Response of Balady Guava Trees Cultivated in Sandy Calcareous Soil to Biofertilization with Phosphate Dissolving Bacteria and / or VAM Fungi

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Abstract: Phosphate dissolving bacteria (*Bacillus megaterium*) and /or vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae, G. fasciculatum* and *G. aggregatum*), were used as biofertilizers for Balady guava (*Psidium guajava L.*) trees, grown in newly reclaimed sandy calcareous soil, during two successive seasons (2007-2008 and 2008-2009). Obtained results showed that, fertilization of guava trees with either VAM, *B. megaterium* or the dual fertilizer (i.e. VAM plus *B. megaterium*), significantly increased growth of the trees, leaf area, leaf nitrogen, phosphorus, potassium, calcium, and magnesium contents, compared to the un-fertilized (control). The highest values of these measurements were recorded for trees fertilized with the dual fertilizer, followed by the trees fertilized with VAM, then those which received *Bacillus megaterium.* In both seasons, fertilization of guava trees with VAM fungi and/or *B. megaterium*, significantly increased fruit weight, fruit number/tree and consequently higher yields (kg./tree) were achieved, compared to the control. The recorded values of the total soluble solids %, L ascorbic acid and pectin in fruits of the inoculated trees were significantly higher than the control. The highest values of these measurements were recorded for trees which received dual fertilizer (VAM plus *B. megaterium*). Titratable acidity was found to be significantly higher in the fruits of the control than the inoculated ones. In both seasons the lowest values of titratable acidity were recorded for the fruits of the trees inoculated with VAM plus *B. megaterium.* [Journal of American Science. 2010;6(9):399-404]. (ISSN: 1545-1003).

Key words: *Psidium guajava*, biofertilization, *Bacillus megaterium*, mycorrhizal, fungi, VAM.

1. Introduction:

Guava (*Psidium guajava L.*) is one of the major horticultural crops throughout the tropical and subtropical zones. In Egypt, guava is a popular fruit, cheap and rich source of vitamin C. Furthermore guava is one of the leading fruit trees in new reclaimed soil in Egypt because of its high adaptability and thrives in these soils.

Like other plants suffer from the common alkalinity (usually above 7) of the Egyptian soils which results to the conversion of applied inorganic phosphorous fertilizer to precipitated form of $Ca_3(PO_4)_2$, which is unavailable to the growing plants (El-Gibaly *et al*.,1977 and Zayed 2005).

Bacillus megaterium produces large amounts of organic acids, which increases the soil acidity and converts the insoluble forms of phosphorus into soluble ones (Zayed, 1997 and Hammad, 1999). Consequently, the use of these bacteria as a biofertilizer in the alkaline soils is very important and essential to increase the availability of soil phosphorus.

Furthermore, the obligate symbiotic microorganisms Vesicular-arbuscular mycorrhizal (VAM) fungi form was associated with plant roots in a host-nonspecific manner (Nelson and Achar, 2001). The principal function of these associations is enhancing the solubility of different nutrients especially phosphorous and the efficiency of its absorption. It has been shown that mycorrhizal plants can absorb and accumulate more phosphate from the soil than non-mycorrhizal plants (Mosse *et al.,* 1981 Marschner, 1995 and Bâ *et al*., 2001). Trees treated with mycorrhiza accumulate more K, Ca, Cu and Mn in the leaf compared with the non-treated trees (Miller *et al.,* 1985). VAM fungi interact with other soil microbes like the free-living nitrogen fixers and phosphate solubilisers to improve their efficiency for the biochemical cycling of elements to the host plants (Linderman, 1988).

The aim of this study was to investigate the effect of the phosphate biofertilizers dissolving bacteria (*Bacillus megaterium*) and/or mycorrhizal (VAM) fungi (*Glomus mosseae, G. fasciculatum* and *G. aggregatum*) on growth, leaf mineral contents, yield, and fruit quality of guava trees *cv.* Balady cultivated in newly reclaimed sandy calcareous soil.

2. Materials and Methods:

The experimental location

This study was carried out during two successive seasons (2007/2008 and 2008/2009) in a private Balady guava trees farm (*psidium guajava L*) at Sedmant Al-Gabal Ahnassia District, Beni Suef Governorate. Forty uniform in vigor, eight years old

Balady guava trees (*P. guajava L*) were selected and the planting space was 4 x 4 m.

Soil type

The soil of the selected experimental farm was sandy calcareous soil. The mechanical and chemical properties of the soil are shown in Table (1).

Table (1): Physical and chemical properties of the used experimental farm soil.

Soil character	Values
Sand %	80.30
Silt %	13.51
Clay %	6.19
Texture	sandy
Organic matter %	0.8
pH (1 :2.5 extract)	8.2
Total $CaCO_3$ %	7.0
Total N%	0.09
Available P (Olsen, ppm)	3.25
Exch. K+ (mg/100g)	18.9
Exch. Ca++ (mg/100g)	22.9

Microorganisms

Strain of *Bacillus megaterium* var. *phosphaticum* efficient in dissolving phosphate and three species of vesicular-arbuscular Mycorrhizal (VAM) fungi (*Glomus mosseae*, *G. fasciculatum* and *G. aggregatum*) were kindly supplied by the Department of Agricultural Microbiology, Faculty of Agriculture, Minia University. Egypt.

Preparation of inocula:

B. megaterium var. *phosphaticum* strain was grown in 200 ml Erlenmayer flasks each containing 100 ml of nutrient broth medium (Allen, 1959) and incubated in shaking incubator at 30°C for 96 h. (giving approximately 16×10^9 cell/ml). This liquid culture was used as phosphate dissolving bacterial inoculum.

VAM inoculum was prepared in fired clay pots of 30 cm in diameter, filled with steam sterilized sandy loam soil. The soil in each pot was inoculated with the spores of the three species of VAM fungi. Five onion seedlings were transplanted in each pot as a host plant. At the end of the growth stage of onion, plants were uprooted. The soil in the pots was mixed together and VAM spores were counted as described by Musandu and Giller (1994). The spore count was found to be 126 spores/10 g. soil. This soil containing mixture of VAM spores, mycelia and chopped roots was kept to be used as VAM inoculum.

Experimental design and treatments

The selected guava trees were divided into four groups each consisted of 10 trees. The groups were treated as follows:

The first group was treated with the prepared phosphate dissolving bacterial biofertilizer.

The second group was treated with the prepared VAM fungal biofertilizer.

The third group was treated with dual biofertilizer (i.e. phosphate dissolving bacteria and VAM fungi)

The fourth group was left without any treatment and used as a control.

Biofertilization was carried out by mixing 200 ml of the prepared bacterial inoculum (in case of the bacterial biofertilization), 1 kg. of the prepared VAM inoculum (in case of the mycorrhizal biofertilization) or both (in case of the dual biofertilization) with 10 kg of farmyard manure. The mixture was added to each tree (according to the experimental design described above) as a circle surrounding the trunk just before irrigation. Each of the un-treated trees (control) received the same amount of farmyard manure (10 kg.). The biofertilizers were applied once at the winter during each season.

All of the selected trees received the common horticultural practices of guava cultures (300 g nitrogen /tree, 160g P_2O_5/tree and 150 g K_2O/tree).

The experiment was arranged in a randomized complete blocks design with five replicates (each replicate comprised two trees).

Sampling and measurements

The following measurements were recorded during both seasons:

1-Shoot length (cm) and leaf area (cm^2) were measured at the last week of July. Leaf area was measured for the mature leaves, which occupied the middle part of spring growths at the four main directions on the tree. Leaf area was calculated according to the equation reported by Ahmed and Morsy (1999) as follow:

Leaf area = 0.72 (L x W) + 2046.

Where L = length of leaf W= width of leaf.

2-The leaves used for leaf area measurements were used were used to determine N, P, K, Ca and Mg contents. The leaves were dehydrated at 80°C overnight, ground to fine powder. Nitrogen was determined by kjeldhal method and phosphorus was determined colourmetrically. Leaf K, Ca and Mg contents were determined using atomic absorption spectrophotommetry (Perkin Elmer 280)

3-Fruit weight (g) and number of fruits per tree were recorded at harvesting time, then the average yield (kg/tree) was calculated.

4-Two fruits from each side of the tree at harvesting time were taken to determine fruit physical properties, *i.e.* fruit longitudinal (cm), fruit diameter (cm) and flesh thickness (cm). The fruit total soluble solids, and titratable acidity were determined according to A.O.A.C. (1990).

L-Ascorbic acid (vitamin C) mg/100g fresh weight was determined by titration with 2-6 Dichlorophenol-Indophenol (A.O.A.C. (1990).

Pectin was extracted and determined by precipitation as calcium pectate, according to Ranganna (1977).

Statistical analysis was carried out according to Snedecor and Cochran (1980) using L.S.D parameter at 5%.

3. Results and Discussion:

Growth of trees

The shoot lengths and leaf area were taken as indication for the growth of guava trees. As shown in Table 2 in both seasons (2007/2008 and 2008/2009) inoculation of guava trees with either VAM, *B. megaterium* or the dual bio-fertilizers significantly increased growth of the trees compared to the control. The highest values of shoot length and leaf area were recorded for trees fertilized with the dual fertilizer followed by trees fertilized with VAM, then those which received *B. megaterium* fertilizer.

The increase in the growth of the biofertilized trees may be due to the ability of *B. megaterium* to produce some growth promoting substances such as IAA, gibberellins and abssicic acid, it is also well known that *B. megaterium* produces organic, inorganic acids and CO_2 which lead to increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, 1977). While, VAM fungi produces hyphae, which are microscopic tubes colonize plant roots and grow out into the soil further than root hairs. Nutrients are taken up by the hyphae to the plant, which lead to a very efficient mobilization and uptake of nitrogen, potassium, magnesium, copper, zinc, boron, sulphur and other elements that are transported to the plant. The VAM hyphae also help in retaining moisture around the root zone of plants (Mosse *et al.*, 1981; Bâ *et al.*, 2001 and Morte *et al.*, 2001).

Table (2): Effect of inoculation with *Bacillus megaterium* and/or mycorrhiza on growth and leaf mineral contents of guava trees cultivated in sandy calcareous soil.

Growth season	Treatments	Growth parameters		Leaf mineral contents				
		Shoot lengths	**L. area (cm^2)**	**N%**	**P%**	**K%**	**Ca%**	**Mg%**
2007/2008	Control	74.3	69.18	1.28	0.121	1.29	1.28	0.270
	Mycorrhiza	92.1	92.75	1.65	0.150	1.44	1.53	0.348
	B.megaterium	89.4	83.87	1.45	0.148	1.34	1.25	0.315
	Dual inoculum*	95.3	95.28	1.70	0.153	1.46	1.55	0.377
	LSD $_{0.05}$	*7.90*	*7.64*	*0.23*	*0.018*	*0.088*	*0.125*	*0.073*
2007/2009	Control	78.4	66.38	1.30	0.124	1.23	1.28	0.242
	Mycorrhiza	96.3	89.17	1.68	0.158	1.39	1.48	0.415
	B. megaterium	95.7	86.75	1.51	0.156	1.31	1.39	0.280
	Dual inoculum*	101.1	94.45	1.80	0.159	1.48	1.53	0.427
	LSD $_{0.05}$	*8.90*	*5.94*	*0.27*	*0.019*	*0.082*	*0.127*	*0.051*

*Dual inoculum (i.e. VAM plus *B. megaterium*),

Gupta *et al.* (2002) reported that VAM fungi interact with other soil microbes like free-living nitrogen fixers and phosphate solubilisers to improve their efficiency for the biochemical cycling of elements to the host plants. This may explain why the highest growth values were detected in trees inoculated with the dual inoculum (VAM combined with *B. megaterium*).

Leaf mineral contents

Data presented in Table (2) indicate that in both seasons (2007/2008 and 2008/2009), bio-fertilization of guava trees with VAM fungi alone or combined with *B. megaterium*, significantly increased the leaf nitrogen, phosphorus, potassium, calcium, and magnesium contents compared to the uninoculated trees. This may be due to the ability of VAM fungi in supplying the host plants with nutrient requirements. Marschner and Dell (1994) stated that mycorrhiza infection is known to enhance plant growth by increasing absorption of nitrogen,

potassium, magnesium, copper, zinc, boron, sulphur, molybdenum and other elements. Pearson and Gianinazzi (1983) reported that the VAM fungi improves plant growth in the low phosphate soils by exploiting large areas of soil and actively transporting the phosphate up to the plants. Gupta *et al.* (2002) stated that VAM inoculation significantly increased the uptake of N, P and K by shoot tissues of mint, but most markedly increased the uptake of P. The VAM-inoculated mint plants depleted the available N, P and K in the rhizosphere soil as compared to un-inoculated control plants, however the extent of nutrient depletion was greater for P than N and K.

Among the tested nutrients only phosphorus increased significantly in leaves of the trees fertilized with *B. megaterium* and no significant increases were observed in the leave contents of the other nutrients (N, K, Ca and Mg) compared to control trees (un-fertilized). The increase in phosphorus content of *B. megaterium* inoculated trees may be due to the ability of these bacteria to produce organic, inorganic acids and CO_2 which lead to an increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, 1977).

Yield and physical properties of the fruits

As shown in Table (3) in both seasons (2007/2008 and 2008/2009) bofertilization of guava trees with VAM fungi and/or *B. megaterium*, significantly increased fruit weight and fruit number/tree and consequently higher yields (kg./tree) were attained as compared to the un-fertilized ones (control). The highest values of these measurements were recorded in the trees biofertilized with VAM combined with *B. megaterium*. Such observation may be due to the ability of VAM fungi to interact with other soil microbes like the free-living nitrogen fixers and phosphate solubilisers to improve their efficiency for the biochemical cycling of elements and supply the host plants with their nutrients requirements. These results are in agreement with those of Bâ *et al.* (2001) and Ibrahim (2009) who reported that the use of *B. megaterium* and VAM fungi as biofertilizers enhanced grapevines growth and increased the fruit yield.

Table (3): Effect of inoculation with *Bacillus megaterium* and/or mycorrhiza on fruit yield and fruit chemical properties of guava trees cultivated in sandy calcareous soil.

Growth season	Treatments	Yield & Its components			Fruit physical properties		
		Fruit weight (g)	**Fruit number**	**Yield (kg/tree)**	**Fruit height**	**Fruit diameter**	**Flesh thickness (cm)**
2007/2008	Control	102.4	112.0	**11.47**	7.72	6.67	1.04
	Mycorrhiza	108.6	118.3	**12.85**	8.25	6.85	1.30
	B. megaterium	107.6	117.7	**12.66**	8.13	6.78	1.23
	Dual inoculum*	109.5	120.8	**13.23**	8.35	6.90	1.40
	LSD 0.05	*5.1*	*6.29*	**1.11**	*0.38*	*0.09*	*0.18*
2008/2009	Control	104.8	104.9	**10.99**	7.66	6.52	1.03
	Mycorrhiza	112.2	119.3	**13.39**	8.35	6.98	1.40
	B. megaterium	112.8	110.5	**12.46**	8.10	6.90	1.28
	Dual inoculum*	115.9	121.0	**14.02**	8.49	7.02	1.45
	LSD 0.05	*5.8*	*9.5*	**1.91**	*0.81*	*0.31*	*0.19*

***** Dual inoculum (i.e. VAM plus *B. megaterium*),

The use of VAM fungi and/or *B. megaterium* as biofertilizers for guava trees led not only to give higher fruit yield, but also enhanced fruit physical properties. As shown in Table 3, values of fruit height, diameter and flesh thickness, were significantly increased in the trees inoculated with VAM fungi and/or *B. megaterium* compared to the unfertilized ones. The highest values of fruit height, diameter and flesh thickness were recorded for trees which received dual inoculum (i.e. VAM plus *B. megaterium*).

Chemical properties of the fruits

Data presented in Table 4 indicate that in both seasons (2007/2008 and 2008/2009) biofertilization of guava trees with VAM and/or *B. megaterium* improved the fruit chemical properties compared to the un-biofertilized ones. The recorded values of total soluble solids, L-Ascorbic acid and

pectin contents in fruits of the fertilized trees fruits were significantly higher than in the un-fertilized ones. The highest values of these measurements were recorded for fruits of the trees, which received the dual fertilizer (VAM plus *B. megaterium*). Titratable acidity was significantly higher in uninoculated trees fruits compared to the inoculated ones.

Table (4): Effect of inoculation with *Bacillus megaterium* and/or mycorrhiza on fruit chemical properties of guava trees cultivated in sandy calcareous soil.

Growth season	Treatments	Fruit chemical properties			
		TSS %	**T. Acidity (%)**	**Vitamin C (mg/100g f w)**	**Ca. pectat (mg/100g d w)**
2007/2008	Control	8.57	1.062	87.8	7.18
	Mycorrhiza	10.53	0.914	110.0	12.45
	B. megaterium	9.80	0.881	104.7	12.55
	Dual inoculum*	10.98	0.866	106.3	13.78
	LSD $_{0.05}$	*1.14*	*0.103*	*13.05*	*4.16*
2008/2009	Control	8.73	1.071	90.3	8.95
	Mycorrhiza	10.08	0.924	107.8	12.33
	B. megaterium	10.27	0.893	102.8	12.73
	Dual inoculum*	11.60	0.888	111.3	14.90
	LSD $_{0.05}$	*1.03*	*0.154*	*12.2*	*2.102*

***** Dual inoculum (i.e. VAM plus *B. megaterium*),

The lowest values of titratable acidity were recorded for the fruits of the trees fertilized with VAM plus *B. megaterium*. These results are in agreement with those of Bâ *et al*. (2000) and Ibrahim (2009).

On the basis of the obtained results it may be concluded that fertilization of guava trees with VAM and/or *B. megaterium* improved growth of the trees, increase leaf mineral contents and fruit yield of high quality. This observation was markedly pronounced in the trees fertilized with dual fertilizer (VAM and *B. megaterium*). Therefore, application of the dual fertilizer (VAM and *B. megaterium*) as biofertilizer for guava trees is highly recommended to enhance growth of the trees and consequently produce high yield of good quality.

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