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RESPONSE OF FLAME SEEDLESS GRAPEVINE CUTTINGS GROWN UNDER HYDROPONIC CULTURE CONDITIONS TO SOME BIOFERTILIZATION TREATMENTS

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ABSTRACT: A hydroponic culture experiment was carried out during two successive seasons (2017 and 2018) on two years old grapevines cv. Flame seedless (*Vitis vinifera* L.) aimed to study the effect of Mycorrhiza, Azospirillum and Azotobacter treatments on growth, leaf chemical composition and cation equilibrium of Flame Seedless grapevines own rooted cuttings. The plants were two years old at the start of the experiment. The experiment was conducted under controlled conditions in Laboratory, with temperature fixed at 25 ± 3C°, relative humidity between 75–85% and 14-hours light exposure. Three microorganisms commonly used in biofertilization, namely: Arbuscular Mycorrhiza, Azospirillum brasilense, and Azotobacter chrococcum were used as biofertilizers. Standard nutrient solution, adapted with grapevines needs, reported by Morard 1995 and Ibrahim (2001) was prepared and used in this experiment. The Flame seedless own rooted cuttings exhibited high growth parameters as a result to biofertilization than those untreated cuttings. However, the higher values of shoot length, number of leaves/shoots, leaf area, chlorophyll contents and growth ratio at nutrient solutions was obtained from the seedlings treated with the three micro-organisms in combination. The chemical analysis of mature leaves and roots indicated that the own rooted cuttings received Mycrrohiza showed significantly higher ratio of P, K, Mg and Fe than other bio-fertilizers treatments. On the other hand, the Azotobacter treatments exhibited higher levels of N and Zn contents. Significant differences were observed between the two cultivars in Ca contents. However, the lowest N. P, K, Mg, Fe and Zn values were obtained from control treatment.

Key words: Hydroponics culture, Grapevines, *Vitis vinifera* L., Flame seedless, Mycrohiza, Azosprillum, Azotobacter.

INTRODUCTION

Grapevines (*Vitis vinifera* L.) belongs to the family Vitaceae, widely grown in the moderate climate of the Mediterranean region and it is well adapted to arid and semi-arid soils, and their grown successfully under Egypt climatic and soil conditions. Some investigators classified the grapevines under salinity resistant plants.

Hydroponics is a recent technology for growing plants using a nutrient solution without soil. Terrestrial plants may grow well in the mineral nutrient solutions only, or in inert medium, such as perlite, gravel, mineral wool, or coconut husk. Actually, hydroponics is an established branch of horticulture **(Douglas, 1975).** The advantage of hydroponic can be summarized in the following points: a). No soil is needed for the hydroponic system. b) The water in this system can be reused. c) It is possible to control the nutrition levels in their entirety. d) No nutrition pollution is released into the environment because of the controlled system, and e) Easy to control both pests and diseases in the system than the soil culture. Therefore, rapid and accurate results can be

achieved via hydroponic technology **(Huett 1994 and Morard 1995).**

Biofertilizers consist mainly of beneficial microorganisms that can release nutrients from raw materials and plant residues in the soil and make them available commercially where specific strains are used as biological fertilizers. They become recently, positive alternatives to chemical fertilizers because they help bring down the costs of chemical fertilizers especially N and P and improve soil fertility by maintaining the physical properties of the soil. They may help in improving crop productivity and quality by increasing the biological N fixation, the availability and uptake of nutrients and stimulating the natural hormones. They are safe for humans, animals and reducing the pollution occurring in our environment.

The target of this study was examining the effect of three microorganisms namely: Mycrrohiza, Azospirillum and Azotbacter on growth and nutritional status of Flame seedless grapevines grown hydroponically under laboratory control conditions.

MATERIALS AND METHODS

This study was carried out during two successive seasons (2017 and 2018) on Flame seedless *(Vitis vinifera* L.) own rooted cuttings. This study was conducted under controlled conditions in a Laboratory. However, the temperature adjusted to 25 ± 3C°, relative humidity ranged between 75 – 85 %- and 14-hours exposure to light.

Plant material: The used own rooted cuttings of the Flame seedless cultivar were two years old. In January, the own rooted cuttings were pruned leaving two spurs/plant, each spur containing four eyes, then they cultivated on sandy soil and irrigated with the referenced nutrient solution which used in this experiment (according to **Morard, 1995**), until the half of February, then the plants were transported to the hydroponic solution culture contained the different used microorganisms.

Nutrient solutions: Standard nutrient solution reported by Ibrahim (2001), supplemented with the nutrients requirements of grapevines was prepared. The nutrient solution contained Macro-nutrients (meq/L): 8.5 NO3, 1.0 H2PO5, 1.3 SO4, 1.0 NH4, 2.1 K, 6.7 Ca, 2.0 Mg and Micro-nutrients (meq/L): 5.9 Fe, 2.0 Mn, 0.05 Mo, 1.50 B, 0.5 Zn, 0.25 Cu. Solution pH was adjusted to 6.5, using HCl or KOH solutions. The three microorganisms (Mycrrohiza, Azospirillum and Azotobacter) were added to the solutions. Aeration system was used for 4 hours/day and the nutrient solution was changed weekly.

Microorganisms strains: The Fungi and bacterial strains used in the experiment were: Arbuscular mycorrhizal fungi (AMF), Azospirillum brasilence strain (AZB), and Azotobacter, as well as their all possible combination. Strains of Azospirillum brasilence strain (AZB), and Azotobacter and Arbuscular mycorrhiza fungi were kindly isolated and propagated at Laboratory of Microbiology, Minia University, Egypt. Strains of Azospirillum were grown on Doberiner medium but Azotobacter was grown on nutrient broth medium. Strains were grown in liquid medium on a rotary shaker at 30 °C and 120 rpm, then the culture were added to the nutrient solutions, three times/year, at a rate of 20 ml per pot, each ml contain 108 cells of Azospirillum or Azotobacter. However, Arbuscular mycorrhiza fungi were developed on onion plants roots, then the onion soil were extracted in water solutions, the solution was added three times yearly to pots in order of 15 ml/pot. However, each 1 mal contained 108 germs.

Experimental work: Under laboratory conditions, the plants were fixed in plastic covers of 10-liter plastic pots, each pot filled with 7L nutrient solution. Each pot was occupied by two plants, the total number of pots used were 35 (five pots for each treatment). This study included the following seven treatments from application of single and combined Mycrrohiza, Azosprillium and Azotobacter, in addition to the control treatment:

1- Control treatment: own rooted cuttings grown in nutrient solution only.

2- Own rooted cuttings grown in nutrient solution supplemented with Mycrrohiza.

3- Own rooted cuttings grown in nutrient solution supplemented with Azosprillium.

4- Own rooted cuttings grown in nutrient solution supplemented with Azotobacter.

5- Own rooted cuttings grown in nutrient solution supplemented with Mycrrohiza + Azosprillium.

6- Own rooted cuttings grown in nutrient solution supplemented with Mycrrohiza + Azotobacter.

7- Own rooted cuttings grown in nutrient solution supplemented with Azosprillium + Azotobacter.

8- Own rooted cuttings grown in nutrient solution supplemented with Mycrrohiza + Azosprillium + Azotobacter.

Each treatment was replicated five times, two plants per each (10 cuttings /treatment).

Measurements of vegetative growth: After the bud burst, the lengths of shoots (in cm) were recorded at 10 days intervals until the end of the experiment (3 Months), and then the growth ratio (cm/day) was calculated. At the end of the experiment the leaf area (cm^2) of the mature leaves was measured by an area meter (Area Meter Cl, 202).

Chlorophyll contents: One gram of fresh tissue was taken from the mature leaves and extracted by grinding in a mortar using 20 ml acetone, a small amount of pure silica quartz and 0.5 g calcium carbonate to neutralize the cellular sap acidity. The extract was filtered using a glass funnel and collected in a conical flask. The residue was re-extracted as described above until it became colorless. The extract was collected in a standard flask and the volume completed to a specific amount by adding acetone. The optical density (O.D.) of the extract was measured at wave lengths 663 and 645 nm to estimate chlorophyll a and b, respectively using a Spectrophotometer (Spectronic 21D). Three replicates for each treatment were employed, and the amount of Chlorophyll a and b and total carotenoids (mg/100g F.W.) were calculated according to the following equations:

Chlorophyll a = (9.784 x E662) – (0.99 x E644)

Chlorophyll b = (21.426 x E644) – (4.65 x E622)

Carotenoids = (4.965 x E440) – 0.268 (Chlo. a+b)

Where E= Optical density at a given wavelength.
Total chlorophyll was estimated by summation of chlorophyll a plus chlorophyll b (mg/ 100 g. F.W).

Foliar diagnoses: The mature leaves were collected at the end of the experiment, as described by **Morard (1995) and Ibrahim (2001).** The Ca, K, and Mg% as well as micro-nutrients were determined by atomic absorption spectrophotometry (Perkin Elmer 280). After overnight dehydration at 80C°, the leaves were grinded to fine powder. However, the total nitrogen was determined by Kjeldhal method and the phosphorus% was determined calorimetrically as described by **Walsh and Beaton (1986).**

Statistical design: The experiment was arranged in a complete randomized design, with five replicates. Each replicate comprised a one pot, each pot occupied by two plants. Data were subjected to analysis of variance and means were compared according to **Snedecor and Cochran, (1990).**

RESULTS AND DISCUSSION

Shoot growth ratio (cm/day)

It is noticed from the obtained data in Table (1) that, treating Flame Seedless cuttings with mycorrhiza, azosprillum and zotobacter was improved the shoot growth ratio (cm/day) significantly during the two seasons compared with untreated cuttings. Regarding the cuttings treated with the three microorganisms alone. The plants treated with mycorrhiza recorded the highest ratio of shoot growth (cm/day) compared to untreated plants or those treated with Azospirillum or Azotobacter bacteria, during the two experimental seasons. It worth to mention that any combination between the three examined microorganisms present higher and significant growth rations rather than the plants received any one alone. However, the combination Mycrrohiza and Azospirillum seems more effective than Azospirillum and Azotobactre, during the two experimental seasons. Furthermore, the highest growth ratios were obtained from the cuttings received the three microorganisms together (mycorrhiza, Azospirillum and zotobacter), during the two experimental seasons.

Shoot and root systems characteristics

Data presented in Table (2) show that inoculation with *Arbuscular Mycorrhiza* Fungi, *Azospirillum brasilense* strain, and *Azotobacter chrococcum*, each one alone or in combinations, significantly increased Flame Seedless vines (shoot lengths, number of leaves/shoot, and leaf area as well as roots fresh and dry weights/plant) compared to the control (un-inoculated one), during the two experimental seasons. The highest values in this connection were obtained from the plants received the three microorganisms in combination (Mycorrhiza, Azospirillum, and Azotobacter) compared to the other treatments under study, during both seasons.

Regarding the effect of each microorganism alone, Mycorrhiza showed superiority to other microorganisms in all vegetative growth characters, during the two experimental seasons. Furthermore, the combined Mycorrhiza with any one of the other two microorganisms showed superiority than the combination of Azospirillum and Azotobacter.

Table 1. Effect of some biofertilizers treatments on Growth ratio (cm/day) of Flame Seedless vines **under hydroponic culture conditions during 2017 and 2018 seasons**

Treatments	10 days		20 days		30 days		40 days		50 days		60 days		70 days		80 days		90 days	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	0.31	0.37	0.66	0.74	0.08	0.56	0.94	1.02	0.58	0.77	0.61	0.55	0.59	0.66	0.51	0.55	0.49	0.50
Mycrrohiza	0.49	0.52	0.93	0.87	0.69	0.72	1.00	1.14	1.31	1.27	0.79	1.22	0.89	0.91	0.89	0.91	0.79	0.82
Azosberlum	0.39	0.42	0.87	0.71	0.63	0.72	0.85	0.94	1.32	1.19	1.07	1.19	0.77	0.88	0.79	0.88	0.71	0.72
Azotobacter	0.31	0.41	0.78	0.72	0.66	0.70	0.95	0.99	1.02	1.00	1.01	1.08	0.73	0.81	0.71	0.87	0.69	0.68
Microhiyza + Azosberlum	0.52	0.59	1.01	0.99	1.02	1.04	1.21	1.31	1.52	1.50	1.53	1.55	1.02	1.11	0.94	0.99	0.83	0.85
Microhiyza + Azotobacter	0.60	0.62	0.91	0.99	0.96	0.99	0.94	0.97	1.12	1.21	1.19	1.22	1.08	1.07	0.92	0.98	0.66	0.65
Azosberlum + Azotobacter	0.51	0.49	0.88	0.91	0.82	0.92	0.91	0.89	1.01	1.29	1.01	1.09	0.96	1.06	0.88	0.87	0.73	0.71
Microhiyza + Azosberlum + Azotobacter	0.73	0.75	1.31	1.32	1.42	1.41	1.51	1.50	1.52	1.49	1.50	1.51	1.32	1.21	1.02	1.07	0.97	0.81
New LSD 5%	0.06	0.05	0.07	0.04	0.08	0.05	0.09	0.07	0.06	0.07	0.07	0.06	0.08	0.06	0.07	0.07	0.05	0.06

Table 2. Effect of some biofertilizers treatments on some growth characteristics of Flame Seedless vines **under hydroponic culture conditions during 2017 and 2018 seasons**

Treatments	Shoot lengths (cm)		No leaves/shoot		Leaf area (cm^2)		Roots fresh weight (g)		Roots dray weight (g)	
	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017
Control	53.4	55.9	10.5	12.3	96.4	99.1	199.9	211.3	79.3	84.5
Mycrrohiza	72.1	70.8	16.7	15.9	101.7	104.3	229.5	228.9	94.7	93.8
Azospirilum	64.5	66.3	14.3	14.7	98.6	99.3	219.8	210.7	87.9	84.3
Azotobacter	61.5	62.5	14.1	14.3	98.7	99.9	209.7	207.3	86.0	82.9
Microhiyza + Azosberlum	79.4	83.6	18.3	19.1	109.1	108.9	252.1	249.3	100.8	99.7
Microhiyza + Azotobacter	69.3	72.0	16.5	15.7	107.4	106.1	243.1	244.9	96.5	97.9
Azosberlum + Azotobacter	67.4	66.5	16.5	15.7	103.1	104.5	241.3	244.7	96.7	96.9
Microhiyza + Azosberlum + Azotobacter	85.4	88.9	21.1	22.3	114.7	118.5	268.1	266.3	109.2	110.7
New LSD 5%	6.8	5.3	3.1	2.7	4.1	3.9	14.1	13.8	4.5	5.1

The obtained results concerning the effect of mycorrhiza, azosprillum, and zotobacter on vegetative growth are in accordance with those obtained by **Smith and Read (1997); Autio *et al.*, (1991); Singh and Sharma (1993); Chirinos *et al.*, (2006); Ibrahim *et al.*, (2010); Swierczynski and Stachowiak (2010) and Mosa *et al.*, (2014).**

Data illustrated in the same Table also shows the effect of mycorrhiza, azosprillum, and zotobacter on the fresh and dray weight of root system of Flame Seedless vines. It is evident from this Table that, root system tacks the same trend as well as the growth system. Whereas, the three microorganisms enhanced the fresh and dray weight of roots, during the two experimental seasons. However, the grape-vines treated with mycorrhiza present superiority in root weight than those treated with azosprillum or zotobacter. The highest fresh and dray roots weights were obtained from the grape-vines received the combination of the three microorganisms together (mycorrhiza, azosprillum, and Azotobacter). Contrary, control plants (un-inoculated) present the lowest roots fresh and dray weight, during the two experimental seasons.

Leaves pigments content

Data concerning the effect of Mycorrhiza Fungi, Azospirillum, Azotobacter and their combinations on leaves main pigments (chlorophylls and total carotenoids mg/100g F.W.) of Flame Seedless vines during 2017 and 2018 seasons are shown in Table (3). It is obvious from the obtained data that, subjected Flame Seedless vines to the three microorganisms individual or in combinations was significantly enhancing the chlorophyll a, chlorophyll b, total chlorophylls, and total carotenoids (mg/100g F.W.) rather than the control treatment, during the two experimental seasons.

Leaves nutritional status

Data presented in Table (4) confirmed that, the treatments with the three microorganisms revealed that Mycorrhiza Fungi, Azospirillum, Azotobacter and their combinations resulted in significant differences in leaves nitrogen, phosphorus, magnesium, and potassium (as %) and Fe, Mn and Zn (as ppm) rather than control treatment. Moreover, the combinations of (Mycorrhiza, Azospirillum, and Azotobacter) followed by the combination (Mycorrhiza with either of Azospirillum or Azotobacter) recorded the highest values of chemical constituents of Flame Seedless cuttings compared to the other treatments under study in both seasons. Regarding individual treatment with Mycorrhiza, Azospirillum, or Azotobacter, Mycorrhiza showed superiority than the two others microorganisms, except the cases of leaves nitrogen content (as %) and Mn (as ppm), whereas Azospirillum showed superiority than the two others microorganisms.

Ibrahim *et al.*, (2010) found that nutritional status of guava trees were enhanced as a results of infected the trees with Mycrrohiza fungi and phosphate dissolving bacteria under salinity and calcareous stress, as compared to the un-inoculated ones**. Shamseldin *et al.*, (2010)** found that Bio-fertilizer inoculation while inoculation with *Azospirillum brasilence* strain W24 improves fruit quantity and quality of Washington navel orange. Furthermore, different types of hydroponic culture with using Mycrrohiza fungi were tested by **Nurbaity *et al.*, (2019)** their results confirmed that Mycrrohizal colonization, mycrrohizal spores, mineral nutrients uptake, and leaves pigments were significantly increasing as a result of reached the nutrient solution with Mycrrohiza spores.

Table 3. Effect of some biofertilizers treatments on main leaf pigments of Flame Seedless vines under hydroponic culture conditions during 2017 and 2018 seasons

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophyll s		Total carotenoids	
	2018	2017	2018	2017	2018	2017	2018	2017
Control	4.6	4.7	1.5	1.6	6.1	6.3	1.3	1.2
Mycrrohiza	5.8	5.7	1.9	1.9	7.7	7.6	1.6	1.7
Azospirilum	5.9	5.8	1.9	2.1	7.8	7.9	1.9	1.9
Azotobacter	4.9	5.1	1.8	1.9	6.7	7.0	1.7	1.8
Mycrrohiza + Azospirilum	6.1	6.3	2.1	2.2	8.2	8.5	1.9	1.9
Mycrrohiza + Azotobactre	6.2	6.2	2.0	2.2	8.0	8.2	2.0	2.2
Azospirilum + Azotobactre	6.2	6.3	1.9	2.1	8.1	8.4	2.0	1.9
Mycrrohiza + Azospirilum + Azotobactre	6.4	6.5	2.2	2.3	8.6	8.8	2.2	2.3
New LSD 5%	0.3	0.3	0.06	0.05	0.4	0.5	0.2	0.3

Table 4. Effect of some biofertilizers treatments on leaves mineral status of Flame Seedless vines under hydroponic culture conditions during 2017 and 2018 seasons

Treatments	N%		P%		K%		Mg%		Ca%		Fe (ppm)		Zn (ppm)		Mn (ppm)	
	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017
Controle	1.61	1.63	0.20	0.21	1.43	1.49	0.55	0.53	1.8	1.7	79	80	44	46	64	65
Microhiyza	1.79	1.78	0.25	0.28	1.55	1.62	0.63	0.65	2.1	2.2	98	99	52	55	72	77
Azosberlum	1.98	1.99	0.20	0.20	1.51	1.50	0.59	0.58	1.9	1.9	84	89	49	49	74	79
Azotobacter	1.86	1.82	0.21	0.21	1.50	1.51	0.57	0.57	1.9	1.8	83	88	48	49	71	73
Microhiyza + Azosberlum	1.99	2.09	0.27	0.28	1.62	1.67	0.71	0.74	2.2	2.1	102	106	53	55	79	81
Microhiyza + Azotobacter	2.01	2.02	0.26	0.28	1.53	1.52	0.71	0.72	2.2	2.2	98	101	51	52	75	75
Microhiyza + Azosberlum + Azotobacter	2.10	2.12	0.31	0.31	1.73	1.74	0.77	0.79	2.3	2.4	110	109	57	59	81	82
New LSD 5%	0.08	0.09	0.02	0.02	0.04	0.03	0.07	0.06	0.07	0.08	5.0	5.0	3.0	2.0	4.0	5.0

Conclusion: The bio-fertilization is economically efficient and sustainable alternative to stimulate growth and nutrition in fruit trees production. The three tested micro-organisms (arbuscular mycorrhiza, azosprillum and zotobacter) revealed a positive influence on vegetative growth and mineral nutrition of grapevines seedling. The Flame Seedless grapevine cuttings received the three micro-organisms in combination present the highest growth ratio (cm/day), number of leaves/plants, leaf area and leaves macro nutrients (N%, P%, K%, Mg% and Ca%), during the two experimental seasons.

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