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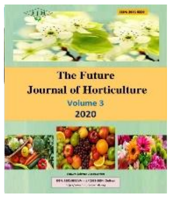

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RESPONSE OF THOMPSON SEEDLESS GRAPEVINES TO FOLIAR APPLICATION OF SOME ANTIOXIDANTS

Heba, F.S. Ibrahim; Ali H. Ali and Gewied A. Gewied

Horticulture Department, Faculty of Agriculture, Minia University, **Egypt.**

***Corresponding author:** hebafawzy2009@yahoo.com **Received:** 20 Dec. 2020 **; Accepted:** 24 Jan. 2021

ABSTRACT: Vegetative growth, yield and berry physical and chemical quality of Thompson Seedless grapevines grown in clay soil under El-Minia region conditions – Egypt in response to three sprays with vitamin E at 40 ppm, citric acid at 500 ppm and folic acid at 500 ppm singly or in combination were investigated during 2019 and 2020 seasons. spraying the three antioxidants were very effective in improving all vegetative growth parameters (in terms of shoot lengths (cm), number of leaves/shoot and leaf area (cm^2), one-year-old wood pruning wood (kg/vine), cane thickness (cm), Yield and berry physical and chemical properties, rather than non- application. However, any combined application of these three antioxidants in was remarkably superior than using each one alone, on all vegetative growth, yield and its components and berry physical and chemical properties of Thompson seedless grapevines. Furthermore, spraying the citric acid in combination with folic acid present superiority effect on all studied parameters than spring citric acid in combination with vitamin E, during the two experimental seasons. The best results with regard to vegetative growth, yield and berry quality of Thompson Seedless grown in clay soil under El-Minia region conditions were obtained when the vines received the three examined antioxidants in combination.

Key words: Grapevines, Thompson Seedless, *Vitis vinifera*, Vitamin E, Citric acid and Folic acid.

INTRODUCTION

Grapes (*Vitis vinifera* L.) are the largest fruit crop on earth, it considers as one of the major horticulture crops throughout the world. Grapevine is one of the most important botanical Genus of Family Vitaceae (**Kanellis & Roubelakis, 1993 and Doring *et al.*, 2015**). However, it is well known that grapevines have great adaptability and thrives in wide range of climatic and soil conditions (**Winkler *et al.*, 1974; Delas 2000; Reynier, 2000; Srinivasan & Mmullins, 2001 and Sluys, 2006**). Grape considered as one of the most important commercial and favorite fruit in Egypt and occupied the second fruit crops, since it is preceded only by citrus crops (Egyptian Ministry of Agriculture and Reclamation, Egypt 2018). Thompson seedless, also called Banaty seedless, is one of the main grapevine cultivars in Egypt, now it has become one of the traditional cultivars, especially in Minia Governorate. Thompson seedless cultivar ripens in the middle of the season; it has a good flavor and distinct taste that make it desirable for the Egyptian consumer. This lead to a remarkable increase area cultivated by this cultivar.

Nowadays, there is a widespread use of the antioxidants. They have an auxinic action, since they have synergistic effect on growth, productivity and provided disease control against most fungi infection of most fruit trees (**Raskin, 1992; Galal & El-Sayed, 1995; Hamada & Al-Hakimi, 2009; Shalata & Newmann, 2011 and Khan *et al.*, 2012**). Folic acid is a form of water-soluble vitamin B. It is important for DNA synthesis and cell division, especially in tissues with high rates of cell multiplication. Furthermore, Folic acid is required for purine and pyrimidine synthesis, amino acid inter-conversions, methylation reactions, and the generation and use of format (**Miller, 2013 and Georgidou *et al.*, 2016**). Citric acid is a natural and organic antioxidant compound has as auxinic action, it provided disease control, cell division and promotion of lipase, synergistic effect on rooting and improving growth, flowering, yield and fruit quality of fruit trees (**Elad, 1992; Ahmed *et al.*, 2003; Abo El-Komsan *et al.*, 2003 and Ahmed & Seleem (2008)**. Vitamin E includes eight fat soluble compounds that include four tocopherols and four tocotrienols (**Evans and Lawrenson, 2017 and Traber & Bruno, 2020**). While, vitamin E was discovered in 1922, isolated in 1935 and first synthesized in 1938, it was given the name tocopherol from Greek words meaning birth and to bear or carry (**Evans and Lawrenson, 2017**). Vitamin E is a fat-soluble antioxidant protecting cell membranes from reactive oxygen species (**Office of Dietary Supplements, US National Institute of Health, August 2019**). Improving nutritional status of vines, berry retention, yield/vine and berries physical and chemical quality of Thompson Seedless grapevines can be achieved through better culture practices. Among the major culture practices must be applied are the application of antioxidants, this was the main objective of this study.

MATERIALS AND METHODS

The present investigation was carried out during two successive seasons 2019 and 2020 on twenty-four own rooted uniform in vigor Thompson Seedless vines, grown under Abo Kourkas distract, El-Minia Governorate-Egypt, where the soil texture is clay, since water table depth is not less than two meters. The chosen vines are ten years old (at the starting of this study) and planted at 2 X 3 meters apart.

Soil analysis

A composite sample of experimental soil was collected and subjected to Physical and chemical analysis according to the procedures outlined by **Ward & Johnston (1962) and Walsh & Beaton (1986)**. The obtained data are shown in Table (1).

Table 1. Physical and chemical analysis of experiment orchard soil

Constituents	Values
Sand %	10.5
Silt %	23.0
Clay %	68.5
Texture	Clay
pH (1:2.5 extract)	7.85
EC (1:2.5 extract) (dsm-1) 1 cm/ 25ºC	1.01
O.M. %	1.48
$CaCO_3$ %	2.01
Total N %	0.15
Available P (Olsen, ppm)	2.22
Available K (ammonium acetate, ppm)	350

Experimental work

The present study included seven treatments from vitamin E at 40 ppm, citric acid at 500 ppm and folic acid at 500 ppm and their combination plus the control treatment. The three antioxidants were sprayed three times yearly (at the starting of vegetative growth, just after berry setting and the third one at one month later). Then, the experiment involved the following eight treatments from these three antioxidants and their combinations. As follows:

1. Control (vines treated with tap water).
2. Spraying vitamin E at 40 ppm.
3. Spraying citric acid at 500 ppm.
4. Spraying folic acid at 500 ppm.
5. Spraying vitamin E at 40 ppm + citric acid at 500 ppm.
6. Spraying vitamin E at 40 ppm + folic acid at 500 ppm.
7. Spraying citric acid at 500 ppm + folic acid at 500 ppm.
8. Spraying vitamin E 40 at ppm + citric acid at 500 ppm + folic acid at 500 ppm.

Each treatment was replicated three times, one vine per each. Then, the present study included twenty-four vines from Thompson Seedless cultivar. The treatments were arranged in a complete randomized block design (CRBD).

Different measurement and determinations

Vegetative growth characters

At the second week of June during both seasons, eight mature leaves opposite the first cluster were picked from each replicate according to **Ibrahim (2010)**, Leaf area (cm^2) was estimated by using an area meter (Area Meter Cl, 202). At the middle of June, the average main shoot length (cm) was recorded as a result of measuring the length of four shoots/vine, one shoots per each direction. The average leaves number/shoot were recorded as a result of counting the number of leaves located at four shoots, one shoots for each direction. Just after carrying out winter pruning, the weight removal of one-year-old pruning wood per each vine was recorded (kg/ vine).

Measurements of berry setting percentage

For calculating berry setting%, before blooming stage four clusters/vine were caged in perforated white paper bags. At the end of berry setting stage, the bags were removed for counting the following: a) Number of attached berries, b) Number of dropped berries, c) Number of dropped flowers. For each cluster, the number of total flowers were calculated (= a+ b + c) per cluster. Berry setting% was mathematically estimated by dividing the number of attached berries by the total number of flowers per cluster multiplying the product by 100 (**Ibrahim, 2010**).

Measurement of yield as well as berry physical properties

The cluster were harvested when the TSS/Total acidity ratio in the juice of the check treatment reached to 23: 25 in the two experimental seasons. The yield per vine was recorded in terms of weight (kg) and number of clusters per vine, and then the yield (kg) per tree was calculated. From each vine, four clusters were randomly picked at maturation date (Last week of June). The following physical and berry characteristics were studied: Berry weight (g), by using sensitivity balance with 0.1g accuracy. Berry length and diameter (cm), by using Vernier caliper with 0.01cm accuracy.

Determination of berries chemical characteristics

After clusters were harvested, 100 berries were taken randomly, the berries were pressed by using Electric Extractor for extracting the juice, the following chemical characteristics were determined: Percentage of total soluble solids (T.S.S %) were determined in juice obtained from each replicate with hand refractometer and expressed as a percentage (Brix), according to **Ranganna (1977)**. Percentage of total treatable acidity (TA), expressed as grams of tartaric acid per 100 grams of juice According to **A.O.A.C, (2000)**.

Percentage of reducing, reducing sugars in berries juice, determined by using Lane and Eynone volumetric method, according to **Ranganna (1977)**.

Statistical analysis of data

All obtained data were tabulated and subjected for the proper statistical analysis; by analysis of variance (ANOVA) using the statistical package MSTATC Program. Comparisons between means were made by New L.S.D at p = 0.05 (**Snedecor and Cochran, 1990**).

RESULTS AND DISCUSSIONS

1. Effect of vitamin E, citric acid and folic acid on vegetative growth parameters

Data presented in Table (2) showed the effect of spraying vitamin E, citric acid and folic acid on average shoot lengths (cm), numbers of leaves/shoot and leaf area (cm^2) as well as average weight of one-year old pruning wood (kg/vine) and cane thickness (cm) of Thompson Seedless grapevines during 2019 and 2020 seasons. The obtained Data obtained in Table (2) displayed that, spraying vitamin E, folic acid and citric acid individually or in combination significantly enhanced all vegetative growth parameters, rather than control treatment. The data took the same trend during the two experimental seasons. The same table shows that, the vines received the mixture of the three antioxidant (vitamin E at 40 ppm plus folic at 500 ppm and citric acid at 500 ppm) present the highest shoot length (96.0 and 96.6 cm), number of leaves/shoot (25.6 & 26.0 leaves), leaf area (128.8 & 124.3 cm^2), pruning wood (2.14 & 2.27 kg/vine) and cane thickness (1.20 & 1.12 cm), followed by those received citric and folic acids each at 500 ppm, during the two experimental seasons respectively. While untreated vines present the lowest vegetative growth parameters (85.5 & 86.0 cm shoot lengths; 16.5 & 16.7

leaves/shoot; 110.2 & 111.0 cm^2 leaf area; 1.70 & 1.82 kg pruning wood/vine and 0,80 & 0.83 cm can thickness), during the experimental seasons respectively.

The positive effect of these three antioxidants on vegetative growth characters of Thompson Seedless grapevines might be attributed to their vital role in protecting the plant cell from senescence, preventing the free radicals from oxidation of lipids as well as their effect in enhancing cell division and building of organic acids and the biosynthesis of organic foods and controlling the incidence of funflal attack (**Elade, 1992 and Rao *et al.*, 2000**). In addition, antioxidants are responsible for enhancing peroxidase and catalase activity that catalize the oxidation of H_2O_2, which is toxic to cell and impairs the resistance of plants agent various diseases (**Rao *et al.*, 2000**). Moreover, the antioxidants, especially vitamin E, citric acid and folic acid, are essential in regulation of metabolism and are responsible for physiological processes such as cytokines and gibberellins (**Samiullah *et al.*, 1988**).

Table 2. Effect of spraying vitamin E, citric acid, folic acid and their combinations on main shoot length (cm), number of leaves/ shoot, leaf area (cm^2), pruning wood (kg/vine) and cane thickness (cm) of Thompson Seedless grapevines, during 2019 and 2020 seasons

Treatments	Shoot length (cm)		Number of leaves/ shoot		Leaf area (cm^2)		Pruning wood (kg/vine)		Cane thickness (cm)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	85.5	86.0	16.5	16.7	110.2	111.0	1.70	1.82	0.80	0.83
Vitamin E. at 40 ppm	87.2	87.6	17.6	17.9	113.3	114.4	1.79	1.90	0.86	0.87
Citric acid at 500 ppm	88.7	89.1	18.9	19.2	114.9	116.1	1.85	2.97	0.91	0.90
Folic acid at 500 ppm	90.1	90.5	20.4	20.5	116.6	117.7	1.92	2.03	0.95	0.94
Vit. E. at 40 ppm + citric acid at 500 ppm	91.6	92.2	22.0	22.1	118.2	119.4	1.98	2.08	1.00	0.99
Vit. E. at 40 ppm + folic acid at 500 ppm	93.1	93.7	23.2	23.4	119.7	121.0	2.03	2.14	1.06	1.03
Citric acid at 500 ppm + Folic acid 500 ppm	94.4	95.1	24.2	24.8	121.3	122.7	2.09	2.21	1.13	1.07
Vit. E. at 40 ppm + citric acid at 500 ppm + Folic acid 500 ppm	96.0	96.6	25.7	26.0	128.8	124.3	2.14	2.27	1.20	1.12
New LSD $_{5\%}$	1.3	1.4	1.0	1.1	1.5	1.6	0.05	0.06	0.04	0.03

2. Effect of vitamin E, citric acid and folic acid on berry setting%, shot berries%, cluster weight (g), cluster numbers and yield (kg/ vine)

Data presented in Table (3) shows the effect of spraying vitamin E, citric acid and folic acid each one alone or in combinations on berry setting %, shot berries%, number of cluster/ vine, average cluster weight (g) and yield (kg/vine) of Thompson Seedless grapevines, during the two experimental seasons (2019 and 2020).

This Table shows that, all examined antioxidants in single or combined application were capable to significantly increase berry setting%, yield and cluster weight and decrease the shot berries %, during the two experimental seasons. However, during the first experimental seasons (2019) all treatments failed to increase the number of clusters/ vine significantly. This effect is logic, since fruiting bud were internally formed in the preceding year. One the other hand, spraying the three antioxidants has a significant effect on the yield/vine at the seam season, this increment can be explained by the important role of the three antioxidants in enhancing the cluster weight and reducing the shot berries % per clusters.

The same Table showed that, all treatments included any combination from the three antioxidants was superior to spraying each one alone. However, the vines received the combined application of the three antioxidants present the highest yield/vine (11.1 & 14.0 kg/vine), cluster weight (423 & 413 g) during the two experimental seasons respectively, and highest cluster number during the second seasons (33.9 cluster/vine). In this respect, using the citric acid + folic acid combination was remarkably and significantly higher than using vitamin E + citric acid, during both experimental seasons.

On the opposite side, untreated vines present the lowest yield (9.0 & 9.5 kg/vine), cluster numbers/vine (25.0 & 26.1) and cluster weight (360 & 365 g), during the two experimental seasons respectively.

The role of the three antioxidants (vitamin E, citric acid and folic acid) in improving the yield and its components a well as berry setting which obtained in the present study was in accordance with the results of some studies such as; **Ali (2000)** on Flame Seedless grapevines; **Ahmed *et al*., (2010)** on Crimson Seedless grapevines; **Ahmed *et al*., (2011)** on Thompson Seedless grapevines; **Abd El-Hammed (2012)** on Early Superior grapevines and **Mekawy (2012)** on Thompson Seedless grapevines. The use of antioxidants as enhancing the productivity of fruit trees especially grapevines well established also in the previous studies. Biostimulants can be lead to improve cluster numbers and weight and also referred as metabolic enhanced (**Georgidou *et al*., 2016** and **Abdelmoniem *et al*., 2019**). Antioxidants application lead to improve mineral uptick and natural growth regulators production by plants (**El- Kady, 2011** and **Georgidou *et al*., 2016**).

Table 3. Effect of spraying vitamin E, citric acid, folic acid and their combinations on berry setting %, shot berries %, number of clusters, cluster weight (g) and yield (kg/vine) of Thompson Seedless grapevines, during 2019 and 2020 seasons

Treatments	Berry setting %		Shot berries %		Number of cluster / vine		Cluster weight (g)		Yield/ vine (kg)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	7.6	8.0	9.0	9.2	25.0	26.1	360	365	9.0	9.5
Vitamin E. at 40 ppm	8.2	8.5	8.0	8.1	25.2	27.2	370	372	9.3	10.1
Citric acid at 500 ppm	8.9	9.1	7.2	7.2	25.6	28.3	378	378	9.7	10.7
Folic acid at 500 ppm	9.4	9.8	6.5	6.2	25.6	29.3	387	386	9.9	11.3
Vit. E. at 40 ppm + citric acid at 500 ppm	10.0	10.2	5.8	5.3	25.6	30.5	395	393	10.1	12.0
Vit. E. at 40 ppm + folic acid at 500 ppm	10.6	10.7	5.0	4.4	25.8	31.6	404	400	10.4	12.6
Citric acid at 500 ppm + Folic acid 500 ppm	11.3	11.2	4.2	3.3	26.1	32.8	414	406	10.8	13.3
Vit. E. at 40 ppm + citric acid at 500 ppm + Folic acid 500 ppm	11.9	11.8	3.3	2.2	26.3	33.9	423	413	11.1	14.0
New LSD $_{5\%}$	0.5	0.4	0.7	0.9	NS	1.0	8.0	6.0	0.2	0.6

3. Effect of vitamin E, citric acid and folic acid on berry physical and chemical properties

The results pertaining to the effect of spraying vitamin E, citric acid and folic acid in single or combined application on berry physical properties (in terms; weight, berry longitudinal and berry equatorial) and chemical properties (in terms; TSS%, Reducing sugars% and total acidity%) of Thompson Seedless during the two experimental seasons are presented in Table (4). The perusal of data reveals that, all treatments of the three antioxidants exerted a significant effect on all berry physical and chemical properties, during the two experimental seasons.

It is clear from this Table that spraying Thompson Seedless with the mixture of the three antioxidants in combination has an announced and significant effect on berry weight, berry longitudinal and berry equatorial, TSS %, reducing sugars % and decrease the total acidity %, compared to the other treatments or control ones. Furthermore, the vines received the combined application of citric acid and folic acid present higher and significant effect on all studied berry physical and chemical properties (except total acidity %) than those received vitamin E and citric acid in combinations. In any case, any combined application of the three antioxidants has superior effect on berry physical and chemical properties than using each one individually. On the opposite side, untreated vines present the lowest berry weight, berry longitudinal, berry equatorial, TSS % and reducing sugars %, during the two experimental seasons respectively. It is also noticed from the obtained data in same Tables and that, folic acid present superiority effect on all physical properties than vitamin E or citric acid, when spraying each antioxidant individually, the data was true in both experimental seasons.

Table 4. Effect of spraying vitamin E, citric acid, folic acid and their combinations on berry physical and chemical properties of Thompson Seedless grapevines, during 2019 and 2020 seasons

Treatments	Berry weight (g)		Berry longitudinal (cm)		Berry equatorial (cm)		TSS %		Reducing sugars %		Total acidity %	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	1.45	1.48	1.60	1.68	1.42	1.40	17.5	17.7	15.8	16.0	0.670	0.655
Vitamin E. at 40 ppm	1.57	1.61	1.65	1.74	1.48	1.46	18.0	18.1	16.3	16.5	0.651	0.640
Citric acid at 500 ppm	1.68	1.73	1.68	1.79	1.53	1.51	18.3	18.4	16.7	16.9	0.636	0.624
Folic acid at 500 ppm	1.78	1.84	1.72	1.83	1.58	1.57	18.7	18.6	17.2	17.3	0.620	0.607
Vit. E. at 40 ppm + citric acid at 500 ppm	1.89	1.94	1.76	1.86	1.62	1.62	19.1	18.9	17.6	17.7	0.602	0.582
Vit. E. at 40 ppm + folic acid at 500 ppm	2.00	2.06	1.81	1.91	1.67	1.67	19.6	19.1	18.1	18.2	0.583	0.566
Citric acid at 500 ppm + Folic acid 500 ppm	2.11	2.17	1.87	1.97	1.72	1.73	20.0	19.4	18.5	18.6	0.566	0.550
Vit. E. at 40 ppm + citric acid at 500 ppm + Folic acid 500 ppm	2.23	2.29	194	2.04	1.78	1.79	20.4	19.8	19.1	19.0	0.550	0.533
New LSD $_{5\%}$	0.10	0.11	0.03	0.03	0.04	0.05	0.3	0.2	0.3	0.4	0.015	0.013

The positive effect of antioxidants on berry physical and chemical properties of grapevines was previously reported by: **Abdelaal *et al.* (2013)** on Thompson seedless; **Abdelaa and Ali (2013)** on Ruby Seedless grapevines; **Mohamed *et al.* (2015) and EL-Boray *et al.* (2015)** on Superior grapevines and **Abo El-Fadle (2017)** on Superior grapevines. Furthermore, similar findings were obtained by other authors on evergreen orchard trees such as; **Maksoud *et al* (2009)** on Chemlali olive fruits; **El-Badawy *et al.*, (2017)** and **Abdelmoniem *et al.* (2019)** on Washington navel orange trees.

However, the important role of the antioxidants on enhancing mineral elements uptick and stimulating some important biological functions in plant cells was able to explain its favorable effect on berry physical and chemical properties, which found in this work. Furthermore, Vitamin E, Citric acid and folic acid are a natural and organic antioxidants compounds have as auxinic action, it can provide disease control, cell division and promotion of lipase, synergistic effect on and improving berries quality of grapevines and other fruit trees (**Elad, 1992; Khiamy, 1999; Ahmed *et al.*, 2003; Abo El-Komsan *et al.*, 2003 and Abdelaal *et al.*, 2013**).

Conclusion

From the results of this study it is recommended to spraying Thompson Seedless grapevines grown in clay soil under El-Minia condition or resembling conditions with the mixture of vitamin E at 40 ppm + citric acid at 500 ppm + folic acid at 500 ppm, three times yearly ((at grow starting, just after berry setting and one month later), in order to improve vines growth and productivity as well as berry physical and chemical properties.

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