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(Original Article)

Influence of Microbial and Synthetic Hormones on the Cluster Quality of Thompson Seedless Grapevines

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Abstract

It is known that there are many problems caused by the use of synthetic hormones on fruit, the most important is that they are not safe for human health in the long term. This work examines some alternatives to synthetic hormones. This research studied the impact of microbial hormones as sources of gibberellins, ascorbic acid, and riboflavin, on the quality of the Thompson grapevine and compared it with synthetic hormones during 2019 and 2020 seasons. The three types of microbial hormones were used alone or combined. synthetic hormones were also used similarly. Some characteristics were measured as indicators of the quality level including yield and the physical and chemical characteristics of berries. The data proved that all treatments, either microbial or synthetic, gave the best effect compared with the control. Microbial hormones had the same effect as synthetic hormones. The best impact was in the combination of the three types of microbial hormones compared with the other treatments.

Keywords: *Ascorbic acid, Aspergillus species, Fusarium species, Gibberellin, Riboflavin.*

Introduction

The grape (*Vitis vinifera* L.,) is one of the most significant fruit crops in the world, used to make raisins and wine. Most grape cultivars grown in Egypt belong to table grapes, all of which are European, and are regarded as a significant source of antioxidants, including vitamins, phenols, flavonoids, anthocyanins, dietary glutathione, and endogenous metabolites (Anastasiadi *et al*., 2010).

Plant growth regulators are compounds that can modify the natural development path by influencing important points in a living plant system. Some physiological impacts include cell elongation, Apical dominance, Parthenocarpy, Abscission, Respiration, Bolting, and Flowering. Numerous studies have shown that plant growth and development could be improved through seed or root inoculation with microbes capable of producing plant growth regulators. However, the effect of Plant growth-promoting rhizobacteria (PGPR) introduced via floral or foliar inoculation of fruit trees, has received little attention.

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Previous studies proved that the treatment with PGPR gave increased growth and yield of apricots, apples, sweet cherries, citrus, raspberries, high-bush blueberries, and mulberries (Abdel-Salam *et al*., 2022).

Several researches have been conducted on the production of organic fruits from vineyards that do not use synthetic hormones, instead promoting the use of bio-stimulants and saving the expenses associated with these pollutants. Using biostimulants is seen to be a viable substitute, especially for poor nations (El-Haddad *et al*., 1993).

According to Saharan and Nehra (2011), plant growth-promoting rhizobacteria (PGPR) are free-living soil rhizosphere and rhizoplane microbes that have positive impacts on plants, both in the rhizosphere and the phyllosphere.

Early yield production with increasing berry size and high quality of clusters are the main characteristic factors for the international markets. So, some farmers overuse synthetic growth regulators, gibberellic acid (GA_3) and cytokinin (BA), to increase berry size.

Plant hormones play a fundamental role in the growth and development of plants, including fruit development, stem elongation, blooming, and seed germination. Gibberellins assist plants in quickly adapting their physiology to ecological changes and changing environmental conditions to meet the demands of an increasing global food demand, particularly in the last few years (Olszewski *et al*., 2002; Fahad *et al*., 2014; Hussain *et al*., 2014). GA_3 has been regularly used in the production of seedless grapes to increase berry and bunch weights, cell division, and cell enlargement as well as to encourage the biosynthesis of proteins and to produce new tissues that improve water and nutrient absorption and induce more vegetative growth. This shifts the balance of competition between reproductive growth and vegetative organs (Soha *et al*.,2018).

Penicillium, *Fusarium*, and *Aspergillus* were the three most on genera sources of gibberellins, according to Tansakul *et al*. (2014).

According to Hafez *et al*. (2010), ascorbic acid is a natural and organic antioxidant compound. One of the plant's main metabolites is ascorbate. This antioxidant shields the plant from oxidative damage brought on by aerobic metabolism, photosynthesis, and various contaminants in combination with other antioxidant system components (Hassanein *et al*., 2009). It functions as an enzymatic cofactor and a plant growth regulator (Gomez and Lajolo, 2008).

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin that plays a significant role in the normal functioning of the human body. The biochemical actions of vitamin C include but are not limited to, histamine detoxification, nitrosamine production, proline hydroxylation, collagen biosynthesis, scurvy prevention, hormonal activation, and antioxidant activity (Walingo, 2005). Additionally, cancer incidence is decreased by vitamin C's antioxidant properties (Lupulescu, 1993). Ascorbic acid has been produced from plant, animal, and microbial sources. This study focused on the use of microorganisms in the production of ascorbic acid. Both Candida and Saccharomyces species have been found to produce erythroascorbic acid (Branduardi *et al*., 2006). In these yeasts, a physiological pathway from D-glucose to erythroascorbic acid has been postulated (Porro and Sauer, 2007).

The micronutrient riboflavin, generally known as vitamin B2, is essential for supporting the health of people, animals, and plants (Sundravel *et al*., 2003).

Riboflavin is a vitamin made by both plants and microorganisms that functions as a coenzyme in numerous physiological processes in both plants and animals as well as in microbes and plants (Dong and Beer, 2000). Currently, vitamin B2 is produced on a large scale both chemically, and biotechnologically. The latter technique is gaining additional significance due to the advantages of biotechnological processes such as cost-effectiveness, less energy required, waste reduction, and therefore the use of renewable feedstocks. (Lim *et al*., 2001).

This study aimed to shed more light on the influence of spraying synthetic and microbial gibberellin (GA_3), ascorbic acid (VC), and riboflavin (B2) on vegetative growth, yield, and berry quality of the Thompson seedless grape cultivar. Also, making the comparison between the effect of microbial gibberellin (GA_3), ascorbic acid (VC), and riboflavin (B2) with synthetic gibberellin (GA_3), ascorbic acid (VC), and riboflavin (B2).

Material and Methods

The experiment was conducted during two consecutive seasons, 2019 and 2020, to study the impact of PGPR and synthetic hormones and compare their effects on the quality of the clusters of the "Thompson Seedless" cultivar, which was cultivated at the Pomology Department, Faculty of Agriculture, Assiut University.

The vines were 15 years old and were planted 2 x 2.5 m apart. Forty-five standardized grapevines were selected in a complete randomized block design (RCBD).

The traditional head training system was used to prune the vines, which included 18 fruiting spurs, four buds per spur, and five replacement spurs per vine. 82 buds were therefore still present on each vine as a whole.

The common agricultural techniques employed in the vineyard, such as irrigation, pest control, and soil fertilization, were applied to all of the vines. A backpack sprayer (25 L) was used to spray vines. At the peak of vine growth, 12 vines might be sprayed with a total of 24 liters.

Preparation of microorganism

Riboflavin

The plants utilized to recover various isolates of *Aspergillus terreus* were maize and Egyptian clover. Every 30 days, the isolates were sub-cultured on potato dextrose agar (PDA) medium while being kept at 4°C.

The capacity of each isolate to synthesize riboflavin in the basal medium was examined (El-Refai *et al.,* 2009).

The high-producer strain was chosen for more research and subsequent CTAB technique extraction of their unique DNA (Lee *et al.,* 1988; Wu *et al.,* 2001).

The riboflavin production medium (basal medium) contains (g/l): glucose, 20.0; NH_4NO_3, 1.0; KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.5; and glycine, 0.2 in distilled water. Before autoclaving, the medium's initial pH was adjusted to 5.2. Using a membrane with a pore size of 0.22 mm, 250 mg/ml chloramphenicol was separately sterilized by membrane filtration and added as a bacteriostatic agent.

An applicator was used to collect spores from colonies of 96-hour cultures on PDA plates, and the spores were then transferred to a tube of sterilized distilled water that contained 0.01% (v/v) tween 80. The tube was vortexed to create a uniform suspension of spores for four to five minutes at room temperature.

The hemocytometer was used to count the spores, and the result was corrected to 3×10^6 spores/ml. Each strain's spore suspension was diluted into a milliliter and added to 250 ml Erlenmeyer flasks containing 50 ml of sterilized basal medium as a fungal inoculum. Incubation with 150 rpm rotary shaking was conducted at 28±2°C. Flasks were checked for the generation of riboflavin after 7 days. The mycelium was recovered by filtering the fungal mycelium through dried, weighed Whatman filter paper (No. 113), washing it with distilled water, and then drying it at 70°C overnight to determine its dry weight.

Gibberellin

Microorganisms and inoculums preparation Diverse Fusarium species, isolated from Egyptian clover, garlic, maize, and onion plants on potato dextrose agar medium (PDA) at 28±1°C with incubation time7 days were used in this research. According to Leslie and Summere (2006), fusarium species were determined based on their macroscopic development on various media and their microscopic features under a light microscope.

Fresh and pure cultures were transferred to pure PDA slants and saved at 4±1°C. Fusarium sp. was grown on PDA medium at 28±1°C for 7 days to prepare the inoculum. To prevent spore collection, fungal hyphae were scraped off growth plates and floated in sterilized distilled water fortified with 0.01% (v/v) Tween 80. They were agitated for 30 minutes until 3 105 spore/ml were reached (Mahmoud and Mostafa, 2017).

Czapek's dextrose fluid medium was used as a production medium for screening Fusarium sp. for the formation of gibberellin. It contained (g/l): glucose (30.0), yeast extract (5), $NaNO_3$, 3.0, KH_2PO_4, 1.0, $MgSO_4.7H_2O$, 0.5, KCl, 0.5, $FeSO_4.7H_2O$, 0.01 and 1000 ml of distilled water with an initial pH regulation of 5.5. Chloramphenicol, 250 mg/ml was incorporated independently as a bacteriostatic agent sterilized by membrane filtering (0.22 mm) after sterilization in an autoclave at 121°C for 20 min. For seven days, the cultures were incubated at 28±1°C with a 150-rpm rotating shaker. According to Shahzad *et al.* (2016), each experiment was carried out independently in triplicate.

Ascorbic acid

Both *Aspergillus flavus* and *Aspergillus tamarii*, ascorbic acid-producing strains, were isolated from the microbiological media Potato Dextrose Broth from BDH Chemicals, and Saboraud Dextrose Agar. Chemicals utilized include indophenol dye and 2,6-dichlorophenol, acetic acid, sodium potassium tartrate, ethylene diamine tetraacetic acid (EDTA), trisodium phosphate, metaphosphoric acid, glucose, galactose, and lactose. The main pieces of apparatus utilized include an autoclave, a hot box oven, an incubator (size 1 Gallenkamp, England), a weighing scale (Mettler Toledo PB 3002, Switzerland), a water bath (Nickel Electro Ltd., England), a pH meter, and a Waring blender. Production and quantification of ascorbic acid by *Aspergillus flavus* and *A. tamarii* was carried out according to the methods of El-Gindy *et al.,* 2005 and Prakash *et al.,* 2011.

Experimental treatments

Treatments were divided into two groups: microbial hormones and synthetic hormones, in addition to the control. For gibberellin treatment, the clusters were sprayed four times: at shoot length (7 cm), full bloom, 6 ml berry diameter, and a month after the previous spray. As for the other compounds, the spraying compounds were added twice, at 6 ml diameter per berry and a month after the previous spray.

PGPR hormones

1- control (T_1).

2-Gibberellin (GA_3) at 50 ppm (T2).

3-Ascorbic acid (VC) at 500 ppm (T3).

4-Riboflavin (B_2) at 200 ppm (T4).

5-Gibberellin (GA_3) at 50 ppm + ascorbic acid (VC) at 500 ppm (T5).

6-Gibberellin (GA_3) at 50 ppm + riboflavin (B_2) at 200 ppm (T6).

7-Ascorbic acid (VC) at 500 ppm + riboflavin (B_2) at 200 ppm (T7).

8-Gibberellin (GA_3) at 50 ppm + riboflavin (B_2) at 200 ppm + ascorbic acid (VC) at 500 ppm (T8).

Synthetic hormones

1- control (T_1).

2-Gibberellin (GA_3) at 50 ppm (T2).

3-Ascorbic acid (VC) at 500 ppm (T3).

4-Riboflavin (B_2) at 200 ppm (T4).

5-Gibberellin (GA_3) at 50 ppm + ascorbic acid (VC) at 500 ppm (T5).

6-Gibberellin (GA_3) at 50 ppm + riboflavin (B_2) at 200 ppm (T6).

7-Ascorbic acid (VC) at 500 ppm + riboflavin (B_2) at 200 ppm (T7).

8-Gibberellin (GA_3) at 50 ppm + riboflavin (B_2) at 200 ppm + ascorbic acid (VC) at 500 ppm (T8).

Each treatment had 3 vines (3 replicates), and every replicate consisted of 2 clusters. Ripening was recorded as and when grapes reached commercial maturity.

Physical characteristics

Two clusters were taken randomly from the yield of each vine, repeated 3 times, and made as a composite sample for physical and chemical determinations. Using an analytical balance, the weight of the cluster and the weight of 100 berries per cluster were calculated in grams. A graduated cylinder was used to calculate the juice volume of 100 berries per cluster in milliliters.

Chemical characteristics: All parameters were measured according to A.O.A.C. (1990).

Total soluble solids (TSS): Using a hand refractometer, the number of soluble solids in fruit juice was calculated.

$$\text{Acidity (\%)} = \frac{\text{Standard solution (N)} \times \text{base solution (ml)} \times 0.075 \times 100}{\text{Total juice (ml)}}$$

Where: The equivalent weight of Tartaric acid = 0.075 and Total juice= 5ml

Reducing sugar %: The amount of reducing and total sugars was calculated using the Lane and Eynon method described in A.O.A.C (1990).

Vitamin C (%): Using 10g of the fresh, the phenol-indophenol dye technique was used to measure the ascorbic acid content. The sample was homogenized by blending it with a 3% metaphosphoric acetic acid extraction solution. With regular indophenols, the filtrate extract was titrated until it reached the pink endpoint A.O.A.C (1990).

Design of experiment

Three replications of each treatment were employed in a randomized complete block design for a combined examination of each season. Using Proc Mixed from the SAS package version 9.2 (SAS 2008), an analysis of variance (ANOVA) was performed, and means were compared using the least significant difference test at a significance threshold of 5% (Steel and Torrie, 1981).

Table 1. Analysis of the tested soil for this study

Properties		Properties	
Sandy (%)	21.15	$CaCO_3$ (%)	3.66
Silt (%)	31.55	Total N (%)	0.14
Clay (%)	48.30	Available P (ppm)	8.25
Texture	Clay	Available K (ppm)	332.15
pH (1:2.5)	8.19	DTPA-extractable	
E.C (1:2.5) (dS/m)	2.26	Fe (ppm)	22.70
Organic matter (%)	1.38	Mn (ppm)	18.31
		Zn (ppm)	4.6

Results

Yield (kg/tree)

The result in Table 2 showed that, in the first season, the most significant effect on the yield weight, whether microbial or synthetic treatments, was in T2 (14.63 kg), followed by T6 (13.27 kg), without significant differences between them, while the minor effect was in T4 (8.77 kg) and the control (8.02 kg), with slight differences between them.

For comparison between the microbial treatment and the synthetic treatment, the data proved that the microbial treatment (15.01 kg) had a greater effect than the synthetic treatment (9.02 kg), with notable variations.

The data showed that the biggest impact in the second season on yield weight in both microbial and synthetic treatments was in all treatments that included gibberellins, namely; T8 (16.61kg), T2 (16.51kg), T6 (16.10kg), and T5 (16.03 kg), with insignificant differences. The control gave the lowest effect (2.08kg).

The data reveals the difference between microbial treatments and synthetic treatments. It proved that the microbial treatment (15.64kg) got the greatest value compared to the synthetic treatment (9.73 kg), with significant differences.

Table 2. Effect of microbial and synthetic hormones on yield (kg/vine) of Thompson seedless grape cultivar in 2019 and 2020 seasons

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	8.02	8.02	8.02 C	2.07	2.07	2.08 D
Gibberellin (T_2)	19.43	9.84	14.63 A	20.11	12.91	16.51 A
Ascorbic acid (T_3)	15.68	9.94	12.81 B	16.84	8.24	12.54 B
Riboflavin (T_4)	11.81	5.73	8.77 C	13.09	6.49	9.79 C
Gibberellin + Ascorbic acid (T_5)	15.93	9.70	12.82 B	19.60	12.47	16.03 A
Gibberellin + Riboflavin (T_6)	16.82	9.72	13.27 AB	20.44	11.76	16.10 A
Ascorbic acid + Riboflavin (T_7)	15.55	9.70	12.63 B	14.99	8.73	11.86 BC
Gibberellin +Riboflavin + Ascorbic acid (T_8)	16.86	9.49	13.17 B	17.98	15.23	16.61 A
Mean	15.01 a	9.02 b	12.02	15.64 a	9.73 b	12.69

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

Cluster weight

The data in Table 3 demonstrated that all treatments, whether microbial or synthetic, had a considerable effect on the cluster weight compared to the control.

In the first season, the highest cluster weight, whether microbial or synthetic, was recorded in sequentially arranged treatments T_6 (636.33g), T_8 (633.17g), and T_2 (629.00 g) without significant differences. Then came the other treatments; there were statistically significant changes, and the control had the lowest value (310.67 g).

In the second season, the highest value was found in T_6 (978.83g) followed by T_2 (867.47g) and T_5 (861.37g) with trivial differences, and the rest of the treatments came after them, and the control had the lowest value (252.33g).

In the comparison between the effects of microbial and synthetic, the data in two seasons proved that the microbial (605.29, 774.31g) had a greater effect than the synthetic (476.63, 595.91g) in both seasons, with significant variations between them. It is worth noting that the results were more favorable in contrast to the first season in the second.

Table 3. Effect of microbial and synthetic hormones on cluster weight (g) of Thompson seedless grape cultivar in 2019 & 2020 season

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	310.67	310.67	310.67 D	252.33	252.33	252.33 D
Gibberellin (T_2)	699.67	558.33	629.00 A	993.60	741.33	867.47AB
Ascorbic acid (T_3)	588.67	509.33	549.00 B	648.67	435.90	542.28 C
Riboflavin (T_4)	601.33	418.33	509.83 C	646.33	505.00	575.67 C
Gibberellin + Ascorbic acid (T_5)	613.00	528. 67	570.83 B	993.67	729.07	861.37AB
Gibberellin + Riboflavin (T_6)	763.00	509.67	636.33 A	1025.00	932.67	978.83 A
Ascorbic acid + Riboflavin (T_7)	536.67	441.00	488.83 C	733.67	461.00	597.33 C
Gibberellin +Riboflavin + Ascorbic acid (T_8)	729.33	537.00	633.17 A	901.23	710.00	805.62 B
Mean	605.29 a	476.63 b	540.96	774.31 a	595.91 b	685.12

* The ascending order starts with (A or a), which implies the highest value and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

100 berries weight

The data in Table 4 demonstrated that the microbial treatments had a favorable outcome on the weight of 100 berries in both seasons in contrast to synthetic treatments, and there were notable distinctions between them. The control had the minimum impact in both seasons.

In the first season, the treatment that included three types of hormones (T_8) had the highest effect (151.33g), followed by the GA_3 (140.67g) treatment with an insignificant variation. Subsequently, the other treatments with significant variations were coming. The control had the least effect (57.33 g).

Table 4. Effect of microbial and synthetic hormones on 100 berries weight (g) of Thompson seedless grape cultivar in 2019 & 2020 seasons

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	57.33	57.33	57.33 E	74.10	74.10	48.00 E
Gibberellin (T_2)	147.33	134.00	140.67 AB	179.20	173.40	145.82 C
Ascorbic acid (T_3)	83.67	66.00	74.83 D	165.17	116.00	115.82 D
Riboflavin (T_4)	83.47	68.40	75.93 D	173.07	124.50	120.08 D
Gibberellin + Ascorbic acid (T_5)	154.33	108.67	131.50 BC	215.53	198.00	184.08 A
Gibberellin + Riboflavin (T_6)	140.83	114.50	127.67 C	203.67	194.70	195.43 A
Ascorbic acid + Riboflavin (T_7)	78.70	67.67	73.18 D	154.83	137.17	122.67 D
Gibberellin +Riboflavin + Ascorbic acid (T_8)	162.67	140.00	151.33 A	190.63	185.67	162.58 B
Mean	113.54 a	94.57 b	104.05	169.53 a	150.44 b	159.98

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

Concerning the second season, the results proved that the highest values were recorded in T_5 and T_6 (184.08, 195.43g), respectively, without significant difference, and T_8 came in second place (162.58g), and the control had the lowest value (48.00g).

Notable, the treatments that included GA_3 had a favorable outcome in contrast to the other treatments, single form or mixed form, without GA_3.

Juice weight

The results in Table 5 of two successive seasons found that the lowest amount was in control, with significant differences with treatments. Microbial treatment had a greater positive effect than synthetic treatments, and there were significant differences between them.

T8 had the most juice (129.87 g) in the first season, followed by T2 (126.00 g) with a negligible difference, and the remaining treatments with substantial differences in order of precedence. The control was 46.07 g.

In contrast, in the second season, the largest levels were discovered in T5 and T6 (184.08, 195.4 g), respectively, and were followed by T8 (162.58 g) with notable variations, and the other treatments with notable variations.

Table 5. Effect of microbial and synthetic hormones on juice weight (g) of Thompson seedless grape cultivar in 2019 & 2020 seasons

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	46.07	46.07	46.07 E	48.00	48.00	48.00 E
Gibberellin (T_2)	136.33	115.67	126.00 AB	152.13	139.50	145.82 C
Ascorbic acid (T_3)	71.67	63.57	67.62 D	143.47	88.17	115.81 D
Riboflavin (T_4)	72.30	66.33	69.32 D	146.50	93.66	120.08 D
Gibberellin + Ascorbic acid (T_5)	139.33	95.33	117.33 BC	195.33	172.83	184.08 A
Gibberellin + Riboflavin (T_6)	128.00	96.10	112.05 C	178.86	212.00	195.43 A
Ascorbic acid + Riboflavin (T_7)	72.33	60.63	66.48 D	137.66	107.66	122.67 D
Gibberellin +Riboflavin + Ascorbic acid (T_8)	144.00	115.73	129.87 A	161.90	163.27	162.58 B
Mean	101.25 a	82.43 b	91.84	145.48 a	128.14 b	136.81

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

TSS%

In Table 6, the data illustrated that there were insignificant differences between the effects of microbial and synthetic hormones on TSS% in the juice of grapes in two seasons in a row.

In the first season, the largest value was in T4 (21.83%), followed by T3 and T7 (21.17% and 21.08%, respectively), and there were insignificant differences between them. Other treatments followed by significant variations, and the lowest value was in T2 (18.41%). It was noticed that the control, T5 and T6 (19.67, 19.25, and 19.33%), had trivial differences between them.

In the second season, the data proved that riboflavin treatment (22.45%) had the largest effect on TSS, followed by T3 and T7 (21.13 and 21.12%, respectively), with insignificant differences between them. The lowest effect was in T5 (19.55%), with a significant difference.

As for the second season, the largest amount was in T4 (22.45%), followed by T3 (21.13%), and T7 (21.12%), without eloquent differences between them. The smallest amount was found in T5 (19.55%), with a significant difference from the other treatments

Table 6. Effect of microbial and synthetic hormones on TSS % of Thompson seedless grape cultivar in 2019 and 2020 seasons

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	19.67	19.67	19.67 C	20.33	20.33	20.33 BC
Gibberellin (T_2)	18.33	18.50	18.41 D	20.83	20.17	20.50 BC
Ascorbic acid (T_3)	21.33	21.00	21.17 AB	21.27	21.00	21.13 AB
Riboflavin (T_4)	22.00	21.67	21.83 A	22.33	22.57	22.45 A
Gibberellin + Ascorbic acid (T_5)	19.33	19.16	19.25 C	19.17	19.93	19.55 C
Gibberellin + Riboflavin (T_6)	19.33	19.33	19.33 C	20.40	20.83	20.62 BC
Ascorbic acid + Riboflavin (T_7)	21.00	21.17	21.08 AB	21.23	21.00	21.12 AB
Gibberellin +Riboflavin + Ascorbic acid (T_8)	20.67	20.67	20.67 B	21.50	20.33	20.92 BC
Mean	20.21 a	20.15 a	20.18	20.88 a	20.77 a	20.83

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

Acidity

The data presented in Table 7 demonstrated that there were trivial differences between the microbial and synthetic hormones in their effects on acidity in both seasons. In the first season, the highest value of acidity was found in T2 (0.553%), followed by T6 (0.532%); there were insignificant differences between them. The rest of the treatments came next, with significant differences between them. While the lowest values were in T3, T4, and T7 (0.343, 0.347, and 0.338%, respectively), without significant differences. Besides that, there were minimal differences between the control and T8 (0.443, 0.448%), respectively.

Table 7. Effect of microbial and synthetic hormones on acidity % of Thompson seedless grape cultivar in 2019 and 2020 seasons

Treatments \ Hormones	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	0.443	0.443	0.443 C	0.270	0.270	0.270 E
Gibberellin (T_2)	0.567	0.540	0.553 A	0.410	0.477	0.443 BC
Ascorbic acid (T_3)	0.353	0.333	0.343 D	0.373	0.340	0.356 D
Riboflavin (T_4)	0.333	0.360	0.347 D	0.410	0.353	0.382 CD
Gibberellin + Ascorbic acid (T_5)	0.517	0.503	0.510 B	0.557	0.483	0.520 AB
Gibberellin + Riboflavin (T_6)	0.550	0.513	0.532 AB	0.526	0.580	0.553 A
Ascorbic acid + Riboflavin (T_7)	0.327	0.350	0.338 D	0.393	0.387	0.390 CD
Gibberellin +Riboflavin + Ascorbic acid (T_8)	0.463	0.433	0.448 C	0.420	0.590	0.505 AB
Mean	0.444 a	0.435 a	0.439	0.420 a	0.435 a	0.428

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

In the second season, there were insignificant differences between microbial and synthetic hormones. While there were significant differences between treatments, the highest values were found in T5, T6, and T8 (0.520, 0.553, and 0.505% respectively), with insignificant differences between them, and the lowest value was in T3 (0.356 %) with a significant difference.

Reducing sugar

As presented in Table 8, the data proved that the microbial treatment had a more positive effect on reducing sugar than the synthetic treatment in both successive seasons. There were significant differences between the values of microbial and synthetic hormones, whereas microbial hormones recorded 16.22 and 18.51%, respectively, and synthetic hormones were 15.61 and 17.18%, respectively, in both seasons.

Concerning data in the first season, the greatest percentage of reducing sugar was in T3 (17.09%), followed by T4 (16.83%) and T8 (16.63%), with statistically significant differences between them, whereas the lowest percentage was in the control (14.04%).

In connection with the data from the second season, the greater percentages were in T2, T4, T6, and T8 (18.96, 18.66, 19.39, and 19.20%), respectively, and there were insignificant differences between them, and the control group had the lowest proportion. (14.14%).

Table 8. Effect of microbial and synthetic hormones on reducing sugar % of Thompson seedless grape cultivar in 2019 & 2020 seasons

Hormones / Treatments	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	14.04	14.04	14.04 D	14.14	14.14	14.14 C
Gibberellin (T_2)	15.94	15.13	15.54 C	19.08	18.84	18.96 A
Ascorbic acid (T_3)	17.47	16.71	17.09 A	17.98	17.03	17.51 B
Riboflavin (T_4)	17.41	16.24	16.83 AB	21.17	16.15	18.66 AB
Gibberellin + Ascorbic acid (T_5)	15.89	15.04	15.46 C	17.78	17.21	17.49 B
Gibberellin + Riboflavin (T_6)	16.26	16.10	16.18 BC	19.52	19.25	19.39 A
Ascorbic acid + Riboflavin (T_7)	16.01	15.14	15.58 C	18.54	16.33	17.44 B
Gibberellin +Riboflavin + Ascorbic acid (T_8)	16.77	16.49	16.63 AB	19.89	18.51	19.20 A
Mean	16.22 a	15.61 b	15.92	18.51 a	17.18 b	17.85

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

Vitamin C

Upon analyzing the data in Table 9, it was proven that microbial hormones had the greatest effect on vitamin C % compared with synthetic hormones in two study seasons. Whereas, the microbial treatment recorded 2.13 and 2.52% and the synthetic treatment recorded 2.01 and 1.81%, respectively, in both consecutive seasons, with statistically significant differences between them.

In the two study seasons, all treatments had a positive effect on V.C. compared to the control, with significant differences among them. The lowest amounts were in the control (1.85 and 1.53%), respectively.

In the first season, the highest amount was in T8 (2.35%), and the other treatments came after with significant differences, while in the second season, the highest percentage was in T3, T4, T7, and T8 (2.50, 2.40, 2.37, and 2.47%), respectively, followed by T5 (2.23%) with insignificant differences, and the other treatments came after with significant differences.

Table 9. Effect of microbial and synthetic hormones on VC% of Thompson seedless grape cultivar in 2019 and 2020 seasons

Hormones / Treatments	Season 1			Season 2		
	Microbial	Synthetic	Mean	Microbial	Synthetic	Mean
Control (T_1)	1.77	1.93	1.85 C	1.53	1.53	1.53 D
Gibberellin (T_2)	2.03	1.93	1.98 BC	2.03	1.87	1.95 BC
Ascorbic acid (T_3)	2.10	2.10	2.10 B	3.03	1.97	2.50 A
Riboflavin (T_4)	2.23	2.07	2.15 B	2.87	1.93	2.40 A
Gibberellin + Ascorbic acid (T_5)	2.23	1.93	2.08 B	2.67	1.80	2.23 AB
Gibberellin + Riboflavin (T_6)	2.13	1.83	1.98 BC	2.13	1.60	1.87 C
Ascorbic acid + Riboflavin (T_7)	2.07	2.07	2.07 B	2.77	1.97	2.37 A
Gibberellin +Riboflavin + Ascorbic acid (T_8)	2.47	2.23	2.35 A	3.10	1.83	2.47 A
Mean	2.13 a	2.01 b	2.07	2.52 a	1.81 b	2.17

The ascending order starts with (A or a), which implies the highest value, and goes down to the letter with the lowest value, and the separation by LSD testing at $P < 0.05$. The identical letters within columns are not significantly different.

In the two study seasons, all treatments had a positive effect on VC compared to the control, with significant differences among them. The lowest amounts were in the control (1.85 and 1.53%), respectively.

In the first season, the highest amount was in T8 (2.35%), and the other treatments came after with significant differences, while in the second season, the highest percentage was in T3, T4, T7, and T8 (2.50, 2.40, 2.37, and 2.47%), respectively, followed by T5 (2.23%) with insignificant differences, and the other treatments came after with significant differences.

Discussion

Merwad *et al*. (2015) proved that the spraying GA_3 increased vegetative growth indices such as cluster weight, berry size and weight, berry length, and diameter, especially when it was applied at the start of vegetative growth. in addition, increased TSS, reduced acidity in the berry juice, and delayed fruit ripening.

Soha *et al*.(2018) demonstrated that the function of cyanobacteria, which is a potent bio-safe and high-PGR product, may provide grapes with the same beneficial effects of chemically produced hormones on yield and quality while posing no health risks or environmental degradation because it has the lowest amount of residual hormones.

Plant growth regulators are messenger molecules that are needed in trace amounts at low concentrations. Their site of action and biosynthesis are different. While GA_3 increased fruit weight, diameter, length, volume, and pulp weight, it decreased fruit set and number as well as bunch and yield weight (Kassem *et al*., 2011).

Ascorbic acid is a form of antioxidant that acts auxinically and synergistically to promote fruit and flower development in fruit trees. Recently, antioxidants have replaced auxins and other chemicals as a means of promoting the growth and fruiting of different fruit trees (Ragab, 2002). Additionally, it has been observed that antioxidants have a beneficial effect in capturing or chelating free radicals, which may lengthen the shelf life of plant cells and stimulate growth factors (Rao *et al*., 2000).

According to Arfan *et al*. (2007), ascorbic acid is necessary for photosynthesis, ethylene biosynthesis, stomatal movement, and the reversal of the effect of ABA on leaf abscission in plants. According to Hayat *et al*. (2008), it improved the antioxidant enzymes' responses to drought and salinity stress in plants. It is in charge of boosting organic hormones, which are crucial for controlling plant growth and development (Senaratna *et al*., 2004). Whereas Zonouri *et al*. (2014) findings, that ascorbic acid can influence some of the physiological reactions of grapes.

In plants, ascorbic acid serves as an antioxidant and an enzyme cofactor. It takes part in a wide range of biological processes, including photosynthesis, cell wall development, and expansion, resistance to environmental stressors, and the manufacture of ethylene, gibberellins, anthocyanins, abscisic acid, and hydroxyl proline. It enhances fruit crop development, blooming, yield, and fruit quality in a synergistic manner. Additionally, it is essential for the integrity and operation of the membrane, the signal transduction system, metabolism, transporter enzyme activation, and the translocation of carbohydrates, the results were the same line as Maksoud *et al*. (2009), and Abdel Salam (2016).

Plant growth, development, and defensive responses are influenced by riboflavin biosynthesis and functional pathways via a variety of methods. Riboflavin is an exogenous vitamin that has been shown to increase plant growth (Peng *et al*., 2002), as well as bacterial, viral, and fungal disease resistance according to Aver'yanov *et al*. (2000), Dong and Beer (2000), and Taheri and Hofte (2006).

Inducing plant growth and resistance to bacterial, viral, and fungal diseases are two benefits of exogenous applications of riboflavin (Li *et al*., 2012).

According to Taheri and Tarighi (2011), riboflavin accelerated the expression of the lipoxygenase (LOX) gene, which is linked to plant maturation and disease resistance. The overexpression of the phenylalanine ammonia-lyase (PAL) gene, a crucial gene linked to plant disease resistance and anthocyanin production, was shown in grapevine in riboflavin-treated plants (Boubakri *et al*., 2013).

Additionally, riboflavin promoted the early buildup of anthocyanin and total soluble solids as well as the elevated expression of genes associated with maturation. According to phenological observations, the treated grape berries proceeded through the color-turning and maturity stages 9 and 7 days earlier, respectively, than the control. Riboflavin may therefore considerably encourage the early maturity of grape berries (Mingtao *et al*., 2019).

Conclusion

This research studied the impact of microbial hormones, which were considered sources of gibberellins, ascorbic acid, and riboflavin, on the quality of the Thompson grapes and compared it with synthetic hormones. The three types of microbial hormones were used in a single or combination of them, and similar treatments were designed for synthetic hormones. Some characteristics were measured as indicators of the quality levels, such as yield and the chemical and physical properties of berries. The data proved that all treatments, either microbial or synthetic, gave the best effect in contrast to the control. Microbial hormones had the same impact as synthetic hormones; and the best impact was in the combination of the three types of microbial hormones compared with the other treatments.

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تأثير الهرمونات الميكروبية والصناعية على جودة عنب طومسون خالي البذور

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الملخص

من المعروف أن هناك العديد من المشاكل الناتجة من استخدام الهرمونات الصناعية على أشجار الفاكهة، أهمها أنها غير آمنة على صحة الإنسان على المدى الطويل. لذلك تناولت معظم الأبحاث الخاصة بالبساتين عامة وبالفاكهة خاصة هذه النقطة لإيجاد حل بديل لاستخدام الهرمونات المخلقة. هذا البحث قام بدراسة تأثير الهرمونات الميكروبية التي تعتبر مصادر للجبرلين وحمض الأسكوربيك والريبوفلافين على جودة عناقيد العنب صنف طومسون ومقارنتها بالهرمونات الصناعية خلال موسمي 2019، 2020.

تم استخدام الأنواع الثلاثة الهرمونات الميكروبية بشكل فردي أو مختلط فيما بينها، وبنفس الطريقة في الهرمونات الاصطناعية. وتم قياس بعض الخصائص كمؤشرات لمستوى الجودة، مثل وزن المحصول والخصائص الفيزيائية والكيميائية للحبات.
أثبتت البيانات أن جميع المعاملات، سواء الميكروبية أو الاصطناعية، أعطت أفضل تأثير مقارنة مع معاملة الكنترول؛ وكان للهرمونات الميكروبية نفس تأثير الهرمونات الاصطناعية وقد يكون أفضل؛ وكان التأثير الأفضل هو المعاملة بالجمع بين الأنواع الثلاثة من الهرمونات الميكروبية مقارنة بالمعاملات الأخرى.

الكلمات المفتاحية: *الجبريلين، الريبوفلافين، الاسبراجلس، حمض الاسكوربك، فيوزاريوم*