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Effect of Green Alga Cells Extract as Foliar Spray on Vegetative Growth, Yield and Berries Quality of Superior Grapevines

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Abstract: The effect of green alga *Chlorella vulgaris* cells extract as foliar spray on nutrient status, growth and yield of superior grapevines was studied and compared with micronutrients foliar fertilizer. Application of algal extract at 25 to 100% had an announced effect on percentages of bud burst and fruiting buds rather than the check treatment. The effect was obviously appeared till 50% concentration, while the promotion was slight with using concentrations above 50%. Growth characters namely leaf area, shoot length and number of leaves/shoot were greatly stimulated in response to application of Algal extract at 25 to 100% rather than the check treatment. There was a slight stimulation when using algal extract at concentrations above 50%. A progressive increase on percentages of N, P and K in the leaves was observed as a result of increasing concentration of algal extract till 50% while with concentrations above 50% the increase was slightly appeared. Yield expressed in weight and number of clusters as well as berry weight were gradually improved in response to increasing algal extract concentrations. Using algal extract above 50% had neglectable effect. Therefore, 50% concentration is suggested to be beneficial from economical point of view. Spraying algal extract at 25 to 100% was accompanied with hastening fruit quality compared to the untreated vines. The advancement was corresponded to increase concentrations of algal extract. Slight promotion was detected on fruit quality as a result of using algal extract above 50% which increased TSS, TSS/Acid ratio and total sugars and decreasing total acidity rather than control.

Key words: Superior grapevines % Micronutrient % Alga cells extract % Vegetative growth and yield

INTRODUCTION

Grape is suggested to be one of the most important fruits for local consumption and export. It is the first fruit crop allover the world with a total cultivated area more than 7919550 hectares produced more than 65.7 million metric tons of fruits according to Food and Agriculture Organization (FAO) [1] statistics. More than 60% of grape is utilized in wine production while the rest (40%) is used in other purposes as table grapes, raisin grapes, fresh juice and canning either solitary or as fruit salad [2].

In Egypt, grape is considered the second major fruit crop after citrus owing to its acreage which attained 160,625 feddan produced 1751880 metric ton fruits out of the total crops area which reached 1118911 feddan produced 6753768 metric ton fruits according to the Annals Reports of statistical Institute and Agricultural Economics Research, 2005.The key for increasing quality of Egyptian grapes exportation to the European markets and reducing the competition with the other producers is by maximizing the early production of table grapes with high berry quality. Supplying the vines with bud breaking chemicals as well as bio-fertilizer extracts containing macro and micronutrients, growth regulators, vitamins and antioxidants proved to be very effective in enhancing fruit repining and improving physical and chemical properties of the grapes.

Superior grapevine successfully gown under Egypt conditions. Such cv. ripens early in June and sometimes in the last week of May under sandy soil conditions, which gave this cultivar a great potentiality for export to foreign markets. A considerable part of Egyptian vineyards usually suffers from the deficiency of most macro and micronutrients due to the great depletion and exhaustion of these nutrients by vines as well as the neglecting of their use by growers. The nutritional status of vines plays an important role in the dynamic activities of metabolism [2]. The deficiency of nutrients in the Egyptian soil became a widespread phenomenon. When this phenomenon is left without solving it causes a

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dangerous disturbance in physiological activities of grapevines that reflects in producing lower yield and poor fruit quality [3].

Kulk [4] and Adam [5] reported the growth promotion in response to application of nitrogen fixer cyanobacterium *Nostoc muscorn* could be attributed to the nitrogenase as well as nitrate reductase activities of algae associated with the surface of plants, or the amino acids and peptides produced in algal filtrate and/or other compounds that stimulated growth of crop plants.

In addition, bio-fertilization is very safe for human, animal and environment to get lower pollution and reduce soil salinity via decrease mineral usage fertilization as well as saving fertilization cost.

Alga extract as a new bio-fertilizer 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.

MATERIALS AND METHODS

This study was carried out during 2006 and 2007 seasons on five years old superior grapevines grown in a private vineyard located at El-Mansoriea, Giza Governorate, where the soil is silty clay loam, well drained and water table not less than two meters deep.

Physical and chemical characteristics of the soil samples (from 0.0 to 90 cm depth) were determined according to the standard procedures outlined by Wilde *et. al.* [6]. The obtained data are shown in Table 1.

The chosen vines were trained according to cane pruning system using modified Y shape supporting system. Pruning was carried out at the second week of December in both seasons. The uniform in vigor vines was spaced at 1.75 m. (between vines) x 2.5 m. (between rows).

Table 1: Physical and chemical analysis of the tested orchard soil

Characters	Value	Characters	Value
Particle size distribution:		**Available macronutrients:**	
Clay	36.3	N%	0.11
Salt	42.5	P ppm.	19.00
Sand	21.2	K ppm.	385.00
Texture grade	Silt clay loam	Mg ppm	1.02
pH (1:2.5 suspension)	7.90	**Available micronutrients:**	
EC (1:2.5) mmohs/cm	0.40	Zn ppm	2.50
Organic matter %	1.85	Fe ppm	5.10
Total carbonate %	1.33	Cu ppm	0.85

Table 2: Major chemical composition and elemental contents of *chlorella vulgaris* cells

Major components of the cells cellextract extract and algal protein amino acid composition		Elements content of the (Major component %)	
Protein	44.6%	**Macro-elements %**	
Fats	7.3%	N	7.10
Carbohydrates	12.8%	P	0.66
Amino acid composition		K	2.15
(g/100 g protein)*		Ca	0.18
Arginine	6.9	Mg	0.34
Histidine	2.0	Ng	0.04
Isoleucine	3.2		
Lucien	9.5	**Micro-elements (ppm)**	
Lysine	6.4	Fe	245.00
Methionine	1.3	Mn	131.20
Phenylalanine	5.5	Zn	111.50
Threonine	5.3	Cu	28.00
Tryptophan	1.5		
Valine	7.0		

* Source El-Fouly *et al.* [7]

Treatments: The present experiment was set in a complete randomized block design in a split-plot arrangement with three replicates each consisted one superior grapevine.

A concentrated slurry of the microalgae *Chlorella vulgaris* (contains about 10% water) was washed with distilled water, re-concentrated by centrifugation, frozen and then re-melted at the room temperature. Then the melted slurry was then centrifuged at 5000 rpm to obtain a clear cells sap. Major components and nutrient content of the algal extract is shown in Table 2.

The vines were treated with the following treatments as foliar sprays three times, the first at 10 days before blooming (1st week of March) then after berry setting (1st week of April) and the last one at 21 days later(the last week of April). The vines were sprayed with the solutions till run off (about one liter/ vine). Treatments were arranged as the follows:-

Control: Tap Water

MN : Micronutrient liquid contains 0.3% Manganese, 0.3% Zinc and 0.3% copper in the from of chelate (Nervaneed) in a concentration of 2 ml/L. in the spray solution.

T_1 : 25% (v/v) alga cells extract in tap water.

T_2 : 50 % (v/v) alga cells extract in tap water.

T_3 : 75 % (v/v) alga cells extract in tap water.

T_4 : 100 % (v/v) alga cells extract.

All vines received the regular agricultural and horticultural practices applied on the vineyard.

Bud Behavior: Observation on bud behavior were carried out at weekly intervals during the period started at the first week of May in 2006 and 2007 seasons. The number of buds that burst and fruiting buds were recorded for each vine to calculate the percentages of both as follows:

The percentage of bud burst was calculated according to the following equation:

$$\text{Bud burst \%} = \frac{\text{Number of burst buds}}{\text{Total number of left buds per vine}} \text{ X } 100$$

The percentage of fruiting buds was calculated according to the following equation:

$$\text{Fruiting buds \%} = \frac{\text{Number of fruiting buds}}{\text{Total number of burst buds per vine}} \text{ X } 100$$

Measurement of Vegetative Growth Parameters:

- The average leaf area (in square centimeters) was estimated during the second week of May in both the two experimental seasons through picking twenty mature leaves from those opposite to the basal clusters on the shoots. Leaf area was measured using the equation reported by Ahmed and Morsy [8].
- Shoot length (cm) and number of leaves/shoot were recorded.

Determination of Leaf Chemical Constituents

Percentage of Nitrogen, Phosphorus and Potassium: Twenty petioles of the same leaves picked measure leaf area at the second week of May according to Kavanci and Ataly [9] and Balo *et al.* [10]. Samples were washed with tap water twice with distilled water followed by oven dried at 70°C and ground in stainless steel mil. The dried samples were digested by using sulphuric acid and hydrogen peroxide, then nitrogen, phosphorus and potassium were determined in the digestions and the results were calculated as percentages of the determined element relative to the dry weight basis.

Total Yield: Harvesting was carried out at the normal commercial harvesting time for this cultivar in the experimental region when total soluble solids percentage reached about 16% in the berries of control vines (the last week of May 2006 and 2007). The number of clusters per vine was recorded. The average weight of each individual cluster was estimated (in grams) and the total yield per vine in kilograms was delivered.

Properties of the Berries: Five clusters were selected randomly from the yield of each replicate as a composite sample for berry juice chemical analysis.

Chemical Composition of Berries: A known weight of the berries were taken from each sample blended by the use of an electric blender, then the following constituents were estimated in the juice according to the corresponding methods:

- Percentage of total soluble solids by using a hand refractometer.
- Total titratable acidity by titration with a known normality solution of sodium hydroxide using phenolphthalein as indicator. Total acidity was calculated as grams of tartaric acid as per 100 grams of juice.
- Total soluble solids/ acid ratio was calculated by dividing (1) by (2).
- Total sugars percentage using Land and Eynon procedure that outlined in A.O.A.C. [11].

Statistical Analysis: All the obtained data were tabulated and statistically analyzed according to Snedecor and Cochran [12] using the L.S.D. test at 5% level to recognize the significance of the differences between various treatment means.

RESULTS AND DISCUSSION

Effect of Algal Extract on Behavior of Buds: Data in Table 3 showed that algal extract treatments had a positive effect on behavior of bud burst % and fruiting buds in the two seasons. Application of algal extract at 50 to 100% had announced effect on behavior of buds. A remarkable and significant promotion was detected on vines received algal extract via foliage rather than those untreated. The stimulation was associated with increasing algal extract concentration. However, increasing concentration from 50 to 100% failed to increase these characters significantly. The maximum values were obtained when the vines treated with algal extract at 100%. The untreated vine gave the minimum values.

The present results are in agreement with those obtained by Kubato *et al*. [13] who worked in Thompson seedless grapevines, Abd El-Hady [14] on Flame seedless grapevines, El-Sayed *et al.* [15] on Roomy grapevines, Omran *et al.* [16] and Abd El-Wahab [17] on early superior grapevine cv.

Table 3: Foliar application with micronutrients (MN) and different concentrations of green alga cells extract on bud burst and fruiting buds (%) of Superior grapevines during 2006 and 2007 seasons

	Season 2006		Season 2007	
Treatments	Bud burst %	Fruiting buds %	Bud burst %	Fruiting buds %
Control	42.30	40.20	41.40	38.70
MN	64.00	61.30	65.30	62.20
T_1	54.00	50.50	56.20	53.00
T_2	63.70	61.00	60.00	59.50
T_3	70.30	70.20	72.00	71.30
T_4	70.10	69.00	71.50	70.90
L.S.D. at 5% level	2.53	3.22	2.30	3.20

Table 4: Foliar application with micronutrients (MN) and different concentrations of green alga cells extract on leaf area, shoot length and number of leaves/shoot of Superior grapevines during 2006 and 2007 seasons

	Season 2006			Season 2007		
Treatments	Leaf area (cm^2)	Shoot Length (cm)	No. of leaves/shoot	Leaf area (cm^2)	Shoot Length (cm)	No. of leaves/shoot
Control	135.00	141.70	21.30	131.50	139.20	19.40
MN	153.70	160.20	25.00	163.80	161.20	24.40
T_1	141.30	149.70	23.70	152.30	140.80	21.00
T_2	151.70	155.30	25.00	154.40	146.30	23.40
T_3	166.10	173.00	28.00	174.00	162.50	26.10
T_4	165.60	172.00	26.70	174.50	170.90	31.90
L.S.D. at 5% level	3.30	5.34	1.20	3.11	60.03	1.45

Table 5: Foliar application with micronutrients (MN) and different concentrations of green alga cells extract on percentage of N,P,K in the leaves of Superior grapevines during 2006 and 2007 seasons

	Season 2006			Season 2007		
Treatments	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	1.10	0.08	0.93	1.03	0.10	0.90
MN	1.35	0.23	1.11	1.30	0.26	0.99
T_1	1.30	0.20	1.18	1.31	0.24	1.13
T_2	1.42	0.17	1.24	1.36	0.19	1.20
T_3	1.56	0.14	1.26	1.50	0.16	1.25
T_4	1.38	0.11	1.21	1.32	0.13	1.17
L.S.D. at 5% level	0.05	0.03	0.01	0.04	0.02	0.05

Effect of Algal Extract on Growth Characters: From date in Table 4, it is concluded that all growth characters (Leaf area, shoot length and number of leaves/shoot) were positively affected by using algal extract at 25 to 100%. Foliar application of algae extract was accompanied with stimulating growth characters significantly compared to untreated vines. The promotion was depended on increasing algal extract concentrations. The increase in such characters was insignificant when algal extract was used at concentrations higher than 50%. The maximum and minimum values were recorded on vines treated with 100 and 0.00% of algal extract respectively. These results were true in the two studied seasons.

These results are in coincidence with those obtained by Khiamy [18] on Flame seedless grapevines and Omran *et.al.* [16] and Madian [19] on Red Roomy grapevines, Ahmed and Abd El-Hameed [20], Mahran [21] who worked to Thompson seedless grapevines and Abd El-Haffez [22].

Effect of Algal Extract on Leaf Chemical Composition: It is evident from the obtained data in Table 5 that there was a material and significant stimulation on these nutrients with using algal extract at 25 to 100% rather than the untreated vines. The promotion was associated with increasing algal extract concentrations and meaningless increase was observed when algal extract was applied at concentration higher than 50%. Foliar application of algae extract at 0.00 and 100% achieved the minimum and maximum values, respectively.

A progressive increase on percentage of N, P and K in the leaves was observed as a result of increasing conc. of algae extract till 75% than the increase was slightly appeared.

In an agreement with the present results those obtained by Gobara *et al.* [23] on Red Roomy grapevines,

Table 6: Foliar application with micronutrients (MN) and different concentrations of green alga cells extract on No. of clusters, cluster weight and total yield/vine of Superior grapevines during 2006 and 2007 seasons

	Season 2006			Season 2007		
Treatments	No. of clusters/vine	Cluster weight/vine (g)	Total yield/vine(kg)	No. of clusters/vine	Cluster weight/vine (g)	Total yield/vine (kg)
Control	25.20	230.00	6.40	29.50	265.30	8.20
MN	32.40	460.60	11.70	39.20	495.00	16.20
T_1	29.10	394.30	9.50	34.00	440.10	12.00
T_2	35.00	534.00	14.00	44.40	579.71	17.60
T_3	39.00	548.10	15.40	49.10	595.20	20.20
T_4	37.30	490.00	13.00	41.30	502.00	16.10
L.S.D. at 5% level	1.20	25.20	0.42	2.53	30.30	0.45

Table 7: Foliar application with micronutrients (MN) and different concentrations of green alga cells extract on chemical properties of superior grapevines during 2006 and 2007 seasons

	Season 2006				Season 2007			
Treatments	T.S.S (%)	Total acidity (%)	T.S.S/acid ratio (%)	Total sugar (%)	T.S.S (%)	Total acidity (%)	T.S.S/acid ratio %	Total sugar %
Control	15.30	0.515	29.70	13.80	16.10	0.575	28.00	16.40
MN	17.50	0.630	27.80	15.50	17.90	0.608	29.40	17.20
T_1	17.20	0.553	27.90	15.70	18.70	0.599	31.20	17.50
T_2	16.90	0.625	25.00	16.40	18.60	0.520	35.80	18.30
T_3	16.60	0.683	24.30	16.90	17.70	0.525	33.70	18.50
T_4	16.20	0.648	25.00	15.60	17.50	0.514	34.00	18.00
L.S.D. at 5% level	1.94	0.020	3.30	0.62	1.65	0.030	5.80	0.71

Khiamy [18] on Flame seedless grapevines, Mahran [21] on Thompson seedless grapevines and Abd El-Wahab [17] on early superior grapevine cv.

Effect of Algal Extract on Yield: It is clear from the obtained date in Table 6 that using algal extract at 25 to 100% significantly improved the yield expressed as weight (kg) and number of clusters per vine compared to unsprayed ones. Application of algal extract at 50 to 100% three times significantly improved the yield especially in the second season of the study. Algal extract treatments had no significant effect on number of clusters in first season. Significant differences on yield were detected as a result of increasing algal extract from 0.00 to 50%, while increasing concentration than 50% failed to cause measurable effect on yield.

Using algal extract above 75% had neglectable effect. Therefore, 75% concentration is suggested to be beneficial from economical point of view. These results were true in both seasons.

These results are in agreement with those obtained by Abada [24], Ahmed *et al*. [25], Omran [16] on Red Roomy grapevines, Khiamy [18] on Flame seedless grapevines Fadl [26] on Early superior grapevines and Abd El-Wahab [17].

Effect of Algal Extract on Chemical Properties: It is worth to mention that chemical properties were greatly varied among concentrations and dates of spraying algal extract Table 7. Spraying algal at 50 to 100% was significantly improved chemical parameters in terms of increasing TSS, total sugars and TSS/acid and reducing total acidity compared to untreated vines. The promotion on chemical properties of berries was attributed to decreasing algal extract concentrations from 0.00 to 100%. Using concentrations higher than 50% had meaningless effect in this connection. Un-spraying gave unacceptable chemical properties, while supplying the vines with algal extract at 50% gave favorable effects on chemical properties parameters. The same trend was obtained in two seasons.

These results are in conformity with those obtained by Abd El-Ghany *et al.* [27], Kamel [28], Gobara *et al*. [23] on Flame seedless grapevines, on Red Roomy grapevines and Ismail-Fatten *et al.* [29] on Thompson seedless grapevines.

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