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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 (٢٠٠٧-٢٠٠٨ and ٢٠٠٨-٢٠٠٩).

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 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 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*.

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 newly reclaimed soil in Egypt because of its high adaptability to thrives in these soils.

Like other plants suffer from the common alkalinity (usually above ٧) of the Egyptian soils which results to the conversion of applied inorganic phosphorous fertilizer to precipitated form of $Ca_{٣}(PO_{٤})_{٢}$, which is unavailable to the growing plants (El-Gibaly *et al.*,١٩٧٧ and Zayed ٢٠٠٥).

Bacillus megaterium produces large amounts of organic acids, which increase soil acidity and convert the insoluble forms of phosphorus into soluble ones (Zayed, ١٩٩٧ and Hammad, ١٩٩٩). 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, ٢٠٠١). 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.*, ١٩٨١ Marschner, ١٩٩٥ and Bâ *et al.*, ٢٠٠١). Trees treated with mycorrhiza accumulate more K, Ca, Cu and Mn in the leaf compared with the un-treated trees (Miller *et al.*, ١٩٨٥). 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, ١٩٨٨).

Response of guava trees to biofertilization

The aim of the this study was to investigate the effect of 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.

MATERIALS AND METHODS

Experimental location

This study was carried out during two successive seasons (٢٠٠٧/٢٠٠٨ and ٢٠٠٨/٢٠٠٩) 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 ٤×٤ 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 ١.

Table ١: Physical and chemical properties of experimental soil.

Soil character	Values
Sand %	٨٠.٣٠
Silt %	١٣.٥١
Clay %	٦.١٩
Texture	sandy
Organic matter %	٠.٨
pH (١ :٢.٥ extract)	٨.٢
Total CaCO٣ %	٧.٠
Total N%	٠.٠٩
Available P (Olsen, ppm)	٣.٢٥
Exch. K+ (mg/١٠٠g)	١٨.٩
Exch. Ca++ (mg/١٠٠g)	٢٢.٩

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 ٢٠٠ ml Erlenmayer flasks each containing ١٠٠ ml of nutrient broth medium (Allen, ١٩٥٩) and incubated in shaking incubator at ٣٠°C for ٩٦ h. (giving approximately ١٦ × ١٠^{٩} cell/ml). This liquid culture was used as phosphate dissolving bacterial inoculum.

VAM inoculum was prepared in fired clay pots of ٣٠ 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 (١٩٩٤). The spore count was about ١٢٦ spores/١٠ g. soil. The 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 ١٠ trees. The groups were treated as follows:

The first group was treated with phosphate dissolving bacterial biofertilizer.

The second group was treated with 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.

Response of guava trees to biofertilization

Biofertilization was carried out by mixing ٢٠٠ ml of the prepared bacterial inoculum (in case of the bacterial biofertilization), ١ kg of VAM inoculum (in case of the mycorrhizal biofertilization) or both (in case of the dual biofertilization) with ١٠ 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 (١٠ kg.). The biofertilizers were applied once at winter during each season.

All selected trees received the common horticultural practices of guava cultures (٣٠٠ g nitrogen /tree, ١٦٠g P_2O_5/tree and ١٥٠ 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:

١-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 (١٩٩٩) as follow:
Leaf area = ٠.٧٢ (L x W) + ٢٠٤٦.
Where L = length of leaf W= width of leaf.

٢-The leaves used for leaf area measurements were used to determine N, P, K, Ca and Mg contents. The leaves were dehydrated at ٨٠°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 spectrophotometer (Perkin Elmer ٢٨٠)

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

٤-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 contents were determined according to A.O.A.C. (١٩٩٠).

L-Ascorbic acid (vitamin C) mg/١٠٠g fresh weight was determined by titration with ٢-٦ Dichlorophenol-Indophenol (A.O.A.C. (١٩٩٠).

Pectin was extracted and determined by precipitation as calcium pectate, according to Ranganna (١٩٧٧).

Statistical analysis was carriedout according to Snedecor and Cochran (١٩٨٠) using L.S.D parameter at ٥٪.

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 ٢ in both seasons (٢٠٠٧-٢٠٠٨ and ٢٠٠٨-٢٠٠٩) 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 dual fertilizer followed by trees fertilized with VAM, then those 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 $CO_٢$ which lead to an increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, ١٩٧٧). 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, molybdenum 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.*, ١٩٨١; Ba *et al.*, ٢٠٠١; Morte *et al.*, ٢٠٠١ and Ibrahim, ٢٠٠٩).

Table ٢: 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 (cm٢)	N%	P%	K%	Ca%	Mg %
٢٠٠٧-٢٠٠٨	**Control**	٧٤.٣	٦٩.١٨	١.٢٨	٠.١٢١	١.٢٩	١.٢٨	٠.٢٧٠
	Mycorrhiza	٩٢.١	٩٢.٧٥	١.٦٥	٠.١٥٠	١.٤٤	١.٥٣	٠.٣٤٨
	B.megaterium	٨٩.٤	٨٣.٨٧	١.٤٥	٠.١٤٨	١.٣٤	١.٢٥	٠.٣١٥
	Dual inoculum*	٩٥.٣	٩٥.٢٨	١.٧٠	٠.١٥٣	١.٤٦	١.٥٥	٠.٣٧٧
	LSD ٠.٠٥	٧.٩٠	٧.٦٤	٠.٢٣	٠.٠١٨	٠.٠٨٨	٠.١٢٥	٠.٠٧٣
٢٠٠٧-٢٠٠٩	**Control**	٧٨.٤	٦٦.٣٨	١.٣٠	٠.١٢٤	١.٢٣	١.٢٨	٠.٢٤٢
	Mycorrhiza	٩٦.٣	٨٩.١٧	١.٦٨	٠.١٥٨	١.٣٩	١.٤٨	٠.٤١٥
	B. megaterium	٩٥.٧	٨٦.٧٥	١.٥١	٠.١٥٦	١.٣١	١.٣٩	٠.٢٨٠
	Dual inoculum*	١٠١.١	٩٤.٤٥	١.٨٠	٠.١٥٩	١.٤٨	١.٥٣	٠.٤٢٧
	LSD ٠.٠٥	٨.٩٠	٥.٩٤	٠.٢٧	٠.٠١٩	٠.٠٨٨	٠.١٢٧	٠.٠٥١

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

Gupta *et al.* (٢٠٠٢) 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 in Table ٢ indicate that in both seasons (٢٠٠٧/٢٠٠٨ and ٢٠٠٨/٢٠٠٩), bio-fertilization of guava trees with VAM fungi alone or combined with *B. megaterium*, significantly increased 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 (١٩٩٤) 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 (١٩٨٣) reported that VAM fungi improves plant growth in low phosphate soils by exploiting large areas of soil and actively transporting the phosphate up to the plants. Also, Gupta *et al*. (٢٠٠٢) 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 the un-inoculated control plants, however the extent of nutrient depletion was greater for P than N and K.

Among the tested nutrients only phosphorus significantly increased in the leaves of the trees fertilized with *B. megaterium* and insignificant increases were observed in the leave contents of the other nutrients (N, K, Ca and Mg) compared to the 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 $CO_٢$ which lead to an increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, ١٩٧٧).

Yield and physical properties of the fruits

Table ٣ shows that in both seasons (٢٠٠٧/٢٠٠٨ and ٢٠٠٨/٢٠٠٩) biofertilization 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 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 Ba *et al*. (٢٠٠١) and Ibrahim (٢٠٠٩) who reported that the use of *B. megaterium* and VAM fungi as biofertilizers enhanced grapevines growth and increased fruit yield.

Table ٣: 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)
٢٠٠٨	Control	١٠٢.٤	١١٢.٠	١١.٤٧	٧.٧٢	٦.٦٧	١.٠٤
	Mycorrhiza	١٠٨.٦	١١٨.٣	١٢.٨٥	٨.٢٥	٦.٨٥	١.٣٠
	B. megaterium	١٠٧.٦	١١٧.٧	١٢.٦٦	٨.١٣	٦.٧٨	١.٢٣
	Dual inoculum*	١٠٩.٥	١٢٠.٨	١٣.٢٣	٨.٣٥	٦.٩٠	١.٤٠
	LSD ٠.٠٥	٥.١	٦.٢٩	١.١١	٠.٣٨	٠.٠٩	٠.١٨
٢٠٠٩	Control	١٠٤.٨	١٠٤.٩	١٠.٩٩	٧.٦٦	٦.٥٢	١.٠٣
	Mycorrhiza	١١٢.٢	١١٩.٣	١٣.٣٩	٨.٣٥	٦.٩٨	١.٤٠
	B. megaterium	١١٢.٨	١١٠.٥	١٢.٤٦	٨.١٠	٦.٩٠	١.٢٨
	Dual inoculum*	١١٥.٩	١٢١.٠	١٤.٠٢	٨.٤٩	٧.٠٢	١.٤٥
	LSD ٠.٠٥	٥.٨	٩.٥	١.٩١	٠.٨١	٠.٣١	٠.١٩

***** 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 higher fruit yield, but also enhanced fruit physical properties. As shown in Table ٣, the values of fruit height, diameter and flesh thickness, were significantly increased in 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 received dual inoculum (i.e. VAM plus *B. megaterium*).

Chemical properties of the fruits

Data presented in Table ٤ show indicate that in both seasons (٢٠٠٧/٢٠٠٨ and ٢٠٠٨/٢٠٠٩) biofertilization of guava trees with

VAM and/or *B. megaterium* improved fruit chemical properties compared to the un-biofertilized ones. The recorded values of total soluble solids, L ascorbic acid and pectin contents 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, received dual fertilizer (VAM plus *B. megaterium*). Titratable acidity was significantly higher in uninoculated trees fruits compared to the inoculated ones.

Table ٤: 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/١٠٠g f w)	Ca. pectat (mg/١٠٠g d w)
٢٠٠٧-٢٠٠٨	**Control**	٨.٥٧	١.٠٦٢	٨٧.٨	٧.١٨
	Mycorrhiza	١٠.٥٣	٠.٩١٤	١١٠.٠	١٢.٤٥
	B. megaterium	٩.٨٠	٠.٨٨١	١٠٤.٧	١٢.٥٥
	Dual inoculum*	١٠.٩٨	٠.٨٦٦	١٠٦.٣	١٣.٧٨
	LSD ٠.٠٥	١.١٤	٠.١٠٣	١٣.٠٥	٤.١٦
٢٠٠٨-٢٠٠٩	**Control**	٨.٧٣	١.٠٧١	٩٠.٣	٨.٩٥
	Mycorrhiza	١٠.٠٨	٠.٩٢٤	١٠٧.٨	١٢.٣٣
	B. megaterium	١٠.٢٧	٠.٨٩٣	١٠٢.٨	١٢.٧٣
	Dual inoculum*	١١.٦٠	٠.٨٨٨	١١١.٣	١٤.٩٠
	LSD ٠.٠٥	١.٠٣	٠.١٥٤	١٢.٢	٢.١٠٢

***** 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 Ba *et al*. (٢٠٠٠) and Ibrahim (٢٠٠٩).

On the basis of the obtained results it could be concluded that fertilization of guava trees with VAM and/or *B. megaterium* improved growth of the trees, increased leaf mineral contents and fruit yield of high quality. This observation was pronounced in 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 trees growth and consequently produce high yield of good quality.

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استجابة أشجار الجوافة البلدي المنزرعة في التربة الرملية الجيرية للتسميد الحيوي بالبكتريا المذيبة للفوسفات مع أو بدون فطريات الميكورهيزا

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في هذه الدراسة تم استخدام البكتيريا المذيبة للفوسفات مع أو بدون فطريات الميكورهيزا كأسمدة حيوية لأشجار الجوافة البلدي النامية في التربة الرملية الجيرية حديثة الأستصلاح بمنطقة سدمنت الجبل – محافظة بني سويف خلال موسمين متتابعين (٢٠٠٧–٢٠٠٨ و ٢٠٠٨–٢٠٠٩).

في كلا الموسمين أدي التسميد الحيوي لأشجار الجوافة بالميكورهيزا أوالبكتيريا المذيبة للفوسفات أو كليهما معاً إلي زيادة معنوية في نمو الأشجار ومساحة الورقة ومحتوي الأوراق من النيتروجين والفوسفور والبوتاسيوم والكالسيوم والماغنسيوم مقارنة بالأشجار الغير مسمدة حيوياً (الكنترول). وقد أعطت الأشجار المسمدة باللقاح المزدوج

(البكتيريا المذيبة للفوسفات والميكورهيزا) أعلي القيم لهذه القياسات ثم االأشجار المسدة بالميكورهيزا فقط يليها الأشجار المسمدة بالبكتريا المذيبة للفوسفات.

في كلا الموسمين أدي التسميد الحيوي لأشجار الجوافة البلدي بفطريات الميكورهيزا مع أو بدون البكتيريا المذيبة للفوسفات إلى زيادة معنوية في وزن الثمار وكذلك عدد الثمار/الشجرة وبالتالي أعطت محصول أعلي مقارنة بالأشجار الغير مسمدة حيوياً. القيم المسجلة لكل من المواد الصلبة الذائبة كنسبة مئوية وفيتامين ج والبكتين في ثمار الأشجار الملقحة كانت أعلي عنها في الأشجار الغير ملقحة. وقد تم تسجيل أعلي القيم لهذه الصفات للأشجار الملقحة باللقاح المزدوج. ومن ناحية أخري أعطت الأشجار الغير ملقحة نسبة أعلي للحموضة في الثمار في كلا الموسمين عنها في الأشجار الملقحة. وقد أعطت الثمار المأخوذة من الأشجار الملقحة باللقاح المزدوج أقل نسبة من الحموضة خلال موسمي الدراسة.