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Effect of Some Antioxidants on Growth, Yield and Bunch Characteristics of Thompson Seedless Grapevine

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Abstract: This study was carried out during two successive seasons at Tanbol region, Wadi EL-Natroon district, Behira Governorate on 5 years old Thompson seedless grapevine cultivar. Three sources of antioxidants, thiamin, ascorbic acid, citric acid and combination between them were used in this study beside control vine. Growth, yield, bunch and berries parameters were significantly affected by foliar application of antioxidant treatments in both seasons. Foliar application of triple combined antioxidants (Thiamin + Ascorbic acid + Citric acid) gave higher shoot length, leaf area, leaf fresh and dry weights, leaf pigments (chlorophylls a and b), total carbohydrates, Indoles, leaf N, P, K contents, yield (kg/vine), bunch weight, bunch length, bunch width, number of berries/bunch, weight and volume of berry, TSS, TSS/Acid ratio and berry total soluble sugars than other antioxidant treatments and control vines in both seasons. The double combined antioxidant treatments gave less stimulatory effect on all parameters, although they were significantly higher than the signal treatment, as well as, foliar application of thiamin either alone or combined with ascorbic acid gave the highest values comparing to foliar application of citric acid treatments in both seasons. On the other side, foliar application of triple combined antioxidant treatments gave the lowest leaf total soluble phenol content, berry firmness and its acidity compared to other antioxidant treatments (double, single or control vine) in both seasons.

Key words: Antioxidants · Thiamin · Ascorbic acid · Citric acid · Thompson seedless · Grapevine

INTRODUCTION

Grapes are the most widely produced fruit in the world. Thompson seedless is one of the most popular grape cultivars in Egypt. The foliar spray practices are widely accepted to improve grape yield and quality all over the world. Antioxidant are designing chemicals, when added in small quantities to a material, react rapidly with the radical intermediates of an auto-oxidation chain and stop it from progressing. Recently, there has been increasing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of variety of chronic disorders [1]. Accordingly attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organism containing them and the mechanisms of their action [2]. Also, plants with high levels of antioxidants, whether constitutive or induced have a greater resistance to such oxidative damage [3].

Antioxidants such as ascorbic acid, citric acid and thiamin have auxinic action and also synergistic effect on flowering and fruiting of fruit trees, recently antioxidants are used instead of auxins and other chemicals for enhancing growth and fruiting of various fruit trees [4]. Also, Maksoud *et al.* [5] found that spraying antioxidants (ascorbic or citric acids) each at 1000 and 2000 ppm alone or combined with bioferilizer enhanced yield and fruit quality of olive trees. Ascorbic acid gave the best yield and bunch quality on Flame seedless grapevine [6].

Tarraf *et al.* [7] and Gamal [8] reported that foliar application of thiamin on lemongrass and sunflower plants increased vegetative growth, total carbohydrates and total nitrogen. Thiamin (vitamin B1) is a necessary ingredient for biosynthesis of the co-enzyme thiamin pyrophosphate; in this latter form it plays an impotent role in carbohydrate metabolism. It is an essential nutrient for plants; it is synthesized in the leaves and in transported to the roots where it controls growth [9].

Blokhina *et al.* [10] stated that ascorbic acid is the most abundant antioxidant that protects plant cells. Ascorbic acid is currently considered a regulator of plant growth and development owing to its effects on cell

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division and differentiation and ascorbic acid is involved in wide range of important functions as antioxidants defense, photo protection and regulation of photosynthesis and growth regulation. Talaat [11] on the sweet pepper detected that foliar application of ascorbic acid increased leaf macronutrients (N, P and K) content.

Therefore, the present investigation aims to study the effect of antioxidants (thiamin, ascorbic acid and citric acid) on growth, yield and bunch characteristics of Thompson seedless grapevine.

MATERIALS AND METHODS

This investigation was carried out during two successive seasons (2006 and 2007) on five years old Thompson seedless grapevine grown under drip irrigation in a commercial vine yard located at Tanbol region, Wadi EL-Natroon district, Behira Governorate. The vines were planted at 2 × 2.5 meters apart (840 vine/feddan) in sandy soil. The tested vines were healthy, nearly uniform in vigor and were pruned in the second week of January in each season at 56 buds/vine (4 fruiting canes x 12 buds plus 4 renewal spurs x 2). All vines received the recommended regular fertilization and other horticulture practices. The tested vines were randomly shared between 8 foliar spray treatments: Ascorbic acid (1000 ppm), Citric acid (1000 ppm) and Thiamin (1000 ppm), in addition to combination between them, beside control treatment (sprayed with water). Each spraying treatment was applied three times on the same vine: the first spraying was applied when the flower clusters reached 8-10 cm in length (at the second week of March); the second spraying was applied at full bloom (at the second week of April); and the third spraying was applied at berry diameter reached about 3-5mm (at the second week of May), as foliar spraying to cover completely the vine foliage. Each treatment was applied on 6 vines shared between 3 replicates. Super film as a wetting agent was added at 0.1% to all spraying solutions. The following parameters were recorded as follows:

Vegetative Growth

Shoot Length: 6 new shoots were randomly chosen per vine and their length was measured at the end of each season.

Leaf Area, Leaf Fresh and Dry Weights and Leaf Chemical Constituents: leaf samples (20 leaves per each replicate of each treatment) were collected from those opposite to the first bunch on each shoot at the first week of July in both seasons. For estimating leaf area using leaf area meter GI-203 AREA METER GID Inc, U.S.A. was used. Moreover, leaf blade samples were taken to determine the content of leaf photosynthetic pigments (chlorophylls a and b, mg/g fresh weight) according to Mckinney [12]. Also, the leaf samples were used to determine the total soluble indols and phenols [13]. The fresh weight of leaf blades was determined and then the samples were thoroughly washed with distilled water and dried at 60°C until constant dry weight, then leaf dry weight was recorded. The blades dry samples of each replicate were finely grinded and following determinations were carried out: Nitrogen (%) with micro-Kjeldahl according to Pregel [14], phosphorus (%) was estimated as described by Chapman and Pratt [15]. While potassium (%) was flame photometrically estimated as described by Brown and Lilleland [16] and total carbohydrates according to AOAC [13].

Yield (Kg/tree) and Fruit Parameters: At harvest time (second week of July), all bunches on the vines were picked. The number of bunches per vine and their total weight, the yield was recorded (kg/vine). The average bunch weight was calculated. Bunch Samples (three bunches) were taken from each replicate and the following parameters were determined: bunch weight (g), length (cm), width (cm) and number of berries/bunch. Also, 100 berries were taken from each replicate and the following berry physical properties were determined: berry weight (g), volume (cm^3) and firmness ($Lb/inch^2$) using pentameter pressure tester. Moreover, the following chemical constituents of berry juice were determined: total soluble solids (TSS%) using a hand refractometer, acidity (%) by titration against 0.1 N sodium hydroxide in presence of phenolphthalein dye, total soluble sugars [13] and TSS/acid ratio was calculated.

Statistical Analysis: The obtained data were tabulated and statistically analyzed according to complete randomized block design [17]. The values of means were compared using LSD methods at 5% level. The percentages were transformed to arcsine to find the binomial percentages according to Steel and Torrie [18].

RESULTS AND DISCUSSION

Vegetative Growth

Shoot Length (cm): Data shown in Table 1 reveal that shoot length was significantly affected by different antioxidant treatments compared to the control vine in

Table 1: Effect of some antioxidant treatments on some vegetative growth parameters of Thompson seedless grapevine during 2006 and 2007 seasons

	Shoot length (cm)		Leaf area (cm^2)		Leaf DW (g)		Leaf FW (g)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	178.83	179.61	167.06	173.19	1.34	1.42	3.71	4.06
Citric acid	175.75	173.67	164.32	166.63	1.32	1.37	3.65	3.91
Thiamin	184.33	189.88	167.92	182.92	1.35	1.50	3.73	4.29
Ascorbic+ citric	212.24	206.50	182.52	197.84	1.49	1.63	4.12	4.64
Ascorbic + thiamin	216.87	222.01	195.51	213.62	1.62	1.76	4.50	5.01
Citric+ thiamin	215.44	209.55	185.45	201.68	1.57	1.66	4.34	4.73
Ascorbic+ citric+ thiamin	223.91	233.68	229.33	235.91	1.75	1.93	5.07	5.51
Control	171.33	166.25	163.83	162.45	1.30	1.34	3.60	3.81
LSD at 5%	3.75	4.52	5.62	7.33	0.06	0.07	0.26	0.28

both seasons. The highest shoot length was obtained with foliar application of triple combined antioxidant treatment (ascorbic acid + citric acid + thiamin), descendingly followed by double combined treatments then single treatments.

The double combined antioxidant treatments gave less stimulatory effect on shoots length, although they were higher than the single treatments. Foliar application of thiamin treatments either alone or combined with ascorbic acid gave the highest value in comparable to foliar application of citric acid treatments either alone or combined with ascorbic acid in both seasons under study.

The present results are in line with those obtained by Gamal [8] on sunflower and Abd El-Aziz *et al.* [19] on *Syngonium*, showed that foliar application of thiamin significantly promoted all growth parameters compared with untreated plants. The effect of antioxidant treatments under study on shoot length may be attributed to the effect of used treatments on cell division and cell elongation [20].

Leaf Area (cm^2) and Leaf Fresh and Dry Weights (g): Table 1 show that leaf area and leaf fresh and dry weights were significantly affected by different treatments in both seasons. The highest leaf area and leaf fresh and dry weights were obtained with foliar application of triple combined treatment (ascorbic acid + citric acid + thiamin) followed in descending order by ascorbic + thiamin, citric + thiamin, ascorbic + citric, thiamin, ascorbic acid, citric acid and control treatment in both studied seasons.

These results are in accordance with those by Tarraf *et al.* [7] on lemongrass plants who reported that application of ascorbic acid promoted the growth of plants. Also,these results are in harmony with those obtained by Youssef and Talaat [21]. They found that thiamin and ascorbic acid significantly promoted vegetative growth of rosemary plant.

Shaddad *et al.* [22] assumed that the effect of ascorbic acid on plant growth might be due to substantial role of ascorbic acid in many metabolic and physiological processes. Also, the increments of leaf area and leaf fresh and dry weights of Thompson seedless grapevine due to thiamin, ascorbic and citric acid treatments may be attributed to the effect of the used treatments on cell division and cell elongation.

Leaf Pigments and Chemical Contents

Leaf Pigments (mg/g FW): Foliar application of antioxidant treatments increased significantly leaf pigments (chlorophylls a and b) content compared with untreated vine (control) in both seasons (Table 2). The highest increase in leaf pigments (chlorophylls a and b) were obtained by foliar application of triple combined antioxidant treatment (ascorbic acid + citric acid + thiamin) compared with other treatments (foliar application of double or single antioxidant treatments).

These results are in agreement with those reported by Blokhina *et al.* [10] who stated that ascorbic acid has a wide range of impotent functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth. Also, these results are in agreement with findings of Smirnoff [23] on the function and metabolisms of ascorbic acid.

Regarding the beneficial effect of antioxidants on photosynthetic pigments, it may be due to its role in increasing the rates of photochemical reduction [24], chloroplast structure, photosynthetic electron transfer as well as photosynthesis [25].

Table 2: Effect of some antioxidant treatments on pigments and some leaf chemical contents of Thompson seedless grapevine during 2006 and 2007 seasons

	Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Total carbohydrates (%)		Total soluble Indoles (mg/g DW)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	0.695	0.706	0.247	0.289	17.55	16.43	5.83	6.85
Citric acid	0.686	0.677	0.238	0.244	15.43	15.50	4.47	4.54
Thiamin	0.724	0.733	0.296	0.308	18.23	17.25	6.57	6.66
Ascorbic+ citric	0.711	0.730	0.287	0.306	19.86	17.75	5.69	5.95
Ascorbic + thiamin	0.753	0.787	0.309	0.316	20.37	20.95	6.71	7.76
Citric+ thiamin	0.731	0.742	0.302	0.311	20.25	18.51	6.65	7.65
Ascorbic+ citric+ thiamin	0.805	0.815	0.316	0.332	23.34	22.15	6.93	7.89
Control	0.666	0.652	0.235	0.222	14.98	14.48	4.33	4.50
LSD at 5%	0.043	0.052	0.021	0.025	2.33	2.52	0.09	0.11

Table 3: Effect of some antioxidant treatments on some leaf chemical contents of Thompson seedless grapevine during 2006 and 2007 seasons

	Total soluble Phenols (mg/g DW)		N (%)		P (%)		K (%)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	0.97	0.99	1.46	1.45	0.29	0.30	1.60	1.66
Citric acid	0.99	1.03	1.46	1.41	0.25	0.33	1.61	1.69
Thiamin	0.90	0.89	1.58	1.54	0.33	0.35	1.69	1.70
Ascorbic+ citric	0.95	0.92	1.60	1.63	0.33	0.33	1.67	1.70
Ascorbic + thiamin	0.88	0.89	1.66	1.68	0.36	0.37	1.73	1.72
Citric+ thiamin	0.88	0.90	1.63	1.67	0.35	0.35	1.73	1.70
Ascorbic+ citric+ thiamin	0.86	0.88	1.86	1.77	0.39	0.44	1.74	1.75
Control	1.06	1.18	1.39	1.36	0.21	0.20	1.55	1.65
LSD at 5%	0.04	0.05	0.12	0.15	0.02	0.04	0.03	0.04

Leaf Total Carbohydrates (%), Total Soluble Indoles and Phenols (mg/g DW) Contents: Leaf total carbohydrates (%) and total soluble indoles contents were significantly increased in response to foliar application of antioxidant treatments (Table 2). Triple combined antioxidant treatment (ascorbic acid + citric acid + thiamin) gave the highest total carbohydrates and total soluble indoles compared to other treatments in both seasons under study. The double combined treatments gave less stimulatory effect on leaf total carbohydrates rate and total soluble indoles, although they were higher than the single treatments. Such increments might be attributed to the significantly increase in photosynthetic pigments content which reflected on photosynthesis process and led to increase in carbohydrates content.

On the other side, control vine gave the highest total soluble phenols value, which followed in a descending order by single treatments, double and triple combined antioxidant treatments in both seasons under study (Table 3).

These results are in agreement with those reported by Maksoud *et al.* [5], Abd El-Aziz *et al.* [19], Youssef and Talaat [21] and Farahat *et al.* [26].

Leaf N, P and K Contents: Data in Table (3) show that leaf N, P and K contents were significantly affected by different antioxidant treatments in both seasons. In general, foliar application of triple combined antioxidant treatments (ascorbic acid + citric acid + thiamin) gave the highest leaf N, P and K contents followed in descending order by foliar application of double, single antioxidant treatments and control vines. Foliar application of thiamin treatment either alone or combined with ascorbic acid gave the highest leaf N, P and K content values compared to foliar application of citric acid either alone or combined with ascorbic acid.

The increment in N concentration due to ascorbic acid and thiamin treatments could be explained by the findings of Talaat [11] who showed that the accumulation of N, P and K by foliar application of ascorbic acid

Table 4: Effect of some antioxidant treatments on yield and some bunch physical characteristics of Thompson seedless grapevine during 2006 and 2007 seasons

	Yield/vine(kg)		Bunch weight (g)		Bunch length (cm)		Bunch Width (cm)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	11.29	11.88	297.11	335.54	21.02	22.41	12.19	12.99
Citric acid	10.61	11.53	279.62	315.15	19.76	21.63	12.04	12.54
Thiamin	11.72	12.65	308.86	352.16	21.65	23.64	12.34	13.71
Ascorbic+ citric	12.91	13.44	333.72	363.83	23.85	25.69	13.59	14.90
Ascorbic + thiamin	13.52	14.10	385.65	382.77	25.85	27.18	14.73	16.03
Citric+ thiamin	13.34	13.92	346.23	370.22	25.07	26.09	14.28	15.13
Ascorbic+ citric+ thiamin	13.84	14.67	392.14	399.41	26.80	27.62	15.00	16.29
Control	9.25	10.33	239.84	278.75	18.99	19.45	11.86	12.20
LSD at 5%	0.29	1.03	9.45	12.94	1.22	0.55	0.52	0.66

Table 5: Effect of some antioxidant treatments on some bunch and berry physical characteristics of Thompson seedless grapevine during 2006 and 2007 seasons

	Number of Berries/bunch		Berry weight (g)		Berry volume (cm^3)		Firmness ($Lb/inch^2$)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	263.12	287.40	1.11	1.15	1.10	1.11	11.41	11.39
Citric acid	254.24	281.00	1.08	1.10	1.06	1.09	11.49	11.42
Thiamin	268.50	290.89	1.12	1.19	1.11	1.12	11.37	11.33
Ascorbic+ citric	292.66	302.39	1.12	1.18	1.10	1.14	11.20	11.26
Ascorbic + thiamin	312.29	311.44	1.19	1.21	1.19	1.18	11.11	11.22
Citric+ thiamin	295.77	307.75	1.15	1.18	1.14	1.15	11.26	11.19
Ascorbic+ citric+ thiamin	316.42	315.45	1.22	1.25	1.20	1.24	11.03	11.11
Control	232.95	249.08	1.03	1.10	1.00	1.08	11.66	11.82
LSD at 5%	2.33	2.31	0.02	0.03	0.02	0.04	N.S.	N.S

may be due to the positive effect of ascorbic acid on root growth which consequently increase nitrate absorption.In this context, the increase in N, P and K concentrations by thiamin, ascorbic and citric acid treatments may be attributed to the postulation of Abd El-Aziz *et al.* [19] and Maksoud *et al.* [5] as they mentioned that foliar spraying with antioxidants may play a role in many metabolic physiological processes.

Yield; Bunch and Berries Parameters: As shown in Tables 4 and 5, yield (kg/vine), bunch weight (g), bunch length (cm), bunch width (cm), number of berries/bunch, berry weight (g), berry volume (cm^3) were significantly affected by different foliar application of antioxidant treatments in both seasons.

In the two seasons, foliar application of triple combined antioxidant treatment (ascorbic acid + citric acid + thiamin) gave the highest yield (kg/vine), bunch and berries physical values followed by foliar application of double combined treatments then single treatments in both seasons. While the lowest values in the two seasons were observed with control vines. Concerning foliar application of single treatment, thiamin gave the best results followed by ascorbic acid then foliar application of citric acid in the two seasons.

Concerning berry firmness ($Lb/inch^2$) there are not significant differences between foliar applications of antioxidant treatments in both seasons, although foliar application of antioxidant triple combined treatment gave the lowest firmness value compared to double, single or control treatments in both seasons.

These results are in parallel with those of El-Sayed *et al.* [6] on Flame seedless and Omar [27] on Red Roomy grapevines, as they pointed out the stimulating effect of antioxidant applications on yield and bunch physical parameters.

Table 6: Effect of some antioxidant treatments on some berry chemical characteristics of Thompson seedless grapevine during 2006 and 2007 seasons

	TSS (%)		Acidity (%)		TSS/acid ratio		Total soluble sugars (%)	
	Seasons		Seasons		Seasons		Seasons	
Treatments	2006	2007	2006	2007	2006	2007	2006	2007
Ascorbic acid	19.53	19.77	0.81	0.78	24.11	25.34	15.39	15.62
Citric acid	19.28	19.51	0.83	0.85	23.22	22.95	15.23	15.45
Thiamin	20.17	20.38	0.77	0.77	26.19	26.46	15.94	16.10
Ascorbic+ citric	20.41	20.73	0.78	0.78	26.16	26.57	16.12	16.41
Ascorbic + thiamin	20.92	21.12	0.76	0.76	27.52	27.78	16.50	16.68
Citric+ thiamin	20.55	20.85	0.76	0.77	27.03	27.07	16.23	16.49
Ascorbic+ citric+ thiamin	21.35	21.70	0.76	0.76	28.09	28.55	16.87	17.35
Control	19.00	19.03	0.87	0.86	21.83	22.12	15.01	15.13
LSD at 5%	0.22	0.43	0.04	0.05	0.58	1.33	0.25	0.47

Berries Chemical Characteristics: Data shown in Table 6 reveal that all studied berries chemical characteristics of Thompson seedless grapevine were affected by foliar application of antioxidant treatments in the two seasons under study. As for the foliar application of antioxidant treatments, application of triple combined antioxidants treatment (ascorbic acid + citric acid + thiamin) resulted in the highest berry TSS, TSS/Acid ratio and total soluble sugars followed in descending order by foliar application of double then single antioxidant treatments in the two seasons under study.

On the other side, control vine gave the highest berry acid value followed by foliar application of single treatments, double treatments then foliar application of triple treatment (ascorbic acid + citric acid + thiamin) in a descending order.

Foliar application of antioxidant treatments (thiamin, ascorbic acid and citric acid, either alone or in combination between them) improved yield and bunch physical and chemical properties, this may be due to the auxinic action of thiamin, ascorbic acid and citric acid on enhancing cell division and cell enlargement, which reflected positively on leaf area [27-29]. Accumulation of dry matter production in bunch and berries can be assumed proportionally to solar radiation intercepted by foliage resulting in more efficiency of photosynthesis process [30]. Therefore, expected increments of carbohydrate supply to berries can explain the improvements in yield, bunch weight, bunch length, bunch width and berries firmness and physical and chemical characteristics in this study.

These results are in agreement with Maksoud *et al.* [5], Abd El-Aziz *et al.* [19], Yossuef and Talaat [21], Farahat *et al.* [26], Omar [27] and Ahmed *et al.* [28, 29]. They stated the same findings.

Finally, using foliar application of antioxidant treatment (ascorbic acid + citric acid + thiamin) gave the best effect on growth, yield and bunch characteristics of Thompson seedless grapevine under the same farm conditions.

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