Physiological Studies on the Effect of Inoculation with Arbuscular Mycorrhizae (AM) Fungi on Superior Grape Rootings under Salt Stress Conditions

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Abstract: This study was carried out to disclose the effect of soil inoculation with arbuscular mycorrhizal fungi under different water salinity levels (1000, 2000 and 3000 ppm) in an attempt to improve vegetative growth parameters, nutritional acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings through two successive seasons (2008 & 2009).The results indicated that increasing levels of water salinity, particularly in case of high salinity concentration (3000 ppm) decreased survival percentage and vegetative growth parameters (i.e. shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2), total leaf area/plant (cm^2), coefficient of wood ripening, shoot and root biomass, total biomass and root/shoot ratio). Leaf total chlorophyll, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur content and shoot total carbohydrate content decreased with increasing salinity concentration. On the contrary, leaf proline amino acid, sodium, and chloride content increased with increasing levels of salinity. Concerning the microbial and enzyme activity in the rhizosphere of Superior grape rootings, it was noticed that populations of total microbial count, spore numbers of AM fungi, the percentage of infection of AM fungi, dehydrogenase enzyme activity in the rhizosphere were also decreased with increasing levels of water salinity. Superior grape rootings strategy for salt stress tolerance could be achieved by AM fungi colonization. AM fungi inoculation benefits the plants by avoiding the undesirable effects of saline water and improving of survival percentage, vegetative growth parameters, nutrient acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings under low to medium level salt concentrations (1000-2000 ppm). However, AM fungi inoculation didn't protect the plants at the highest salt concentration (3000 ppm) used in this experiment.

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1. Introduction

Plantation of the grape cultivars in Egypt has been progressively developed in the last few years. However, a great acreage is located at the new reclaimed soils which have many problems including salinity. The concentration and composition of dissolved constituents in water determine its quality for irrigation (Miller *et al* 1990).

Salinity is an environmental stress that results in negative effects on plant survival and considered as the most important biotic factor limiting plant growth and yield by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects (Shannon *et al*., 1994). High salinity causes both hyperosmotic and hyperionic stress effects and the consequence of these can be plant demise (Niu *et al*., 1995; Yeo 1998 and Glenn *et al*., 1999). The most harmful effects is the increase in osmotic stress due to high salt concentration in soil solution and consequently the decrease in the soil-water potential (Saad El-Dien *et al*., 1992), reduction in assimilates partitioning to roots (Gaser, 1992) and imbalance in overall concentrations of the ions due to ion toxic effect on physiological processes (Valia & Potiel, 1997), such as growth inhibitors (Tat, 1977), nucleic acid metabolism (Salem, 1981), photosynthesis (Prior *et al.*, 1992), respiration rate (Walker, 1994) and change of enzyme activity (Lio, 1996).

One of the natural and technological ways which has been among the most studied subjects for the last decades to reduce the salinity damages in agricultural crops was the inoculation with Arbuscular mycorrhizae fungi.

AM fungi can benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stress, increasing resistance to pests and tolerance to environmental stresses (e.g., drought, salinity), and improving soil properties (Bethlenfalvay *et al*., 1988; Bethlenfalvay and Linderman, 1992; Copeman *et al*., 1996; Cordier *et al*., 1996 and Al-Karaki, 2000).

Mycorrhizal fungi also play a vital role in alleviating the effects of salinity (Nasim, 2005). By improved nutrient acquisition, AM fungi compensate

for the nutritional imbalances imposed by salinisation. AM fungi also play a positive role in protecting plants from pH extremes. Many studies have demonstrated that inoculation with AM fungi improved growth of plants under a variety of salinity stress conditions (Ruiz-Lozano *et al*., 1996; Al-Karaki *et al*., 2001 and Feng *et al*., 2002). To some extent, these fungi have been considered as bio-ameliorators of saline soils (Azcón-Aguilar and Barea, 1997; Singh *et al*., 1997 and Rao, 1998).

The goal of this study is to disclose the effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels (1000, 2000 and 3000 ppm) in an attempt to improve vegetative growth parameters, nutritional acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings.

2. Material and Methods

This study was conducted during 2008 and 2009 seasons in the shade house of the Horticultural Research Institute, Giza, Egypt. Uniform and healthy 240 own rooted one-year-old Superior rootings were chosen. The rootings were planted through the first week of March in polyethylene bags filled with 5 kg of a medium containing clean sand carefully washed with tap water several times to remove any soluble salts. All bags had bottom holes to allow excess water drainage. Field capacity and wilting point of the sand medium were: 6.5% and 2.3%, respectively, while the electric conductivity (E.C.) of irrigation tap water was 0.85 m mhos/cm (544 ppm). The plants were irrigated with saline water treatments twice a week to keep moisture content of the planting medium about 70% of the field capacity throughout the period of the experiment from the first of May till the end of October. Leaching of accumulated salts was done every 15 days by tap water up to the end of the experiment.

The applied treatments were as follows:

1) Irrigation with tap water at 544 ppm salinity (control)
2) Inoculation with arbuscular mycorrhizal fungi (AM)
3) Irrigation with saline water at 1000 ppm
4) Irrigation with saline water at 1000 ppm + AM
5) Irrigation with saline water at 2000 ppm
6) Irrigation with saline water at 2000 ppm + AM
7) Irrigation with saline water at 3000 ppm
8) Irrigation with saline water at 3000 ppm + AM

Salinity in the irrigation water was Strogonov stock solution chloride consisting of: 78 gm NaCl, 10 gm $MgSO_4$, 9 gm $CaCO_3$, 2 gm $MgCl_2$, 1 gm $CaSO_4$ mixture dissolved in one litre (Strogonov, 1964) to yield a balance of cations and anions with a value of SAR reaching 6.0 and for preparing 1000, 2000 and 3000 ppm concentrations.

Mycorrhizal spores were originally extracted from the Egyptian soil. Spores of AM-mycorrhizae including Genera Glomus, Gigaspora and Acaulospora were added before planting. Extraction and counting of identified mycorrhizal spores were carried out according to the method described by (Massoud, 1999). Fifty grams per bag of mixed spores (250 spores/gram) of AM fungi genera were prepared and mixed with soil, then the rootings were planted (Massoud 2005).

All treatments were fertilized with a nutrient solution (Hoagland and Arnon, 1950) at half weekly intervals till the end of the growing season.

Each treatment was comprised of 30 plants distributed in 3 replicates (10 plants/ replicate) in completely randomized design.

The following parameters were determined.

1. Morphological studies:

- Survival percentage

Number of survived plants was counted in the end of experimental season.

- Vegetative growth parameters

Shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2) of the apical 5th and 6th leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA. were recorded in both seasons. Total leaf area/plant (cm^2) was determined by multiplying total number of leaves per plant by average leaf area. Coefficient of wood ripening was calculated by dividing length of the ripened part of the shoot by total length of the shoot according to Bouard (1966).

- Plant biomass

Shoot biomass (g dry weight), root biomass (g dry weight), total biomass (g dry weight) and root/shoot ratio were recorded.

2. Chemical studies:

- Leaf total chlorophyll content (SPAD). This was measured by using nondestructive Minolta chlorophyll meter SPAD 502 (Wood *et al.*, 1992).
- Leaf proline content (mg/g) was colorimetrically estimated on fresh weight basis according to the method of Batels *et al.* (1973).
- Shoot total carbohydrate content (%) (Smith *et al*., 1956).
- Leaf mineral content: N (%) (Pregl, 1945), P (%) (Snell and Snell 1967), K (%) (Jackson, 1967) and Ca, Mg, S, Cl, and Na percentages were estimated according to Evenhuis (1978).

3. Microbiological studies:-

Samples were taken for carrying out the following determinations:

- Total microbial count (-x10^5 colony forming unit (cfu)/g soil) according to (Esher and Jensen 1972).
- Number of AM (spore/g soil) according to (Massoud, 2005).
- AM infection (%) according to (Massoud, 2005).
- Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) according to Salman (1967).

Statistical analysis:

The completely randomized design was adopted for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Chocran (1980). Averages were compared using the new L.S.D. values at 5% level.

3. Results and Discussion

1. Morphological studies:

- Survival percentage

As shown in Table (1), it is obvious that increasing salt concentration gradually decreased survival percentage. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of survival percentage compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly improved in survival percentage as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results show that survival percentage of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite significant values of survival percentage were medium salinity (1000-2000 ppm). The highest obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

These results are in harmony with Kilany *et al*., (2006) who found that water stress due to salinity by raising salt concentration in the irrigation water effectively depressed the percentage of survival.

However, Arbuscular mycorrhizal (AM) fungi improve survival percentage of tomato plants for long-term under salt stress (Copeman *et al*., 1996).

- Vegetative growth parameters

Data presented in (Table 1) show the effect of irrigation with saline water and soil inoculation with Aarbuscular mycorrhizae fungi on the vegetative growth parameters (i.e. shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2), total leaf area/plant (cm^2) and coefficient of wood ripening) of Superior grape rootings during 2008 and 2009 seasons.

All of the studied vegetative growth parameters were significantly decreased with increasing levels of salinity, particularly in case of high salinity concentration (3000 ppm) compared to control which recorded the highest values for these parameters regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that it caused significant increases in all studied vegetative growth parameters as compared with non-AM plants which took an adverse trend in both seasons.

A significant interaction was observed between saline water and inoculation with AM fungi, it is clear from the results that vegetative growth parameters of non-AM plants significantly decreased with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was detected for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of growth parameters were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) were shown to have that the lowest values in both seasons of the study.

- Plant biomass

The results concerning dry biomass production in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 2).

In the salinity treatments; there was a decline in plant biomass with increasing salinity level. Shoot biomass, root biomass, total biomass and root/shoot ratio recorded the lowest values in plants grown under the highest salinity concentration (3000 ppm) as compared to control regardless of AM fungi inoculation status.

Concerning the effect of inoculation with AM fungi, it is obvious that the shoot biomass, root biomass, total biomass and root/shoot ratio was higher in AM plants than those of non-AM plants grown under both saline and non-saline conditions in both seasons.

Table (1): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on survival (%) and some vegetative growth characteristics of Superior grape rootings (2008 and 2009 seasons)

		Survival (%)		Average shoot length (cm)		Average shoot diameter (cm)		No. of leaves/shoot		Average leaf area/shoot (cm^2)		Total leaf area/plant (cm^2)		Coefficient of wood ripening	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	94.0	97.6	74.8	78.7	0.82	0.85	26.7	28.3	121.8	126.7	3259.5	3592.6	0.83	0.89
	(A2) 1000 ppm	83.0	87.1	70.2	73.6	0.80	0.83	25.7	27.1	117.7	122.1	3025.6	3309.0	0.81	0.86
	(A3) 2000 ppm	57.0	56.6	60.8	65.0	0.76	0.78	23.4	24.5	109.0	114.7	2552.6	2811.1	0.74	0.80
	(A4) 3000 ppm	43.2	45.2	49.4	50.4	0.61	0.59	16.6	17.7	93.9	99.1	1562.6	1758.8	0.70	0.74
new L.S.D. (A) =		**9.0**	**6.5**	**6.0**	**6.6**	**0.04**	**0.04**	**2.5**	**2.3**	**7.2**	**6.9**	**483.8**	**478.2**	**0.04**	**0.03**
(B) : Soil inoculation	(B1) non-AM	65.8	69.1	61.6	64.4	0.72	0.73	22.2	23.6	108.0	112.9	2427.4	2694.9	0.75	0.80
	(B2) AM	72.8	74.1	66.0	69.5	0.77	0.79	24.1	25.3	113.2	118.4	2772.8	3040.8	0.79	0.84
new L.S.D. (B) =		**6.4**	**4.6**	**4.3**	**4.7**	**0.03**	**0.03**	**1.8**	**1.6**	**5.1**	**4.9**	**340.7**	**336.8**	**0.03**	**0.02**
(AXB) : Interaction	A1 B1	92.6	96.5	72.1	75.7	0.80	0.82	25.6	27.0	117.5	122.5	3012.8	3311.1	0.81	0.86
	B2	95.4	98.6	77.4	81.7	0.84	0.87	27.8	29.6	126.0	130.8	3506.2	3874.1	0.86	0.91
	A2 B1	79.7	84.4	67.8	70.8	0.78	0.81	24.7	25.9	113.7	119.3	2805.5	3088.5	0.78	0.83
	B2	86.2	89.9	72.5	76.4	0.81	0.85	26.7	28.3	121.6	124.9	3245.7	3529.4	0.83	0.89
	A3 B1	52.5	53.7	58.7	62.8	0.74	0.76	22.5	24.1	107.0	112.4	2410.8	2708.8	0.73	0.79
	B2	61.5	59.5	62.8	67.2	0.77	0.79	24.3	24.9	110.9	117.0	2694.4	2913.3	0.76	0.81
	A4 B1	38.4	41.9	47.6	48.3	0.57	0.54	15.8	17.2	93.7	97.2	1480.5	1671.2	0.68	0.72
	B2	47.9	48.6	51.1	52.5	0.65	0.63	17.5	18.3	94.1	100.9	1644.8	1846.5	0.71	0.76
new L.S.D. (AXB) =		**12.7**	**9.1**	**8.5**	**9.3**	**0.06**	**0.05**	**3.5**	**3.2**	**10.1**	**9.7**	**681.4**	**673.5**	**0.06**	**0.04**

Table (2): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on shoot biomass, root biomass, total biomass and root/shoot ratio of Superior grape rootings (2008 and 2009 seasons)

		Shoot biomass (g dry weight)		Root biomass (g dry weight)		Total biomass (g dry weight)		Root/shoot ratio	
		2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	12.36	12.52	19.36	20.15	31.72	32.67	1.57	1.61
	(A2) 1000 ppm	12.08	12.23	18.60	19.54	30.68	31.76	1.54	1.60
	(A3) 2000 ppm	11.56	11.65	17.11	17.68	28.67	29.33	1.48	1.52
	(A4) 3000 ppm	10.94	11.04	15.69	16.14	26.62	27.18	1.43	1.46
new L.S.D. (A) =		**0.36**	**0.33**	**1.68**	**1.60**	**2.07**	**1.94**	**0.06**	**0.05**
(B) : Soil inoculation	(B1) non-AM	11.58	11.68	17.09	17.80	28.66	29.48	1.47	1.52
	(B2) AM	11.89	12.03	18.29	18.95	30.18	30.98	1.54	1.57
new L.S.D. (B) =		**0.26**	**0.24**	**1.19**	**1.13**	**1.46**	**1.37**	**0.05**	**0.04**
(AXB) : Interaction	A1 B1	12.19	12.23	18.53	19.43	30.72	31.66	1.52	1.59
	B2	12.52	12.81	20.19	20.87	32.71	33.68	1.61	1.63
	A2 B1	11.89	11.98	18.03	18.93	29.92	30.91	1.52	1.58
	B2	12.26	12.47	19.17	20.14	31.43	32.61	1.56	1.62
	A3 B1	11.44	11.57	16.39	16.93	27.83	28.50	1.43	1.46
	B2	11.68	11.73	17.83	18.42	29.51	30.15	1.53	1.57
	A4 B1	10.78	10.95	15.39	15.91	26.17	26.86	1.43	1.45
	B2	11.09	11.12	15.98	16.37	27.07	27.49	1.44	1.47
new L.S.D. (AXB) =		**0.51**	**0.47**	**2.37**	**2.26**	**2.91**	**2.73**	**0.09**	**0.07**

The interaction effect was shown to be significant. It is apparent from the results that AMF inoculation has benefited the plants under low to medium level salt concentrations (1000-2000 ppm). However, AMF didn't protect the plants at the highest salt concentration. The highest significant values of plant biomass were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

The reduction observed on growth parameters at increasing salinity levels can, in some instances, be attributed to salinity-induced adverse change in leaf water relations reducing photosynthesis, dehydration of proteins and protoplasm to a lower extent (Nieves *et al*., 1991 and Tozlu *et al*. 2000) and this may also be because of osmotic effect of salt on root and toxic effect of accumulated ions on the plant tissues (Lea-Cox and Syvertsen 1993 and Storey 1995).

Several mechanisms for the explanation of AM role have been proposed: AM plants have an improved ability for growth and tolerance to salt stress. Ruiz-Lozano *et al*. (1996) concluded that the mechanisms underlying AM plant growth improvement under saline conditions were based on physiological processes (increased carbon dioxide exchange rate, transpiration, stomatal conductance and water use efficiency) rather than on nutrient uptake (N or P). In addition, Feng *et al*., (2002) showed that arbuscular mycorrhizal fungus improved the resistance capacity to osmotic stress by increasing soluble sugar and electrolyte concentrations in plants roots.

Many studies have indicated that AM fungi contribute to plant growth via enhancement of mineral nutrient uptake (Bethlenfalvay *et al*., 1988; Marschner and Dell, 1994 and Ruiz-Lozano and Azcon 2000).

2. Chemical studies:

- Leaf total chlorophyll content

It's clear from data of Table (3) that increasing salt concentration gradually decreased chlorophyll content. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of leaf total chlorophyll compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that it significantly increased the leaf total chlorophyll content as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi; the results clearly show that leaf total chlorophyll content of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was shown for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of leaf total chlorophyll content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

The adverse effects of water salinity on total chlorophyll content in the leaves can be attributed to its negative action on interrupting and reducing the availability of water and nutrients particularly magnesium, destroying the building and conductance tissue and decreasing the biosynthesis of pigments and photosynthesis (Nijjer, 1985). In this concern, Gaser (1992) stated that irrigation with saline water greatly affected plant photosynthesis process, via inhibiting pigment formation. Also, Murkute *et al.*, (2006) recorded that chlorophyll decreased under stress due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments.

However, the previous increase of total chlorophyll content in the leaves in mycorrhizal plants could be ascribed due to the cytokinin-like substances secreted by fungi, which enhance the chloroplast development (Marks and Kozlowski 1973). In addition, the increase in total chlorophyll content in the leaves in mycorrhizal plants could be attributed to the ability of AM to secrete the cytokinen like substances (Nawar *et al*., 1988).

- Leaf proline content

The data in Table (3) showed that the irrigation with saline water significantly increased the proline content in the leaves. The capacity of the plant to accumulate proline under saline conditions is positively correlated with salt concentration in the irrigation water. Leaf proline content recorded the highest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

As regards to the effect of inoculation with AM fungi, it is clear that the soil inoculation with AM fungi had no effect on leaf proline content under both salinty and non-salinity conditions in both seasons.

The interaction effect in this respect was significant. However, the lowest significant values of leaf proline content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded that the highest values in both seasons of the study.

Table (3): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on leaf content of total chlorophyll and proline and shoot content of total carbohydrate of Superior grape rootings (2008 and 2009 seasons)

		Leaf total chlorophyll content (SPAD)		Leaf proline content (mg/g F.W.)		Shoot total carbohydrate content (%)	
		2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	37.2	28.3	0.07	0.09	22.4	23.8
	(A2) 1000 ppm	36.5	26.7	0.08	0.10	21.7	22.9
	(A3) 2000 ppm	34.4	25.1	0.11	0.12	20.4	21.7
	(A4) 3000 ppm	30.9	23.3	0.13	0.14	19.6	20.7
new L.S.D. (A) =		**1.3**	**1.2**	**0.04**	**0.02**	**0.6**	**0.5**
(B) : Soil inoculation	(B1) non-AM	34.1	24.9	0.10	0.12	20.7	22.0
	(B2) AM	35.4	26.7	0.09	0.11	21.3	22.6
new L.S.D. (B) =		**0.9**	**0.9**	**N.S**	**N.S**	**0.5**	**0.4**
(AXB) : Interaction	A1 B1	37.0	26.3	0.08	0.09	21.8	23.1
	B2	37.4	30.3	0.05	0.08	23.0	24.4
	A2 B1	35.8	25.7	0.09	0.10	21.4	22.6
	B2	37.2	27.6	0.07	0.09	22.0	23.2
	A3 B1	33.2	24.6	0.11	0.12	20.2	21.6
	B2	35.5	25.6	0.10	0.12	20.5	21.8
	A4 B1	30.2	23.1	0.13	0.15	19.4	20.5
	B2	31.6	23.4	0.12	0.13	19.8	20.8
new L.S.D. (AXB) =		**1.8**	**1.7**	**0.05**	**0.03**	**0.9**	**0.7**

Increasing proline content in the leaves with increasing water salinity might be attributed to the increase of hydrolytic enzymes caused by chloride salts and salinity (Klyskov and Rakova, 1964). Furthermore, leaf proline content has been used as an evaluation parameter for selecting salinity and drought resistant varieties (Batels *et al.*, 1973). In addition, plants build up proline in the tissues to maintain osmotic balance with the soil solution (Salisbury and Ross, 1992). In this connection, El-Said *et al*. (1995) and Abbas (1999) suggested that proline functions as a source of solute for intera-cellular osmotic adjustments under saline condition.

- Shoot total carbohydrate content

As shown in Table (3), it is obvious that increasing salt concentration gradually decreased shoot content of total carbohydrates. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of shoot content of total carbohydrates compared to the other treatment while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly improved in shoot content of total carbohydrates as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results show that total carbohydrate content in the shoots of non-AM plants significantly declined with increasing salinity level, particularly in case of the highest salinity concentration (3000 ppm), while the opposite trend was found with AM plants under low and medium salinity (1000-2000 ppm). The highest significant values of shoot carbohydrate content were obtained from the AM plants under non-salinity conditions while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

These results are in harmony with Kilany *et al*., (2006) who found that water stress due to salinity by raising salt concentration in the irrigation water effectively depressed the synthesis of carbohydrates.

However, Arbuscular mycorrhizal (AM) fungi improve physiological processes, like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Rosendahl and Rosendahl 1991).

- Leaf mineral content

The results concerning leaf mineral content in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 4).

The data showed that the irrigation with increased salinity level up to 3000 ppm significantly decreased the nitrogen, phosphorus, potassium, calcium, magnesium and sulfur content in the leaves as compared with non- salted ones. On the contrary, sodium, and chloride content in the leaves recorded the highest values in plants grown under the highest salinity concentration (3000 ppm) as compared to the control regardless of AM fungi inoculation.

As regards the effect of inoculation with AM fungi, it is clear that this resulted in an increase in leaf N, P, K, Ca, Mg and S content as compared with the untreated plants. On the contrary, the addition of AM fungi reduced leaf Na, and Cl content under both salinity and non-salinity conditions in both seasons.

The interaction effect in this connection was found to be significant. It is clear from the results that AM fungi inoculation increased leaf N, P, K, Ca, Mg and S content and decreased leaf Na, and Cl content under non-salinity conditions, while non-AM plants grown under high saline conditions (3000 ppm) took the opposite trend., it caused an obvious reduction in leaf N, P, K, Ca, Mg and S content while it was responsible for enhancing leaf Na, and Cl content in both seasons of the study.

The reduction occurring in N, P, K, Ca, Mg and S content of the leaves under salt stress might be attributed to the increase in osmotic pressure, thereby reducing the water and nutrients uptake. These results were confirmed by Gaser (1999), Hassan *et al*. (1999), Sivritepe (2000) and Stevens and Walker (2002).

Some mechanisms have been suggested to explain the role of AM inoculation: AM can improve salt tolerance through inducing osmotic adjustment (Duke et al., 1986), AM capability of dissolving weakly soluble soil minerals by releasing acids (Leyval, and Berthelin, 1989), improve and balance nutrition in plants could also increase salt tolerance (Marschner, 1995), reduce the negative effects of Na and Cl by maintaining membrane integrity (Mancuso and Rinaldelli, 1996 and Rinaldelli and Mancuso, 1996) that would facilitate compartmentalization within vacuoles, and selective ion intake. In this respect, Cantrell and Linderman (2001) suggested that improved mineral nutrient absorption by AM fungi in plants grown under saline conditions might reduce the negative effects of Na and Cl and retain them in roots without being translocated to the shoots by maintaining vacuolar membrane integrity and retaining in intracellular AM fungal hyphae or was compartmentalized in the root cell vacuoles which prevented these ions from interfering in the metabolic pathways of growth.

Table (4): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on leaf mineral content of Superior grape rootings (2008 and 2009 seasons)

		N (%)		P (%)		K (%)		Ca (%)		Mg (%)		S (%)		Cl (%)		Na (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	2.16	2.22	0.36	0.39	1.46	1.54	2.63	2.75	0.63	0.69	0.32	0.35	1.08	1.02	0.38	0.43
	(A2) 1000 ppm	2.07	2.16	0.34	0.37	1.42	1.51	2.56	2.66	0.60	0.66	0.29	0.32	1.15	1.10	0.42	0.47
	(A3) 2000 ppm	1.97	2.00	0.28	0.33	1.35	1.43	2.44	2.51	0.51	0.59	0.22	0.25	1.26	1.21	0.53	0.57
	(A4) 3000 ppm	1.88	1.92	0.25	0.27	1.28	1.37	2.21	2.30	0.44	0.51	0.16	0.20	1.54	1.44	0.63	0.65
new L.S.D. (A) =		**0.13**	**0.11**	**0.03**	**0.01**	**0.06**	**0.04**	**0.06**	**0.05**	**0.06**	**0.04**	**0.04**	**0.03**	**0.12**	**0.09**	**0.07**	**0.06**
(B) : Soil inoculation	(B1) non-AM	1.97	2.02	0.29	0.32	1.35	1.44	2.43	2.51	0.52	0.58	0.23	0.25	1.31	1.23	0.52	0.56
	(B2) AM	2.07	2.13	0.32	0.36	1.40	1.49	2.49	2.60	0.57	0.63	0.27	0.30	1.21	1.15	0.46	0.50
new L.S.D. (B) =		**0.10**	**0.08**	**0.02**	**0.01**	**0.04**	**0.03**	**0.05**	**0.04**	**0.04**	**0.03**	**0.03**	**0.02**	**0.09**	**0.07**	**0.05**	**0.04**
(AXB) : Interaction	A1 B1	2.06	2.14	0.34	0.37	1.43	1.50	2.58	2.70	0.60	0.66	0.29	0.31	1.13	1.07	0.42	0.46
	B2	2.25	2.31	0.38	0.41	1.49	1.57	2.67	2.79	0.65	0.71	0.34	0.38	1.03	0.97	0.34	0.39
	A2 B1	2.01	2.08	0.32	0.35	1.40	1.48	2.50	2.57	0.57	0.62	0.26	0.29	1.21	1.15	0.46	0.51
	B2	2.14	2.23	0.35	0.39	1.45	1.54	2.62	2.75	0.62	0.69	0.31	0.34	1.09	1.05	0.37	0.43
	A3 B1	1.95	1.97	0.27	0.31	1.33	1.41	2.43	2.48	0.49	0.57	0.20	0.23	1.28	1.23	0.55	0.58
	B2	1.99	2.03	0.29	0.34	1.36	1.45	2.45	2.53	0.53	0.60	0.24	0.26	1.24	1.19	0.51	0.55
	A4 B1	1.84	1.89	0.23	0.25	1.26	1.35	2.19	2.27	0.42	0.48	0.15	0.18	1.61	1.48	0.64	0.67
	B2	1.91	1.94	0.26	0.29	1.30	1.39	2.23	2.33	0.46	0.53	0.17	0.21	1.46	1.39	0.61	0.63
new L.S.D. (AXB) =		**0.19**	**0.16**	**0.04**	**0.02**	**0.08**	**0.05**	**0.09**	**0.07**	**0.08**	**0.05**	**0.06**	**0.04**	**0.17**	**0.13**	**0.10**	**0.08**

In addition, Zhu, (2003) recorded that improved plant nutrition by AM fungi allows cells to more effectively regulate and separate flowing ions which its pump in the plasma membrane and tonoplast of root cells.

The results are in agreement with those obtained by Duponnois *et al*., (2005) and Al-Karaki, (2006) who explained that the higher mineral nutrient acquisition in AM compared to non-AM plants likely occurred because of increased availabilities or transport (absorption and/or translocation) by AM fungi hyphae.

3. Microbiological studies:-

Data concerning the effect of saline water and soil inoculation with Aarbuscular mycorrhizae fungi on microbial and enzyme activity in the rhizosphere of Superior grape rootings during 2008 and 2009 seasons are shown in Table (5) and Figure (1, 2, 3 and 4).

- Total microbial count

It's clear from data of Table (5) and Figure (1) that increasing salt concentration gradually decreased total microbial count. Irrigation with high saline water (at 3000 ppm) significantly recorded the lowest values of total microbial count compared to the other treatments while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that, it was found that soil inoculation with AM significantly increased in total microbial count as compared with non-AM plants in both seasons.

A significant interaction was observed between saline water and soil inoculation with AM fungi, the results revealed that total microbial count of non-AM plants significantly decreased with increasing salinity level, particularly in case of the high salinity concentration (3000 ppm), while the opposite trend was shown for AM plants under low and medium salinity concentrations (1000-2000 ppm). The highest significant values of total microbial count were obtained from the AM plants under non-salinity conditions recording (117 & 134 $x10^5$cfu/g soil) for both seasons respectively, and resulting in an increase over control by (1.40 & 1.39) fold for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values in both seasons of the study.

The results are in agreement with those obtained by (Godeas *et al*., 1999) who explained that the increase in populations of rhizospheric microorganisms in the roots of most plants are influenced by a combined inoculation of microorganism and AM fungi.

- Number of AM

The results concerning number of AM spores / soil in Superior grape plants in response to salinity and AM inoculations are presented in (Table, 5) and Figure (2).

In the salinity treatments; there was a decline in number of AM spores in soil with increased salinity level. Number of AM spores in soil recorded the lowest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

Concerning the effect of inoculation with AM fungi, it is clear that the number of AM spores in soil was higher in AM plants than those of non-AM plants grown under both salinity and non-salinity conditions in both seasons.

The interaction effect was significant. It can be shown from the results that AMF inoculation benefits the plants under low to medium levels of salt concentrations (1000-2000 ppm). However, AMF didn't protect the plants at the highest salt concentration. The highest significant values of number of AM spores in soil were obtained from the AM plants under non-salinity conditions recording (760 & 893 spores/g soil) for both seasons respectively, and resulting in an increase over control by (20.54 & 15.95) fold for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

These findings are in line with those obtained by (Turk *et al*., 2006) who pointed out that AM-mycorrhizae colonize plant roots and mainly in the surrounding soil extending the roots depletion zone around the root system.

- AM infection

Data concerning the effect of saline water and soil inoculation with Aarbuscular mycorrhizae fungi on percentage of AM infection of Superior grape rootings during 2008 and 2009 seasons are shown in Table (5) and Figure (3).

It's obvious that increasing salt concentration gradually decreased percentage of AM infection. Irrigation with high saline water at 3000 ppm significantly recorded the lowest values of total microbial count compared to the other treatments, while the untreated plants had the highest values regardless of AM fungi inoculation.

As for the inoculation with AM fungi, it was found that soil inoculation with AM significantly increased the percentage of AM infection as compared with non-AM in both seasons.

Table (5): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on microbial and enzyme activity in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

		Total microbial count (-x10^5cfu/g soil)		Number of AM (spore/g soil)		AM infection (%)		Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day)	
		2008	2009	2008	2009	2008	2009	2008	2009
(A) : Water salinity	(A1) control	101	115	399	475	41.3	48.8	72	80
	(A2) 1000 ppm	88	99	328	433	33.3	44.9	63	71
	(A3) 2000 ppm	45	54	292	377	29.6	39.7	32	40
	(A4) 3000 ppm	25	30	214	333	21.7	34.6	18	23
new L.S.D. (A) =		**31**	**26**	**129**	**124**	**13.7**	**10.7**	**23**	**21**
(B) : Soil inoculation	(B1) non-AM	52	60	22	37	2.6	5.9	37	44
	(B2) AM	78	89	594	772	60.3	78.0	55	63
new L.S.D. (B) =		**22**	**19**	**91**	**87**	**9.7**	**7.6**	**17**	**15**
(AXB) : Interaction	A1 B1	84	97	37	56	5.2	8.3	60	68
	B2	117	134	760	893	77.4	89.2	84	93
	A2 B1	67	74	23	47	2.3	6.2	48	56
	B2	109	123	632	819	64.3	83.5	78	85
	A3 B1	39	46	17	28	1.7	5.8	28	34
	B2	52	61	567	726	57.4	73.6	37	45
	A4 B1	19	23	11	16	1.2	3.4	14	17
	B2	32	37	417	649	42.2	65.8	23	28
new L.S.D. (AXB) =		**43**	**37**	**181**	**174**	**19.3**	**15.1**	**33**	**29**

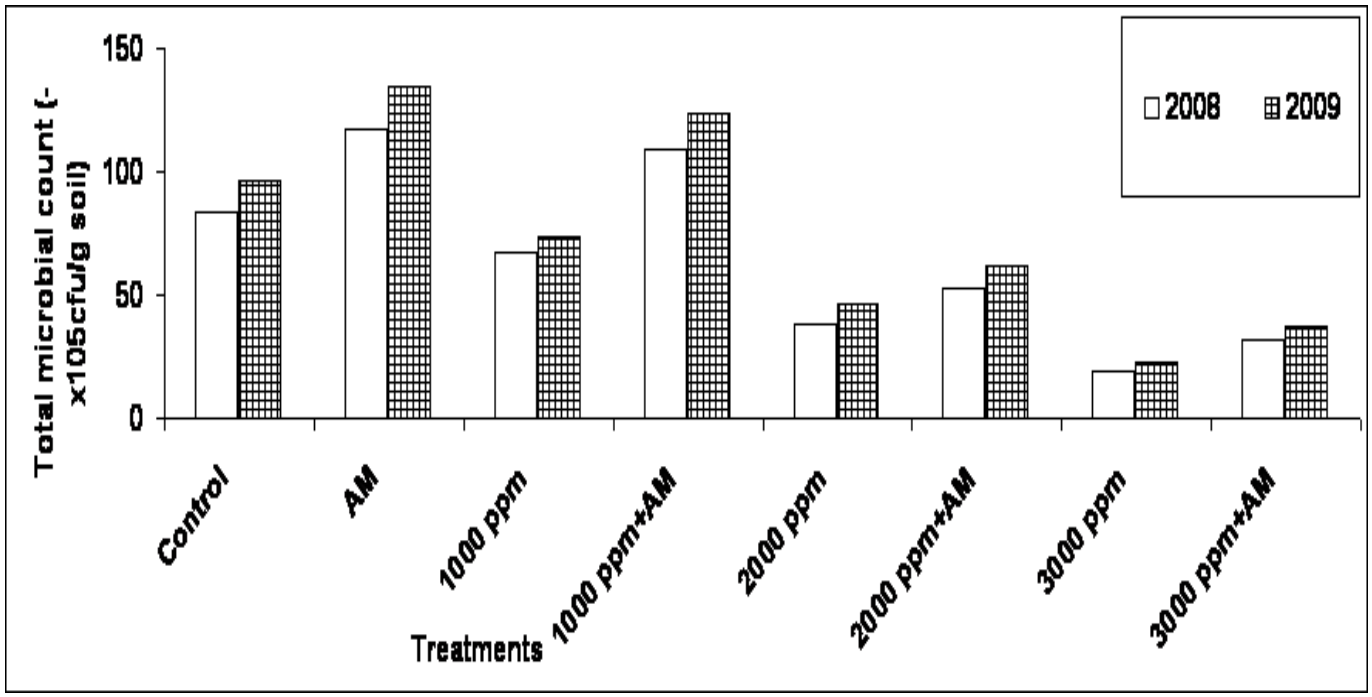

Fig (1): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on total microbial count (-x105cfu/g soil) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

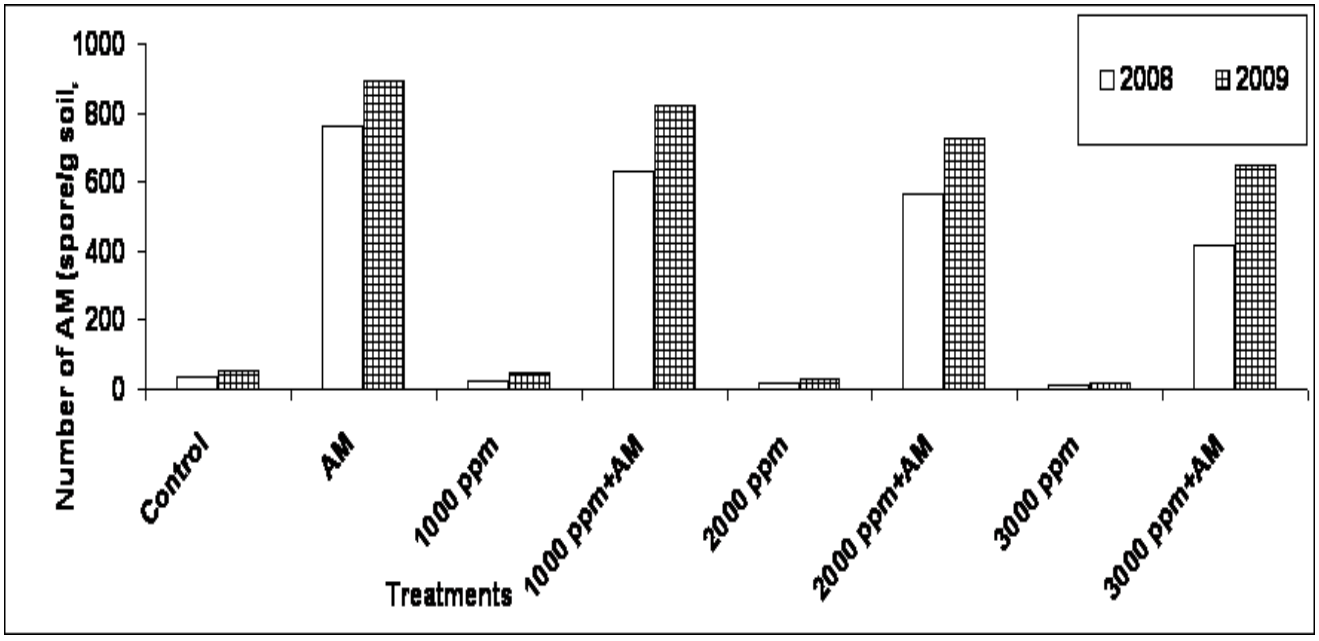

Fig (2): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on number of AM (spore/g soil) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

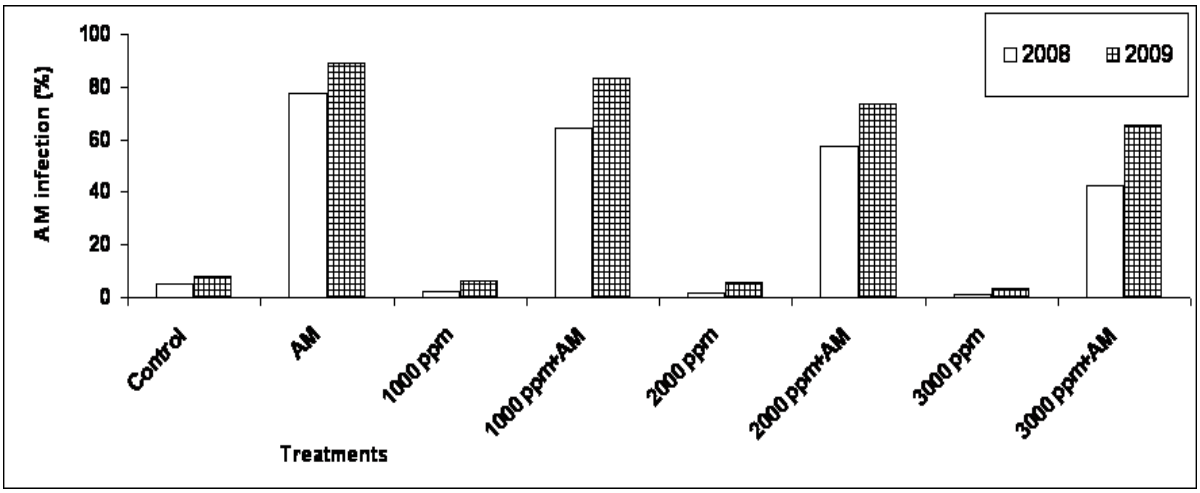

Fig (3): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on AM infection (%) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

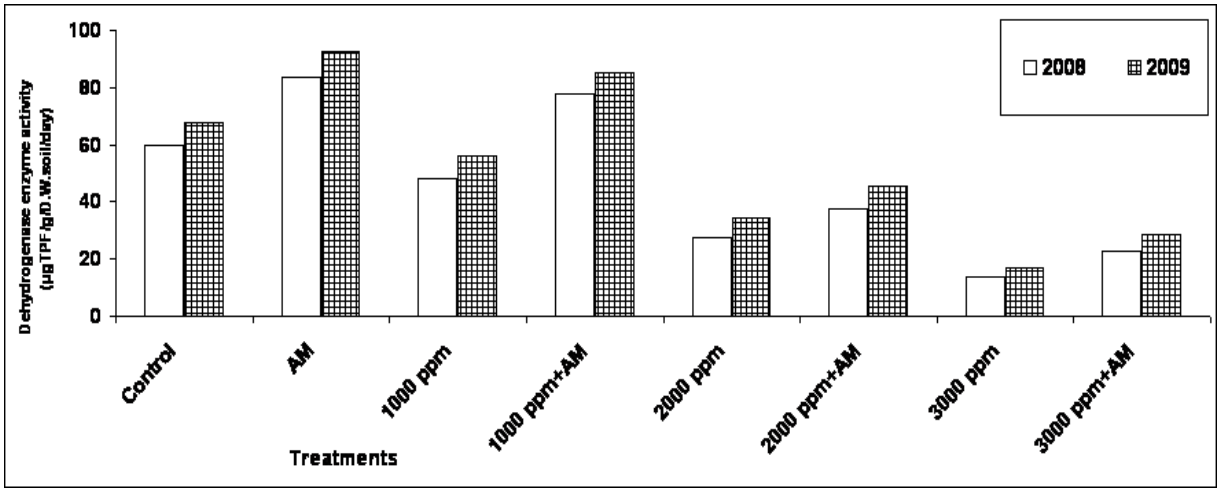

Fig (4): Effect of soil inoculation with arbuscular mycorrhizal (AM) fungi under different water salinity levels on dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) in the rhizosphere of Superior grape rootings (2008 and 2009 seasons)

A significant interaction was observed between saline water and soil inoculation with AM fungi, it is clear from the results that percentage of AM infection of non-AM plants significantly declined with increasing salinity level, particularly in case of the high salinity concentration (3000 ppm), while the opposite trend was found for AM plants under low and medium salinity (1000-2000 ppm). The highest significant values of percentage of AM infection were obtained from the AM plants under non-salinity conditions for both seasons, while non-AM plants grown under high saline conditions (3000 ppm) recorded that the lowest values in both seasons of the study.

Previous researches have shown that salinity may reduce mycorrhizal colonization by inhibiting the germination of spores (Hirrel, 1981), finding of adverse conditions for sporulation and development of spores under unfavorable rhizosphere conditions (Duke *et al*. 1986), reducing the number of arbuscules (Pfeiffer and Bloss, 1988) and inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen *et al*., 1998).

- Dehydrogenase enzyme activity

Data shown in Table (5) and Figure (4) revealed the existence of dehydrogenase enzyme activity among treatments giving an indication of microbial activity in the soil inoculated with arbuscular mycorrhiza (AM) at different concentrations of salinity.

In the salinity treatments; there was a decline in activity of dehydrogenase enzyme with increasing salinity level. Number of AM spores / soil recorded the lowest values in plants grown under high salinity (3000 ppm) compared to control regardless of AM fungi inoculation.

As regards the effect of inoculation with AM fungi, it is clear that the activity of dehydrogenase enzyme was higher in AM plants than that of non-AM plants grown under both salinity and non-salinity conditions in both seasons.

The interaction effect was found to be significant. The highest significant values of activity of dehydrogenase enzyme were obtained from the AM plants under non-salinity conditions recording (84 & 93 µgTPF/g/D.W.soil/day) for both seasons respectively, while non-AM plants grown under high saline conditions (3000 ppm) recorded the lowest values recording (14 & 17 µgTPF/g/D.W.soil/day) for both seasons of the study.

The increase in dehydrogenase enzyme activity was attributed to the intense activity of microflora as a mixture of biomass than each individual one. The highest increase in microbial respiration was recorded with the mixture of microorganism in the soil (Massoud, 2005).

In conclusion, it seems that Superior grape rootings strategy for salt stress tolerance could be achieved by AM fungi colonization. AM fungi inoculation benefits the plants by avoiding the undesirable effects of saline water and improving of survival percentage, vegetative growth parameters, nutrient acquisition and microbial and enzyme activity in the rhizosphere of Superior grape rootings under low to medium levels of salt concentrations (1000-2000 ppm). However, AM fungi inoculation didn't protect the plants at highest salt concentration (3000 ppm) used in the study.

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