The Beneficial Effects of Using Chitosan and Glutathione on the Fruiting of Red Roomy Grapevines

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Abstract: During 2015 and 2016 seasons, Red Roomy grapevines were sprayed three times at growth start, just after berry setting and one month later with glutathione at 0.025 to 0.1 and/or chitosan at 0.05 to 0.2%. The goal was examining the effect of these treatments on growth vine nutritional status, berry setting as well as berries colouration and quality of the berries. Single and combined applications of glutathione at 0.025 to 0.1 % and chitosan at 0.05 to 0.2 % had an obvious promotion on all growth aspects, vine nutritional status, berry setting, yield, berries colouration and quality of the berries relative to the control treatment. Combined applications were superior than using each material alone. Using chitosan at 0.05 to 0.2 % was considerably favourable than using glutathione at 0.025 to 0.1 % in improving all growth aspects, nutrients, berry setting, yield, berries quality. A slight promotion on these characteristics was observed among the higher two concentrations of glutathione namely 0.05 and 0.1 % and chitosan namely 0.1 and 0.2 %. Three sprays at growth start, just after berry setting and one month later of glutathione at 0.05 % and chitosan at 0.1 % was responsible for improving yield, berry setting and berries colouration and quality of Red Roomy grapevines.

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1. Introduction

Chitosan (acetyl glucosamine) is a natural biopolymer combined derived by deacetylation of chitin a major component of the shells of crustacean such as crab, shrimp and crawfish (Sanford, 2002). It is an ecologically sound alternative for controlling different disorders and has received much interest for applications in agriculture because of its non-toxicity bioactivity (Muzzarelli et al., 2012). It is able to form a film outer surface of fruits and reduces respiration rate by adjusting the permeability of CO₂ and O₂ and reducing fruit metabolism and extending shelf-life (Elsabee and Abdou, 2013). Since, it is responsible for forming protective barrier on the fruit surface it inhibits decay and induces defense against different diseases (Zeng et al., 2010). It is able enhance antoxidative ability and reduce water loss (Shi et al., 2013).

Glutathione (cysteine + glutamic acid+ glycine) is the most important non protein thiol present in plants. It is essential in sulfur metabolism and defense against most stresses. It is important pool of reduced sulfur and it regulates sulfur uptake at root level. Reduced glutathione, the major water soluble antioxidant in photosynthestic and non – photosynthestic tissues, reacting directly or indirectly with reactive oxygen species, contribute to maintain the integrity of cell structure and the proper functions of various metabolic pathways. In addition to its effects on expression of defense genes glutathione may also be involved in redox control of cell division and enhanced growth of plants (Mulleineaux and Rausch, 2005).

Chitosan was found by Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2007); Dang *et al* (2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017) to enhance growth aspects, tree nutritional status, yield and fruit quality of different fruit crops.

The results of Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017) emphasized the beneficial effects of glutathione on growth, tree nutritional status, yield and fruit quality of fruit crops.

The goal of this study was elucidating the effect of glutathione and/or chitosan on growth aspects, vine nutritional status, berry setting, yield, berries colouration and quality of Red Roomy grapes.

2. Materials and Methods

This study was carried out during 2015 and 2016 seasons on thirty uniform in vigour 10-years old Red Roomy grown grapevines in a private vineyard located at Abu Korkas district, Minia Governorate where the texture of the soil is clay, well drained and water table not less than two meters deep. All the selected vines are planted at 2×2 m apart. The chosen vines (60 vines) were head pruned during the middle of January in both seasons using

spur pruning method. Vine load was 72 eyes for all the selected vines on the basis of 20 fruiting spurs × 3 eyes plus 6 replacement spurs × two eyes. Surface irrigation system was followed using Nile water containing 160 ppm EC.

The main target of this study was examining the effect of single and combined applications of glutathione and chitosan on growth, vine nutritional status, yield and quality of the berries of Red Roomy grapevines. In addition, this study aimed at selecting the best combination of glutathione and chitosan that would help produce a high quality economical yield.

Mechanical, physical and chemical analysis of the tested soil were carried out at the start of the experiment according to the procedures of **Wilde** *et al.*, (1985), and the results are shown in Table (1).

Table ((1)):	Anal	ysis	of	the	tested	vineyard	soil:
								-/	

Constituents	Values
Particle size distribution:	
Sand %	7.0
Silt %	21.5
Clay %	71.5
Texture	Clay
pH (1:2.5 extract)	7.95
EC (1:2.5 extract) (dsm^{-1}) 1 cm / 25°C.	0.97
O.M. %	2.01
CaCO ₃ %	2.41
Total N %	0.11
Available P (Olsen, ppm)	3.11
Available K (ammonium acetate, ppm)	405.9

Except those dealing with the present treatments (glutathione and chitosan), all the selected vines (60 vines) received the usual horticultural practices which are commonly used in the vineyard.

This study included the following ten treatments from glutathione and chitosan:

- 1. Control.
- 2. Glutathione at 0.025 %.
- 3. Glutathione at 0.05 %.
- 4. Glutathione at 0.1 %.
- 5. Chitosan at 0.05 %.
- 6. Chitosan at 0.1 %.
- 7. Chitosan at 0.2 %.
- 8. Glutathione + Chitosan at low conc.
- 9. Glutathione + Chitosan at mid. conc.
- 10. Glutathione + Chitosan at high conc.

Each treatment was replicated three times, two vines per tree. The total vines selected for achieving this experiment was 60 vines. Glutathione and chitosan were sprayed three times at growth start (3rd week of Apr.), just after berry setting (2rd week of June) and at one month later (3rd week of July).

Triton B as agent was added to all spraying solutions. Spraying was done till runoff.

Randomized complete block design (RCBD) was followed, where the experiment consisted of ten treatments, each treatment was replicated three times, two vines per each.

The following measurements were recorded during the two experimental seasons:

1- Measurements of vegetative growth characteristics:

At the last week of May, the main shoot length and leaf area were recorded as follows:

1-Average main shoot length (cm.) as a result of measuring the length of the labeled ten main shoots per vine and then the average was estimated.

2- Average leaf area (cm^2) as a result of measuring the diameter of twenty mature leaves from those opposite to the basal clusters on the main shoots.

Leaf area (cm²) was measured using the following equation as outlined by **Ahmed and Morsy (1999)**.

Leaf area $(cm^2) = 0.45 (0.79 \times d^2) + 17.77$, where d is the maximum diameter of leaf, then the average leaf area was registered.

3- Wood ripening coefficient was measured by dividing the length of brownish part of the cane by the total length of cane just before pruning date (1st week of January) (**Bouard, 1966**).

4- For each vine five canes were selected just before Winter pruning (1st week of January) for measuring the cane thickness (mm) by using Vernier caliper.

2- Measurements of leaf total carbohydrates and leaf pigments:

The leaf content of total carbohydrates was determined according to methods previously outlined (A.O.A.C., 2000).

Samples of five mature and fresh leaves from those leaves opposite to the basal clusters on each shoot were taken at the last week of May during both seasons and cut into small pieces and 0.5 g weight from each sample was taken, homogenized and extracted by 25% acetone in the presence of little amounts of Na₂CO₃ then filtered. The residue was washed several times with acetone until the filtrate became coulorless (Fadl and Seri El-Deen, 1978). The extract was completed to a known volume (20 ml) with acetone 85%. A portion of this extract was taken for the determination of chlorophylls A and B colourimetrically (as mg/ 100 g F.W) and acetone (85 % V/V) was used as a blank. The optical density of the filtrate was determined at the wave length of 662, 664 and 440 nm to determine chlorophylls A and total carotenoids, and B respectively. Concentration of each pigment was calculated by

using the following equations according to **von** Wettstein (1957).

Cl. A = (9.784 × E 662) – (0.99× E 664) = mg / g. F.W.

Cl. B = $(21.426 \times E \ 664) - (4.65 \times E \ 662) = mg$ / g. F.W.

Total carotenoids = $(4.965 \times E \ 440 - 0.268)$ (chlorophyll a + chlorophyll b).

Where E = optical density at a given wave length. Each value was multiplied by 100. Total chlorophylls was estimated by summation of chlorophyll a + chlorophyll b (mg/ 100 g. / F.W.).

3- Measurements of leaf chemical composition:

Twenty leaves picked from the main shoots opposite to the basal clusters (according to **Balo** *et al.*, **1988**) for each vine were taken at the last week of May (veraison stage) in both seasons. Blades of the leaves were discarded and petioles were saved for determination of N, P, K and Mg (as percentages). Petioles were oven dried at 70°C and grinded then 0.5 g weight of each sample was digested using H₂SO₄ and H₂O₂ until clear solution was obtained (according to **Wilde** *et al.* **1985**). The digested solutions were quantitatively transfered to 100 ml volumetric flasks and completed to 100 ml by distilled water. Thereafter, leaf contents of N, P, K, Mg, Ca, S, Zn, Fe, Mn and Cu were determined as follows:

1-Nitrogen % by the modified micro-Kjeldahl method as described by Chapman and Pratt (1987).

2- Phosphorus % by using Olsen method as reported by Wilde *et al.*, (1985).

3- Potassium % by using flame photometer as outlined by (Chapman and Pratt (1987).

4- Mg and Ca% by titration against EDTA (Cottenie *et al.*, 1982).

5-S% by using nephelometer apparatus (Chapman and Pratt, 1987).

6-Micronutrients namely Zn, Fe and Mn by using atomic absorption.

4- Measurements of berry setting %:

It was calculated by caging five clusters per vine in perforated white paper bags before blooming stage. At the end of berry setting stage, the bags were removed for counting the following:

a) The number of attached berries.

- b) The number of dropped berries.
- c) The number of dropped flowers.

d) The number of total flowers (a + b + c) per cluster. Berry setting % was estimated by dividing number of attached berries by total number of flowers per cluster and multiplying the product by 100.

5- Measurements of yield and both physical- and chemical characteristics of the berries: 5-1 Yield: Harvesting took place when T.S.S. / acid in the berries of the check treatment (using N as 100% inorganic N) reached at least 25:1 (at the last week of June in both seasons) (according to **Winkler** *et* **al.**, (1974) and **Weaver**, (1976). The yield per vine expressed in weight (kg.) and number of clusters per vine was recorded.

5-2 Berries quality:

Five clusters from each vine were taken at random for determination of the following physical and chemical characteristics.

1- Cluster dimensions (length and shoulder, cm.)

2- Percentage of berries colouration by dividing namely of red berries by the total number of berries/cluster and multiplying the product by 100.

3- Average berry weight (g.)

4- Average berry dimensions (longitudinal and equatorial, in cm) and berry shape index value was estimated.

5- Percentage of shot berries by dividing number of shot berries by total number of berries per cluster and multiplying the product by 100.

6- Percentage of total soluble solids in the juice by using hand refractometer.

7- Percentage of reducing sugars in the juice was determined by Lane and Eynon (1965) volumetric method as described in A.O.A.C. (2000).

8- Total anthocyonins in the juice was determined by using (Fulcki and Francis, 1968) method.

9- Percentage of total acidity (as g tartaric acid/ 100 ml juice) by titration against 0.1 NaOH using phenolphthalein as an indicator A.O.A.C. (2000).

All the obtained data were tabulated and statistically analyzed using New L.S.D. at 5 % for made all comparisons among the investigated treatment means according to **Mead** *et* **al.**, (1993).

3. Results and Discussion

1- Effect of single and combined applications of glutathione and chitosan on some vegetative growth characteristics:

It is clear from obtained data in Table (2) that treating the vines three times with glutathione at 0.025 to 0.1 % and/or chitosan at 0.05 to 0.2 % significantly enhanced the six growth aspects namely the main shoot length, number of leaves/shoot, leaf area, wood ripening coefficient, cane thickness and pruning wood weight/vine relative to the control. The promotion was associated with increasing concentrations of glutathione from 0.025 to 0.1% and chitosan from 0.05 to 0.2%. Combined applications of glutathione and chitosan significantly increased these growth aspects than using each material alone. Using chitosan was significantly superior than using glutathione in stimulating these growth traits. Increasing concentrations of glutathione from 0.05 to 0.1 % and chitosan from 0.1 to 0.2 % had no significant promotion on these growth traits.

The maximum values of main shoot length (119.0 & 119.8 cm), number of leaves/shoot (26.3 & 27.4 leaf), leaf area (98.6 & 100.4 cm), wood ripening coefficient (0.95 & 0.91), cane thickness (1.48 & 1.50 cm) and pruning wood weight (2.71 & 2.72 kg) were recorded on the vines that received three sprays of a mixture of glutathione at 0.1 % and chitosan at 0.2 % during both seasons, respectively. The untreated vines produced the minimum values of main shoot length (106.36 & 107.1 cm), number of leaves/shoot (16 & 15 leaf), leaf area (87.3 & 88.0 cm²), wood ripening (0.66 & 0.65), cane thickness (1.00 & 1.02 cm) and pruning wood weight (1.95 & 1.99 kg/vine) during both seasons, respectively.

The beneficial effects of chitosan on enhancing antioxidants, enzyme, hormones the resistance to diseases and microorganisms, levels of ABA which plays a key role in the regulation of water use due to the closure of stomata, availability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and in descending order water loss, transpiration the accumulation of harmful free ridicules (**Hadwiger** *et al.*, **2002**) could explain the present results.

The results of chitosan are in harmony with those found by Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2007); Dang *et al* (2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017)

The higher content of glutathione from glycine, cycteine and glutamic as well as its action on enhancing sulfur metabolism and defense gene and reducing reactive oxygen species (ROS) could explain the present results (**Mullineaux and Raush**, **2005**). These results concerning the positive action of glutathione on growth are in harmony with **Abdelaal** *et al* (2012); **Gad El-Kareem** (2012); **Ahmed** *et al* (2012) and (2013); **El-Khawaga and Mansour** (2014) and **Madany** (2017) emphasized the beneficial effects of glutathione on growth, tree nutritional status, yield and fruit quality of fruit crops.

2- Effect of single and combined applications of glutathione and chitosan on leaf pigments:

Data in Table (3) obviously reveal that varying glutathione and chitosan treatments significantly altered the leaf pigments namely chlorophylls a & b, total chlorophylls and total chlorophylls and total

carotenoids rather single and combined applications significantly were responsible for enhancing these plant pigments relative to the control. There was a gradual promotion on these leaf pigments with increasing concentrations of glutathione concentrations from 0.025 to 0.1 % and chitosan from 0.05 to 0.2 %. Using glutathione was significantly preferable than using chitosan in enhancing these plant pigments. Using both materials together significantly increased these leaf pigments combined to using material alone in enhancing these leaf pigments. No significant differences were observed on these leaf pigments among the higher two concentrations of glutathione namely 0.05 and 0.1 % and chitosan from 0.1 to 0.2 %. Treating the vines with glutathione at 0.1 % and chitosan at 0.2 % gave the maximum values of chlorophyll a (1.87 & 1.89 mg/1 g F.W), b (1.27 & 1.28 mg/1 g F.W), total chlorophylls (3.14 & 3.16 mg/ 1g F.W) and total carotenoids (1.51 & 1.52 mg/1 g F.W) during both seasons, respectively. The lowest values were recorded on untreated vines. Similar results were announced during 2015 and 2016 seasons.

The enhancing effect of chitosan on uptake of water and different nutrients surely reflected on enhancing the biosynthesis of plant pigments (Hadwiger *et al.*, 2002). The promotion effect of chitosan on plant pigments was supported by Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2007); Dang *et al* (2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017) the results of chlorophylls a & b, total chlorophylls and total chlorophylls and total carotenoids.

The increase in amino acids / photosynthetic process (Mullineaux and Rausch, 2005) could explain the promoting effect of glutathione on the biosynthesis of plant pigments. These results are in agreement with those obtained by Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017).

3- Effect of single and combined applications of glutathione and chitosan on the leaf content of N, P, K, Mg and S as (%) and Zn, Fe and Mn (as ppm):

Tables (4 & 5) and Figures (11 to 18) show the effect of single and combined applications of glutathione and chitosan on the leaf content of N, P, K, Mg and S (as %) and Zn, Fe and Mn (as ppm) of Red Roomy grapevines during 2015 and 2016 seasons.

One can state from the obtained data that subjecting Red Roomy grapevines three times with glutathione and/or chitosan was significantly followed by stimulating N, P, K, Mg, S, Zn, Fe and Mn relative to the control treatment. The stimulation on these nutrients was in proportional to the increase in concentrations of each material. Employing chitosan at 0.05 to 0.2 % significantly was accompanied with enhancing these nutrients than using glutathione. Combined applications were significantly superior than using each material. Negligible promotion on these nutrients were observed among the higher two concentrations of each material. Using the higher concentrations of glutathione namely 0.1 and chitosan namely 0.2 % gave the highest values of N (2.22 & 2.23 %), P (0.41 & 0.35%), K (1.62 & 1.58 %), Mg (0.77 & 0.79 %), S (0.83 & 0.84 %), Zn (66.3 & 66.4 ppm), Fe (71.6 & 72.0 ppm) and Mn (70 & 70 ppm) during both seasons, respectively. The untreated vines produces the lowest values. The results were true during both seasons.

The positive action of glutathione and chitosan on enhancing root development and up take pf nutrients could explain the present results **Hadwiger** *et al* (2002); **Eweis** *et al* (2006); **Xu** *et al* (2007); Liu *et al* (2007); **Dang** *et al* (2010); **Meng** *et al* (2010); **Hadwiger** (2013); **Saied and Radwan**, (2017).

Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017).

4- Effect of single and combined applications of glutathione and chitosan on the percentage of berry setting, yield as well as cluster weight and dimensions:

Data concerning the effect of single and combined applications of glutathione and chitosan on the percentage of berry setting, yield as well as cluster weight and dimensions (length & shoulder) of Red Roomy grapevines during 2015 and 2016 seasons are shown in Table (5).

The evident from the obtained data that supplying the vines with glutathione at 0.025 to 0.1% and / or chitosan at 0.05 to 0.2 5 significantly was followed by improving berry setting %, yield expressed in weight (kg) and number of clusters per vine and weight, length and shoulder of cluster relative to the control treatment. There was a progressive promotion on these parameters with increasing concentrations of each material. Significant differences on these parameters were observed between all concentrations and materials except among the higher two concentrations of each material, therefore from economical point of view it is necessary to use the material. Combined were favourable than using each material alone in this respect. Using chitosan significantly preferable than using chitosan in improving berry setting, yield and cluster characteristics.

From economical point of view, using glutathione at 0.05 plus chitosan at 0.1 % resulted in the highest yield. Under such promised treatment, yield per vine reached 10.3 and 14.0 during both seasons, respectively. The untreated vine gave yield reached 8.2 and 7.8 during both seasons, respectively. The percentage of increment on the yield due to application of the previous treatment over the check treatment reached 25.6 and 79.5 % during both seasons, respectively. These results were nearly the same during both seasons.

The beneficial effect of glutathione and chitosan on berry setting might be attributed to their positive action on growth, vine nutritional status and pigments. The promotion on the yield was attributed to their positive action non berry setting and cluster weight and dimensions. These results regarding the effect of glutathione on promotion berry setting, yield and cluster weight and dimensions are in concordance with the results of Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017)

The promoting effect of chitosan on berry setting, yield and cluster weight was emphasized by Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017).

5- Effect of single and combined applications of glutathione and chitosan on the percentage of berries colouration:

Table (6) show the effect of single and combined applications of glutathione and chitosan on the percentage of berries colouration of Red Roomy grapevines during 2015 and 2016 seasons.

It is revealed from the obtained data that subjecting Red Roomy grapevines to glutathione at 0.025 to 0.1 % and/or chitosan at 0.05 to 0.2 % significantly enhanced berries colouration relative to the control treatment. Using chitosan was significantly superior than using glutathione in enhancing berries colouration. A mixture of glutathione and chitosan was significantly preferable in enhancing berries colouration than using material alone. Meaningless promotion on berries colouration was observed among the higher two concentrations of each material. A progressive promotion was noticed with increasing concentrations of each material. Economically point of view and for solving the irregular berries colouration problem, it is useful to use the two materials together at the medium concentrations. The berries coloration reached the highest values (74.3 & 75.7 %) in the vines that received both materials together at the higher

concentration. The lowest berries colouration (54.1 & 55.0 %) was occurred on the untreated vines during both seasons, respectively. These results were true during both seasons.

The enhancing effect of glutathione and chitosan on berries colouration might be attributed to their positive action on enhancing the leaf area and photosynthesis (**Mullineaux and Rausch, 2002**).

These results regarding the promoting effect of glutathione on berries colouration are in harmony with those obtained by Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017).

The results of berries colouration are in the same line with the present results concerning the effect of chitosan on enhancing berries colouration Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2007); Dang *et al* (2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017).

6- Effect of single and combined applications of glutathione and chitosan on the percentage of shot berries:

The results regarding the effect of single and combined applications of glutathione and chitosan on the percentage of shot berries in the cluster of Red Roomy grapevines during 2015 and 2016 seasons are shown in Table (6).

It is reveal from the obtained data that percentages of Red Roomy grapevines was significantly controlled by using glutathione at 0.025 to 0.1 % and/or chitosan at 0.05 to 0.2 % over the control treatment. Employing chitosan was significantly accompanied with depressing shot berries % relative to the application of glutathione. The reduction was related to the increase of both materials. A significant reduction was observed on shot berries % with using both materials together rather than using any material alone. Increasing concentrations of glutathione from 0.05 to 0.1 % and chitosan from 0.1 to 0.2 % failed to show significant reduction or seed undeniable phenomenon. The lowest values o shot berries % (1.9 & 1.8 %) were recorded on the vines received the two materials together at higher concentrations during both seasons, respectively. The untreated vines produced shot berries % in the clusters reached (9.0 & 9.2 %)during both seasons, respectively. These results were true during both seasons.

The effect of glutathione on enhancing cell division and photosynthesis process as well as reducing reactive oxygen species could result in reducing shot berries in clusters (**Mullineaux and Raasch, 2002**). The great stimulation on vine nutritional status can give another explanation.

The effect of chitosan on enhancing photosynthesis and vine nutritional status can give explanation of the reducing effect on shot berries in the clusters (**Nge** *et al.*, **2006**).

These results regarding the reducing effect of glutathione on shot berries are nearly in the same line with those obtained by Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017).

The results of Hadwiger *et al* (2002); Eweis *et al* (2006); Xu *et al* (2007); Liu *et al* (2007); Dang *et al* (2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017) supported the results of chitosan on controlling shot berries.

7- Effect of single and combined applications of glutathione and chitosan on some physical and chemical characteristics of the berries:

Data in Tables (6 & 7) and Figures (27 to 34) show the effect of single and combined applications of glutathione and chitosan on berry weight and dimensions (longitudinal and equatorial), T.S.S. %, total sugars %, total acidity %, total anthocyanins and T.S.S./acid in the berries of Red Roomy grapevines during 2015 and 2016 seasons.

It is clear from the obtained data that treating Red Roomy grapevines three times with glutathione at 0.025 to 0.1 % and/or chitosan at 0.05 to 0.2 % significantly was favourable than the control treatment in improving quality of the berries in terms of increasing weight, longitudinal and equatorial of berry, T.S.S.%, total sugars %, total anthocyanins and T.S.S./acid and decreasing total acidity % relative to the check treatment. The promotion on quality of the berries was related to the increase in concentrations of glutathione and chitosan without significant promotion among the higher two concentrations of glutathione and chitosan. Using chitosan significantly was preferable than using glutathione in enhancing physical and chemical properties of the berries.

These results regarding the effect glutathione and chitosan on promoting berries quality might be ascribed to their positive action on enhancing leaf pigments and total anthocyanins in the berries. Another explanation is their effect in enhancing photosynthesis process (Mullineaux and Rausch, 2005).

The results of Abdelaal *et al* (2012); Gad El-Kareem (2012); Ahmed *et al* (2012) and (2013); El-Khawaga and Mansour (2014) and Madany (2017) supported the beneficial effects of glutathione on berries quality.

These results regarding the promoting effect of chitosan on berries quality are in harmony with those

obtained by Hadwiger et al (2002); Eweis et al (2006); Xu et al (2007); Liu et al (2007); Dang et al

(2010); Meng *et al* (2010); Hadwiger (2013); Saied and Radwan, (2017)

Treatment	Main shoot length (cm.)		No. of leaves/s	No. of leaves/shoot		Leaf area (cm.) ²		Wood ripening coefficient		:55	Pruning wood weight/vine (kg)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	106.36	107.1	16.0	15.0	87.3	88.0	0.66	0.65	1.00	1.02	1.95	1.99
Glutathione at 0.025 %	108.0	108.7	17.0	18.0	89.0	89.8	0.70	0.69	1.08	1.09	2.05	2.10
Glutathione at 0.05 %	109.9	110.6	18.0	19.0	91.0	91.7	0.74	0.73	1.16	1.17	2.16	2.21
Glutathione at 0.1 %	110.0	110.8	18.0	19.0	91.3	91.8	0.75	0.74	1.17	1.19	2.18	2.22
Chitosan at 0.05 %	111.9	112.7	20.0	21.0	93.0	93.9	0.80	0.77	1.24	1.26	2.31	2.33
Chitosan at 0.1 %	113.8	114.5	22.0	22.0	95.0	96.0	0.84	0.81	1.31	1.33	2.41	2.44
Chitosan at 0.2 %	114.0	114.7	23.0	22.0	95.3	96.3	0.85	0.81	1.32	1.34	2.42	2.46
Glutathione + Chitosan at low conc.	115.7	116.5	25.0	25.0	97.0	98.3	0.89	0.85	1.40	1.41	2.55	2.59
Glutathione + Chitosan at mid. conc.	118.8	119.5	26.0	27.0	98.5	100.0	0.94	0.90	1.47	1.49	2.69	2.71
Glutathione + Chitosan at high conc.	119.0	119.8	26.3	27.4	98.6	100.4	0.95	0.91	1.48	1.50	2.71	2.72
New L.S.D. at 5%	1.4	1.6	1.0	1.0	1.4	1.6	0.04	0.04	0.05	0.03	0.06	0.07

Table (2): Effect of single and combined applications of glutathione and chitosan on some vegetative growth
characteristics of Red Roomy grapevines during2015 and 2016 seasons

Table (3): Effect of single and combined applications of glutathione and chitosan on some leaf pigments and
percentages of N and P in the leaves of Red Roomy grapevines during2015 and 2016 seasons

Treatment	Chlorop (mg/1g F	hyll a '.W)	Chlorop (mg/1g F	hyll b ".W)	Total chl (mg/1g F.	orophylls .W)	Total caroteno (mg/1g F	ids '.W)	Leaf N	N %	Leaf I	P %
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	1.11	1.18	0.91	0.95	2.01	2.13	1.00	1.01	1.61	1.55	0.14	0.13
Glutathione at 0.025 %	1.20	1.28	0.95	1.00	2.15	2.28	1.06	1.07	1.69	1.63	0.17	0.17
Glutathione at 0.05 %	1.29	1.36	1.00	1.07	2.29	2.43	1.12	1.13	1.79	1.77	0.20	0.20
Glutathione at 0.1 %	1.30	1.37	1.02	1.08	2.32	2.45	1.13	1.14	1.80	1.79	0.21	0.21
Chitosan at 0.05 %	1.41	1.50	1.10	1.12	2.51	2.62	1.20	1.21	1.89	1.88	0.24	0.25
Chitosan at 0.1 %	1.55	1.61	1.14	1.16	2.69	2.77	1.29	1.31	1.99	1.98	0.28	0.28
Chitosan at 0.2 %	1.57	1.63	1.15	1.17	2.72	1.80	1.30	1.32	2.01	2.00	0.29	0.29
Glutathione + Chitosan at low conc.	1.74	1.76	1.20	1.22	2.99	2.98	1.39	1.41	2.11	2.13	0.34	0.33
Glutathione + Chitosan at mid. conc.	1.85	1.88	1.26	1.27	3.11	3.15	1.50	1.51	2.21	2.22	0.40	0.34
Glutathione + Chitosan at high conc.	1.87	1.89	1.27	1.28	3.14	3.16	1.51	1.52	2.22	2.23	0.41	0.35
New L.S.D. at 5%	0.05	0.03	0.02	0.03	0.05	0.06	0.03	0.04	0.05	0.07	0.02	0.03

Treatment	Leaf K %		Leaf %	Mg Leaf S %		S %	Leaf Zn (ppm)		Leaf Mn (ppm)		Leaf Fe (ppm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	1.16	1.14	0.50	0.49	0.55	0.53	49.7	47.9	50.3	50.9	51.7	52.0
Glutathione at 0.025 %	1.22	1.18	0.53	0.54	0.59	0.60	52.0	50.9	53.6	54.0	54.8	55.0
Glutathione at 0.05 %	1.30	1.22	0.57	0.58	0.64	0.64	54.3	53.3	56.0	56.9	57.9	58.0
Glutathione at 0.1 %	1.31	1.23	0.58	0.59	0.65	0.65	55.0	53.4	56.6	57.0	58.0	58.4
Chitosan at 0.05 %	1.41	1.40	0.62	0.64	0.69	0.68	58.0	56.0	60.0	60.0	61.0	62.0
Chitosan at 0.1 %	1.50	1.45	0.67	0.67	0.74	0.73	61.0	59.0	62.9	63.0	64.0	65.0
Chitosan at 0.2 %	1.51	1.46	0.68	0.68	0.75	0.74	61.3	59.3	63.9	63.3	64.3	65.6
Glutathione + Chitosan at low conc.	1.56	1.50	0.72	0.73	0.79	0.78	64.0	62.9	67.0	67.0	68.0	68.6
Glutathione + Chitosan at mid. conc.	1.61	1.57	0.76	0.78	0.82	0.83	66.0	66.0	69.9	69.9	71.0	71.9
Glutathione + Chitosan at high conc.	1.62	1.58	0.77	0.79	0.83	0.84	66.3	66.4	70.0	70.0	71.6	72.0
New L.S.D. at 5%	0.04	0.02	0.02	0.03	0.03	0.02	1.9	2.1	2.2	2.3	2.4	2.3

Table (4): Effect of single and combined applications of glutathione and chitosan on the leaf content of K, Mg and S (as %) and Zn, Mn and Fe (as ppm) in the leaves of Red Roomy grapevines during2015 and 2016 seasons

Table (5): Effect of single and combined applications of glutathione and chitosan on the percentages of berry setting, yield as well as cluster weight and dimensions of Red Roomy grapevines during2015 and 2016 seasons

Treatment	Berry setting %		No. of clusters/vine		Yield/vine (kg.0		Cluster weight (g.)		Cluster length (cm)		Cluster shoulder (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	5.9	5.7	25.0	24.0	8.2	7.8	327.0	325.0	19.4	19.0	12.3	12.0
Glutathione at 0.025 %	6.9	6.7	25.0	26.0	8.5	8.7	340.0	335.0	20.0	19.9	12.7	12.4
Glutathione at 0.05 %	8.0	7.7	25.0	28.0	8.8	9.7	350.0	345.0	20.6	20.5	13.1	12.8
Glutathione at 0.1 %	8.1	7.8	26.0	29.0	9.1	10.0	351.0	346.0	20.7	20.6	13.2	12.9
Chitosan at 0.05 %	9.0	8.9	26.0	31.0	9.4	11.0	362.0	356.0	21.2	21.1	13.6	13.5
Chitosan at 0.1 %	10.0	9.9	26.0	33.0	9.7	12.1	372.0	366.0	21.7	21.6	14.1	14.0
Chitosan at 0.2 %	10.1	10.0	26.0	34.0	9.7	12.5	373.0	367.0	21.8	21.8	14.2	14.1
Glutathione + Chitosan at low conc.	11.0	11.1	26.0	36.0	10.0	13.6	385.0	377.0	22.3	22.4	14.6	14.6
Glutathione + Chitosan at mid. conc.	11.9	12.1	26.0	36.0	10.3	14.0	395.0	388.0	23.0	22.9	15.0	15.0
Glutathione + Chitosan at high conc.	12.0	12.3	26.0	36.0	10.3	14.0	396.0	389.0	23.2	23.0	15.1	15.1
New L.S.D. at 5%	0.9	0.7	NS	2.0	0.6	0.8	10.0	9.4	0.4	0.4	0.4	0.3

Treatment	Berries colouration %		Shot berries %		Av. Berry weight (g)		Av. Berry longitudinal (cm)		Av. Berry equatorial (cm)		T.S.S. %	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	54.1	55.0	9.0	9.2	5.00	4.99	2.14	2.11	1.80	1.77	18.0	18.1
Glutathione at 0.025 %	54.5	55.5	8.0	8.0	5.20	5.19	2.20	2.19	1.86	1.83	18.5	18.7
Glutathione at 0.05 %	55.6	56.0	7.0	6.9	5.39	5.40	2.25	2.24	1.91	1.90	18.9	19.1
Glutathione at 0.1 %	55.7	56.5	6.9	6.8	5.40	5.41	2.26	2.25	1.92	1.91	19.0	19.2
Chitosan at 0.05 %	61.4	62.0	5.0	4.9	5.60	5.61	2.31	2.30	1.97	1.98	19.5	19.6
Chitosan at 0.1 %	66.0	67.9	4.0	3.9	5.79	5.80	2.36	2.35	2.02	2.03	20.0	20.1
Chitosan at 0.2 %	66.3	68.0	3.9	3.8	5.80	5.81	2.37	2.36	2.03	2.04	20.1	20.2
Glutathione + Chitosan at low conc.	69.9	71.0	3.0	2.9	5.99	6.00	2.44	2.45	2.10	2.11	20.6	20.8
Glutathione +												
Chitosan at mid. conc.	74.0	75.5	2.0	1.9	6.14	6.16	2.50	2.51	2.15	2.16	21.1	21.3
Glutathione +												
Chitosan at high	74.3	75.7	1.9	1.8	6.15	6.17	2.51	2.52	2.16	2.17	21.2	21.4
conc.												
New L.S.D. at 5%	0.4	0.3	0.7	0.9	0.11	0.14	0.05	0.05	0.04	0.04	0.4	0.4

Table (6): Effect of single and combined applications of glutathione and chitosan on the percentages of berries colouration and shot berries and some physical and chemical characteristics of the berries of Red Roomy grapevines during2015 and 2016 seasons

Table (7): Effect of single and combined applications of glutathione and chitosan on some chemical characteristics of the berries of Red Roomy grapevines during 2015 and 2016 seasons

Treatment	Total sug	ars %	Total aci	dity %	Total anthocyanins (mg/1g F.W)		T.S.S./acid	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	16.9	17.0	0.719	0.718	2.11	2.14	25.03	25.21
Glutathione at 0.025 %	17.4	17.3	0.691	0.694	2.20	2.24	26.77	26.95
Glutathione at 0.05 %	17.8	17.7	0.670	0.669	2.31	2.41	28.21	28.55
Glutathione at 0.1 %	17.9	18.0	0.669	0.667	2.32	2.42	28.40	28.79
Chitosan at 0.05 %	18.4	18.4	0.647	0.645	2.50	2.54	30.14	30.39
Chitosan at 0.1 %	18.8	18.8	0.629	0.621	2.61	2.66	31.80	32.37
Chitosan at 0.2 %	18.9	18.9	0.628	0.620	2.62	2.67	32.01	32.58
Glutathione + Chitosan at low conc.	19.4	19.5	0.600	0.594	2.74	2.75	34.33	35.02
Glutathione + Chitosan at mid. conc.	19.8	19.9	0.580	0.572	2.85	2.87	36.38	37.24
Glutathione + Chitosan at high conc.	19.9	20.0	0.577	0.570	2.86	2.88	36.74	37.54
New L.S.D. at 5%	0.3	0.3	0.016	0.015	0.07	0.08	0.92	0.96

Conclusion:

Three sprays at growth start, just after berry setting and one month later of glutathione at 0.05 % and chitosan at 0.1 % was responsible for improving yield, berry setting and berries colouration and quality of Red Roomy grapevines.

References

- Abdelaal, A.M.K.; Masoud, A.A.B. and Mohamed, A.Y. (2012): Response of Taimour mango trees to application of the antioxidant glutathione. Menufiya J., Agric. Res. Vol. (3): 303-310..
- 2. Ahmed, F. F. and Morsy, M. H. (1999): A new method for measuring leaf area in different fruit species. Minia J. of Agric. Res. & Develop., Vol. (19) pp 97-105.
- 3. Sanford, P.A. (2002): Commercial sources of chitin and chitosan and their utilization. Advances in chitin siene, 6, 35-42.
- Saied, H.H.M. and Radwan, E.M.A. (2017): Insight into the effect of chitosan on growth and fruiting of succary mango trees. J. Product & Dev. 22(3): 781-793.
- 5. Ahmed, F.F.; Al-Wasfy, M.M. and Madian, A.M. (2012): Fruiting of Zaghloul date palms in

response to foliar application of the antioxidant glutathione. Minia J. of Agric. Res. & Develop. 32(4): 1123-1140.

- Association of Official Agricultural Chemists (A.O.A.C.) (2000): Official Methods of Analysis (A.O.A.C), 12th Ed., Benjamin Franklin Station, Washington D.C., U.S.A.pp.490-510.
- Balo, E.; Prilesszky, G.; Happ, I.; Kaholami, M, and Vega. L. (1988): Soil improvement and the use of leaf analysis for forecasting nutrient requirements of grapes. Potash Review (Subject 9, 2nd suite, No. 61: 1-5).
- Bouard, J. (1966): Recherches physiologiques sur la vigne et en particulier sur laoutment des serments. Thesis Sci. Nat. Bardeux, France p. 34.
- 9. Chapman, H.D. and Pratt, P.E. (1987): Methods of Analysis for Soil, Plant and Water. Univ. California, Div. Agric. Sci. 172-173.
- Cottenie, A.; Cerloo, M.; Kiekens, L.; Velgle, G. and Amerlynuck, R. (1982): Chemical analysis of plant and soil. 34-51. Laboratory of Analytical and Agroch. State Univ. Belgium, Gent.
- Dang, Q. F., Yan, J. Q., Li, Y., Cheng, X. J., Liu, C. S., & Chen, X. G. (2010). Chitosan acetate as an active coating material and its effects on the storing of Prunus avium L. *Journal of food science*, 75(2).
- 12. El-Khawaga, A.S. and Mansour, A.G.M. (2014): Promoting productivity of Washington Navel orange trees by using some crop seed sprout extracts, silicon and glutathione. Middle East Journal of Applied Sciences, 4(3): 779-785.
- Eweis, M., Elkholy, S. S., & Elsabee, M. Z. (2006). Antifungal efficacy of chitosan and its thiourea derivatives upon the growth of some sugar-beet pathogens. *International Journal of Biological Macromolecules*, 38(1), 1-8.
- Fadl, M.S. and sari- El- Dean, S.A. (1978): Effect of N. Benzyl adenine on photosynthetic pigments and total soluble sugars on olive seedlings growth under saline. Condition. Res. Bulletion No. 843. Fac. Agric. Ain Shams Univ. Egypt.
- Fulcki, T. and Francis, F.J. (1968): Quantitative methods for anthocyanins II Determination of total anthocyanins and degradative index for berry juice J. Food Sci. 33: 78-83.
- Gad El- Kareem, M.R. (2012): Improving productivity of Taimour mango trees by using glutatione, silicon and vitamin B. Minia J. of Agric. Res. & Develop 32 (7): 1105-1121.
- Hadwiger, L. A. (2013): Multiple effects of chitosan on plant systems: solid science or hype. Plant Sci. 208, 42–49.
- Hadwiger, L. A., Klosterman, S. J., & Choi, J. J. (2002). The mode of action of chitosan and its

oligomers in inducing plant promoters and developing disease resistance in plants. *Advances in chitin science*, *5*, 452-457.

- Lane, J. H. and Eynon, L. (1965): Determination of reducing sugars by means of Fehlings solution with methylene blue as indicator A.O.AC. Washington D.C.U.S.A. pp.490- 510.
- Liu, J., Tian, S., Meng, X., & Xu, Y. (2007). Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biology and Technology*, 44(3), 300-306.
- 21. Madany, M.H.G. (2017): Response of succary mango trees to foliar applications of glutathione and boric acid. M.Sc. Thesis Fac. of Agric. Minia Univ. Egypt.
- 22. Mead, R.; Currnow, R. N. and Harled, A. M. (1993): Statistical Methods in Agriculture and Experimental Biology. Second Ed. Chapman & Hall London.pp 10-44.
- Meng, X., Yang, L., Kennedy, J. F., & Tian, S. (2010). Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. *Carbohydrate Polymers*, *81*(1), 70-75.
- 24. Mulleineaux, P.M. and Rausch, T. (2005): Glutathione, photosynthesis and the redox regulation of stress responsive gen expression photosynthesis. Res. 47: 459-474.
- 25. Peach, K and Tracey, I.M.V. (1968): Modem Methods of Plant Analysis, Vol. lip. 37-38.
- von Wettstein, D. (1957): Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. Experimental Cell Research, 12(3): 427-506.
- Weaver, R. J. (1976): Grape Growing. A Wiley Interscience Publication John Wiley & Davis, New York, London, Sydney, Tronto pp. 160-175.
- Wilde, S. A.; Corey, R. B.; Layer, J. G. and Voigt, G. K. (1985): Soils and Plant Analysis for Tree Culture. Mohan Primlani, Oxford & IBH Publishing Co., New Delhi, India, p 1- 142.
- 29. Winkler, A.J.; Cooke, A.J. Kliewer, W.M. and Lider, L.A. (1974): General Viticulture. California Univ. Press, Berkley pp. 60 - 74.
- Xing, K., Zhu, X., Peng, X., & Qin, S. (2015). Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. *Agronomy for Sustainable Development*, 35(2), 569-588.
- 31. Xu, J., Zhao, X., Han, X., & Du, Y. (2007). Antifungal activity of oligochitosan against Phytophthora capsici and other plant pathogenic fungi in vitro. *Pesticide Biochemistry and Physiology*, 87(3), 220-228.

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