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Role of Mycorrhizal Fungi in Reducing the Harmful Effects of Salinity on Grapevines Seedlings Grown Hydroponically

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Abstract: The effect of salinity stress on 'Flame Seedless' and 'Superior Seedless' grapevines seedlings (*Vitis vinifera L.*) grown hydroponically under salinity stress conditions, as well as the effectiveness of inoculation with Arbuscular Mycorrhizae Fungi (AMF) in reducing the harmful effects of salinity stress were investigated during 2022 and 2023 seasons. The investigation was carried out under Laboratory conditions, whereas the temperature was fixed at 25 ± 3 and relative humidity at 80 ± 5, referenced nutrient solution adapted with grapevines needs was used. The obtained results confirmed that, all vegetative parameters, fresh and dry weight of shoot and root systems, eaves main pigments and mineral status of both cultivars were negatively affected by increasing the salinity level in the nutrient solution. However, inoculated the seedlings with AMF significantly reduced these harmful effects of both cultivars. During both experimental seasons, the response of Flame Seedless seedlings to AMF inoculations was more perspicuous rather than those of Superior Seedless seedlings, these findings were true in most studied traits.

Key words: Grapevines, Salinity stress, Flame Seedless, Superior, Hydroponic culture, and Arbuscular Mycrrohiza Fungi (AMF).

1. Introduction

Grapevine (*Vitis vinifera* L.) is considered to be one of the major fruit crops throughout the world based on hectares cultivated and economic value **(Reynier, 2000)**. However, the Soilless culture technique is defined as any method of growing plants without the use of soil as a medium of root growing, however the mineral nutrients absorbed are supplied to the roots throw the irrigation water **(Ibrahim, 2001 and El-Kazzaz, 2017)**.

Soilless systems are alternative to soil cultivation in case of soil or water insufficiency, or salinization **(Ibrahim, 2001)**. Soilless system techniques have been integrated into horticultural production, maximizing the efficiency of water uses and nutrients **(Van Zelm *et al.*, 2020)**. This technique is adapted to fruit trees and its success depends on the proportion of the root colonizing the substrate with regard to the total amount of tree roots. Then, the time used until the roots colonized the substrate is one of the major factors affecting the success and efficiently of soilless culture **(Steven 2005 and Renu *et al.*, 2016)**.

The term of mycorrhizae refers to the symbiotic relationship between a group of fungi and the roots of vascular plants. These fungi live in the culture medium in a symbiotic relationship with the roots of many plants. These mutualistic relationships are beneficial, not harmful, due to their important role in plant nutrition and improving nutrient absorption. These fungi rely on the energy they take from the host plant in exchange for supplying the plant with the phosphorus and other nutrients it needs. Mycrrohiza divided into main two types: Ectomycorrhiza and Endomycorrhiza also called vesicular-arbuscular mycorrhiza (VAM). Arbuscular Mycrrohiza Fungi (AMF) has a lot of beneficial effects on plant growth and predictability such as, the antagonist's effect (biological control) on other soil harmful microorganisms, it conceder as hormone producer and promoting plant growth, It is well known that AMF increasing mineral availability and uptake, it widely used as bio-fertilizers all over the world, and we can conserve at least 50% of mineral fertilizers by using AMF inoculation, as well as it is low cost rather than the other fertilizers and it is well known that AMF is Eco-friendly fertilizer **(Ibrahim, 2020; Metawe, 2020; Abd Elhakem, 2025 and Hendy, 2025)**.

Flame Seedless cultivar conceded as one of the popular grapes cultivar. It successfully grown under different Egyptian soil and climate conditions, and it is one of the earlier ripens cultivars (**Ibrahim *et al*., 2020**). Whereas, this cultivar has a medium favorable berry size, red color, crispy and well balanced flavor (**Metawie, 2020; Mohamed, 2023 and Abdel-Hakem, 2025**). Superior Seedless cultivar successfully grown under El-Minia conditions, it considered as popular grapevine cultivar, ripe early during Early June. Superior Seedless considered as one of the prime cultivars, main popular and the most profitable for the consumers. The efficacy of AMF as bio-fertilizer has been proven in many field trails results that have shown significant improvements in growth and productivity.

2. Material and Methods

A laboratory investigation aimed evaluate the positive role of AMF in elevated the harmful effect of salinity stress on two main grapevines cultivars, using the hydroponic culture technique was carried out during two successive experimental seasons 2022 and 2023 on these two grapevines cultivars.

2.1. Plant materials

The present study included two mains own rooted grapevines cultivars namely Flame seed less and Superior Seedless were grown in the nursery of Faculty of Agriculture Minia University. The seedlings had one-year-old at the beginning of study, chosen so that the size and vigour was similar as possible. During early February to Early March, the seedlings were grown in referenced nutrient solution adapted in grapevines needs (Ibrahim 2001). The seedlings transferred to the pots field with the referenced nutrient solution and the treatments were done.

2.2. Nutrient solution

A referenced nutrient solution made of the macro and micro mineral elements necessary for the optimally growth of grapevines was used (Ibrahim 2001). The composition of this nutrient solution was as follows: Macronutrients (meq/L): 8.5 NO_3, 1.0 H_2PO_5, 1.3 SO_4, 1.0 NH_4, 2.1 K, 6.7 Ca and 2.0 Mg. Micronutriments (meq/L): 5.9 Fe, 2.0 Mn, 0.05 Mo, 1.50 B, 0.5 Zn, 0.25 Cu. The pH was adjusted to 6.5 by using HCl or KOH solutions. However, the nutrient solution used was replaced every ten days **(Wootton-Beard, 2024)**.

2.3. Salinity treatments

NaCl was used to adjust the nutrient solution salinity in growing pots. The level of NaCl adjusted to: 500, 1000, 1500, 2000 and 2500 ppm.

2.4. Arbuscular mycorrhizal fungi (AMF) preparation

The Arbuscular mycorrhizal fungi (AMF) used in these study (AMF) was kindly isolated, purified and propagated in Laboratory of Microbiology Faculty of Agriculture, Minia University - Egypt, according to the methods outlined by **Ranganayaki *et al.* (2006)**. The number of AMF spores was adjusted to 10^8 spores / each ml. this suspension was used in order to 50 ml per pot.

2.5. Experimental Implementation

The seedlings were fixed in plastic covers of 10-liter plastic pots. Each pot was filled with 7 Liters of nutrient solution. Each pot was occupied by four seedlings. The seedlings were pruned so that each seedling had one spur with 4 eyes. After growth, the number of shoots was fixed to 2 shoots per seedlings. The following salinity and AMF inoculations treatments were done for the two cultivars: Control: 500 ppm salinity without AMF inoculation; 1000 ppm NaCl without AMF; 1000 ppm NaCl + AMF; 1500 ppm NaCl without AMF; 1500 ppm NaCl + AMF; 2000 ppm NaCl without AMF; 2000 ppm NaCl + AMF; 2500 ppm NaCl without AMF and 2500 ppm NaCl + AMF.

Each treatment was represented by one pot, and each pot containing four seedlings, each replicate presented by one seedling. The nutrient solution used was replaced every 10 days. An air pump was installed into the nutrient solution, it worked on 3 times a daily, every time running for 40 minutes. The laboratory temperature was set at 25 ± 3 and relative humidity at 85 ± 5, during the experimental period.

2.6. Experimental design and statistical analysis

Complete randomized design (CRD) was followed, where the experiment consisted two cultivars, each one treated with 9 treatments, each treatment included one pot occupied by four seedlings. The obtained data were tabulated and subjected to statistical analysis, according to **Snedecor and Cochran (1990)**.

2.7. Different measurement and determinations

Vegetative growth parameters: Each week the main shoot length (cm) was recorded, at the end experimental during both seasons, the numbers of leaves per shoot were recorded. Then, twelve mature leaves from each plant located at the middle part of main shoots were picked (according to Ibrahim, 2010) for estimated in Leaf area (cm2) measuring, by using the equation reported by **(Ahmed and Morsy, 1999)**.

Fresh and dry weight of shoot and root system for each plant were done at the end of experiment during each season.

Determination of plant pigments: Samples of six mature and fresh leaves from the middle part of shoots (which used in measuring leaf area) were taken and the blades only were used in chlorophylls and total carotenoids determination, according to **Ward and Johnston (1962)**. By using the following equations:

Chlorophyll A = (9.784 x E 662) – (0.99 x E 644) = mg/100 g F.W.

Chlorophyll B = (21.426 x E 644) – (4.65 x E 622) = mg/100 g F.W.

Total carotenoids= (4.965 x E440) – 0.268 (Chlorophyll a+b) = mg/100 g F.W.

Where E = optical density at a given wavelength. However, the total chlorophyll was estimated by the summation of chlorophylls a and b (mg/100 g. F.W).

Foliar diagnoses: The same six adult leaves which used in measuring leaf area, were taken from each pot plants in order to determining the mineral contents, during each season, according to (Martin-Preval *et al.*, 1984). The petioles only were used to determining the contents of mineral nutrients. The petioles washed with distillated water, dried, grounded and 0.5 g weight fine powder was used N. P, K, Ca and Mg as well as micronutrients (Fe, Mn and Zn) determination according to **Martin-Preval *et al.* (1984)**.

3. Results and Discussion:

3.1. Fresh weight of shoot system (g)

During the two seasons, a gradual decreasing in fresh weight of shoot system of ‘Flame Seedless’ and ‘Superior Seedless’ was observed, as a result of increasing the NaCl concentration in nutrient solution from 500 ppm to 2500 ppm (Table 1). It is clear from this Table that increasing the level of salinity lead to a sharp and significant decreasing of fresh weigh of both examined cultivars. Then, the seedlings grown under high salinity level (2500 ppm) present the lowest fresh weight of shoot system (240.2 & 226.6 for Flame Seedless and 128.6 & 131.4 g for Superior Seedless). Contrary, the seedling grown in 500 ppm nutrient solution present the highest fresh weight (581,4 & 589.4g for Flame Seedless and 631.8 & 658.2 g for Superior Seedless) during the two experimental seasons respectively. However, this decrement was more evident on ‘Superior Seedless’ cultivar rather than on ‘Flame Seedless’ cultivar (Table 1).

The obtained results in the above-mentioned table and figures showed that inoculating the seedlings of the two cultivars with AMF had a positive and significant effect on reducing the harmful effect of high salinity in the growth medium (nutrient solution), this was positively reflected in increasing the fresh weight of the vegetative system of both cultivars under study. The results showed also that, the response of the Flame Seedless cultivar to inoculation with AMF was more pronounced than that of the Superior Seedless cultivar.

Table (1). Effect of AMF inoculations on fresh weight of shoot system of ‘Flame Seedless’ and ‘Superior Seedless’ grapevines grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Fresh weight of shoot system (g)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	581.4	631.8	589.4	658.2
1000 ppm NaCl	478.0	531.0	489.0	537.8
1500 ppm NaCl	409.8	295.4	415.8	281.0
2000 ppm NaCl	295.8	256.2	307.8	241.8
2500 ppm NaCl	240.2	128.6	226.6	131.4
1000 ppm NaCl + AMF	572.2	553.8	616.0	580.4
1500 ppm NaCl + AMF	471.0	389.8	476.2	474.6
2000 ppm NaCl + AMF	207.8	219.4	335.4	209.0
2500 ppm NaCl + AMF	182.4	139.4	186.4	138.6
New LSD at 5%	**15.2**	**17.3**	**16.4**	**18.8**

3.2. Dry weight of shoot system

A gradual decreasing in shoot system dry weight of ‘Flame Seedless and Superior Seedless cultivars in relation to increasing the nutrient solution salinity (from 500 to 2500 ppm) were observed, during the two experimental seasons. The salinity level in nutrient solution from 500 ppm to 2500 ppm (Table 2). Whereas, the seedlings grown under high salinity level (2500 ppm) present the lowest dry weight of shoot system (60.1 & 56.9 g for Flame Seedless and 32.2 & 32.9 g for Superior Seedless), during the two experimental seasons respectively. However, this decrement was more evident on Superior Seedless cultivar rather than on Flame Seedless cultivar (Table 2). The obtained results showed that inoculating the seedlings of the two cultivars with AMF had a positive and significant effect on reducing the harmful effect of high salinity in the nutrient solution (Table 2). This positively reflected in increasing the dray weight of the vegetative system of both cultivars under study, compared to non-inoculated seedlings. The results showed also that, the response of the ‘Flame Seedless’ to inoculation with AMF was more pronounced than this of the ‘Superior Seedless’ cultivar. Considering the seedlings grown in a nutrient solution containing 1000 ppm sodium chloride and inoculated with mycorrhizae fungi, neither cultivar recorded any significant differences over the two seasons of the experiment compared to those grown in a nutrient solution containing 500 ppm sodium chloride. This demonstrates the ability and efficiency of this fungus in giving these two cultivars the ability to tolerate of salinity stress. The tolerance to salt stress of both cultivars gradually decreased with increasing salinity concentration in the medium, but was always better than that of the seedlings grown under the same salinity level without inoculation with mycorrhizae fungi.

Table (2). Effect of AMF inoculations on dry weight of shoot system of 'Flame Seedless' and 'Superior Seedless' grapevines grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Dry weight of shoot system (g)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	146.3	157.9	147.4	164.6
1000 ppm NaCl	120.5	133.3	122.3	134.5
1500 ppm NaCl	103.4	74.2	104.8	70.5
2000 ppm NaCl	74.5	64.3	76.9	60.7
2500 ppm NaCl	60.1	32.2	56.5	32.9
1000 ppm NaCl + AMF	144.2	138.5	154.0	145.1
1500 ppm NaCl + AMF	118.7	99.8	126.6	101.7
2000 ppm NaCl + AMF	57.9	54.8	83.8	52.3
2500 ppm NaCl + AMF	45.6	34.9	41.6	34.7
New LSD at 5%	**8.2**	**10.3**	**9.1**	**9.9**

3.3. Fresh and dry weight of root system

The results recorded in Tables (3 and 4) shows the effect of increasing salinity stress in the growth medium (nutrient solution) on the root system fresh and dry weight of two grapevine cultivars (Flame Seedless and Superior Seedless), grown under hydroponic conditions in the laboratory. These obtained results clearly demonstrate the that, in the case of the control (500 ppm salinity), Superior Seedless seedlings showed a superior in both fresh and dry root weight compared to the Flame Seedless cultivar. When the sodium chloride concentration in the solution was gradually increased from 500 ppm to 2500 ppm, there was a gradual and significant decrease in fresh and dry weight of root system for both cultivars, during the two experimental seasons.

However, the harmful effect of increasing the salinity concentration in the growth medium was more pronounced on 'Superior Seedless' than those on the 'Flame Seedless'. The seedlings grown in a highly salinity nutrient solution recorded the lowest both fresh and dry weight of roots. Contrary, the seedlings grown in a lowest salinity (500 ppm) nutrient solution recorded the highest fresh and dry root weights. These results were true for both study seasons.

The obtained data also show that inoculating the seedlings of both cultivars with AMF had a positive and significant effect on reducing the harmful effect of salinity on root growth, represented by a significant decrease in the fresh and dry weight of both examined cultivars root stem (Table 11). This positive effect of AMF was more pronounced in Flame Seedless than those of Superior Seedless cultivar, these findings were true during both experimental seasons.

The negative effect of salinity stress on grapevines fresh and dry weigh as well as the positive role of AMF inoculation in reducing the harmful effects of salinity and improving grapevines or other fruit trees growth parameters were mentioned by some authors such as: **Bernstein, 1977; Ibrahim 2016; Shani & Ben-Gal (2005); Papadakis *et al.*, (2007); Mastrogiannidou *et al.*, (2016) and Al-Taey & Al-Ameer (2023)** on salinity harmful effects on fruit trees grown. Furthermore, the positive role of AMF in elevating the harmful effects of salinity was confirmed by: **Gosling *et al.*, (2006); Bennewitz & Hlusek (2006); Dasgan *et al.*, (2008); Medina** and **Azcon (2010); Sedlacek *et al.*, (2012); Nurbaity *et al.*, (2019); Ibrahim *et al.*, (2020); Lin, *et al.*, (2021) and Metawe 2020 and Hendy 2025.**

Table (3). Effect of AMF inoculations on fresh weight of root system of 'Flame Seedless' and 'Superior Seedless' grapevines grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Fresh weight of root system (g)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	309.7	337.3	312.3	319.5
1000 ppm NaCl	252.9	261.1	276.2	258.7
1500 ppm NaCl	215.4	202.3	219.4	212.5
2000 ppm NaCl	99.7	89.5	105.6	97.3
2500 ppm NaCl	81.0	67.1	91.0	82.1
1000 ppm NaCl + AMF	262.7	275.5	284.4	267.7
1500 ppm NaCl + AMF	124.6	209.3	218.3	209.4
2000 ppm NaCl + AMF	108.4	93.7	145.3	122.1
2500 ppm NaCl + AMF	92.6	71.2	108.5	101.3
New LSD at 5%	**10.2**	**9.4**	**9.1**	**10.5**

Table (4). Effect of AMF inoculations on dry weight of root system of 'Flame Seedless' and 'Superior Seedless' grapevines grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Dry weight of root system (g)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	96.0	104.5	96.8	99.1
1000 ppm NaCl	78.4	80.9	85.6	81.2
1500 ppm NaCl	66.8	62.7	68.0	65.9
2000 ppm NaCl	30.9	28.6	32.7	29.2
2500 ppm NaCl	24.1	20.8	28.2	25.4
1000 ppm NaCl + AMF	81.4	85.4	88.2	82.9
1500 ppm NaCl + AMF	38.6	64.9	67.7	64.9
2000 ppm NaCl + AMF	33.6	29.1	42.0	35.9
2500 ppm NaCl + AMF	29.7	24.1	34.2	28.2
New LSD at 5%	**3.7**	**5.2**	**4.1**	**4.4**

Salinity stress cause a severe harmful effects on fruit trees growth and nutrition statues as well as soil erosion particularly in arid and semiarid regions (**Kumar, *et al.*, 2010; Himabindu *et al.*, 2016; Hashem *et al.*, 2018; Jia *et al.*, 2019 and Boorboori & Lackoova 2025**). High salinity level growth medium (nutrient solution) can reduces the osmotic potential of the culture medium, and also suppresses the activities related to nutrients in plants, and then it leads to unfavourable Na/Ca and Na /K ratios (**Romero-Munar *et al.*, 2019 and Boorboori & Lackoova 2025**). Increasing the salinity level causes osmotic, and ionic stress in plants. This lead to reducing plant growth and productivity by disrupting important biochemical, and physiological processes (**Abd-Allah *et al.*, 2015**, **Fall *et al.*, 2018,** and **Litalien & Zeeb, 2020**).

This decreasing of roots growth as a result of increasing salinity level in growth medium is the first response of seedlings to excessive salinity in medium (**Greenway & Munns, 1980** and **Mengel,**

2007). Salt present in the rhizosphere zone, led to slower growth of primary roots. Salinity caused inhibition of cell division and root cells elongation, while lateral roots grow (**Marschner, 1995 and Bernstein *et al.*, 2013**). However, salinity toxic effects on root growth and development are related to inhibition of endogenous phytohormones levels, such as IAA and IBA (**Mengel, 2007; Egamberdieva 2009; Khalil *et al.*, 2011; Egamberdieva *et al.*, 2016 and Hashem *et al.*, 2018**). The harmful effects of salinity of salinity on shoot and root growth may be explained also by decreasing the capacity of assimilates which supply to the root system and other growing tissues, which is significantly affect plant growth and particularly under long-term salinization (**Chinnusamy *et al.*, 2005 and 2005 Chen *et al.*, 2020**).

3.4 Leaves photosynthesis pigments

The results illustrated in Tables (4, 5 and 6) showed the negative effects of increasing the salinity level as well as the positive role of AMF inoculations on leaves photosynthesis pigments (Chlorophylls and carotenoids) of Flame Seedless and Superior Seedless cultivars grown hydroponically, during 2022 and 2023 seasons.

3.4.1. Chlorophyll A contents

The obtained data during the two seasons as illustrated in Table (4) declare that, regardless the cultivar, increasing the concentration of NaCl in nutrient solution from 500 ppm to 2500 ppm has a marked decreases effect on chlorophyll a contents compared to control treatment during both experimental seasons. These observations were true for the two examined cultivars in both experimental seasons. However, this decrement was parallel to increasing of salinity level. However, this harmful effect was more pronounced for Superior Seedless than Flame Seedless cultivar. The seedlings grown in nutrient solution contained 2500 ppm NaCl present the lowest chlorophyll a contents, during 2022 and 2023 seasons respectively.

It is clear from the obtained data also that inoculating the seedlings of both cultivars with AMF significantly stimulated the synthesis of chlorophyll a, even under salinity stress. This positive effect was more pronounced in Flame Seedless plants than Superior Seedless cultivar. In the same context, the AMF inoculated seedlings of both cultivars grown in nutrient solution contained 1000 ppm NaCl not present any significant differences in chlorophyll a contents in comparison to control treatment (500 ppm). In all cases, the comparison between AMF inoculated seedlings and un-inoculated one (at the same level of salinity) has always been better for inoculated one. These findings were true for both cultivars and experimental seasons.

Table (5). Effect of AMF inoculations on chlorophyll a contents of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Chlorophyll A (mg/100 g F.W.)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	7.2	7.8	6.8	7.3
1000 ppm NaCl	6.1	6.0	5.2	5.7
1500 ppm NaCl	5.3	5.1	4.1	4.0
2000 ppm NaCl	3.3	2.8	3.6	3.0
2500 ppm NaCl	2.7	2.2	3.3	2.5
1000 ppm NaCl + AMF	7.3	7.7	6.6	6.7
1500 ppm NaCl + AMF	6.2	6.1	5.3	5.1
2000 ppm NaCl + AMF	4.5	4.2	4.2	4.0
2500 ppm NaCl + AMF	4.1	4.0	3.8	3.4
New LSD at 5%	**0.6**	**0.5**	**0.4**	**0.4**

3.4.2. Chlorophyll b contents

During the two experimental seasons the results showed that, regardless the cultivar used, increasing the concentration of NaCl in nutrient solution from 500 ppm to 2500 ppm has led to a significant decrease in chlorophyll b contents compared to control treatment. Under low salinity level (500 ppm), ‘Superior Seedless’ present higher contents of chlorophyll b in there leaves in comparison to ‘Flame Seedless’ cultivar, in both experimental seasons. Contrary, under higher level of salinity (2500 ppm) ‘Flame Seedless’ present higher levels in chlorophyll b in thier leaves than those of ‘Superior Seedless’ cultivar, during 2022 and 2023 seasons. Then, chlorophyll b decrement was related to increasing of NaCl concentration in nutrient solution. However, this harmful effect was more pronounced in ‘Superior Seedless’ compared to ‘Flame Seedless’ during the two experimental seasons.

It is clear from the obtained data that inoculating the seedlings of both cultivars with AMF significantly stimulated the synthesis of chlorophyll b, even under salinity stress. This positive effect was more pronounced in Flame Seedless than in Superior Seedless cultivar, these data were true during the two experimental seasons. Furthermore, non-significant differences were obtained in chlorophyll b contents between the AMF-inoculated seedlings grown in nutrient solution contained 1000 ppm NaCl and the control (500 ppm) seedlings. These data were true during the two seasons for the two cultivars. In all cases, the comparison between AMF-inoculated seedlings and un-inoculated one (at the same level of salinity) has always been better for inoculated one. These findings were true for both cultivars and experimental seasons.

Table (6). Effect of AMF inoculations on chlorophyll b contents of ‘Flame Seedless’ and ‘Superior Seedless’ grapevines leaf area grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Chlorophyll B (mg/100 g F.W.)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	2.5	2.6	2.1	1.9
1000 ppm NaCl	1.7	1.9	1.8	1.7
1500 ppm NaCl	1.6	1.6	1.4	1.4
2000 ppm NaCl	1.9	2.1	1.2	1.3
2500 ppm NaCl	1.2	0.9	1.0	0.8
1000 ppm NaCl + AMF	2.4	2.6	2.0	2.2
1500 ppm NaCl + AMF	1.9	1.9	1.6	1.5
2000 ppm NaCl + AMF	1.8	1.7	1.3	1.2
2500 ppm NaCl + AMF	1.6	1.4	1.2	1.1
New LSD at 5%	**0.3**	**0.3**	**0.2**	**0.3**

3.4.3. Total chlorophyll contents

Data illustrated in Table (7) shows the effect of increasing the nutrient solution salinity from 500 ppm to 2500 ppm on total chlorophyll contents. In adaption, the same table declares the positive effect of AMF-inoculation on reducing the harmful effect of salinity on the total chlorophyll. In both examined cultivars, nearly the leaves total chlorophyll contents have taken the same tendency that obtained in chlorophyll a and chlorophyll b.

The obtained data declared that increasing the level of salinity in nutrient solution from 500 ppm to 2500 ppm was parallel to decreasing the total chlorophyll contents in the leaves of both examined cultivars. This decrement was more pronounced in Superior Seedless than Flame Seedless cultivar. Inoculated the seedlings of both examined cultivars with AMF significantly reduced the total chlorophyll in the leaves of both cultivars. However, the response of Flame seedless was more pronounced than Superior Seedless cultivar. Un-inoculated 'Superior Seedless' seedlings grown in 2500 ppm nutrient solution present the lowest total chlorophyll contents followed by Flame Seedless grown in the same solution. These data were true during the two experimental seasons respectively.

Table (7). Effect of AMF inoculations on total chlorophyll contents of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Total chlorophyll (mg/100 g F.W.)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	9.5	10.4	8.9	9.2
1000 ppm NaCl	7.8	7.9	7.0	7.4
1500 ppm NaCl	6.9	6.7	5.5	5.4
2000 ppm NaCl	5.2	4.9	4.8	4.3
2500 ppm NaCl	3.9	3.1	4.3	3.3
1000 ppm NaCl + AMF	9.7	10.3	8.6	8.9
1500 ppm NaCl + AMF	8.1	8.0	6.9	6.6
2000 ppm NaCl + AMF	6.3	5.9	5.5	5.2
2500 ppm NaCl + AMF	5.7	5.4	5.0	4.5
New LSD at 5%	**0.43**	**0.50**	**0.44**	**0.56**

3.4.4. Total carotenoids contents

Table (8) showed that, the adult leaves of Flame Seedless and Superior cultivars inoculated or un-inoculated with AMF and grown hydroponically under salinity stress present lower total carotenoids content than those grown in nutrient solution at 500 ppm NaCl (control). Total carotenoid contents taken similar tendency as chlorophyll contents. It is clear from this table that increasing nutrient solution salinity level was parallel to decreasing the total carotenoids contents in leaves. However, the seedlings of each cultivar grown in 2500 ppm NaCl produced the lowest carotenoids contents in their adult leaves, during the two experimental seasons respectively. On the opposite side seedlings grown in 500 ppm (control) produced the highest contents of total carotenoids. These data were true for the two cultivars, during the two seasons respectively.

Generally, during the two experimental seasons, leaves total carotenoid contents significantly increase as a result of inoculated the seedlings of both cultivars (Flame Seedless and Superior Seedless) with AMF in comparison with un-inoculated one, under the same salinity level. However, Flame seedless shows more adapted with AMF inoculation rather than Superior Seedless cultivar. Even when inoculated the seedlings of both cultivars with AMF, the leaves total carotenoids contents were significantly decreased as a result of increasing nutrient solution salinity. Under the same level of salinity, this attrition rate of carotenoids was less than that of un-inoculated seedling.

Table (8). Effect of AMF inoculations on total carotenoids contents of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Total carotenoids (mg/100 F.W.)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	2.6	2.4	2.5	2.5
1000 ppm NaCl	2.6	1.7	2.4	2.3
1500 ppm NaCl	1,8	1.7	1.7	1.9
2000 ppm NaCl	1.1	1.0	1.2	1.0
2500 ppm NaCl	0.9	0.8	1.0	0.9
1000 ppm NaCl + AMF	2.6	2.3	2.5	2.3
1500 ppm NaCl + AMF	2.4	2.1	2.4	1.9
2000 ppm NaCl + AMF	2.1	1.6	2.2	1.5
2500 ppm NaCl + AMF	1.7	1.4	1.8	1.1
New LSD at 5%	**0.2**	**0.1**	**0.1**	**0.2**

Some interested previous investigations have showed the effect of AMF inoculation on different plant grown under salinity stress. These studied demonstrated that inoculated the plants with AMF under salinity stress led to a higher photosynthetic capacity (**Chandrasekaran *et al.*, 2019; Liang & Shi 2021** and **Liang *et al.*, 2021**). This positive effect of AMF can have explained by the different roles of AMF in improving transpiration rate, water status of leaves, strengthen of photosynthesis machinery, main pigment contents, photochemistry and non-photochemistry of PSII, and the role of AMF in carbon uptake and transfer to mycorrhizae (**Hashem *et al.*, 2018; Zai *et al.*, 2021 and Ait-El-Mokhtar *et al.*, 2020**). The coexistence of AMF in salty environments affects age-related changes (ARCs) in metabolism of leaves and partially prevents leaf aging, this lead to the better metabolites accumulation (**Shtark *et al.*, 2019**). Furthermore, in this concern plant inoculated with AMF and grown under salinity stress had a leaf cells protect of saline by preventing cell wall detachment, reducing plasma membrane damage, inhibiting chloroplasts degradation, and protect of chloroplast structure (**Liang *et al.*, 2021; Evelin *et al.*, 2013 and Evelin *et al.*, 2019**). AMF inoculated Osmolyte accumulation can protect CO_2 fixing enzymes (**Talaat and Shawky, 2014**), which gains the plant a higher PSII efficiency. Consequently, these lead to increasing the photosynthetic capacity under salinity stress.

3.4.5. Effect on leaves macro nutrients content's

Data presented in Tables (9, 10, and 11) shows that leave N, P, K, and Mg contents (%) of 'Flame Seedless' and 'Superior Seedless' seedlings significantly decreased as a result of increasing salinity level in nutrient solution. This decrement was correlated with increasing the salinity level in the nutrient solution. High the salinity level low N, P, K, and Mg % were obtained in the leaves of both cultivars. These findings were true during the two experimental seasons. It is clear from these tables that non AMF-inoculation seedlings grown in higher salinity nutrient solution (2500 ppm) present the lowest N, P, K, and Mg contents, during the two experimental seasons. Treated the seedlings of both cultivars with AMF significantly enhancing leaves N, P, K, and Mg percentages compared to non-inoculated one. This positive effect of AMF significantly reduced in relation to increasing the salinity level in nutrient solution.

Under normal conditions plant absorb the Nitrogen in form of nitrate (NO_3^-), and ammonium (NH_4^+). The absorption of both cations can hinders by salinity interferes by immobilizing them (**Frechilla *et al.*, 2001 and Kumar *et al.*, 2010**). Under salinity stress conditions, the ammonium cation uptake is challenged by sodium cations, and the Nitrate anion uptake inhibited by chlorine, which causes the low flux of ammonium and nitrate from growth medium to hair roots, this led to a decrease in nitrate reductase enzyme activity in plants (**Marschner 1995** and **Hoff *et al.*, 1992 and Kumar *et al.*, 2010**).

It is well documented that, the salts are plant nutrients and led to K deficiencies, recurring problem in salt-affected soil. The excess of salts in the growth medium caused a competition in the absorption, transfer and distribution of nutrients. This may be lead to unbalance in the plant mineral composition. Thus can affecting the plant physiological characteristics (**Rabie & Almadini, 2005 and Munns & Tester, 2008**). Some investigations were carried out under saline environments indicated that an abundance of sodium and chlorine ions obstruct the solubility and absorption of other nutrients, such as N, P, K, Ca, Mg, Fe and Zn in plants **(Evelin *et al.*, 2019; Hasegawa *et al.*, 2000 and Zai *et al.*, 2021)**. Sodium cation can absorb by roots through non-selective cation channels in the plasma membrane. Salinity also interfere with phosphorus absorption, because of its by other cations such as zinc, magnesium, and Calcium (**Evelin *et al.*, 2019**). Phosphorus is an essential macro nutrient in all plants. Decreasing the P contents in plant causing the stoppage of plant growth and leaves death (**Taiz *et al.*, 2015**). Salts accumulated in soil caused unavailability of water to plant roots, thus led to decline the osmotic pressure in plant cells and cell dehydration (**Ait-El-Mokhtar *et al.*, 2020**).

Table (9). Effect of AMF inoculations on Nitrogen % of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area grown hydroponically under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Nitrogen %			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	1.88	1.94	1.90	1.99
1000 ppm NaCl	1.59	1.42	1.57	1.43
1500 ppm NaCl	1.33	1.30	1.42	1.23
2000 ppm NaCl	1.15	1.07	1.24	1.10
2500 ppm NaCl	0.79	0.64	0.87	0.71
1000 ppm NaCl + AMF	1.89	1.67	1.89	1.73
1500 ppm NaCl + AMF	1.65	1.40	1.69	1.37
2000 ppm NaCl + AMF	1.57	1.41	1.33	1.25
2500 ppm NaCl + AMF	1.32	1.10	1.32	1.05
New LSD at 5%	**0.22**	**0.27**	**0.16**	**0.21**

Table (10). Effect of AMF inoculations on total phosphorus of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area in hydroponic culture under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Phosphorus %			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	0.32	0.34	0.35	0.32
1000 ppm NaCl	0.21	0.22	0.23	0.22
1500 ppm NaCl	0.19	0.18	0.19	0.17
2000 ppm NaCl	0.15	0.12	0.14	0.12
2500 ppm NaCl	0.13	0.11	0.12	0.10
1000 ppm NaCl + AMF	0.30	0.28	0.29	0.27
1500 ppm NaCl + AMF	0.28	0.22	0.28	0.24
2000 ppm NaCl + AMF	0.20	0.21	0.22	0.20
2500 ppm NaCl + AMF	0.19	0.17	0.21	0.18
New LSD at 5%	**0.03**	**0.04**	**0.03**	**0.02**

Table (11). Effect of AMF inoculations on potassium of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area in hydroponic culture under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Potassium %			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	1.48	1.45	1.42	1.43
1000 ppm NaCl	1.29	1.30	1.29	1.29
1500 ppm NaCl	0.87	0.81	0.82	0.74
2000 ppm NaCl	0.63	0.50	0.60	0.47
2500 ppm NaCl	0.52	0.41	0.55	0.40
1000 ppm NaCl + AMF	1.49	1.44	1.40	1.38
1500 ppm NaCl + AMF	1.32	1.21	1.33	1.20
2000 ppm NaCl + AMF	1.17	1.09	1.15	1.09
2500 ppm NaCl + AMF	0.84	0.78	0.85	0.7 8
New LSD at 5%	**0.19**	**0.17**	**0.21**	**0.14**

3.4.6. Effects on leaves micro-nutrients content

The data recorded in Tables (12, 13 and 14) shows the effect of increasing sodium chloride (NaCl) concentration (from 500 to 2500 ppm) on the content of micronutrient elements (iron, manganese and zinc) in the adult leaves of the Flame Seedless and Superior Seedless cultivars, as well as the response of these two cultivars to inoculation with AMF with regard to reducing the harmful effect of growth medium salinity on the leaves content of these elements micro nutrients. The obtained results show that: Increasing the concentration of sodium chloride in the nutrient solution (from 500 to 2500 ppm) had a clear and significant effect on reducing the content of these three micronutrients in the leaves of both cultivars under study (Flame Seedless and Superior Seedless). Seedlings of both cultivars grown in highly salinity nutrient solution (2500 ppm) recorded the lowest concentration of the three micronutrients under study. Contrary the seedlings of both cultivars grown in lower salinity nutrient solution (500 ppm) present the highest level of the three micro-nutrients (Fe, Zn and Mn), during the two experimental seasons (2022 and 2023).

Regarding seedlings inoculation with AMF during the two experimental seasons, the results shown in Tables (21, 22 and 23) Illustrates the essential role played by AMF inoculating of both cultivars seedlings in reducing the harmful effect of high salinity in the root growth medium. The results show that all treatments running out with AMF inoculation for both cultivars (Flame Seedless and Superior Seedless) led to a significant increase in the leaf content of these three microelements. The results followed the same trend during both study seasons (2022 and 2023).

Table (12). Effect of AMF inoculations on Iron (ppm) of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area in hydroponic culture under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Iron (ppm)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	143	122	151	132
1000 ppm NaCl	130	117	135	109
1500 ppm NaCl	98	77	89	66
2000 ppm NaCl	51	44	56	41
2500 ppm NaCl	39	30	41	31
1000 ppm NaCl + AMF	141	131	144	134
1500 ppm NaCl + AMF	120	112	115	101
2000 ppm NaCl + AMF	91	78	89	73
2500 ppm NaCl + AMF	75	70	80	74
New LSD at 5%	**9**	**6**	**8**	**7**

Table (13). Effect of AMF inoculations on Zinc (ppm) of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area in hydroponic culture under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Zinc (ppm)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	73	78	71	77
1000 ppm NaCl	61	52	60	57
1500 ppm NaCl	51	48	55	40
2000 ppm NaCl	33	29	36	30
2500 ppm NaCl	32	20	35	21
1000 ppm NaCl + AMF	70	72	72	68
1500 ppm NaCl + AMF	69	61	68	59
2000 ppm NaCl + AMF	57	49	53	48
2500 ppm NaCl + AMF	40	35	44	36
New LSD at 5%	**4**	**6**	**3**	**5**

Table (14). Effect of AMF inoculations on manganese (ppm) of 'Flame Seedless' and 'Superior Seedless' grapevines leaf area in hydroponic culture under varying levels of salinity, during 2022 and 2023 seasons

Treatments	Manganese (ppm)			
	First season 2022		Second Season 2023	
	Flame Seedless	Superior Seedless	Flame Seedless	Superior Seedless
Control	47	42	45	40
1000 ppm NaCl	33	31	35	30
1500 ppm NaCl	27	26	28	25
2000 ppm NaCl	20	18	22	19
2500 ppm NaCl	18	17	19	14
1000 ppm NaCl + AMF	42	40	42	39
1500 ppm NaCl + AMF	33	32	35	29
2000 ppm NaCl + AMF	30	23	31	24
2500 ppm NaCl + AMF	25	20	26	21
New LSD at 5%	**2**	**4**	**2**	**3**

Plants inoculated with AMF shows more accumulate of soluble carbohydrates, that can reduce the excessive salt exposure harm effecting, throughout stabilizing the structure and protein complexes activation, maintaining the activity of mature leaves, and caused a balance in energy transfer (**Dodd & Perez-Alfocea, 2012** and **Diao *et al.*, 2021**). Mycorrhizal plants characterized by higher concentration of trehalose, it can be caused an increase in Trehalose-6- phosphate phosphatase enzyme, and Trehalose-6-phosphate synthase enzyme activities. This effect led to decreased TRE activity (trehalose degrading enzymes phosphorylase) (**Garg & Bhandari, 2016** and **Boorboori & Lackoova 2025**). This effects can have explained the mechanism of AMF in reducing the harmful effects of salinity on absorption and accumulation of these three micro-nutrients. Furthermore, Proline increasing in plants can be attributed to some factors such as: inactivation of proline dehydrogenase (enhanced to breakdown the proline) and a greater glutamate dehydrogenase enzyme activity (contributed the glutamate synthesis, precursor of proline), a higher level of proline carboxylate synthase, and increasing in proline carboxylate synthase expirations (**Garg & Baher, 2013 and Boorboori & Lackoova 2025**). However, some studies looked at the role of AMF in relation to proline concentration in plants under salinity stress have been contradictory, and so some investigation have found a higher content of proline in plants inoculated with AMF, contrary investigators have shown a lower proline in inoculated plants (**Shekoofeh *et al.*, 2012; Yooyongwech *et al.*, 2016**). proline is an indicator of stress tolerance and conceder as stress marker, so the decreasing of proline synthesis in AMF inoculated plants possibly as a result of AMF mediated stress reducing (**Evelin *et al.*, 2019**). Some organic acids (malic, citric, acetic, oxalic and fumaric, acids) are significantly increase in AMF inoculated plants. AMF play an important role in regulation of organic acids concentrations and metabolism, its tolerance to salinity (**Guo *et al.*, 2010** and **Sheng *et al.*, 2011**).

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