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EFFECT OF HUMIC ACID, BIO-FERTILIZERS AND MICRO-ELEMENTS ON LEAF MINERAL CONTENTS OF KING RUBY GRAPEVINES

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ABSTRACT

The present investigation was carried out during the seasons of 2011 and 2012 to study the effect of soil application of humic acid (at 0.75, 1.5 and 3 g / vine) with or without bio-fertilizers (*Serratia sp*. + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) at 7.14 ml / vine and micro-elements ($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) / vine on mineral contents of King Ruby leaf petioles**.**

Results obtained showed that adding humic acid at 3 g\vine with bio-fertilizers at 7.14 ml\vine and micro-elements ($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) / vine, activated the absorption of macro-elements since it produced significantly the highest values of N, P and K content in leaf petioles when compared with all of the other treatments, N values were 3.83 and 3.71 %, P values were 0.47 and 0.42 % and K values were 2.49 and 2.59 % in 2011 and 2012 seasons, respectively. Concerning micro-elements, it was observed that adding humic acid at 3 g\vine with bio-fertilizers and micro-elements also activated micro-elements uptake by grapevines, it recorded in 2011 and 2012, respectively the highest values, Fe was (131.00 and 128.43 ppm), Zn (45.40 and 47.70 ppm) and Mn was (150.07 and 143.37 ppm). On the other hand, the control treatment gave the lowest values of macro and micro-elements if compared to those resulted in the other treatments in both seasons of the study.

INTRODUCTION

In Egypt, grapes are considered one of the important fruit crop. The total planted area with grape cultivars reached about 171973 fed. Yet, the fruitful ones are about 154369 fed. produced about 1320801 ton**.** King Ruby cultivar is considered one of the most important commercial grape cultivars, the fruitful planted area reached about 3370 fed. produced about 38263 ton., according to the Ministry of Agriculture Statistics of 2011. This cultivar has a great importance either for the local market or export needs. Therefore, the grape growers gave a great attention to all cultural practices, especially fertilization program to provide the cultivated grapevines with their optimum nutrient requirements.

Humic acid is a principal component of humic substances, which are the major organic constituents of soil. It is produced by biodegradation of dead organic matter. It is not a single acid; rather, it is a complex mixture of many different acids containing carboxyl and phenolate groups so that the mixture behaves functionally as a dibasic acid or, occasionally, as a tribasic acid. Humic acids can form complexes with ions that are commonly found in the environment creating humic colloids.

According to Mayhew (2004), humic substances have demonstrated the ability to: chelate (bind) soil nutrients, improve nutrient uptake, reduce the need for nitrogen fertilizer, remove toxins from soils, stimulate soil biological activity, solubilize minerals, improve soil structure; and improve water holding capacity.

Bio-fertilizers are microbial inoculants (preparations containing living micro organisms), which enhance production by improving the nutrient supplies and their crop availability. There are a number of inoculants with possible practical application in crops, where they can serve as useful components of integrated plant nutrient supply systems, may help in increasing crop productivity by increasing biological N fixation availability or uptake of nutrients through convert insoluble P in the soil into forms available to plants or increasing absorption, stimulation of plant growth through hormonal action or antibiosis or by decomposition of organic residues (Wani and Lee, 1995).

Micro-elements play essential roles in vegetative and fruit development. These elements are more available at lower soil pHs, 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. Mode of action for micro-elements was explained by Larue and Johnson (1989). Iron (Fe) complexes with proteins to form important enzymes in the plant and is associated with chloroplasts, where it has some roles in the synthesizing chlorophyll. Zinc (Zn) has been identified as component of almost 60 enzymes, therefore, it has a role in many plant functions, and it has a role as an enzyme in producing the growth hormone IAA. Manganese (Mn) participates in several important processes including photosynthesis, and metabolism of both nitrogen and carbohydrate.

MATERIALS AND METHODS

The present investigation was carried out during two successive seasons (2011 and 2012).The experiment was conducted on 17 years old King Ruby grapevines planted on sandy soil under drip irrigation system at 2 × 3 meters (2 m within rows and 3 m between rows) in a private farm (El – Egeizy vineyard) located at El-Sadat district, Minufiya governorate, Egypt. Mixed pruning method under parron trellis system leaving 4 cordons per vine. Pruning was done at 5 January in the first season and 20 January in the second season. Leaving about 88 eyes / vine (on the basis of 3 fruiting spurs / 3 arms / cordon × 6 eyes + 2 renewal spurs / cordon × 2 eyes).

Ninety six vines were chosen for this study, uniform in vigor as possible, all the chosen vines received the cultural management such as, fertilization, irrigation, diseases and pest control that commonly performed in that district. The experimental design was complete randomized blocks design. Vines subjected to sixteen treatments, each treatment was replicated three times with two vines per each, as shown in Table 1. Also borders were left around and between each treatment as well as between blocks. The physical and chemical properties of the experimental soil are presented in Table 2.

Table 1: Tested treatments on King Ruby grapevines in 2011 and 2012 seasons.

Symbols	Applied treatments
T_1	Control (applied with tap water only)
T_2	Bio-fertilizers*
T_3	Micro-elements**
T_4	Bio-fertilizers + Micro-elements
T_5	0.75 g\vine Humic acid
T_6	0.75 g\vine Humic acid + Bio-fertilizers
T_7	0.75 g\vine Humic acid + Micro-elements
T_8	0.75g\vine Humic acid + Bio-fertilizers+ Micro-elements
T_9	1.5 g\vine Humic acid
T_{10}	1.5 g\vine Humic acid + Bio-fertilizers
T_{11}	1.5 g\vine Humic acid + Micro-elements
T_{12}	1.5g\vine Humic acid+ Bio-fertilizers + Micro-elements
T_{13}	3g\vine Humic acid
T_{14}	3g\vine Humic acid + Bio-fertilizers
T_{15}	3g\vine Humic acid + Micro-elements
T_{16}	3g\vine Humic acid + Bio-fertilizers + Micro-elements

* **Bio-fertilizers:(*Serratia sp*. + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) at 7.14 ml / vine.**

** **Micro-elements:($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) / vine.**

All treatments were applied as soil application, added in four corners under dripping position around vine at 25 cm distance from the vine trunk.

These treatments were applied at 4 stages:

1) At the opening of 80 % of buds (3 and 9 March in 2011 and 2012, respectively).
2) One week before flowering (2 and 10 April in 2011 and 2012, respectively).
3) After 7 days of fruit set stage (29 April and 4 May in 2011 and 2012, respectively).
4) At veraison stage (17 and 22 June in 2011 and 2012, respectively).

This investigation was carried out to study the effect of soil application of humic acid at the rate of 0.75, 1.5 and 3 g\vine with or without bio-fertilizers (*Serratia sp*. + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) at 7.14 ml\vine and micro-elements ($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) \ vine on mineral content of leaf petioles of King Ruby grapevines.

Before the experiment soil samples were taken to determine the properties of experimental soil at three depths from soil surface, 0 to 30, 30 to 60 and 60 to 90 cm. Such samples in each category were completely mixed and subjected to mechanical and chemical analysis to measure certain properties of soil as included in Table 2.

Table 2: Values of mechanical and chemical properties of the experimental vineyard soil.

Sample Depth (cm)	Mechanical analysis %					Chemical analysis					Available (ppm)		
	Coarse sand	Fine sand	Silt	Clay	Texture	EC (1:5) dSm^{-1}	pH (1:2.5)	Sp (%)	$CaCO_3$ (%)	OM (%)	N	P	K
0-30	4.32	50.49	26.44	18.75	Sandy clay loamy	1.62	7.88	49	2.69	1.19	48.2	3.9	277
30-60	4.07	48.94	27.30	19.69		1.25	7.97	52	2.51	0.95	43.8	3.1	242
60-90	3.88	49.03	27.95	20.14		1.16	8.01	57	2.10	0.78	36.3	2.8	238

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

Preparation of Humic acid:

Compost prepared from rice straw, farmyard manure, rock phosphate, bentonite and urea were digested with 0.5 N KOH for 48 h at room temperature in the ratio of 1/10 (W\V) (Compost\Water). Separation of the solute form the undigested residues were then carried out by filtration with 100 Mesh screen. The supernatant was acidified at pH 2 with concentrated H_2SO_4 and left settling for 24 h in the dark in order to allow humic acid flocculation. Humic acid precipitated was collected. The preparative was dried at room temperature, then oven dried at 70 ^{o}C till a constant weight and grinded. (Vallini *et al.,* 1990). Chemical analysis of humic acid was recorded in Table 3

Table 3: Chemical analysis of humic acid

Characteristics	Values
EC	1.13 (dSm^{-1})
pH	2.8
OM	52.03 (%)
C	30.25 (%)
C \ N ratio	14.14 (%)
Macro-elements (%)	
Total N	2.14
Total P	0.27
Total K	3.16
Micro-elements (ppm)	
Total Fe	393
Total Zn	213
Total Mn	168

Preparation of bio-fertilizers inoculants :

Serratia sp, grown on pepton – glycerol media (Grimont and Grimont, 1984), *Pseudomonas fluorescens* grown on king's media (Alef, 1995), *Bacillus Polymyxa* grown on nutrient broth media (Dowson, 1957) and *Trichoderma* species grown on Potato dextrose media (ATTC,1992) were incubated for 2-3 days at 28 $^{\circ}$C to maintain populations of $3x10^8$ 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).

Measurements:

Chemical analysis of leaf petioles

The contents of N, P, K, Fe, Zn and Mn leaf petioles were determined after two weeks from the last addition in the two seasons of study. Samples of 12 leaf petioles per each replicate were taken from mature leaves opposite to basal clusters (Nijjar, 1985). The leaf petioles were oven dried at 70 ^oC till a constant weight and grinded.

1) Macro-elemrents

1.1- Leaf nitrogen content: The modified Micro-kjeldahl method of Parnars and Wagner as described by Jones *et al.* (1991) was employed for total nitrogen determination according to AOAC (1984).

1.2- Leaf phosphorus content: Total phosphorus was determined spectrophotomitrically by Milton Roy spectronic 120 at wavelength of 725 nm using Stannous Chloride Reduced Molybdophosphoric Blue Colours method in sulphoric system as described by Jackson (1973).

1.3- Leaf potassium content: Total Potassium was estimated flamephotometrically using Ienway Flamephotometer model Corning 400 according to the method described by Peterburgski (1968).

2) Micro-elements

Total Fe, Zn and Mn were estimated using atomic absorption spectrophotometer (A Perkin-Elmer, Model 2380.USA) according to the methods of Chapman and Pratt (1982).

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) using GenStat Eleventh Edition Package. 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 analyses.

RESULTS AND DISCUSSION

The obtained data for mineral analysis of leaf petioles are shown as follow:

1- Nitrogen, Phosphorus and potassium (%) content in leaf petioles.

As for effect of humic acid**,** data in Table (4) showed that adding humic acid at 3 g\vine increased nutrients uptake as it gave the highest significant increase in N, P and K content in leaf petioles, N values were 3.47 and 3.42 %, whereas P values were 0.41 and 0.39 % and K values were 2.03 and 2.08 % in 2011 and 2012 seasons, respectively as compared with that of control, where the same values were 2.93 and 2.82 % for N content, 0.31 and 0.33 % for P content and 1.34 and 1.30 % for K content in 2011 and 2012 seasons, respectively.

Table 4: Effect of humic acid, bio-fertilizers + micro-elements and their interaction on N, P and K % content in leaf petioles of King Ruby grapevines during 2011 and 2012 seasons.

			N %		P %		K %	
			2011	**2012**	**2011**	**2012**	**2011**	**2012**
Effect of humic acid (A)								
Control (applied with tap water only)			2.93	2.82	0.31	0.33	1.34	1.30
0.75 g \ vine			3.33	3.36	0.38	0.37	1.84	1.89
1.5 g \ vine			3.42	3.35	0.39	0.38	1.94	1.96
3 g \ vine			3.47	3.42	0.41	0.39	2.03	2.08
New LSD at 5%			0.05	0.14	0.003	0.005	0.02	0.05
Effect of bio - fertilizers + micro - elements (B)								
Control			2.99	2.95	0.32	0.33	1.40	1.33
Bio-fertilizers			3.36	3.30	0.38	0.38	1.88	1.91
Micro-elements			3.19	3.15	0.36	0.36	1.70	1.70
Bio-fertilizers + Micro-elements			3.61	3.54	0.43	0.40	2.18	2.28
New LSD at 5%			0.04	0.12	0.004	0.006	0.04	0.03
Effect of the interaction (AB)								
Humic acid	**Bio-fertilizers + Micro-elements**							
0 g	T_1	Control	2.79	2.64	0.28	0.31	1.18	1.10
	T_2	Bio-fertilizers	2.88	2.72	0.30	0.32	1.24	1.18
	T_3	Micro-elements	2.92	2.82	0.31	0.33	1.34	1.29
	T_4	Bio + Micro	3.14	3.10	0.35	0.35	1.59	1.62
0.75 g	T_5	Control	3.01	3.22	0.32	0.33	1.39	1.30
	T_6	Bio-fertilizers	3.44	3.42	0.40	0.39	1.96	2.06
	T_7	Micro-elements	3.18	3.15	0.37	0.36	1.72	1.76
	T_8	Bio + Micro	3.68	3.63	0.43	0.41	2.28	2.43
1.5 g	T_9	Control	3.08	2.93	0.33	0.34	1.47	1.39
	T_{10}	Bio-fertilizers	3.53	3.49	0.41	0.39	2.11	2.14
	T_{11}	Micro-elements	3.30	3.25	0.37	0.37	1.83	1.83
	T_{12}	Bio + Micro	3.78	3.72	0.45	0.41	2.37	2.48
3 g	T_{13}	Control	3.08	3.02	0.34	0.34	1.57	1.53
	T_{14}	Bio-fertilizers	3.60	3.55	0.43	0.40	2.19	2.25
	T_{15}	Micro-elements	3.38	3.38	0.39	0.39	1.88	1.94
	T_{16}	Bio + Micro	3.83	3.71	0.47	0.42	2.49	2.59
New LSD at 5%			0.09	0.27	0.008	0.01	0.07	0.08

Bio= Bio-fertilizers(*Serratia sp.* + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) at 7.14 ml / vine.

Micro= Micro-elements ($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) / vine.

Regarding with effect of bio-fertilizers + micro- elements, data from the same table clearly showed that adding bio-fertilizers and micro-elements together stimulates absorption of nutrients due to provide ideal conditions for the absorption as it significantly produced the highest values of N, P and K content in leaf petioles comparing with other treatments, such values were 3.61 and 3.54 % for N content, 0.43 and 0.40 % for P content and 2.18 and 2.28 % for K content in 2011 and 2012 seasons, respectively. The untreated vines gave a lowest significant values, These values were 2.99 and 2.95 %, 0.32 and 0.33 % and 1.40 and 1.33 % for N, P and K, respectively in 2011 and 2012 seasons.

In case of effect of interaction treatments between humic acid and bio-fertilizers + micro- elements, the concerned data in Table (4) showed that the combination treatment T_{16} (The combination between humic acid at 3 g\vine, bio-fertilizers and micro-elements) activated the absorption of N, P and K from the rhizosphere as it produced significantly the highest values of N, P and K content in leaf petioles when compared with all the other treatments. The N values were 3.83 and 3.71 %, the P values were 0.47 and 0.42 % and K values were 2.49 and 2.59 % in 2011 and 2012 seasons, respectively. While, the lowest values in that respect were recorded by the control T_1, which recorded values of 2.79 and 2.64 %, 0.28 and 0.31 % 1.18 and 1.10 % for N, P and K, respectively in both seasons of the study. Moreover, as for the effect of the interaction on nitrogen content in leaf petioles, the results also indicated that the difference between the values of the combination treatment T_{16} and T_{12} (The combination between humic acid at 1.5 g\vine, bio-fertilizers and micro-elements) was insignificant comparing with the interaction effect of the rest combinations, T12 gave the values (3.78 and 3.72 %) in 2011 and 2012 seasons, respectively.

The effect of organic amendments such as compost and humic acid with or without bio-fertilizers on grapevines was the subject of several studies carried out by El-Mansi (2007), Ferrara *et al.* (2007), Megawer (2009) and Gawad Shaheen *et al.* (2012). They all confirmed that the application of such amendments enhanced the absorption of macro-elements as they gave the highest values of N, P and K content in leaf petioles as compared with that of control. In similar line, Khalil, (2012) working on the effect of bio-fertilizers on Flame seedless grapevines found that all microbial fertilization treatments significantly increased N, P and K content in leaf petioles when compared with control treatment. In the contrary, Abd El-Monem *et al.* (2008) found that humic acid reduced N content in the leaves especially when added with bio-fertilizers, while P and K content were not affected.

There is some evidence that humic products may increase the nutrient and water uptake by plant roots (Russo and Berlyn, 1990), This enhanced uptake of water and nutrients may be an effect of increase root surface area or increase cell membrane permeability caused by the humates (Rauthan and Schnitzer, 1981). Moreover, Maggioni *et al.*(1987) indicated that humic and fulvic acids can influence the nutrient absorption, due to their effect on the K^+ and Mg^{2+} dependent ATPase. In addition, Humic acids contribute to plant nutrition improving N and K availability, soil structure, water-air retention capacity, increasing soil microbial population, and acts as a buffer solution in cation exchange capacity and pH (Anderson, 1979 and Magdoff & Weil, 2004). Compost amendments are an attractive way to incorporate organic matter in the soil as it has beneficial properties, including mobilization of mineral phosphates (Wickramatilake *et al.*, 2010). Moreover, Organic amendments supply C, N, P and energy for microorganisms in soil (Tabatabai and Dick, 2002).

Phosphorus solubilization by microorganisms is one of the most important processes in the soil. In the presence of carbohydrates, microorganisms, produce organic acids and thus changing the pH around, or they produce acid or alkaline phosphatases which break the phosphates groups in organic matter (Mikanova and Novakovà, 2002). Also, The genera *Pseudomonas*, *Bacillus* and *Rhizobium* sp. have as primary mechanism the production of organic acids and acid phosphatase for the mineralization of organic phosphorus in soil (Caballero *et al.*, 2007). Addition of organic matter into the soil enhances microbial diversity as well as its biomass; numerous authors had demonstrated the increase in functional groups as mycorrhizal fungi and beneficial rhizosphere bacteria (Heargreaves *et al.,* 2008).

2- Iron, Zinc and Manganese (ppm) content in leaf petioles.

As for effect of humic acid, results in Table (5) indicated that adding humic acid at 3 g\vine stimulates the absorption of micro – elements as it gave the highest significant increase in leaf contents of Fe, Zn and Mn compared with all other rates of humic acid, values of Fe were114.71 and 115.23 ppm, whereas Zn were 36.86 and 38.27 ppm and Mn were 138.82 and 129.86 ppm in the first and second seasons of study, respectively. The control gave significantly the lowest values, these values were 98.75 and 101.38 ppm for Fe content, 26.74 and 26.96 ppm for Zn content and 123.43 and 114.37 ppm for Mn content, respectively in 2011 and 2012 seasons.

In case of effect of bio-fertilizers + micro- elements, data presented in Table (5) showed that adding bio-fertilizers and micro- elements together activates micro-elements uptake by grapevines as it gave the highest significant values in Fe, Zn and Mn content in leaf petioles compared with other treatments, Fe content values were 123.98 and 122.64 ppm, Zn values were 41.77 and 44.17 ppm and Mn values were145.89 and 138.12 ppm during 2011 and 2012 seasons, respectively. The control gave significantly the lowest values, these values were 94.32 and 98.20 ppm for Fe, 24.45 and 24.46 ppm for Zn and 120.78 and 111.00 ppm for Mn in both seasons of study, respectively.

Concerning the effect of the interaction treatments between humic acid and bio-fertilizers + micro- elements. Results in Table (5) once again pointed to the superiority of treatment T_{16}, it was cleared that the highest values of Fe, Zn and Mn were resulted in leaf petioles of grapevines under T_{16} treatment (The combination between humic acid at 3 g\vine, bio-fertilizers and micro-elements) in both seasons, since it recorded the highest values of Fe 131.00 and 128.43 ppm, Zn 45.40 and 47.70 ppm and Mn 150.07 and 143.37 ppm. during 2011 and 2012, respectively The concerned results also indicated that the difference between the values of the treatment T_{12} (The combination between humic acid at 1.5 g\vine, bio-fertilizers and micro-elements) and T_{16} was insignificant in the two tested seasons. The values of T_{12} were for Fe 128.53 and 126.57 ppm, Zn 43.70 and 46.83 ppm and Mn 149.73 and 141.20 ppm in 2011 and 2012 seasons, respectively. On the other hand, the control treatment gave the lowest values if compared to other treatments. The values of control were 88.40 and 92.63 ppm for Fe, 21.53 and 19.60 ppm for Zn and 113.87 and 104.73 ppm for Mn in the two seasons, respectively.

Table (5): Effect of humic acid, bio-fertilizers + micro-elements and their interaction on Fe, Zn and Mn (ppm) content in leaf petioles of King Ruby grapevines during 2011 and 2012 seasons.

			Fe (ppm)		Zn (ppm)		Mn (ppm)	
			2011	**2012**	**2011**	**2012**	**2011**	**2012**
Effect of humic acid (A)								
Control (applied with tap water only)			98.75	101.38	26.74	26.96	123.43	114.37
0.75 g \ vine			109.39	110.42	33.32	34.79	133.47	125.43
1.5 g \ vine			112.82	113.48	34.08	36.39	137.24	127.42
3 g \ vine			114.71	115.23	36.86	38.27	138.82	129.86
New LSD at 5%			0.96	2.11	1.35	1.26	0.97	1.18
Effect of bio - fertilizers + micro - elements (B)								
Control			94.32	98.20	24.45	24.46	120.78	111.00
Bio-fertilizers			102.88	105.19	28.71	29.57	127.68	118.06
Micro-elements			114.48	114.48	36.08	38.22	138.61	129.90
Bio-fertilizers + Micro-elements			123.98	122.64	41.77	44.17	145.89	138.12
New LSD at 5%			2.46	2.28	1.10	1.33	2.05	1.76
Effect of the interaction (AB)								
Humic acid	**Bio-fertilizers + micro-elements**							
0 g	T_1	Control	88.40	92.63	21.53	19.60	113.87	104.73
	T_2	Bio-fertilizers	91.80	94.73	22.23	21.57	114.67	109.27
	T_3	Micro-elements	102.20	104.97	28.37	29.70	128.20	115.80
	T_4	Bio + Micro	112.60	113.20	34.83	36.97	137.00	127.67
0.75 g	T_5	Control	93.80	97.30	23.77	24.37	119.90	110.90
	T_6	Bio-fertilizers	104.17	107.87	29.97	31.00	128.50	118.40
	T_7	Micro-elements	115.80	114.13	36.43	38.60	138.70	132.20
	T_8	Bio + Micro	123.80	122.37	43.13	45.20	146.77	140.23
1.5 g	T_9	Control	96.53	100.40	24.73	24.47	123.23	112.37
	T_{10}	Bio-fertilizers	107.10	109.17	30.47	32.73	132.70	121.37
	T_{11}	Micro-elements	119.10	117.77	37.43	41.53	143.30	134.73
	T_{12}	Bio + Micro	128.53	126.57	43.70	46.83	149.73	141.20
3 g	T_{13}	Control	98.53	102.47	27.77	29.40	126.10	116.00
	T_{14}	Bio-fertilizers	108.47	109.00	32.17	32.97	134.87	123.20
	T_{15}	Micro-elements	120.83	121.03	42.10	43.03	144.23	136.87
	T_{16}	Bio + Micro	131.00	128.43	45.40	47.70	150.07	143.37
New LSD at 5%			4.78	4.86	2.52	2.85	4.03	3.57

Bio= Bio-fertilizers(*Serratia sp.* + *Bacillus polymyxa* + *Pseudomonas fluorescens* + *Trichoderma viride* + *Trichoderma harzianum*) at 7.14 ml / vine.

Micro= Micro-elements ($FeSO_4.7H_2O$ at 0.35 g + $ZnSO_4.7H_2O$ at 0.18 g + $MnSO_4.H_2O$ at 0.18 g) / vine.

These results are in agreement with those reported by Sanchez *et al.* (2006) and Ashoori *et al.* (2013) .

Humic acids are important constituents of soils in that respect they could affect water retention, contribute to cation exchange capacity, and serve as a nutrient reserve for living organisms, plants and microbes. The ability of humic acids to act as a nutrient reserve comes from having a high exchange capacity, and the capability to form water soluble complexes with metal ions ,e.g. Fe, thus possibly enhancing the absorption of some ions by roots (Schnitzer,1967). Also, they show a chelating activity that provides the plant with microelements (especially iron) and make it more readily available for plant uptake (Stevenson, 1991).

The humic substances have been related, by several authors, with improving agronomic parameters like stimulating root development (Vaughan and MacDonald, 1976) and nutrient uptake (Vaughan *et al.*, 1985 and Ortega & Fernandez, 2007). Low molecular weight fractions induced morphological changes in plants, similar to those caused by indole-3-acetic acid (IAA) (Muscolo *et al.*, 1993). In addition, humic substances can serve as carrier of micronutrients or growth factors; a theory even is proposed on which humic substances can act as a direct stimulator of plant growth by entering into the plant tissue, resulting in various biochemical effects at the cell wall, membrane, or in the cytoplasm (Magdoff and Weil, 2004).

Concerning bio-fertilizers, a recent list of mechanisms has been suggested by many investigators. Carvajal Liliana *et al.* (2009) and Harman (2011) reported that *Trichoderma harzianum* increased nutrient availability and Stimulate plant nutrient uptake. *Pseudomonas fluorescens* derives its name from its ability to produce fluorescent pigments under iron-limiting conditions (Baysse *et al.,* 2003).

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تأثير حمض الهيوميك والأسمدة الحيوية والعناصر الصغرى على محتوى أوراق العنب الكينج روبى من العناصر المعدنية.

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أجريت هذه الدراسة خلال موسمي 2011 و2012 بمزرعة خاصة (العجيزى) بمدينة السادات – محافظة المنوفية على شجيرات عنب صنف الكينج روبى عمرها 17 عاما، منزرعة فى تربة رملية على مسافة 2 × 3 م وتروى بنظام الرى بالتنقيط ، وقد استهدف هذا البحث دراسة تأثير حمض الهيوميك مع أو بدون الأسمدة الحيوية والعناصر الصغرى على محتوى أعناق أوراق العنب الكينج روبى من العناصر الكبرى مثل النتروجين والفوسفور والبوتاسيوم وكذلك العناصر الصغرى مثل الحديد والزنك والمنجنيز.

أظهرت نتائج الدراسة أن إضافة حمض الهيوميك بمعدل 3 جم / كرمه مع الأسمدة الحيوية والعناصر الصغرى (المعاملة 16) أدى الى تنشيط امتصاص العناصر الكبرى حيث أظهر زيادة معنوية فى محتوى أعناق الأوراق من عناصر النتروجين والفوسفور والبوتاسيوم مقارنة بالكنترول وكانت قيم النتروجين 3.83 – 3.71 % وقيم الفوسفور 0.47 – 0.42 % وقيم البوتاسيوم 2.49 – 2.59 % خلال موسمى 2011 و 2012 على التوالى ، أما بالنسبة للعناصر الصغرى ، فقد لوحظ أيضا أن هذه المعاملة أدت إلى تنشيط امتصاص العناصر الصغرى حيث أعطت أعلى قيم لعناصر الحديد والزنك والمنجنيز ، وكانت قيم الحديد 131.00 – 128.43 جزء فى المليون وقيم الزنك 45.40 – 47.70 جزء فى المليون وقيم المنجنيز 150.07 -143.37 جزء فى المليون خلال موسمى 2011 و 2012 على التوالى من جهة أخرى فقد أظهرت معاملة الكنترول أقل قيم للعناصر الكبرى والصغرى مقارنة بباقى المعاملات فى كلا موسمى الدراسة.

قام بتحكيم البحث

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