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(Original Article)

Minimizing Mineral-N Fertilization Superior Seedless Grapevines by Using Humic and Fulvic Acids

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Abstract

Nowadays a great attention is focused on minimizing the intensive amounts of mineral nitrogen fertilization by applying organic amending such as humic and fulvic acids which considered the main fraction of humic substances and fulvic acid could activate growth and product of plants. To achieve this aim, an experiment was conducted on 14 years old Superior seedless grapevines in 2020, 2021 and 2022.

Application of mineral-N at 50 to 75% of recommended dose of nitrogen (RND) along 25% of humic acid or/and fulvic acid. The experimental vines were set up in a randomized complete design with four treatments and three replications two vines per each. The gained results could be briefing as follow:

Using RDN via 50 to 75% mineral plus 25% humic acid and/or fulvic acid significantly increased, leaf area, pruning wood weight, and leaf total chlorophyll as well as leaf nutrient composition resembled to use RDN via non-organic-N fertilizer alone. Combination fertilization handling significantly increase the yield and improved the cluster and berry characteristics compared to use RDN through mineral source alone.

Therefore, applying humic or fulvic acids improved the productivity and quality of Superior seedless grape, and proved that it could be used as alternative organic N fertilization in the future. It could be consummated that fertilized vines with 75% of nitrogen needed plus 25% humic acid or/and 25% fulvic acid improved the vegetative growth, status of nutritional, yield and cluster attributes and berry traits of Superior seedless grape-vines under this experiment circumstances

Keywords: *Humic acid, Fulvic acid, Superior Seedless, Grapevines, Fertilization*

Introduction

Grapes is representing the third fruit crop in Egypt. The total fruiting area grapes in Egypt attained about 221709 fed. producing 1626259 tons (according to

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the statistics of Food and Agriculture Organization (FAOSTAT, 2019). Moreover, Superior seedless table grape is considered one of the extreme important table grapes varieties in Egypt, as it has very good qualities and high price being early maturing and harvesting than the several varieties, and it very leading for the Arabian and European export markets. So, these days it receives greet interest to increase its production and qualitative.

Fertilization is one of the important managements for increasing the yield. The efficiency of nitrogen fertilizer under field conditions and flood irrigation rarely exceeds 50% (Sahrawat, 1979). Therefore, the organic production of fruits through used organic and biological fertilization with lack of the use of chemical fertilizers as well as stimulants has become an urgent necessity (El-Salhy, 2004 and Calleja-Cervantes *et al*., 2015).

Therefore, applying organic such as Humic acids (HA) which considered the main fractions of humic substances (HS) and the most active components of soil and compost organic matter (Ferrara and Brunetti, 2010). Humic acid have been shown to activate plant growth and yield through action on mechanisms included in: photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities and cell respiration, then be useful to increase the production and berry quality traits of Superior seedless grape (Chen *et al.*, 2004; Asgharzode and Babaeian, 2012; Abdelaal *et al*., 2013; Ibrahim and Ali, 2016; Akin, 2018 and Abdel-Rahman *et al*., 2021).

The addition of humic substances increases the organic matter content of the soil without going for any humification process it to happen because it is already present as humified material. They are responsible for enhancing water holding capacity, aeration of soil, soil workability, resistance to drought and N uptake (Kabeel *et al*., 2008 and Yang *et al*., 2019).

Fulvic acid (FA) is mostly important compound of organic and natural materiality in hydrous arrangement as well as on interest for plant and soil. It is leading to increase microbial activity and activates nutrient transmission as chelating agent and enhances vegetative characteristics, nutritional status and leaf pigments. Fulvic acid has oxygen content twice that of humic acids and they have many carboxyl and hydroxyl groups much than humic acid (Aiken *et al*., 1985 and Chen *et al*., 2004). Using of humic and fulvic acids as a partial standing by mineral N fertilizers were responsible to enhance growth and fruiting of different grape cultivars was a wide field for many authors (Ferrara and Brunetti, 2010; Ahmed *et al*., 2011; Abdelaal *et al*., 2013; Abdel-Hameed *et al*., 2014; El-Boray *et al*., 2015; Mohamadineia *et al*., 2015; Ibrahim and Ali, 2016; Mostafa *et al*., 2017; Akin, 2018 and Abdel-Rahman *et al*., 2021).

Then the goal of this study was selecting the best inorganic N proportion applied with humic acid and/or fulvic acid as well as detecting the best N management in Superior seedless vineyards.

Materials and Methods

Experimental field

This study was carried out during three successive seasons in 2020, 2021 and 2022, on 14 years old Superior seedless grapevine grown at the experimental orchard Faculty of Agriculture, South Valley University, Qena Governorate 30.42°N, 31.48 E, Egypt, some physical and chemical properties of experimental soil at 0.0-90 cm depth are presented in Table (1) according to the procedures of Black *et al*. (1965) and Carter (1993).

Table 1. Analysis of the tested soil

Constituents	Values	Constituents	Values
Particle size distribution:		Total N %	0.09
Sand %	8.5	P ppm (Oslen)	3.3
Silt %	21.0	K ppm (ammonium acetate)	410.0
Clay %	70.5	**Available micronutrients (EDTA, ppm):**	
Texture	Clay	Fe	1.8
pH (1:2.5 extract)	8.0	Zn	2.1
EC (1:2.5 extract) mmhos/l cm 25° cm	0.75	Mn	1.9
Organic matter %	1.9	Cu	0.7
Total $CaCO_3$ %	1.9		

All selected vines were planted at 2 x 3 m. The vines were trained according to the traditional cane pruning system on arbors (Spanish Type). Winter pruning system was carried out in the end of December using cane pruning system leaving 96 buds (8 fruiting canes x 10 buds + 8 renewal spurs x two buds). The vines received the usual horticultural practices, concerning irrigation fertilization, pests and weeds control except those dealing with the present treatments. In addition, the best 40 bunches in the two seasons were left on each vine.

Twenty-four healthy vines, with no visual nutrient deficiency symptoms and at almost uniform in their vigor were chosen and divided into four different treatments including the check. The experimental vines were arranged in a complete randomized block design with three replications per treatment, two vines in each.

Thus, the treatments were as follow:

1- Check (100% mineral-N).

2- 75% mineral-N + 25% humic acid (50 g/vine).

3- 75% mineral-N + 25% fulvic acid (50 g/vines).

4- 50% mineral-N + 25% humic acid and 25% fulvic acid.

Each treatment had the recommended N level (80g N/vine/year). Ammonium nitrate (33.5% N) as a mineral source was applied at three times growth start,

immediately after berry set and at two months later. The humic and fulvic acid as added once at first week of March.

The following parameters were determined during the three studied seasons to evaluate the effects of these treatments on growth and fruiting.

Vegetative growth parameters

Leaf area (cm^2): Sample of ten mature leaves from each replicated was removed from the growing shoot top (6^{th} or 7^{th} leaf) for estimation the leaf area using the following equation: Leaf area (cm^2) = 0.587 (L×W), where L = length of leaf blade and W = width of leaf blade according to (Montero *et al*., 2000) and the leaf area (cm^2) was calculated.

Weight of pruning wood was recorded immediately after pruning (December, 15) and was expressed as kg/vine.

Leaf chlorophyll content was estimated by using chlorophyll meter (SPAD 502 plus) using four leaves/replication from the fourth terminal expended leaf of the shoot (Yadava, 1986).

Leaf mineral contents: Samples of 30 leaves for each replication were removed from the full mature leaves from the shoots top in mid-July and leaf petioles were dismissed from the blades. The petioles were washed with tap water, distilled water, air-dried, oven dried at 70°C to constant weight, then ground in a stainless-steel mill. Wet digestion was done by using concentrated sulphoric acid and hydrogen peroxide for overnight. Percentages of N, P and K (on dry weight basis) were determined in the digestion according to Wilde *et al*. (1985).

Yield components

At harvest time (when TSS of berry juice in the check treatment reached 14-15% brix), the clusters were harvested, weighed and yield/vine (kg) was recorded. Two clusters were taken at random from yield of each vine and the following characteristics were determined. Cluster weight (g) and berry weight (g), then cluster compactness coefficient according to Winkler *et al*. (1974).

In addition, berry quality in terms of berry weight, TSS, total titratable acidity and reducing sugars % according to AOAC (1985). Data were tabulated and statistically analyzed according to (Gomez and Gomez., 1984 and Snedecor and Cochran., 1990) using COSTAT program. The individual comparisons between the obtained values were carried out using LSD at 5% level.

Results

Growth vegetative characteristics

It can be stated from the obtained data in Tables (2 to 5) that using different doses of nitrogen fertilization and humic or fulvic acids application on some traits of vegetative growth and nutritional status i.e., number and area of leaves and weight, pruning wood as well as content leaf of chlorophyll and minerals (NPK) of Superior seedless grapevines in 2020, 2021 and 2022 seasons. Obtained data

declared that the results go in the same direction took similar trend during the three studied seasons

Generally, data in these tables declared that the applying of the required N via 75 or 50% of the recommended dose of nitrogen (RDN) as mineral N along with using 25% as humic acid or and fulvic acid significantly increased such traits comparing to use RDN as a mineral N fertilizer alone. The maximum values of leaf area, pruning wood weight, Leaf chlorophyll content and leaf mineral contents were recorded on the vines that were fertilized via 75% mineral-N and 150 g humic acid. The highest leaf area (191.8 cm^2), pruning wood weight (2.111 kg/vine), total chlorophyll (45.77 SPAD value), leaf N (2.17%), leaf P (0.302%) and leaf K (1.49% as an av. of the three studied seasons). On otherwise, the less reading o these traits (167.9 cm^2), 1.821 kg/vine, 37.42 SPAD value, 1.87%, 0.245% and 1.26% av. the three studied seasons) were recorded on the vines treated with 100% mineral N (control), respectively. Then, the increment percentage of leaf area, pruning wood weight, total chlorophyll and leaf N P K % were (14.23, 15.93, 22.31, 16.04, 23.27 and 18.26% as an av. the three studied seasons) due to fertilize via 75% mineral-N and 50 g humic acid/vine compared to the check treatment, respectively. Therefore, N fertilization with Humic or fulvic acids as a partly replacement inorganic partial substitute for mineral ones significantly increases the vegetative growth due to improve the total surface area of leaves of grapevines.

Table 2. Effect of humic and fulvic acids application on pruning wood and leaf area of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat. \ Charact.		Pruning wood weight				Leaf area			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	1.660	1.928	1.875	1.821	169.5	168.6	165.5	167.9
T_2	Humic acid 50 g	1.951	2.226	2.156	2.111	195.1	193.1	188.2	191.8
T_3	Fulfic acid 50 g	1.863	2.146	2.101	2.037	190.9	188.4	184.3	187.9
T_4	Humic acid 50g + Fulfic acid 50 g	1.815	2.085	2.030	1.977	186.3	182.8	178.4	182.9
	LSD 0.05%	0.06	0.075	0.073		5.62	5.84	5.99	

Table 3.Effect of humic and fulvic acids application on total leaf area/shoot and total chlorophyll (SPAD value) of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat. \ Charact.		Total leaf area/shoot				Total Chlorophyll SPAD value			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	3627.30	4164.48	4021.62	3937.78	37.52	36.42	38.31	37.42
T_2	Humic acid 50 g	4868.41	5590.1	5382.7	5280.43	45.92	44.56	46.84	45.77
T_3	Fulfic acid 50 g	4499.10	5158.00	4939.24	4865.45	42.38	41.22	43.42	42.34
T_4	Humic acid 50g + Fulfic acid 50 g	4303.53	4953.92	4727.6	4661.68	40.56	39.33	41.53	40.47
	LSD 0.05%	168.18	201.25	184.86		1.44	1.94	1.57	

Table 4. Effect of humic and fulvic acids application on leaf N and P of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat.	Charact.	N%				P%			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	1.78	1.86	1.81	1.82	0.256	0.261	0.248	0.255
T_2	Humic acid 50 g	2.13	2.21	2.16	2.17	0.301	0.308	0.296	0.302
T_3	Fulfic acid 50 g	2.05	2.15	2.11	2.10	0.288	0.296	0.284	0.289
T_4	Humic acid 50g + Fulfic acid 50 g	1.91	1.99	1.93	1.94	0.276	0.285	0.276	0.279
	LSD 0.05%	0.07	0.06	0.08		0.016	0.017	0.016	

Table 5. Effect of humic and fulvic acids application on leaf K and yield/vine of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat.	Charact.	K%				Yield/vine (kg)			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	1.27	1.26	1.25	1.26	12.58	13.33	13.02	12.98
T_2	Humic acid 50 g	1.51	1.48	1.48	1.49	14.69	15.61	15.22	15.17
T_3	Fulfic acid 50 g	1.48	1.46	1.45	1.46	14.42	15.33	15.05	14.93
T_4	Humic acid 50g + Fulfic acid 50 g	1.35	1.34	1.32	1.34	13.83	14.65	14.23	14.24
	LSD 0.05%	0.07	0.06	0.06		0.60	0.56	0.58	

Yield and cluster characteristics

Data presented in Tables (5, 6 & 7) showed that using different doses of nitrogen fertilization and humic or fulvic acids on yield/vine, cluster weight, berry weight, and compactness coefficient of Superior Seedless grapevines in 2020, 2021 and 2022 seasons. It is visible from the data that the results go in the same direction during the three studied seasons. Using nitrogen fertilization as combination form mineral humic or fulvic acids plus significantly increased the yield/vine and cluster weight and decreased compactness coefficient of cluster compared to application of N as 100% mineral fertilization.

Moreover, fertilized by combined forms induce the uppermost values of these characteristics and minimal values of compactness coefficient than checked treatment. The heaviest yield and cluster weight as well as berry weight and least values of cluster compactness coefficient were detected due to fertilize via 75% mineral-N and humic acid.

The recorded highest values of yield/vine (15.17 kg/vine), cluster weight (474.9g), 25 berries weight (92.27g) and least cluster compactness coefficient (6.17) as an av. the three studied seasons due to fertilize via 75% mineral-N and 50 humic acid, respectively. Contrarily, these values on checked vines were (12.98 kg), (413.3 g), (80.95g) and (6.70), respectively. Then, the identical percentage increase increment percentages for these studied characteristics over check treatment were (16.87%), (14.90 %) and (13.98 %) as well as the decrement percentage of cluster compactness coefficient was (7.91 %) as an av. the three

studied seasons, respectively. In general, it could be concluded that combined humic acid with mineral-N fertilization had positive effects on productivity of Superior seedless grapevines.

Table 6. Effect of humic and fulvic acids application on cluster weight and cluster length of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat. \ Charact.		Cluster weight (g)				Cluster length (cm)			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	401.2	423.8	414.5	413.3	16.7	17.2	16.9	16.9
T_2	Humic acid 50 g	460.3	488.1	476.3	474.9	18.1	18.5	18.1	18.2
T_3	Fulfic acid 50 g	437.2	464.5	456.1	452.6	17.6	18.1	17.8	17.8
T_4	Humic acid 50g + Fulfic acid 50 g	418.8	443.8	431.3	431.3	17.3	17.8	17.5	17.5
	LSD 0.05%	14.56	16.25	15.64		0.58	0.59	0.55	

Table 7. Effect of humic and fulvic acids application on compactness coefficient and 25 berries weight of Superior Seedless grapevines during 2020, 2021 and 2022 seasons

Treat. \ Charact.		Compactness coefficient				25 berries weight (g)			
		2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	6.81	6.74	6.56	6.70	78.81	80.91	83.12	80.95
T_2	Humic acid 50 g	6.18	6.25	6.08	6.17	90.91	91.72	94.18	92.27
T_3	Fulfic acid 50 g	6.36	6.31	6.18	6.28	86.90	88.62	91.22	88.91
T_4	Humic acid 50g + Fulfic acid 50 g	6.25	6.19	5.96	6.13	85.16	85.53	87.95	86.21
	LSD 0.05%	0.18	0.16	0.15		3.52	3.38	3.61	

Chemical constituents of berry juice

Data of various berry characteristics as affected by different studied treatments during 2020 to 2022 seasons are presented in Table (8). The data indicated that using double form of fertilization significantly improved the Superior Seedless grapes quality in terms of increasing total soluble solids and reducing sugar and decreasing total acidity compared to checked treatment (100% mineral N).

The highest values of juice total soluble solids and reducing sugars contents were (14.71 and 11.35%) as an av. of the three studied seasons recorded on vines fertilized with 75% mineral-N and humic acid. On other side, the lower values of these reading were recorded on vines that fertilized by (100% mineral N checked treatment) which gave (13.43%) and (10.22%) as an av. of the three studied seasons, respectively.

Hence, the percentage of increases of these juice contents were recorded due to using fertilization via 75% mineral-N plus humic acid over the check treatment attained (9.53 & 11.06%), respectively. The least values of acidity was recorded on vines that fertilization by 75% mineral-N plus humic acid was (0.492%) compared to 0.552% as an av. three studied seasons on check vines. Hence such

amending induces decrement percentage in total acidity attained (10.87%) as an av. of the three studied seasons.

Regarding to the sitting effects, it could be recommended that applying vines with 50 to 75% of nitrogen requirements plus humic or/and fulvic acids promoted the nutritional status of vine, hence increase the vegetative growth. These beneficial effects improved the yield, cluster attributes and berry quality of Superior Seedless grapevines under the circumstances of this experiment.

Table 8. Effect of humic and fulvic acids application on TSS%, reducing sugar % and total acidity % of Superior Seedless grapes during 2020, 2021 and 2022 seasons

Charact. / Treat.		TSS %				Reducing sugar %				Total acidity %			
		2020	2021	2022	Mean	2020	2021	2022	Mean	2020	2021	2022	Mean
T_1	Control	13.28	13.63	13.39	13.43	10.25	10.28	10.12	10.22	0.561	0.543	0.551	0.552
T_2	Humic acid 50 g	14.51	14.93	14.70	14.71	11.41	11.36	11.28	11.35	0.495	0.486	0.496	0.492
T_3	Fulfic acid 50 g	14.11	14.52	14.30	14.31	10.86	10.79	10.81	10.82	0.524	0.516	0.518	0.519
T_4	Humic acid 50g + Fulfic acid 50g	13.85	14.31	14.11	14.09	10.71	10.68	10.63	10.67	0.532	0.520	0.531	0.528
	LSD 0.05%	0.41	0.37	0.33		0.33	0.31	0.36		0.011	0.011	0.09	

Discussion

Nitrogen fertilization is the most important way to increase the crop productivity. Nitrogen plays a key job in the feeding of fruit trees. It is a necessary element for chlorophyll, protoplasm and nucleic acids (Nijjar, 1985).

Humic acid effects are majority labor via actions i.e., soil fertility, raise the microbial population, higher cation exchange capacity (CEC), and soil structure improvement. In addition, biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Pinton, 2000 and Chen *et al.,* 2004).

Fulvic acid had highly beneficial on soil and plant. It is important to increase microbial activity and promote nutrient uptake induce increasing the vegetative growth and status of nutritional trees. These positive effects significantly raise the yield and promoted the fruit quality (Chen *et al*., 2004 and Ahmed *et al*., 2011).

Using humic and fulvic acids as organic nitrogen improve the growth and berry characteristics due to the reliable role of them on enhancing soil structure aggregation the water-holding capacity, soil organic matter and humid substances may raise the obtainable of nutrients and decrease soil pH and salinity (Nijjar, 1985; Lee *et al*., 2004 Zhang *et al*., 2010; Asgharzade and Babaeian 2012 and Suh *et al*., 2014). Moreover, they stimulate the facilitation and translocation of most nutrients, that synthesis carbohydrate and protein and nutrient movement, encouraging, cell division and has evolved of meristematic tissues. In addition, it induces opposition of plant to root diseases and controlling vegetative growth of

tree, then, improving its productivity (Gaur *et al*., 1980, Suba Rao, 1984 and Kannaiyan, 2002).

Current study showed that the application of 75% mineral-N plus humic or fulvic acids result significantly increased the leaf area about 14.23 or 11.91%, chlorophyll 22.31 & 13.14%, N 16.04 & 13.30% and K 16.77 & 14.63%. Moreover, these applications significantly increased yield/vine about 16.87 & 15.02% and beery weight 13.98 & 9.83%, as well as significantly improved TSS about 9.53 & 6.55% and decreased acidity 10.87 & 5.98%. Hence these treatments lead to increase the yield and hasting ripening with good berry quality which lead increase backable yield for exporting. Above mentioned results were in accordance with those obtained by Abdel-Monem *et al*., (2008); Ferrara and Brunetti (2010); Ahmed *et al*. (2011); Abdelaal *et al* (2013); Abdel-Hameed *et al*. (2014); El-Boray *et al*. (2015); Mohamadineia *et al*., (2015); Ibrahim and Ali., (2016); Mostafa *et al*. (2017); Akin., (2018); Popescu and Popescu (2018) and Abdel-Rahman *et al*. (2021). They concluded that humic acid or fulvic acid applied along 75% of suitable N could be improve the growth aspects, yield and fruit quality of different grape cultivars.

Conclusion

Therefore, it can be recommended that using 75% of nitrogen requirements plus humic acid or fulvic acid improve the vine nutrient status, yield and fruit quality leading to an increase of the packable yield. In addition, improve some soil physical and chemical properties, as well as it minimizes the out-pot costs and environmental pollution which could be resulting via excess of mineral fertilizers.

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تقليل التسميد النيتروجيني المعدني في بساتين العنب باستخدام حمض الهيوميك والفولفيك

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الملخص

أجريت هذه الدراسة خلال ثلاث مواسم متتالية 2020 و2021 و2022 على كروم العنب السوبريور اللابذري بكلية الزراعة، جامعة جنوب الوادي، محافظة قنا، جمهورية مصر العربية. بهدف دراسة تأثير إحلال حمض الهيوميك والفولفيك بدلاً من التسميد النيتروجيني المعدني على نمو وإثمار عنب السوبريور. وقد تم إضافة التسميد المعدني على ثلاث مرات بينما أضيف حمض الهيوميك والفولفيك مرة واحدة أول مارس. ويمكن تلخيص أهم النتائج فيما يلي:

أدي إضافة 75% من السماد المعدني مع 25% من حمض الهيوميك أو الفولفيك إلى حدوث زيادة معنوية في صفات النمو الخضري والمحصول وتحسين خصائص حبات العنب السوبريور.

من نتائج هذه الدراسة يمكن التوصية بإضافة الجرعة الموصي بها من السماد النيتروجيني في الصورة الثنائية (75% معدني + 25% حمض هيوميك أو حمض فولفيك) وذلك للحصول على نمو خضري جيد ومحصول عال مبكر ذو عناقيد وحبات ذات خصائص ممتازة تتفق مع سوق التصدير والقدرة التنافسية بالأسواق الخارجية فضلاً عن تقليل تكاليف الإنتاج والتلوث البيئي.