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Effect of some Antioxidants Spraying on Growth and Fruiting of Flame Seedless Grapevines

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Abstract

This study was conducted during 2018, 2019 and 2020 seasons, on 10 years old Flame Seedless grapevines, grown on the experimental vineyard of the Research Station, Matana, Esna, Luxor, Egypt to study the effect of antioxidants on vegetative growth, nutrient status, and fruiting of Flame Seedless grapevine. The experiment was arranged in a complete randomized block design with four treatments and three replications two vine per each.

The obtained results could be summarized as follow:

Spraying either citric acid, ascorbic acid or vitamin B_{12} significantly increased, pruning wood weight and leaf area as well as leaf total chlorophyll and leaf nutrient composition compared to spray water ones (check treatment). No significant differences on these traits due to spray with any ones compared to either. All antioxidants treatments significantly increased the yield and improved the cluster and berry traits compared to sprayed water. It is evident from the foregoing results that spraying with either ascorbic acid or citric acid or vitamin B_{12} at 250 ppm three times improved the vegetative growth, yield and berry quality.

***Keywords**: Antioxidants, Fruiting, Berry quality, Grapevines, Vitamin.*

Introduction

Grapes are considered the first major fruit crop in its production all over the world for been nice taste, excellent flavor and high nutritional value. In Egypt, it is ranked third among fruit crops after citrus and mangoes. The total planted area attained about 221709 fed., about 1626259 tons. It is of the most important export horticultural crop and its export value is about 10% while the quantity is about 3% of total horticultural export (MALR, 2019). Flame Seedless cultivar is one of the most important commercial and early produced in Egyptian market hence it has a great importance either to local or exported markets (El-Salhy *et al.*, 2017).

One of the goals of pomologists is to increase the production of grapes to satisfy the requirements for local consumptions and foreign markets export. The increase could be achieved by importing cultural practices i.e. fertilization, irrigation and protected cultivation of biotic and abiotic stresses (Nijjar, 1985 and Novello and de Palma, 2008).

Antioxidants play an important role in plant defense against oxidative stress induced by unfavorable conditions. Application of them is accompanied with enhancing alpha keto glutaric acid biosynthesis which is united with ammonia to for amino acids and proteins (Samiullah *et al*., 1988 and Tzeng and De Vay, 1989). Using vitamins is favourable in the biosynthesis of proteins, vitamins antioxidants enzyme and natural hormones as well as controlling diseases. They are responsible for enhancing cell division and building of most organic foods. The positive action of these antioxidants in chelating hazard radicals could results in extending the shelf-life of cells and producing healthy plants (Rao *et al*., 2003).

Vitamins participates a viral role in plant growth and development indirectly by enhancing the endogenous levels of various growth factors such as cytokinins and gibberellins (Foyer and Lelandias, 1993). Physiological processes such as nutrient uptake, absorption of water, translocation of organic foods, building of natural hormones, respiration, photosynthesis as well as chlorophyll and protein synthesis depend more or less on the availability of vitamins. Vitamins are known as important factors responsible for enhancing growth and fruiting of fruit crops. Vitamins have many important functions in plants. It is responsible for protecting cell walls from death and preventing reactive oxygen species (ROS) in favor of prolonging the age of cells, enhancing photosynthesis and the building of most organic foods in the plants (Samiullah *et al.,* 1988).

The beneficial effects of antioxidants on improving the growth and nutrient status as well as yield and fruit quality were emphasized by Khrenovskov and Kameneva (2001), Ahmed *et al*. (2002), Ahmed and Abd El-Hameed (2004), Nashed (2006), Farahat (2008), Ahmed and Seleem-Basma (2008), Abd El-Kariem (2009), Abada and Abdel-Hameed (2010), Refaai (2011), Ahmed *et al*. (2011), Abdelaal (2012), Abdelaal and Aly (2013), Abada (2014), Ebrahiem (2015), Abd El-Galil (2015), Mohamed-Attiat (2016), Sayed (2017) and Gamea-Marwa (2018). They found from their investigation that sprayed ascorbic acid, citric acid, and selenium were favourable to enhance the growth and fruiting of grapevines.

The aims of this investigation was testing the effect of antioxidants on growth, vine nutritional status and fruiting of Flame Seedless grapevines.

Materials and Methods

This study was carried out in three successive seasons of 2018, 2019 and 2010 on Flame Seedless grapevine. The vines were grown in the experimental vineyard of Research Station Agriculture Matana, Esna, Luxor, Egypt, where the soil is clay. Some properties of the orchard soil were determined according to Wilde *et al*. (1985) and are present in Table (1).

Table 1. Some physical and chemical properties of the soil of the experiment site.

Soil properties	Values	Soil properties	Values
Sand %	7.5	Total N (%)	0.13
Silt %	13.5	Total K (%)	0.17
Clay %	79.0	$NaHCO_3$ extractable P (ppm)	7.8
Texture	Clay	NH_4OAC-extractable K (ppm)	463
$CaCO_3$ (%)	1.21	DTPA extractable Fe (ppm)	10.75
Organic matter (%)	1.35	DTPA extractable Mn (ppm)	12.39
pH (1:1 suspension)	7.98	DTPA extractable Zn (ppm)	1.82
ECe (dS/m^{-1})	0.97	DTPA extractable Cu (ppm)	1.63

The vines were 10 years old at the starting of this experiment and spaced at 2x3 meters apart. Twenty-four vines of healthy with no visual nutrients deficiency symptoms, nearly uniform in shape, size and productivity were chosen and devoted by this study. The chosen vines were received the usual agriculture practices that are used in the vineyard including irrigation and pest control. The vines were trained according to the T. shape system and pruned during the second week of December by leaving 15 fruiting spurs with 4 buds each plus six replacement spurs with 2 buds each.

The experiment included four treatments of antioxidants sprays as follows:

1- Spraying with distilled water (checked treatment).
2- Spraying with vitamin B_{12} (V. B_{12} at 250 ppm).
3- Spraying with citric acid at 250 ppm
4- Spraying with ascorbic acid at 250 ppm.

The experiment was set up as a complete randomized block design. Each treatment was replicated three times, two vines per each. Solution of vitamin B_{12}, citric acid and ascorbic acid concentrations were prepared with distilled water and then sprayed thrice after berry set, one and two months later.

Regular agricultural and horticultural practices which are used in vineyard including pruning, hoeing, fertilization, irrigation and pest control. Ammonium nitrate (33.5% N) was applied at three equal doses at growth start, after berry set and 45 days later. Potassium sulphate was added at two equal patches after berry set and one month later, where calcium superphosphate (15.5% P_2O_5) was added with organic manure in the first week of January.

The following parameters were determined to evaluate the effects of different antioxidant treatments on growth, nutrient status, yield and berry quality.

1- Vegetative growth Parameters:

All vegetative growth traits i.e. main shoot thickness (cm), number of leaves/ shoot, leaf area and total chlorophyll were measured in the middle of July.

The average leaf area (cm^2): Twenty leaves from those opposite to basal clusters were measured according to the following equation that was reported by **Ahmed and Morsy (1999)**

Leaf area = 0.56 (0.79 x w^2) + 20.01, where, w = the maximum leaf width.

Weight of pruning wood (kg) was estimated by weighing the removal one year old wood after pruning.

2- Leaf nutritional status:

Samples of 30 leaves for each replication were collected from the first full mature leaves from shoots top in mid of July and leaf petioles were separated from the blades. The petioles were washed with tap water, distilled water, air-dried, oven-dried at 70°C to constant weight, then ground in a stainless steel mill. Wet digestion was done by using concentrated sulphoric acid and hydrogen peroxide for overnight. Percentages of N, P and K (on dry weight basis) were determined in the digestion according to Wilde *et al*. (1985). Total chlorophylls were determined in leaves according to Von Wettstein (1957).

3- Yield:

At harvest date (mid of June), the yield per vine in terms of weight (kg) and number of clusters per vine was recorded.

4- Cluster and berry characteristic:

Two clusters were randomly taken from each vine to determine the cluster and berry traits such as cluster weight and cluster compactness coefficient. Berry quality such as berry weight and dimension as well as reducing sugar percentages, total soluble solids and total acidity (expressed as gm tartaric acid per 100 ml juice), berry properties were evaluated according to A.O.A.C. methods (1985). Total anthocyanin content of berry skin was determined according to Rabino and Mancinelli, 1986). All the obtained data were tabulated and analyzed according to Gomez and Gomez (1984) and Snedecor and Cochran (1990). Differences between various treatment means were compared by Duncan's multiple range tests at 5% level of probability to Duncan (1955).

Results

1- Growth vegetative characteristics:

Data in Tables (2 and 3) showed the effect of antioxidants (V. B_{12}, citric acid and ascorbic acid) on leaf area, pruning wood weight, leaf chlorophyll content and leaf mineral contents (N, P, K) of Flame Seedless grapevine in 2018, 2019 and 2020 seasons. Obtained data clarified that the results took similar trend during the three studied seasons. Data in pre mentioned tables showed that sprayed either V. B_{12}, citric acid or ascorbic acid as an antioxidant source significantly increased such traits compared to water-sprayed ones (check treatment). The maximum values of leaf area, pruning wood weight, leaf chlorophyll content and leaf mineral contents were recorded on the vines that were sprayed ascorbic acid. No significant differences were recorded due to spray with any antioxidant compared to other. On other hand, the lowest values of the growth traits were recorded on the vines that were sprayed with water (check treatment). The highest leaf area (135.2 cm^2), pruning wood weight (1.57 kg/vine), total chlorophyll (5.16%), leaf N (1.85%), leaf P (0.131%) and leaf K (1.22% as an av. of the three studied seasons) were obtained due to spray with ascorbic acid. On other hand, the lowest values of these traits were recorded on the unsprayed (119.5 cm^2, 1.46 kg/vine, 4.63 mg/g, 1.80%, 0.117%, 1.11% as an av. of the three studied seasons, respectively. Then, the increment percentage of leaf area, pruning wood weight, total chlorophyll and leaf NPK% were (13.14, 7.53, 11.45, 9.44, 11.97 and 9.91% as an av. the three studied season) due to spray with ascorbic acid compared to the check treatment, respectively. Therefore, it could be concluded that all the antioxidant used in the vineyard have indirectly and positively effect on grapevines growth, where had a significantly increased the total leaf surface area, nutritional status and vegetative growth of vines.

Table 2. Effect of antioxidants spraying on leaf area, wood pruning weight and total chlorophyll of Flame Seedless grapevines during 2018, 2019 and 2020 seasons.

Treat. / Season	Leaf area (cm^2)				Wood pruning weight (kg)				Total chlorophyll (mg/g F.W)			
	2018	2019	2020	Mean	2018	2019	2020	Mean	2018	2019	2020	Mean
Cont.	120.3B	118.1B	120.2A	119.5	1.42B	1.44B	1.51B	1.46	4.58C	4.65C	4.66 C	4.63
V. B_{12}	130.1A	132.4A	134.6A	132.4	1.49A	1.51A	1.58A	1.53	4.80B	4.88B	4.90 B	4.86
Citric A.	131.5A	133.7A	135.9A	133.7	1.51A	1.53A	1.61A	1.55	4.93AB	5.01AB	5.04AB	4.99
Ascorbic A.	132.8A	135.3A	137.5A	135.2	1.53A	1.55A	1.63A	1.57	5.09A	5.18A	5.20A	5.16

Values followed by the same letter in the same column are not significantly different at 0.5% level of probability.

Table 3. Effect of antioxidants spraying on leaf N, P and K% of Flame Seedless grapevines during 2018, 2019 and 2020 seasons.

Treat. / Season	N %				P %				K %			
	2018	2019	2020	Mean	2018	2019	2020	Mean	2018	2019	2020	Mean
Cont.	1.76B	1.82B	1.82B	1.80	0.118B	0.116B	0.118B	0.117	1.11B	1.10B	1.11B	1.11
V. B_{12}	1.90A	1.93A	1.94A	1.92	0.130A	0.129A	0.128A	0.129	1.17A	1.16A	1.18A	1.17
Citric A.	1.92A	1.95A	1.97A	1.95	0.131A	0.128A	0.130A	0.130	1.20A	1.19A	1.20A	1.20
Ascorbic A.	1.94A	1.98A	1.99A	1.97	0.133A	0.130A	0.131A	0.131	1.22A	1.21A	1.23A	1.22

Values followed by the same letter in the same column are not significantly different at 0.5% level of probability.

2- Yield and cluster characteristics:

Data presented in Tables (4 & 5) showed that the effect of spraying with either V. B_{12}, citric acid or ascorbic acid on yield/vine, cluster weight, berry weight, and compactness coefficient of Flame Seedless grapevine in 2018, 2019 and 2020 seasons. Spraying with V. B_{12}, citric acid or ascorbic acid as an antioxidants source significantly increased the yield/vine and cluster weight and decreased compactness coefficient of cluster compared to water-sprayed one. Moreover, sprayed by ascorbic acid gave the heaviest yield/vine, cluster weight and berry weight and least values of compactness coefficient comparing checked treatment. The heaviest yield and cluster weight as well as berry weight and least values of cluster compactness coefficient were detected due to spray with ascorbic acid.

The obtained highest values of yield/vine (12.21 kg), cluster weight (420.3 g), berry weight (2.79 g) and least cluster compactness coefficient (6.10) as an av. the three studied seasons due to spray with ascorbic acid, respectively. No significant differences were recorded due to spray with any antioxidant compared to other. Contrarily, these values on checked vines were (10.12 kg), (371.4 g), (2.48 g) and (6.71), respectively. Hence, the corresponding increment percentages for these traits over check treatment were (20.65, 13.17 and 12.50%) as well as the decrement percentage of cluster compactness coefficient was (9.09%) as an av. the three studied seasons, respectively. No significant differences were recorded due to spray with ascorbic acid, citric acid or V. B_{12}. In general, it could be concluded that using any antioxidants had positive effects on productivity of Flame Seedless grapevines.

3- Chemical constituents of berry juice:

Data of berry characteristics as affected by different antioxidants during 2018, 2019 and 2020 seasons are presented in Tables (5 & 6). The data indicated that spraying with any antioxidant source significantly improved the Flame Seedless grapes quality in terms of increasing total soluble solids, reducing sugars and anthocyanin contents and decreasing total acidity compared to checked treatment. The highest total soluble solids, reducing sugars and anthocyanin contents were (17.3%), (15.49%) and (1.32 mg/100g) as an av. of the three studied seasons, obtained from vines spayed with any ascorbic acid. No significant differences were recorded due to spray with antioxidant compared to others. Contrary, the least values of these traits were recorded on vines that sprayed with water (checked treatment) which gave (15.6%), (14.12%) and (1.16) as an av. of the two studied seasons, respectively. Hence, the increment percentage of these attributers due to spray with ascorbic acid over the check treatment attained (10.89%, 9.70% & 13.79%), respectively. The least values of acidity was recorded on vines that spraying with ascorbic acid was 0.486% compared to 0.585% as an av. three studied seasons on checked vines. Hence, such amending induces decrement percentage in total acidity attained (16.92%) as an av. of the three studied seasons.

Table 4. Effect of antioxidants spraying on yield/vine, cluster weight and cluster compactness coefficient of Flame Seedless grapevines during 2018, 2019 and 2020 seasons.

Treat. / Season	Yield/vine (kg)				Cluster weight (g)				Cluster compactness coefficient			
	2018	2019	2020	Mean	2018	2019	2020	Mean	2018	2019	2020	Mean
Cont.	8.71B	10.65B	11.00B	10.12	362.8B	372.0B	378.3B	371.4	6.72A	6.67A	6.73A	6.71
V. B_{12}	9.66A	12.15A	12.55A	11.45	402.7A	412.0A	418.3A	411.0	6.48B	6.34B	6.40B	6.41
Citric A.	9.76A	12.50A	12.99A	11.75	406.8A	417.0A	421.8A	415.2	6.06C	6.25BC	6.32BC	6.21
Ascorbic A.	9.76A	13.19A	13.67A	12.21	411.2A	422.6A	427.1A	420.3	6.05C	6.10C	6.16C	6.10

Values followed by the same letter in the same column are not significantly different at 0.5% level of probability.

Table 5. Effect of antioxidants spraying on berry weight, TSS and reducing sugar of Flame Seedless grapes during 2018, 2019 and 2020 seasons.

Treat. / Season	Berry weight (g)				TSS				Reducing sugar %			
	2018	2019	2020	Mean	2018	2019	2020	Mean	2018	2019	2020	Mean
Cont.	2.44B	2.50B	2.50B	2.48	15.5B	15.8B	15.5B	15.6	14.10B	13.81B	14.45B	14.12
V. B_{12}	2.66A	2.73A	2.76A	2.72	16.9A	17.1A	16.8A	16.9	15.19A	14.93A	15.38A	15.17
Citric A.	2.70A	2.77A	2.80A	2.76	17.1A	17.3A	17.0A	17.1	15.37A	15.11A	15.55A	15.34
Ascorbic A.	2.73A	2.80A	2.83A	2.79	17.3A	17.5A	17.2A	17.3	15.51A	15.25A	15.70A	15.49

Values followed by the same letter in the same column are not significantly different at 0.5% level of probability.

Table 6. Effect of antioxidants spraying on total acidity and anthocyanin (mg/100 g) of Flame Seedless grapes during 2018, 2019 and 2020 seasons.

Treat. / Season	Total acidity %				Anthocyanin (mg/100 g)			
	2018	2019	2020	Mean	2018	2019	2020	Mean
Cont.	0.612 A	0.553 A	0.589 A	0.585	1.16 B	1.12 B	1.15 B	1.16
V. B_{12}	0.553 B	0.506 B	0.540 B	0.533	1.26 A	1.28 A	1.24 A	1.26
Citric A.	0.526 BC	0.475 BC	0.505 BC	0.502	1.29 A	1.30 A	1.27 A	1.29
Ascorbic A.	0.509 C	0.459 C	0.491 C	0.486	1.31 A	1.34 A	1.30 A	1.32

Values followed by the same letter in the same column are not significantly different at 0.5% level of probability.

On the account of the present results, it could be concluded that spraying vines with ascorbic acid, citric acid or V. B_{12} improved the growth and nutritional status, as well as, yield, cluster attributes and berry quality of Flame Seedless grapevines under the circumstances of this experiment.

Discussion:

Antioxidants play an important role in plant defense against oxidative stress as well as the biosynthesis of most organic foods and activation of cell division process. Ascorbic acid (vitamin c) is known as a growth-regulating factor which influences many biological processes. It acts as a co-enzyme in the enzymatic reactions by which carbohydrates, proteins are metabolizes and involved in photosynthesis and respiration (Robinson, 1973). Ascorbic acid is currently considered to be a regulator on plant growth and development owing to their effects on cell division and differentiation. It is involved in a wide range of important functions as antioxidant defense, photo protection and growth (Blokhina *et al*., 2003).

The positive action of antioxidants in catching or chelating the free radicals which could result in extending the shelf life of plant cells and stimulating growth aspects is reported (Rao *et al.* 2003). In the mean time, ascorbic acid is considered a regulator of plant growth. Also, citric acid plays an essential role in signal transduction system, membrane stability and functions, activating transporter enzymes, metabolism and translocation of carbohydrates (Smirnoff, 1996).

Antioxidants participate in plant growth and development by enhancing of natural hormones such as cytokinins and gibberellins that stimulate growth factors various physiological processes such as nutrient uptake, respiration, photosynthesis, plant pigments as well as protein and hormones biosynthesis depend on availability of antioxidants (Oretili, 1987 and Elade, 1992). Using vitamins is favourable in the biosynthesis of proteins, vitamins antioxidants enzyme and natural hormones as well as controlling diseases. They are responsible for enhancing cell division and building of most organic foods. The positive action of these antioxidants in chelating hazard radicals could results in extending the shelf-life of cells and producing healthy plants. Their positive action on controlling the incidence of pests attack did not neglect (Rao *et al*., 2003).

These results of the present investigation agree with those of Khrenovskov and Kameneva (2001), Ahmed *et al*. (2002), Ahmed and Abd El-Hameed (2004), Nashed (2006), Farahat (2008), Ahmed and Seleem-Basma (2008), Abd El-Kariem (2009), Abada and Abd El-Hameed (2010), Refaai (2011), Ahmed *et al*. (2011), Abdelaal (2012), Abdelaal and Aly (2013), Abada (2014), Ebrahiem (2015), Abd El-Galil (2015), Mohamed-Attiat (2016), Sayed (2017) and Gamea-Marwa (2018). They found from their investigation that sprayed ascorbic acid, citric acid, and selenium were favourable to enhance the growth and fruiting of grapevines.

Conclusion

Therefore, it could be concluded that spraying with ascorbic acid citric acid or vitamin B_{12} at 250 ppm three times, improve the vine nutrient status, yield and fruit quality leading to an increase of the packable yield.

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تأثير رش مضادات الأكسدة علي نمو وإثمار كروم العنب الفليم اللابذري

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الملخص

أجريت هذه الدراسة خلال مواسم ٢٠١٨ و ٢٠١٩ و ٢٠٢٠ علي كروم العنب القليم عديم البذور المنزرعة بالمزرعة البحثية بمحطة البحوث الزراعية بالمطاعنة – اسنا – الأقصر. بهدف دراسة تأثير رش مضادات الأكسدة علي النمو الخضري وحالة العناصر الغذائية والمحصول وخصائص العناقيد والحبات.

وقد أظهرت النتائج:

- أدي رش حمض الاسكوربيك أو الستريك أو فيتامين B_{12} إلي زيادة مؤكدة في وزن خشب التقليم ومساحة الأوراق ومحتوي الأوراق من الكلوروفيل وعناصر النيتروجين والفوسفور والبوتاسيوم مقارنة بعدم رش مضادات الأكسدة (المقارنة).
- سبب رش أي من مضادات الأكسدة تحسين صفات العنقود والثمار مقارنة بالرش بالماء (معاملة المقارنة).
- أظهرت النتائج تفوق رش حمض الأسكوربيك مقارنة باستخدام كل من حمض الستريك أو فيتامين B_{12}.
- لا توجد فروق معنوية بين رش حمض الستريك أو فيتامين B_{12}.

من نتائج هذه الدراسة يمكن التوصية بأهمية رش حمض الاسكوربيك أو حمض الستريك أو فيتامين B_{12} ثلاث مرات بتركيز ٢٥٠ جزء في المليون وذلك لتحسين النمو الخضري والحالة الغذائية لشجيرات العنب صنف فليم عديم البذرة مع إنتاج محصول عال ذو خصائص ثمرية جيدة.