



Article

Increasing the Add Value of Crimson Seedless and Red Globe Grapevines Varieties Treated by a Bio-stimulant Substance to Produce Stirred Yoghurt

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Abstract: The feasibility of applying the bio-stimulant substance Bio-Tech (NABTA-Bio) on growth, yield and quality of eight-year-old Red Globe and Crimson Seedless cv. grapevines grafted on Freedom rootstock was studied in this experiment in order to increase the add value for stirred yoghurt production for human health importance. This trial took place for the two successive seasons 2019 and 2020. Five treatments were applied whether as foliar spray or soil addition of Bio-Tech in different concentrations. Results revealed that vines treated with Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray) gave the highest values in all growth parameters and chemical characteristics of berries for both cultivars in addition to its positive effect when added to stirred yoghurt as a grape juice at two concentrations of 8 and 12 % through increasing the chemical, rheological and microbial properties of the yogurt during the storage period. Data showed a decrease in the acidity and microbial content and an increase in the total solids, protein and the content of phenolic substances and antioxidants when stored for 5 days with a reduction when stored for 10 days in the processed Crimson seedless more than in Red Globe cultivar. Also K, P, Mg and Fe % were increased in both cultivars varieties compared to the control, with superiority for Crimson seedless over Red Globe cv. Moreover, the stirred yoghurt made from Crimson seedless grape juice treated with Bio-Tech as bio-stimulant, whether at 8 or 12% had the highest sensory evaluation than the yogurt made from the untreated grapes.

Key words: Bio-Tech, bio-stimulant, biofertilizers, Red Globe, Crimson Seedless, stirred yoghurt.

INTRODUCTION

Red grapes (*Vitis vinifera* L.) are very popular for local consumption and export markets due to the taste and red pigments. Red Globe cv. is a seeded grape, pink to red skin color and large round berries with plump clusters, firm and crunchy, moderate sweet taste with a moderate vigor, it used as fresh consumption and for grape juice production, as well as it is the most popular seeded grape in Egypt, Asian and the middle east markets (Olmo and Koyama, 1981; Omran, 2011).

Crimson seedless grapevine cv. is a late-season red seedless grape with a firm crispy berries and juicy pulp, sweet flavor which developed in the USDA Fruit Genetics and Breeding Research Unit, Fresno, CA. USA. In addition, it is globally preferred due to its quality parameters as high sugar content, greater nutritional properties and with a variety of consumption as fresh, dried raisins and processed products (Río-Segade *et al.*, 2013; Samaan and Nasser 2020) or added to other products (Dokoozlian and Peacock, 2001).

Indeed, red table grape production meets challenges in enhancing the quality and coloration to satisfy different consumer standards. Therefore, using the plant bio-stimulants or microorganisms provides the plants with variable properties needed to increasing its value (Petoumenou and Patris, 2021)

The bio-stimulant product Bio-Tech (NABTA-Bio) is a mixture of biofertilizers which consists of photosynthetic bacteria (*Cyanobacteria*), Lactic acid Bacteria, Nitrogen fixing bacteria (*Azotobacter chroococcum*) and yeast (*Saccharomyces cervicisae*, L.)

• Photosynthetic organisms (*Cyanobacteria*)

They have been considered as autotroph, also known as Cyanophyta and considered as a phylum of gram-negative bacteria which able to produce organic substances from inorganic nutrients based on energy harvested from light or via photosynthesis (Sinha and Häder, 2008; Schmidt *et al.*, 2013). The photosynthetic microbes exist in sub- soil surface, rocks and bryophytes Hamard *et al.*, (2021). It seems important to ecosystem, C and N metabolism in plants, CO₂ fixation under a wide range of environmental conditions, regulating enzyme activity and many biological processes. They used successfully for carbohydrates production which explains the stimulatory effect of enhancing crop productivity (Jassey *et al.*, 2022).

• Lactobacilli or lactic acid bacteria (LAB)

It is naturally found in a wide range of soil which could isolate easily. Their abundance points to their importance for sustainable agriculture development. LAB is a safe microorganisms work as a liquid biofertilizer for foliar spray or soil application to improve the organic matter and soil fertility in organic agriculture, so it is considered safe for environment and human health Lamont *et al.* (2017). Further, LAB can directly affect boosting yield through increases the solubility of fertilizers, facilitating nutrient uptake of roots in order to promote plant growth, control disease and increase plant resistance to a biotic stress due to its functional role in producing organic acids, siderophores, cytokinens and promote nitrification (Raman *et al.*, 2022).

• Nitrogen fixing bacteria (*Azotobacter chroococcum*)

Nitrogen fertilization is considered an important and limiting factor for different grapevine cvs. Hence, this category of biofertilizers belongs to plant growth promoting rhizobacteria (PGPR) which is considered an ideal alternative bio-stimulants for chemical fertilization in organic and conventional production (Rouphael and Colla, 2020). This strain is positively enhancing soil fertility, producing a variety of hormones, availability of nutrients and uptake this led to growth promoting and improving crop quality and productivity (Ibrahim *et al.*, 2020). As well as it could be employed as eco-friendly supplement for sustainable agriculture by the application alone or with another inoculants (Kumar *et al.*, 2022).

• **Yeast (*Saccharomyces cervicisae*, L.)**

Active dry yeast has a positive effect due to activating photosynthesis process through producing natural hormones, vitamins and enzymes, beside stimulating cell division and protein synthesis (**El-Halaby *et al.*, 2015**). Moreover, soil addition of yeast stimulates all growth characters with a significant effect on berry physical characteristics, TSS%, acidity, anthocyanin, leaf content of pigments, leaf area, number of leaves/shoot, leaf content of N %, shoot length and diameter of Red globe cultivar (**El-Morsy *et al.*, 2017**).

Furthermore, to develop functional stirred yoghurt of acceptable quality based on mixing yoghurt and grapes, the chemical, rheological, physical and organoleptic characteristics of grapes and the formulated yoghurt were determined as well.

Generally, grapes are natural, high efficient bioreactors being able to produce various bioactive secondary metabolites, including natural antioxidants including phenol, flavonoid, and anthocyanin (**Honda *et al.*, 2002**) that enable to scavenge free radicals in human bodies and alleviate inflammation, thus have health benefits on prevention of many degenerative and chronic diseases such as coronary heart disease, diabetes, Alzheimer's, cataracts, arthritis and ageing (**Miller *et al.*, 2000**). Phenolic compounds have been found at concentrations as high as 260-920 mg/kg in grapes and 1.8-3.2 mg/L (**Gamez-meza *et al.*, 2011**). Moreover, grapes contain phytochemicals that may reduce heart disease and some varieties are good to excellent sources of vitamin C.

Furthermore, it was found that consumption of dairy products is associated with beneficial health effects beyond their nutritional values. Fermented milk products have positive health image due to the beneficial viable bacteria and yoghurt has been already recorded as a healthful product. Yoghurt contains healthy bacteria known as probiotics, which keep your digestive and immune system functioning well. It is also a good source of protein and nutrients important for bone health. Yoghurt helps prevent osteoporosis, reduce the risk of high blood pressure and active cultures help the gut (**Lourens and Bennie, 2001**).

The objective of the present work is to assess the promising role of applying Bio-Tech (NABTA-Bio), as a liquid source containing various bio-stimulants strains, on yield and quality of two red grape cvs and evaluate its potential use as healthy ingredients in enriching the antioxidant properties of stirred yoghurt.

MATERIALS AND METHODS

This experiment took place in a private vineyard located at Belbes, Sharqiya governorate for the two successive seasons 2019 and 2020 with a preliminary season 2018, on eight-year-old Red Globe and Crimson Seedless cvs. grapevines grafted on Freedom rootstock. Sixty uniform vines (5 treatments x 3 replicates x 4 vines/replicate). All vines grown in a sandy loam soil, spaced at 2 x 3 meters apart and irrigated by the drip irrigation system, Red Globe were cane pruned (6 canes x 12 buds with a total bud load of 72 buds/vine) beside 6 spur x 2 buds/spur and Crimson Seedless were cane pruned (10 canes x 10 buds with a total bud load of 100 buds/vine) beside 10 spur x 2 buds/spur and both were trellised by Gable system and free from any visual infections and received regularly the recommended horticultural practices.

Five treatments were applied whether as soil addition or foliar spray till run off by partial substitute 50 % of the mineral fertilizers by using bio-stimulant product Bio-Tech (NABTA-Bio) in addition to the control vines were sprayed with tap water for both cultivars Red Globe and Crimson Seedless. The bio-stimulant Bio-Tech (NABTA-Bio) was applied four times during the growing season as follow: the first at shoot length 25 cm, second at pre-bloom, third at fruit set (5mm berry diameter) and the fourth at veraison. The experiment included 5 treatments as follows:

- 1- Control (100% mineral fertilizers) (T1)

- 2- Bio-Tech (5L/ fed) (soil addition) (T2)
- 3- Bio-Tech (5L/ fed) (foliar spray) (T3)
- 4- Bio-Tech (2.5L/ fed) (soil addition) + Bio-Tech (2.5 L/ fed) (foliar spray) (T4)
- 5- Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray) (T5)

This bio-stimulant Bio-Tech (NABTA-Bio) is a commercial product analyzed by Agriculture Microbiology Dep. Biofertilizers Production unit. Agricultural and biology division, National Research Center, Dokki, Giza, Egypt. Its composition is displayed in Table (1).

Table 1. Bio-Tech (NABTA-Bio) composition analysis

Components	Results analysis in 1 ml
Photosynthetic bacteria	30 x 10 ⁷ cell
Lactic acid bacteria	10 x 10 ⁸ cell
Nitrogen fixing bacteria	22 x 10 ⁶ cell
Yeast	44 x 10 ⁸ cell

Table 2. Physical and chemical analysis of the vineyard soil

Physical	
Sand (%)	71.2
Silt (%)	2.1
Clay (%)	26.7
Texture	Sandy loam
Chemical	
Organic carbon (%)	0.07
pH	7.75
EC (dsm ²)	1.06
Water holding capacity (%)	35.0
Ca CO ₃ (%)	0.98
N (ppm)	160.2
P ₂ O ₅ (ppm)	9.5
K ₂ O (ppm)	178.1
Ca (mq/L)	2.36
Mg (mq/L)	1.98
Fe (mq/L)	5.84
Zn (mq/L)	3.04
Mn (mq/L)	4.22
Cu (mq/L)	1.36
Na (mq/L)	5.9

The following measurements were carried out during the growing seasons:

1. Yield

At harvest time (maturity) in both seasons, yield of selected vines was determined as Kg/ vine for all treatments.

- a. Yield (kg)
- b. Cluster weight (g),
- c. Berry weight (g),
- d. Berry size (cm³)

2. Chemical characteristics of the berries

At harvest date when TSS% berry juice reached about 16-18 % in both cultivars according to (Tourkey *et al.*, 1995) Clusters from each treatment were randomly collected to determine the following measurements:

- a. TSS % was determined using hand refractometer.
- b. Total acidity % was measured according to A.O.A.C. (2000).
- c. TSS/acid ratio
- d. Anthocyanin (mg /100 g f.w.) as described by Yilidz and Dikmen (1990).

3. Physical and chemical characteristics of leaves

- a. Leaf area (cm²): samples of 30 leaves taken from the fifth to the seventh positions from the apex were randomly collected from each treatment from the fruiting shoots for leaf area determination at harvest using leaf area meter, Model CI 203, U.S.A.
- b. Shoot length (cm): it was determined by measuring the fruiting shoots at harvest.
- c. Total chlorophyll content (SPAD): were measured at harvest in the mature leaves of the 5th to the 7th positions from the apex from the fruiting shoots by using the nondestructive Minolta chlorophyll meter model SPAD 502 (Castelli *et al.*, 2008).
- d. Macro nutrients content: Nitrogen % was estimated by micro-keldahl according to Pregel (1945), phosphorus % was determined by a colorimetric method as described by Temminghoff and Houba (2004) and potassium % was measured according to Balo *et al.* (1988).

4. Statistical analysis

The experiment was arranged in a randomized complete block design, using new LSD at 5% method (Mead *et al.*, 1993).

Experimental product

Two concentrations 8 and 12 % of the grape juice for both cultivars from the best treatment Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray) and fresh buffalo yoghurt was used and grape juice was added as a puree for the preparing the following treatments:

- A: Yoghurt with 12% grape juice of Red globe without Bio-Tech (control)
- B: Yoghurt with 12% grape juice of Crimson seedless without Bio-Tech (control)
- C: Yoghurt with 8% grape juice of Red globe with Bio-Tech

D: Yoghurt with 12% grape juice of Red globe with Bio-Tech

E: Yoghurt with 8% grape juice of Crimson seedless with Bio-Tech

F: Yoghurt with 12% grape juice of Crimson seedless with Bio-Tech

The yoghurt was manufactured according to the protocol proposed by **Tamime and Robinson, (1999)** with some modification as shown in Figure 1.

1. Chemical analysis of yoghurt

The buffalo milk containing (14.88% TS, 5% Fat) was obtained from the Dairy Science Department, Faculty of Agriculture, Cairo University, Egypt for yoghurt production. The yoghurt was manufactured according to the protocol proposed by **Tamime and Robinson, (1999)** with some modification as shown in Fig (1). Total solids (TS), protein, fat, titratable acidity and pH values of milk and yoghurt were determined according to the method described by **A.O.A.C, (2000)**. The total phenolic content of yoghurt after adding grape juice for both cultivars Red globe (GJR) and Crimson seedless (GJC) were evaluated using Folin-Ciocalteu reagent in accordance with the modified method described by **Lafka *et al.*, (2007)**. Total phenolic content was calculated by a calibration curve prepared with gallic acid as a standard and expressed as mg gallic acid equivalent (GAE/g). The antioxidant activity of ASP was determined using DPPH radical scavenging method as described by **Matthus (2002)**. The radical scavenging activity of the tested samples was expressed as a percentage inhibition of DPPH, which calculated according to the following formula:

$$\text{Inhibition, \%} = [(A^{\text{control}} - A_0^{\text{sample}}) / A^{\text{control}}] \times 100$$

Where: **A**, the absorbance at 515 nm of the control sample; **A₀**, the final absorbance of the test sample at 515 nm.

Minerals content were determined as described by **Hankinson (1975)** using Atomic absorption spectrophotometer No. 3300 (PerkinElmer, US instrument Division Norwalk, CT, USA).

2. Microbiological analysis

Total bacterial count for yoghurt after adding GJR and GJC samples were done using plate count agar media according to the method described by **APHA (2004)**. Detection and enumeration of yeasts and moulds were carried out according to **Marshall (1992)**, using acidified potato dextrose agar (pH 3.5) using sterile lactic acid solution (10 % conc.). All results were expressed as log₁₀colony forming unit (log cfu/g) for sample. The detection of coliform group was done according to the method suggested by **BSI (1993)** using violet red bile agar (VRBA).

3. Rheological characteristics of yoghurt

Texture profile measurements of yoghurt after adding GJR and GJC samples were done as mentioned by **Lobato-Calleros *et al.* (1997)** using a Universal Testing Machine (TMS-Pro), Food Technology Corporation, Sterling, Virginia, USA, equipped with (250 lbf) load cell and connected to a computer programmed with Texture Pro™ texture analysis software (program, DEV TPA withhold). The texture profile parameters were Hardness, Cohesiveness, Gumminess, Chewiness, Adhesiveness and Springiness.

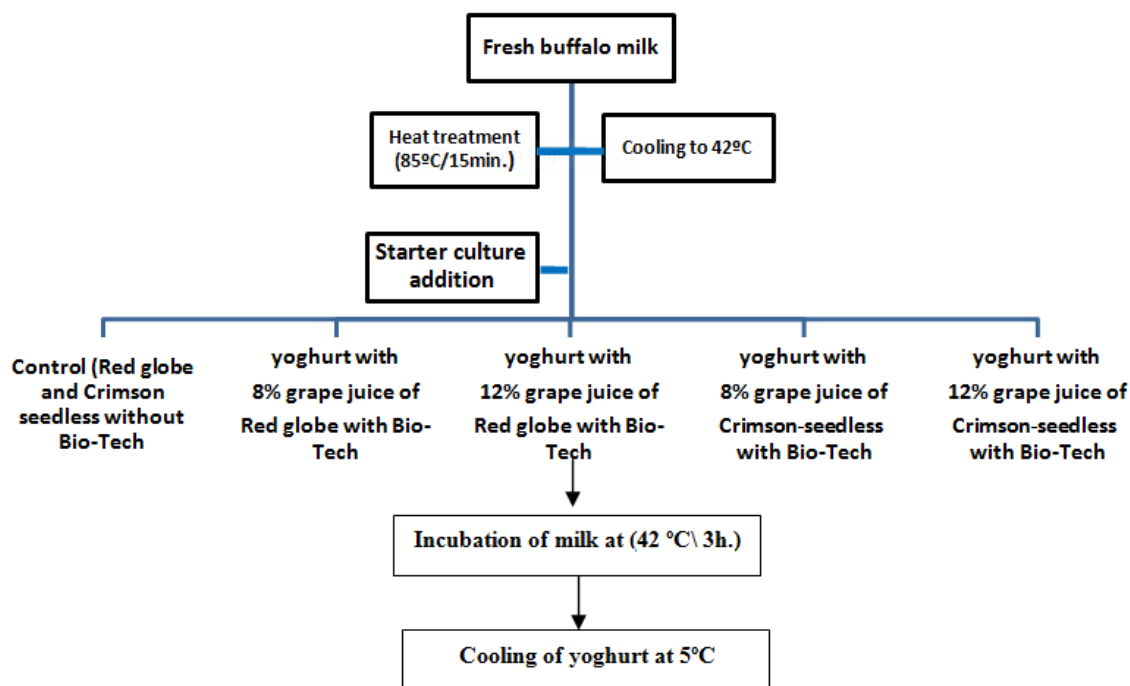


Fig. 1. Technological process of yoghurt samples with grape juice treated by anti-heat stress treatments stored for 10 days at $5\pm 1^{\circ}\text{C}$.

4. Sensory evaluation

Fresh set and flavored yoghurt from the different treatments were evaluated for their sensory attributes by ten well-trained members of the staff of the Food Technology Research Institute. The panelists were judged the samples for appearance (out of 10 points), flavor (out of 50 points), Body & texture (out of 30 points) and color (out of 10 points) as described by **El-Etriby *et al.*, (1997)**.

5. Statistical analysis

The data obtained were exposed to proper statistical analysis according to statistical analysis system user's guide (**SAS, 1996**).

RESULTS AND DISCUSSION

I. Effect of spraying different concentrations of Bio-Tech on yield and its attributes

1. Yield

Yield (kg/ vine), cluster weight (g), berry weight (g) and berry size (cm³)

The results presented in Table (3) revealed that, yield, cluster weight, berry weight and size were significantly affected by the conducted treatments in both cultivars and seasons. Vines fertilized with the fifth treatment, Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5 L/ fed) (foliar spray) (T5), resulted significantly the maximum yield and had the best effect on all cluster characters followed by the fourth treatment Bio-Tech (2.5L/ fed) (soil addition) + Bio-Tech (2.5 L/ fed) (foliar spray) (T4) for both cultivars and both seasons as compared with the control vines. Similar results were obtained from **Lamont *et al.* (2017)** who stated that lactic acid bacteria (LAB) has a highly effect on improvement the vine growth, yield and quality of grape cultivars through its ability to live in the plant endosphere, which is responsible to enhance plant production by improving nutrient availability, acting as a bio control agent, alleviating biotic stresses, and directly stimulating plant growth.

In addition, photosynthetic bacteria enhance cluster size by promoting synthesis and translocation of carbohydrates on grape fruits as they play a central role in carbon fixation as well as oxygen production and able to produce organic substances from inorganic nutrients based on energy harvested from light (Schmidt *et al.*, 2013).

Furthermore, on Thompson seedless grapevines Alhawezy and Ibrahim (2018) stated that the number of clusters/vine, cluster weight, yield increased by application of yeast, and there were significant differences between treatments comparing to control vines.

In addition, Hassan and Salem (2020) proved that treating Flame seedless grape vines with biofertilizers application (N-fixing bacteria) achieved the best results in term of yield, cluster weight, berry weight and size. Also, El-Salhy *et al.* (2021) found that using 2-4 g/L yeast was effective in producing high yield with good cluster and berry quality of Flame Seedless grapevines.

Table 3. Effect of spraying different concentrations of Bio-Tech on yield and its attributes for Red Globe and Crimson Seedless cvs. in both 2019& 2020 seasons

Treatments	Red globe								
	Yield (Kg/vine)		Cluster weight (g)		Berry weight (g)		Berry size (cm ³)		
	2019	2020	2019	2020	2019	2020	2019	2020	
Control (100% mineral fertilizers)	20.3	19.8	882.3	851.5	12.0	13.6	10.6	11.9	
Bio-Tech (5L/ fed) (soil addition)	21.0	21.5	935.8	952.4	14.5	15.9	12.5	13.0	
Bio-Tech (5L/ fed) (foliar spray)	22.5	23.0	913.7	920.9	13.9	15.4	12.1	13.5	
Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	22.4	22.7	973.1	987.2	15.0	16.6	12.7	13.3	
Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	23.6	24.3	1026.0	1056.3	15.7	18.3	13.5	14.5	
New L.S.D at 5%	0.8	0.9	20.2	21.5	0.5	0.5	0.4	0.5	
Treatments	Crimson seedless								
	Control (100% mineral fertilizers)	10.5	10.3	418.0	412.5	2.8	3.2	1.5	1.9
	Bio-Tech (5L/ fed) (soil addition)	13.7	14.2	546.0	568.6	4.3	4.5	2.6	2.8
	Bio-Tech (5L/ fed) (foliar spray)	12.5	13.8	498.7	552.0	4.1	4.4	2.2	2.5
	Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	15.4	15.9	616.3	636.1	4.7	4.9	3.0	3.3
	Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	16.8	17.4	673.4	696.5	5.0	5.2	4.2	4.8
	New L.S.D at 5%	0.9	1.0	34.2	36.7	0.1	0.1	0.3	0.3

2. Chemical characteristics of berries

Total soluble solids (TSS%), total acidity (TA%), TSS/acid ratio and anthocyanin (mg/100g FW)

An obvious increase in berry TSS % and TSS/acid ratio were due to the double application with high rates of Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5 L/ fed) (foliar spray) (T5) whereas the least values were attributed to 100% mineral fertilization treatment as shown in Table (4). Also, (T5) scored an effective reduction in total acidity % in both seasons for the both cultivars which is could be ascribed to the yeast positive effect on these parameters. Similarly, Alhawezy and Ibrahim, (2018) reported that the best results were obtained by spraying yeast which caused significant increase in total soluble solid (TSS), decreased titratable acidity (TA), with highest value of TSS/TA ratio compared with the untreated vines. In another trial similar results were recorded by Radwan *et al.* (2019) found

that foliar spray with active dry yeast is very necessary treatment in increasing TSS % and reducing acidity % of Superior Seedless berries. The positive impact could be due to the yeast effect on photosynthesis processes and the increment of the promoter hormone cytokinin. The same trend was observed by **Hassan and Salem (2020)** on Flame seedless grape vines with applying the biofertilizers (N-fixing bacteria) which achieved the best results in significantly producing the highest percentage of TSS, TSS/acid ratio and reducing juice acidity.

Likewise, anthocyanin pigment in berry skin recorded the highest values with vines received the double application of Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5 L/ fed) (foliar spray) (T5). Similarly, **El-Morsy *et al.* (2017)** found soil application by yeast extract significantly increased the anthocyanin accumulation when applied on Red globe grapevines. In another trial done on Flame Seedless grapevines, vines treated with 2-4 g/L yeast decreased juice acidity as well as increasing TSS, and anthocyanin contents (**El-Salhy *et al.*, 2021**).

Table 4. Effect of spraying different concentrations of Bio-Tech on chemical characteristics of berries for Red Globe and Crimson Seedless cvs.in both 2019&2020 seasons.

Treatments	Red Globe								
	TSS %		TA %		TSS /acid ratio		Anthocyanin (mg/100g FW)		
	2019	2020	2019	2020	2019	2020	2019	2020	
Control (100% mineral fertilizers)	16.3	16.5	1.12	1.21	18.3	13.6	40.1	42.4	
Bio-Tech (5L/ fed) (soil addition)	17.5	18.6	0.65	0.72	26.9	25.8	48.4	51.7	
Bio-Tech (5L/ fed) (foliar spray)	17.0	17.2	0.93	0.90	18.4	19.1	45.9	46.2	
Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	18.4	18.8	0.49	0.54	37.6	36.1	55.3	58.1	
Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	19.5	20.0	0.28	0.35	69.7	57.1	60.1	62.0	
New L.S.D at 5%	0.8	0.9	0.11	0.09	20.4	18.8	2.2	1.9	
Treatments	Crimson seedless								
	Control (100% mineral fertilizers)	16.8	17.5	1.1	1.1	15.9	15.3	36.9	38.2
	Bio-Tech (5L/ fed) (soil addition)	17.9	18.7	0.65	0.72	27.5	25.9	48.4	51.7
	Bio-Tech (5L/ fed) (foliar spray)	17.3	18.1	0.69	0.76	21.8	20.2	42.7	46.8
	Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	18.9	19.1	0.49	0.54	38.6	35.4	55.3	58.1
	Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	19.5	20.0	0.28	0.35	69.6	57.1	60.1	62.0
	New L.S.D at 5%	0.4	0.5	0.01	0.01	11.5	12.4	0.4	0.3

3. Physical and chemical characteristics of leaves

1. Leaf area (cm²), shoot length (cm) and total chlorophyll content (SPAD)

Significant differences among treatments in leaf area, shoot length and chlorophyll content were recorded in Table (5). Results indicated that combined both Bio-Tech (5L/ fed) as (soil addition) with Bio-Tech (5 L/ fed) as (foliar spray) showed the best vegetative growth parameters for both red cultivars in both successive seasons. On the other hand, the control vines had the least values in this respect. These results were recorded before in a trial done by **Ahmed *et al.* (2017)** it was found that leaf area and shoot length, were significantly affected by treating the vines with 50% mineral N (ammonium nitrate) and 50% bacterial strains over the application of N as 100 % inorganic N alone. In this line, treating Flame seedless grape vines with biofertilizers application (N-fixing bacteria) achieved the best

results concerning the vegetative growth parameters as shoot length and leaf area, producing significantly the highest values (Hassan and Salem, 2020). Similar results in using biofertilization (*Azotobacter chroococcum*) Ibrahim *et al.* (2020) declared that chlorophyll contents exhibited the highest values when applied on own rooted cuttings of Flame seedless than the untreated cuttings.

Table 5. Effect of spraying different concentrations of Bio-Tech on Leaf area (cm²), shoot length (cm) and chlorophyll (SPAD) for Red Globe and Crimson Seedless cvs. in both 2019 & 2020 seasons

Treatments	Red Globe						
	Leaf area cm ²		Shoot length cm		Chlorophyll (SPAD)		
	2019	2020	2019	2020	2019	2020	
Control (100% mineral fertilizers)	135.6	149.3	156.9	162.4	30.5	32.7	
Bio-Tech (5L/ fed) (soil addition)	169.1	170.9	173.3	175.6	37.9	38.1	
Bio-Tech (5L/ fed) (foliar spray)	160.8	160.9	168.2	171.8	36.6	37.8	
Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	172.4	176.3	188.6	188.7	39.5	41.3	
Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	185.9	197.5	196.5	198.3	43.8	45.9	
New L.S.D at 5%	10.5	11.1	6.0	7.0	2.1	2.3	
Treatments	Crimson seedless						
	Control (100% mineral fertilizers)	155.3	143.7	140.1	145.5	29.4	30.0
	Bio-Tech (5L/ fed) (soil addition)	169.8	164.6	155.9	162.6	34.7	36.1
	Bio-Tech (5L/ fed) (foliar spray)	165.4	159.2	153.7	158.0	33.4	35.7
	Bio-Tech (2.5L/ fed) (soil addition)+ Bio-Tech (2.5 L/ fed) (foliar spray)	174.5	172.5	164.6	177.3	36.7	38.3
	Bio-Tech (5L/ fed) (soil addition) + Bio-Tech (5L/ fed) (foliar spray)	189.9	183.3	177.9	189.7	40.8	41.5
	New L.S.D at 5%	11.4	11.0	8.0	7.1	1.8	2.0

2. N%, P%, and K% in leaf petiole

The positive effect of applying the bio-stimulant Bio-Tech (5L/ fed SA.) along with Bio-Tech (5L/ fed FS.) (T5) is clearly increasing the leaf pedicel content of N%, P%, K% for the both red cultivars while the fourth treatment Bio-Tech (2.5L/ fed SA.) along with Bio-Tech (2.5 L/ fed FS.) came in the second position as compared with the control treatment which ranked last in its effect (Figure, 2). The active dry yeast positive effect on nutritional status of the vines was attributed to its own different micro and macro nutrients, vitamins, amino acids, enzymes, carbohydrates, auxins and cytokinins. Moreover, yeast applications are probably responsible for facilitating leaf stomata opening (Kamelia *et al.*, 2000b) to release carbon dioxide which improves net photosynthesis rate. In addition, it is a natural source for most of nutritional elements as well as, other growth promoting substances (Abd El-Galil *et al.*, 2003) this in turn may leads to an increase in the uptake of elements as a result of increasing root distribution. Moreover, a positive effect was also detected on NPK seasonal changes especially N and P. when yeast extract at 2.5 gm/L, was sprayed as natural bio-stimulant (El-Boray *et al.*, 2007). Additionally, El-Halaby *et al.* (2015) evaluated the effect of spraying active dry yeast on Flame Seedless grapevines stating that due to its high content of minerals, led to induce an improving in the N, P and K percentage. Furthermore, on Flame seedless grapevines El-Salhy *et al.* (2017) using 25% mineral plus 75% yeast and bio-power as bio-form significantly increased leaf nutrient composition and minimized the environmental Pollution compared with untreated vines.

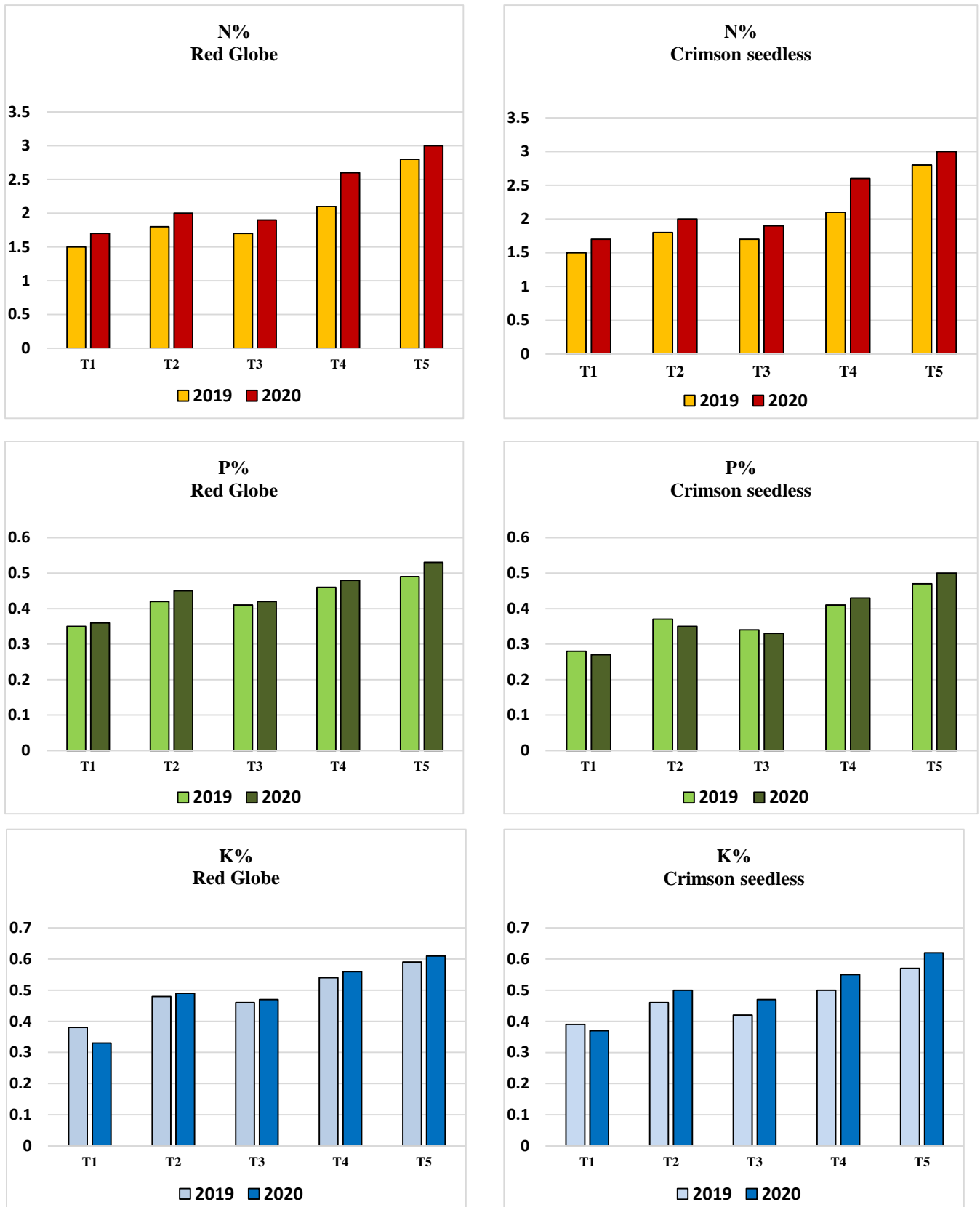


Fig. 2. Effect of spraying different concentrations of Bio-Tech on the chemical characteristics (N, P and K %) of leaf petioles for Red Globe and Crimson Seedless cvs.in both 2019&2020 seasons.

II. Experimental product

1. Chemical composition of yoghurt after adding grape juice

The changes of composition in different yoghurt treatments as affected by adding grape juice at 8 and 12 % for both cultivars presented in (Table 6) were not significant. However, the total solids (TS) was slightly higher in yoghurt with grape juice at 8 and 12 % for Crimson seedless with Bio-Tech (16.45 and 16.77), than Red globe total solids at the same concentrations (16.36 and 16.68) and the control (16.08, 16.18) respectively. In addition, the nutritional value of yoghurt with grape juice was better in terms of fat, protein and ash content; these results were due to the chemical composition of grapes treated with Bio-Tech. Moreover, the acidity of yoghurt was decreased, as stated by **Fayed *et al.* (2016)** who was reported that, the high rate of production of lactic acid in yoghurt was observed at the initial 12 days due to the high bacterial metabolic activity with the consumption of lactose.

In general, the pH was lower in yoghurts fortified with control grape juice when compared with grape juice of Red globe (GJR) and Crimson seedless (GJC) treated with Bio-Tech. **Leeand Lucey (2015)** mentioned that, the increased solids content in yoghurt milk as a result of fortification also creates increased buffering that requires additional anti-heat stress treatments development by starter culture to achieve a similar pH target. The increasing in pH values of yoghurt fortified with grapes ranged from 4.62 to 4.89. Ash increased from 0.77 to 0.88 %.

Table 6. Chemical Composition of yoghurt with adding grapes

Samples	T.S %	Protein%	Fat%	Ash%	Titratable acidity	pH values
A	16.08 ^d	4.14 ^c	3.23 ^c	0.77 ^a	1.77 ^a	4.62 ^c
B	16.18 ^c	4.15 ^c	3.23 ^c	0.78 ^a	1.77 ^a	4.62 ^c
C	16.36 ^b	4.26 ^b	3.37 ^b	0.80 ^a	1.73 ^b	4.75 ^b
D	16.68 ^a	4.33 ^a	3.55 ^a	0.86 ^a	1.65 ^c	4.80 ^a
E	16.45 ^b	4.27 ^b	3.46 ^b	0.86 ^a	1.68 ^c	4.82 ^a
F	16.77 ^a	4.34 ^a	3.69 ^a	0.88 ^a	1.60 ^c	4.89 ^a

Mean values with different small letters are statistically significant ($P \leq 0.05$).

A: Yoghurt with 12% grape juice of Red globe without Bio-Tech (control)

B: Yoghurt with 12% grape juice of Crimson seedless without Bio-Tech (control)

C: Yoghurt with 8% grape juice of Red globe with Bio-Tech

D: Yoghurt with 12% grape juice of Red globe with Bio-Tech

E: Yoghurt with 8% grape juice of Crimson seedless with Bio-Tech

F: Yoghurt with 12% grape juice of Crimson seedless with Bio-Tech

2. Changes in phenolic compounds and total antioxidant activity of yoghurt after adding grape juice

Data presented in Table (7), indicated that the grapes treated Red globe, Crimson seedless with bio-Tech was a good source of total phenolic content (TPC) and pronounced free radical scavenging activity. The TPC of grapes treated Red globe, Crimson seedless with bio of yoghurt was higher than control. However, TPC generally and antioxidant activity were dropped after 10 days of storage (Table 7). The level of phenolic compounds and the free radical scavenging capacity of the resultant yoghurt increased with the increase of added grapes treated. These results are in agreement with those obtained by

(Ashoush *et al.*, 2013 and Saleh *et al.*, 2018). These results suggest that addition of both grapes treated with bio can increase the nutritional value of yoghurt by enhancing its antioxidant capacity. Thus, the enrichment with both grapes treated with bio increases health benefits by increasing antioxidant properties as reported by (Jette *et al.*, 2010).

Table 7. Changes in phenolic compounds and total antioxidant activity of yoghurt with adding grapes.

Parameters*	A		B		C		D		E		F	
Storage periods (days)	5	10	5	10	5	10	5	10	5	10	5	10
Phenolic compounds (mg/100gm)	4.90 _d	1.28 _d	7.01 _c	3.99 _c	15.30 _b	11.90 _b	17.70 _a	13.50 _a	16.20 _b	12.80 _b	18.86 _a	14.62 _a
Total antioxidant activity (%)	25.62 _d	10.36 _d	35.50 _c	19.32 _c	76.87 _b	40.62 _b	95.02 _a	66.40 _a	86.30 _b	48.28 _b	96.50 _a	81.64 _a

Mean values with different small letters are statistically significant ($P \leq 0.05$).

* See footnote Table 6

3. Minerals content of yoghurt after adding grape juice

Data presented in Table (8) illustrate the major minerals of control and yoghurt with adding both grapes with bio. The adding grapes 8, 12% Crimson seedless with bio had significantly higher potassium content (205.37, 205.70mg/100g) whereas the control had only (176.00 mg/100g). The same trend of results was recorded with respect to Ca, P, Mg and Fe contents. Richness of date with different minerals (Chaira *et al.*, 2007; El-Sohaimy and Hafez, 2010; Hasnaoui *et al.*, 2010; Trabzuni *et al.*, 2014) was responsible for such significant increase in the prepared products.

Table 8. Mineral contents (mg/100g) of yoghurt with adding grapes

Samples*	Ca	K	P	Mg	Fe
A	183.04 _d	176.00 _d	171.21 _d	12.03 _c	0.47 _c
B	187.07 _c	204.99 _c	174.86 _c	18.56 _b	0.79 _b
C	183.45 _b	205.26 _b	175.25 _b	18.79 _b	0.81 _a
D	184.41 _a	205.50 _a	176.12 _a	19.01 _a	0.82 _a
E	183.98 _b	205.37 _b	175.72 _b	18.90 _b	0.82 _a
F	184.87 _a	205.70 _a	176.65 _a	19.14 _a	0.83 _a

Mean values with different small letters are statistically significant ($P \leq 0.05$)

* See footnote Table 6.

4. Microbiological analysis of yoghurt after adding grape juice

Microbiological analysis of yoghurt with adding grapes (Table 9) showed that, total count increased during storage periods, samples A and B had the higher total count than the other treatments. It can be seen clearly from Table 9 that yeast and mold counts increased progressively during storage. Çon *et al.* (2016) found that, the high yeast and mold count could be attributed to contamination from air, the fruit marmalade, molasses and the 1 day old culture used for yogurt manufacture. Treatments E and F had

the lowest total count and yeast & mold than all treatments during storage periods; these results may be due to decreasing of acidity and the effect of the percentage of anti-heat stress treatment in grapes.

Table 9. Microbiological analysis of yoghurt after adding grape juice

Parameters*	A		B		C		D		E		F	
Storage periods(days)	5	10	5	10	5	10	5	10	5	10	5	10
Total count(log ¹⁰)	8.18 ^a	8.48 ^a	8.20 ^a	8.52 ^a	7.31 ^b	7.44 ^b	7.32 ^b	7.42 ^b	7.13 ^c	7.20 ^c	7.06 ^c	7.12 ^c
Molds & Yeast(log ¹⁰)	ND**	4.22 ^a	ND	4.25 ^a	ND	3.44 ^b	ND	3.46 ^b	ND	2.65 ^c	ND	2.50 ^c

* See footnote Table 6

**ND: Not detected.

Mean values with different small letters are statistically significant ($P \leq 0.05$).

5. Rheological characteristics of yoghurt after adding grape juice

Yoghurt made with grapes treated by anti-heat stress treatments (Table 10), it was observed the differences between treatments of the yogurts fortified with the different dairy ingredients. Results indicated that, except of the springiness criterion, other texture parameters, namely hardness, cohesiveness, gumminess and chewiness exhibited proportionally higher values. The texture of samples treated by bio changed, these observations may be due to increasing of total solids while, springiness of yoghurt samples behaved opposite trend. Similar findings were reported by **Pollard *et al.* (2013)**.

Table 10. Changes in Rheological characteristics of yoghurt after adding grape juice

Samples*	Hardiness (N)	Chewiness (N/m)	Cohesiveness (-)	Springiness (mm)	Gumminess (N)
A	0.7 ^b	1.97 ^c	0.24 ^c	12.06 ^a	0.2 ^c
B	0.7 ^b	1.69 ^c	0.27 ^c	12.10 ^a	0.2 ^c
C	0.9 ^b	2.52 ^b	0.32 ^b	11.35 ^b	0.3 ^b
D	0.9 ^b	2.55 ^b	0.38 ^b	11.25 ^b	0.4 ^a
E	1.8 ^a	3.76 ^a	0.52 ^a	11.20 ^b	0.4 ^a
F	1.9 ^a	4.21 ^a	0.54 ^a	11.08 ^b	0.4 ^a

N: Newton m: meter mm: millimeter

Mean values with different small letters are statistically significant ($P \leq 0.05$).

* See footnote Table 6

6. Sensory evaluation of yoghurt after adding grape juice

Sensory attributes were evaluated as flavor, texture, color and overall acceptability. The results for the experimental yogurts are given in (Table 11). The yoghurt samples with grapes containing anti-heat stress treatments had a higher scores of overall acceptability compared with the other treatments. These results may be due to the physical characteristics of grapes treated by anti-heat stress treatments.

Texture of yoghurt is influenced by various factors such as quality and composition of milk and its protein and fat content, heat treatment, combination of lactic acid bacteria used, acidification rate, and storage time (**Sodini *et al.*, 2014**).

Similarly, sensory evaluation scores on the flavor, the body and texture, and the color and appearance of the yogurts fortified with casein hydrolysis decreased by storage time (Zhao *et al.*, 2016). It was observed that, samples E and F had the highest scores of sensory evaluation while, samples A and B had the lowest scores.

Table 11. Sensory evaluation of yoghurt after adding grape juice

Sample*	Storage periods (days)	Appearance (10)	Flavor (50)	Body & Texture (30)	Color (10)	Overall acceptability
A	5	8 ^b	46 ^a	27 ^a	9 ^a	90 ^a
	10	6 ^b	42 ^b	26 ^b	6 ^b	80 ^b
B	5	9 ^a	47 ^a	27 ^a	8 ^a	91 ^a
	10	7 ^b	41 ^b	25 ^b	6 ^b	79 ^b
C	5	9 ^a	47 ^a	27 ^a	9 ^a	92 ^a
	10	8 ^b	44 ^b	26 ^b	7 ^b	85 ^b
D	5	9 ^a	47 ^a	27 ^a	9 ^a	92 ^a
	10	8 ^b	45 ^b	27 ^a	7 ^b	87 ^b
E	5	9 ^a	48 ^a	28 ^a	9 ^a	94 ^a
	10	8 ^b	46 ^b	26 ^b	7 ^b	87 ^b
F	5	9 ^a	49 ^a	29 ^a	8 ^a	95 ^a
	10	8 ^b	46 ^b	28 ^a	7 ^b	89 ^b

Mean values with different small letters are statistically significant ($P \leq 0.05$).

* See footnote Table 6

CONCLUSION

From this study, we conclude that replacing 50% of mineral fertilizers partially with the safety application of the bio-stimulant Bio-Tech at (5L/ fed) as (soil addition) along with Bio-Tech (5 L/ fed) as (foliar spray) for Red Globe and Crimson seedless grape cvs. Proved its beneficial effects in improving vegetative and reproductive growth performances besides their chemical characteristics of berries and leaves as compared with other treatments in this investigation. In addition to preserving human health, reducing soil and environmental pollution in both conventional and organic farming for sustainable agriculture. Moreover, it was recommended to enrich the yogurt with the grape juice of Crimson seedless variety at 8 or/and 12%, treated with Bio-Tech as bio-stimulant substance can be used as a functional ingredient in yoghurt due to its flavor, natural, antioxidant and fibers content in addition to its nutritive constitute and health promoting characteristics, improving the rheological characteristics and total acceptability of yogurt. Therefore, grapes treated with Bio-Tech bio-stimulant can be used successfully to obtain a new fermented functional yogurt product with high sensory composition, properties and acceptable sensory attributed comparable with the untreated grapes. So, it could be recommended in the manufacture acceptability comparable to untreated grapes samples.

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