* var. *phosphaticum* was grown on medium of Bunt and Rovira (1955) modified by Abdel-Hafez (1966) for 72 h at 30˚C. While, *Paenibacillus polymyxa* was propagated in nutrient broth medium and incubated on a rotary shaker (180 rpm) at 30˚C for 5 days (Reyes *et al*., 2006). Inoculants of equal volumes of the suspensions were obtained (about 10^8 cells/ml from each) Thirty-day old tomato transplants were individually soaked in heavy cell suspension of phosphate solubilizing microorganisms for 5 min Arabic gum (10%) was added as an adhesive agent prior to inoculation. The same previous density of cell suspension was used with sterilized peat moss at the rate of 2:1 (V/W) under aseptic conditions. Each inoculum was used at a rate of 1200 g/fed and thoroughly mixed with rock phosphate.

The Treatments Were Designed as Follows:

Control (Check Treatment):

Application of calcium superphosphate (15.5 % P_2O_5) at the recommended dose of mineral P fertilizers (70 kg P_2O_5 Fed^{-1}) without inoculation with phosphate solubilizing microorganisms neither the calcium superphosphate nor the transplants.

RP:

Application of rock phosphate (28.30% P_2O_5) with the recommended dose of mineral P fertilizers (70 kg P_2O_5 Fed^{-1}) without inoculation with phosphate solubilizing microorganisms. Chemical analysis of rock phosphate is illustrated in Table (B).

RP + PP:

Application of rock phosphate and *Paenibacillus polymyxa* was inoculated to transplants and rock phosphate as described previously.

RP + BMP:

Application of rock phosphate and *Bacillus megaterium* var. *phosphaticum* was inoculated to transplants and rock phosphate as described previously.

RP + PP + BMP:

Application of rock phosphate and *Paenibacillus polymyxa* and *Bacillus megaterium* var. *Phosphaticum* were inoculated to transplants and rock phosphate as described previously.

Ammonium sulphate (20.5% N) as N-fertilizer was added at a rate of 400 kg/fed. in three equal doses, i.e., at 30, 45 and 60 days after transplanting, for all treatments. Potassium sulphate (48 % K_2O) was applied at a rate of 200 kg K_2O fed^{-1} at two times. The first portion took place before transplanting, whereas, the second part was added one month later. Other standard agricultural practices for growing tomato were carried out as recommended by the Ministry of Agriculture.

Table B: **The chemical properties of Egyptian rock phosphate.

ELEMENT	P_2O_5	CaO	Fe_2O_3	SiO_2	F	SO4	$CaCo_3$	Humidity
RESULT (%)	28.30	44.61	1.48	6.91	2.70	1.20	11.15	3.64%

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The transplants were set up into the field on 21st and 23rd of March in 2008 and 2009 spring seasons, respectively. The area of the experimental plot was 20 m^2 consisted of five ridges, each ridge was 4 m length and 1 m width. The plant distance was 30 cm apart on one ridge.

The Experimental Design:

The experiment was laid out in a complete randomized block design with four replicates

Studied Characteristics:

Microbial activities of plant's rhizhosphere after 50 days from transplanting were conducted. The samples were analyzed for dehydrogenase activity according to the method described by Casida *et al*. (1964), while phosphatase activity was determined according to Drobnikova (1961). In addition, rhizosphere samples were analyzed for available phosphorus according to A.P.H.A, (1992).

After 65 days from transplanting, five plants per plot were randomly chosen to measure plant height, stem diameter, number of branches/plant, number of leaves/plant and leaf area using the disk method according to Koller (1972). Photosynthetic pigments (chlorophyll and carotenoids) were determined, in the fourth leaf, calorimetrically as described in A.O.A.C. (1990). Total nitrogen, phosphorous, potassium and magnesium was determined in the fourth leaf, according to Chapmen and Pratt (1961), total sugars and total carbohydrates were determined, in the fourth leaf, according to Thomas and Dutcher (1924) and Dubois *et al*. (1956), respectively, while crude protein was determined according A.O.A.C. (1990).

Fruit setting (number of fruits per plants / number of flowers per plant using ten labelled plants from the 2nd and 3rd ridges in each plot), early yield (as the first two pickings), total yield per plant, plot and feddan were recorded. Fruit juice of randomly selected ripe-fruits was used to measure total soluble solids by using a hand refractometer, while both vitamin C content and titratable acidity were determined according A.O.A.C. (1990).

Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.) software. The comparison among means of the different treatments was determined as illustrated by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Microbial Activities:

Data in Table (1) show the enzymatic activities in rhizosphere of tomato plants. Dehydrogenase activity (DHA), represents the energy transfer, therefore, it is considered as an index of overall microbial activity in the soil. Rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* resulted in the highest values of DHA followed in decreasing order by *P. polymyxa* without significant difference between them during the both seasons. Inoculation with *P. polymyxa* significantly increased DHA than *B. megaterium* treatment, only in the second season, while the two treatments were not significantly different in the first season despite the trendy for higher DHA with *P. polymyxa*. This may be due to that *P. polymyxa* plays an important role as plant growth promoting rhizobacteria via P-solubilization (Muthukumar and Udaiyan, 2006), producing plant growth promoters such as IAA and cytokinins (Timmusk *et al*., 1999). This might lead to accumulate available nutrients and stimulate the microorganisms in rhizosphere soil. In this respect, the biological activity of the microorganisms would have helped the soil status to become a ready to serve zone for essential nutrients to plant's root system (Premsekhar and Rajashree, 2009).

Concerning phosphatase activity, the maximum values of phosphatase activity were significantly recorded with the application of rock phosphate with *Paenibacillus polymyxa* and *Bacillus megaterium*. This may be attributed to its contents of the potential phosphate solubilizing bacteria. Using soluble P-fertilizer in uninoculated treatment decreased phosphatase activity to the minimum than those treatments fertilized with insoluble-P as rock phosphate. This may be due to the P cycle enzyme activities which are inversely related to P-availability so that 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). On the other hand, there were positive effects of inoculations to produce higher phosphatase especially with *P. polymyxa*. These results are in harmony with those obtained by Khan and Khan (2006).

Data in Table (1) also show that significant increases in available P were observed in inoculated tomato treatments compared to uninoculated ones. The highest significant available P in rhizosphere was recorded with rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* treatment. There were no significant differences between *P. polymyxa* and *B. megaterium* regarding this aspect.

Table 1: Some microbial activities in rhizosphere of tomato plants as affected by rock phosphate with phosphate solubilizing microorganisms in the two seasons (2008 and 2009).

Treatments	Dehydrogenase activity (µl DHA/g soil/day)		Phosphatase activity (µg inorganic phosphorus/g dry soil/day)		Available phosphore in rhizosphere (ppm)	
	2008	2009	2008	2009	2008	2009
Cont	27.61	33.71	19.30	17.50	61.9	66.1
RP	28.85	31.97	23.85	25.03	80.9	75.1
RP + PP	39.28	56.45	36.61	38.39	118.1	115.2
RP + BMP	38.41	45.90	30.93	34.40	104.7	109.7
RP + PP + BMP	41.41	60.54	47.91	45.47	152.7	159.2
LSD at 5%	3.45	4.19	2.67	2.74	11.6	17.6

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate. PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

These results may be due to the observed high rates of phosphatase activities (Table 1) which could be reflected on the available P. Similar results were reported by Mehasen *et al*. (2002) who found that available-P content was increased when the plants were inoculated with P-solubilizer and amended with rock phosphate. Han *et al*. (2006) also reported that inoculation with phosphate solubilizer in the presence of rock-P increased the availability of phosphorus in the soil. These observations are also in harmony with those obtained by

Zaghloul *et al*. (2002) and Abou-Aly *et al*. (2006). Similarly, *B. Megaterium* from tea rhizosphere was able to solubilize phosphate and thus it helps the plant growth promotion as reported by Saharan and Nehra (2011).

Vegetative Characteristics:

Plant height was not significantly different among all treatments in the first season although it was significantly slightly shorter by phosphate solubilizing microorganisms combined with rock phosphate treatments compared the control in the second season (Table 2). On the contrary, other growth parameters such as stem diameter, number of branches, number of leaves and leaf area/plant had significantly higher values under application of phosphate solubilizing microorganisms combined with rock phosphate treatments compared to control, the highest significant values recorded with rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* followed by *P. polymyxa*. This could be attributed to the highest values of available P compared to other treatments (Table 1). The effect of phosphate solubilizing bacteria on growth may be due to the activity of P solubilization caused by the used strain and increased further mineral availability uptake (Table 4). Also, Premsekhar and Rajashree (2009) and El-Tantawy and Mohamed (2009) showed that the increase in growth characters might be due to the fact that phosphate solubilizing bacteria inoculated plants were able to absorb nutrients from solution at faster rates than uninoculated plants resulting in accumulation of more N, P and K in the leaves. These results agree, to a great extent, with those obtained by Castagno *et al*. (2011), who found that the isolates (phosphate-solubilizing strains) obtained in this study showed a significant in vitro plant-growth promoting activity. 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 P-solubilization, production of growth promoting plant hormones especially cytokinins and production of antibiotics as well as improvement of soil porosity. In addition, *B. megaterium* from tea rhizosphere was able to solubilize phosphate and enhanced the plant growth promotion (Saharan and Nehra, 2011).

Table 2: Effect of rock phosphate with phosphate solubilizing microorganisms on vegetative growth of tomato plant in the two seasons (2008 and 2009).

Treatments	Plant height (cm)		Stem diameter (cm)		No. of branches/ plant		No. of leaves / plant		Leaf area/ plant (cm^2)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Cont	40.13	42.70	0.98	0.87	6.25	7.15	28.15	29.25	1917.6	2020.6
RP	41.16	39.40	1.00	1.11	6.70	7.18	27.17	26.5	2513.6	2743.8
RP + PP	39.75	38.85	1.15	1.20	7.70	8.20	29.80	30.75	2518.3	2540.4
RP + BMP	38.25	40.75	1.10	1.18	9.20	9.85	29.40	31.90	2286.9	1982.3
RP + PP + BMP	37.90	38.45	1.35	1.31	11.25	12.15	32.70	33.18	2845.5	2903.2
LSD at 5%	5.11	3.90	0.13	0.10	1.64	2.10	1.95	1.57	176.8	154.1

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate.
PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

Photosynthetic Pigments and Chemical Constituents:

Results in Tables (3&4) show that maximum enhancement of chlorophyll and carotenoid contents (Table 3) as well as chemical constituents (N, P, K, Mg, total sugars and carbohydrates and crude protein) (Table 4) of tomato plants was observed in plants treated with rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium*.

The enhancing effect of rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* on chlorophyll content may be due to that rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* increased total sugar (Table 4) and the latter are essential for chlorophyll formation. In this respect, Abou-Aly and Gomaa (2002) stated that biofertilizers increased both nutrient content and leaf chlorophyll concentration than control. Also, Han *et al*. (2006) found that the integrated treatment of P-solubilizers and application of rock-P significantly increased leaf photosynthesis over the control. Similar results were reported by El-Tantawy and Mohamed (2009) who found that the biofertilizers increased P availability and other nutrients.

In this study, the higher availability of P for plant growth due to the application of *P. polymyxa*, *B. megaterium* or rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* led to large increase in number and area of leaves (Table 2) as well as the photosynthetic pigments (Table 3) and both parameters might be reflected on the rate of photosynthesis which is sufficient to support plant growth and production. This may explain the increase in total carbohydrates recorded in the plants (Table 4). Castagno *et* *al*. (2011) and Biswas *et al*. (2009) reported that the use of these bacteria as bioinoculants could be a sustainable practice to facilitate the nutrient supply to Lotus tenuis plants and preventing negative side-effects such as eutrophication.

Table 3: Effect of rock phosphate with phosphate solubilizing microorganisms on photosynthetic pigments of tomato plants in the two seasons (2008 and 2009).

Treatments	Chlorophyll (mg/g F.W) (a)		(b)		(a+b)		Carotenoids (mg/g F.W)		Chl.(a+b) /Carot.	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Cont	0.430	0.420	0.370	0.364	0.800	0.784	0.250	0.280	3.200	2.800
RP	0.450	0.525	0.385	0.355	0.835	0.880	0.310	0.317	2.694	2.776
RP + PP	0.640	0.620	0.420	0.455	1.060	1.075	0.340	0.360	3.118	2.986
RP + BMP	0.635	0.590	0.390	0.375	1.025	0.965	0.325	0.337	3.154	2.864
RP + PP + BMP	0.890	0.870	0.495	0.511	1.385	1.381	0.395	0.388	3.506	3.559
LSD at 5%	0.050	0.06	0.040	0.050	0.110	0.130	0.050	0.053	0.180	0.220

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate.
PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

Table 4: Effect of rock phosphate with phosphate solubilizing microorganisms on some nutrients and biochemical constituents of tomato leaves in the two seasons (2008 and 2009).

Treatments	N (mg/g DW)		P (mg/g DW)		K (mg/g DW)		Mg (mg/g DW)		Total sugars (Mg/g DW)		Total carbohydrates (mg/g DW)		Crude protein (mg/g DW)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Cont	31.54	33.72	4.80	4.45	38.40	39.20	4.15	4.25	22.60	26.72	420.5	430.7	197.13	210.75
RP	35.70	34.50	4.95	4.85	41.70	42.20	4.20	4.12	21.33	28.40	435.7	425.8	223.13	215.63
RP + PP	37.91	38.65	5.84	5.70	45.70	46.10	5.10	5.35	29.12	33.75	550.8	518.7	236.94	241.56
RP + BMP	36.75	35.45	5.20	4.90	44.75	46.80	4.80	4.75	30.40	34.90	490.5	488.5	229.69	221.52
RP + PP + BMP	42.11	43.70	6.90	6.40	47.50	48.90	5.95	5.80	33.60	35.50	610.9	625.4	263.19	273.13
LSD at 5%	1.28	0.95	0.06	0.08	2.12	1.45	0.22	0.28	2.72	2.12	42.7	29.1	9.78	4.11

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate.
PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

Yield and Yield Components:

Data illustrated in Table (5) show that application of phosphate solubilizing microorganisms combined with rock phosphate significantly increased fruit set percentage which might the reflected on early and total yields compared with the control. Among biofertilizer treatments, rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* showed significantly the highest values compared to inoculation with *P. polymyxa* or *B. megaterium*. Moreover, early and total yield were significantly increased in response to inoculation of phosphate solubilizing microorganisms combined with rock phosphate compared to control. The application of rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* as P-solubilizer produced the highest early and total yields compared to the other treatments. Similar results were reported by El-Tantawy and Mohamed (2009), Mohamed and Ibrahim (2011) and Premsekhar and Rajashree (2009).

Table 5: Effect of rock phosphate with phosphate solubilizing microorganisms on fruit setting and yield of tomato plant in the two seasons (2008 and 2009).

Treatments	Fruit setting (%)		Early yield (kg/plant)		Total yield (kg/plant)		Total yield (kg/plot)		Total yield (ton/fed.)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Cont	28.89	29.42	0.37	0.31	1.59	1.44	104.9	95.0	19.94	18.06
RP	26.75	21.46	0.39	0.38	1.33	1.40	87.7	92.4	16.68	17.56
RP + PP	33.87	33.71	0.44	0.51	1.93	1.86	127.3	122.7	24.20	23.32
RP + BMP	31.74	34.35	0.41	0.59	1.76	1.77	116.1	116.8	22.07	22.20
RP + PP + BMP	39.42	36.42	0.64	0.89	2.21	2.35	145.8	155.1	27.71	29.47
LSD at 5%	2.40	2.47	0.06	0.08	0.06	0.071	2.51	2.5	1.02	1.02

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate.
PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

These increases may be attributed to the beneficial effects of P-solubilizer that can decompose organic and insoluble phosphates by utilizing organic compounds as carbon and energy source and produce organic acids that solubilize insoluble phosphate, which improved crop yield. Furthermore, rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* had beneficial effects on dehydrogenase and phosphatase activities (Table 1), vegetative characters (Table 2), improved photosynthetic pigments (Table 3), and increased nutrients, total sugar and carbohydrates contents (Table 4) which eventually must have been reflected on the yield in this study.

According to data in Table (6), vitamin C, total soluble solids, and titratable acidity, as chemical constituents of fruits, were significantly increased with the different inoculation treatments. Inoculation with *P. polymyxa* and *B. megaterium* improved the fruit quality than uninoculanted treatments. In addition, the same positive trend was recorded in the treatment that received rock phosphate with *Paenibacillus polymyxa* and *Bacillus megaterium*. These results are in harmony with those obtained by Khan and Khan (2006) found that treatment with the phosphate solubilizing microorganisms tested resulted in significant increase in total soluble solids of tomato. In this respect, it was reported that application of rock-phosphate with P-solubilizers especially *B. megaterium* increased sugarcane yield and improved juice quality (Rai, 2006) and improved fruit quality of squash (Abou-Aly *et al.* , 2006) and wheat (Babana and Antoun, 2006).

Table 6: Effect of rock phosphate with phosphate solubilizing microorganisms on chemical constituents of tomato fruits in the two seasons (2008 and 2009).

Treatments	Vitamin C mg/100g FW		Total soluble solids (%)		Titratable acidity (%)	
	2008	2009	2008	2009	2008	2009
Cont	120.25	122.75	3.10	3.12	0.209	0.215
RP	125.70	121.50	3.05	3.17	0.205	0.217
RP + PP	133.17	135.40	4.05	4.00	0.210	0.235
RP + BMP	128.40	131.72	3.75	3.50	0.225	0.230
RP + PP + BMP	137.70	138.95	4.45	4.20	0.241	0.294
LSD at 5%	5.12	4.71	0.22	0.21	0.070	0.090

Cont: uninoculated treatment used mineral phosphate fertilizer (calcium super phosphate).
RP: Rock phosphate.
PP: *Paenibacillus polymyxa*.
BMP: *Bacillus megaterium* var *phosphaticum*.

Based on the present study, it could be concluded that microbial inoculation, in general, has a simulative impact upon growth, productivity and quality of tomato plants. This effect was more obvious with dual inoculation of bacteria (phosphate solubilizing microorganisms) to rock phosphate since it gave the most positive synergetic effect compared with other treatments as chemical calcium super phosphate fertilizer or natural rock phosphate individually. The positive effect was clearly reflected on fruit setting, high early and total yield and good fruit quality. Through that a new environmental friendly alternative for P fertilization is being locally developed that can overcome soil problems regarding P fixation and contribute to environmental saving through reducing chemically synthesized P fertilizers. The positive response of tomato plants to different inoculation with P-solubilizer, in particularly to rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium*, indicate that there is a marked useful and promising effect of both systems. Generally, phosphate solubilizing microorganisms play an important role in plant nutrition and allow the use of cheaper crude P sources such as rock-phosphate instead of superphosphate. The relative importance of these findings to organic farming systems is very clear especially with the other plant growth promoting effects of those microbes which make them important contributor to biofertilization of agricultural crops.

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