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Response of Guava Transplants to Soil and Foliar Spray with Some Bio-Stimulants

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THIS research aimed to study the effect of some bio-stimulants on growth and nutritional status of guava transplants in order to reduce the mineral fertilization during seasons of 2011 and 2012. Results revealed that all treatments significantly increased vegetative growth *i.e.,* (stem height and diameter, number of shoots, number of leaves and leaf area) in both seasons. Results, also, indicated that leaf photosynthetic pigments content (chlorophyll a, b and carotenoids) were increased as well as leaf mineral contents (N, P, K, Ca, Mg, Fe, Mn and Zn) were improved by the different treatments. Therefore, it could be concluded that, all investigated bio – stimulants improve growth and nutritional status especially foliar application of 20 g Kotengin + 40 g phosphorine + 40 g Rhizobacterin/ plant +20 g K_2SO_4/ plant followed by foliar application of 20 g Kotengin + 40 g Rhizobacterin per plant + PK applied of control.

The guava "*Psidium guajava* L." is believed to be native to the areas between Mexico and Peru. It has spread to all over the tropical and subtropical countries, Chandler (1958). Guava fruits are the cheapest and richest source in vitamin "C", as well as it contains small amounts of vitamin "A", "B", carbohydrates, oils and proteins, Godeston and Chain (1946).

Guava trees occupy 38873 feddans, which in turn produced about 314438 metric tons fruits. (Statistics of Ministry of Agriculture, A. R. E. 2012).

Bio-fertilizers are important for plant production as they play an vital role in increasing vegetative growth, yield and fruit quality (Haggag and Azzazy, 1996) on mango seedling (Soliman, 2001) on guava and banana plants (Abd-Rabou, 2006) on avocado plants, (Ahmed *et al*., 1999 and Osman *et al*., 2010) on olive plants, (Chokha *et al*., 2000, El-Geuoshy, 2011 and Bakry *et al*., 2013) on sweet orange. Shaban and Mohsen (2009) also showed that, all bio-fertilizers were effective in improving vegetative growth and nutritional status of sweet orang transplants. Bio-fertilization is biological preparations containing primarily patent strains of micro- organisms in sufficient populations. The multi - strain bio-fertilizers might contain different strains of symbiotic associative phosphate - solubilizing micro-organisms, silicate dissolving micro- organisms, blue green algae and VAM (Saber, 1993). They proved to eliminate the use of pesticides

sometimes and rebalance the ratio between plant nutrients in soils. It is worthy to state that, bio-fertilizers do not replace mineral fertilizers, but significantly reduce their rate of applications. Bio-fertilizers are very safe for human, animal and environment. Since, they reduce at the lower extent the great environmental pollution. Phosphorine is a bio-fertilizer product containing active microorganisms hydrolyzing the insoluble phosphate into soluble one under high soil pH and greater percentage of calcium carbonate, consequently partially overcomes the phosphate blocking and/or unavailability. In addition, Rhizobacterin as new bio-fertilizers have greater amount of symbiotic bacteria and non symbiotic bacteria responsible for nitrogen fixation. Application of both achieved the following advantages:

1-Reduce plant requirements of nitrogen by 25%; 2- Improve the availability of various nutrients for plant absorption; 3-Increase the resistance of plants to root disease; 4-Reduce the environmental pollution induced by the application of chemical fertilizers; 5-Improve the productivity of the trees (Ishac, 1989).

Bio-fertilizers are now available commercially. Specific strains are used as biological fertilizers, for nitrogen, phosphorus and silicate dissolving such as N-fixing bacteria and yeasts. The use of these materials encourages yield and keeps the environment clean.

The present study aimed to throw some light on the beneficial effect of replacing mineral N, P and K soil application with foliar application with some bio-fertilizers namely Novatrene and Biomagic on growth and nutritional status of guava transplants.

Material and Methods

The present investigation was carried out during two successive seasons of (2011 and 2012) at nursery of Hort., Fac. of Agric., Benha Univ., Egypt. A uniform and healthy one-year- old seedlings of guava *"Psidium guajava, L."* were carefully selected and used as plant material. In both seasons of study and during the first week of February, those seedlings were transplanted individually each in a plastic pot of 35 cm in diameter that previously had been filled with specific weight of growing medium consisting of clay and sand at equal proportion (V: V). Mechanical and chemical analysis of growing media were done as shown in Table (1 a&b) according to the methods described by Jackson, (1967) and Israelsen & Hansen (1962).

TABLE (1-a). Physical properties of soil media used for growing guava seedlings (%).

Partial distribution		
Total sand	Silt	Clay
65.00 %	10.00 %	25 %

TABLE (1-b). Chemical properties of soil media used for growing guava seedlings.

Soluble cations mg/L				Soluble anions mg/L				Ca Co_3	pH	EC
Mg^{++}	Ca^{++}	K^{+}	Na^{+}	$HCO3^{-}$	$CO3^{--}$	$SO4^{--}$	Cl^{-}			
2.10	8.80	0.60	7.70	3.00	-	9.20	6.90	1.30	7.72	1.90

The bio-fertilizers (BF) used in this study were produced by soil microbiology unit, Desert Res. Center. This experiment involved eight treatments:

- Mineral NPK fertilization program as control was annually added at the rate of 40g ammonium sulphate, 40g superphosphate and 20 g potassium sulphate per pot (plant). Whereas, the corresponding amount of each NPK fertilizer was fractionized into five equal doses to be soil applied monthly from mid March till mid July during both season plus water spray (50cm^3/ transplant).
- Foliar spray of urea at 1% + Orthophosphoric acid at 100 ppm P_2O_5 + K_2SO_4 at 1% (50cm^3/ transplant).
- Foliar spray of diluted Novatrene 1.0 conc.: 150 water (v:v) (50cm^3/ transplant).
- Foliar spray of Biomagic at 7.5g /L (50cm^3/ transplant).
- Foliar spray of diluted Novatrene solution (1.0: 150 v:v) at the rate of 50cm^3/ plant plus soil application of Kotengin at 20 g/ plant + phosphorine at 40 g/ plant + Rhizobacterin at 40 g/ plant +K_2SO_4 at 20 g/ plant.
- Foliar spray of diluted Novatrene solution (1.0: 150 v:v) at the rate of 50cm^3/ plant plus soil application of Kotengin at 20 g/ plant + one liter Biovit (prepared by dissolving one liter of commercial Biovit in 50 liter water) + K_2SO_4 at 20 g/ plant.
- Foliar spray of diluted Biomagic (7.5g /L water) at the rate of 50cm^3/ plant plus soil application of Kotengin at 20 g/ plant + phosphorine at 40 g/ plant + Rhizobacterin at 40 g/ plant +K_2SO_4 at 20 g/ plant.
- Foliar spray of diluted Biomagic (7.5g /L water) at the rate of 50cm.3/ plant plus soil application of Kotengin at 20 g/ plant + one liter Biovit (prepared by dissolving one liter of commercial Biovit in 50 liter water) + K_2SO_4 at 20 g/ plant.

Bio-fertilizers (Kotengin, Biomagic, Phosphorine, Rhizobacterin and Biovit each was applied once/ year in March. Foliar application with urea, H_3PO_4, K_2SO_4, Novatrene and Biomagic solutions were periodically sprayed 5 times at one month interval starting from mid March till mid July every season.

The response of guava seedlings to differential treatments were investigated throw determining of the following measurements:

Vegetative growth measurements

At the last week of October during both seasons, as the experiment was ended, the effect of different treatments on some vegetative growth measurements were evaluated *i.e.* increment percentage in stem height, stem

diameter, average number of shoots/ plant, average number of leaves/ plant and average leaf area (cm^2).

Chemical analysis

Photosynthetic pigments (chlorophyll a, b and carotenoids) and leaf mineral content (N, P, K, Ca, Mg, Fe, Mn and Zn) were determined as described by A. O. A. C. (1990).

Statistical analysis

Data were subjected to analysis of variance according to (Snedecor and Cochran, 1977). In addition, significant differences among means were differentiated according to the Duncan's, multiple test range (Duncan, 1955).

Results and Discussion

Vegetative growth measurements

Increment percentage in both stem height and diameter, average number of shoots/ plant, average number of leaves and leaf area were studied as influenced by the differential bio-fertilizers (Kotengin, Biomagic, Phosphorine, Rhizobacterin, Biovit and Novatrien) treatments during 2011& 2012 seasons are presented in Table 2.

As shown from Table 2 all investigated bio- fertilizer treatments increased significantly the abovementioned five growth parameters as compared with control. However, such response varied obviously from one treatment to another, in spite of all growth parameters followed in most cases, the same trend during both experimental seasons. Anyhow, foliar spray of diluted Biomagic (7.5g /L water) at the rate of $50cm^3$/ plant plus soil application of Kotengin at 20 g/ plant + one liter Biovit + K_2SO_4 at 20 g/ plant (8^{th} treatment) was the most effective and ranked statistically 1^{st}, whereas it resulted in the greatest average stem (height & thickness), number of shoots per transplant, number of leaves per shoot and average leaf area during the two experimental seasons. On the contrary, the least values of all investigated growth parameters were significantly exhibited by (control). In addition, other treatments were in between the aforesaid two extremes.

This result goes in line with the findings of Izquierdo *et al*. (1993) and Chokha *et al*. (2000) on growth measurements of bio-fertilized Volkamer lemon and Mosambi sweet orange, respectively as they gave support to the obtained result particularly as the benefit effect of Biomagic application was concerned. Also, this result goes partially in line with those found by Bakry *et al*. (2013) on Washington navel orange trees and Khamis *et al*. (2012) on sweet orange stated that, the addition of bio- fertilizers increased vegetative growth measurements.

On the other hand, the noticeable positive effect of six investigated bio – fertilizers may be attributed to the improvement in soil physical and chemical properties induced by the additional N source like as Kotengin, Biomagic,

Phosphorine, Rhizobacterin, Biovit and Hummer which reflected positively on various nutrient absorption.

TABLE 2. Effect of mineral NPK fertilizers and their combinations with some bio – fertilizers soil and foliar spray applied on vegetative growth of guava transplants during 2011 & 2012 seasons.

Treat.	Increment (%) in stem height		Increment (%) in Stem diameter		No. of Shoots per transplant		No. of leaves per transplant		Leaf area (cm^2)	
	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season
1	25.00H	23.06G	16.99H	17.00H	1.00E	1.00G	50.00G	52.06E	22.50H	21.71H
2	47.05G	47.52F	27.61G	28.64G	1.33E	1.67F	83.62F	82.32D	33.72F	32.29G
3	48.23F	48.94F	30.69F	30.60F	2.00D	2.00EF	84.44F	86.31D	25.61G	34.71F
4	50.26E	51.16E	33.55E	34.17E	2.33D	2.33E	90.49E	91.29CD	40.59E	39.33E
5	62.39B	63.66B	48.52B	43.03B	4.33B	5.00B	121.70B	85.12D	55.49B	52.59B
6	53.87D	54.50D	36.57D	38.33D	3.00C	3.00D	101.90D	10.00BC	42.60D	44.38D
7	57.56C	58.34C	38.37C	40.02C	4.00B	4.00C	109.00C	108.21B	49.06C	49.07C
8	67.21A	66.53A	50.00A	47.36A	5.00A	6.00A	126.58A	125.76A	57.95A	57.81A

Values within each column followed by the same letter/s are not significantly different at 5 % level.

Nutritional status (leaf photosynthetic pigments and mineral composition).

Leaf N, P, K, Ca, Mg, Fe, Mn, Zn and photosynthetic pigments (chlorophyll a, b and carotenoids) contents were determined as indicators of nutritional status of guava transplants in response to different bio-fertilizer treatments. Data obtained are presented in Tables 3 and 4.

As shown in Tables 3 and 4 leaf macro and micro elements content (N, P, K, Ca, Mg %, Fe, Mn, and Zn ppm) and leaf photosynthetic pigments were increased significantly by all bio- fertilizers treatments as compared to control. Such trend was true during the both seasons of study. Anyhow, foliar spray of diluted Biomagic (7.5g /L water) at the rate of $50cm^3$/ plant plus soil application of Kotengin at 20 g/ plant + one liter Biovit + K_2SO_4 at 20 g/ plant (8^{th} treatment) was the most effective and exhibited statistically the highest leaf macro and micro nutrient elements content during both seasons. On the other hand, foliar spray of diluted Novatrene solution (1.0: 150 v:v) at the rate of $50cm^3$/ plant plus soil application of Kotengin at 20 g/ plant + phosphorine at 40 g/ plant + Rhizobacterin at 40 g/ plant +K_2SO_4 at 20 g/ plant (5^{th} treatment) ranked statistically 2^{nd}.

This result goes in line with Abd El-Migeed *et al*. (2007) on Washington navel orange. Moreover, findings of Osman *et al*. (2010) on two olive cultivars were in partial agreement with the present results in this respect regarding the simulative effect of some bio fertilizers.

The obtained results regarding leaf macro and micro nutrient contents of fruit trees were supported by the findings of Omar (2006) who reported that, olive transplants which received N as basal dressing led to increase leaf N, Ca, Mg and Fe content. El - Geuoshy (2011) on sweet orange trees found that, trees which were fertilized with the highest level of NPK soil application combined with bio - fertilizers significantly showed the highest values of leaf mineral contents (N, P, K, Ca, Mg, Fe, Zn and Mn). Bakry (2007) and Bakry *et al*. (2013) showed that, sweet orange leaves contained more N, P and K as a result of bio - fertilizers soil applications which encourage the leaves to gain more chlorophyll and more dry matter. Also, these results are in harmony with those found by Mohamed *et al*. (2010), Fawzi *et al*. (2010) and Abdou (2010) reported that, the highest leaf pigments content was obtained by bio-fertilizer stimulants of "Le- Conte" pear trees.

TABLE 3. Effect of mineral NPK fertilizers and their combinations with some bio – fertilizers soil applied on leaf macro nutrient elements contents of guava transplants during 2011 & 2012 seasons.

Treat.	Leaf N %		Leaf P %		Leaf K %		Leaf Ca %		Leaf Mg %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1 (Control)	1.60G	1.60G	0.116E	0.130D	1.41E	1.42C	1.50E	1.53G	0.433E	0.477D
T2	1.73F	1.75F	0.150D	0.153C	1.45E	1.45C	1.56E	1.55G	0.493D	0.520D
T3	2.12E	2.20D	0.157D	0.180B	1.65D	1.69B	1.72D	1.70F	0.610C	0.660C
T4	2.30D	2.00E	0.183C	0.190B	1.66D	1.69B	1.75D	1.75E	0717B	0.740BC
T5	2.80A	2.83B	0.273A	0.300A	2.98A	2.85A	2.07B	2.10B	0.820A	0.847AB
T6	2.40C	2.45C	0.193C	0.200B	1.67D	1.68B	1.83C	1.90C	0.720B	0.747BC
T7	2.48B	1.29H	0.187C	0.200B	1.75C	1.68B	1.84C	1.85D	0.733B	0.673C
T8	2.85A	2.95A	0.240B	0.310A	2.81B	2.85A	2.21A	2.17A	0.827A	0.863A

Values within each column followed by the same letter/s are not significantly different at 5 % level.

TABLE 4. Effect of mineral NPK fertilizers and their combinations with some bio – fertilizers soil applied on leaf micro nutrient elements and photosynthetic pigments contents of guava transplants during 2011 & 2012 seasons.

Treat.	Fe (ppm)		Zn (ppm)		Mn (ppm)		Chlorophyll (A) mg/ g f. w.		Chlorophyll (B) mg/ g f. w		Carotene (mg/ g f. w)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1 (Control)	136.3G	138.7G	47.67E	59.67D	72.50E	73.00E	4.57H	4.65H	1.82F	1.83E	2.43F	2.48C
T2	143.3F	144.7F	52.33D	53.33E	84.50D	85.00D	4.72G	4.73G	1.86F	1.87E	2.48F	2.52C
T3	147.3E	150.3E	57.67C	59.33D	86.00D	86.00D	4.85F	4.86F	1.92E	1.95D	2.58E	2.60BC
T4	152.0D	154.7D	65.00B	66.00C	87.33D	85.83D	4.96E	4.95E	1.99D	2.01C	2.70D	2.73B
T5	185.0B	188.0B	71.33A	77.00B	115.0B	113.5B	7.20B	7.26B	2.91B	2.93A	2.84B	3.85A
T6	162.7C	162.7C	63.33B	66.67C	95.00C	97.50C	6.12D	6.21D	2.17C	2.25B	2.73CD	2.77B
T7	165.0C	166.0C	65.33B	68.00C	96.00C	82.50D	6.35C	6.40C	2.20C	2.25B	2.79C	2.79B
T8	197.7A	202.0A	73.67A	80.00A	122.5A	123.0A	7.42A	7.47A	2.98A	2.99A	3.92A	3.93A

Values within each column followed by the same letter/s are not significantly different at 5 % level.

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استجابة شتلات الجوافة للتسميد الارضى والرش الورقى ببعض منشطات النمو

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فى هذا البحث تم اختبار بعض الاسمدة الحيوية من حيث تاثيرها على النمو والحالة الغذائية لشتلات الجوافة بهدف تقليل استخدام الاسمدة المعدنية ومن ثم تقليل التلوث للمياه الجوفية والمنتجات الزراعية خلال موسمين متتاليين هما ٢٠١١ - ٢٠١٢.
وقد اوضحت النتائج المتحصل عليها ان كل المعاملات المستخدم فيها التسميد الحيوى قد حسنت من جميع قياسات النمو الخضرى لشتلات الجوافة مثل النسبة المئوية لارتفاع وقطر الساق وعدد النموات الجانبية لكل شتلة وعدد الاوراق لكل شتلة ومساحة الورقة كما حسنت من محتوى الاوراق من العناصر الغذائية مثل النيتروجين والفوسفور والبوتاسيوم والكالسيوم والماغنسيوم والحديد والمنجنيز والزنك كما حسنت من محتوى الاوراق من صبغات التمثيل الضوئى مثل كلوروفيل ا و ب والكاروتينات.

كما اوضحت النتائج ان اكثر المعاملات فاعلية فى هذا الشان هى معاملة شتلات الجوافة بالرش الورقى ب (٧,٥ جم / لترماء بيوماجيك مع التسميد الارضى للشتلات ب٢٠ جرام كوتنجن + ١ لتر بيوفيت + ٢٠ جم كبريتات بوتاسيوم لكل شتلة) يليها معاملة الشتلات بالرش الورقى بالنوفاترين مع التسيد الارضى ب ٢٠ جم كوتنجن + ٤٠ جم فوسفورين +٤٠ جم ريزوباكترين + ٢٠جم كبريتات بوتاسيوم لكل شتلة).