

Article



Response of Early Sweet Grapevines to Foliar Applying with Royal Jelly and Glutathione

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Abstract: Vegetative growth aspects and leaves nutritional status of 8year-old Early Sweet grapevines grown in clay soil in response to applying various concentrations of royal jelly (100, 200 and 400 ppm) and/or glutathione at (250, 500 and 1000 ppm) separately or in combination, three times during 2020 and 2021 in a privet Farm located in a privet Farm located at West Mallawy Center-Minia Governorate. Applying glutathione was more effective than the royal jelly. Elevating two highest levels of all treatments did not significantly affected the studied parameters. However, the dual addition was more effective than the solo one and the best findings with regard to vegetative growth, leaf pigments and leaf chemical content were obtained when the vines sprayed with medium concentration royal jelly (200 ppm) and glutathione (500 ppm) thrice from economical point during two seasons.

Key words: Royal jelly, glutathione, vegetative, nutritional statues and Early Sweet grapevines

1. Introduction

The grapevine (*Vitis vinifera* L.) is recognized as the most economically significant crop on a global scale and is the second most important crop in Egypt. Table grapes are the most frequently grown grape varieties in Egypt, and they are all composed of European grape cultivars (**Mohamed** *et al.*, **2019**). The area allocated for grape cultivation totaled 85,240 ha, yielding 1,435000 tons (**FAO**, **2023**). Grapes are considered a major horticultural commodity for export. The volume of this product constitutes roughly 3% of total horticultural exports, whereas its export value accounts for approximately 10%. Annual exports of Egyptian grapes have reached around 131 thousand tons, with expectations for future increases (**M.A.L.R.**, **2019**).

The global marketing process for table grapes is heavily impacted by berry quality. Producers often utilize mineral fertilizers to enhance fruit size, leading to various health concerns. Some of these

methods remain in use due to their substantial impact on fruit diameter enhancement, subsequently leading to increased yield. Therefore, pursuing safer alternative agricultural practices is essential to achieve this objective. The application of bio-stimulants serves as a supplement to enhance grape productivity and reduce reliance on chemical fertilizers (**Masoud** *et al.*, **2024**).

To enhance the growth and productivity of trees in challenging conditions, recent endeavors have focused on identifying safe, natural materials suitable for application in the horticultural sector. In contemporary times, there has been an increased focus on employing natural and environmentally friendly stimulants to enhance both the yield and the quality of Early Sweet grapevines. Bio-stimulants enhance the efficiency of resource use (fertilizers, nutrients, and water) by modulating biochemical, molecular, and plants physiological processes, thereby improving photosynthesis, plant growth, quality, and tolerance to biotic and abiotic stresses (**Zulfiqar** *et al.*, **2019; Rouphael and Colla, 2020**).

Royal jelly is a nutrient-dense natural substance with substantial potential application in cosmetics, medicine, and as a health-enhancing diet. This bee product comprises essential elements, including minerals (such as Mg, K, Ca, P, Fe, S & Mn), proteins, hormones, lipids, flavonoids, neurotransmitters, and polyphenols, which contribute to the notable biological and therapeutic properties of royal jelly (**Oršolić** *et al.*, **2024**), vitamins such as E, B5, B9, B6, & B12 (**Wang**, **2016** and **Balkanska** *et al.*, **2017**).

Currently, there is a significant amount of interest in the external supplementation of vitamins and substances to enhance growth and development of plant. This is due to the fact that these products are natural, such as glutathione, (GSH) is a tripeptide composed of L-glutamyl, L-cysteinyl, and glycine, which is utilized in antioxidants and bio-stimulants that play a role in regulating the reduction and oxidation processes within the cell of plant. Glutathione has a variety to plays a role in plant growth and development that other antioxidants are unable to contribute or perform to, such as detoxification, oxidation balance and stabilization within the cell of plant (**Noctor** *et al.*, **2012**). Additionally, it protects the thiol groups in proteins, preventing oxidative denaturation under stress. Additionally, it functions as a substrate for glutathione S-transferase, glutathione peroxidases, and glutaredoxin, which are implicated in floral development and plant defense signaling (**Rouhier** *et al.*, **2008 and Hasanuzzaman** *et al.*, **2017**). The application of glutathione at varying concentrations and frequencies positively influenced the quality and yield of most fruit crops (**Ahmed** *et al.*, **2018; Dawood** *et al.*, **2020**).

This study aimed to examine the effects of different levels of royal jelly and glutathione on Early Sweet grapevine development, leaf chemical status in the Minia area.

2. Materials and Methods

2.1. Plant materials

This research was conducted in a privet Farm located at West Mallawy Center-Minia Governorate, on 8-year-old Early Sweet grapevines grafted onto Paulsen rootstock and planted at 2 m (between vine) x 3 m (between rows) in clay soil, irrigated by a surface irrigation system using Nile water. To study the impact of foliar sprinkle with royal jelly and glutathione at various levels on Early Sweet grapevines. The cane pruning system was implemented using the Gable shape supporting technique. The vine load for all the selected vines was modified to 6 fruiting spurs plus 10 eyes and 6 renewal spurs with 2 eyes, resulting in a total of 72 buds left on each vine. The chosen 30 vines were picked as homogeneous in vigor as feasible and dedicated to this investigation and received the standard horticulture techniques often employed in the vineyard.

The physical and chemical examination of the examined soil is detailed in Table A as per **Wilde** *et al.* (1985).

Soil	Pa	rticle size di	stribution (ECppm (1:2.5	pH	Organic matter	CaCO ₃		
Years	Sand	silt	Clay	T. class	extract)	(1:2.5 extract)	matter %	%	
	1.97	36.87	61.16	Clay	296	7.56	2.22		
2020	Soil nutrients								
/2021	Total N (%)	Available P (ppm)	Available K (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	2.29	
	0.11	5.36	497.5	2.6	3.2	3.8	0.8		

Table (A). Analysis of the tested soil

2.2. Examined designs and treatments

The experiment consisted of ten treatments organized in a Randomized Complete Block design, with every treatment reproduced thrice, with one vine each replication, including the control treatment, applications of glutathione and royal jelly separately and in combination. The treatments were arranged as follows:

- 1) Control (spray with tap water).
- 2) Royal jelly (100 ppm).
- 3) Royal jelly (200 ppm).
- 4) Royal jelly (400 ppm).
- 5) Glutathione (250 ppm).
- 6) Glutathione (500 ppm).
- 7) Glutathione (1000 ppm).
- 8) Royal jelly (100 ppm) + glutathione (250 ppm).
- 9) Royal jelly (200 ppm) + glutathione (400 ppm).
- 10) Royal jelly (400 ppm) + glutathione (1000 ppm)

Royal jelly and glutathione were applied via spraying three times: at the onset of growth, immediately after berry setting, and one month thereafter. Chemical analysis of royal jelly presented in Table (B).

2.3. Measurements

Across the investigative seasons (2020 and 2021), the subsequent data were recorded:

Characteristics of vegetative development as main shoot length (cm), number of leaves/shoot, leaf area (cm²) as per **Ahmed and Morsy (1999**), Leaf area = $0.56 (0.79 \times W^2) + 20.01$, where W represents the maximum leaf width, cane thickness (mm) and pruning wood weight/vine (kg).

- Chemical analysis of leaves: pigments: utilizing the **Von Wettstein** (**1957**) methodology: chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids as well as the concentrations of N, P, and K (%), together with the levels of Zn, Fe, and Mn (ppm) in the leaf petioles linked to the basal clusters, were examined using the methodologies of **Cottenie** *et al.* (**1982**) and **Balo** *et al.* (**1988**) in the first week of July.

2.4. Statistical analysis

All gathered data were systematically arranged into tables and subjected to statistical analysis following the technique of **Mead** *et al.* (1993), with treatment means compared utilizing the new LSD test at a significant level of 5%.

Constituents	Values mg/ 100 g F.W.
Dry matter	34.7
Portents	48.2
Carbohydrate	37.8
Lipids	10.4
Ash	2.0
Sugar	23.0
Glucose	4.0
Fructose	4.0
Sucrose	5.0
Nutrients (ppm)	
K	220
Mg	105
Ca	112
Fe	50
Р	118
S	44
Mn	32
Si	5
Vitamins (mg/ 100 g F.W.)	
Vitamins B ₁	0.4
Vitamins B ₂	0.3
Vitamins B ₅	0.4
Vitamins B ₆	0.3
Vitamins B ₈	0.3
Vitamins B ₉	0.4
Vitamins B ₁₂	0.3
Α	0.4
С	0.9
D	0.5
K	0.4
Е	0.3
Essential amino acids	1100

Table (B). Royal jelly chemical analysis of according to Townsend and Lucas (1966)

3. Results and Discussion

3.1. Characteristics of growth

The data shown in Table (1) clearly indicate that administering Early Sweet grapevines thrice with royal jelly and/or glutathione considerably enhanced main shoot length, number of leaves/shoot, leaf area, average pruning weight, and cane thickness in comparison to the control vines. A gradual enhancement of these parameters occurred with increasing level of royal jelly and glutathione. Elevating levels of royal jelly from 200 to 400 ppm and glutathione from 500 to 1000 ppm did not significantly enhance the height of the main shoot, number of leaves, leaf area, average pruning weight, and cane thickness. The application of glutathione was markedly more effective in enhancing these measurements than the use of royal jelly. The combined application of royal jelly and glutathione considerably enhanced the traits compared to the application of each substance individually. From an economic perspective, optimal results were achieved by administering of medium concentration royal jelly (200 ppm) and glutathione (500 ppm) thrice to the grapevines. Under this treatment, the main shoot length, number of leaves, leaf area, average pruning weight, and cane thickness attained (118.1 - 118.6 cm), (19.2 - 19.3), (112.5 - 113.5 cm²), (2.15 - 2.13 kg) and (12.7 - 12.6 mm) during the 2020 and 2021 seasons, respectively.

The elevated levels of royal Jelly, comprising macro and micronutrients, plant pigments, vitamins, amino acids, antioxidants, organic substances and natural hormones (Townsend and Lucas, 1966; Nation and Robinson, 1971), undoubtedly contribute to the enhancement of cell division and

photosynthesis, thereby promoting the growth of robust plants. These findings align with those acquired by El-Sayed *et al.* (2017); Hussein (2019); Sabry *et al.* (2021); Ali and Ahmed (2023).

The glutathione compound actively participates in different metabolic processes within the plant, thereby enhancing growth, this has a favorable impact the vegetative system's functionality. This results in improved characteristics of the vegetative system due to cell expansion. Glutathione consists of small oxidizing and reducing molecules that contribute to the synthesis of salicylic acid and the plant's defense signals. Salicylic acid aids in the maintenance of auxins, inhibits the enzyme IAA oxidase, and promotes the increase of cytokinins and gibberellins, thus promoting plant growth by encouraging division in meristematic areas (**Gharib and Hegazi, 2010**). This could be explained by glutathione's involvement in cell elongation and division, as it functions as an antioxidant that safeguards cells from degradation and preserves them in their active state. These findings regarding glutathione's beneficial impact on development are consistent with **Saied (2019); and Abdel-Wahed (2022); Ali and Ahmed (2023).**

Characteristics	Main shoots height (cm)		Leaves number/shoot		Leaf area (cm²)		Pruning wood weight (kg)/vine		Cane thickness (mm)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Control	111.4	112.0	13.0	13.4	106.0	107.5	1.80	1.85	11.1	11.3
Royal jelly (100 ppm)	114.0	114.5	15.1	15.4	108.1	109.5	1.90	1.93	11.6	11.7
Royal jelly (200 ppm)	115.6	115.9	16.6	16.8	109.7	111.0	1.99	2.00	12.0	12.0
Royal jelly (400 ppm)	116.7	117.0	17.8	17.9	110.9	112.1	2.06	2.04	12.2	12.1
Glutathione (250 ppm)	115.4	116.0	16.5	16.8	109.6	110.8	1.98	1.99	11.9	12.0
Glutathione (500 ppm)	116.9	117.0	17.8	18.1	111.1	112.2	2.07	2.05	12.3	12.4
Glutathione (1000 ppm)	118.1	118.4	19.0	19.1	112.4	113.4	2.13	2.10	12.4	12.5
Royal jelly (100 ppm) + glutathione (250 ppm)	116.7	117.3	17.9	18.0	111.0	112.1	2.06	2.06	12.3	12.3
Royal jelly (200 ppm) + glutathione (500 ppm)	118.1	118.6	19.2	19.3	112.5	113.5	2.15	2.13	12.7	12.6
Royal jelly (400 ppm) + glutathione (1000 ppm)	119.3	119.7	20.4	20.3	113.7	114.6	2.21	2.17	12.9	12.7
New LSD at 5%	1.3	1.2	1.3	1.2	1.4	1.3	0.08	0.06	0.3	0.2

 Table (1). Vegetative growth of Early Sweet grapevines as influenced by foliar applying with royal jelly and glutathione throughout 2020 and 2021 seasons

3.2. Leaf pigments mg/100 g F.W.

It is obvious from the data in Table 2 that spraying the both of royal jelly and /or glutathione at various levels significantly enhanced chlorophylls a, b, total chlorophylls and total carotenoid in the leaves of Early Sweet grapevines in comparison to the control treatment. The most suitable material in this context was glutathione, and the untreated vines held the final position in this regard. Promotion of these constituents was deemed ineffective as concentrations of royal jelly increased from 200 to 400 ppm and 500 to 1000 ppm of glutathione. The highest values were observed in the vines treated with glutathione at a concentration of 1000 ppm. The combination between two materials was more effective than solo application. At the elevated concentrations of 400 ppm royal jelly combined with 1000 ppm glutathione and 200 ppm royal jelly with 1000 ppm glutathione, no observable enhancement in chlorophyllase content and total carotenoid was noted. The control treatments yielded the lowest levels of chlorophyll a, b, total and total carotenoid (4.0-3.8, 1.1-1.0, 5.1-4.8 and 1.2-1.1 mg/100g FW),

respectively, across the two seasons. The leaf pigments measured in the other treatments showed a moderate range of values. The results remained consistent across both seasons.

Chlorophylls are acknowledged as remarkable natural compounds on our planet, essential for the process of photosynthesis (**Mochizuki** *et al.* **2010; Dalal and Tripathy, 2012**). This form of vegetation primarily relies on the absorption of light rays by chlorophyll, particularly chlorophyll A. This mechanism is vital for the production of organic substances required for the growth and development of plants (**Popescu and Popescu, 2014**). Chlorophyll and carotene, which are essential for plant physiological activity, are present in the leaves of the majority of plant species (**Peter** *et al.* **2010; Croft** *et al.* **2017**). The enhancement of photosynthesis during growth phases through the application of foliar royal jelly can be attributed to the increase in pigments. The results obtained are in accordance with those of **Abdel-Rahman** *et al.* **(2019); Hussein (2019); Ali and Ahmed (2023).**

Glutathione comprises glutamate, cysteine, and glycine, with cysteine contributing to the sulfur bond as a sulfur donor that protects cells from free radicals. Additionally, glutathione plays a crucial role in photosynthesis (**Bianucci**, 2008). When glutathione was applied to the leaves, the amount of total chlorophyll increased because the magnesium levels rose, which is essential for chlorophyll synthesis (**Amini** *et al.*, 2011). The elevation of amino acids and the photosynthetic process, as noted by **Mullineaux and Rausch** (2005), could clarify the way glutathione enhances the production of plant pigments. The results align with those obtained by **Saied** (2019); **Shoug** (2022); **Ali and Ahmed** (2023).

Characteristics	Chlorophyll a mg/100 g FW		Chlorophyll b mg/100 g FW		Total chlorophyll mg/100 g FW		Total carotenoid mg/100 g FW	
	2020	2021	2020	2021	2020	2021	2020	2021
Control	4.0	3.8	1.1	1.0	5.1	4.8	1.2	1.1
Royal jelly (100 ppm)	5.1	4.8	1.5	1.5	6.6	6.3	2.2	2.0
Royal jelly (200 ppm)	5.6	5.4	1.8	1.9	7.4	7.3	2.6	2.3
Royal jelly (400 ppm)	5.8	5.7	1.9	2.1	7.7	7.8	2.8	2.5
Glutathione (250 ppm)	5.4	5.3	1.8	1.9	7.2	7.2	2.5	2.4
Glutathione (500 ppm)	5.7	5.8	2.2	2.2	7.9	8.0	2.9	2.8
Glutathione (1000 ppm)	5.8	6.0	2.3	2.4	8.1	8.4	3.0	3.0
Royal jelly (100 ppm) + glutathione (250 ppm)	5.8	5.7	2.0	2.3	7.8	8.0	2.9	2.7
Royal jelly (200 ppm) + glutathione (500 ppm)	6.1	6.2	2.4	2.6	8.5	8.8	3.2	3.0
Royal jelly (400 ppm) + glutathione (1000 ppm)	6.3	6.5	2.5	2.7	8.8	9.2	3.4	3.1
New LSD at 5%	0.3	0.4	0.2	0.3	0.4	0.6	0.3	0.3

Table (2). Leaf pigments content of Early Sweet grapevines as influenced by foliar applying with
royal jelly and glutathione throughout 2020 and 2021 seasons

3.3. The nutritional quality of leaves

Presented data in Table (3) denote that leaf N, P, K%, Zn, Fe and Mn ppm greatly impacted by the foliar administration of royal jelly and / or glutathione as compared to control treatment (sprayed with tap water) in the two seasons. Consequently, the greatest N, P, K%, Zn, Fe and Mn ppm mean values (1.90-1.85%, 0.27% in both seasons, 1.22-1.26%, 61.4-62.9 ppm, 60.9-60.4 ppm and 62.4-

62.5 ppm) were achieved from 1000 ppm glutathione. The superiority of applying 500 ppm concentration was regard to vines as individual economic addition. Meaning that glutathione was more effective than the royal jelly. The interaction between royal jelly and glutathione concentrations indicated that minerals in leaves increased in proportion to an increase in concentration, with no significant difference observed between the two maximum concentrations. The data revealed that the control group exhibited the lowest values of nutrient in leaves. In contrast, the application of 400 ppm royal jelly combined with 1000 ppm glutathione yielded the highest mean values (1.97-1.91%, 0.31-0.30%, 1.30-1.35%, 64.3-65.3 ppm, 62.7-62.4 ppm and 64.3-64.5 ppm). This was comparable to the results obtained from 200 ppm royal jelly and 500 ppm glutathione, which recorded values of (1.92-1.88%, 0.30-0.29%, 1.27-1.31%, 62.5-63.3 ppm, 61.1-60.6 ppm and 62.7 - 62.9 ppm).

The promotion of leaf pigments and nutrients by Royal Jelly may be attributed to its increased concentration of plant pigments, and different minerals as (N, Mg, P, Zn, K, Fe, Cu and Mn), with increased levels of antioxidants and amino acids (**Wang, 2016**), as well as its ability to facilitate the assimilation of water and root development (**Townsend and Lucas, 1966**). Our findings are consistent with those attained by **Wassel** *et al.* (2015); El-Sayed *et al.* (2017); Hussein (2019); Ali and Ahmed (2023).

The beneficial effect of glutathione on promoting root development and nutrient uptake may account for the observed results. Also, can be attributed to its essential function in regulating and sustaining the intercellular redox system and chloroplast metabolism, where it offers the ability to buffer redox, which is essential for efficient photosynthesis, and takes part in the management of oxidants generated during capturing light and then transporting electrons (Kocsy *et al.*, 2001; Mulleineaux and Raucsh, 2005). These outcomes are consistent with those seen by Ahmed *et al.* (2018); Saied (2019); Hameed and Abdel-Wahed (2022).

Characteristics	Leaf N%		Leaf P%		Leaf K%		Leaf Zn ppm		Leaf Fe ppm		Leaf Mn ppm	
Treatments	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Control	1.60	1.64	0.15	0.14	1.07	1.08	51.0	50.8	50.6	49.5	51.6	52.0
Royal jelly (100 ppm)	1.71	1.70	0.19	0.19	1.12	1.15	56.1	56.3	55.8	54.5	56.7	57.0
Royal jelly (200 ppm)	1.79	1.76	0.22	0.23	1.16	1.21	58.5	58.8	57.7	56.5	58.9	58.9
Royal jelly (400 ppm)	1.84	1.80	0.23	0.25	1.19	1.24	60.3	60.9	59.3	58.3	60.6	60.5
Glutathione (250 ppm)	1.179	1.77	0.23	0.22	1.17	1.20	58.3	58.6	57.6	56.7	58.8	59.2
Glutathione (500 ppm)	1.86	1.82	0.27	0.27	1.22	1.26	60.5	60.8	59.4	58.7	60.8	61.0
Glutathione (1000 ppm)	1.90	1.85	0.28	0.29	1.24	1.30	61.4	62.9	60.9	60.4	62.4	62.5
Royal jelly (100 ppm) + glutathione (250 ppm)	1.86	1.83	0.27	0.26	1.23	1.26	60.5	60.9	59.4	58.7	60.9	61.0
Royal jelly (200 ppm) + glutathione (500 ppm)	1.92	1.88	0.30	0.29	1.27	1.31	62.5	63.3	61.1	60.6	62.7	62.9
Royal jelly (400 ppm) + glutathione (1000 ppm)	1.97	1.91	0.31	0.30	1.30	1.35	64.3	65.3	62.7	62.4	64.3	64.5
New LSD at 5%	0.06	0.05	0.02	0.03	0.04	0.05	2.0	2.2	1.7	1.9	1.8	1.7

Table (3). Leaves content of minerals of Early Sweet grapevines as influenced by foliar applyingwith royal jelly and glutathione throughout 2020 and 2021 seasons

4. Conclusion

Under Minia governorate and comparable conditions, suppling Early Sweet grapevines with a mixture containing 200 ppm royal jelly and 500 ppm glutathione three times at the onset of growth, immediately after berry setting, and one month thereafter was the most effective treatment from an economic perspective for enhancing growth and leaves chemical content.

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إستجابه العنب "إيرلى سويت" للرش الورقى بتركيزات مختلفة من الغذاء الملكى والجلوتائيون على حسن على - حمدى إبراهيم محمود - مينا نبيل إبراهيم حنا قسم البساتين – كلية الزراعة – جامعة المنيا – مصر

الملخص العربى

إستجابه الصفات الخضرية والمحتوى الكيميائى لكروم العنب "إيرلى سويت" (٨ سنوات) إلى الرش الورقى بمستويات مختلفة من الغذاء الملكى (١٠٠، ٢٠٠، ٢٠٠ جزء فى المليون) و الجلوتاثيون (٢٥٠، ٥٠٠، ١٠٠ جزء فى المليون) ثلاث مرات بصوره فرديه أو مجتمعه معا، المزروعه فى تربه طينيه بمزرعه خاصة غرب مركز ملوى بمحافظة المنيا. وجد أن الرش بالجلوتاثيون كان أكثر فعاليه من الرش بالغذاء الملكى، و لم يلاحظ أى تأثير معنوى عند أعلى تركيزين لجميع المعاملات. و مع ذلك سجل الرش بمزيج منهما قيماً أعلى من الرش فى صوره فرديه و سجلت أفضل القيم للصفات الخضريه و محتوى الأوراق من الصبغات و المعادن من وجه النظر الإقتصاديه عند المستويات المتوسطه بتركيز ٢٠٠ جزء فى المليون غذاء ملكى + ٥٠٠ خلال كلا الموسمين.

الكلمات المفتاحيه: غذاء ملكي، جلوتاثيون، نمو خضري، حاله معدنيه، عنب "إيرلي سويت".



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