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IMPROVING PRODUCTIVITY AND QUALITY OF FLAME SEEDLESS GRAPEVINES (*Vitis vinifera* L.) BY USING CHITOSAN AND SALICYLIC ACID

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ABSTRACT: This study was achieved during the two successive seasons 2017 and 2018. The experiment was conducted at private farm in Talha village, Minia Governorate (240 km southern Cairo city) – Egypt. The main target of this study was examining the effect of spraying of salicylic acid (SA) at 0, 1000, 2000 and 3000 ppm or/and chitosan at 0, 500, 1000 and 2000 ppm on leaves mineral content, yield, beery physical properties and beery chemical characteristics of Flame Seedless grapevines (*Vitis vinifera* L.) grown in heavy clay soil. Increasing chitosan or/and salicylic acid concentrations resulted in a gradual and significant promotion in leaves mineral contents, yield (kg/vine and number of clusters/vine), berry physical and chemical. However, no significant differences were observed neither between the two highest concentrations of chitosan (1000 and 2000 ppm) nor between the two highest concentrations of salicylic acid (2000 and 3000 ppm). The combined application of chitosan and salicylic acid was significantly preferable than using each martial alone in this respect. Furthermore, the vines received the two higher concentrations from chitosan (2000 ppm) and salicylic acid (3000ppm) gave the higher value in most studied parameters. However, no significant differences were observed between the two highest combined concentrations of chitosan and salicylic acid (2000 ppm chitosan + 3000 ppm salicylic acid) and (1000 ppm chitosan + 2000 ppm salicylic acid).

Key words: Grape, Flame Seedless, chitosan, salicylic acid, yield, berries quality.

INTRODUCTION

It is well known that grapevines (*Vitis vinifera* L.) have great adaptability and thrives in wide range of climatic and soil conditions **(Winkler *et al.*, 1974; Delas 2000; Reynier 2000 and Sluys 2006)**. Grapevines are fairly adaptable plants, flourishing around the globe in the temperate bands between 20°C and 50°C Latitude, north or south of the Equator **(Winkler *et al.*, 1974; Reynier, 2000 and Srinivasan & Mmullins, 2001)**.

Flame seedless grapevine considered as one of the most popular grape cultivars successfully grown under Egyptian conditions. This cultivar ripens early in the middle of June and sometimes in the last week of May when grown in new Egyptian reclamation sandy soils. It has a great opportunity for export to foreign reign markets due to its early ripening **(Saad, 2014 and Abd- Elwahab, 2015).** However, in Minia region it faces some problems such as yield decline and inferior of berries quality which in turn negatively affect marketing of such grapevine cv.

Salicylic acid is widely used in organic synthesis and function as a plant hormone. It is derived from the metabolism of salicin (**Vazirimeh & Rigi, 2014 and Zou *et al.*, 2014**). It is a phenolic compound photosynthesis and is found in plants with role in plant growth development, photosynthesis, transpiration as well as uptake and transport of nutrients (**Raskin, 1992; Conrath *et al.*, 1995; Hayat & Ahmed, 2007 and Harvath *et al.*, 2007**). Under non-stress conditions, SA is present in plant tissues in quantities of several mg to several µg in one g of fresh weight. Its level substantially increases in plants exposed to stress (**Hayat & Ahmed, 2007 and Dat *et al.*, 1998**).

Chitosan is produced by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans, such as crabs and shrimp, and cell walls of fungi. Chitin is the second most prominent biopolymer after cellulose found in nature (**Rinaudo 2006**). Chitosan is a semi-synthetic commercial amino-polysaccharide derived by deacetylation of the naturally occurring biopolymer chitin. Chitin is the most abundant of the renewable polysaccharides in the marine environment and one of the most abundant on Earth after cellulose (**Rinaudo 2006; Muzzarelli & Muzzarelli 2009; Suseno *et al.*, 2014**). Chitin is an important source of carbon and nitrogen for marine organisms and its ecological importance in the marine environment is nowadays recognized. The main sources exploited for chitin and chitosan production at industrial scale are marine crustaceans, the shells of shrimps and crabs and the bone plate of squids (**Kean and Thanou 2011; Kim 2014; Younes and Rinaudo 2015; Dima *et al.*, 2017; Nechita, 2017; Philibert *et al.*, 2017 and Sandford, 1989).**

Therefore, the objective of this study was examining the effect of spraying both chitosan and salicylic acid individually or in combination on productivity and quality attributes of Flame seedless table grape.

MATERIALS AND METHODS

The present study was conducted during two successive seasons 2017 and 2018 on thirty vines uniform in vigor ten years old Flame Seedless grapevines grown in private vineyard located at Talha village, Minia Distract Minia Governorate, where the soil texture is heavy clay and well drained water since water table depth is not less than two meters. The chosen vines are planted at 2 X 2.5 meters apart. The selected vines were head pruned the first week of January in both seasons using spur pruning method. The vine load was 76 eyes per vine (on the basis of 16 fruiting spurs X 4 eyes plus 6 replacement spurs X two eyes). Surface irrigation system using Nile water was adopted. The chosen vines are subjected to regular horticulture practices that were commonly applied in the orchard including fertilization, irrigation, and pest management.

Soil characters

The soil where the present experiment carried out was heavy clay soil (table 1). A composite sample was collected and subjected to Physical and chemical analysis according to the procedures outlined by **Ward & Johnston (1962) and Wilde *et al.*, (1985**), the data are shown in Table (1).

Table.1. Physical and chemical analysis of vineyard soils

Constituents	Values
Sand %	5.40
Silt %	14.82
Clay %	80.49
Texture	Clay
EC (1: 2.5 extract) mmhos / cm / 25 C	0.95
Organic matter %	2.62
pH (1: 2.5 extract)	7.8
Total $CaCO_3$ %	1.79
N %	0.09
Available P (Olsen, ppm)	6.10
Exch. K^+ (mg/100g)	422.10
Exch. Ca^{++} (mg/100g)	20.8

Treatments design

In order to study the single and combined effect of salicylic acid and chitosan on Flame Seedless grapevine, four concentrations of each material were used individually and in combination: The two materials were sprayed three times during the vegetative growth cycle, the first one at vegetative growth start, the second just after berry setting and last spray was applied after one month of the second one. This study included the following ten treatments from chitosan and salicylic acid, arranged as flowed:

1- Control (0.0 ppm salicylic acid and 0.0 ppm chitosan).
2- Salicylic acid at 1000 ppm
3- Salicylic acid at 2000 ppm
4- Salicylic acid at 3000 ppm
5- Chitosan at 500 ppm.
6- Chitosan at 1000 ppm.
7- Chitosan at 2000 ppm.
8- 1000 ppm salicylic acid and 500 ppm chitosan.
9- 2000 ppm salicylic acid and 1000 ppm chitosan.
10- 3000 ppm salicylic acid and 2000 ppm chitosan.

Each treatment consisted of three replicates, one vine was used per each replicate. Triton B (2 ml/ l) as a wetting agent was added to all solutions of chitosan and salicylic acid.

Experimental design

Treatments were arranged in a complete randomized block design (RCBD) with three replicates per each treatment, one vine per each replicate was used.

Measurements

Leaf mineral content, yield as well as physical and chemical characters of berries were measured during the two experimental seasons.

1- Determination of N, P, K, Ca and Mg in leaves

Twenty leaves picked from the main shoots opposite to the basal clusters **Martin-Préval *et al.*, (1984)** for each vine were taken at the middle of June. The blades were separated and discarded and the petioles only were used for mineral elements determination, according to **(Martin-Préval *et al.*, 1984)**.

- Nitrogen was determined by the modified microkejldahl method as described by **(Martin-Préval *et al.*, 1984).**

- Phosphorus was determined by using colorimetric method, described by **Wild *et al.*, (1985)**, by measuring the optical density of phosphor-molibdo-vanadate complex by Spectro-photometrically at 430 nm.

- Potassium was flam-photometrically determined by using the method outlined by **Martin-Préval *et al.*, (1984).**

2- Measurement of yield as well as physical and chemical properties of berries

The clusters were harvested when TSS/ acid in the berries of the check treatment reached 24 – 25 (According to **Winkler *et al.*, 1974)**.

- The yield per vine was recorded in terms of weight (kg/vine) and number of clusters per vine.

- Four clusters were taken random from the yield of each vine as a composite sample for determination of the following physical and chemical parameters: cluster length (cm), cluster width (cm), cluster weight (g), berry weight (g), berry dimensions (longitudinal and equatorial (cm), percentage of total soluble solids (TSS %) in the juice and percentage of total acidity, according to **(A.O.A.C, 2000)**.

- Percentage of reducing sugar in the juice by using **Lane and Eynone (1960)** volumetric method

- Total anthocyanin content (Mg/100g F.W.) was extracted from one-gram berry skin (fresh weight) with 100 ml of Acidified methanol (0.1% HCL). The solution was filtered through a centered glass funnel G-3 and absorbance was measured at wavelength 520 nm by Spekol 11 spectrophotometer **(Geza et al., 1983).**

Statistical analysis

All the obtained data were analyzed statistically by using analysis of variance (ANOVA) using the statistical package MSTATC Program. Comparisons between means were made by the F-test and least significant differences (LSD) at p = 0.05 (**Sendecore and Cochran, 1980**).

RESULTS AND DISCUSSION

1- Effect on leaves mineral contents

As shown in Table (2). It's clearly noticed that treating Flame Seedless grapevines with salicylic acid at (1000 pppm, 2000 and 3000 ppm) and chitosan at (500 ppm, 1000 ppm and 2000 ppm) significantly affected on enhancing leaves N%, P%, K%, Mg% and Ca% contents over the checked treatments compared with control treatment.

Moreover, spraying salicylic acid or chitosan each one alone, had a significant promotion on leaves mineral contents, this result was parallel with increasing the concentrations of salicylic acid from 1000 ppm to 3000 ppm or chitosan from 500 ppm to 2000 ppm Furthermore, the same Table show a relative superiority effect of chitosan compared with salicylic acid on the five examined elements (N, P, K, Mg and Ca), during the two experimental seasons. However, the combination of both salicylic acid and chitosan was more pronounced effect on leaves content of nitrogen, phosphorus, potassium, magnesium and calcium than using each material alone in both seasons 2017 and 2018.

The positive effect of chitosan on enhancing leaf mineral contents in Flame Seedless grapevines might be attributed to their essential content in macro and micro nutrients such as N, P, Ca, K, Zn, Fe and Mn, as well as its favorable effect on nutrient and water uptake, also its positive effect on bio-stimulation effect on photosynthesis and mineral uptake that lead to increased macro and micro elements in leaves as in the previous studies (**Van *et al.*, 2013; Suseno *et al.*, 2014: Petriccione *et al.*, 2015; Ahmed *et al.*, 2016; Agbodjato *et al.*, 2016; El-Kenawy 2017** and **Ayed 2018**).

A clear involvement of salicylic acid in the control of nutrient assimilation might be expected. Moreover, salicylic acid contributes in the control of redox status of plants, most likely by regulating the synthesis of the antioxidant glutathione, which protects plant against oxidative stress that follows many nutritional deficiencies (**Freeman *et al.*, 2005; Shao *et al.*, 2007**). Furthermore, the chelating properties of chitosan also make it an excellent source of macro and micronutrients (**Rabea *et al.*, 2003; Divya & Jisha 2018 and Rahman *et al.*, 2018**).

Table 2. Single and combined effect of salicylic acid (SA) and chitosan on leaves N%, P%, K%, Mg% and Ca% of Flame seedless grapevines, during 2017 and 2018 seasons

Treatments	N %		P %		K %		Mg%		Ca%	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	1.59	1.55	0.14	0.15	1.67	1.72	0.41	0.40	1.36	1.35
SA at 1000ppm	1.67	1.65	0.17	0.19	1.83	1.79	0.44	0.51	1.44	1.42
SA at 2000ppm	1.73	1.74	0.20	0.22	1.88	1.87	0.46	0.56	1.46	1.46
SA at 3000ppm	1.78	1.78	0.21	0.23	1.90	1.91	0.52	0.57	1.47	1.48
Chitosan 500ppm	1.76	1.73	0.22	0.22	1.89	1.88	0.49	0.53	1.51	1.53
Chitosan 1000ppm	1.84	1.79	0.26	0.25	1.94	1.95	0.55	0.57	1.59	1.62
Chitosan 2000ppm	1.88	1.82	0.27	0.26	1.97	1.99	0.57	0.58	1.59	1.63
SA 1000ppm + Chitosan 500ppm	1.83	1.80	0.26	0.26	1.95	1.96	0.62	0.66	1.59	1.61
SA 2000ppm + Chitosan 1000ppm	1.91	1.87	0.29	0.31	2.00	2.04	0.71	0.73	1.78	1,77
SA 3000ppm + Chitosan 2000ppm	1.96	1.91	0.30	0.32	2.03	2.07	0.73	0.74	1.79	1.79
New LSD at 5%	0.06	0.05	0.02	0.02	0.04	0.04	0.07	0.09	0.10	0.11

2- Effect on cluster numbers, cluster weight and yield/vine

It was clearly noticed that in Table (3) increasing in cluster numbers/vine, cluster weight (g) and yield/vine (kg) of Flame Seedless grapevines during 2017 and 2018 seasons as a response of spraying different concentrations of salicylic acid or/and chitosan. As noticed treating Flame Seedless grapevines with salicylic acid at (1000, 2000 and 3000 ppm) and chitosan at (500, 1000 and 2000 ppm) significantly affected on enhancing number of cluster/vine (only in the second season), cluster weight (g) and yield/vin (kg) over the check treatments. There was a gradual promotion on these three parameters with increasing the concentration use of salicylic acid and/or chitosan. However, neither increasing the concentration of salicylic acid nor increasing the concentration of chitosan had a significant effect on the number of clusters during the first season. This effect is logic, since fruiting bud in grapevines were internally formed in the preceding year.

It is evident from the same Table that, all the treatments of the two materials had a significant effect on cluster weight (g) and yield/vine in comparison with control vines, during both experimental seasons of this study.

The vines received the higher concentration of salicylic acid companied with chitosan produced the highest cluster weight (390 and 400 g) and yield /vine (10.9 and 14.8 kg). While, untreated vines produced the lowest cluster weight (341 and 350 g) and yield/vine (9.2 and 9.1 kg), during the two experimental seasons respectively.

In accordance of our results **Rahman *et al.*, (2018)** as they fund that treating strawberry plants by 500 ppm of chitosan solution provided the highest fruit yield (42% higher than untreated control) in Strawberry Festival cultivar than that of untreated control.

3- Effect on berry physical properties

The single and combined effect of salicylic acid or/and chitosan on berry physical properties in terms of berry weight and dimensions of Flame Seedless grapes during 2017 and 2018 seasons are illustrated in Table (4).

It was clearly noticed that increasing of the concentration of each material was capable to increase berry weight, berry longitudinal and berry equatorial (cm) in comparison with control treatment in both seasons. This improvement of berry physical properties was parallel with increasing salicylic acid and chitosan concentrations.

It is clear that spraying chitosan at 500 to 2000 ppm was significantly superior to spraying salicylic acid at 1000 to 3000 ppm. However, the combined application of salicylic acid and chitosan was significantly preferable than using each martial alone. Furthermore, the vines received the two highest concentration of salicylic acid (3000 ppm combined with chitosan at 2000 ppm roduced the highest berry weight (3.88 & 4.00 g), berry longitudinal (1.96 & 2.00 cm) and berry equatorial (1.79 & 1.80 cm) followed by those received salicylic acid at 2000 ppm combined with chitosan at 1000 ppm (3.82 & 3.93 g for berry weight, 1.94 & 1.98 cm for berry longitudinal and 1.78 & 1.79 cm for berry equatorial), during the two experimental seasons respectively. Also, the same table indicated that increasing salicylic acid concentration from 2000 ppm to 3000 ppm or/and chitosan from 1000 ppm to 2000 ppm had no significant effect on berry physical properties, in both experimental seasons. On the other hand, untreated vines gave the minimums weight of berry (3.41 & 3.50 g), berry longitudinal (1.71 & 1.75 cm) and berry equatorial (1.55 & 1.57 cm) during both experimental seasons respectively.

Table 3. Single and combined effect of salicylic acid (SA) and chitosan on number of clusters, cluster weight (g), and yield/vine (kg) of Flame seedless grapevines, during 2017 and 2018 seasons

Treatments	Number of clusters/vines		Cluster weight (g)		Yield/vine (kg)	
	2017	2018	2017	2018	2017	2018
Control	27.0	26.0	341.0	350.0	9.2	9.1
SA at 1000ppm	27.0	28.8	352.0	363.0	9.5	10.2
SA at 2000ppm	28.0	30.0	362.0	372.0	10.1	11.2
SA at 3000ppm	28.0	31.0	369.0	380.0	10.3	11.7
Chitosan 500ppm	28.0	32.0	360.0	374.0	10.0	12.0
Chitosan 1000ppm	28.0	35.0	369.0	384.0	10.3	13.4
Chitosan 2000ppm	28.0	36.0	375.0	392.0	10.5	14.1
SA 1000ppm + Chitosan 500ppm	28.0	34.0	372.0	385.0	10.4	13.1
SA 2000ppm + Chitosan 1000ppm	28.0	36.0	382.0	394.0	10.7	14.2
SA 3000ppm + Chitosan 2000ppm	28.0	37.0	390.0	400.0	10.9	14.8
New LSD at 5%	NS	1.5	8.0	9.0	0.3	0.8

Table 4. Single and combined effect of salicylic acid (SA) and chitosan on berry physical properties of Flame seedless grapes, during 2017 and 2018 seasons

Treatments	Berry weight (g)		Berry longitudinal (cm)		Berry equatorial (cm)	
	2017	2018	2017	2018	2017	2018
Control	3.41	3.50	1.71	1.75	1.55	1.57
SA at 1000ppm	3.50	3.60	1.75	1.80	1.60	1.63
SA at 2000ppm	3.62	3.71	1.80	1.86	1.65	1.68
SA at 3000ppm	3.67	3.78	1.82	1.88	1.66	1.70
Chitosan 500ppm	3.63	3.70	1.81	1.87	1.70	1.67
Chitosan 1000ppm	3.72	3.81	1.86	1.93	1.71	1.70
Chitosan 2000ppm	3.78	3.87	1.87	1.94	1.72	1.73
SA 1000ppm + Chitosan 500ppm	3.71	3.82	1.88	1.92	1.76	1.77
SA 2000ppm + Chitosan 1000ppm	3.82	3.93	1.94	1.98	1.78	1.79
SA 3000ppm + Chitosan 2000ppm	3.88	4.00	1.96	2.00	1.79	1.80
New LSD at 5%	0.07	0.08	0.03	0.03	0.02	0.03

4- Effect on berry chemical characteristics

Effect of salicylic acid or/and chitosan on berry chemical characteristics in terms of total soluble solids, acidity, total acidity, TSS/acidity ratio, reducing sugars, and total anthocyanin contents of Flame seedless grapes during 2017 and 2018 seasons are illustrated in Table (5).

As it shown both salicylic acid and chitosan had a significant effect on TSS%, TSS/acid ratio, reducing sugars, and total anthocyanin contents compared with control in Flame seedless grapes during the two experimental seasons. On the other hand, spraying salicylic acid and chitosan gradual and significant decreased in the total acidity% during the two experimental seasons compared to untreated vines.

The promotion on TSS%, TSS/acid ratio, reducing sugars and total anthocyanin contents of the berries as well as the decrease in total acidity was associated with increasing the concentration of salicylic acid or/and chitosan. However, no-significant promotion was attributed by increasing salicylic acid from 2000 to 3000 ppm as well as increasing the concentrations of chitosan from 1000 ppm to 2000 ppm neither using each material alone nor in combination. Also, it was clearly that the combined application of salicylic acid and chitosan was significantly preferable than using each martial alone in this respect.

The illustrated data also showed that, the vines received the higher concentrations of salicylic acid (3000 ppm) combined with chitosan at 2000 ppm gave the higher TSS% (20.3 & 20.4 %), TSS/acidity ratio (33.0 & 33.2) and lower total acidity (0.615 & 0.614%) followed by those received salicylic acid at 2000 ppm combined with chitosan 1000 ppm during the two experimental seasons respectively.

Regarding the effect of each material alone, it is clear that spraying chitosan at 500 ppm to 2000 ppm was significantly superior to spraying salicylic acid at 1000 ppm to 3000 ppm However, the combined application of salicylic acid and chitosan was significantly preferable than using each martial alone in this respect.

Salicylic acid significantly reduced the quality loss in peach (**Wang *et al.*, 2006, Abbasi *et al.*, 2010 and Wang *et al.*, 2010**) and loquat fruit (**Cai *et al.*, 2005**). Furthermore, salicylic acid and its derivatives are widely used to enhance pre- and postharvest quality of fruit such as Peaches (**Wang *et al.*, 2006**), increase reducing sugars of pomegranate (**Mohamed 2017**), increasing TSS% and reducing sugars of Bartlett pear as in (**Meng *et al.*, 2010**) and banana fruits during ripening (**Srivastava and Dwivedi 2000**) as well as Delicia Peach fruit during conservation (**Abbasi *et al.*, 2010**).

In addition to salicylic acid effects, some field trails have shown the positive effect of chitosan on chemical and physical properties of fruit **among then: Romanazzi *et al.*, (2017)** and **Rahman *et al.*, (2018)** reported that total polyphenols in several fruits had increased due to chitosan-coating that activated the key enzyme such as phenylalanine ammonia lyase (PAL) in the phenol synthesis pathway. Chitosan treatment of litchi fruit delayed changes in contents of anthocyanin, flavonoid and total phenolic by delaying the increase in PPO activity, and inhibited post-harvest decay partially (**Zhang *et al.*, 2003** and **Rahman *et al.*, 2018**).

Table 5. Single and combined effect of salicylic acid (SA) and chitosan on berry chemical characteristics of Flame seedless grapes, during 2017 and 2018 seasons

Treatments	TSS (%)		Total acidity (%)		TSS/ acid ratio		Reducing sugars %		Anthocyanins (mg/100 g)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	18.0	18.2	0.719	0.727	25.0	25.0	14.9	14.5	24.9	24.0
SA at 1000ppm	18.5	18.6	0.699	0.700	26.5	26.6	15.5	15.0	25.6	25.0
SA at 2000ppm	19.1	19.1	0.673	0.675	28.4	28.3	15.9	15.4	26.2	25.0
SA at 3000ppm	19.4	19.3	0.657	0.657	29.5	29.4	16.2	15.6	26.6	26.4
Chitosan 500ppm	19.1	19.2	0.674	0.672	28.2	28.6	16.0	15.6	26.3	26.1
Chitosan 1000ppm	19.6	19.7	0.653	0.652	30.0	30.2	16.4	15.9	26.9	27.1
Chitosan 2000ppm	19.8	20.2	0.636	0.635	31.0	31.0	16.5	16.0	27.2	27.5
SA 1000ppm + Chitosan 500ppm	19.7	19.7	0.650	0.651	30.3	30.3	16.6	16.1	27.1	27.0
SA 2000ppm + Chitosan 1000ppm	20.1	20.1	0.632	0.632	31.8	31.8	17.1	16.5	27.7	27.8
SA 3000ppm + Chitosan 2000ppm	20.3	20.4	0.615	0.614	33.0	33.2	17.4	16.7	28.1	28.3
New LSD at 5%	0.4	0.4	0.018	0.019	1.30	1.69	0.4	0.3	0.5	0.6

Conclusion

It could be recommended that spraying Flame seedless grapevines by salicylic acid at 3000 ppm combined with spraying chitosan at 2000 ppm three times during growing season (at the beginning of vegetative growth, just after berry setting and one month later), could be promising method to improve leaves growth and chemical composition, also improving productivity and quality of Flame seedless table grape.

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