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Effect of Chelated Fe, Zn and Mn Soil application with Spraying GA3 and Ascorbic Acid on Growth, Yield and Fruit Quality of Flame Seedless Grapevines under Calcareous Soil Conditions

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Abstract: The present study was conducted during two successive seasons to study the effect of soil application of chelated Fe, Zn and Mn as well as foliar spray of GA_3 and ascorbic acid on improving the vegetative growth and productivity of Flame seedless grapes under calcareous soil conditions. Mixture chelated Fe, Zn and Mn were applied to soil at 100,150 and 200 ppm in the beginning of opening bud stage and repeated 2 times - one month intervals alone or in combination with spraying ascorbic acid 500 ppm each 15 days intervals after berry set, 4 times to compare with the control (water only) and to form 7 treatments for this study. Moreover, GA_3 was used for all treatments except the control to elongate cluster (20 ppm when cluster length reached 8-10 cm) and to reduce excessive fruit set (5 ppm at full bloom) as well as to improve berry size (2 times at 30 ppm when berry reached 6 - 8 mm and after one week from the first spray). The result showed that all treatments improved the vegetative growth (leaf area, chlorophyll and mineral content) as well as yield and fruit quality than the control. Moreover, the yield (kg/vine) was increased as a result to improve cluster weight due to increase berry weight and cluster dimension. Furthermore, all treatments improved also the fruit quality such as TSS, TSS/acid ratio and anthocyanin than the control. However, the treatments decreased the acidity and berry firmness comparing with the control.

Generally, the treatment of soil application of chelated Fe, Zn and Mn at 200 ppm combined with spraying GA_3 and ascorbic acid is recommended to improve growth, yield and fruit quality of Flame seedless grapevines under calcareous soil conditions.

Keywords: micronutrients; ascorbic acid; calcareous soil; Flame seedless; grapes.

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Introduction

Grapes are considered as one of the most important fruits in worldwide.The harvested area in Egypt reached approximately 66262 ha which produced about 1378815 tons[1] . 'Flame seedless' is a popular table grape cultivar that recently introduced in Egypt and consider as a promising variety because its qualities for local market and export[2]. It grown successfully under Egypt environmental conditions and has progressively developed in the last few years. Vast acreage of Flame seedless grapevine is being cultivated in the newly reclaimed areas along the desert roads in North and Middle Egypt[3].

On the other hand, Flame seedless grapevines are suffering under new reclaimed semi-arid areas from many problems, especially under calcareous soil conditions such as a lack of nutrients and organic matter in soil as well as high soil pH. The high soil pH effect on soil nutrient availability and chemical reactions and caused the loss or fixation of almost all nutrients, where, Fe, Zn and Mn deficiencies are common under these soil conditions which has a free calcium carbonate (Ca CO_3) in the profile more than 8 %. The carbonates, due to their relatively high solubility, reactivity and alkaline character, buffer the pH of most calcareous soils within the range of 7.5 to 8.5. Therefore, Fe, Zn and Mn deficiencies can be corrected through foliar and soil application of chelates[4].

Concerning soil application of microelements under calcareous soil conditions, until now little studies are found, however, the soil application with the chelated Fe, Zn and Mn to the vines at bud opening stage can be used compared with foliar application which conducts later on full expended leaves.

Furthermore, excessive fruit set can reduce the quality of 'Flame seedless' table grapes, resulting in compact, tightly filled clusters with small berries that are prone to bunch rot at harvest. Bloom sprays for 'Flame seedless' grapes increase berry weight but have little effect on berry shape. Therefore, spraying GA_3 at different concentrations on seedless grape cultivars once ortwice at full bloom or berry set increased cluster weight, berry weight and the yield per vine[5,6,7] .

Moreover, Flame seedless grapevines growth is affected by adverse environmental under the calcareous soils in semi-arid areas. Therefore, antioxidant compounds like ascorbic acid (vitamin C) have great benefits on grapevines growth under these conditions. In this respect, antioxidant have auxinic action, since they have synergistic effect on growth and productivity of most fruit trees[8,9] . In addition, antioxidants play a definite role in protecting the plant cells from the free radicals which are producing during plant metabolism and caused oxidation of lipids and the components of plasma membrane that is accompanied with the loss of permeability of cells[10,11,12]. Furthermore, ascorbic acid spraying accelerate berry color changes, grape maturation and improve berry color at harvest[8,13,14] .

The present study aim to improve growth, yield and fruit quality of Flame seedless grapevines under calcareous soil conditions by reach to three goals: (1) Correct Fe, Zn and Mn deficiencies with soil application of chelates. (2) Increase cluster weight by foliar application of GA_3 (3 applications: to elongate cluster, reduce fruit set and improve berry size). (3) Enhance color formation of Flame seedless grapes using safe compound, low cost source and organic antioxidant such as ascorbic acid and to protect also the grapevines from adverse environmental conditions which effect on growth and productivity.

Experimental

Plant materials and vineyard site: The present study was conducted during two successive growing seasons of 2013 and 2014 on nine years old own-rooted 'Flame seedless' grapevines (*Vitis vinifera* L.) grown in a commercial vineyard located near Nubaria city at Cairo-Alexandria Desert Road (80 Km before Alexandria city), Egypt. The vines were planted in a calcareous soil (Table, 1) under drip irrigation system, spaced at 1.5 x 3 m, trained to quadrilateral cordon, spur pruned and supported by Gable trellis.

Table (1): Physical and chemical properties of the experimental soil.

Particle size distribution (%)			Texture	Ca CO_3 (%)	EC (1:1) dSm^{-1}	pH	Available nutrients							
							Cations (meq/L)				Anions (meq/L)			
Sand	Silt	Clay					K^+	Na^+	Mg^{++}	Ca^{++}	SO_4^{--}	Cl^-	HCO_3^-	CO^{--}
74.56	15.31	10.13	Sandy loam	12 %	2.00	7.58	0.78	4.0	7.6	10	17.74	2.0	2.5	0

Treatments and experimental design: The vines were treated with seven treatments as follows:

T1 = Mixer chelates Fe+Zn+Mn 100 ppm.
T2 = Mixer chelates Fe+Zn+Mn 150 ppm.

T3 = Mixer chelates Fe+Zn+Mn 200 ppm.
T4 = T1 + 500 ppm ascorbic acid.
T5 = T2 + 500 ppm ascorbic acid.
T6 = T3 + 500 ppm ascorbic acid.
T7 = Water only (control).

Where:

a) Mixer of chelates with ratio (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% was added to drip irrigation system in the beginning of opening bud stage and repeated 2 times at monthly intervals. While, EDTA is ethylene diamine tetraacetic acid and EDDHA is ethylene diamine di hydroxyphenyl acetate.
b) Ascorbic acid was conducted as foliar application at 500 ppm, 4 times after berry set each 15 days intervals as follows: (1) 15 days after berry set. (2) When berry size in pea stage (6-8 mm). (3) At veraison stage when approximately 10 % of the berries on 50 % of the clusters had softened and red color. (4) 15 days fter veraison.
c) Experimental vines except the control (T7) were treated with GA_3 during 4 applications[5]: (1) When clusters length reach about 8 -10 cm with spraying GA_3 at 15 ppm to elongate the cluster. (2) At full bloom, with spraying GA_3 at 5 ppm for flower thinning to reduce excessive fruit set. (3) When berry size reached about 6-8 mm, with spraying GA_3 at 30 ppm, 2 times one week interval to improve berry size. In addition, surfactant agent at 40 cm/100 L water was added to all foliar treatments in order to obtain best penetration results.

The vines of this experiment were arranged in randomized complete block design (RCBD) and each treatment was done with three replicates (1 replicate = 3 vines).

Growth vigor parameters and leaf mineral content:

1. Leaf area (cm^2): three leaves were taken from the middle of growing shoots after reach to maximum expended mature leaf and measured by the automatic area meter LI-3000 (supplied by LI-COR GmbH, 61352 Bad Homburg, Germany).
2. Leaf content of chlorophyll: fifty leaves were collected on 1*st* May in both seasons and chlorophylls (a) and (b) were determined by spectrophotomer at 660, 640 mm wavelength for chlorophyll (a) and (b), respectively[15].
3. Leaf mineral content: were determined in dry leaf samples which collected at the harvest of each season (first week of June) and were taken from the sixth node from the base of shoots. Nitrogen (%) was measured by Micro-Kjeldahl[16]. Also, phosphorus (%) was determined as described by Murphy and Riely[17]. While, potassium (%) was measured according to Brown and Lilleland[18]. Fe, Zn and Mn contents were estimated by atomic absorption spectrophotometer (2-8200 Series, Hitachi, Japan) using specific lamp for specific nutrient.

Yield and cluster characteristics:

At harvest date (1*st* week of June) in both seasons, clusters per each vine were collected and weighed to determine the yield (kg/vine) and samples of 4 clusters were randomly taken from each vine to estimate the following cluster characteristics: (1) Cluster weight (g). (2) Cluster dimensions; length and width (cm). (3) No. of berries per cluster. (4) Cluster compactness (No. of berries per cluster/length).

Fruit quality:

In both seasons, a random sample of 100 berries per each replication was collected to determine: (a) Fruit physical properties: (1) Berry dimensions; length and width (cm). (2) Weight of 100 berries (g). (3) Berry firmness ($Ib/inch^2$). (b) Fruit chemical properties: (1) T.S.S % using hand refractometer . (2) Titratable acidity as tartaric acid equivalent was determined by titration with 0.1 N Na OH[19]. (3) T.S.S/ acid ratio. (4) Total anthocyanin (mg cyanidin / 100 g FW)[20] .

Statistical analysis:

Data were analyzed by analysis of variance (ANOVA), and means were compared using Duncan's test at $p < 0.05$ to determine the significance of differences between the conducted treatments[21].

Results and Discussions:

Results in Table (2) present the effect of trace elements, GA_3 and ascorbic acid treatments on growth parameters (leaf area and total chlorophyll) as well as mineral nutrient status of NPK of 'Flame seedless' grapevines under calcareous soil conditions during two succesive seasons. It's cleared from results that all micronutrients, GA_3 and ascorbic acid treatments increased leaf area (ranged from 66.5 to 77.1 cm^2 in 1^{st} season and from 71.3 to 82.8 cm^2 in the 2^{ed} season) compared with the control (45.9 and 48.5 cm^2 in both seasons).

Table (2): Effect of micronutrients, GA_3 and ascorbic acid on growth parameters and NPK mineral status of Flame seedless grapevines under calcareous soil conditions.

Treatments	Leaf area (cm^2)		Total chlorophyll (mg/100g FW)		N (%)		P (%)		(K) %	
	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season
T1	66.5 a	71.3 a	40.3 c	47.5 c	2.11 d	2.16 d	0.33 d	0.31 d	1.90 d	1.97 d
T2	69.2 a	74.1 a	41.1 c	43.8 c	2.14 cd	2.19 cd	0.34 cd	0.33 cd	1.93 cd	1.99 cd
T3	67.4 a	76.0 a	41.5 c	49.4 c	2.20 cd	2.24 cd	0.34 cd	0.35 cd	1.98 cd	2.03 cd
T4	70.9 a	72.2 a	43.8 bc	52.3 bc	2.29 bc	2.35 bc	0.36 bc	0.36 bc	2.07 bc	2.14 bc
T5	73.0 a	78.3 a	46.0 ab	54.6 ab	2.38 ab	2.44 ab	0.37 ab	0.38 ab	2.15 ab	2.23 ab
T6	77.1 a	82.8 a	48.6 a	58.2 a	2.51 a	2.57 a	0.39 a	0.41 a	2.26 a	2.34 a
T7	45.9 b	48.5 b	38.7 d	40.1 d	1.88 e	1.91 e	0.27 e	0.25 e	1.68 e	1.74 e

T1= chelated Fe+Zn+Mn 100 ppm*. **T2**= chelated Fe+Zn+Mn 150 ppm. **T3**= chelated Fe+Zn+Mn 200 ppm. **T4**= T1+ AA 500 ppm** **T5**= T2+ AA 500 ppm **T6**= T3+ AA 500 ppm. **T7**= Control***.
*Chelates of Fe+Zn+ Mn (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% as soil application.
**AA= ascorbic acid was sprayed 4 times (after berry set each 15 day intervals). ***Experimental vines except the control (T7) were treated with GA_3.
Means within a column followed by different letter (s) are statistically different at 5 % level.

Regarding to total chlorophyll, all micronutrients, GA_3 and ascorbic acid treatments raised it than the control during two years. On the other side, there were significant differences between treatments in leaf content of chlorophyll, where, T5 and T6 had higher content of chlorophyll (46.0 and 48.6 mg/100g FW in the first season, 54.6 and 58.2 mg/100g FW in the second season) than T1, T2, and T3 (40.3, 41.1 and 41.5 mg/100g FW in 1^{st} year, 47.5, 43.8 and 49.4 mg/100g FW in 2^{ed} year, consequently). Meanwhile, the lowest value in leaf content of chlorophyll recorded by control (38.7 and 40.1 mg/100g FW in both seasons).

The effect of soil application of Zn, Fe and Mn treatments at different doses alone or in combination with spraying GA_3 and ascorbic acid increasing growth parameters (leaf area and total chlorophyll) could be attributed to enhance the formation and transportation of indol acetic acid (IAA) as well as stimulating cell division and the biosynthesis of carbohydrates[6] . In this respect, ascorbic acid (vitamin C) have great benefits on grapevines growth under calcareous soil conditions because its auxinic action which considered synergistic effect on growth and productivity[9,22] . This means that the increase in leaf area may be ascribed to the hormonal action of micronutrients, GA_3 and ascorbic acid which increased the endogenous hormonal level of treated grapevines [23] . This positive effect on the growth parameters are in agreement with other study reported that spraying Thompson seedless grapevines with Fe, Zn and Mn in chelated form at 0.05 % improved their growth parameters[24] . Similar results were noticed when Flame seedless grapevines treated four times with spraying Zn plus Fe in chelated form at 0.05 % [25] . Moreovere, single or combined foliar application of three micronutrients (Zn, Fe and Mn) in chelated form at 0.05 % were very effective in improving growth characters such as leaf area of Red Roomy grapevines[26] . Similar findings were found by foliar application using microelements, GA_3 and ascorbic acid on white banaty seedless grapevines[6] as well spraying extract of micronutrients and vitamins on ‘Perlette’ grapes[23].

Concerning the effect of micronutrients, GA_3 and ascorbic acid treatments on NPK mineral status of Flame seedless grapevines under calcareous soil conditions during two seasons, all treatments recorded high concentration of N, P and K than the control. As for N leaf content, T6 had high value (2.51 and 2.57 % in both seasons) followed by T5 (2.38 and 2.44%) and T4 (2.29 and 2.35%), then T1, T2 and T3 came in the next order (ranged from 2.11 to 2.20% in the 1^{st} year and 2.16 to 2.36% in the 2^{ed} year), whereas, the control was the lowest N leaf concentration (1.88 and 1.91%).

Regarding to P leaf content, T6 achieved the highest value (39 and 0.41% in both years), then came T5 (0.37 and 0.38%) and T4 (0.36%), where, T1, T2 and T3 took next place (reached from 0.33 to 0.34% in the 1^{st} season and 0.31 to 0.35% in the 2^{ed} season),while, the control was lowest value (0.27 and 0.25%).

As for K leaf content, T6 achieved high concentration (2.26 and 2.34%) as well as T5 (2.15 and 2.23%), followed by T4 (2.07 and 2.14%), then T1, T2 and T3 (in the range from 1.90 to 1.98% in first season and from 1.97 to 2.03% in the second season). The control vine had lowest value of K% (1.68 and 1.91%). Generally, the increment of N, P and K which was occurred by micronutrients, GA_3 and ascorbic acid treatments has the same trend. In this respect, T6 was more effective treatment in improving NPK mineral status.

The differences in NPK uptake could be explained to micronutrients, GA_3 and ascorbic acid treatments effect which gives different absorption capability or tendency for some specific minerals[3]. The present results are in harmony with those obtained by Gobara and Khiamy on Flame seedless[25,27].; Abd El- Hady and Ebrahim-Alia as well as Madian on Red Roomy grapes[28,29] and Mahran on White Banaty grapevines[30] .

Table (3): Effect of micronutrients, GA_3 and ascorbic acid on Fe, Zn and Mn mineral status of Flame seedless grapevines under calcareous soil conditions.

Treatments	Fe (ppm)		Zn (ppm)		Mn (ppm)	
	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season	1^{st} season	2^{ed} season
T1	122 e	129 e	26 c	28 c	75 c	77 c
T2	125 de	132 de	29 b	30 b	76 c	79 c
T3	131 cd	138 cd	30 b	32 b	83 b	85 b
T4	135 bc	141 bc	33 a	36 a	85 b	88 b
T5	139 b	145 b	35 a	37 a	89 ab	90 ab
T6	148 a	155 a	36 a	39 a	93 a	96 a
T7	92 f	96 f	19 d	20 d	61 d	63 d

T1= chelated Fe+Zn+Mn 100 ppm*. **T2**= chelated Fe+Zn+Mn 150 ppm. **T3**= chelated Fe+Zn+Mn 200 ppm. **T4**= T1+ AA 500 ppm** **T5**= T2+ AA 500 ppm **T6**= T3+ AA 500 ppm. **T7**= Control***.
*Chelates of Fe+Zn+ Mn (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% as soil application.
**AA= ascorbic acid was sprayed 4 times (after berry set each 15 day intervals). ***Experimental vines except the control (T7) were treated with GA_3.
Means within a column followed by different letter (s) are statistically different at 5 % level.

Table (3) shows the effect of micronutrients, GA_3 and ascorbic acid on Fe, Zn and Mn mineral status of Flame Seedless grapevines under calcareous soil conditions. All treatments had great influence on Fe, Zn and Mn leaf content compared with the control. Regarding to Fe leaf content, T6 achieved high content (148 and 155 mg/kg in both years) and in the second order came T4and T5 (135 and 139 mg/kg in the 1^{st} year and 141 and 145 mg/kg in the 2^{ed} year, respectively), followed by T1,T2 and T3 (ranged from 122 to 131 mg/kg in the 1^{st} season and from 129 to 138 mg/kg in the 2^{ed} season) compared with the control (92 and 95 mg/kg in both seasons).

Concerning Zn leaf content, the treatments effect significantly on its concentration compared to control. The treatments of micronutrients combined with GA_3 and ascorbic acid (T4, T5 and T6) had great impact on Zn leaf content (33, 35 and 36 mg/kg, 36, 37 and 39 mg/kg in the two years, respectively), however, treatment of medium and high microelement alone (T2 and T3) took second place (29 and 30 mg/kg in 1^{st} year and 30 and 32 mg/kg in 2^{ed} year), followed by treatment of low microelement alone (T1) that recorded 26 and 28 mg/kg in both years. While, the control had lowest effect on Zn leaf content (19 and 20 mg/kg in both seasons).

As for Mn leaf concentration, the vines responded significantly to all treatments in compare to control. T5 and T6 had great effect on Mn leaf content (89 and 93 mg/kg, 90 and 96 mg/kg in the two seasons, consequently), followed by T3 and T4 (83and 85 mg/kg in 1^{st} season, 85 and 88 mg/kg in 2^{ed} season), however, T1and T2 came in third order (75 and 76 mg/kg in 1st season season, 77 and 79 mg/kg in 2ed season). Whereas, the control was lowest impact on Mn leaf content (61 and 63 mg/kg in both seasons).

In general, T6 was more effective treatment that improve status of Fe, Zn and Mn together through all micronutrients, GA_3 and ascorbic acid treatments.

It is clear that leaf mineral status of Fe, Zn and Mn was improved by soil application of chelated microelements due to facing the negative influence of high soil pH under calcareous soil conditions that effect on soil nutrient availability and chemical reactions and caused the loss or fixation of almost all nutrients, where, Fe, Zn and Mn deficiencies are common under these soil conditions as a result to a free calcium carbonate (Ca CO_3) in the profile more than 8% [4]. Therefore, soil application of chelates in this experiment is effective and can be corrected Fe, Zn and Mn deficiencies reflected on improving growth parameters and mineral status which raised consequently yield & cluster characteristics as well as berry physical and chemical properties.

Moreover, soil application of chelates supplies microelements to the vine starting from bud opening stage compared with foliar spray of chelates which conducts when the leaves take its full size, and become mature, therefore, the foliar application supplies microelements late than the soil application that effect finally on yield and fruit quality under calcareous soil conditions. From the results, GA_3 and ascorbic acid have great benefits on improving nutrient statue of grapevines grown under calcareous soil conditions because their auxinic action that has synergistic effect on growth and productivity[9,22]. On the other side, Norrie *et al* . reported that foliar application of microelement extract had no effect on mineral contents of grapevine leaves[31] .

Table (4): Effect of micronutrients, GA_3 and ascorbic acid on yield and cluster characteristics of Flame seedless grapevines under calcareous soil conditions.

Treatments	Yield (kg/vine)		Cluster weight (g)		Cluster length (cm)		Cluster diameter (cm)		Cluster compactness (No. of berries/ cluster length)	
	1st season	2ed season	1st season	2ed season	1st season	2ed season	1st season	2ed season	1st season	2ed season
T1	11.9 c	13.7 c	625 b	614 b	20.3 a	21.0 a	14.2 d	14.8 de	9.09 b	9.13 b
T2	12.3 c	14.9 c	633 ab	637 b	21.0 a	22.2 a	14.5 cd	15.5 cd	8.72 b	9.24 b
T3	13.4 c	15.5 c	647 ab	641 b	20.7 a	21.2 a	14.6 cd	15.5 cd	8.74 b	9.11 b
T4	14.0 bc	17.9 bc	670 ab	678 ab	21.1 a	21.9 a	15.8 bc	16.7 bc	8.59 b	9.14 b
T5	15.4 ab	19.3 ab	705 ab	686 ab	21.2 a	22.1 a	16.4 b	17.3 b	8.80 b	8.84 b
T6	16.8 a	21.4 a	718 a	730 a	21.8 a	23.2 a	17.7 a	18.6 a	8.31 b	8.29 b
T7	8.6 d	10.8 d	391 c	368 c	16.6 b	17.3 b	13.1 e	13.8 e	12.19 a	13.26 a

T1= chelated Fe+Zn+Mn 100 ppm*. **T2**= chelated Fe+Zn+Mn 150 ppm. **T3**= chelated Fe+Zn+Mn 200 ppm. **T4**= T1+ AA 500 ppm** **T5**= T2+ AA 500 ppm **T6**= T3+ AA 500 ppm. **T7**= Control***.
*Chelates of Fe+Zn+ Mn (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% as soil application.
**AA= ascorbic acid was sprayed 4 times (after berry set each 15 day intervals). ***Experimental vines except the control (T7) were treated with GA_3.
Means within a column followed by different letter (s) are statistically different at 5 % level.

Data in Table 4 show the effect of micronutrients, GA_3 and ascorbic acid treatments on the yield (kg/vine) and cluster characteristics (cluster weight, length , diameter and compactness) of Flame seedless grapevines under calcareous soil conditions during the two seasons of this study. The results clear that all treatments increased the yield than the control in the both seasons. In this respect, the highest yield (16.8 and 21.4 kg/vine in the two seasons, respectively) was achieved by the treatment of high micronutrients concentration (200 ppm) combined with GA_3 and ascorbic acid (T6) followed by the low and medium concentration of microelements (100 and150 ppm) combined with GA_3 & ascorbic acid (T4 and T5) which recorded 14.0 and 15.4 kg/vine in the first year as well as 17.9 and 19.3 kg/vine in the second year, consequently. Meanwhile, the treatments of micronutrients 100,150 and 200 ppm alone (T1, T2 and T3) came in the third order and gave 11.9, 12.3, 13.4 & 14.0 kg/vine in the first season, 13.7, 14.9, 15.5 and 17,9 kg/vine in the second season, respectively. While, the control was the lowest yield (8.6 and 10.8 kg/vine).

Concerning the cluster weight, all treatments improved it than the control. The high concentration of microelements with GA_3 and ascorbic acid (T6) recorded high cluster weight in the 1[St] and 2[ed] year (718 and 730 g, respectively) and the other treatments took the second place (from 625 to 705 g and 614 to 686 g in the two seasons, consequently). Whereas, the control was the lowest value in the two years (391 and 368 g, respectively).

Regarding to cluster dimension, all application raised the cluster length compared with the control during the two seasons. It reached by treatments 20,3 to 21.8 cm and 21.0 to 23.2 in first season and the second season, respectively. While, the control was 16.6 and 17.3 cm in the two seasons, respectively. However, the cluster diameter effected by each treatment. The treatment of high micronutrients concentration (200 ppm) combined with GA_3 and ascorbic acid (T6) achieved high cluster diameter (17.7 and 18.6 cm for both seasons) followed by the low and medium concentration of microelements (100 and 150 ppm) combined with GA_3 and ascorbic acid (T4 and T5) which have 15.8 and 16.4 cm in the 1[St] year and 16.7 and 17.3 cm in 2[ed] year. Moreover, the treatments of micronutrients 100, 150 and 200 ppm alone (T1, T2 and T3) recorded the third place and were 14.2 to 14.6 cm and 14.8 to 15.5 cm in both years, consequently. While the control has the smallest cluster diameter during the two seasons (13.1 and 13.8 cm).

Concerning the cluster compactness, result show that the control has excessive compact cluster (12.9 and 13.26 berries/cm in both seasons) compared with all treatment that have 8.31 to 9.09 and 8.29 to 9.13 berries/cm in both years.

Generally, the results of yield (kg/vine) and cluster characteristics (cluster weight, length , diameter) could be cleared the response of grapevines under calcareous soil conditions to micronutrients, GA_3 and ascorbic acid treatments that improved vegetative growth and nutritional status of grapevines and as a result reflected on increasing cluster characteristics (cluster weight, length , diameter) and yield/vine. Furthermore, the soil application of microelements combined with spraying ascorbic acid had significant variation on the weight of cluster and the yield/vine due to the auxinic action of ascorbic acid. In this respect, ascorbic acid have great benefits on grapevines growth under calcareous soil conditions because its auxinic action which considered synergistic effect on growth and productivity[9,22]. In addition, GA_3 had great effect of on cluster weight and yield due to its role in enhancing berry weight and dimensions. All of these factors together could explained the present results. The results are in accordance with other studies on White Banaty seedless grapes[6,32] , on Thompson seedless [24,33,34,35] , on Delight grapevines[34] , on Roumi Red and Gharibi grapevines grown in calcareous soil[35] , on Crimson seedless[5] and on Flame seedless grapevine[7,36] .

Table (5): Effect of Micronutrients, GA_3 and ascorbic acid on berry physical properties of Flame seedless grapevines under calcareous soil conditions.

Treatments	Berry length (cm)		Berry diameter (cm)		weight of 100 berries (gm)		No. of berry per cluster		Flesh firmness (Ib/inch²)	
	1st season	2ed season	1st season	2ed season	1st season	2ed season	1st season	2ed season	1st season	2ed season
T1	2.29 b	2.40 b	2.52 b	2.59 b	329 a	312 b	176 b	190 b	4.59 b	5.34 b
T2	2.32 b	2.45 b	2.55 b	2.65 ab	347 a	319 b	182 b	194 b	3.41 c	4.27 c
T3	2.35 b	2.48 b	2.57 b	2.67 ab	342 a	325 b	178 b	192 b	4.20 bc	4.31 c
T4	2.47 ab	2.51 b	2.71 ab	2.70 ab	366 a	331 ab	190 b	198 b	3.91 c	4.15 c
T5	2.50 ab	2.56 ab	2.75 ab	2.66 ab	370 a	343 ab	187 b	195 b	3.96 c	4.11 c
T6	2.57 a	2.69 a	2.82 a	2.80 a	391 a	374 a	180 b	191 b	3.20 d	3.26 d
T7	1.82 c	1.90 c	2.00 c	1.97 c	157 b	140 c	235 a	246 a	6.68 a	6.91 a

T1= chelated Fe+Zn+Mn 100 ppm*. **T2**= chelated Fe+Zn+Mn 150 ppm. **T3**= chelated Fe+Zn+Mn 200 ppm. **T4**= T1+ AA 500 ppm** **T5**= T2+ AA 500 ppm **T6**= T3+ AA 500 ppm. **T7**= Control***.
*Chelates of Fe+Zn+ Mn (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% as soil application.
**AA= ascorbic acid was sprayed 4 times (after berry set each 15 day intervals). ***Experimental vines except the control (T7) were treated with GA_3.
Means within a column followed by different letter (s) are statistically different at 5 % level.

It can be seen from Table (5) the effect of micronutrients, GA_3 and ascorbic acid treatments on the berry physical properties (berry dimensions, weight of 100 berries and number of berries per cluster) of 'Flame seedless' grapevines under calcareous soil conditions during the two studied seasons. It's clear from the obtained results that all treatments increased berry length compare to the control. In addition, the treatment of high micronutrients concentration (200 ppm) combined with GA_3 and ascorbic acid (T6) has bigger berry length (2.57 and 2.69 cm in two years) than the treatments of micronutrients 100,150 and 200 ppm alone (T1, T2 and T3) which were 2.29, 2.32 and 2.35 cm in the first year, 2.40, 2.45 and 2.48 cm in the second year, respectively. On the other side, the control produced a smallest berry length (1.82 and 1.90 cm in the two seasons, respectively).

The same trend was observed in response of berry diameter to treatments comparing with the control, T6 recorded high berry diameter (2.82 and 2.80 cm in both studied seasons) compared to T1, T2 and T3 (2.52, 2.52 and 2.57 cm in 1^{st} year, 2.59, 2.65 and 2.67 cm in 2^{ed} year, consequently). While, control was the smallest berry diameter (2.00 and 1.97 cm in the two seasons, consequently).

Concerning the effect of treatments on weight of 100 berries, results were in parallel with berry dimension (berry length and diameter). T6 gave heavier weight of 100 berries (391 and 374 g in the both studied seasons) comparing with T1, T2 and T3 (329, 347 and 342 g in 1^{st} year, 312, 319 and 325 in 2^{ed} year). Whereas, control produced smallest weight of 100 berries (157 and 140 g in the two seasons). Furthermore, results point out that all treatments had a positive effect on reducing number of berries per cluster (176 to 190 in the first year and 190 to 198 in the second year) than the control (235 and 246 in both seasons).

Regarding to flesh firmness, results show that control produced firmer berry (6.68 and 6.91 Ib/inch^2 in both years) than all treatments which varied significantly in this parameter. Where, low micronutrients 100 ppm alone (T1) came in the second order (4.59 and 5.34 Ib/inch^2 in two seasons) followed by T3, T2, T4 and T5 (ranged from 3.41 to 4.20 Ib/inch^2 in 1^{st} year and 4.11 to 4.31 Ib/inch^2 in 2^{ed} year). on the other side, the treatment of high micronutrients 200 ppm combined with GA_3 and ascorbic acid (T6) recorded the lowest flesh firmness (3.20 and 3.26 Ib/inch^2 in 2 years).

In General, the results of berry physical properties (berry dimensions, weight of 100 berries and number of berries per cluster) could be illustrate the positive effect of micronutrients on these parameters as a result to promote the cell division and biosynthesis of carbohydrates[37].

Furthermore, the increasing of cluster weight in response to the application of ascorbic acid might be attributed to its positive action on berry weight and dimensions. Moreover, GA_3 plays an important role in stimulating cell elongation process that enhance berry weight and dimensions. All these effects could explained the present results.

The current results are in agreement with those conducted on Thompson seedless grapes[24, 35] , on Roumi Red and Gharibi grapevines grown in calcareous soil[35], and on 'Perlette' grapevines [6,23] which reported that foliar application of chelated iron, zinc and manganese or extracts included micronutrients increased berry dimensions and weight due to improve the nutritional status of vines. Furthermore, Wassel *et al.,* mentioned that there was auxinic action for ascorbic acid on increasing berry weight and dimensions thus enhanced cluster weight and using ascorbic acid at 500 ppm did not differ statistically than the effect of 1000 ppm in this concern[6] . Meanwhile, Qadir *et al.*, mentioned that the cluster weight and berry weight of 'Flame seedless' grapes were significantly increased after the vines sprayed with GA_3 at bloom and fruit setting[36] . Moreover, spraying GA_3 on the other seedless grape cultivars at full bloom increased cluster weight, berry weight and the yield per vine [5,6,33,34] , since the application of gibberellic acid (GA_3) during bloom reduces the excessive fruit set of seedless table grape cultivars that improve berry dimensions and weight.

Data illustrated in Table (6) showed the effect of Micronutrients, GA_3 and ascorbic acid on berry chemical properties [total soluble solids % (T.S.S), acidity %, T.S.S/acid ratio and total anthocyanin] of Flame Seedless grapevines under calcareous soil conditions during the two seasons. All treatments significantly increased T.S.S in comparison with the control during the two seasons. There were significant differences in T.S.S % between treatments, whereas, the highest T.S.S % was achieved by T6 (21.5 and 21,7% in both seasons) followed by T2, T4 and T5 (ranged from 19.3 to 20.1 % in 1^{st} season and from 19.2 to 20.6 % in 2^{ed} season) then came T3 in the third order (16.9 and 18.5 % in both seasons). The last order was the control (14.9 and 15.2% in the two years).

Table (6): Effect of Micronutrients, GA_3 and ascorbic acid on berry chemical properties of Flame Seedless grapevines under calcareous soil conditions.

Treatments	T.S.S %		Acidity %		T.S.S / acid ratio		Anthocyanin (mg/100 g FW)	
	1st season	2ed season	1st season	2ed season	1st season	2ed season	1st season	2ed season
T1	19.3 bc	19.0 bc	0.49 b	0.50 b	39.4 b	38.0 b	37.49 c	40.13 c
T2	19.8 ab	20.6 ab	0.45 c	0.45 c	44.0 b	45.7 b	42.71 bc	40.20 c
T3	16.9 c	18.5 c	0.48 b	0.49 b	35.2 b	37.8 b	40.21 bc	41.48 bc
T4	20.1 ab	20.4 ab	0.49 b	0.48 b	41.0 b	42.5 b	44.31 ab	42.54 ab
T5	19.3 ab	19.2 ab	0.31 e	0.33 e	62.3 a	58.2 a	46.83 a	45.76 a
T6	21.5 a	21.7 a	0.39 d	0.40 d	55.1 a	53.3 a	47.60 a	46.35 a
T7	14.9 d	15.2 d	0.53 a	0.54 a	28.1 c	28.2 c	30.16 d	39.93 d

T1= chelated Fe+Zn+Mn 100 ppm*. **T2**= chelated Fe+Zn+Mn 150 ppm. **T3**= chelated Fe+Zn+Mn 200 ppm. **T4**= T1+ AA 500 ppm** **T5**= T2+ AA 500 ppm **T6**= T3+ AA 500 ppm. **T7**= Control***.
*Chelates of Fe+Zn+ Mn (2:1:1) from Fe EDDHA 6% + Zn EDTA 13 % + Mn EDTA 13% as soil application.
**AA= ascorbic acid was sprayed 4 times (after berry set each 15 day intervals). ***Experimental vines except the control (T7) were treated with GA_3.
Means within a column followed by different letter (s) are statistically different at 5 % level.

Regarding to acidity %, all treatments significantly reduced acidity % compared to the control in the both years. The control recorded 0.53 and 0.54 % in the two seasons, respectively. whereas, T1, T3 and T4 came in the second place and reduced the acidity to 0.49, 0.48 and 0.49 % in first season, 0.50, 0.49 and 0.48% in the second season, consequently. While, T6 was more effective in reducing the acidity (0.39 and 0.40 % in both years) followed by T2 (0.45 % in both years). On the other hand, the lowest acidity % was achieved by T5 (0.31 and 0.33 % in both years).

As for maturity index (MI) which defined as T.S.S/acid ratio, micronutrients, GA_3 and ascorbic acid treatments affected significantly T.S.S/acid ratio compared with control in the two years. The results reported significant variations in this parameter. While, T5 and T6 had the greatest T.S.S/acid ratio (62.3 and 55.1 in 1st season, 58.2 and 53.3 in 2ed season, consequently) followed by T1-T4 (39.4, 44.0, 35,2 and 41.0 in 1st year and 38.0, 45,7, 37,8 and 42.5 in 2ed year, respectively). On the other side, the control was the lowest T.S.S/acid ratio (28.1 and 28.2 in both seasons).

Concerning total anthocyanin in berry fresh weight, all treatments increased it significantly in the berries compared with the control during the two seasons of this research. Furthermore, there were significant differences in total anthocyanin content of berries among treatments, whereas T5 and T6 increased the total anthocyanin (46.83 and 47.60 mg/100g FW in 1st year , 45.76 and 46.35 mg/100g FW in 2ed year, respectively) than T1, T2 and T3 (37.49, 42.71 and 40.21 mg/100g FW in 1st season, 40.13, 40.20 and 41.48 mg/100g FW in 2ed season, consequently). On the other hand, the control had the lowest value of total anthocyanin (30.16 and 39.93 mg/100g FW in both years).

The effect of micronutrients, GA_3 and ascorbic acid on increasing T.S.S attributed to enhanced level of leaf chlorophyll in the treated grapevines ultimately resulted in increased rate of photosynthesis and accumulation of carbohydrate reserves in the vines[23] . Furthermore, a high variability in this experiment was found in the color of berries due to influence of micronutrients, GA_3 and ascorbic acid on increasing K leaf mineral status, whereas it improves color[20,38] . In general, all of these factors effect on anthocyanin berry content.

The result are in parallel with those of Abada who reported that foliar application of yeast extract and some micronutrients increased T.S.S of 'Red Roomy' grapevines[26] . Moreover, Wassel *et al.,* found that spraying ascorbic acid was very effective in improving the berry chemical qualities of white banaty seedless grapevines in terms of increasing T.S.S and T.S.S/acid ratio and reducing acidity[6] . Concerning effect of GA_3 on berry chemical properties, Abd-Allah *et al.,* confirmed that spraying Flame seedless grapevines with GA_3 increased T.S.S and reduced the acidity compared with the control (untreated vines) and that raised T.S.S/acid ratio[7]. On the other hand, Qadir *et al.,* on 'Flame seedless', Saad *et al*., on Thompson seedless and Delight, Dokoozlian and Peacock on 'Crimson seedless', who reported that spraying GA_3 had no effect on T.S.S. and acidity of berries[5,34,36] .

Conclusion:

For improving yield and fruit quality of ‘Flame seedless’ grapes under calcareous soil conditions of Egypt, It is recommended to apply mixture chleated microelements at 200 ppm (Fe 100 ppm, Zn and Mn 50 ppm) as soil application in the beginning of opening bud stage and repeated 2 times - one month intervals combined with spraying GA_3 (to elongate the cluster, flower thinning and berry sizing) and ascorbic acid at 500 ppm as foliar application (15 days intervals after berry set, 4 times).

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