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Quality attributes of *Flame seedless* grapes as affected by some bio-stimulants

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Abstract : This study was undertaken during 2014 and 2015 seasons to investigate the beneficial effects of different bio-stimulants as foliar application on chlorophyll, nutritional status, yield and physico-chemical characteristics of grapevines cv. '*Flame seedless*'. Multiple types of bio-stimulants such as a): microbiota [*Bacillus megaterium, Bacillus subtilis, Azospirillum lipoferum*, and yeast (*Saccharomyces cerevisiae*)] b): algae [*Scenedesmus obliquus*, *Spirulina platensis* and seaweed (*Ascophyllum nodosum*)] were evaluated. However, control treatment was water spray. Furthermore, all treatments with reference to the standard applied GA_3 treatments. Each bio-stimulant was sprayed one time at five grapevine-phonological growth stages. The obtained results showed that the majority of these responses were significantly higher than control and some than the standard GA_3 treatment. Generally, using algae extract of *Ascophyllum nodosum* at 4g/L led to clear enhancements in the majority of the tested vegetative and fruiting parameters of *Flame seedless* grapevines.
Key words: Grapevine, *Flame seedless*, Bio-stimulant, Vegetative growth, chlorophyll, Nutritional status, Yield and Quality parameters.

Introduction:

Grape is one of the widely grown fruit crops in the world [1]. It occupies top position in the world with respect to area and production. However, in Egypt it ranks 2nd after citrus with a harvested area that reached approximately 72190 ha and a total production about 1596169 tons [2].

Flame seedless is one of the most important cultivars cultivated in the Egyptian vineyards for both exportation and local market. It originated in Fresno, California by the U. S. Dept. of Agriculture in 1973, vines are moderate of vigour and very productive. They respond well to gibberellic acid treatment [3].

GA_s are used for cluster elongation, flower thinning, also to increase berry size and make it uniformity in seedless table grapes [4,5,6]. However, it has some hazards as decreasing fertility [7] and delaying maturity [8,9,10]. In this respect, some studies were accomplished for producing organic fruits from vineyards through avoiding the application of chemicals and synthetic hormones as well as encouraging the application of bio-stimulants as well as afford the costs of chemical and synthetic hormones, which considered as pollutants. The utilization of bio-stimulants is considered as a promising alternative, particularly for developing countries [11].

Plant bio-stimulant is any microorganism or substance based on natural resources, in the form in which it is supplied to the user, applied to plants, seeds or soil and any other substrate with the intention to stimulate

natural processes of plants to benefit their nutrient use efficiency and/or their tolerance to stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use [12].

Bacillus species used as bio-stimulants probably have direct effects on plant growth through the synthesis of plant growth hormones [13].

Azospirillum synthesizes and metabolizes GAs in vitro and in plant. The production of different GA compounds and metabolism of exogenously applied GA are reported for different *Azospirillum* species [14].

Yeast (*Saccharomyces cervicisae*, L.) is considered as one of the promising bio-stimulant for many crops [15]. The positive effect of yeast application could be due to one or more merits: yeast aids in activating photosynthesis process through enhancing the release of carbon dioxide [16], or/and yeast contains some natural growth regulators, i.e. auxin (IAA) [17], GA_3 [18,19] and cytokinins [20]. Also, the yeast was found to encourage the uptake of various nutrients as N, P and K and some common amino acids [21].

Algal extract is a bio-stimulant containing N, P, K, Ca, Mg and S as well as Zn, Fe, Mn, Cu, MO and Co, some growth regulators, polyamines and vitamins applied to improve nutritional status, vegetative growth, yield and fruit quality in different orchard as well as vineyards [22].

Seaweed extract being organic and biodegradable in nature is considered as an important source of nutrition for sustainable agriculture [23]. Seaweeds contain various trace elements (Fe, Cu, Zn, Co, Mo, Mn & Ni), vitamins, amino acids and plant growth hormones which cause many beneficial effects on plant growth and development [24,25,26]. The extract of seaweeds has been reported to induce many positive changes in treated plants such as improved crop yield, increased nutrient uptake, resistance to frost and stress conditions, increased postharvest shelf life, increased seed germination and reduced incidence of fungal and insect attack [24]. Foliar applications of seaweed extract has been reported to influence growth, productivity and fruit quality of some fruit crops including '*Red roomy*' [27], '*Superior seedless*' [28,22] and '*Thompson seedless*' [29] grapes.

The purpose of the present study is to investigate the impact of some bio-stimulants on the vegetative growth, nutritional status, yield and quality of *Flame seedless* grapes with reference to the standard applied GA_3 treatments.

Materials and Methods

This study was carried out through two successive seasons of 2014 and 2015 on own rooted *Flame seedless* grapevines in a private vineyard at El-Bostan district, Beheira Governorate, Egypt. Vines were 10 years old at the beginning of the investigation, planted 1.5 × 3 m apart in sandy soil with a drip irrigation system of two laterals each had two 4L emitter used for irrigation and fertigation. Selected vines were uniform in growth vigour, trellised with a modified Y trellising system, and quadrilateral cordon trained by leaving about 80 eyes/vine. Normal management practices recommended by the national Ministry of Agriculture were adopted.

The present investigation was set as a Completely Randomizes Block Design with twelve foliar treatments adopted each comprising three replicates each made of two vines. Same vines were used in both seasons for same treatments included in this investigation.

The applied foliar treatments were as follows:

1. Control (water spray).
2. Standard treatment (GA_3) the commercial product Gibro Acid (10% GA_3) (Orbital Agrochemicals) has been used.
3. Microbiota culture extracts

The plant growth promoting bacteria (PGPB) *Bacillus megaterium, Bacillus subtilis, Azospirillum lipoferum* in addition to the yeast strain *Saccharomyces cerevisiae* were obtained from the culture collection of the Microbiology Dept., Fac. Agric., Cairo Univ., Egypt. Nutrient Agar medium [30] was used for cultivation and subculturing of *B. megaterium* and *B. subtilis* and this medium fortified with 1% glucose was used as well for

cultivation and subculturing the yeast (*S. cerevisiae*). *A. lipoferum* was propagated and maintaind in malic acid medium [31]. All bacterial candidates were preserved in 1/1 glycerol/ broth culture mixture at - 40° C.

Bacterial and yeast inocula preparation: One liter of 48hr-old culture of each examined bacterial or yeast strain was inoculated in an autoclave plastic container (20-liter Nestle® mineral water container) containing 9 liters of the specific medium of the inocula strain. An air pump was used for cultures aeration through sterilized filter during an incubation period at 30° C for 72 hrs. Population density of each inocula culture was standardized to 10^8 cells. ml^{-1} using sterilized broth media applying the direct microscopic counting method.

4. Algal bio-stimulants

a) *Scenedesmus obliquus* (Green Algae)
b) *Spirulina platensis* (Blue Green Algae).

They were grown in the Algae Biotechnology Unit, Fertilization Technology Department, National Research Centre (NRC), Dokki, Cairo, Egypt. Both *S. obliquus* and *S. platensis* were produced within three open ponds with a final capacity of 75 m^3. Continuous centrifugation (Westifalia Separator) was employed for harvesting algal bulk which contains 75-80 % moisture) and then after freeze for 48 hrs at -25° C. Prior centrifugation, algal slurry was drastically stressed by hyper nutritional doses to meet obligatory nutrient accumulation within algal cells. The frozen bulk was then re-melted at the room temperature, homogenized and an aerobically fermented 72 hrs. The fermented biomass was then homogenized and filtered till it used [32]. Major components of the used algal extracts are shown in Table (1).

Table (1): **Chemical composition of algal bio-stimulants**

Contents	***Spirulina platensis***	***Scenedesmus obliquus***
Macro Nutrients (%)		
N	13.30	7.42
P_2O_5	2.22	1.94
K_2O	2.13	1.86
MgO	0.11	0.04
Na	0.07	0.02
CaO	0.23	0.43
Micro Nutrients (ppm)		
Fe	4644	3361
Cu	21	23
Zn	954	751
Mn	1300	441
Essential amino acids (mg/g dry weight)		
Isoleucine	4.62	3.13
Leucine	6.2	5.01
Lycine	2.95	3.13
Methionine	4.1	3.39
Phenylalanine	4.98	2.87
Threonine	4.12	3.12
Valine	3.65	2.08
Histidine	4.09	3.06

c) Seaweed (*Ascophyllum nodosum,* Brown Algae). The commercial product CYTOLAN® CONCENTRATED POWDER based on *A. nodosum* (100% seaweed extract) was obtained from (Opal trading and service co.) Major components of powder are shown in Table (2).

Table (2): Chemical composition of CYTOLAN® CONCENTRATED POWDER

Contents	*Ascophyllum nodosum*
Total Nitrogen (N) (%)	1
Phosphorus Pentoxide (P_2O_5) (%)	0.2
Potassium Oxide (K_2O) (%)	10
C/N ratio	10/1
Mg (%)	0.42
Ca (%)	0.17
Fe (ppm)	0.06
Mn (ppm)	0.05
Cu (ppm)	0.003
S (%)	1.16
As (ppm)	50
Phytohormones (ppm)	600
Carbohydrates (%)	35
Alginic Acid (%)	3
Mannitol (%)	9
Organic matter (%)	45 – 55

Application timing: The afore mentioned treatments were all sprayed at five phonological stages i.e. cluster length 10-13 cm, 50 – 60 % bloom (cap fall) and when average berry diameter was 6-7 mm, 7-8 mm and 8-9 mm.

Rates of application and doses: On each spraying date, each vine was sprayed with adopted treatment with a hand pressure sprayer till runoff (approximately 3L/vine).

GA_3 was applied at; 10,10,20,30 and 40 ppm for the five previously mentioned phonological stages respectively.

Microbiota bio-stimulants were sprayed with each culture separately at the same time of GA_3 application. Whereas, the resultant cultures were containing 10^8 cells. ml^{-1} for *B. megaterium, B. subtilis, A. lipoferum* and Yeast (*S. cerevisiae*), respectively.

However, *S. obliquus*, *S. platensis* and *A. nodosum* extracts were sprayed at two rates (2 and 4g /L/ Vine) after diluted with tap water to one Liter immediately before spraying.

The following parameters were assessed for this study:

1. Vegetative growth determinations:

Four new shoots were randomly chosen per vine to measure the following parameters e at the end of the growing season:

a. Shoot Length (cm)

b. Shoot girth (mm) by using a digital vernier caliper

c. Number of leaves per shoot.

d. 20 leaves sample (6^{th} leaf from the apical) for each vine were collected to measured average leaf area (cm^2) in mid-July according to [33], by using the following equation Leaf Area (cm^2) = 0.45 (0.79 x maximum diameter) + 17.77.

2. Leaf chemical analysis:

a. Total Chlorophyll was calorimetrically determined in fresh leaf samples according to [34].

b. Further, In mid-July a 20 leaves sample include blade and petiole (6th leaf from the apical) for each vine were collected to determined leaf contents of macro and micro nutrients. N (%) using the modified micro-kjeldahl method as lined by [35]. P (%) was estimated as described by [36]. While K and Ca (%) determined by using flame photometer according to [37]. In addition, micro nutrients Fe, Zn and Mn (ppm) determined by using atomic absorption (Model, spectronic 21 D) as described by [38].

3. Yield and cluster parameters:

Four clusters per vine were harvested at the ripening stage when juice TSS% reach to 16% in control treatment to determine the average of: Yield/vine (kg), Cluster length (cm), Cluster width (cm) and Cluster weight (g).

4. Berry physical parameters:

Hundred berries/cluster were used to determine the average of: Berry length (mm), Berry diameter (mm), Berry size (cm^3), Berry weight (g),Juice volume of 100 berry (cm^3) and Berry Firmness (g/cm^2), by using a texture analyzer instrument; Fruit Hardness Tester, No.510-as a small cylinder (3 mm in diameter) penetrates into a distance of 3 mm inside the berry with a speed of 0.2 mm / second, then the resistance of berry to this penetration force was recorded and taken as an expression of berry firmness (g/cm^2).

5. Berry chemical analysis:

Total soluble solids (TSS %) in juice was determined by using a hand refractometer. Then TSS/acidity ratio was measured further total anthocyanin (mg/100g fresh weight) was measured according to [39].

Statistical analysis: Obtained data was subjected to analysis of variances (ANOVA) according to [40] using MSTAT program. Duncan Multiple Range test [41] was used to compare between means at probability of 5 %.

Results and Discussion

1. Effect of treatments on vegetative growth parameters of *Flame seedless* grapevines

Average shoot length was significantly affected by different treatments in both seasons compared with control. Insignificant differences between vines treated with *S. platensis* (4g/L), *S. obliquus* (4g/L) and control were noticed in the 2nd season only (Table, 3). In addition, *A. nodosum* (4g/L) treatment resulted in significantly the longest shoot (247.17 and 252.17 cm in both seasons respectively), compared with control vines and the rest of treatments. Whereas control vines and those treated with *S. platensis* (4g/L) resulted significantly the shortest shoot 180.77 and 181.53 cm in the 1st and the 2nd seasons, respectively (Table ,3).

Data in Table (3) also show that insignificant differences in shoot girth observed between all treatments and control in the 1st season. While in the 2nd season, it was clear that all treatments significantly increased this parameter compared with control with statistically equal magnitude.

It is evident from Table (3) that control untreated vines attained significantly the lowest number of leaves per shoot compared with all treatments (23.42 and 25.60 leaves/shoot). *A. nodosum* (4g/L) resulted in the highest magnitude of this parameter (31.83 and 32.76 leaves/shoot in both seasons, respectively) with insignificant differences from all treatments except *S. obliquus* at both rates, Yeast (*S. cerevisiaen*) and *S. platensis* (2g/L) in the 1st season and *S. platensis* (4g/L) in the 2nd one.

Data in Table (3) show that leaf area was affected evidently by different treatments in both seasons. *A. nodosum* (4g/L) treatment resulted in significantly the largest leaf area (169.24 and 173.97 cm^2). Insignificant differences were attributed to *B. megaterium* in both seasons, *S. obliquus* (4g/L) in the first season and *B. subtilis* in the second season. However, the smallest leaves area was attributed to control (133.59 and 130.07 cm^2) and *S. platensis* (2g/L) treatment that were statistically equal.

Table (3): Effect of bio-stimulants on vegetative growth parameters of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	Shoot length (cm)		Shoot girth (mm)		No. leaves/shoot		Leaf area (cm^2)	
	2014	2015	2014	2015	2014	2015	2014	2015
Control (water spray)	180.77i	183.38fg	0.95a	0.95b	23.42e	25.60c	133.59g	130.07f
Standard treatment (GA_3)	232.53c	235.30bc	1.01a	1.22a	30.75ab	32.01ab	157.57c	157.21c
B. megaterium	186.95gh	190.38f	1.05a	1.26a	31.58ab	32.68ab	166.44ab	172.18a
B. subtilis	213.77f	216.69e	1.04a	1.24a	31.08ab	32.35ab	161.71bc	171.27ab
A. lipoferum	225.10d	228.46cd	1.02a	1.23a	29.00abc	30.26abc	151.95d	143.98e
Yeast (*S. cerevisiae*)	189.77g	191.66f	0.98a	1.19a	25.50cde	26.76abc	148.88de	149.24de
S. obliquus (2g/L)	213.17f	215.70e	1.00a	1.21a	26.75cde	28.10abc	150.92d	154.12cd
S. obliquus (4g/L)	184.93h	187.79fg	1.01a	1.22a	25.33de	26.68bc	166.83ab	165.32b
S. platensis (2g/L)	217.20e	220.45de	1.01a	1.22a	28.00bcd	29.10abc	135.27g	133.64f
S. platensis (4g/L)	185.42h	181.53g	0.99a	1.19a	31.67ab	32.76a	141.40f	144.93e
A. nodosum (2g/L)	238.93b	243.17b	1.01a	1.22a	30.75ab	32.18ab	144.98ef	144.28e
A. nodosum (4g/L)	247.17a	252.17a	1.02a	1.23a	31.83a	32.76a	169.24a	173.97a

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Concerning to vegetative growth characters of *Flame seedless* grapevines, the previous finding was in parallel with those of [22] on *Superior seedless* cv., [42] on '*Feteasca alba*' cv., [43] on '*Perlette*' cv. and [44] on *Early superior* cv. They reported that, using algae and seaweed extracts stimulated vegetative growth parameters significantly compared to untreated vines. The promotion was depended on increasing extract concentrations. The increase in vegetative characters ascribed to the hormonal action of algae and seaweed extracts, which increased the endogenous hormonal level of treated grapevines [26,43,45].

The obtained results of the effect of foliar application of yeast (*S. cerevisiae*) on vegetative growth characters are agree with [46] on *Ruby seedless* cv., [47] on *Thompson seedless* and *Flame seedless* cvs. They reported that, foliar application of yeast significantly increased vegetative growth compared with the untreated vines (control). The detected enhancements in vegetative growth due to yeast extract application was due to an increase in levels of endogenous hormones, i.e. IAA and GA_3 in treated plants which leads to more cell division and elongation. In addition to the physiological roles of vitamins and amino acids found in the yeast extract which results in an increase in the metabolic processes and due to its effect in activating photosynthesis process [48].

Results indicated that treated vines with microbiota (*B. megaterium, B. subtilis, A. lipoferum*) had a positive effect on vegetative growth parameters in comparison to untreated vines. These results are agree with [49] on *Superior seedless* grapevines, [50] on *Crimson seedless* grapevines and [51] on *Flame seedless* grapevines. They reported that using microorganisms as bio-stimulants on grapevines stimulated vegetative growth parameters such as shoot length, shoot diameter, number of leaves per shoot and leaf area in comparison with untreated vines.

2. Effect of bio-Stimulants treatments on leaf total chlorophyll content of *Flame seedless* grapevines

Total leaf chlorophyll content was significantly affected by different conducted treatments in both seasons (Table, 4). Data clarify that *S. platensis* sprayed at 2 or 4g/L resulted in the highest leaf chlorophyll content in both seasons. Statistically equal results were attributed to *S. obliquus* (4g/L) and *B. subtilis* (2g/L) in the 2nd season only. On the other hand, control untreated vines registered significantly the lowest value of leaf chlorophyll content in both seasons. Insignificant differences in this respect were due the standard treatment and *A. nodosum* (2g/L) treatment in the 1st seasons.

The effect of *S. platensis* on leaf chlorophyll content may be due to its' content of has N [23]. Moreover, algae extract may have a role through its content of cytokinins which delays the aging of leaves by reducing the degradation of chlorophyll. In addition, algal extract as a bio-regulator affecting the balance between photosynthesis and respiration processes in plants [52,53]. [25] also reported a 12% increase in the chlorophyll contents in 'Fuji' apple leaves with a consequent increase in the photosynthesis and respiration rates attributed to using of algal extract.

Table (4): Effect of bio-stimulants on chlorophyll contents of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	Total Chlorophyll (mg.g^{-1})	
	2014	2015
Control (water spray)	2.61g	2.65e
Standard treatment (GA_3)	2.71efg	2.89d
B. megaterium	2.86cd	3.04bc
B. subtilis	2.72ef	3.13ab
A. lipoferum	2.84cd	3.04bc
Yeast *(S. cerevisiae)*	2.78de	2.97cd
S. obliquus (2g/L)	2.72ef	2.93cd
S. obliquus (4g/L)	2.93bc	3.12ab
S. platensis (2g/L)	3.01ab	3.16ab
S. platensis (4g/L)	3.06a	3.22a
A. nodosum (2g/L)	2.65fg	2.85d
A. nodosum (4g/L)	2.72ef	3.02bc

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

3. Effect of bio-Stimulants treatments on leaf content of macro and micro – nutrients of *Flame seedless* grapevines

Leaf N content was significantly altered by various applied treatments when compared with control. (Table, 5). *A. nodosum* (4g/L) induced statistically the highest leaf N content 2.25 and 2.45 % in both seasons, respectively. Statistically equal results were attributed to *A. nodosum* (4g/L) and the standard treatment GA_3 in the 1st season only. While, in the 2nd season they were attributed to both *A. lipoferum* and *B. subtilis*. While, utreated vines exhibited the lowest leaf N content 1.43 and 1.48 % in both seasons, respectively with insignificant differences from that exhibited by *S. obliquus* (4g/L) in the first season only.

B. megaterium resulted statistically the highest leaf P content 0.36 and 0.32 % in both seasons, respectively. Whereas control attained the lowest leaf content of P 0.22 and 0.19 % in both seasons, respectively (Table, 5). No significant differences were observed between control and all conducted treatments except *B. megaterium* in both seasons and *S. platensis* (2g/L) in the 2nd season only as both were significantly higher in this respect.

Results in Table (5) also indicate that leaf K content was significantly affected by all treatments in both seasons. *S. platensis* (4g/L) treatment in the first season and *S. obliquus* (4g/L) treatment in the 2nd season resulted in the highest significant percentage of K in leaves. Statistically equal percentages were attributed due to *S. obliquus* (4g/L), *A. nodosum* in its two rates, *S. platensis* (4g/L), standard GA_3 and *B. megaterium* in the 2nd season only. Furthermore, *S. obliquus* (2g/L) resulted in the lowest percentage of K content in leaves 0.75 and 0.60 % in both seasons, respectively. Insignificant differences were achieved by control treatment, *B. subtilis* and yeast (*S. cerevisiae*) in the 1st season. While in the 2nd season Insignificant differences were achieved by control and *S. obliquus* (2g/L).

As shown in Table (5) the all of conducted treatments significantly increased the Ca content in leaves compared with control except for the Yeast (*S. cerevisiae*) in both seasons and the *S. obliquus* (2g/L) treatment in the 2nd season as their effect was statistically equal to control. The standard treatment GA_3 resulted in the significantly the highest leaf Ca content in both seasons with insignificant differences attributed to *S. obliquus*

(4g/L), *S. platensis* (4g/L) and both *A. nodosum* treatments in the 1st season. In the 2nd season however, only the *A. nodosum* (4g/L) treatment achieves statistically equal results to that of the standard GA_3 treatment.

Fe leaf content was significantly increased by all of the adopted treatments compared with control by different treatments in both seasons (Table, 6). *A. nodosum* (4g/L) resulted in significantly the highest Fe leaf content in both seasons. With respect to the leaf Zn content, all adopted treatments significantly increased this parameter compared with the control and the standard GA_3 treatment which were statistically equal. Both the *B. subtilis* and the *A. nodosum* (4g/L) treatments induced significantly the highest percentages.

As shown in Table (6) data point out that, leaf content of Mn was significantly increased by all of the applied treatments when compared with the control. Yeast (*S. cerevisiae*) treatment recorded in the highest significant leaf amounting to 90.31 and 90.90 ppm in both seasons

Table (5): Effect of bio-stimulants on macro nutrients of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	N %		P %		K %		Ca %	
	2014	2015	2014	2015	2014	2015	2014	2015
Control (water spray)	1.43g	1.48g	0.22b	0.19c	0.76e	0.62f	1.48e	1.61e
Standard treatment (GA_3)	2.16abc	2.32b	0.26b	0.25abc	0.92bc	0.85abcd	1.97a	2.28a
B. megaterium	1.81f	2.01d	0.36a	0.32a	0.98b	0.85abcd	1.83c	2.13bc
B. subtilis	1.98e	2.45a	0.30ab	0.27abc	0.77e	0.71e	1.61d	2.07c
A. lipoferum	2.12bcd	2.50a	0.28ab	0.26abc	0.87cd	0.83bcd	1.90b	2.13bc
Yeast *(S. cerevisiae)*	1.76f	1.88e	0.27b	0.28abc	0.82de	0.79d	1.51e	1.63e
S. obliquus (2g/L)	2.01de	2.32b	0.29ab	0.26abc	0.75e	0.60f	1.60d	1.73e
S. obliquus (4g/L)	1.47g	1.60f	0.26b	0.26abc	0.98b	0.92a	1.95ab	2.02c
S. platensis (2g/L)	2.07cde	2.19c	0.30ab	0.29ab	0.87cd	0.82cd	1.81c	1.89d
S. platensis (4g/L)	1.82f	1.96d	0.23b	0.21bc	1.14a	0.91ab	1.95ab	2.06c
A. nodosum (2g/L)	2.19ab	2.27b	0.27b	0.26abc	0.92bc	0.85abcd	1.95ab	2.15bc
A. nodosum (4g/L)	2.25a	2.45a	0.28ab	0.26abc	0.93bc	0.87abc	1.95ab	2.25ab

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Table (6): Effect of bio-stimulants on micro nutrients of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	Fe (ppm)		Zn (ppm)		Mn (ppm)	
	2014	2015	2014	2015	2014	2015
Control (water spray)	121.7k	122.2k	23.46f	24.18f	60.67l	61.31l
Standard treatment (GA_3)	130.9e	131.4e	24.52f	25.25f	86.80d	87.39d
B. megaterium	128.8f	129.4f	33.50b	34.43b	77.78g	78.38g
B. subtilis	135.1c	135.8c	37.67a	38.59a	68.23j	68.83j
A. lipoferum	125.8g	126.4g	26.07e	26.71e	72.77h	73.36h
Yeast *(S. cerevisiae)*	123.7i	124.3i	33.04bc	33.74bc	90.31a	90.90a
S. obliquus (2g/L)	124.7h	125.3h	29.25d	30.03d	87.00c	87.58c
S. obliquus (4g/L)	131.9d	132.5d	34.14b	34.92b	70.75i	71.34i
S. platensis (2g/L)	122.7j	123.3j	31.98c	32.66c	89.81b	90.40b
S. platensis (4g/L)	125.8g	126.4g	28.31d	29.04d	62.70k	63.29k
A. nodosum (2g/L)	141.2b	141.9b	34.22b	35.00b	79.78f	80.38f
A. nodosum (4g/L)	149.4a	150.1a	36.95a	37.71a	82.78e	83.36e

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Concerning leaf content of both macro and micro - nutrients and total chlorophyll. Results are in a line with those of [44] on '*Early superior*' cv., [43] on '*Perlette*' cv., where they postulated that spraying seaweed and algae extracts increased the total chlorophylls, N, P, K, Fe, Zn and Mn in the leaves in comparison with untreated treatment. [54] on grapevine *Karaerik* cv showed that spraying algae and algae extracts had no significant effect on nutrient uptake. The beneficial effects of seaweed (*A. nodosum)* extract on *Fame seedless* grapevines might be attributed its' own content of essential amino acids, minerals, vitamins, organic foods, amino acids and natural plant hormones namely IAA, GA_3 and cytokinins [55].

As for the results induced by yeast on macro they are in parallel with those of [56] on *Thompson seedless* and [57] on *Flame seedless* grapevines. They both illustrated that, treatments with yeast (*S. cerevisae*) caused significant increases in N, P, K, Fe, Zn and Mn of leaf petiole and chlorophyll content in leaves.

The effects of microbiota (*B. megaterium, B. subtilis, A. lipoferum*) on increasing the aforementioned contents were previously cited cited by [49] on *Superior seedless* grapevines, [50] on *Crimson seedless* grapevines, [51] on *Flame seedless* grapevines. They reported that, application of microbiota had a positive effect on leaf chlorophyll content and leaf nutritional status.

4. **Effect of bio-Stimulants treatments on yield and cluster parameters of *Flame seedless* grapevines**

All of the applied treatment significantly increased the yield when compared with control. However, the effect of both Yeast *(S. cerevisiae)* and *B. subtilis* in the 1st season and *A. lipoferum* in the 2nd were insignificantly different from control. Significantly the most superior yield was dedicated to *A. nodosum* (4g/L) treatment amounting to 18.70 and 20.98 kg/vine in both seasons, respectively (Table, 7).

As shown in Table (7) statistically the shortest clusters were due to control in both seasons of the investigation. *S. platensis* (2g/L) treatment resulted in statistically equal magnitudes to control. All remaining treatments enhanced this parameter statistically with various degrees. Significantly, the longest clusters were due to sprays with *S. platensis* at (4g/L) in both seasons. The attained length was insignificantly different from those resulting from all of the remaining treatments except *B. megaterium, A. lipoferum* and Yeast (*S. cerevisiae*).

Cluster width was significantly altered by different treatments with various degrees in both seasons (Table, 7). Significantly, the narrowest clusters were found on control untreated vines. All treatments except that of the standard treatment (GA_3), *A. lipoferum*, *S. obliquus* (2g/L) and *S. platensis* (2g/L) in the 1st season showed statistically equal effects. They were all equal statistical and significantly higher than control. Whereas in the 2nd season, only the yeast treatment was statistically equal to control. All the remaining treatments were significantly higher than control.

Data presented in Table (7) also reveal that compared with control and the remaining treatments, *A. nodosum* at (4g/L) resulted in the significantly the heaviest cluster weight amounting to 747.90 and 839.14 g in both seasons, respectively. Whereas, the lightest clusters were those born on control vines (632.12 and 655.07 g) in both seasons, respectively. *B. subtilis* and yeast (*S. cerevisiae*) in the 1st season and *A. lipoferum* in the 2nd one was resulted in clusters of statistically equal weights.

Table (7): Effect of bio-stimulants treatments on yield and cluster parameters of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	Yield/vine (kg)		Cluster Length (cm)		Cluster Width (cm)		Cluster Weight (g)	
	2014	2015	2014	2015	2014	2015	2014	2015
Control (water spray)	15.80h	16.38i	16.67f	16.67f	7.08c	9.50c	632.12h	655.07j
Standard treatment (GA_3)	18.43b	19.27d	23.50abc	23.50abc	9.92a	12.17ab	737.34b	770.63e
B. megaterium	16.52g	19.64c	21.17bcde	21.17bcde	8.58abc	11.67ab	660.88g	785.48c
B. subtilis	15.95h	16.78h	22.67abcd	22.67abcd	7.42c	12.00ab	638.00h	671.10i
A. lipoferum	17.32d	16.44i	20.00de	20.00de	10.08a	12.00ab	692.73d	657.72j
Yeast *(S. cerevisiae)*	15.88h	18.77e	20.83cde	20.83cde	8.67abc	10.75bc	635.01h	750.94f
S. obliquus (2g/L)	16.84e	19.49c	22.33abcd	22.33abcd	9.67ab	13.17a	673.49e	779.67d
S. obliquus (4g/L)	16.59fg	19.36d	23.83abc	23.83ab	8.25abc	12.67ab	663.58fg	774.25e
S. platensis (2g/L)	16.77ef	17.28g	18.67ef	18.67ef	9.75ab	11.83ab	670.83ef	691.19h
S. platensis (4g/L)	16.61fg	18.36e	24.33a	24.33a	7.67bc	12.83a	664.32fg	734.45g
A. nodosum (2g/L)	17.79c	20.57b	23.17abc	24.00ab	8.75abc	12.00ab	711.74c	822.68b
A. nodosum (4g/L)	18.70a	20.98a	24.00ab	24.00ab	9.08abc	12.67ab	747.90a	839.14a

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

5. Effect of bio-Stimulants treatments on berry physical parameters of *Flame seedless* grapevines

The standard GA_3 treatment and *B. subtilis* registered significantly the longest berries amounting to 14.82 and 17.42 mm in the both seasons, respectively. The magnitude attained was statistically equal to all treatments except for control, both *S. platensis* treatments, *A. nodosum* (2g/L) in both seasons and the *B. megaterium* treatment in the 2nd season. All those treatments resulted in significantly shorter Table, 8).

As shown in Table (8), the berry diameter was affected by the adopted treatments in manner similar to that of the length and as with similar significance detected.

As clear from Table (8) that firmest berries were due to the standard GA_3 treatment amounting to 13.83 & 16.58 g/cm^2 for both seasons respectively. Significantly softer berries compared with the standard GA_3 were due to control, *A. lipoferum* and *A. nodosum* (4g/L) treatments in the 1st season. Whereas the remaining treatments achieved berries of statistically equal firmness. In the 2nd season however, the *S. obliquus* (4g/L) and the the *A. nodosum* (2g/L) treatment resulted in berries that were statistically equal firmness to the standard GA_3 treatment. Whereas as all the remaining treatments resulted in significantly softer berries.

Results in Table (9) illustrate that berry size was affected significantly by treatments in both seasons. *S. obliquus* (4g/L) produced significantly the largest berries (2.11 and 2.3 cm^3) in both seasons, respectively with insignificant difference the remaining treatments except *S. platensis* (2g/L) and control treatments in the 1st season which were significantly smaller. While in the 2nd one, the Yeast (*S. cerevisiae*) and *A. nodosum* (4g/L) treatments resulted in berries with statistically equal size to that of the *S. obliquus* (4g/L) treatment. Whereas, all of the remaining attained statistically smaller berries.

As shown in Table (9) data reveal that all treatments increased significantly the average berry weight compared with control except for the *S. platensis* (2g/L) in both seasons and the *A. nodosum* (2g/L) in the 2nd season only as their effects were insignificantly different from control. *S. obliquus* (4g/L) resulted in significantly the heaviest berries in the 1st season with insignificant difference from the *S. obliquus* (4g/L), the Standard treatment (GA_3) and the Yeast (*S. cerevisiae*). In the 2nd season however, significantly the heaviest berries were due to the *S. obliquus* (4g/L) treatment with insignificant differences from the standard treatment, *B. subtilis, A. lipoferum,* Yeast (*S. cerevisiae*), *S. obliquus* (2g/L) and *A. nodosum* (4g/L).

Juice volume/100 berries was significantly affected by different treatments in both seasons (Table, 9). Compared with control and the remaining treatments *A. nodosum* (4g/L) induced the largest juice volume/100 berries was 178.17 and 181.73 cm^3 in both seasons, respectively. Insignificant differences were attributed to the Standard treatment (GA_3) and yeast (*S. cerevisiae*) in the 2nd season only. However, the lowest value of juice volume/100 berries was attributed to control 59.85 and 117.10 cm^3 in both seasons, respectively.

Table (8): Effect of bio-stimulants on berry length, diameter and firmness of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	Berry Length (mm)		Berry Diameter (mm)		Berry Firmness (g/cm^2)	
	2014	2015	2014	2015	2014	2015
Control (water spray)	10.82d	11.25c	11.14c	11.35d	10.50c	12.08c
Standard treatment (GA_3)	14.82a	17.01a	14.63a	15.81a	13.83a	16.58a
B. megaterium	12.71abcd	13.23b	12.55abc	13.02cd	13.83a	13.17bc
B. subtilis	13.99abc	17.42a	13.78ab	16.48a	13.50ab	14.00bc
A. lipoferum	13.23abcd	16.13a	13.01abc	15.80a	10.67c	13.75bc
Yeast *(S. cerevisiae)*	13.07abcd	16.55a	13.02abc	15.70a	12.42abc	13.00bc
S. obliquus (2g/L)	13.64abc	15.97a	13.60ab	15.50ab	13.08ab	12.58bc
S. obliquus (4g/L)	14.25ab	15.80a	13.94ab	15.04ab	12.58abc	14.75ab
S. platensis (2g/L)	12.00bcd	12.23bc	12.21bc	11.87cd	13.17ab	13.33bc
S. platensis (4g/L)	12.12bcd	13.49b	12.17bc	13.59bc	13.75a	12.92bc
A. nodosum (2g/L)	11.56cd	13.40b	11.33c	12.80cd	13.00ab	13.50bc
A. nodosum (4g/L)	13.75abc	17.04a	13.96ab	16.53a	11.42bc	14.17abc

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Table (9): Effect of bio-stimulants treatments on berry size, weight and juice volume of *Flame seedless* grapevines 2014 and 2015 seasons

Treatment	Berry Size (cm^3)		Berry Weight (g)		Juice Volume/100 berry (cm^3)	
	2014	2015	2014	2015	2014	2015
Control (water spray)	1.47b	1.22h	0.75g	1.22d	59.85i	117.10h
Standard treatment (GA_3)	1.98ab	2.40cd	1.42abcd	2.55a	113.75e	177.77ab
B. megaterium	1.89ab	2.08de	1.03def	2.41b	84.97g	151.78d
B. subtilis	1.98ab	2.33bc	1.74bcde	2.71ab	149.33b	174.88bc
A. lipoferum	1.82ab	2.25f	1.71f	2.17ab	136.33c	141.36e
Yeast *(S. cerevisiae)*	2.04ab	2.28ab	1.61abc	2.93ab	143.23bc	178.07ab
S. obliquus (2g/L)	1.92ab	2.18cde	1.64cde	2.48ab	125.47d	170.77c
S. obliquus (4g/L)	2.11a	2.36a	1.58a	3.02ab	125.40d	170.37c
S. platensis (2g/L)	1.49b	1.37g	0.78g	1.48d	70.50h	125.55g
S. platensis (4g/L)	1.91ab	1.68cde	1.32def	2.53c	104.50f	134.81f
A. nodosum (2g/L)	1.86ab	1.45ef	0.83ef	2.31cd	69.83h	129.76g
A. nodosum (4g/L)	2.08a	2.22ab	1.95ab	2.82ab	178.17a	181.73a

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Generally, foliar application of bio-stimulants especially at its highest rates was statistically far behind the control concerning yield, cluster parameters and physiological parameters of berries of *Flame seedless* grapevines in both seasons of the study. Seaweed extract had the highest values in most studied parameters.

Results on yield and cluster parameters are in parallel with those obtained by [58] on *Thompson seedless* grape, [59] on *Trakya ilkeren* grape, [44] on *Early superior* grape and [43], on *Perlette* grape. They reported that foliar

spraying of algal extracts specially seaweed extract increased yield and cluster weight per vine. Increase in yield due to seaweed-treated vines, are thought to be associated with the hormonal substances present in the algae extracts, especially cytokinins [60,61,62]. Cytokinins in vegetative plant organs are associated with nutrient partitioning, whereas in reproductive organs, high levels of cytokinins may be linked with nutrient mobilization (better mobilization photosynthesis

Concerning physical parameters of berries seaweed extract increase berry size, berry weight and berry firmness. The obtained results are agree with [59,63] on *Trakya ilkeren* grape and [43] on grapes cv. '*Perlette*'. They showed that, treated vines with foliar application of seaweed extract had an increasing effect on berry size, berry weight and berry firmness in comparison with untreated vines. The increase in physical parameters of berries is ascribed to the increased of chlorophyll contents of leaves, which increased photosynthesis and ultimately overall health of vine.

6. Effect of bio-Stimulants treatments on berry chemical analysis of Flame seedless grapevines

Results in Table (10) illustrate that TSS was affected by applied treatments in both seasons. *A. nodosum* (4g/L) treatment recorded significantly the highest percentage 18.20 % and 18.45 % in both seasons, respectively. Insignificant differences were attained between *A. nodosum* (4g/L) and *A. nodosum* (2g/L) in both seasons, and *S. plantensis* (4g/L) in the 1st season only. *B. megaterium* induced statistically the lowest percentage of TSS in both seasons with insignificant differences with standard treatment GA_3, *B. subtilis*, *A. lipoferum* and *S. obliquus* (2g/L) in both seasons.

TSS/Acid ratio was affected by different treatments in both seasons (Table, 10). *A. nodosum* at 2 or 4 g/L resulted in the highest value of TSS/Acidity ratio in both seasons, While, *B. megaterium* was resulted in the lowest ratio of TSS/Acidity in both seasons.

Skin anthocyanin content varied with varies degrees of significane as a resulted of applying the adopted treatments (Table, 10). *A. nodosum* (4g/L) resulted in significantly the highest content (29.57 and 31.58 mg/100g f/wt. in both season, respectively). Whereas, control scored the lowest content (20.44 and 21.07 mg/100g f/wt. in both seasons, respectively). Insignificant differences were attained between control and both *Bacillus* treatments in the two seasons of the investigation. In the 2nd season however, insignificant differences from control were attained by both *A. lipoferum* and yeast (*S. cerevisiae*) treatments.

Table (10): Effect of bio-stimulants on berry chemical analysis of *Flame seedless* grapevines in 2014 and 2015 seasons

Treatment	TSS (%)		TSS/Acidity ratio		Anthocyanin (mg/100g f/wt.)	
	2014	2015	2014	2015	2014	2015
Control (water spray)	16.20bc	16.25c	31.15d	31.25d	20.44f	21.07e
Standard treatment (GA_3)	15.60cde	15.80cd	29.43e	29.26e	25.99b	27.00bc
B. megaterium	14.00e	14.50d	23.33h	23.39h	21.55ef	21.98e
B. subtilis	14.45de	14.75d	25.35g	25.00g	21.35ef	22.02e
A. lipoferum	15.00cde	15.40cd	27.78f	27.50f	22.89de	23.31de
Yeast *(S. cerevisiae)*	16.20bc	16.30c	32.40cd	31.35d	22.39de	23.24de
S. obliquus (2g/L)	15.40cde	15.60cd	29.06ef	28.36ef	24.02cd	24.63d
S. obliquus (4g/L)	16.00bcd	16.45c	34.78b	36.56b	24.05cd	25.44cd
S. platensis (2g/L)	16.30bc	16.35c	33.27bc	34.79c	25.53bc	27.36bc
S. platensis (4g/L)	16.75abc	16.82bc	33.50bc	34.33c	26.14b	27.86b
A. nodosum (2g/L)	17.60ab	17.85ab	37.45a	37.98ab	28.08a	29.10b
A. nodosum (4g/L)	18.20a	18.45a	36.40a	38.44a	29.57a	31.58a

Mean separation within each column by Duncan multiple range (0.05); Means with similar letters are insignificantly different

Regarding the chemical parameters of berries algal extract increased TSS, TSS/acidity ratio in juice and anthocyanin content in skin. Results obtained are in harmony with those by [22] they indicated that foliar

application of algal extract on '*Superior seedless*' grapevines significantly improved TSS synthesis. Similarly, [29,64] also observed that application of bio-stimulants improved the TSS/acidity ratios in '*Red roomy*' and '*Thompson seedless*' grapes.

The enhancements in the tested chemical properties are suggested to be due to actions and effects of nutrients, vitamins, and growth regulators contained by these extracts [22,65,66] which are reflected on fruit quality. Also, the increase in leaf total chlorophyll content was reflected on increasing rate of photosynthesis rate and accumulation of carbohydrates reserves which lead to positive effect on fruit quality. In addition, Increase in TSS and TSS/acidity ratio may be related to enzymes which are present in seaweed extract that enhanced the synthesis of different proteins, acids and sugars.

Regarding the increase in the anthocyanin content, it could also be attributed to the increase in the leaf chlorophyll which in turn is expected to enhance the photosynthetic activity. Carbohydrates play a vital role in the development of fruit colour [67].

Conclusion

In conclusion, our results show that, beneficial responses were attributed to the adopted treatments. The majority of these responses were significantly higher than control and some than the standard GA_3 treatment. In general, using algae extract *A. nodosum* (4g/L) led to clear enhancements in the majority of the tested vegetative and fruiting parameters of *Flame seedless* grapevines. These enhancements might be due to increasing the photosynthetic capacity and or its' rich content of nutrients and hormones.

These findings needs further investigation to optimize their effects as it will be of crucial importance for both conventional and organic viticulture.

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