Journal of Horticultural Science & Ornamental Plants 1 (2): 39-48, 2009
ISSN 2079-2158

Response of Citrus Rootstocks and Transplants to Biofertilizers

A.E.A. Shaban and A.T. Mohsen

Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: This experiment was carried out during 2006/2007 and 2007/2008 seasons at the nursery of the faculty of agriculture Cairo University Giza Egypt. The study was arranged in two experiments the first aimed at evaluate the effect of biofertilizers namely phosphorine, nitrobine and microbine on growth of sour orange and volkamer lemon citrus rootstocks. The second experiment was conducted to studying the effect of these biofertilizers on growth of Valencia orange budded on these rootstocks. Single or combined applications of Phosphorine, microbine or nitrobine were used at 5, 10 or 20 g per seedling of Sour orange and Volkamer lemon rootstocks. Biofertilizer treatments were carried out on one month old seedling in the first experiment and repeat again in the second experiment on one year old of Sour orange and Volkamer lemon seedling budded with Valencia orange. Combined application of the three biofertilizers at 20 g per seedling from each biofertilizer recorded the best results for seedling height, thickness, number of leaves per seedling, leaf area, root length, root dry weight, seedling dry weight, leaf N, P and K content of Sour orange and Volkamer lemon rootstocks. Also this treatment recorded the highest budding success percentage, scion length, number of leaves per scion, leaf chlorophyll content, leaf N, P and K content of Valencia orange budded on both Sour orange and Volkamer lemon rootstocks. Microbine and nitrobine treatments improved vegetative growth, leaf N and K content while phosphorine induced root growth and leaf P content. All studied growth parameters were higher with Volkamer lemon than sour orange rootstock.

Key words: Citrus rootstock · Sour orange · Volkamer lemon · Phosphorine · Nitrobine · Microbine

INTRODUCTION

For producing good citrus transplants we need to get a good growth for seedling rootstocks this commonly by giving them the optimum cultural practices such as fertilization. Also, supplying the rootstocks with their nutrient requirements help to shortage the period for getting the transplant. Bio-fertilization is considered an important factor in reducing the used rates of chemical fertilizers which appear to be safely for environment, improving soil fertility and increasing soil productivity [1-3]. The most important sweet orange grown in such area is the Late Valencia which had higher demand for marketing a nd export. The used rootstocks in Egypt are very limited in range Sour orange rootstock has been extensively used as it compatible with most the commonly-grown commercial cultivars [4]. Also, Volkamer lemon is one of the promising citrus rootstock. It has significant effective characters that reflected positively on the scions budded on it regarding their rate of growth and its suitability for the unfavorable environmental conditions [5]. Phosphorine is a biofertilizer which contains a phosphate dissolving bacteria "*Bacillus megaterium*" which hydrolyze the insoluble phosphorus into soluble one [6]. Nitrobine is a biofertilizer which contains nitrogen fixing bacteria "*Azospirillum sp*" they are known to fix atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. Microbine biofertilizer contains nitrogen fixing bacteria "*Azotobacter sp*, *Azospirillum sp,* and *Pseudomonas sp*" phosphate dissolving bacteria "*Bacillus megaterium*" and "*Vesicular Arbuscular Mycorrhiza*"*VAM*" [7]. The benefit of using bio-fertilization as chemical fertilizer substitution is due to the activities of Azospirillum and Azotobacter to fix N_2 gas from soil atmosphere (non-symbiotic N fixer) to become ammonium N, and due to the effect of Phosphorylase as an enzyme produced by Aeromonas to dissolve fixed-P in the soil and also due to the increase in Particle soil aggregation and soil aeration done by Aspergillus [8]. Azospirillum is one of the non-symbiotic N fixer which increases root number 15-20% [9] enzyme activities at root zone, concentrations of IAA, and soil aeration [10]. Azotobacter, a non-symbiotic N fixer increased crop yield

Correspoding Author: A.E.A. Shaban, Department, Pomology Faculty of Agriculture, Cairo University, Giza, Egypt

up to 30%, and produced plant growth promoting substances such as IAA, gibberellins, and cytokinins [8, 11]. Thus, the objective of this study was to induce the growth of sour orange and Volkamer lemon citrus rootstocks by using different rates of Phosphorine, microbine and nitrobine. Also, to evaluate the comparative effect of these bio-fertilizers on growth of Valencia orange scions budded on both Sour orange and Volkamer lemon citrus rootstocks.

MATERIALS AND METHODS

The present experiment was carried out during 2006/2007 and 2007/2008 seasons at the nursery of the faculty of agriculture Cairo University Giza Egypt. The study arranged in two experiments the first aimed at studying the response of sour orange and volkamer lemon citrus rootstocks to phosphorine, nitrobine and microbine biofertilizers. Seeds of both sour orange and volkamer lemon rootstocks were sowing in seedbed contained mixture of sand plus peat moss (3:1) in glass house during the first week of February (2006 and 2007). After seed germination (two month from sowing date in April 2006 and 2007) the healthy seedling from each rootstock were planted individually in plastic bags (20X35 cm) containing sand: peat moss (3:1). The three biofertilizers (phosphorine, nitrobine and microbine) which used in this study were produced by the General Organization for Agricultural Equalization found (GOAEF), ministry of Agriculture Egypt.

The seedlings of Sour orange and Volkamer lemon rootstocks received the illustrated thirteen biofertilizers treatments in Table 1.

The experiment was arranged in a randomized complete blocks design with three replicates and each replicate was represented by 15 seedlings.

Each biofertilizer treatment (the dose of biofertilizer per seedling) was divided into two equal doses; the first dose was mixed with the media (sand and peat moss) in the plastic bags before planting the seedling in it. The second dose was added to the seedling one month later (first week of May 2006 and 2007). The seedling received their normal agricultural practices including their mineral nutrition (50, 20 and 8 g/seedling/ season of NPK respectively). After one year from growing the seedling the following parameters were measured in April 2007 and 2008: seedling height (cm) from the surface of the soil to highest growing point. Seedling thickness (cm) above the soil surface with 5 cm. Number of leaves per seedling was counted and leaf area (cm^2) was measured by using leaf area meter (Model CI-203, USA). Also, root length (cm) of seedlings was measured. Dry matter of seedling (vegetative growth) and root system (g) fresh weight of foliage and roots was oven dried at 65 C and weighted. Leaf mineral contents of nitrogen (N), phosphorus (P) and potassium (K) were estimated in mature leaves of sour orange and volkamer lemon rootstocks. Ten mature leaves were collected from the middle part of one year old of sour orange and volkamer lemon rootstocks in April 2007 and 2008. Leaves samples were washed with water then oven dried at 70 C till constant weight. After that 0.3 g of dried leaves was taken for determine nitrogen phosphorus according to A.O.A.C [12] and potassium was determined by flame photometry.

Experiment II: This experiment was done to studying the effects of phosphorine, nitrobine and microbine biofertilizers on enhancing growth of Valencia orange budded on Sour orange and Volkamer lemon rootstocks and shorted the period for producing healthy budded plants.

Table 1: Biofertilizer treatments which were used in the two experiments in this study

Treatments	Phosphorine (P)	Nitrobine (N)	Microbine (M)	P+N+M
(1)Control				
2	5 g/ plant			
3	10 g/ plant			
4	20 g/ plant			
5		5 g/ plant		
6		10 g/ plant		
7		20 g/ plant		
8			5 g/ plant	
9			10 g/ plant	
10			20 g/ plant	
11				5+5+5 g/ plant
12				10+10+10 g/ plant
13				20+20+20 g/ plant

The budded plants received the same biofertilizers treatments which were added to the seedling rootstocks in 2006 and 2007 seasons as shown in Table 1. Each biofertilizer treatment (the dose of biofertilizer per budded seedling) was divided into two equal doses; the first dose was added just before budding operation and the second dose was added to the budded seedling one month later (first week of May 2007 and 2008). Buds of Valencia orange were budded on one year old seedlings of sour orange and volkamer lemon rootstocks in the first week of April in 2007 and 2008 seasons by using T budding method. Budding success percentage of Valencia orange was recorded at 45 days from budding date. Scion length (cm), number of leaves per scion and leaf area (cm^2) was measured six month after grafting. Leaf chlorophyll content was determined by using samples of six leaves per replicate from the middle part of scion shoots by using chlorophyll meter (Model SPAD - 502). Also, leaf mineral contents of nitrogen (N), phosphorus (P) and potassium (K) were estimated in mature leaves of Valencia orange scions which were budded on both rootstocks. Ten mature leaves were collected from the middle part of six month old of Valencia scions in October 2007 and 2008. Leaves samples were washed with water then oven dried at 70 C till constant weight. After that 0.3 g of dried leaves was taken for determine nitrogen and phosphorus according to A.O.A.C [12] and potassium was determined by flame photometry.

Data were tabulated and statistically analyzed according to Snedecor and Cochran [13] and mean values were compared by Duncan's multiple range test at 5% [14].

RESULTS AND DISCUSSION

Experiment I: Response of Sour Orange and Volkamer Lemon Seedlings to Biofertilizers

Rootstock Growth: Data presented in Tables 2, 3, 4 and 5 show the effect of phosphorine, nitrobine and microbine on seedling height, thickness, number of leaves per seedling, leaf area, seedling dry weight, root length and root dry weight of Sour orange and Volkamer lemon rootstocks. Regardless of biofertilizers treatments, Volkamer lemon recorded higher significant values for all studied growth parameters than sour orange rootstock. Concerning the effect of biofertilizers treatments, combined application of the three biofertilizers at high level or medium level (20 or 10 g per seedling from each biofertilizer) significantly stimulated all growth parameters compared with the control which recorded the lowest significant values in this respect. Interaction effect between rootstocks and biofertilizers showed that, combined application of the three biofertilizers at high level proved to be the best treatment for improving growth parameters of Sour orange and Volkamer lemon rootstocks. Also, single application at high level of 20 g per seedling of any biofertilizer recorded higher values than mid or low level. All vegetative growth parameters were improved with application of microbine or nitrobine rather than phosphorine. On the other hand, phosphorine treatments produced longer root length and higher root dry weight comparing with microbine or nitrobine. In general, the lowest values of all studied growth parameters of sour orange and Volkamer lemon rootstocks were recorded with the control. From the above mentioned result it could be concluded that combined application of the three biofertilizers at high level of 20 or 10 g per seedling from each biofertilizer was favorable for improving growth than single application. So, using these biofertilizers treatments accelerated growth of rootstocks, shorted the period required for reaching the budding stage, increased percentage of seedlings ready for budding as well as producing healthy budded plants. Improving of vegetative growth with microbine and nitrobine may be due to containing hese biofertilizers on nitrogen fixing bacteria *Azospirillum sp, Azotobacter sp. and pseudomonas sp.,* which are known to fix atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. The enhancement of root growth by phosphorine may be related to the role of phosphorus in root growth, this biofertilizer containing on phosphate dissolving bacteria *"Bcillus megaterium"* which hydrolyze the insoluble phosphorus into soluble one which involved in root growth. Results of this study are in agreement with Haggag and Azzazy [15] who reported a significant positive effect of microbine on growth of mango seedlings. Moreover, inoculation of citrus, mango, banana, guava, grape, papaya, pineapple and pomegranate with *Azospirillum sp* increased vegetative growth parameters [16]. Combination of phosphorine, nitrobine and potassine increased vegetative growth of Eggazi and Koroneiki olive cultivars [17]. Yousif and Marzouk [18] reported that inoculation of one year old of Guava trees with phosphorine at 5g per pot showed significant increase in dry mater. Also, the effect of microbine on growth of Avocado and Mango seedlings was cleared by Abd-Rabou [19].

Nitrogen, Phosphorus and Potassium in Leaf of Rootstocks as Affected by Biofertilizers: Effect of biofertilizer treatments on leaf N, P and K contents of Sour orange and Volkamer lemon rootstocks are presented in Tables 5 and 6.

Table 2: Effect of some biofertilizers on seedling height and thickness (cm) of sour orange and volkamer lemon rootstocks in 2006/2007 and 2007/2008 seasons

	Seedling height (cm)						Seedling thickness (cm)					
	2006/2007 season			2007/2008 season			2006/2007 season			2007/2008 season		
Biofertilizers treatments (g per seedling)	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean
Control	33.5 n	43.6 gh	38.5 H	36.8 n	45.0 h-j	40.9 H	0.40 j	0.40 j	0.40 G	0.41 hi	0.42 hi	0.41 E
Phosphorine 5 g	33.8 mn	44.0 f-h	39.9 GH	39.0 lm	46.0 hi	42.5 G	0.40 j	0.40 j	0.40 G	0.40 i	0.43 g-i	0.41 E
Phosphorine 10 g	36.1 l	44.2 e-h	40.1 FG	40.1 kl	46.7 gh	43.4 FG	0.44 h-j	0.43 ij	0.43 FG	0.44 f-i	0.44 f-i	0.44 C-E
Phosphorine 20 g	40.2 jk	46.3 de	43.2 CD	41.2 k	48.1 fg	44.6 F	0.46 g-i	0.43 ij	0.44 F	0.45 f-i	0.45 e-i	0.45 C-E
Nitrobine 5 g	35.0 l-n	43.5 gh	39.2 F-H	40.0 kl	47.0 gh	43.3 FG	0.48 f-i	0.43 ij	0.45 EF	0.44 f-i	0.45 e-i	0.44 C-E
Nitrobine 10 g	39.2 k	46.0 d-f	42.6 CD	43.2 j	49.2 ef	46.2 E	0.49 f-h	0.45 g-j	0.47 EF	0.44 f-i	0.46 e-h	0.45 C-E
Nitrobine 20 g	41.3 i-k	46.0 d-f	43.6 C	47.0 gh	52.0 c	49.3 C	0.49 f-h	0.48 f-i	0.48 DE	0.44 f-i	0.49 d-f	0.46 CD
Microbine 5 g	36.0 lm	45.2 e-g	40.6 EF	44.2 ij	50.9 c-e	47.5 D	0.48 f-i	0.55 c-e	0.51 CD	0.43 g-i	0.50 c-e	0.46 CD
Microbine 10 g	36.0 lm	48.0 cd	42.0 DE	48.0 fg	52.0 c	50.0 C	0.50 e-g	0.56 b-d	0.53 C	0.46 e-h	0.50 c-e	0.48 C
Microbine 20 g	42.1 h-j	50.0 bc	46.0 B	50.0 de	57.0 b	53.5 B	0.52 d-f	0.58 a-c	0.55 BC	0.48 d-g	0.51 d-g	0.47 C
Combined application at low level (15 g)	36.8 l	48.1 cd	42.4 CD	38.0 mn	46.3 gh	42.1 GH	0.57 b-d	0.59 a-c	0.58 AB	0.52 cd	0.55 bc	0.53 B
Combined application at mid. level (30 g)	49.7 bc	51.6 a	50.6 A	51.0 c-e	60.0 a	55.5 A	0.59 a-c	0.61 ab	0.60 A	0.55 bc	0.59 ab	0.57 B
Combined application at high level (60 g)	50.0 bc	55.5 b	52.2 A	51.2 cd	60.0 a	55.6 A	0.60 a-c	0.63 a	0.61 A	0.62 a	0.62 a	0.62 A
Mean	39.2 B	46.9 A		43.7 B	50.7 A		0.48 B	0.50 A		0.46 B	0.49 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level .So = sour orange Vl = volkamer lemon.

Table 3: Effect of some biofertilizers on number of leaves per seedling and leaf area (cm2) of sour orange and volkamer lemon rootstocks in 2006/2007 and 2007/2008 seasons

	Number of leaves per seedling						Leaf area (cm2)					
	2006/2007 season			2007/2008 season			2006/2007 season			2007/2008 season		
Biofertilizers treatments (g per seedling)	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean
Control	40.5 m	54.5 ef	47.5 F	44.5 o	56.2 j	50.5 L	21.1 j	22.3 g-j	21.7 F	21.2 l	23.2 g-k	22.2 G
Phosphorine 5 g	40.8 m	55.0 ef	47.9 F	47.1 mn	57.5 ij	52.1 JK	21.2 ij	23.5 d-g	22.3 EF	22.8 h-l	23.7 f-k	23.2 FG
Phosphorine 10 g	45.1 jk	55.2 ef	50.1 E	48.5 lm	58.3 hi	53.4 IJ	23.1 e-j	23.2 e-i	23.1 DE	23.1 g-k	23.6 f-k	23.3 FG
Phosphorine 20 g	48.6 hi	57.8 d	53.2 CD	49.6 l	60.1 gh	54.8 H	23.2 e-j	23.6 d-g	23.3 C-E	24.5 e-g	25.2 de	24.9 DE
Nitrobine 5 g	42.3 lm	54.3 f	48.3 F	48.4 lm	58.7 hi	53.5 HI	22.4 g-j	23.4 d-h	22.8 EF	23.9 e-j	24.1 e-h	24.0 D-F
Nitrobine 10 g	47.2 ij	57.5 d	52.3 CD	52.2 k	61.5 fg	56.9 G	23.6 d-g	26.2 a-c	24.9 B	24 e-i	26.2 cd	25.1 CD
Nitrobine 20 g	49.6 gh	57.5 d	53.5 C	56.8 ij	65.0 d	60.9 E	24.6 c-f	27.0 ab	25.8 AB	25.2 d-f	27.1 bc	26.1 BC
Microbine 5 g	43.5 kl	56.5 de	50.0 E	53.4 k	63.5 de	58.4 F	21.4 h-j	24.0 d-f	22.7 EF	22.3 j-l	23.6 f-k	22.9 FG
Microbine 10 g	43.5 kl	60.0 c	51.7 D	61.2 fg	65.0 d	63.1 D	22.5 g-j	24.0 d-f	23.2 DE	22.4 i-l	25.4 de	23.9 EF
Microbine 20 g	50.9 g	62.5 b	56.7 B	62.0 e-g	68.1 c	65.0 C	23.2 e-i	26.3 a-c	24.7 BC	23.5 g-k	26.3 cd	24.9 DE
Combined application at low level (15 g)	44.5 k	60.0 c	52.2 CD	45.9 no	57.8 ij	51.8 KL	22.5 f-i	26.3 a-c	24.5 B-D	22.1 kl	22.3 j-l	22.2 G
Combined application at mid. Level (30 g)	60.1 c	68.1 a	64.1 A	61.7 e-g	73.0 b	67.3 B	25.3 b-d	28.1 a	26.7 A	25.2 d-f	29.1 a	27.1 AB
Combined application at high level (60 g)	60.5 bc	68.2 a	64.3 A	63.0 ef	75.0 a	69.0 A	25.1 d-e	28.2 a	26.6 A	26.2 cd	28.7 ab	27.4 A
Mean	47.4 B	59.0 A		53.4 B	63.0 A		23.0 B	25.0 A		23.5 B	25.2 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level .So = sour orange Vl = volkamer lemon.

Table 4: Effect of some biofertilizers on root length (cm) and root dry weight (g) of sour orange and volkamer lemon rootstocks in 2006/2007 and 2007/2008 seasons

	Root length (cm)						Root dry weight (g.)					
	2006/2007 season			2007/2008 season			2006/2007 season			2007/2008 season		
Biofertilizers treatments (g per seedling)	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean
Control	19.2 i	21.3 hi	20.2 H	18.9 m	22.2 l	20.5 G	4.8 i	5.3 hi	5.0 F	4.7 h	5.5 gh	5.1 E
Phosphorine 5 g	27.2 c	28.1 bc	27.6 C	25.4 g-i	29.8 e	27.6 B	6.8 c-f	7.1 b-e	6.9 BC	6.3 e-g	7.4 a-d	6.8 B
Phosphorine 10 g	28.0 bc	32.0 a	30.0 B	30.1 de	33.0 a-c	31.5 A	7.1 b-e	8.0 ab	7.5 AB	7.6 a-c	8.2 a	7.9 A
Phosphorine 20 g	32.0 a	33.0 a	32.5 A	31.8 b-d	33.6 a	32.6 A	8.0 ab	8.3 a	8.1 A	8.0 a	8.4 a	8.2 A
Nitrobine 5 g	21.0 hi	22.0 g-i	21.6 GH	21.0 l	22.4 j-l	21.8 F	5.2 hi	5.5 g-i	5.3 EF	5.5 gh	5.6 gh	5.5 DE
Nitrobine 10 g	23.2 e-h	24.0 d-h	23.6 E-G	22.3 kl	24.0 i-k	23.1 E	5.8 f-i	6.1 e-h	5.9 DE	5.7 f-h	6.1 e-g	5.9 CD
Nitrobine 20 g	23.0 f-h	23.4 e-h	23.2 FG	22.6 j-l	26.0 f-h	24.3 DE	5.7 f-i	6.0 e-h	5.8 D-F	5.9 e-g	6.5 d-g	6.2 B-D
Microbine 5 g	25.0 c-g	25.0 c-g	25.0 D-F	24.1 ij	25.2 hi	24.6 D	6.2 e-h	6.2 e-h	6.2 CD	6.0 e-g	6.3 e-g	6.1 B-D
Microbine 10 g	25.2 c-f	26.2 c-e	25.7 C-E	24.8 hi	27.0 fg	25.8 C	6.3 e-h	6.5 e-g	6.4 CD	6.2 e-g	6.7 c-f	6.4 BC
Microbine 20 g	26.1 c-f	27.1 cd	26.6 CD	24.6 hi	27.3 f	25.9 C	6.6 d-g	6.7 d-f	6.6 CD	6.1 e-g	6.8 b-e	6.4 BC
Combined application at low level (15g)	23.4 e-h	24 d-h	23.7 E-G	22.0 l	26.1 f-h	24.0 DE	5.8 f-i	6.2 e-h	6.0 DE	5.5 gh	6.5 d-g	6.0 CD
Combined application at mid. Level (30 g)	31.0 ab	32.8 a	31.7 AB	31.0 c-e	33.2 ab	32.2 A	7.7 a-d	8.2 ab	7.9 A	7.8 ab	8.3 a	8.0 A
Combined application at high level (60 g)	31.6 a	33.0 a	32.3 A	31.3 c-e	33.4 a	32.3 A	7.9 a-c	8.3 a	8.1 A	7.9 a	8.3 a	8.1 A
Mean	25.8 B	27.0 A		25.4 B	27.9 A		6.5 B	6.8 A		6.4 B	6.9 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level .So = sour orange Vl = volkamer lemon

Table 5: Effect of some biofertilizers on seedling dry weight (g) and nitrogen (%) in leaf of sour orange and volkamer lemon rootstocks in 2006/2007 and 2007/2008 seasons

	Seedling dry weight (g)						Leaf nitrogen (%)					
	2006/2007 season			2007/2008 season			2006/2007 season			2007/2008 season		
Biofertilizers treatments (g per seedling)	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean
Control	16.1 n	17.9 g-k	17.0 F	17.2 k	19.6 e-h	18.4 E	1.4 ef	1.7 b-f	1.6 DE	1.3 g	1.3 g	1.3 F
Phosphorine 5 g	16.3 mn	18.0 f-j	17.1 EF	17.8 jk	19.9 d-g	18.8 E	1.3 f	1.7 b-f	1.5 E	1.3 g	1.4 fg	1.35 EF
Phosphorine 10 g	16.5 l-n	18.1 f-j	17.3 D-F	18.1 i-k	20.2 c-g	19.1 DE	1.5 d-f	1.7 b-f	1.6 DE	1.4 fg	1.4 fg	1.4 EF
Phosphorine 20 g	17.4 h-m	18.7 d-g	18.0 CD	18.4 h-k	19.5 f-h	18.9 E	1.5 d-f	1.7 b-f	1.6 DE	1.5 e-g	1.6 d-g	1.5 D-F
Nitrobine 5 g	16.3 mn	17.9 g-k	17.1 EF	18.1 i-k	20.2 c-g	19.1 DE	1.6 c-f	1.7 b-f	1.6 DE	1.5 e-g	1.5 e-g	1.5 D-F
Nitrobine 10 g	17.2 i-n	18.6 e-h	17.9 C-E	19 g-j	20.9 b-e	19.9 CD	1.6 c-f	1.8 a-f	1.7 B-E	1.6 d-g	1.5 e-g	1.5 D-F
Nitrobine 20 g	17.6 g-l	18.8 c-g	18.2 BC	20 d-g	21.7 b	20.8 BC	1.9 a-f	2.1 a-d	2.0 A-D	1.7 c-g	1.8 c-f	1.7 CD
Microbine 5 g	16.7 k-n	18.4 f-i	17.5 C-F	19.2 g-i	21.4 bc	20.3 BC	2.0 a-e	2.0 a-e	2.0 A-D	1.5 e-g	1.8 c-f	1.6 C-E
Microbine 10 g	17.1 j-n	19.2 c-f	18.1 B-D	20.3 c-g	21.7 b	21.0 B	2.0 a-e	2.2 a-c	2.1 A-C	1.8 c-f	2.0 a-d	1.9 A-C
Microbine 20 g	18.2 f-j	19.8 c-e	19.0 B	20.8 b-f	23.2 a	22.0 A	2.3 ab	2.2 a-e	2.1 A-C	1.9 b-e	2.0 a-d	1.9 A-C
Combined application at low level (15 g)	17.2 i-n	19.2 c-f	18.2 BC	17.5 k	21 b-d	19.2 DE	2.2 a-c	2.0 a-c	2.2 A	1.7 c-g	1.9 b-e	1.8 CD
Combined application at mid. level (30 g)	19.9 b -d	21.1 ab	20.5 A	21.1 b-d	24.0 a	22.5 A	2.4 a	2.3 ab	2.3 A	2.0 a-d	2.3 ab	2.1 AB
Combined application at high level (60 g)	20.0 a-c	21.2 a	20.6 A	21.3 b-d	24.3 a	22.7 A	2.4 a	2.3 ab	2.3 A	2.1 a-c	2.4 a	2.2 A
Mean	17.4 B	18.9 A		19.1 B	21.3 A		1.8 B	1.9 A		1.6 A	1.7 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level .So = sour orange Vl = volkamer lemon.

Table 6: Effect of some biofertilizers on leaf contents of Phosphorous and potassium (%) of sour orange and volkamer lemon rootstocks in 2006/2007 and 2007/2008 seasons

	Phosphorous (%)						Potassium (%)					
	2006/2007 season			2007/2008 season			2006/2007 season			2007/2008 season		
Biofertilizers treatments (g per seedling)	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean	So.	Vl.	Mean
Control	0.11 c	0.14 fg	0.12 G	0.12 i	0.15 hi	0.13 D	1.0 gh	0.9 h	0.95E	0.9 a	0.9 a	0.91 F
Phosphorine 5 g	0.19 c-f	0.22 cd	0.20 CD	0.21 d-g	0.27 a-c	0.24 B	1.0 gh	1.1 fg	1.05 DE	1.0 a	1.0 a	1.00 EF
Phosphorine 10 g	0.21c-e	0.28 ab	0.24 AB	0.22 c-f	0.29 ab	0.25 AB	1.2 ef	1.1 fg	1.15 CD	1.0 a	1.0 a	1.00 EF
Phosphorine 20 g	0.22 cd	0.33 a	0.27 A	0.26 b-d	0.32 a	0.29 A	1.2 ef	o.9 h	1.07 D	1.1 a	1.1 a	1.10 DE
Nitrobine 5 g	0.16 e-g	0.19 c-f	0.17 D-F	0.15 hi	0.17 f-i	0.16 CD	1.3 de	1.2 ef	1.25 BC	1.3 a	1.0 a	1.16 CD
Nitrobine 10 g	0.16 e-g	0.19 g	0.17 D-F	0.16 g-i	0.18 f-h	0.17 CD	1.3 de	1.3 de	1.30 B	1.4 a	1.2 a	1.30 C
Nitrobine 20 g	0.19 c-f	0.21 c-e	0.20 C-E	0.16 g-i	0.18 f-h	0.17 CD	1.4 cd	1.3 de	1.35 B	1.5 a	1.4 a	1.45 B
Microbine 5 g	0.15 fg	0.18 d-f	0.16 EF	0.18 f-h	0.20 e-h	0.19 C	1.6 ab	1.5 bc	1.55 A	1.5 a	1.4 a	1.45 B
Microbine 10 g	0.18 d-f	0.22 cd	0.20 C-E	0.18 f-h	0.20 e-h	0.19 C	1.7 a	1.6 ab	1.65 A	1.6 a	1.5 a	1.55 AB
Microbine 20 g	0.21 c-e	0.24 bc	0.22 BC	0.20 e-h	0.26 b-d	0.23 B	1.7 a	1.6 ab	1.65 A	1.6 a	1.6 a	1.60 A
Combined application at low level (15 g)	0.16 e-g	0.19 c-f	0.17 D-F	0.17 f-i	0.19 f-h	0.18 C	1.5 bc	1.6 ab	1.55 A	1.2 a	1.2 a	1.25 C
Combined application at mid. level (30 g)	0.23 b-d	0.32 a	0.27 A	0.26 b-d	0.32 a	0.29 A	1.6 ab	1.6 ab	1.60 A	1.5 a	1.4 a	1.45 B
Combined application at high level (60 g)	0.22 cd	0.31 a	0.26 A	0.25 b-e	0.32 a	0.28 A	1.7 a	1.6 ab	1.65 A	1.5 a	1.5 a	1.50 AB
Mean	0.18 B	0.22 A		0.19 B	0.23 A		1.40 A	1.33 B		1.32 A	1.24B	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level .So = sour orange Vl = volkamer lemon.

Concerning N content, leaf of Volkamer lemon contained higher N than Sour orange. The highest N content was recorded with combined application of the three biofertilizers at high level (20 g per seedling); this was noticed for both two tested rootstocks. Single application of microbine or nitrobine was more effective in increasing leaf N content than phosphorine treatments. Also, increasing biofertilizer level from 5 to 20 g per seedling improved leaf nutritional status. On the contrary, the lowest values of leaf N content were detected with the control and single application of phosphorine.

Regarding P content, it was noticed that leaf of Volkamer lemon contained higher P than Sour orange and the differences between them were significant. Combined application of the three biofertilizers at high or mid levels (20 or 10 g per seedling) or single application of phosphorine at 20 g per seedling recorded higher leaf P content than the other treatments. On the other side, the lowest leaf P content of Sour orange and Volkamer lemon was detected with the control.

In addition K content, it was noticed that leaf of Volkamer lemon recorded higher significant values of K than Sour orange. In respect to the effect of biofertilizer treatments on leaf K content of Sour orange and Volkamer lemon, the highest values were recorded with nitrobine at 20 g per seedling followed by combined application of the three biofertilizers at high level. In general, microbine treatments recorded higher values of leaf K content than nitrobine. On the other hand, the lowest values of leaf K content were observed with the control and phosphorine treatments; this was noticed with both Sour orange and Volkamer lemon. Results of the mineral nutritional status

Table 7: Effect of biofertilizers on budding success (%) and scion length (cm) of Valencia orange budded on sour orange and volkamer lemon rootstocks in 2007 and 2008 seasons

	Budding success (%)						Scion length (cm)					
	2007 season			2008 season			2007 season			2008 season		
Biofertilizers treatments (g per seedling)	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean
Control	80.0 e	83.3 d	81.6 G	80.0 e	83.3 d	81.6 F	21.2 l	24.6 jk	22.9 I	21.9 o	26.1 kl	24..0 K
Phosphorine 5 g	80.0 e	83.3 d	81.6 G	80.0 e	83.3 d	81.6 F	24.0 k	26.0 ij	25.0 H	24.6 m	26.3 k	25.4 J
Phosphorine 10 g	83.3 d	86.6 c	84.9 E	86.6 c	86.6 c	86.6 C	24.9 jk	28 gh	26.4 FG	25.2 lm	28.6 hi	26.9 HI
Phosphorine 20 g	86.6 c	86.6 c	86.6 D	86.6 c	86.6 c	86.6 C	25.0 jk	31.0 de	28.0 E	26.0 kl	30.0 fg	28.0 FG
Nitrobine 5 g	80.0 e	83.3 d	81.6 G	80.0 e	83.3 d	81.6 F	23.3 k	28.5 f-h	25.9 GH	23.5 n	29.0 gh	26.2 I
Nitrobine 10 g	83.3 d	86.6 c	84.9 E	86.6 c	86.6 c	86.6 C	27.3 hi	30.0 ef	28.6 E	28 h-j	29.8 fg	28.9 E
Nitrobine 20 g	90.0 b	90.0 b	90.0 C	93.3 b	93.3 b	93.3 B	30.0 ef	31.0 de	30.5 D	30.2 f	32.0 e	31.1 D
Microbine 5 g	83.3 d	83.3 d	83.3 F	83.3 d	83.3 d	83.3 E	28.0 gh	27.0 hi	27.5 EF	27.6 ij	27.5 j	27.5 GH
Microbine 10 g	86.6 c	86.6 c	86.6 D	83.3 d	86.6 c	84.8 D	29.0f-h	32.6 cd	30.6 CD	30.0 fg	32.8 d	31.4 D
Microbine 20 g	90.0 b	93.3 a	91.6 B	93.3 b	93.3 b	93.3 B	31.0 de	33.0 c	32.0 C	32.0 e	33.4 d	32.7 C
Combined application at low level (15 g)	83.3 d	83.3 d	83.3 F	83.3 d	83.3 d	83.3 E	28.3f-h	29.5 e-g	28.9 E	28.0 h-j	29.0 gh	28.5 EF
Combined application at mid. level (30 g)	93.3 a	93.3 a	93.3 A	93.3 b	96.6 a	94.9 A	32.1 cd	35.4 b	33.7 B	33.0 de	37.0 b	35.0 B
Combined application at high level (60 g)	93.3 a	93.3 a	93.3 A	93.3 b	96.6 a	94.9 A	35.0 b	38.6 a	36.7 A	34.6 c	39.2 a	36.9 A
Mean	85.6 B	87.1 A		86.3 B	87.8 A		27.5 B	30.3 A		28.0 B	30.8 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level Vo/So (Valencia orange budded on sour orange) Vo/Vl (Valencia orange budded on volkamer lemon)

in relation to biofertilizer treatments indicated that, leaf mineral contents of N, P and K were increased by increasing level of the three biofertilizers and combined them together. Microbine and nitrobine treatments were more effective in increasing N and K than phosphorine which increased leaf P content. The improving of leaf nutritional status by application of biofertilizers may be due to the effect of biofertilizers on enhancing availability of nutrients [20]. Moreover, Gaudry [21] mentioned that bacteria of *Azotobacter* and *Azospirillium* could produce Indole acetic acid and cytokinin, which increase the surface area per unit root length and were responsible for root hair branching with an eventual increase of nutrients from soil. Also, using biofertilizers to improve nutritional status of different fruit crops were reported by Izquierdo *et al.* [22] on Volkamer lemon seedling; Singh and Sharma [23] on sweet orange;; Abou Taleb *et al.* [24] on Olive; El-Sharkawy and Mehisen [25] on Guava; Abd-Rabou [19] on Avocado and Mango.

Experiment II: Response of Budded Seedlings to Biofertilizers:

Budding Growth: Data presented in Table 7 revealed that budded Valencia orange on Volkamer lemon gave a higher significant percent of budding success percentage than Sour orange rootstock. Combined application of the three biofertilizers at mid and high levels gave the highest significantly budding success percentage; this was noticed with both rootstocks. Interaction between rootstocks and biofertilizers treatments indicated that, Valencia orange budded on seedling of Volkamer lemon which inoculated with combined application of the three biofertilizers at high or mid levels had the highest budding success percentage.

Concerning the effect of biofertilizers on growth behaviors of Valencia orange budded on sour orange and Volkamer lemon data presented in Tables 7 and 8 revealed that, budded Valencia orange on Volkamer lemon gave the highest significantly values of scion length, number of leaves per scion and leaf area in both studied seasons.

Combined application of the three biofertilizers at high level (20 g per seedling) gave the highest significant values of scion length and number of leaves per scion. Meanwhile, the lowest values of scion length and number of leaves per scion were detected with the control. Interaction between rootstocks and biofertilizers treatments showed the highest values of scion length and number of leaves per scion with combined application of the three biofertilizers at high level (20 g per seedling); this was noticed with both rootstocks. Concerning leaf area, the highest values was observed with nitrobine at 20 g per seedling for scions growing on Sour orange. Concerning leaf area of the scions growing on Volkamer lemon, the highest values was detected with combined application of the three biofertilizers at low level (5 g per seedling) in both seasons. On the other hand the lowest leaf area was recorded with single application of phosphorine at 5 g per seedling. In general, single application of microbine or nitrobine or combined application of the three biofertilizers at high level were

Table 8: Effect of biofertilizers on number of leaves per scion and leaf area (cm2) of Valencia orange budded on sour orange and volkamer lemon rootstocks in 2007 and 2008 seasons

	Number of leaves per scion						Leaf area (cm2)					
	2007 season			2008 season			2007 season			2008 season		
Biofertilizers treatments (g per seedling)	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean
Control	19.2 n	23.0 l	21.1 H	23.0 h-j	25.1 fg	24.0 E	21.9 kl	27.6 a-c	24.7 E-G	22 gh	27.0 ab	24.5 D-F
Phosphorine 5 g	19.1 n	21.2 m	20.1 I	20.0 l	22.0 jk	21.0 G	16.8 n	21.8 kl	19.3 I	17.0 j	21.9 hi	19.4 G
Phosphorine 10 g	23.0 l	23.0 l	23.0 G	24.3 f-h	23.1 h-j	23.7 E	25.0 f-h	23.6 ij	24.3 GH	25.1 b-e	23.4 e-h	24.2 EF
Phosphorine 20 g	24.0 jk	27.8 de	25.9 D	25.2 fg	27.0 e	26.1 D	23.2 j	27.6 a-c	25.4 C-E	23.0 f-h	27.1 a	25.0 C-E
Nitrobine 5 g	21.0 m	22.5 l	21.7 H	21.2 kl	24.0 g-i	22.6 F	26.3 de	22.5 jk	24.4 F-H	26.7 ab	23.9 d-g	25.30 B-E
Nitrobine 10 g	22.8 l	25.0 hi	23.9 EF	23.0 h-j	24.1 g-i	23.5 EF	24.6 hi	24.8 gh	24.7 E-G	25.1 b-e	24.5c-f	24.8 C-F
Nitrobine 20 g	27.0 ef	28.1 cd	27.5 C	27.0 e	29.0 cd	28.0 C	27.1 b-d	28.2 ab	27.6 A	27.2 a	27.1 a	27.1 A
Microbine 5 g	23.0 l	24.0 jk	23.5 FG	22.8 ij	24.6 fg	23.7 E	23.4 j	26.9 c-e	25.1 D-F	24.3 c-f	27.0 ab	25.6 B-D
Microbine 10 g	23.2 kl	24.3 ij	23.7 EF	24.1 g-i	24.5 fg	24.3 E	21.2 l	26.5 c-e	23.8 H	22.2 gh	26.9 ab	24.5 D-F
Microbine 20 g	26.0 g	29.0 c	27.5 C	27.0 e	29.6 bc	28.3 C	26.3 de	25.9 e-g	26.1 BC	27.0 ab	26.2 a-c	26.6 AB
Combined application at low level(15 g)	23.0 l	25.8 gh	24.4 E	23.0 h-j	25.6 f	24.3 E	19.0 m	28.3 a	23.6 H	20.0 i	27.2 a	23.5 F
Combined application at mid. level(30 g)	26.1 fg	31.0 b	28.5 B	28.0 de	32.0 a	30.0 B	26.8 c-e	24.7 hi	25.7 B-D	27.1 a	24.3 c-f	25.7B-D
Combined application at high level(60 g)	31.0 b	32.0 a	31.5 A	30.8 ab	32.1 a	31.4 A	26.0 d-f	26.7 c-e	26.3 B	26.2 a-c	25.8 a-d	26.0 A-C
Mean	23.7 B	25.9 A		24.5 B	26.3 A		23.6 B	25.7 A		24.0 B	25.5A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level Vo/So (Valencia orange budded on sour orange) Vo/Vl (Valencia orange budded on volkamer lemon)

more effective in inducing growth of Valencia orange budded on Sour orange and Volkamer lemon rootstocks than control or phosphorine treatments. The growth enhancement by biofertilizers may be due the production of growth regulators as well as to N fixation. In this concern, Bashan and Levanony [26] reported that plant inoculation with nitrogen fixer such as *Azospirillum* affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plants, enhancement in uptake NO3, NH4, PO4 and K by nitrogen fixing bacteria was proposed to cause an increase in foliar dry mater, accumulation of minerals in stem and leaves, increase in root system and finally resulted in higher growth. Abd El-Aziz [27] reported that the vegetative growth of Williams banana were enhanced by application of biogein, nitrobein and microbein biofertilizers. Also, Hammam [28] indicated that fertilizing Williams and Cavendish banana plants with microbein produced the maximum height and girth of pseudostem and total leaf area. Abd El-Samad *et al.* [29] revealed that, growth of Sour orange, Volkamer lemon and Cleopatra mandarin were improved by biofertilizers. Also, they added that Valencia orange budded on Volkamer lemon rootstock had higher values of stem length and diameter than those on Sour orange. Moreover, Aseri *et al.* [30] enhanced growth of pomegranate by using biofertilizers.

Leaf chlorophyll: Data presented in Table 9 clearly show that budded Valencia orange on Sour orange recorded higher significant leaf chlorophyll content than those budded on Volkamer lemon; this was noticed in both seasons. Combined application of the three biofertilizers at high level or single application of nitrobine at 20 g per seedling recorded the highest leaf chlorophyll content of Valencia orange budded on Sour orange or Volkamer lemon rootstocks. Concerning the interaction between rootstocks and biofertilizer treatments results showed that, the application with nitrobine at 20 g per seedling or combined application of the three biofertilizers at high or mid levels (20 or 10 g per seedling) recorded the highest values of leaf chlorophyll content. Nitrobine was more effective in increasing leaf chlorophyll content than microbine. On the other hand, the lowest leaf chlorophyll content was observed with the control and phosphorine at 5 g per seedling. The important role of biofertilizers on enhancing the formation of leaf chlorophyll might be attributed to their action on increasing availability of water and minerals. The results of Ahmed and El-Dawwey [31]; El-Sayied [32] and Ragab [6] supported the stimulating effect of biofertilizers on chlorophyll. Also, Tiwary *et al* [33] recorded higher chlorophyll content in leaves of Banana inoculated with N2-fixing bacteria. Moreover, Kohler *et al.* [34] reported that the high chlorophyll content might have resulted from enhanced plant growth.

Table 9: Effect of biofertilizers on leaf chlorophyll (mg/100 g f.w) and nitrogen (%) contents of Valencia orange budded on sour orange and volkamer lemon rootstocks in 2007 and 2008 seasons

	Leaf chlorophyll						Leaf nitrogen (%)					
	2007 season			2008 season			2007 season			2008 season		
Biofertilizers treatments (g per seedling)	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean
Control	42.0 fg	38.0 ij	40.0 GH	41.3 ij	37.0 l	39.1 I	1.6 b	1.8 ab	1.7 CD	1.7 b	1.9 ab	1.8 B
Phosphorine 5 g	41.9 fg	36.0 k	38.9 I	42.0 i	37.1 l	39.5 I	1.6 b	1.7 ab	1.6 D	1.7 b	1.9 ab	1.8 B
Phosphorine 10 g	42.1 fg	37.0 jk	39.5 HI	43.0 h	39.0 k	41.0 H	1.6 b	1.8 ab	1.7 CD	1.8 ab	1.9 ab	1.8 B
Phosphorine 20 g	43.0 ef	37.9 j	40.4 FG	45.0 f	41.0 j	43.0 E	1.7 ab	1.8 ab	1.7 CD	1.8 ab	2.0 ab	1.9 AB
Nitrobine 5 g	46.1 c	42.0 fg	44.0 DE	47.0 e	42.0 i	44.5 D	1.8 ab	1.8 ab	1.8 B-D	1.7 b	1.9 ab	1.8 B
Nitrobine 10 g	49.2 b	48.1 b	48.6 C	49.3 b	46.3 e	47.8 B	1.8 ab	1.9 ab	1.8 A-D	1.8 ab	1.9 ab	1.8 B
Nitrobine 20 g	53.0 a	49.0 b	51.0 A	53.2 a	48.3 cd	50.7 A	2.0 ab	2.0 ab	2.0 A-D	1.9 ab	2.0 ab	1.9 AB
Microbine 5 g	42.0 fg	40.0 h	41.0 F	43.0 h	41.0 j	42.0 G	2.0 ab	2.1 ab	2.0 A-D	1.8 ab	1.8 ab	1.8 B
Microbine 10 g	44.0 de	43.0 ef	43.5 E	43.3 gh	41.0 j	42.1 FG	2.1 ab	2.2 ab	2.1 A-C	1.9 ab	2.0 ab	1.9 AB
Microbine 20 g	44.0 de	45.0 cd	44.5 D	48.2 d	44.0 g	46.1 C	2.2 ab	2.3 a	2.2 AB	2.0 ab	2.1 ab	2.0 AB
Combined application at low level(15 g)	41.3 g	39.2 hi	40.2 F-H	44.1 fg	41.3 ij	42.6 EF	1.9 ab	2.0 ab	1.9 A-D	1.7 b	1.9 ab	1.8 B
Combined application at mid. level(30 g)	53.0 a	46.1 c	49.5 B	52.7 a	48.1 d	50.4A	2.0 ab	2.1 ab	2.0 A-D	2.1 ab	2.2 ab	2.1 AB
Combined application at high level(60 g)	53.0 a	49.0 b	51.0 A	52.8 a	49.2 bc	51.0 A	2.3 a	2.4 a	2.3A	2.1 ab	2.3 a	2.3 A
Mean	45.7 A	42.3 B		46.5 A	42.7 B		1.8 A	1.9 A		1.8 A	1.9 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level Vo/So (Valencia orange budded on sour orange) Vo/Vl (Valencia orange budded on volkamer lemon)

Table 10: Effect of biofertilizers on leaf Phosphorous and potassium contents (%) of Valencia orange budded on sour orange and volkamer lemon rootstocks in 2007 and 2008 seasons

	Leaf Phosphorous (%)						Leaf potassium (%)					
	2007 season			2008 season			2007 season			2008 season		
Biofertilizers treatments (g per seedling)	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean	Vo/So	Vo/Vl	Mean
Control	0.12 i	0.41 hi	0.13 F	0.13 i	0.15 g-i	0.14 E	1.10 hi	1.21 e-g	1.15 G	1.05 gh	1.10 e-g	1.07 FG
Phosphorene 5 g	0.15 ghi	0.16 f-i	0.16 EF	0.14 hi	0.18 e-i	0.16 DE	1.21 e-g	1.15 f-h	1.18 E-G	1.02 h	1.12 ef	1.07 FG
Phosphorene 10 g	0.18 e-h	0.19 d-h	0.19 C-E	0.19 e-i	0.21 d-h	0.20 CD	1.22 ef	1.23 d-f	1.22 DE	1.06 gh	1.13 ef	1.09 FG
Phosphorene 20 g	0.25 abc	0.26 ab	0.25 AB	0.25 a-e	0.28 a-d	0.26 AB	1.31 b-d	1.22 ef	1.26 CD	1.12 ef	1.21 c	1.16 D
Nitrobiene 5 g	0.19 d-h	0.19 d-h	0.19 C-E	0.16 g-i	0.18 e-i	0.17 DE	1.02 i	1.02 i	1.02 H	1.03 h	1.02 h	1.02 H
Nitrobiene 10 g	0.20 c-g	0.20 c-g	0.20 CD	0.17 f-i	0.19 e-i	0.18 C-E	1.13 gh	1.03 i	1.08 H	1.03 h	1.03 h	1.03 H
Nitrobiene 20 g	0.19 d-h	0.20 c-g	0.19 CD	0.18 e-i	0.21 d-h	0.19 CD	1.25 c-e	1.24 c-e	1.25 CD	1.15 de	1.22 c	1.18 CD
Microbiene 5 g	0.18 e-h	0.19 d-h	0.18 C-E	0.16 g-i	0.18 e-i	0.17 DE	1.03 i	1.13 gh	1.08 H	1.06 gh	1.05 gh	1.05 GH
Microbiene 10 g	0.18 e-h	0.21 b-f	0.19 CD	0.17 f-i	0.19 e-i	0.18 C-E	1.22 ef	1.22 ef	1.22 D-F	1.20 cd	1.24 c	1.22 C
Microbiene 20 g	0.21 b-f	0.23 b-e	0.22 BC	0.22 c-g	0.24 b-f	0.23 BC	1.25 c-e	1.35 ab	1.3 BC	1.21 c	1.32 b	1.26 B
Combined application at low level (15 g)	0.16 f-i	0.19 d-h	0.17 DE	0.19 e-i	0.21 d-h	0.20 CD	1.12 hi	1.23 d-f	1.16 FG	1.12 fg	1.15 de	1.11 E
Combined application at mid. level (30 g)	0.24 a-d	0.26 ab	0.25 AB	0.27 a-d	0.29 a-c	0.28 AB	1.32 bc	1.35 ab	1.33 AB	1.33 b	1.41 a	1.37 A
Combined application at high level (60 g)	0.25 a-c	0.29 a	0.27 A	0.31 ab	0.32 a	0.31 A	1.36 ab	1.43 a	1.39 A	1.35 b	1.45 a	1.40 A
Mean	0.19 B	0.21 A		0.19 B	0.21 A		1.19 A	1.22 A		1.13 B	1.18 A	

Within the column for biofertilizers treatment means, the row for rootstocks means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at the 5% level Vo/So (Valencia orange budded on sour orange) Vo/Vl (Valencia orange budded on volkamer lemon)

Leaf Mineral Contents of Valencia Orange Budded on Sour Orange and Volkamer Lemon as Affected by Biofertilizers: Leaf N, P and K contents of Valencia orange scions budded on Sour orange and Volkamer lemon rootstocks are presented in Tables 9 and 10.

Concerning leaf N content the Valencia orange on Volkamer lemon recorded higher values than those on Sour orange however the differences between them were insignificant. The highest N content was recorded with combined application of the three biofertilizers at high level (20 g per plant). Single applications of microbine or nitrobine were more effective in increasing leaf N content than phosphorine treatments which recorded nearly the similar result of the control. Leaf of Valencia orange on Volkamer lemon contained higher P than those on Sour orange and the differences were significant in both seasons. Regardless of rootstocks, the highest leaf P content was observed with combined application of the three biofertilizers at high level (20 g per plant) followed by mid level (10 g per plant) then single application of phosphorine at 20 g per plant. Interaction between

rootstocks and biofertilizers showed the highest values of leaf P content with combined application of the three biofertilizers at high level (20 g per plant). Leaf of Valencia orange on Volkamer lemon contained higher K than those on Sour orange and the differences were significant in the second season only. Combined application of the three biofertilizers at high level (20 g per seedling) recorded the highest values. From the above mentioned results it could be concluded that leaf nutritional status of Valencia orange on Volkamer lemon or Sour orange were improved with the combined application of three biofertilizers at high level. Also, Volkamer lemon rootstock was more effective in increasing leaf mineral contents than Sour orange this may be related to the effect of Volkamer on stimulating growth of Valencia orange than the Sour orange rootstock. The great availability and release of N, P and K due to the application of biofertilizers were announced by Ahmed and El-Dawwey [31] on Olive and Chokha and Sharma [35] on Sweet orange; Abd El-Samad *et al.* [29] on Citrus and Al-Ashkar [36] on Banana.

Results of this study cleared the importance role of microbine, nitrobine and phosphorine in improving growth of Sour orange, Volkamer lemon rootstocks and scions of Valencia orange budded on them.

REFERENCES

1. Abd El-Ghany, B.F., M.O. Salem and M.A.F. El Sibaie, 1997. Effect of bacterial inoculation, P and organic fertilization on microbiological properties, growth and production of fenugreek plant under saline calcareous soil conditions. Desert Inst. Bull., Egypt, 47: 1-13.
2. El Kholy, A.F., 1998. Essentiality of bio-fertilizers with special references to biological nitrogen fixation (BNF). Egypt J. Soil Sci., 38: 339-352.
3. David, G., 2002. Tree fruits production with organic farming methods. Center for Sustaining Agriculture and Natural Recourses. Washington State Univ., Wenatchee, AUS. (www.yahoo.com).
4. Castle, W.S., O.P. Tueker, H.A. Krezdorn and C.O. Youtsey, 1993. Rootstock for Florida Citrus. Rootstock Selection: The First Step to Success. 2nd (Ed.), Uni. of Florida, pp: 14-21.
5. Salem, S.E., S.S. Moustafa, M.A. Abdel-Rahman and L.F. Gunidy, 1994. Evaluation of Valencia orange tree on sour orange and Volkamer lemon under sandy soil conditions. Bull. Fac. Agric., Cairo Univ., 45: 827-838.
6. Ragab, M.A., 1999. Effect of six biofertilizers on growth and uptake of some nutrients in Chemlali olive seedling. Minia J. Agric. Res. and Dev., 19 : 45-65.
7. Shehata, W.A.M., 2008. Studies on bio-fertilization of olive transplants. M.Sc. Thesis, Fac. Agric., Cairo Univ. Egypt.
8. Goenadi, D.H., R. Saraswati, Y. Away, I. Herman, Y. Santoso, I. Sukin, I. Haryanto, A.A. Rais, L. Rocmalia, M.S. Arifin, D.S. Damardjati, L.P. Santi, dan S. Gunawan., 1999. Produksi Biofertilizer untuk Efisiensi Penggunaan Pupuk dalam Budidaya Tanaman yang Aman Lingkungan. Laporan Akhir Penelitian Riset Unggulan Kemitraan II 1996/67-1998/99. Unit Penelitian Bioteknologi Perkebunan bekerja sama dengan PTP Nusantara I/III/IV/VII/VIII/XIV. hlm. 214.
9. Okon, Y., S. Sang and A. Blum, 1989. Promotion root growth in Sorgum bicolor inoculated with *Azospirillum brasiliense*. p. 196-200. In T. Hattori, Y. Ishida, Y. Maruvama, R.Y. Morita, and A. Uchida (Eds.). Recent Advances in Microbial Ecology. Japan Sci. Soc. Press.
10. Fallik, E., Y. Okon and M. Fricher, 1988. The effect of *Azospirillum brasiliense* inoculation on metabolic enzyme activity in maize root seedlings. Symbiosis, 7-18.
11. Ghazi, N.A.K., 2006. Nursery inoculated of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with sterile water. Sci. Hortic., 109: 1-7.
12. A.O.A.C. 1985. Official Methods of Analysis. 14th (Ed.) Washington.
13. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods, 7th (Ed.) The Iowa State Univ. Press, Amer., lowa., USA.
14. Duncan, D.B., 1955. Multiple ranges and multiple F test. Biometrics, 11: 1-42.
15. Haggag, L.F. and M.A. Azzazy, 1996. Evaluation of Microbein as a multi-strains biofertilizer for production of improved Mango seedling with appropriate vigour for grafting in shorter time. Ann. of Agric. Sci. Ain Shams Univ. Cairo, 41: 321-331.
16. Chundawet, B.S., 2001. Integrated nutrient management in tropical and subtropical fruits. Indian J. Hort., 58: 59-69.
17. Abou El-Khashab, A., A.A. Safia and S.T. Wafaa, 2005. Eggazi and Koroneiki olive trees as affected by organic and biofertilizers, calcium citrate and potasseine. Ann. Agr. Sci. Ain Shams Univ., 13: 419-440.

18. Yousif, A.M. and H.A. Marzouk, 2005. Comparative studies of using mineral and Biofertilizers on growth and yield of guava. J. Agric. Adv. Res., 10: 283-292.
19. Abd-Rabou, F.A., 2006. Effect of microbine and phosphorine and effective micro-organisms (EM) as bio-stimulants on growth of avocado and mango seedlings. Egypt. J. Appl. Sci., 21: 673-693.
20. Frankenberger, W.T. and M.J.R. Arshad, 1995. Phytohormones in soils (Microbial production and function). Marcel Dekker, Inc. Publishers, New York, USA.
21. Gaudry, J.F.M., 2001. Nitrogen assimilation by plants: physiological, biochemical and molecular aspects. Published by Science Publishers, Inc. Enfield. NH, USA, pp: 185-197.
22. Izquierdo, I., M. Lescaille, B. Sandrino, M.J. Garcia, E. Canizares, J. Azcuy and M.E. Rodriguez, 1993. Effect of biofertilizer combinations on the availability of soil NPK to citrus Volkameriana seedlings. Sevilla-Espana-19-26 September, pp: 711-719.
23. Singh, C. and B.B. Sharma, 1993. Leaf nutrient composition of sweet orange as affected by combined use of bio and chemical fertilizers. South Indian Hortic, 41: 131-134.
24. Abou-Taleb, A.S., A.M. Abou El-Khashab and W.T. Saeed, 2004. Growth and productivity of olive trees as affected by some bio and mineral nitrogen fertilizers. Minofiya J. Agric. Res., 29: 933-963.
25. El-Sharkawy, M.M. and S.M.A. Mehaisen, 2005. Response of guava trees to bio-fertilization. Minofiya J. Agric. Res., 30: 673-688.
26. Bashan, Y. and H. Levanony, 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can. J. Microbial., 36: 591-608.
27. Abd El-Aziz, A.B.K., 2002. Physiological studied on biofertilization of banana plants cv. Williams. Ph.D. Thesis, Fac. Agric. Minia Univ., Egypt.
28. Hammam, M.S., 2003. Effect of biofertilization on growth and fruiting of Cavendish and Williams bananas. Egypt, J. Hort, 30: 67-81.
29. Abd El-Samad, G.A., M.A. Galal, M.M. El-Badry and S.M. Hussein, 2006. Response of Valencia orange budded on some citrus rootstocks to bio-fertilization and growing media. The second Conference on Farm Integrated Pest Management, 16-18 Jan. 2006, Fac. Agric. Fayoum Univ. Egypt.
30. Aseri, G.K., N. Jain, J. Panwar, A.V. Rao and P.R. Meghwal, 2008. Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) in indian thar desert. Scientia Horticulturae, 117: 130-135.
31. Ahmed, F.F. and G.M. El-Dawwey, 1992. Effect of phosphorene (as source of phosphate dissolving bacteria) in enhancing growth and supplying of Chemlali olive seedlings with available phosphorus. Minia J. Agric. Res. and Dev., 14 : 37-54.
32. El-Sayied, A.I., 1996. Response of some fruit seedlings to soil inoculation with Mycorrhizae fungi. M. Sc. Thesis, Fac. Of Agric. Moshtohor. Zagazig Univ. Egypt.
33. Tiwary, D.K., M.A. Hassan and P.K. Chattopadhyey, 1999. Leaf nutrient and chlorophyll and content in banana under the influence of *Azotobacter* and *Azospirillum* inoculation. Environ. and Ecology, 17: 346-350.
34. Kohler, J., F. Caravaca, L. Carrasco and A. Rolden, 2007. Interaction between a plant growth promoting rhizobacterium, an AM fungus and phosphate-solublizing fungus in the rhizosphere of *Lactuca sativa*. Appl. Soil Ecol., 35: 480-487.
35. Chokha, S. and B.B. Sharma, 1993. Leaf nutrient composition of sweet orange as affected by combined use bio and chemical fertilizers. South Indian Horticulture, 41: 131-134.
36. Al-Ashkar, R.A., A.E.M. Mansour and M.M. Merwad, 2007. Effect of some organic and biofertilization treatments on growth and productivity of Grandnian banana plants. Egypt. J. Appl. Sci., 22: 276-301.