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Effect of Some Stimulative Substances on Growth of Two Citrus Rootstocks

[1]Abeer T. Mohsen, [1]M.A. Abdel-Mohsen, [2]Magdy A. Ibrahim and [2]Amany S. Mostafa

[1]Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Citrus Research, Horticulture Research Institute. ARC. Giza

Abstract: The present study was conducted in the greenhouse of the nursery of Horticulture Research Institute, Giza, Egypt on Sour orange (*Citrus aurantium* L.) and Volkamer lemon (*Citrus volkameriana*) rootstocks seedlings during the growing season of 2011 and 2012. The study aimed to the assess the positive influence of using phosphorus, active dry yeast, algae (diatoms) and combinations between them on improving the growth of those rootstocks. The obtained results showed an improvement in the vegetative growth characteristics. Moreover, transplants treated with combination between active dry yeast, algae and phosphorus showed the highest significant performance of stem length, stem diameter, dry weight of roots and leaf area. Also, leaves nitrogen, phosphorus and potassium content increased significantly with the addition of combination between active dry yeast, algae and phosphorus. Therefore, it is recommended to use the combination between active dry yeast, algae and phosphorus in order to produce transplants with good vegetative characteristics and to shorten the time the seedling needs to be fit for grafting.

Key words: Citrus rootstocks · Sour orange · Volkamer lemon · Phosphorus · Active dry yeast-Algae · Growth characters

INTRODUCTION

Citrus is one of the most important world fruit crops grown in many tropical and subtropical countries, Citrus occupies a prominent position in the fruit industry of the world, as well as in Egypt. It is the first popular fruit in Egypt and the most important fruit crops regarding both production and consumption because of the high nutritional value of their licenses and the length of displayed markets [1]. Moreover, it occupies about 518694 feddans which represent 33.97% of total fruit cultivated area in Egypt during 2012 [2]. During the past decades the Egyptian citrus industry has continued to increase. The number of nurseries has also increased to meet the demand caused by replanting, tree loss to aging and virus and virus-like diseases and industry expansion to new land [3].

Rootstocks are of vital importance in the quality and quantity of production and survival of citrus plants. Sour orange (*Citrus aurantium L.*) is a universal rootstock for citrus and widely used in the Mediterranean region [4]. Sour orange rootstock is reported to be suitable for heavy moist soil, gives good yield and quality fruits, but with smaller fruit size, thin and smooth skin, high TSS and acidity [5]. Volkamer lemon)*Citrus volkameriana* (is used as rootstock for citrus, due to its tolerance and its acceptable resistance of a large scale of citrus disease. It has significant effect on growth due to its suitability for the unfavorable environmental (climatic and soil) conditions [4].

Phosphorus (P) is one of the most important elements for plant growth and metabolism. It plays a key role in many plant processes such as energy metabolism, the synthesis of nucleic acids and membranes, photosynthesis, respiration, nitrogen fixation, enzyme regulation [6]. Further, bio fertilizer as yeast or algae extract are recommended for increasing the growth parameters of many plants [7-9]. Increasing of vegetative growth potential is one of the positive effects of yeast application [10]. Applying of active dry yeast was very effective in improving the growth, leaf area and leaf content of N, P and K [7]. The various positive effects of applying active dry yeast to different plants were attributed to its high content nutrients, high percentage of proteins, large amount of vitamin B and the natural plant growth hormones [8]. Algae extract contains N, P, K,

Corresponding Author: Abeer T. Mohsen, Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt.

Ca, Mg, S, Zn, Fe, Mn, Cu, Mo, Co, some growth regulators, polyamines and vitamins. It is applied to improve nutritional status, vegetative growth, yield and fruit quality in different orchard [11].

In this concern, this study was carried out to determine the optimal combinations that lead to increase the growth of root system and plant nutritional statue which reflect directly on the vegetative growth and consequently reduce the time needed in the nursery for seedling production.

MATERIALS AND METHODS

The present study was carried out during two consecutive seasons 2011 and 2012 in the nursery of horticulture research institute, Giza, Egypt.

Experiment Layout: Phosphoric acid (85 -90 %) as a source of phosphorus, diatoms as a source of algae and active dry yeast as a source of yeast were used in this investigation to enhance growth of citrus rootstock. A preliminary experiment was conducted to determine the range of the concentrations of algae and phosphorus to be investigated in this study. Uniform and healthy seedlings of two rootstocks were divided into two groups for each rootstock to study each of the two. Each group was divided into 4 sub groups each containing 6 seedlings (replicates). Algae was studied at four concentrations (1.2, 2.4, 3.6 and 4.8 gm per seedling) other group was treated with four phosphorus concentrations (0.6, 1.2, 1.8 and 2.4cm per seedling).

According to the preliminary data, three concentrations for phosphorus (1.2, 1.8 and 2.4cm phosphoric acid (85-90 %) per seedling), one concentration for algae (4g) were chosen. The yeast was used at 0.5g yeast plus 0.5g sugar to 250cm of water and left for 12 hours to ferment. Uniform seeds of Sour orange and Volkamer lemon were planted in January of both seasons of study. Two months after planting, 480 uniform and healthy seedlings of each rootstock were chosen and transplanted individually in black polyethylene bags with dimensions 15 x 35cm, filled with sand : peat moss (3:1) and kept under greenhouse.

Sixteen treatments for each rootstock were applied. Each treatment replicated three times, each with 10 seedlings. The seedlings were treated every 10 days until the end of the experiment (10 months for each season). Besides, seedling of all treatments were fertilized with recommended nutrient does every week till the end of growing season according to ministry assessment of agriculture recommended dose.

Growth Parameter: The following morphological and chemical parameters were recorded at the end of the experiment in both seasons.

Morphological parameter: Stem length (cm), stem diameter (cm) and leaf area (cm^2) as average and total and dry weight of roots after drying at 70°C.

To measure average and total leaf area, twenty mature leaf sample of each replicate were taken at the middle portion of shoots and by using a cork borer twenty disks (1cm diameter) of each were taken. Dry weight of each disk was measured then average leaf area was calculated according to the equation of Bleasdale [12].

- Leaf area = dry wt. of leaves x diameter of disk / dry wt. of disk.
- And total leaf area collected as follows:
- Total leaf area = number of leaves x leaf area.

Chemical Parameters: On both seasons, 20 mature leaves from the middle portion of shoots of each replicate were taken and washed several times with tap water followed by distilled water to remove any residues that might affect the results and then dried at 70c for dry matter estimation. Dried samples were finely grounded with a porcelain morter and pestle and stored in small light bags for determination of N, P and K [13]. Content of nitrogen in leaves was determined by the modified micro- kjeldahel method as described by Van Shouwenburg and Walinga [14], leaf phosphorus content was determined colormetrically according to the method of Jackson [15] and using the flame photometer method (Corning 410) according to Piper [16] to determined potassium content.

Statistical Analysis of Data: The periodical measurements were arranged in split analysis. The obtained data was subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [17] with 32 treatments, each treatment comprised of 3 replicates. Mstat-C program was used to calculate least significant difference (LSD) to compare between means of treatments according to Waller and Duncan [18] at probability of 0.05 using MSTAT software package. The data was tabulated and represented graphically by Excel program where appropriate.

RESULTS AND DISCUSION

Morphological Parameters: Stem length increment of Sour orange and Volkamer rootstocks under study was influenced by growth stimulative substances in the two seasons (Table 1). In general, average stem length was insignificantly different between the two rootstocks in season 2011 but in season 2012 stem length was longer in Volkamer than Sour orange. Stem length was significant longer by yeast application compared with phosphorus (P) or algae application. However, stem length increased linearly by increasing P dose in both rootstocks. Furthermore, stem length was enhanced with application with combination of three stimulative substances followed by bilateral combination then application with either substances alone, while the longest stem length was obtained with seedling receiving the highest dose of P plus algae and yeast during the two seasons of study. Interaction data appeared significant longest stem in both seasons were detected on Sour orange or Volkamer lemon seedling that were supplied with 2.4 cm phosphoric acid plus algae and yeast in both seasons. On other hand the shortest length were recorded with both rootstocks under control treatment.

Generally, average stem diameter was significant affected by citrus rootstocks in the two seasons, Sour orange showed significant thicker shoot during both seasons (Table, 2). However, stem diameter was increased linearly and significantly by increasing P dose in both seasons. Furthermore, stem diameter was enhanced with application of combination of three stimulative substances followed by combination of two substances then application with either substances alone. The largest stem diameter was obtained with seedling received the highest dose of P plus algae and yeast during the two seasons of study. Interaction data revealed significant stem thickness in both seasons in Sour orange seedling that were supplied with 2.4 cm phosphoric acid plus algae and yeast in both seasons. On other hand the smallest diameter was recorded with both control rootstocks.

The effect of stimulus substances used in this study on the rootstock stem length and diameter may be due to the role of these materials as illustrated; where phosphorus (P) is one of the most important elements for plant growth and metabolism. It plays a key role in many plant processes such as energy metabolism, the synthesis of nucleic acids and membranes, photosynthesis, respiration, nitrogen fixation, enzyme regulation, energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the other [6,19]. Also, P fertilizer caused a significant increase of some growth measurements specially stem height and diameter [20 - 24]. However, all plants growth parameters were significantly promoted by Algae applications. The highest values of plant height and stem diameter, number of leaves/plant and leaf area were obtained by algae application [25]. Also, Muller [26] found that, diatoms

Table 1: Effect of some stimulative substances on stem length of two citrus rootstocks during 2011 and 2012 seasons.

	Stem length (cm)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	16.00 m	15.67 m	15.84 K	11.00 p	13.67 o	12.34 J
P_1 (1.2cm)	27.00 l	30.00 i	28.50 J	26.00 n	29.00 j-l	27.50 I
P_2 (1.8cm)	29.00 j	30.67 hi	29.84 I	27.33 l-n	29.67 i-k	28.50 HI
P_3 (2.4cm)	30.00 i	32.00 fg	31.00 G	29.00 j-l	33.00 c-e	31.00 EF
Algae	29.00 j	28.00 k	28.50 J	28.67 k-m	27.00 mn	27.84 I
Yeast	30.00 i	30.67 hi	30.34 H	29.33 i-k	31.00 f-i	30.17 FG
P_1 + Algae	31.67 g	32.00 fg	31.84 F	31.00 f-i	33.00 c-e	32.00 DE
P_1 + Yeast	32.00 fg	33.00 e	32.50 E	33.00 c-e	35.00 ab	34.00 BC
P_2 + Algae	31.33 gh	30.33 i	30.83 G	29.67 i-k	29.33 i-k	29.50 GH
P_2 + Yeast	32.00 fg	32.00 fg	32.00 F	31.00 f-i	30.00 h-k	30.50 FG
P_3 + Algae	32.67 ef	31.33 gh	32.00 F	31.67 e-h	32.00 d-g	31.84 DE
P_3 + Yeast	33.33 de	32.00 fg	32.67 E	32.67 c-f	33.00 c-e	32.84 CD
Algae + Yeast	34.00 cd	32.67 ef	33.34 D	30.67 g-j	34.00 bc	32.34 D
P_1 + Algae + Yeast	34.33 bc	33.33 de	33.83 C	33.67 b-d	35.00 ab	34.34 AB
P_2 + Algae + Yeast	35.00 ab	34.33 bc	34.67 B	34.00 bc	35.33 ab	34.67 AB
P_3 + Algae + Yeast	35.33 a	35.33 a	35.33 A	35.00 ab	36.00 a	35.50 A
Mean	30.79 *n.s*	30.83 *n.s*		29.61 *B*	31.00 *A*	

Table 2: Effect of some stimulative substances on stem diameter of two citrus rootstocks during 2011 and 2012 seasons.

	Stem diameter (cm)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	2.11 t	2.34 s	2.23 L	1.99 u	2.05 u	2.02 M
P_1 (1.2cm)	5.17 m	4.98 n	5.08 I	5.00 q	5.21 o	3.61 I
P_2 (1.8cm)	5.81 hi	5.51 l	5.66 H	5.11 p	5.42 mn	3.77 H
P_3 (2.4cm)	5.89 gh	5.75 ij	5.82 FG	5.73 hi	5.64 j	5.69 F
Algae	4.50 q	4.69 p	4.60 K	4.10 t	4.33 r	4.22 L
Yeast	5.73 i-k	5.66 jk	5.70 H	5.52 kl	5.35 n	5.44 G
P_1 + Algae	5.91 gh	5.77 i	5.84 F	5.81 gh	5.81 gh	5.81 E
P_1 + Yeast	6.11 f	5.93 g	6.02 E	5.95 ef	5.96 ef	5.96 D
P_2 + Algae	4.83 o	4.37 r	4.60 K	4.23 s	4.35 r	4.29 K
P_2 + Yeast	5.16 m	4.74 op	4.95 J	4.93 q	4.29 rs	4.61 J
P_3 + Algae	5.89 gh	5.64 k	5.77 G	5.55 k	5.45 lm	5.50 G
P_3 + Yeast	6.12 f	5.91 gh	6.02 E	5.83 g	5.68 ij	5.76 E
Algae + Yeast	6.38 e	6.19 f	6.29 D	6.00 e	5.89 fg	5.95 D
P_1 + Algae + Yeast	6.65 d	6.45 e	6.55 C	6.35 d	6.00 e	6.18 C
P_2 + Algae + Yeast	6.90 c	6.83 c	6.87 B	6.70 c	6.29 d	6.50 B
P_3 + Algae + Yeast	7.16 a	7.02 b	7.09 A	7.00 a	6.48 b	6.74 A
Mean	5.65 *A*	5.49 *B*		5.36 *A*	5.26 *B*	

(algae) improved growth of stems. Furthermore, yeast effect on vegetative growth characteristics, increasing diameter and length [27]. Whereas, active dry yeast instead of using chemicals is getting much important due to its vital role in improving the nutritional status of the plants, producing healthy plants and enhancing the yield and quality of fruit trees [10]. Vegetative growth such as stem length, significantly increased when Washington navel orange trees were sprayed with active dry yeast [8].

Sour orange produced significantly higher average and total leaf area than Volkamer in both seasons. Any as the used stimulative substances enhanced significantly leaves parameters compared with control. The highest dose of P enhanced rootstock average and total leaf area compared with medium or low P dose. Application of algae or yeast has positive effect on average and total leaf area but yeast application was more effective in this respect. Average and total leaf area were enhanced with combined application of three stimulative substances followed by bilateral combination then application of either substances only. Significant highest average and total leaf area in both seasons was produced with seedling of Sour orange which received 2.4 cm phosphoric acid plus algae and yeast in both seasons. On other hand the lowest value of average and total leaf area was recorded with control of both rootstocks under study (Table, 3 and 4).

These results are in line with Mekonnen *et al.*[28] and Rahimi and Pouzesh [29], they found that using phosphorus fertilizers increased leaf area. These previous benefits may be due to the function of phosphorus in plants. P is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next [19]. However, growth and leaf area was improved also by application of algae [30]. Furthermore, active dry yeast is an effective substances for achieving better in leaf area [31, 32]. Whereas, the positive action of active dry yeast on leaf area and its contents of various nutrients could be attributed to its content of cytokinins and vitamin B as well as its important role on building up carbohydrates. These previous benefits of these materials surely reflected on enhancing both cell division and cell enlargement [9].

On the average the Sour orange produced significant higher root dry weight in both seasons of the investigation. The stimulative substances resulted in, significant increments in root dry weight in seedling treated with the combination of P at highest dose algae and yeast. Furthermore, root dry weight per seedling was linearly increased significantly with the increase in P dose. Yeast application appeared more effective on total leaf area compared with phosphorus at medium and low dose

Table 3: Effect of some stimulative substances on average leaf area of two citrus rootstocks during 2011 and 2012 seasons.

	Average leaf area (cm2)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	2.08 u	2.23 t	2.16 N	2.08 p	2.92 o	2.92 M
P_1 (1.2cm)	7.92 o	8.39 m	8.15 J	6.63 k	5.94 n	5.94 K
P_2 (1.8cm)	8.10 n	8.62 jk	8.36 I	6.95 hi	6.25 m	6.27 I
P_3 (2.4cm)	8.58 kl	8.94 hi	8.76 GH	7.15 fg	6.55 kl	6.57 G
Algae	7.14 rs	7.10 s	7.12 M	5.98 n	6.02 n	6.06 L
Yeast	8.17 n	8.57 kl	8.37 I	6.85 ij	6.25 m	6.25 IJ
P_1 + Algae	8.50 lm	8.93 hi	8.72 H	7.05 gh	6.63 k	6.65 G
P_1 + Yeast	8.87 i	9.08 g	8.97 F	7.42 e	6.95 hi	6.95 E
P_2 + Algae	7.40 q	7.22 r	7.31 L	6.44 l	5.96 n	5.99 K
P_2 + Yeast	7.54 p	7.57 p	7.55 K	6.79 j	6.19 m	6.23 J
P_3 + Algae	8.71 j	8.94 hi	8.82 G	6.93 hi	6.50 l	6.52 H
P_3 + Yeast	9.00 gh	9.25 f	9.13 E	7.18 f	6.85 ij	6.86 F
Algae + Yeast	9.23 f	9.52 e	9.38 D	7.55 d	7.04 gh	7.05 D
P_1 + Algae + Yeast	9.50 e	9.84 d	9.67 C	7.69 c	7.39 e	7.40 C
P_2 + Algae + Yeast	10.03 c	9.94c	d 9.98 B	7.94 b	7.77 c	7.82 B
P_3 + Algae + Yeast	10.45 a	10.28`b	10.37 A	8.11 a	7.95 b	7.96 A
Mean	8.20 *B*	8.40 *A*		6.80 *A*	6.45 *B*	

Table 4: Effect of some stimulative substances on total leaf area of two citrus rootstocks during 2011 and 2012 seasons.

	Total leaf area (cm2)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	31.15 q	29.80 q	30.48 N	21.40 r	26.12 r	23.76 K
P_1 (1.2cm)	163.75 mn	167.73 m	165.74 K	137.06 m	112.86 p	124.96 I
P_2 (1.8cm)	183.71 k	181.08 k	181.08 J	157.61 jk	127.18 n	42.40 H
P_3 (2.4cm)	206.07 i	196.61 j	201.34 H	171.63 gh	148.51 l	160.07 F
Algae	157.09 o	139.67 p	148.38 M	119.61 o	112.32 p	115.97 J
Yeast	197.39 j	185.70 k	191.55 I	155.41 k	127.09 n	141.25 H
P_1 + Algae	201.20 ij	205.40 i	203.30 GH	166.95 hi	146.00 l	156.48 F
P_1 + Yeast	212.69 h	217.76 gh	215.23 F	178.05 f	162.17 ij	170.11 E
P_2 + Algae	165.28 mn	144.45 p	154.87 L	126.68 n	103.71 q	115.20 J
P_2 + Yeast	173.50 l	158.97 no	166.24 K	138.08 m	115.67 op	126.88 I
P_3 + Algae	217.75 gh	196.61 j	207.18 G	159.47 jk	138.59 m	149.03 G
P_3 + Yeast	234.36 f	222.00 g	228.18 E	174.67 fg	157.51 jk	166.09 E
Algae + Yeast	249.28 e	234.88 f	242.08 D	193.71 d	171.33 gh	182.52 D
P_1 + Algae + Yeast	259.78 d	249.31 e	254.55 C	202.51 c	184.81 e	193.66 C
P_2 + Algae + Yeast	281.06 c	265.00 d	273.03 B	214.41 b	202.25 c	208.33 B
P_3 + Algae + Yeast	306.71 a	293.93 b	300.32 A	227.25 a	214.77 b	221.01 A
Mean	202.57 *A*	193.06 *B*	`	159.03 *A*	140.68 *B*	

application or algae application (Table, 5). However, dry weight of root was enhanced with application with combination of three stimulative substances followed by bilateral combination then application with one substances alone. Interaction data showed significant by highest root dry weight in both seasons with Sour orange seedling that were supplied with 2.4 cm phosphoric acid plus algae and yeast in both seasons. On other hand the lowest leaf area recorded with both rootstocks under control treatment.

These results were in the same direction with Rafael *et al*. [33] they noticed that, phosphorus fertilization promoted the root dry weight. However, yeast extract increased the vegetative parameters resulted in roots dry weight [34]. Also, Hafez *et al.* [35] found that, the effect of foliar spray of yeast on vegetative growth measurements of olive resulted in significant increases in root dry weight. Further, algae improved vegetative growth plant height, stem diameter, leave numbers per plant and plant dry weight [36].

Table 5: Effect of some stimulative substances on root dry weight of two citrus rootstocks during 2011 and 2012 seasons.

Total root dry weight(g)						
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	1.87 x	1.86 x	1.87 N	1.92 q	1.89 q	1.91 J
P_1 (1.2cm)	10.77 j	7.80 q	9.29 J	7.73 m	6.64 o	7.19 H
P_2 (1.8cm)	11.43 h	8.79 p	10.11 H	8.11 jk	7.32 n	7.72 G
P_3 (2.4cm)	11.74 g	9.43 n	10.59 G	9.00 g	8.04 j-l	8.52 F
Algae	7.27 t	6.03 w	6.65 M	7.75 m	5.99 p	6.87 I
Yeast	11.45 h	8.73 p	10.09 H	8.20 ij	7.26 n	7.73 G
P_1 + Algae	11.66 g	9.43 n	10.55 G	9.00 g	7.83 lm	8.42 F
P_1 + Yeast	12.12 ef	10.11 l	11.12 E	9.41 f	8.58 h	9.00 E
P_2 + Algae	7.49 s	6.32 v	6.91 L	7.69 m	6.66 o	7.18 H
P_2 + Yeast	7.66 r	6.98 u	7.32 K	7.90 k-m	7.20 n	7.55 G
P_3 + Algae	9.47 n	9.28 o	9.38 I	9.49 f	7.72 m	8.61 F
P_3 + Yeast	12.05 f	9.99 m	11.02 F	9.57 f	8.40 hi	8.99 E
Algae + Yeast	12.52 d	10.40 k	11.46 D	9.91 e	8.97 g	9.44 D
P_1 + Algae + Yeast	12.77 c	11.19 i	11.98 C	10.36 d	9.94 e	10.15 C
P_2 + Algae + Yeast	12.96 a	11.70 g	12.33 B	11.28 b	10.44 d	10.86 B
P_3 + Algae + Yeast	13.56 b	12.23 e	12.90 A	11.85 a	10.81 c	11.33 A
Mean	10.42 *A*	8.77 *B*		8.70 *A*	7.73 *B*	

Table 6: Effect of some stimulative substances on leaf nitrogen content of two citrus rootstocks during 2011 and 2012 seasons.

Nitrogen (%)						
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	1.08 n	1.01 n	1.05 J	1.05 n	1.05 n	1.05 H
P_1 (1.2cm)	1.90j g-j	1.64 m	1.77 G-I	1.62 m	1.90 g-j	1.76 FG
P_2 (1.8cm)	1.85 i-k	1.65 m	1.75 I	1.67 lm	1.97 e-h	1.82 EF
P_3 (2.4cm)	1.98 e-h	1.67 lm	1.83 F-H	1.80 i-l	1.88 g-j	1.84 EF
Algae	1.83 i-k	1.67 lm	1.75 HI	1.78 i-l	1.63 i-l	1.71 G
Yeast	1.93 g-i	1.89 g-j	1.91 C-E	1.62 m	1.83 h-k	1.73 G
P_1 + Algae	1.99 e-h	1.82 i-k	1.91 C-F	1.73 k-m	1.95 f-h	1.84 EF
P_1 + Yeast	2.00 f-g	1.96 f-h	1.98 G-I	1.87 g-k	2.10 c-e	1.99 C
P_2 + Algae	1.88 h-j	1.89 g-j	1.89 C	1.75 j-m	1.94 f-h	1.85 EF
P_2 + Yeast	1.92 g-i	1.67 m	1.80 D-F	1.73 k-m	2.00 d-g	1.87 D-F
P_3 + Algae	1.83 i-k	1.83 i-k	1.83 E-G	1.80 i-l	2.05 d-f	1.93 C-E
P_3 + Yeast	2.11 b-d	1.78 j-k	1.95 CD	1.87 g-k	2.12 b-d	2.00 C
Algae + Yeast	2.15 bc	2.00 d-g	2.08 B	1.78 i-l	2.13 b-d	1.96 CD
P_1 + Algae + Yeast	2.08 c-e	1.74 k-m	1.91 CD	2.22 a-c	2.23 a-c	2.23 B
P_2 + Algae + Yeast	2.20 ab	2.07 c-f	2.14 B	2.22 a-c	2.25 ab	2.24 B
P_3 + Algae + Yeast	2.28 a	2.18 a-c	2.23 A	2.35 a	2.32 a	2.34 A
Mean	1.94 *A*	1.78 *B*		1.80 *B*	1.96 *A*	

Chemical Parameters: Sour orange had a significant higher leaf N content than Volkamer in the first season of study. Contrast results were obtained in the second one. N content increased linearly with increasing P dose during both season. Specially in first season, yeast had more positive effects on leaf nitrogen content compared with algae application. The combined treatment increased significantly N leaf content compared with the single treatment. Furthermore, tri-combination treatments enhanced leaf N content and seedling supplied P_3 with algae plus yeast had the highest leaf N content. Furthermore, interaction data appeared highest leaf N content with seedling of Sour orange or Volkamer supplied with 2.4 cm (P_3) phosphoric acid plus algae and yeast in both seasons. On other hand the lowest leaf area recorded with both rootstocks under control treatment (Table, 6).

Also, under this investigate, phosphorus contents was insignificant by affected by citrus rootstocks and stimulative substances treatments on season 2011 and

Table 7: Effect of some stimulative substances on leaf phosphorus content of two citrus rootstocks during 2011 and 2012 seasons.

	Phosphorus (%)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	0.22 no	0.17 o	0.19 J	0.18 m	0.14 m	0.16 H
P_1 (1.2cm)	0.34 f-k	0.26 mn	0.30 G-I	0.30 ij	0.24 l	0.27 G
P_2 (1.8cm)	0.33 g-k	0.33 h-k	0.33 F-G	0.33 g-i	0.30 ij	0.31 EF
P_3 (2.4cm)	0.38 d-g	0.35 f-j	0.37 CD	0.36 e-h	0.37 e-g	0.36 CD
Algae	0.28 lm	0.26 mn	0.27 I	0.24 kl	0.26 j-l	0.25 G
Yeast	0.29 k-m	0.33 h-l	0.31 F-H	0.30 ij	0.32 g-i	0.31 EF
P_1 + Algae	0.33 g-k	0.35 f-j	0.34 D-F	0.36 e-h	0.36 e-g	0.36 CD
P_1 + Yeast	0.35 f-j	0.37 e-h	0.36 DE	0.37 e-g	0.36 e-g	0.36 CD
P_2 + Algae	0.30 j-m	0.27 m	0.29 HI	0.27 j-l	0.29 i-k	0.28 FG
P_2 + Yeast	0.31 i-m	0.33 g-k	0.32 F-H	0.31 h-j	0.30 ij	0.31 F
P_3 + Algae	0.36 e-i	0.38 d-h	0.37 C-E	0.36 e-g	0.33 f-i	0.35 DE
P_3 + Yeast	0.38 d-h	0.37 e-h	0.37 CD	0.38 d-f	0.34 f-i	0.36 CD
Algae + Yeast	0.41 b-e	0.39 c-f	0.40 BC	0.40 de	0.38 d-f	0.39 C
P_1 + Algae + Yeast	0.42 a-d	0.44 a-c	0.43 AB	0.45 a-c	0.40 c-e	0.43 B
P_2 + Algae + Yeast	0.44 a-c	0.45 ab	0.45 A	0.47 ab	0.42 b-d	0.44 AB
P_3 + Algae + Yeast	0.46 ab	0.47 a	0.46 A	0.48 a	0.45 ab	0.47 A
Mean	0.35 *n.s*	0.35 *n.s*		0.35 *A*	0.33 *B*	

2012. It was showed in Table 7 that Sour orange and Volkamer had the same leaf P content in first season while leaf of Sour orange had the highest percentage in second one. Seedling under control treatment appeared the lowest significant content of P during both seasons. Phosphorus content per leaf was significantly affected by increasing P-rate. Application of yeast or algae alone improved the percentage of P in leaf and this increment enhanced when it was with P treatments. Furthermore, leaf phosphorus content (%) was significantly highest by using the highest dose of P plus algae and yeast on two seasons between two rootstocks.

Potassium content was significant affected by citrus rootstocks in the two seasons. Generally, Volkamer or Sour orange interchanged recording higher leaf k content in two season of study. In addition, highest dose of P plus algae and yeast was resulted in significantly higher K content. It also increased linearly with increased P dose during both season. Yeast had more positive effect in leaf nitrogen content compared to algae application. The combination treatment increased significantly K leaf content compared with the single treatment. Further, the tri-combination of algae plus yeast with P in highest dos produced significant highest K content compared with others bilateral combination. Furthermore, interaction data showed that highest leaf K content was in seedling of Sour orange or Volkamer lemon supplied with 2.4 cm phosphoric acid plus algae and yeast in both seasons. On other hand, the lowest leaf content recorded with both rootstocks was in control treatment (Table, 8).

Increased P fertilizer rate resulted in improving leaf N, P and K content [37, 38, 22]. Enhancement leaf N, P and K content with application is attributed to yeast or algae extract contain many different nutrients by increasing soil organism's activity and so increasing availability of nutrient elements and consequence their uptake and indirectly by improving root growth and these enhancing in root absorption [9,11,39,35]. This was confirmed by Mansour [7] and El-Quesni *et al.* [25] they noted that, applying yeast and algae was very effective in improving leaf N, P and K content.

It was cleared from the previous data that, enhanced leaf citrus rootstocks content of N, P and K, which improving by apply stimulative substances (phosphorus, yeast and algae), improved growth parameters such as stem length, stem diameter, leaf area and dry weight of root. These positive effects in growth was due to the role of this element in plant [40 - 42].Whereas, Nitrogen has many functions in plant life. Being a part of proteins, N is an important constituent of protoplasm. It is responsible for the biosynthesis of enzymes, nucleoproteins, amino acid, amines, amino sugars, polypeptides, chlorophylls and encourages cell divisions [40]. While, phosphorus is necessary for many life processes such as photosynthesis, synthesis and breakdown of carbohydrates and the transfer of energy within the plant [41]. Further, potassium is necessary for basic physiological functions such as formation of sugars and starch, synthesis of proteins and cell division and growth [42].

Table 8: Effect of some stimulative substances on leaf potassium content of two citrus rootstocks during 2011 and 2012 seasons.

	Potassium (%)					
	Season 2011			Season 2012		
Stimulative Substances	Sour orange	Volkamer lemon	Mean	Sour orange	Volkamer lemon	Mean
Control	0.94 n	0.95 n	0.94 K	0.96 q	0.89 r	0.92 I
P_1 (1.2cm)	1.25 m	1.34 lm	1.30 J	1.39 op	1.34 p	1.37 H
P_2 (1.8cm)	1.30 lm	1.54 ij	1.42 I	1.50 lm	1.53 ki	1.51 F
P_3 (2.4cm)	1.52 ij	1.67 gh	1.59 F	1.65 g-i	1.64 hi	1.65 E
Algae	1.29 lm	1.38 kl	1.33 J	1.41 no	1.39 op	1.40 GH
Yeast	1.53 ij	1.44 jk	1.48 HI	1.57 jk	1.46 mn	1.52 F
P_1 + Algae	1.70 fg	1.66 gh	1.68 DE	1.65 gh	1.60 ij	1.63 E
P_1 + Yeast	1.83 cd	1.80 d-f	1.82 C	1.71 d-f	1.69 e-g	1.70 D
P_2 + Algae	1.50 ij	1.51 ij	1.51 GH	1.42 no	1.45 mn	1.44 G
P_2 + Yeast	1.66 gh	1.59 hi	1.63 EF	1.53 kl	1.54 kl	1.53 F
P_3 + Algae	1.64 gh	1.51 ij	1.58 FG	1.67 f-h	1.56 jk	1.62 E
P_3 + Yeast	1.72 f-g	1.72 f-g	1.72 D	1.75 cd	1.68 f-h	1.72 D
Algae + Yeast	1.81 de	1.80 d-f	1.80 C	1.78 c	1.74 c-e	1.76 C
P_1 + Algae + Yeast	1.81 de	1.93 bc	1.87 C	1.83 b	1.75 cd	1.79 C
P_2 + Algae + Yeast	1.86 cd	1.99 ab	1.93 AB	1.88 b	1.87 b	1.88 B
P_3 + Algae + Yeast	1.92 bc	2.05 a	1.99 A	1.96 a	1.95 a	1.96A
Mean	1.58 *B*	1.62 *A*		1.60 *A*	1.57 *B*	

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