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INFLUENCE OF FULVIC ACID PLUS SOME MICROELEMENTS AND MICROORGANISMS ON YIELD AND QUALITY CHARACTERISTICS OF SUPERIOR SEEDLESS GRAPEVINES

EL-Borai, M.S.[1]; M.F. Mostafa[1]; A.D. Shaltout [2] and K.H. Hassan [3]
[1] Pomology Dept., Fac. of Agric., Mansoura Univ.
[2] Pomology Dept., Fac. of Agric., Ain Shams Univ.
[3] Agric. Res. cent., Giza, Egypt.

ABSTRACT

The present investigation was conducted during 2012, 2013 and 2014 seasons on 10-year-old Superior grapevines cultivar grown in a sandy soil, planted at 2 m within rows and 3 m between rows and irrigated using drip irrigation system at a private vineyard located at El-Khatatba, Menoufiya governorate, Egypt. The obtained data was discussed in details for the last 2 seasons only. The main objective of this investigation was to study the effect of fulvic acid foliar application 9 ml/liter /vine, either alone or in combination with micro-elements (Fe SO_4. $7H_2O$ at 0.36 g + Zn SO_4.$7H_2O$ at 0.18 g + $MnSO_4$. H_2O at 0.18 g) and bio-fertilizers (*Trichoderma viride* + *Trichoderma harzianum* + *Serratia sp*. + *Pseudomonas fluorescens* + *Bacillus polymyxa*) at 7.14 ml/liter/vine (10^4/ml per fungi and 10^9/ml per bacteria viable cell) on vegetative growth, total chlorophyll content, yield, physical and chemical characteristics of cluster and quality of berries, total carbohydrates, total N and C/N ratio in canes of Superior seedless grapevines. Results revealed that the combined treatment of fulvic acid, micro-elements plus bio-fertilizers induced indispensable positive effects for enhancing vegetative growth, total chlorophyll content, yield, physical and chemical characteristics of cluster and quality of berries, total carbohydrates, total N and C/N ratio in canes of Superior grapevines. A stronger effect may probably support the hypothesis of that humic substances have different effects on plant adequately supplied with nutrients, in sustainable or organic viticulture for production of organic products, which can be a noteworthy alternative to synthesized chemicals fertilizers to produce a healthy product-free from their toxic residues and fit for export. Foliar spray applications of these products can have prospects for optimal economical use in terms minimizing the cost of production and in turn increased the income of vineyards.

Keywords: Fulvic acid; Microelements; Yield; Quality; Canes; Seedles.

INTRODUCTION

Grape (*Vitis vinifera*, L) is one of healthy and most important fruit crops. Its varieties have been adapted to different climates around the world, the area of grapevines cultivated exceeding 18.802 million feddan (OIV, 2006). Many cultivars have been developed for wine and table consumption as used in a wide variety of products; fresh fruit, preserves, juice, wine and raisins (Creasy and Creasy, 2009). In addition that, nutritional value of consuming fresh berries, which containing natural sugars, potassium and iron, which make the grape one of the very hygienic and popular fruits for many people all over the world.

In Egypt, Superior cultivar is an early-ripening cultivar, which ripens on the first to mid June. Some grape growers suffer greatly from the remarkable depression in the level of bud fertility, which is negatively reflected on the yield of

this variety. C/N ratio can directly helps to explain fruit bud formation (Winkler *et al*, 1965).

A great attention is focused on minimizing the intensive amounts of mineral nitrogen fertilization especially under sandy soils, which are naturally poor either in nutrient elements or organic matter through using supplementary organic N fertilizers as well as bio-fertilizers, which increased nutrient use efficiencies of crops in particular of fruit crops when such inoculants were added to either organic matter or soil (Sanga Khora and Weerakera, 1999).

In this respect, organic fertilization improves vegetative growth, nutritional status and reduces the residuals of nitrate and nitrite in grape berries and in turn using organic fertilizer will be promising in the long run for grapevines (Kassem and Marzouk, 2002 and Farag, 2006). Foliar fertilization can be absorbed from 8 to 20 times as efficient as ground application (Anonymous, 1985).

Fulvic acid is very active because of its low molecular weight, it has necessary and ability to readily bond minerals and elements into its molecular structure causing them dissolve and become mobilized fulvic complexes, Fulvic acid usually carries 70 or more mineral and trace elements as part of its molecular complexes, (Aiken *et al*., 1985). Fulvic acids are key ingredients of high quality foliar fertilizers. As they can help the penetration to the plant parts, stimulate the uptake of elements from plant surfaces into plant tissues. Once applied to leaves, fulvic acids transport trace minerals directly to metabolic sites within plant cells. Hence, foliar spray applications at specific plant growth stages, containing mineral chelated can be used as a primary technique for maximizing plants productive capacity (Chen *et al*., 2004).

Micro-elements are nutrient that applied in very low concentrations to the grapevine, but they play an essential role in vegetative and fruit development. These elements are more available at lower soil pH, less available in leached sandy soils or are readily leached where the cation exchange capacity is low and the metal cations of zinc, manganese and iron are readily fixed by most soils. Therefore, soil application with synthetic chelated, which are usually quite effective can overcome these problems (Chen and Barak, 1982).

Biofertilizers are environment friendly, decreased agricultural costs with maximum output. These biofertilizers play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone production, ammonia excretion and controlling various plant diseases. Biofertilizers enhance nutritional status of leaves could be related to the role of effective microorganisms in improving the availability of nutrients and to the modifications of root growth, morphology and physiology through hormonal exudates of biofertilizers bacteria and fungi resulting in more efficient absorption of available nutrients (EL-Gamal, 1996 and Eissa, 2003).

The present study was undertaken to investigate the effects of fulvic acid foliar application either alone or in combination with micro-elements (Fe, Zn and Mn) and bio-fertilizers (*Trichoderma viride* + *Trichoderma harzianum* + *Serratia sp*. + *Pseudomonas fluorescens* + *Bacillus polymyxa*) on vegetative growth, total chlorophyll content, yield, physical and chemical characteristics of cluster and

quality of berries, total carbohydrates, total N and C/N ratio in the canes of Superior seedless grapevines cultivar.

MATERIALS AND METHODS

The present investigation was conducted during three successive seasons of 2012, 2013 and 2014. The work in the first year considered as a preliminary trial. The 10-year- old Superior grapevines were grown in a sandy soil, spaced at 2 x 3 meters apart (2m within rows and 3m between rows) and irrigated using drip irrigation system, in a private vineyard located at El-Khatatba, Menoufiya governorate, Egypt. Cane-pruned and trellised by using the Spanish baron supporting system. The vines were pruned on the first week of December in both seasons of study 96 eyes (8 canes x 12 buds/cane).

For this study, sixty three vines were chosen nearly uniform in vigor, all the chosen vines received the same cultural management that commonly performed in that district such as, fertilization, irrigation, diseases and pest control. The factorial experiment used was complete randomized block design.

Each three vines acted as a replicate and each three replicates were treated by one of the following treatments. Seven agricultural treatments were as follows:

T_1: Control (only water foliar spray).
T_2: Fulvic acid at 9 ml/liter /vine.
T_3: Micro-elements ($FeSO_4.7H_2O$ at 0.36 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g)/ liter /vine.
T_4: Bio-fertilizers (*Trichoderma viride* + *Trichoderma harzianum* + *Serratia sp*. + *Pseudomonas Fluorescens*+ *Bacillus polymyxa*) at 7.14 ml/liter /vine (10^4/ml per fungi and 10^9/ml per bacteria viable cell).
T_5: Fulvic acid (T_2) + Micro-elements (T_3).
T_6: Fulvic acid (T_2) + Biofertilizers (T_4).
T_7: Fulvic acid (T_2) + Micro-elements (T_3) + Biofertilizers (T_4).

- All treatments were applied as foliar spray.

All previous treatments were sprayed at the following 4 stages:

1- ^{1}st stage was when new shoots reached about 15-20 cm length (first week of March)
2- ^{2}nd stage was when clusters flower attained yellowish discoloration (one week before blooming).
3- ^{3}rd stage was one week after fruit setting stage (second week of April).
4- ^{4}th stage was when berries diameter reached 8-10 mm (first week of May).

Before commence of the experiment, soil samples were taken to determine mechanical and chemical properties of the experimental soil at three depths from surface, (0-30, 30-60 and 60-90cm). Such samples in each category were completely mixed and subjected to mechanical and chemical analysis to measure certain properties of soil as included in Table1.

Table 1. Mechanical and chemical properties of the experimental vineyard soil.

Soil characters		0-30 cm	30-60 cm	60-90 cm
Mechanical analysis (%)	Coarse sand	8.72	4.13	3.94
	Fine sand	69.63	70.91	71.20
	Silt	12.48	13.84	14.49
	Clay	9.17	11.12	11.57
	Texture class	Sandy	Sandy	Sandy
Chemical analysis (%)	EC (1:5) $dS.m^{-1}$	1.19	1.31	1.25
	pH (1:2.5)	8.16	8.05	8.09
	SP (%)	32	34	39
	OM (%)	0.59	0.46	0.29
	Total $CaCO_3$ (%)	6.73	4.18	3.77
Available (mg/kg)	N	21.5	18.6	14.3
	P	2.92	3.05	2.75
	K	183	171	167
	Zn	1.03	0.84	0.67
	Fe	2.17	1.79	1.54
	Mn	1.61	1.12	1.05

EC = Electrical conductivity of soil saturation extract.
Sp = Saturation percent.
OM = Organic matter.

Preparation of fulvic acid:

Compost (prepared from rice straw, farmyard manure, rock phosphate, bentonite and urea) was digested with 0.5N KOH for 48 h at room temperature in the ratio of 1/10 (W\V). Separation of the solute form the undigested residues were then carried out by filtration by 100 mesh screen. The supernatant was acidified at pH 2 with concentrated H_2SO_4 and left for 24 h in the dark in order to allow humic acid flocculation. Fulvic acid collected by filtration. (Vallini *et al*. 1990). Fulvic acid data analyses were recorded in Table 2.

Table 2. Chemical analysis of fulvic acid.

Trait	Value
pH	2.8
EC (dS/m)	8.68
Organic-C (%)	2.81
Available –N (ppm)	210
Available –P (ppm)	7.4
Total –K (%)	2.26

Preparation of microorganisms:

Serratia sp, are grown on peptone-glycerol media (Grimont and Grimont,1984), *Pseudomonas fluorescens* grown on king's media and *Bacillus polymyxa* grown on nutrient broth media (Alef, 1995) and *Trichoderma* species grown on Potato dextrose media (ATTC, 1992) were incubated for 2-3 days at 28 oC to maintain populations of 3 x 108 colony forming unit ml^{-1} (CFU\ml). All microbial strains were kindly provided from Dept. of Microbiology, Soils, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC) Giza, Egypt

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1- Measurements during vegetative growth stage:

Growth vigor of vine was estimated by the measurement of certain growth indices carried out on each of the tested vines during the two experimental seasons after two weeks from the last addition in the two seasons of study (last week of May) such indices included:

1- Average shoot length (cm) :

Shoot length was calculated by measuring the average length of 4 shoots / vine (shoot from each side)

2- Average shoot diameter (cm):

The same previous shoots were used to determine average diameter of the middle of the second basal internode by using veriner caliper.

3- Average leaf surface area (cm^2/leaf):

Representative sample of four mature leaves per each treated vine (6th or 7th leaf from the top of the same previous shoots) that were taken from the different vine sides and used for leaf surface area measurements according to the following equation (Montero *et al*. 2000):

$$\textbf{Leaf surface area } (cm^2/leaf) = 0.587\ (L \times W)$$

Where, L = Length of leaf blade, W = Width of leaf blade.

4- Leaf chlorophyll pigments (mg/g fresh weight):

Total chlorophylls content were determined in the previous leaves used for leaf surface area determination, it was estimated by taken 4 mature leaves from each side vine, as a representative sample at the 6th or 7th leaf from the shoot tip. Fresh leaf sample of 0.05 g was used, soaked in 10 ml methanol for 24 hours in cool chamber after adding a trace from sodium carbonate (Robinson and Britz, 2000), then leaf chlorophyll pigments were determined spectrophotometrically. The amount of chlorophyll present in the extract was calculated using the following equations introduced by (Arnon, 1949).

$$\textbf{Ch.A} = 16.5\ OD_{665} - 8.3\ OD_{650}$$

$$\textbf{Ch.B} = 33.8\ OD_{650} - 12.5\ OD_{665}$$

$$\textbf{Total chlorophyll} = 25.5\ OD_{650} + 4.0\ OD_{665}$$

Where, OD = Optical density at wave length of 650 and 665 nm

$$\textbf{Total chlorophyll (mg/g fresh weight)} = \frac{\textbf{Total chl. x Volume of solution}}{\textbf{Weight of sample x 1000}} \times 100$$

II- Measurements at harvest time:

1- Yield (kg/vine):

At harvest time, when the soluble solids content percentage in berry juice reached to 16 %, (10 and 7 June in 2013 and 2014, respectively) the yield expressed in weight (kg) was estimated by multiplying number of clusters per vine by the average weight of cluster.

2- Cluster characteristics:

Number of clusters was recorded. A sample of 12 clusters/treatment,4 clusters from each replicate were harvested and transported to the laboratory of

Pomology Dept., Mansoura University to determine physical and chemical properties of clusters and berries.

2.1- Average cluster weight (g):

This was estimated in grams by using an electrical sensitive balance.

3- Berry chemical characteristics:

3.1- Soluble solids content percentage (SSC %):

This was measured by using a carlzeiss according to (Chen and Mellenthim, 1981).

3.2- Titratable acidity (%)

It was determined by titrating 5 ml of clear juice against NaOH (0.1 N) after the addition of a few drops of phenolphthalein (ph.th) as an indicator. The total titratable acid was expressed as mg of tartaric acid in 100 ml juice. The formula used in this respect was from AOAC (1984).

$$\text{Tartaric acid in mg/100 ml juice} = \frac{\text{ml NaOH} \times \text{N. NaOH} \times 0.075}{\text{ml juice}} \times 100$$

Where:

0.075 = Milliequivalent weight of tartaric acid

N = Normality of Na OH (0.1 N)

3.3- Soluble solids content / acid ratio

This ratio was calculated by dividing the percentage of SSC on total titratable acidity.

3.4- Nitrate and nitrite content in berries (ppm)

It was determined according to the method described by Singh (1988).

III. Measurements after harvest:

1- Total carbohydrates in canes (%):

For determination of total carbohydrates in canes, , the canes per each replicate were cut, oven dried at 70 °C till a constant weight and finely grinded, then, 0.1 g of samples was submerged over night in 10 ml of 80 % (v/v) ethanol at 25 °C with periodic shaking. The ethanolic mixture was filtered and the ethanolic filtrate was made up to known volume. Carbohydrates first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 620 nm, standard curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of glucose. Amount of carbohydrates present in 100 g of the sample = mg of glucose/volume of test sample x 100 (Hedge and Hofreiter, 1962).

2- Total nitrogen in canes (%):

For determination of total nitrogen in canes, the canes per each replicate were cut, oven dried at 70 °C till a constant weight and finely grinded. Then, 0.2 g crude dried powder from each sample was wet digested with a mixture of concentrated sulphuric acid and perchloric acid, then heated until become clear solutions (Peterburgski, 1968). After digestion, the clear solution was quantitatively transferred into 50 ml measuring flask with distilled water and kept for determinations. The modified micro-kjeldahl apparatus of Parnars and Wagner as described by Jones *et al*. (1991) was used for total nitrogen determination according to the method of AOAC (1984).

3- C/N ratio in canes:

The obtained ratio was calculated by dividing the percentage of total carbohydrates on total nitrogen.

Statistical analysis:

The obtained data of this study were statistically analyzed according to the technique of analysis of variance (ANOVA) for the complete randomized blocks design according to the method described by Gomez and Gomez (1984). The treatment means were compared using the New Least Significant Differences (New LSD) according to the producers outlined by Waller and Duncan (1969). A significance level of 5% was used for all statistical analysis.

RESULTS AND DISCUSSION

Shoot length (cm):

As shown in Table 4 all tested treatments significantly increased average shoot length in both seasons of the study as compared to that of control treatment (T_1). The combined application (Fulvic acid + Microelements + Bio-fertilizers) recorded a significant increase of average shoot length (193.00 & 213.90 cm) comparing to other treatments under the study in the two seasons, respectively. The other tested treatments recorded intermediate values in both seasons. The lowest values of shoot length were observed at the control treatment (129.00 and 138.80 cm).

These results agreed with the findings of Ferrara *et al*., (2007), Abd El-Wahab, (2011), Shaheen *et al*., (2012) and Ahmed *et al*., (2011), who stated that using the suitable N via 60 to 80 % inorganic form plus 5 to 10 cm^3 / vine/ season Fulvic acid and Spirulina platensis algae significantly stimulated main shoot length compared with using N completely via inorganic form or with using inorganic N at percentage lower than 60 %. Reducing percentage of inorganic N from 100 to 60 % and at the same time increasing percentages of organic and bio-fertilizers from 0.0 to 10 cm^3/ vine/ season were accompanied by a gradual stimulation on such growth character. Abd El-Hameed *et al*., (2014) using the suitable N in form of inorganic N 30 to 75% besides organic and biofertilization with Fulvic acid and (EM) each at 10 to 20 ml/vine/year significantly stimulated shoots length rather than using N as inorganic N at 30 75% alone.

This positive effect of combination can be attributed to the nutrient contents in their extracts with special emphasis to biological function of nitrogen in plant life. It is being a part of proteins, enzymes, amino acids, polypeptides and many other biochemical compounds in plant system. Therefore, it is required for survival of each plant cell (Mengel and Kirkby, 1987). Moreover, applied compost tea to the plant foliage, provides beneficial micro-organisms and nutrients to the surface of the plant as well as assists the plant to suppress certain diseases and increases nutrients availability (Biocycle, 2004).

Table 3. Effect of organic, microelements and bio-fertilizers on the vegetative growth of Superior seedless grapevines during 2013 & 2014 seasons.

Treatment	Shoot length (cm)		Shoot diameter (cm)		Leaf surface area (cm^2)	
	2013	2014	2013	2014	2013	2014
Control	129.00	138.80	1.10	0.95	119.10	122.40
Fulvic acid	175.00	188.70	1.40	1.53	127.00	128.60
Microelements	150.00	166.60	1.27	1.40	134.50	135.00
Bio-fertilizers	170.00	183.10	1.53	1.60	141.80	143.30
Fulvic acid + Microelements	181.70	196.50	1.60	1.73	153.70	158.00
Fulvic acid + Bio-fertilizers	183.00	203.60	1.67	1.87	146.10	147.10
Fulvic acid + Microelements + Bio-fertilizers	193.00	213.90	1.80	2.23	160.00	162.40
New LSD at 5 %	5.50	5.90	0.17	0.19	4.30	3.90

Bio-fertilizers = (*Trichoderma viride* + *Trichoderma harzianum* + *Serratia sp.* + *Pseudomonas fluorescens* + *Bacillus polymyxa*) at 7.14 ml/liter /vine.
Micro-elements = ($FeSO_4.7H_2O$ at 0.36 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g)/ liter /vine.

Shoot diameter (cm):

It is obvious from data in Table 4 that there were significant differences between all treatments rather than control treatment concerning average shoot diameter, sole and combined applications greatly increased average shoot diameter, (Fulvic acid + Microelements + bio-fertilizers) and (Fulvic acid + bio-fertilizers) treatments significantly increased average shoot diameter (1.80 and 2.23 cm) and (1.67 &1.87cm) in both seasons, respectively. The sole treatments had in between values. Control vines had lowest values of average shoot diameter (1.10 & 0.95cm) during the two seasons of study, respectively.

These results are in accordance with those obtained by Ferrara *et al*. (2007), Abd El-Wahab, (2011) and El-Sabagh *et al*., (2011) on Thompson seedless grapevines, they studied the possibility of reducing the amount of mineral fertilizers by using different sources of bio- fertilizers and they found that the highest values of canes diameter was that of vines received mineral nitrogen fertilization combined with bio fertilizers. Likewise, Ahmed *et al*., (2011) revealed that the applications of both humic acid (HAs) generally increased shoot diameter. Shaheen *et al*., (2012) reported that vines received compost in presence of bio-fertilizers and humic acid significantly increased canes diameter of Crimson seedless grapevines.

The positive effect may be due to the great availability of nutrients and hence stimulating cell division and cell enlargement as well as presence of natural hormones associated with biofertilizers application (Nijjar, 1985).

Leaf surface area (cm^2):

It is clear from Table 4 that all tested treatments significantly increased average leaf surface area compared to the control treatment. So, treating vines with treatment T_7 (Fulvic acid + Microelements + Bio- fertilizers) gave the highest significant values of average leaf surface area (160.00 & 162.40 cm^2) comparing to other treatments during both seasons of the study respectively, followed by T_5 (Fulvic acid + Microelements treatment). However, the untreated vines had the

lowest values of leaf surface area (119.10 & 122.40 cm^2), during the two seasons of study, respectively.

Our data go in line with those reported by El-Sabagh *et al*., (2011) who illustrated that adding bio-fertilizers caused an increment in leaf area of Thompson seedless grapevines. In the same line, Khalil, (2012) on Flame seedless grapevines found that highest leaf area was obtained with vines fertilized with 100% of the recommended mineral fertilization plus bio-fertilizers. In addition, Megawer, (2009) working on Superior grapes, Shaheen *et al*. (2012) on Crimson seedless grapes and Ali *et al*., (2013) on Thompson seedless grapevines, stated that adding humic acid with bio-fertilizers effectively maximized leaf surface area. Abd El-Hameed *et al*., (2014) stated that using the suitable N in form inorganic N 30 to 75% besides organic and biofertilization with Fulvic acid and (EM) each at 10 to 20 ml/vine/year significantly stimulated leaf area rather than using N as inorganic N at 30 75% alone.

The beneficial effect of organic fertilizers on leaf surface area of plants could be related to providing energy from micro-organisms activity, increasing nutrient supply and improving the efficiency of macro- elements as well as its ability to meet some micro-nutrients requirements (El-Nagar, 1996).

The positive merits of using of fulvic acid and biofertilizers on vegetative growth might be attribute to the following reasons they effectively enhanced availability of nutrients, antioxidants, natural hormones such as IAA, GA3 and cytokines, vitamin B, and enzymes such as nitrogenase (Abd El- Hameed *et al*. 2014)

Leaf chlorophyll pigments:

Data concerning leaf chlorophyll pigments (mg/g FW) are presented in Table 5 it could be concluded that single and combined applications significantly increased chlorophyll A, chlorophyll B and total leaf chlorophyll pigments content comparing with control treatment, vines foliar spraying with the combined of (Fulvic acid + Microelements + bio-fertilizers) was significantly increased to the total leaf chlorophyll pigments content (chlorophyll A, chlorophyll B and total chlorophyll (A + B) (0.397 & 0.412, 0.216 & 0.236 and 0.612 & 0.652 mg/g FW) in both seasons of this study, respectively. Otherwise, the control treatment had insignificant values.

These results are in agreement with those obtained with Ferrara *et al*., (2007) and Ferrara and Brunetti (2010), who illustrated that application of humic acid was able to increase chlorophyll contents values in Italia grapevines leaves. In addition, Ali *et al*., (2013) found that humic acids with bio-fertilizers had significant effective role in increasing total leaf chlorophyll content in Thompson seedless grapevines leaves. Abd El- Hameed *et al*., (2014) using the suitable N in form inorganic N 30 to 75% besides organic and biofertilization with Fulvic acid and (EM) each at 10 to 20 ml/vine/year significantly stimulated chlorophyll a & chlorophyll b and total chlorophyll (a+ b) rather than using N as inorganic N at 30 to 75% alone.

Table 4. Effect of organic, microelements and bio-fertilizers on chlorophyll A, B and total chlorophyll in leaves of Superior seedless grapevines during 2013 & 2014 seasons.

Treatment	Chlorophyll A (mg/g FW)		Chlorophyll B (mg/g FW)		Total chlorophyll (mg/g FW)	
	2013	2014	2013	2014	2013	2014
Control	0.351	0.367	0.185	0.197	0.536	0.565
Fulvic acid	0.354	0.373	0.189	0.202	0.543	0.575
Microelements	0.365	0.384	0.192	0.205	0.557	0.589
Bio-fertilizers	0.373	0.390	0.199	0.210	0.573	0.599
Fulvic acid + Microelements	0.388	0.404	0.210	0.224	0.597	0.628
Fulvic acid + Bio-fertilizers	0.377	0.401	0.205	0.221	0.582	0.621
Fulvic acid + Microelements + Bio-fertilizers	0.397	0.416	0.216	0.236	0.612	0.652
New LSD at 5 %	0.007	0.007	0.006	0.005	0.006	0.010

Bio-fertilizers = (*Trichoderma viride + Trichoderma harzianum + Serratia sp. + Pseudomonas fluorescens + Bacillus polymyxa*) at 7.14 ml/liter /vine.
Micro-elements = ($FeSO_4.7H_2O$ at 0.36 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g)/ liter /vine.

Measurements after harvest:

Yield/vine (kg):

Data presented in Table 5 showed that treating vines with (Fulvic acid + Bio-fertilizers) alone or combined with Micro-elements significantly increased yield per vine in the two seasons as compared with other treatments or the control. In both seasons, it can be noticed, that yield per vine (11.64 &12.88 kg/vine) and (12.33 & 13.45 kg/vine) respectively, were recorded when vines treated with (Fulvic acid + Bio-fertilizers) and (Fulvic acid + Micro-elements + Bio-fertilizers), compared to untreated vines which gained (8.04 & 8.16 kg/vine).

Table 5. Effect of organic, microelements and bio-fertilizers on yield per vine and cluster weight of Superior seedless grapevines during 2013 & 2014 seasons.

Treatment	Yield/vine (kg)		Cluster weight (g)	
	2013	2014	2013	2014
Control	8.04	8.16	524.10	508.60
Fulvic acid	11.47	11.95	593.80	581.90
Microelements	9.19	9.79	551.80	600.00
Bio-fertilizers	11.30	11.81	607.00	568.80
Fulvic acid + Microelements	10.89	11.18	616.30	578.50
Fulvic acid + Bio-fertilizers	11.64	12.88	592.10	560.00
Fulvic acid + Microelements + Bio-fertilizers	12.33	13.45	649.97	605.00
New LSD at 5 %	0.47	0.38	32.84	21.59

Bio-fertilizers = (*Trichoderma viride + Trichoderma harzianum + Serratia sp. + Pseudomonas fluorescens + Bacillus polymyxa*) at 7.14 ml/liter /vine.
Micro-elements = ($FeSO_4.7H_2O$ at 0.36 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g)/ liter /vine.

Similar results were reported by Akgül *et al*., (2007) reported that, in Sultani Çekirdeksiz grape cultivar, zinc fertilizer in the form of $ZnSO_4.7H_2O$ at 0.50 and 0.25% dose levels increased grape yield and improved quality

characteristics of berries. Megawer, (2009) on Superior seedless grapevines and Shaheen *et al*., (2012) on Crimson seedless grapevines mentioned that yield of tested grapevines increased by using humic acid and bio- fertilizers. Abd El-Hameed *et al*., (2014) using the suitable N via mineral N at 60 to 75% of the suitable N with Fulvic acid and (EM) each at 15 ml was very effective in improving the yield comparing with using N completely via mineral N or when mineral N was applied at percentage lower 60%.

Humic substances have different effects on plants showed evidence of stimulation on plant growth by humic substances and consequently increased yield by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water, and nutrient uptake, enzyme activities. (Chen *et al*. 2004).

Physical and chemical characteristics of clusters and berries.

Cluster weight (g):

Data in Table 5 clearly showed differences in cluster weight as affected by all treatments during the two seasons of study, in this point view, treating vines with (Fulvic acid + Microelements + Bio-fertilizers) produced the highest increase of clusters weight 649.97 g in the ^{1}st season and 605.00 g in the ^{2}nd season, followed by (Fulvic acid + Microelements) 616.30 & 578.50.0 g in the ^{1}st and ^{2}nd seasons, respectively.

These results are in harmony with those reported by Megawer, (2009) on Superior seedless grapevines and Shaheen *et al*., (2012) on Crimson seedless grapevines, they all mentioned that cluster weight of tested grapevines increased by using humic acid and biofertilizers. Moreover, El-Sabagh *et al*., (2011) and Khalil, Hoda (2012) studied the effect of replacing the excessive application of mineral fertilizers partially with bio-fertilizers, they found that the highest values of cluster weight were recorded by vines treated with bio-fertilizers. Shamseldin *et al*., (2010) found that bio-fertilizer inoculation with strain of *Pseudomonas fluorescens* on Washington navel orange trees had increased fruit weight by a rate of (33.25% and 31.6%) in the first and second seasons.

As for the effect of organic and bio-fertilizers on improving both physical and chemical properties of the grapes, the beneficial effect of organic and bio-fertilizers on fruit quality could attributed to the effect of nutrient content of the vines, which accelerated the formation of carbohydrates (Ezz, 1999). The great availability of nutrients is stimulating cell division and cell enlargement as well as natural hormones in producing larger fruits (Nijjar, 1985).

Soluble solids content (%), Titratable acidity (%) and SSC/acid ratio:

Soluble solid content in Table 6 generally revealed significant increase at the tested treatments in comparison to control treatment. (Fulvic acid + Bio-fertilizers) and (Fulvic acid + Microelements+ Bio-fertilizers) combined treatments recorded the highest significantly increased of SSC%, where recorded (17.10 and 17.50%) and (17.50 and 17.20%) for the two seasons of study, respectively.

Likewise, similar effects were recorded concerning SSC/ acid ratio measurements. On the other hand, as for total titratable acidity, the same treatments showed the lowest values as compared to other treatments (0.63 and 0.59%) and (0.61 and 0.60%) in the two seasons of study, respectively. The combined foliar application of these materials improved the chemical quality of berries in terms of increasing total soluble solids content (%), total soluble solids/