The Influence of Some Antioxidants Foliar Applications on Cleopatra Mandarin Rootstock Seedlings Performance

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ABSTRACT

This study was conducted on Cleopatra mandarin seedlings under a greenhouse conditions in citrus nursery, Horticulture Research Institute, Giza, Egypt. during two successive seasons (2022-2023), hydrogen peroxide (H_2O_2) at a concentration of 500 mg/L, vitamin E (tocopherol) at concentration of 2 mg/L and selenium (Na_2SeO_3) at a concentration of 0.3 mg/L as antioxidants, either individually or in combination, in order to shortening the growth period of Cleopatra mandarin rootstock seedlings to the grafting stage and achieving the highest economic return. Obtained results revealed a considerable enhancement in all tested parameters of leaf area, number of leaves/seedling, leaves and shoots dry matter percentage, percentage of increase in both seedling stem (length and diameter), grafting success percentage, leaf pigments contents, leaf indole and phenol contents, mineral content of leaves and antioxidant enzymes activities as compared with the control. While, the optimum results were obtained from the combination treatments Hydrogen peroxide (H_2O_2) at 500 mg/L + Selenium (Na_2SeO_3) at 0.3 mg/L.

Keywords: Cleopatra mandarin- Seedlings- Antioxidant- Hydrogen peroxide- Vitamin E and Selenium.

INTRODUCTION

Citrus *(Citrus spp.)* is a widely grown and commercially popular fruit crop (Wu et al., 2011). It is one of the most popular fruit crops in Egypt, the total area is about 529405 Fed., their fruitful acreage reached over 486271 Fed., and yielding approximately 5142829 tons. (According to Ministry of Agriculture and Land Reclamation Statistics Annual Report, 2023). The Cleopatra mandarin *(C.reshni Tlort.ExTan)* rootstock is considered one of the important citrus rootstocks that are distinguished by the quality of the fruits of the grafted varieties. Also, it tolerates the conditions of new lands in Egypt, such as salinity and calcium carbonate. However, it is criticized for its slow growth, which leads to a long period of time required to reach the grafting stage. Therefore, it is necessary to carry out some procedures to reduce this period of time and reach a good, strong-growing seedling suitable for grafting in a short period of time

Vegetative growth in citrus seedlings is influenced by various factors including temperature, light intensity, water availability, and nutrient supply, which is very important to optimize citrus seedlings growth. Reactive oxygen species (ROS) or the free radicals are created when oxygen interacts with specific molecules It causes injury to cellular membranes, organelles and nucleic acids and reduces enzyme activity and photosynthesis pigments (Valko et al., 2007, Halliwell, 2011 and Ye, 2015). Antioxidants are compounds have given electrons that can scavenge free radicals, and minimizing or avoiding damage from free-radical processes (Halliwell, 2012; Ramirez et al., 2013 and Aline et al., 2023).

Recent research has shown that Hydrogen peroxide (H_2O_2) can act as a strong signaling molecule that mediates a variety of physiological and biochemical processes in plants. Plant growth, growth regulators, antioxidant enzyme activity, fruit output, and plant quality can all benefit from a lower dose of hydrogen peroxide treatment (Deng et al., 2012 and Orabi et al., 2015). Exogenous hydrogen peroxide treatments caused the plants to develop root system, increasing the area where all minerals could be absorbed and boosts plant antioxidant (Zhang et al., 2011 and Nik et al., 2020).

Vitamin E (tocopherol) is an essential vitamin for plants and functions as antioxidant playing a pivotal role in plant growth (Falk and Munné- Bosch, 2010). The chloroplast are the primary site of vitamin E biosynthesis in the cytoplasm and vitamin E functions as a signaling molecule in plants, regulating signaling from the chloroplast to the nucleus and maintaining the redox state (Wang et al., 2016). During a variety of biotic and abiotic stressors, vitamin E can be elevated to high levels (Bao et al., 2020).

Conversely, plants with low vitamin E levels are more vulnerable to various stressful treatments, indicating that vitamin E plays a critical role in plant adaptation to their environment.

Selenium (Se) helps to inhibit the damage caused by climate changes such as drought, salinity, heavy metals, and extreme temperature through its oxidative activity. Also, regulates antenna complex of photosynthesis, protecting chlorophylls by raising photosynthetic pigments (Chen et al., 2022). Selenium can lessen the effects of oxidative stress brought on by other elements that boost plants' antioxidant systems (Gupta and Gupta, 2017). Selenium is generally thought to function as an antioxidant at low concentrations, and as a pro-oxidant at higher concentrations. The growth-promoting effect of Selenium may be attributable to its antioxidant properties (Maria et al., 2010).

The current study aimed to investigate the effect of antioxidant treatments and the possible mechanisms of action of antioxidants (hydrogen peroxide, vitamin E and selenium) on decrease the necessary period time until Cleopatra mandarin seedlings are suitable for grafting.

MATERIALS AND METHODS

This experiment was conducted on potted seedlings of citrus rootstocks Cleopatra mandarin *(C. reshni Tlort. Ex Tan)* in a greenhouse clad with single skin plastic film of the citrus nursery, Horticulture Research Institute, Agriculture Research Center, Giza governorate, Egypt during two consecutive seasons 2021-2022 and 2022/2023 to enhance and accelerate growth of Cleopatra mandarin rootstock for reaching suitable stage for grafting through investigating their response to foliar spray with different forms of antioxidant. The experiment was designed in a randomized complete block design with three replicates and each replicate represented by twenty seedlings. So, the estimated total number of seedlings used in the experiment was four hundred eighty seedlings are produced from one citrus rootstock × eight treatments × three replicates × twenty seedlings. In both studied seasons, at mid-March, Cleopatra mandarin rootstock seedlings that were uniform and healthy, were carefully chosen, the seedlings were grown individually in plastic bags filled with the growing media consists of (sand + compost of plant origin) at 1:1 ratio The bags were regularly irrigated with tap water once every two days, then the selected seedlings of each category were randomly subjected to spraying with the antioxidants treatments. Every treatment was repeated nine times as a spray (monthly) starts from May to January. In order to graft with Murcott tangerine on Cleopatra mandarin rootstock, seedlings were carefully selected during both examined seasons in January of the following year based on their consistency, health, and suitability for the graft process. During the two consecutive seasons of 2022–2023, agricultural techniques, such as irrigation and fertilization were carried out in accordance with land reclamation recommendations and the Citrus Res. Dept. of the Ministry of Agriculture.

The experiment included eight treatments as follow:

T_1: Control (spraying with water)
T_2: Hydrogen peroxide (H_2O_2) at 500 mg/L.
T_3: Vitamin E (Tocopherol) at 2 mg/L.
T_4: Selenium (Na_2SeO_3) at 0.3 mg/L.
T_5: Hydrogen peroxide (H_2O_2) at 500 mg/L + Vitamin E (Tocopherol) at 2 mg/L.
T_6: Hydrogen peroxide (H_2O_2) at 500 mg/L + Selenium (Na_2SeO_3) at 0.3 mg/L.
T_7: Vitamin E (Tocopherol) at 2 mg/L + Selenium (Na_2SeO_3) at 0.3 mg/L.
T_8: Hydrogen peroxide (H_2O_2) at 500 mg/L + Vitamin E (Tocopherol) at 2 mg/L + Selenium (Na_2SeO_3) at 0.3 mg/L.

1) Vegetative growth measurements.

At the end of both seasons, the effectiveness of the researched treatments was assessed using the results of the following measurements:

a) Ten enlarged leaves from each replicate's spring cycle-roughly six months old- were selected from the center of the seedling. Using a portable area meter – model LI 3000 made in U.S.A, the leaf area was calculated in (cm^2).

b) Number of leaves / seedling.

c) Leaves and shoots dry matter percentage: random samples (5g) were taken in September and dry matter percentage was assessed after drying at 70^{o}c for 24 hour.

d) Percentage of increase in (fresh or dry) weight (g) both of shoots or roots samples were collected at the experiment beginning and end by using the formulae:

Increase in (fresh or dry) weight of (vegetative or roots) (%) = (Fresh or dry) weight of (vegetative or roots) at experiment end **– (**Fresh or dry) weight of (vegetative or roots) at experiment beginning/(Fresh or dry) weight of (vegetative or roots) at experiment beginning) x 100

e) Percentage of increase in both seedling stem length (cm) and diameter (cm) samples were collected at the end of the following three times according to the formulas which described by (El-Harouny et al., 2023).

- First time: May and July.
- Second time: August and October.
- Third time: November and January.

Increase in seedling stem length (%) = (Length at time end - Length at time beginning/ Length at time beginning) x 100

Increase in seedling stem diameter (%) = (Diameter at time end - Diameter at time beginning / Diameter at time beginning) x 100

Grafting success percentage:

Grafting was performed during 15 of February of each season of study, for Cleopatra mandarin seedlings with stem diameter over from 1/2 inch, which represent suitable diameter for budding citrus rootstocks (Albrecht et al., 2017). Grafting success percent was recorded 15 days after grafting using the following equation (Patel et al., 2010):

Grafting Success (%) = (Number of sprouted grafts / Total Number of grafts) **x** 100

The total number of grafts represented the number of Cleopatra mandarin rootstock seedlings that attained the stem thickness appropriate for grafting on February 15 from for both seasons.

2) Bio-chemical contents:

a) Leaf pigments contents:

Chlorophylls a & b contents as mg/100 g leaves fresh weight were estimated. Fresh leaves samples (5g) were extracted with dimethyl formaldehyde solution and placed overnight at (5°C). Chlorophyll A and B as well as carotenoid were measured by spectrophotometer at wave lengths 663, 647 and 470 nm respectively according to the equation which described by (Norani 1982).

b) Leaf indole and phenol contents:

Total indole was determined as mg/100g dry weight according to Larsen (1962) and total phenols was determined as mg /100g dry weight by using the Folin calorimetric method by (Horwitz , 2006).

3) Mineral content of leaves:

0.5 gram of dried leaves samples was digested using the H_2SO_4 and H_2O_2 as described by (Cottenie, 1980). The digested samples were used to determine the following minerals:

a) Nitrogen using the micro Kjeldahl technique, as described by Bremner and Mulvaney (1982)

b) Phosphor was quantified by spectrophotometer according to Murphy and Riely (1962) technique.

c) According to Temminghoff, and Houba, (2004), a flame photometer was used to assess potassium content.

d) Total Carbohydrates (g/100g D.W.): were determined in dried samples of leaves and shoots according to the method of (Dubois et al., 1956).

4) Enzymes activities study:

At end the experiment, a sample of five leaves per replicate was chosen in order to assess the antioxidant enzymes activities as below:

a) Catalase (CAT) activity was measured using the methodology outlined by (Gallego et al., 1996).

b) Superoxide dismutase (SOD) activity was measured in accordance with (Wang et al., 2004).

c) Glutathione peroxidase (POD) activity was calculated using the methodology outlined by (Zhang et al., 1995).

5) Economical evaluation:

The cost of spraying chemicals, labor and constant cost (electricity for irrigation, fertilizers, pesticides and grafting) was estimated to calculate the net income of the studied treatments with consideration of the form gate price of the grafted seedlings through 2022 and 2023 seasons respectively to assess the profitability of the present treatments as (Hudson and Gregorion, 2010).

6) Statistical analysis:

Randomized complete block designs with eight treatments; three replicates for each treatment were established. The obtained data each season were subjected to one-way analysis of variance (ANOVA) according to Snedecor and Cochran (1967) using M-STAT program. Means values represented the various investigated treatments were compared by the Duncan's multiple range test (Duncan, 1955) at 0.05 level of significance.

RESULTS AND DISCUSSION

1) Vegetative growth measurements:

a) Leaf area, number of leaves/seedling, leaf and shoot dry matter percentage:

According to the data illustrated in **Table (1)**, antioxidant treatments significantly increased leaf area, number of leaves per seedling and leaf dry matter for all Cleopatra mandarin rootstock seedlings, however, Hydrogen peroxide at 500 mg/L + Selenium at 0.3 mg/L (T_6) recorded the highest significant leaf area, No. of leaves/seedling and leaf dry matter percentage compared to the control (T_1) for both seasons while (T_4 and T_8) holds the greatest significant shoot dry mater for both seasons respectively. Regarding the percentage increase in average leaf area , number of leaves per seedling and leaf dry matter Hydrogen peroxide at 500 mg/L + Selenium at 0.3 mg/L (T_6) shows the greatest values for both seasons by about 87.15% – 138.46% - 120.55% and 80.25% - 215.19 - 91.28% for both seasons, respectively. However, combination treatment of both hydrogen peroxide at 500 mg/L + Vitamin E at 2 mg/L + Selenium at 0.3 mg/L (T_8) showed the highest percentage of shoots dry matter percentage of about 80.75% – 70.77% for both seasons respectively as compared with control treatment (T_1).

Table (1). Effect of antioxidant treatments on measurements vegetative growth of Cleopatra mandarin rootstock seedlings during the two successive seasons.

Treatments	Leaf area (cm^2)	Number of leaves / seedling	Leaf dry matter percentage	Shoots dry matter percentage
		1st season		
T_1: Control (spraying with water)	8.95 d	13.00 f	12.46 c	19.12 d
T_2: Hydrogen peroxide.	12.94 bc	19.52 d	17.59 b	25.64 c
T_3: Vitamin E.	11.37 cd	16.00 e	16.41 bc	27.78 bc
T_4: Selenium.	15.13 ab	25.31 c	26.53 a	33.14 a
T_5: Hydrogen peroxide + Vitamin E.	12.73 bc	20.47 d	20.23 ab	29.35 b
T_6: Hydrogen peroxide + Selenium.	16.75 a	31.00 a	27.48a	27.66 bc
T_7: Vitamin E + Selenium.	15.33 ab	24.30 c	21.46ab	29.73 b
T_8: Hydrogen peroxide + Vitamin E + Selenium.	16.83 a	29.00 b	26.58a	34.56 a
		2nd season		
T_1: Control (spraying with water)	9.67 d	11.00 f	13.31c	20.97 d
T_2: Hydrogen peroxide.	13.57 bc	20.46 d	18.71 b	26.60 c
T_3: Vitamin E.	11.75 cd	17.00 e	15.97 bc	28.56 bc
T_4: Selenium.	15.46 a-c	25.58 bc	24.59 ab	34.63 a
T_5: Hydrogen peroxide + Vitamin E.	13.56 bc	21.00 d	19.81 b	30.30 b
T_6: Hydrogen peroxide + Selenium.	17.43 a	34.67 a	25.46 a	28.83 bc
T_7: Vitamin E + Selenium.	15.64 ab	24.33 c	20.64 b	30.00 b
T_8: Hydrogen peroxide + Vitamin E + Selenium.	17.68 a	30.33 b	26.46 a	35.81 a

At the 5% level, there is no significant difference between the mean that have the same letter.

b) Fresh and dry weight of vegetative and root growth:

Data in **Figs. (1-2)** showed that, antioxidant treatments had a significant impact on the fresh and dry weight of vegetative and root growth of Cleopatra mandarin rootstock seedlings. The treatment with hydrogen peroxide at 500 mg/L + Selenium at 0.3 mg/L (T_6) significantly induced the highest significant percentage of increase in fresh and dry weight of vegetative growth which in turn caused a notable increase in all parameters by 169.23 - 178.48 % and

511.76 - 477 % compared the control treatment (T_1) respectively. As for the percentage of increase in roots fresh and dry weight, hydrogen peroxide at 500 mg/L + Vitamin E at 2 mg/L + Selenium at 0.3 mg/L (T_8) treatment overcame the control treatment (T_1) by 343.04 - 368.00 % and 145.45 - 180.00 % for the two seasons respectively.

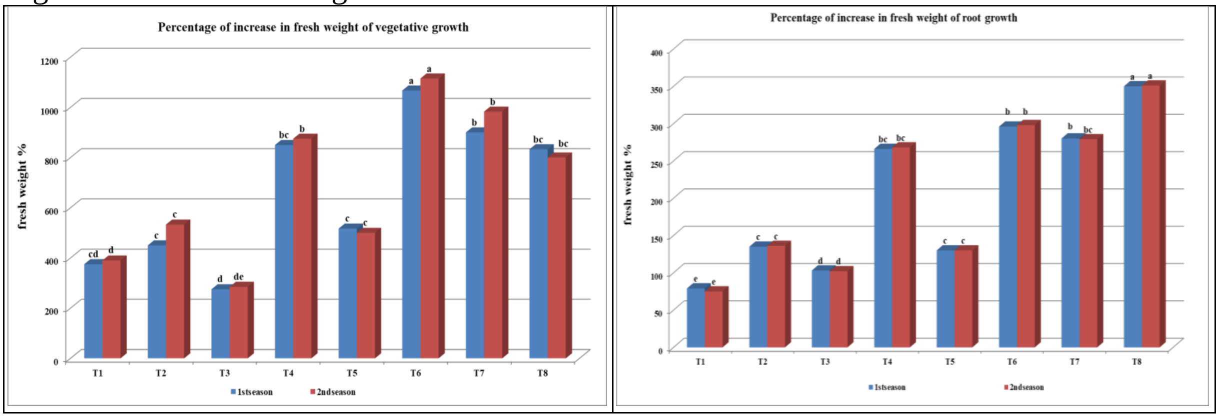

Fig. (1). Effect of antioxidant on percentage of increase in fresh weight of vegetative and root growth

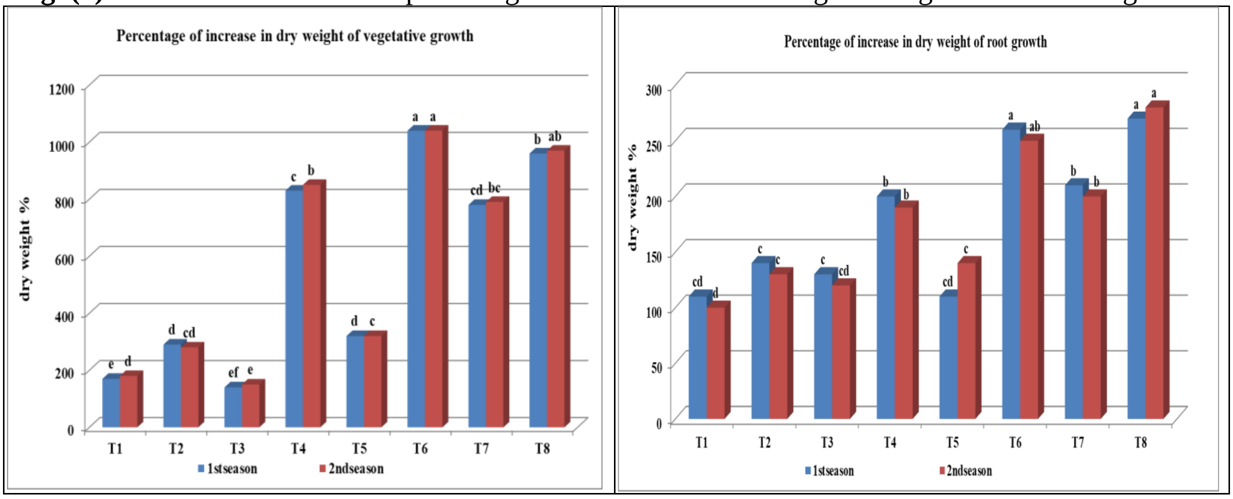

Fig. (2). Effect of antioxidant on percentage of increase in dry weight of vegetative and root growth.

T_1: Control (spraying with water)
T_2: Hydrogen peroxide.
T_3: Vitamin E.
T_4: Selenium.
T_5: Hydrogen peroxide + Vitamin E.
T_6: Hydrogen peroxide + Selenium.
T_7: Vitamin E + Selenium.
T_8: Hydrogen peroxide + Vitamin E + Selenium.

c) Stem diameter and length:

The responses of the examined growth metrics, specifically plant height and stem diameter, during both seasons are illustrated in **(Figs. 3-4)**. obtained results indicate that while the control treatment (T_1) had the lowest value, the Cleopatra mandarin rootstock seedlings that received an exogenous application of hydrogen peroxide at 500 mg/L + selenium at 0.3 mg/L (T_6) treatment showed the most percentage increase in stem length in both seasons. Furthermore, the treatment that increased the percentage of stem diameter the most was hydrogen peroxide at 500 mg/L + Vitamin E at 2 mg/L + Selenium at 0.3 mg/L (T_8) followed by hydrogen peroxide at 500 mg/L + Selenium at 0.3 mg/L (T_7) and hydrogen peroxide at 500 mg/L + Selenium at 0.3 mg/L (T_6) where the differences were mostly significant.

Regarding the percentage increase in stem diameter and length, obtained results indicate that, the variation in activity and growth over the three designated periods may be attributed to differing environmental circumstances, including temperature and relative humidity, as well as the disparity in the duration of daylight versus nighttime (Ahammed et al., 2021). Seedling growth was determined by measuring the percentage of increment in both seedling stem length and diameter at the end of each of the three studied period. Obtained results indicate that, the percentage of increase of the stem length of Cleopatra mandarin rootstock seedlings were more pronounced throughout the 1st period of growth (May-July) followed by 2nd period (Aug.-Oct.) while the increase percentage through the 3rd period (Nov.-Jan.) was scare, the percentage of increment in seedling stem diameter follows the same pattern as for stem length. The decline in seedling growth in the 2nd period (Aug. - Oct.) may be related to the great increase in temperature during August, September and October months, which reach inside the green house an average of 51°C for both seasons for about 5 to 6 hours /day, which represent unfavorable temperature for seedling growth. Increase leaf temperature from 25 to 40°C cause considerable reduction in CO_2 assimilation (Ribeiro et al., 2004). However, reduction in growth during the 3rd period (Nov. - Jan.) may be due to the decrease in temperature and shortening in day length for November, December and January in reaction to lower temperatures and a shorter photoperiod (length of days), plants often grow more slowly throughout the winter.

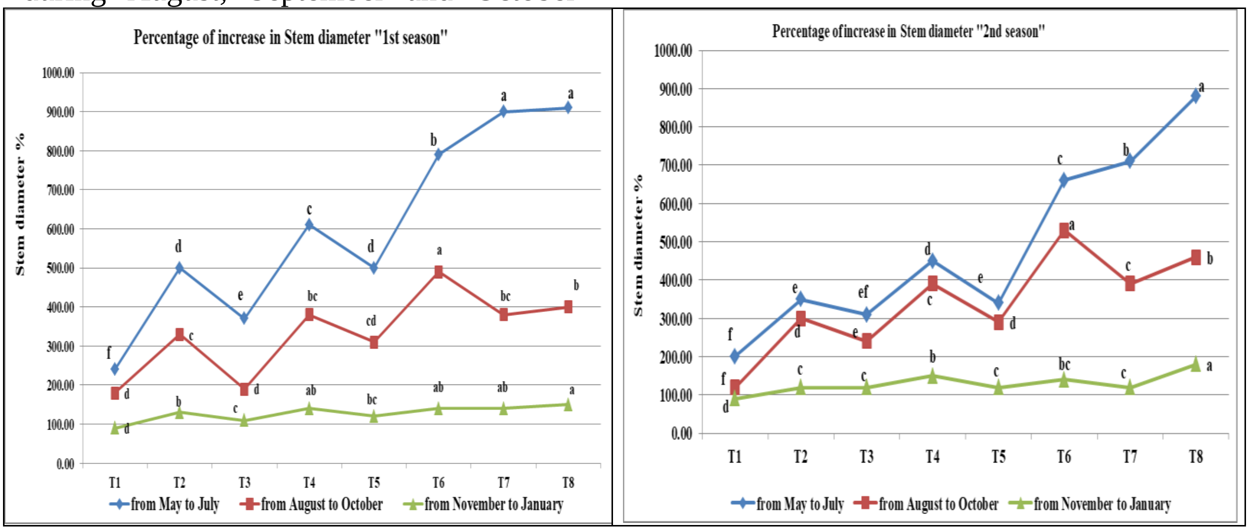

Fig. (3). Effect of antioxidant on percentage of increase in stem diameter.

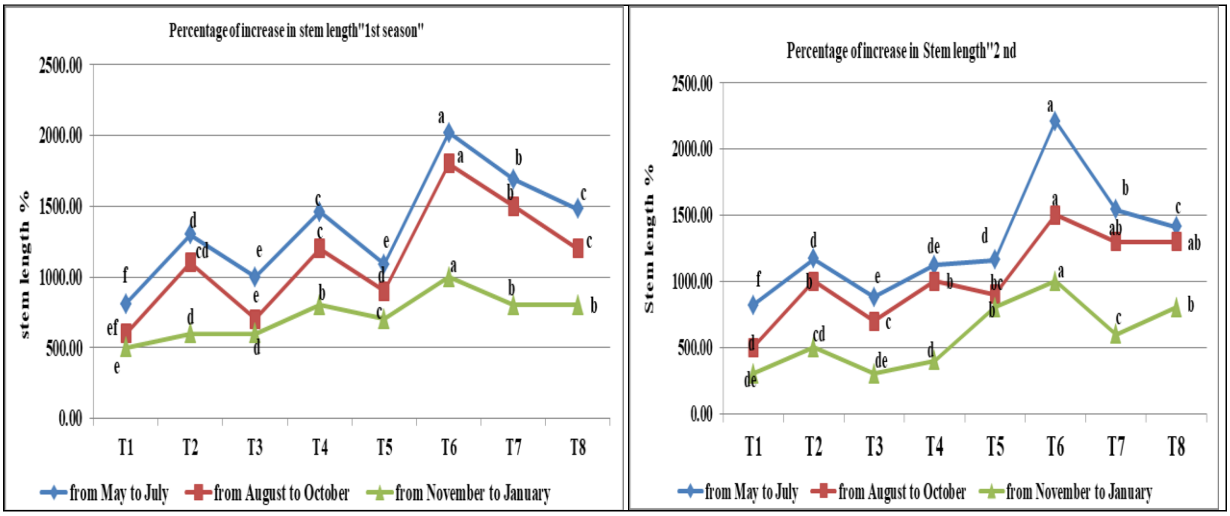

Fig. (4). Effect of antioxidant on percentage of increase in stem length.

T_1: Control (spraying with water)	**T_5**: Hydrogen peroxide + Vitamin E.
T_2: Hydrogen peroxide.	**T_6**: Hydrogen peroxide + Selenium.
T_3: Vitamin E.	**T_7**: Vitamin E + Selenium.
T_4: Selenium.	**T_8**: Hydrogen peroxide + Vitamin E + Selenium.

d) Grafting success (%):

For grafting success, reported data in **Table (2)** reveal that, foliar applications of antioxidant treatment increased significantly the percentage of grafting success for Cleopatra mandarin rootstock for both seasons, and the highest significant percentage of success was recorded for Cleopatra mandarin rootstock at Hydrogen peroxide + Vitamin E + Selenium (T8) (83.33 and 85.00%) followed by Hydrogen peroxide + Selenium (T_6) (73.33 – 75.00%) and Vitamin E + Selenium (T_7) (61.66 – 63.33%) which are suitable for grafting at mid of February for both seasons respectively compared with control treatment (spraying with water) which recorded lowest significant grafting success percentage (6.66 – 8.33%). Moreover, the beneficial effect of antioxidant spray application on vegetative growth of Cleopatra mandarin rootstock seedlings could be explained on antioxidant improved many physiological processes such as regulated the plant growth, differentiation and metabolism of plants and increasing physiological availability of water and nutrients (Zahra et al., 2016). In addition Barakat, (2003) reported that, The increase in growth of plant in response to antioxidant treatment relative to untreated plants might be a result from increased levels of endogenous hormones consequently stimulation of cell division and/or cell enlargement and subsequently growth.

Table (2). Effect of antioxidant treatments on grafting success percentage of Cleopatra mandarin rootstock seedlings during the two successive seasons.

	Grafting success percentage	
Treatments	**1st Season**	**2nd Season**
T_1: Control (spraying with water)	8.33 F	6.66 G
T_2: Hydrogen peroxide.	43.33 D	45.00 E
T_3: Vitamin E.	31.66 E	33.33 F
T_4: Selenium.	56.66 C	53.33 CD
T_5: Hydrogen peroxide + Vitamin E.	45.00 D	48.33 DE
T_6: Hydrogen peroxide + Selenium.	73.33 A	75.00B
T_7: Vitamin E + Selenium.	61.66 B	63.33 C
T_8: Hydrogen peroxide + Vitamin E + Selenium.	83.33 A	85.00 A

At the 5% level, there is no significant difference between the mean that have the same letter.

2) Leaf chemical contents.

Leaf pigments content (mg/100g F.W.):-

The data in **Table (3)** show that antioxidant treatments significantly increase leaf chlorophyll a, chlorophyll b, total carotenoids, and total chlorophyll content in both seasons when compared to the control treatment. The obtained results showed that T_6 treatment significantly induced the highest chlorophyll a and b in the 1st season (0.82 and 0.77 mg/100g F.W.) as well as in the 2nd season of study (0.84 and 0.83mg/100g F.W.) respectively followed by T_4 treatment through 2022 season (0.80 and 0.70 mg/100g F.W.) as well as through 2023 season (0.81 and 0.72 mg/100g. F.W.) than the other treatments and control. Furthermore, total chlorophylls were the highest with T_8 (2.44 and 2.47 mg/100g F.W.) respectively for both seasons. Carotenoids leaf content recorded the highest values (1.01 and 1.01 mg/100g. F.W.) in the 1st season and (1.02 and 1.03 mg/100g F.W.) in the 2nd season respectively with T_7 and T_8 treatments with significant differences than the rest treatments and control.

This result may be attributed, to alteration in amino acid balance and consequently change in the quality of proteins which are the main element in chlorophyll production, moreover, Hydrogen peroxide (H_2O_2) protects chloroplast ultrastructure to preserve photosynthetic pigments and stimulate photosynthesis (Li et al., 2016c). However, Selenium helps to protecting chlorophylls by raising photosynthetic pigments (Gupto and Gupto, 2017 and Chen et al., 2022).

Table (3). Effect of antioxidant treatments on leaf pigments contents of Cleopatra mandarin rootstock seedlings during the two successive seasons.

Attributes / Treatments	Chlorophyll (a) (mg/100g F.W.)	Chlorophyll (b) (mg/100g F.W.)	Total chlorophylls (mg/100g F.W.)	Carotenoids (mg/100g F.W.)
		1st season		
T_1: Control (spraying with water)	0.49 e	0.33 h	1.76 e	0.50 f
T_2: Hydrogen peroxide.	0.63 c	0.49 e	1.99 c-e	0.43 g
T_3: Vitamin E.	0.56 d	0.36 g	1.88 de	0.64 d
T_4: Selenium.	0.80 a	0.70 b	2.25 a-c	0.86 b
T_5: Hydrogen peroxide + Vitamin E.	0.61 cd	0.46 f	2.09 b-e	0.58e
T_6: Hydrogen peroxide + Selenium.	0.82 a	0.77 a	2.40 ab	0.73 c
T_7: Vitamin E + Selenium.	0.70 b	0.54 d	2.12 a-d	1.01 a
T_8: Hydrogen peroxide + Vitamin E + Selenium.	0.74 b	0.61 c	2.44 a	1.01 a
		2nd season		
T_1: Control (spraying with water)	0.51 e	0.32 h	1.80 c	0.51f
T_2: Hydrogen peroxide.	0.64 c	0.50 e	2.03 bc	0.46fg
T_3: Vitamin E.	0.57 d	0.37 g	1.90 c	0.66 d
T_4: Selenium.	0.81 a	0.72 b	2.30 ab	0.87 b
T_5: Hydrogen peroxide + Vitamin E.	0.63 c	0.47 f	2.11 a-c	0.60 de
T_6: Hydrogen peroxide + Selenium.	0.84 a	0.83 a	2.43 a	0.77 c
T_7: Vitamin E + Selenium.	0.71 b	0.57 d	2.13 a-c	1.02 a
T_8: Hydrogen peroxide + Vitamin E + Selenium.	0.75 ab	0.62 c	2.47 a	1.03 a

At the 5% level, there is no significant difference between the mean that have the same letter.

Leaf content of phenol and indole (mg/100g F.W.):-

Data in **Table (4)** displays how various treatments impact the chemical composition of Cleopatra mandarin rootstock seedlings leaves over two seasons, control (T_1) treatments has the least leaf content of indole (0.26 – 0.27 mg/100g F.W.) and the highest phenol content (1.47 – 1.46 mg/100g F.W.) respectively. Otherwise, T_4 and T_6 treatments get the highest leaf indole content through the 1st season (0.69 – 0.65) and 2nd season (0.69 – 0.67 mg/100g F.W.) as well as the least phenole content in 2022 season (0.64 – 0.84) and in 2023 season (0.62 – 0.83 mg/100g F.W.) Obtained results is in agreement with the findings that the plant's antioxidant compounds are mainly phenolic including compounds such as tocopherols, carotenoids, phenolic acids and flavonoids. Secondary plant-derived metabolites, including phenolic compounds, have a potent potential to clear free radicals that exist in all parts of the plant, such as the leaves, fruits, seeds and roots, on the other hand, Vitamin E can convert free radicals into less reaction compounds play a pivotal role in decrease total phenol Which has a positive effect on plant growth (Sozen et al., 2019 and Orabi et al., 2015).

Table (4). Effect of antioxidant treatments on leaf growth promoters and inhibitors of Cleopatra mandarin rootstock seedlings during the two successive seasons.

Treatments	Phenol (mg/100g F.W.)	Indole (mg/100g F.W.)
	1st season	
T_1: Control (spraying with water)	1.47 a	0.26 e
T_2: Hydrogen peroxide.	1.22 c	0.38 d
T_3: Vitamin E.	1.41 b	0.30 e
T_4: Selenium.	0.64 g	0.69 a
T_5: Hydrogen peroxide + Vitamin E.	1.21 c	0.44 c
T_6: Hydrogen peroxide + Selenium.	0.84 f	0.65 a
T_7: Vitamin E + Selenium.	1.04 e	0.56 b
T_8: Hydrogen peroxide + Vitamin E + Selenium.	1.09 d	0.59 b
	2nd season	
T_1: Control (spraying with water)	1.46 a	0.27 f
T_2: Hydrogen peroxide.	1.18 c	0.39 d
T_3: Vitamin E.	1.39 b	0.33 e
T_4: Selenium.	0.62h	0.69 a
T_5: Hydrogen peroxide + Vitamin E.	1.15 d	0.45 c
T_6: Hydrogen peroxide + Selenium.	0.83 g	0.67 a
T_7: Vitamin E + Selenium.	1.01f	0.56 b
T_8: Hydrogen peroxide + Vitamin E + Selenium.	1.08 e	0.59 b

At the 5% level, there is no significant difference between the mean that have the same letter.

3) Leaf content of minerals:-

The present results **(Table 5)** illustrated the leaf mineral content; the highest nitrogen (N) and potassium (K) content through both seasons of study was recorded for T_4, T_6 and T_8. In addition to, T_8, T_6, T_7 and T4 treatments have higher phosphorous (P) and total carbohydrates in leaves and stem in 2022 and 2023 seasons respectively with significant differences than the other treatments. The increase in N percentage on Cleopatra mandarin rootstock seedlings could be explained by, the positive effects of antioxidants on root growth, which in turn increased nitrate absorption (Li et al., 2016). As we know that N, P, K and total carbohydrates in leaves and stem play important roles directly or indirectly in cell division, cell enlargement and differentiation because all nutrients are the key constituents of many metabolically active compounds that regulate various physiological functions (Sozen et al., 2019). K alone has a crucial function in the activation of over 50 enzymes (Chen et al., 2022). In this study, higher nutrient uptake may be one of the reasons for improved plant growth and development. Exogenous H_2O_2 treatments caused the plants to develop a robust root system, increase all minerals could be absorbed (Zhang et al., 2011).

Table (5). Effect of antioxidant treatments on leaf mineral content and total carbohydrates of Cleopatra mandarin rootstock seedlings during the two successive seasons.

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Total carbohydrates in leaves (g/100g D.W.)	Total carbohydrates in stem (g/100g D.W.)
			1st season		
T_1: Control (spraying with water)	1.49 g	0.06 f	0.64 f	8.93 f	14.81e
T_2: Hydrogen peroxide.	1.94 c	0.09 e	0.93 d	14.14 de	16.07 d
T_3: Vitamin E.	1.69 f	0.08 ef	0.76 ef	15.51d	22.82 c
T_4: Selenium.	2.54 a	0.14 c	1.12 b	25.50 c	35.96 ab
T_5: Hydrogen peroxide + Vitamin E.	1.81 e	0.12 cd	0.82 e	16.61 d	23.68 c
T_6: Hydrogen peroxide + Selenium.	2.38 b	0.17 b	1.28 a	25.76 c	33.77 b
T_7: Vitamin E + Selenium.	2.27 bc	0.16 b	1.03 c	30.54 a	37.01 a
T_8: Hydrogen peroxide + Vitamin E + Selenium.	2.44 ab	0.19 a	1.22 ab	27.38 b	35.93 ab
			2nd season		
T_1: Control (spraying with water)	1.51 g	0.07 e	0.68 e	9.58 e	15.93e
T_2: Hydrogen peroxide.	1.92 e	0.10 d	0.96 c	14.62 d	16.58 d
T_3: Vitamin E.	1.71 f	0.09 d	0.80 d	15.74 d	23.04 c
T_4: Selenium.	2.57 a	0.15 c	1.15 b	25.96 c	36.57 a
T_5: Hydrogen peroxide + Vitamin E.	1.87 e	0.13 c	0.85 d	16.75 d	24.08 c
T_6: Hydrogen peroxide + Selenium.	2.40 c	0.19 a	1.26 a	26.94 bc	34.85 b
T_7: Vitamin E + Selenium.	2.31 d	0.17 b	1.08 b	31.67 a	37.38 a
T_8: Hydrogen peroxide + Vitamin E + Selenium.	2.48 b	0.20 a	1.23 a	28.87 b	36.31 a

At the 5% level, there is no significant difference between the mean that have the same letter.

4) Enzymes activity:-

The data in **Table (6)** revealed that treated Cleopatra mandarin rootstock seedlings had increased activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH) enzymes as a mode of defense against any stress. The relationship between antioxidant responses in plants and enzymes increased activity has been examined across a wide range of species, demonstrating that, stress causes oxidative damage via reactive oxygen species (ROS) buildup, activating sophisticated antioxidant defense mechanisms from through enzymes increased activity (Farmer and Mueller, 2013). In terms of enzymatic defenses, the activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) is increased to neutralize ROS. Non-enzymatic defenses rely on proline, ascorbate (AsA), and glutathione (GSH) to scavenge ROS (Apel and Hirt, 2004). Cleopatra mandarin had higher proline and total AsA levels under stress, but it incurred more oxidative damage because to inadequate enzymatic coordination (Wang et al., 2022). In this regard, data from **Table (5)** showed that spraying Cleopatra mandarin rootstock seedlings with hydrogen peroxide at a concentration of 500 mg/L, vitamin E at a concentration of 2 mg/L, and selenium at a concentration of 0.3 mg/L, either individually or in combination, results in a significant increase in the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH) enzymes in leaves when compared to untreated seedlings. This finding could be related to the fact that antioxidant application enhances the activities of antioxidant enzymes as part of the plant's defense mechanism, by regulating antioxidative metabolism in plants during stress, maintaining higher antioxidant activities, and improving the efficiency of the ascorbate-glutathione cycle in detoxifying (Sozen et al., 2019).

Table (6). Effect of antioxidant treatments on antioxidant enzymes activities (u/g. min) of Cleopatra mandarin rootstock seedlings during the two successive seasons.

Treatments	Catalase enzyme	Superoxide dismutase enzyme	Glutathione peroxidase enzyme
T_1: Control (spraying with water)	0.90 ef	20.25 e	55.00 f
T_2: Hydrogen peroxide.	2.10 d	25.60 d	71.75 d
T_3: Vitamin E.	1.40 e	23.50 de	60.00 e
T_4: Selenium.	3.50 b	39.80 b	70.00 d
T_5: Hydrogen peroxide + Vitamin E.	2.70 c	30.50 c	75.00 c
T_6: Hydrogen peroxide + Selenium.	4.30 a	44.75 ab	88.60 bc
T_7: Vitamin E + Selenium.	3.60 b	40.30 b	95.50 a
T_8: Hydrogen peroxide + Vitamin E + Selenium.	3.80 ab	46.00 a	90.00 b

At the 5% level, there is no significant difference between the mean that have the same letter.

5) Economical evaluation:-

Economically speaking, foliar spraying hydrogen peroxide, selenium, vitamin E, or their combination shortened the time it took for seedlings to become suitable for grafting, which in turn decreased the amount of time they spent in nurseries and, ultimately, the cost of producing citrus seedlings. This decrease is noticeable for a Cleopatra mandarin seedling that achieved grafting after approximately nine months of antioxidant treatment **Table (7)**. In contrast, under control treatment, it takes twenty-four months for the seedlings to be suitable for grafting. This increases the costs of producing Cleopatra mandarin seedlings, including labor costs during that time as well as fertilizer, irrigation, and pest control costs. The present treatments in **Table (7)** showed that, the highest net income was gained from the combination of Hydrogen peroxide + Selenium + Vitamin E (T_8) and Hydrogen peroxide + Selenium (T_6) followed by Vitamin E + Selenium (T_7) and Selenium (T_4). On the other hand, the other treatments showed low net income while control treatment showed negative net income.

Table (7). Economical evaluation of the present treatments on net income of grafted Cleopatra mandarin rootstock seedlings.

Treatments	Chemicals (LE)	Labors (LE)	Chemicals + labors (LE)	Constant cost (LE)	Total cost (LE)	No. of grafted seedlings	Seedlings price (LE)	Net income (LE)
T_1: Control (spraying with water)	——	85	85	120	205	7	119	-86
T_2: Hydrogen peroxide.	14	85	99	120	219	26	442	223
T_3: Vitamin E.	18	85	103	120	223	19	323	100
T_4: Selenium.	68	85	153	120	273	34	578	305
T_5: Hydrogen peroxide + Vitamin E.	32	85	117	120	237	27	459	222
T_6: Hydrogen peroxide + Selenium.	82	85	167	120	287	44	748	461
T_7: Vitamin E + Selenium.	86	85	171	120	291	37	629	338
T_8: Hydrogen peroxide + Vitamin E + Selenium.	100	85	185	120	305	47	799	494

CONCLUSION

So, we can recommend nursery growers of Cleopatra mandarin rootstock to foliar spray the seedlings with Hydrogen peroxide (H_2O_2) at 500 mg/L + Vitamin E (Tocopherol) at 2 mg/L + Selenium (Na_2SeO_3) at 0.3 mg/L. The foliar spray should be propitious to the growing season (from May to January) as monthly dosage to increase grafting success percentage, vegetative and root growth followed by higher net income.

REFERENCES

Ahammed, G. J., Guang, Y., Yang, Y. and Chen, J. (2021). Mechanisms of elevated CO-induced 2 thermotolerance in plants: the role of phytohormones. Plant Cell Rep., 40(12): 2273-2286.

Albrecht, U., Zekri, M. and Williamson, J. (2017). Citrus Propagation IFAS Extension HS-1309Vol. No. 5 1-6

Aline, R., Connor, H., Jeremy, B., Seema, S., Jithesh, V., Julie, S., Nate, B., Melissa, W., Katarzyna, G., Nicole, R., Buan, R. and Roston, L. (2023). The effects of exogenously applied antioxidants on plant growth and resilience. Phytochem Rev. 22:407–447.

Apel, K., and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55(1):373-399

Barakat, H. (2003). Interactive effects of salinity and certain vitamin on gene expression and cell division. Int. J. Agric. Biol., 3: 219-225.

Bao, Y., Magallenes-Lundback, M., Deason, N., and DellaPenna, D. (2020). High throughput profiling of tocochromanols in leaves and seeds of Arabidopsis and Maize. Plant Methods 16:14.

Bremner, J. M. and Mulvaney, C. S. (1982). Nitrogen-total. Methods of soil analysis: part 2 chemical and microbiological properties, 2nd ed.; Page, A.L., Miller, R.H., Kenney, D.R., Eds.; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.

Chen, F., Wang, L., Zhang, D., Li, S. and Zhang, X. (2022).Effect of an Established Nutritional Level of Selenium on Energy Metabolism and Gene Expression in the Liver of Rainbow Trout. Biological Trace Element Research , 200:3829–3840.

Cottenie, A. (1980). Soils and plant testing as a basis of fertilizer recommendation. FAO Soil Bull., 3812.

Deng, X.P., Cheng, Y.J., Wu, X.B., Kwak, S.S., Chen, W. and Egrinya, A. (2012). Exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. Aust. J. Crop Sci., 6: 1572–1578.

Duncan, D.B. (1955). Multiple range and Multiple F test. Biometrics, 11: 1-42.

Dubois, M., Giller, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorometric method for determinations of sugars and related substance. Analytical Chemistry, 28:350-356.

El-harony, S. B., Hussein, S. A. and Amjad, M. E. (2023).Effect of LED Lighting on Horticultural Traits and Pathogenicity in Citrus Nurseries. Journal of Horticultural Science & Ornamental Plants, 15 (2): 70-82.

Falk, J., and Munné-Bosch, S., (2010). Tocochromanol functions in plants:Antioxidation and beyond. J. Exp. Bot., 61: 1549–1566.

Farmer, E. E. and Mueller, M. J. (2013). ROS-mediated lipid peroxidation and RES-activated signaling. Annual Review of Plant Biology, 64(1): 429-45

Gallego, S. M., Benavídes, M. P. and Tomaro, M. L. (1996). Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Science, 121: 151–159.

Gupta, M. and Gupta, S. (2017). An overview of selenium uptake, metabolism, and toxicity in plants. Front. Plant Sci. 7:2074.

Halliwell, B., (2011). Free radicals and antioxidants – quo vadis? Trends Pharmacol.Sci., 32(3): 125–130.

Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. Nutr. Rev., 70(5): 257–265.

Horwitz, W. and Albert, R., (2006). A useful index of method performance with respect to precision. J AOAC Int., 89 (4):1095–1109.

Hudson, R. and Gregoriou, A. (2010). Calculating and comparing security returns is harder than you think. A comparison between logarithmic and simple returns SSRN, SSRN, 1549328.

Larsen, P, Harbo, A, Klungsour, S. and Asheim, T. (1962). The biogenesis of some indol compounds in Acetobacterxylinum. Physiol. Plant, 15: 552-565.

Li, W., Liu, H., Ashraf, U., Li, GK., Li, YL. and Lu, W. (2016c). Exogenous gamma-aminobutyric acid (GABA) application improved early growth, net photosynthesis, and associated physio-biochemical events in maize. Plant Sci., 7:919.

Maria, Z., Maria, F., Stanisław, W., Halina, M., Andrzej, K., Zbigniew, M. and Helinä, H. (2010). Effect of selenium on macro and microelement distribution and physiological parameters of rape and wheat seedlings exposed to cadmium stress. Plant Soil 329:457–468.

Murphy, J. and Riely, J. P. (1962).A modified single solution method for the determination phosphate in natural waters. Anal. Chem. Acta , 27:31-36.

Nik, N., Nashriyah, M., Khamsah, S. M., Noor, A. B., Nornasuha, Y., Mohammad, H. S., Khairil, M., Ahmad, F. and Mohammad, M. (2020). The effects of hydrogen peroxide on plant growth, mineral accumulation, as well as biological and chemical properties of *Ficus deltoidea*. Agronomy, 10: 599. doi: 10.3390.

Nornai, R. (1982). Formula for determination of chlorophyllous Pigments extracted with N.N dimethyl formamid. Transplant physiol., 69:1371-1381.

Orabi, S.A., Dawood, M.G. and Salman, S.R. (2015). Comparative study between the physiological role of hydrogen peroxide and salicylic acid in alleviating the harmful effect of low temperature on tomato plants grown under sand-ponic culture. Sci. Agric., 9: 49–59.

Patel, R.K., Babu, K.D., Singh, A., Yadav, D.S., and De, L.C. (2010). Soft wood grafting in Mandarin (C. Reticulata Blanco): A novel vegetative propagation technique. Inter. J. Fruit Sci., 10 (1): 54-64.

Ramirez, L., Bartoli, C.G. and Lamattina, L. (2013). Glutathione and ascorbic acid protect Arabidopsis plants against detrimental effects of iron deficiency. J Exp Bot, 64:3169–3178.

Ribeiro, R.V., Machad, E. C. and Oliveira, R. F., (2004) Growth-and leaf-temperature effects on photosynthesis of sweet orange seedlings infected with Xylella fastidiosa. Plant Pathol., 53: 334-340.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, Oxford and J.B.H pup co. Publishing 6th Edition.

Sozen, E., Demirel, T. and Ozer, N. K., (2019) Vitamin E: Regulatory role in the cardiovascular system. IUBMB Life, 71: 507–515.

Temminghoff, E. E. and Houba, V. J. (2004) Plant analysis procedures. Kluwer Academic Publishers: Dordrecht, The Netherlands, pp. 94–96.

Valko, M., Leibfritz, D., Moncol, J., Cronin, MT., Mazur, M. and Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. ,39(1): 44–84.

Wang, Y. H., Ying, Y., Chen, J. and Wang, X. C. (2004). Transgenic arabidopsis overexpressing Mn-SOD enhanced salt-tolerance. Plant Science, 167:671–677.

Wang, M. M., Toda, K., and Maeda, H. A. (2016). Biochemical properties and subcellular localization of tyrosine aminotransferases in Arabidopsis thaliana. Phytochemistry 132: 16–25. doi: 10.1016/j.phytochem.2016.09.007

Wang, Y., Zhang, H., Hou, P., Su, X., Zhao, P., Zhao, H., and Liu, S. (2022)

Exogenous spermidine enhances the photosynthetic and antioxidant capacity of citrus leaves under heat stress. Plant Signaling & Behavior, 17(1):2086372.

Wu, Q.S., Zou, Y.N., Peng, Y.H. and Liu, C.Y. (2011). Root morphological modification of mycorrhizal citrus (Citrus tangerine) seedlings after application with exogenous polyamines. The J. Anim. Plant Sci., 21(1):20-25.

Ye, W., Zhang, J., Townsend, M. and Tew, D. (2015). Oxidative stress, redox regulation and diseases of cellular differentiation. Biochim. Biophys. Acta, 1850(8): 1607–1621.

Zahra, Z., Mansureh, G.,Gianluigi, B. and Ali, T., (2016). Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. International Journal of PharmTech Research, 9(9): 86-96.

Zhang, J., Cui, S., Li, J. and Kirkham, M. B., (1995). Protoplasmic factors, antoxidant responses, and chilling resistance in maize. Plant Physiology and Biochemistry 33: 567–575.

Zhang, X.L., Jia, X.F., Yu, B., Gao, Y. and Bai, J.G. (2011). Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. Sci. Hortic., 129: 656–662.

الملخص العربى

تأثير التسميد الورقي ببعض مضادات الأكسدة على أداء شتلات اليوسفي الكليوباترا

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تم اجراء دراسه على شتلات يوسفي كليوباترا تحت الصوب في مشتل الموالح بمعهد بحوث البساتين بالجيزة مصر. تمت خلال موسمين متتاليين (2022-2023)، من أجل بحث تأثير بيروكسيد الهيدروجين بتركيز 500 ملجم/لتر وفيتامين هـ (توكوفيرول) بتركيز 2 ملجم/لتر والسيلينيوم (سيلينيوزات صوديوم) بتركيز 0.3 ملجم/لتر كمضاده للأكسدة، إما بشكل فردي أو مجتمعة ، بغرض تقصير الفترة اللازمه لنمو شتلات أصل يوسفي كليوباترا للوصول إلى مرحلة التطعيم وتحقيق أعلى عائد اقتصادي. أظهرت النتائج التي تم الحصول عليها عن تحسن كبير في جميع المعايير المختبرة لمساحة الورقة، وعدد الأوراق/الشتلة، ونسبة المادة الجافة للأوراق والفروع، ونسبة الزيادة في كل من ساق الشتلة (الطول والقطر)، ونسبة نجاح التطعيم، ومحتوى الصبغات الورقية، ومحتوى الإندول والفينول في الأوراق، ومحتوى العناصر في الأوراق وأنشطة إنزيمات مضادات الأكسدة مقارنة بالكنترول. في حين تم الحصول على أفضل النتائج من المعالجات المركبة بيروكسيد الهيدروجين بتركيز 500 ملجم/لتر + السيلينيوم بتركيز 0.3 ملجم/لتر.