

Alleviatory Effects of Salt Stress by Mycorrhizal Fungi and Gibberellic Acid on Chamomile Plant

Ali, E.F.^{1,2*}, Hassan, F. A. S.^{1,3}

¹Current Address: Biology Department Faculty of Science Taif University Saudi Arabia

²Horticulture Department Faculty of Agriculture Assuit Univ., Egypt

³Horticulture Department Faculty of Agriculture, Tanta Univ., Egypt

Abstract: A pot experiment was conducted to study the alleviation of salinity effects by GA_3 or inoculation with arbuscular mycorrhizal fungi (AMF) and their effects on growth, flower yield and volatile oil content of chamomile plant. Salinity concentrations were 0, 3 and 6 dSm^{-1} NaCl and GA_3 was used at 0, 75 and 150 mgL^{-1} . Salinity treatments significantly decreased plant height, branch number and relative water content (RWC) compared with the control. The volatile oil percentage was increased while the volatile oil yield was decreased with increasing salinity level. Salinity treatments also reduced chlorophyll content and membrane stability index (MSI) however, total soluble sugars (TSS), proline content and antioxidant enzyme activities (CAT, SOD and POX) were increased relative to the control. N, P, K, percentages were reduced with increasing salinity concentrations. Meanwhile, sodium was gradually increased with increasing salinity level and hence Na:K ratio was increased in salt stressed plants. GA_3 or AMF treatments alleviated the abovementioned negative effects of salinity. The increment of antioxidant enzymes activities and accumulation of proline as a result of GA_3 or AMF treatments are suggested to involve as part of the defense against salinity in chamomile plant. To alleviate the negative effects of salinity on chamomile plant, treatment of GA_3 at 150 mgL^{-1} or AMF inoculation treatment was recommended.

Keywords: Salinity; Chamomile; Mycorrhiza; GA_3 ; Chlorophyll; Proline; Antioxidant Enzymes

1. Introduction

Chamomile (*Matricaria chamomilla*, L) plant, belongs to Asteraceae family, is native to Europe and Western Asia (Renuka, 1992) and has been cultivated in arid and semi-arid regions in different countries all over the world. It is one of the highest consuming medicinal plants which has been largely recognized because of its medicinal compounds (Farkoosh et al., 2011). Chamomile flowers have volatile oil and the most important constituents of it are chamazulen and bisabolol that are used widely in pharmaceutical and flavoring industries (Glambosi and Holm, 1991). Chamomile has become a prominent plant because of its medically uses for calming, relaxation, soothing, sore nipples, and exhaustion as well as being useful as a carminative, sedative, antiseptic, anti-inflammatory, sedative and spasmolytic activity (Avallone et al., 2000). Chamomile plant is classified as a tolerant to moderately salt tolerant crop (Dadkhah, 2010).

The major problem facing different counties in Arab lands is salinity (Ruiz-Lozano et al., 2001) and therefore, the sustainability of agriculture production in many areas of the world is at risk due to soil salinization (Rengasamy, 2006). Salinity not only affects the establishment, growth and development of plants but also decreases the productivity (Giri et al., 2003). Salinity reduced the vegetative growth characters and flower yield of chamomile plant however volatile oil content was increased at the same level of salinity (Dadkhah, 2010). Salt stress negatively affected the vegetative growth characteristics and dry weight of several plants (Shoresh et al., 2011; Asrar and Elhindi, 2011).

Salinity stress also decreased relative water content (RWC) and chlorophyll content (Tuna et al., 2008) however; total

soluble sugars, proline content and membrane permeability were increased (Shoresh et al., 2011; Celik and Atak, 2012). Salinity induces oxidative stress in plants and hence the formation of reactive oxygen species (ROS) were induced (Bernstein et al., 2010). The most common mechanism for detoxifying ROS synthesized during stress response is the induction of ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) (Agarwal and Pandey, 2004; Bernstein et al., 2010).

Arbuscular mycorrhizal fungi (AMF) are beneficial microorganisms in plant growth and development. AMF have been known as one of the most widespread plant strategies to enhance the tolerance of environmental stresses (Brachmann and Parniske, 2006). AMF have been found to improve growth and volatile oil content of chamomile (Farkoosh et al., 2011) and fennel (Kapoor et al., 2004). The yield of several plants was increased due to AMF application (Giri et al., 2003; Sannazzaro et al., 2007; Colla et al., 2008).

AMF affect host plant positively on growth, pigments, and phosphorous content, flower quality and thereby alleviates the stress (Asrar and Elhindi, 2011). AMF plants contained lower levels of ABA in compared to untreated plants, suggesting that they were less stressed than non-colonized plants (Aroca et al., 2013). It has been suggested that salt stress alleviation by AMF results from a combination of nutritional, biochemical and physiological effects (Evelin et al., 2009). AMF have been shown to promote salinity tolerance by various mechanisms, such as enhancing nutrient acquisition and maintenance of the K^+ : Na^+ ratio (Smith and Read, 2008; Asrar and Elhandi, 2011) and altering the hormonal profiles (Aroca et al., 2013). Under saline conditions, AMF have been shown to have a positive

influence on the composition of mineral nutrients of plants (Giri and Mukerji, 2004; Giri et al., 2007). Otherwise, AMF can reverse the effect of salinity on K^+ : Na^+ ratio and prevent inhibition of protein synthesis (Colla et al., 2008).

Plants inoculated with AMF maintained relatively higher water content (Sheng et al., 2008), had higher chlorophyll content (Giri et al., 2003; Colla et al., 2008) and accumulated more proline (Sharifi et al., 2007) compared with uninoculated plants. Moreover, the increase in total carbohydrates is found to be positively correlated with AMF treatment (Porcel and Ruiz-Lozano, 2004). Several studies suggested that AMF treatment improved integrity and stability of the membrane (Garg and Manchanda, 2008) and enhanced the activities of antioxidant enzymes (Ghorbanli et al., 2004). On the other hand, Aroca et al. (2013) revealed that AMF can alleviate salt stress in plants however the intimate mechanisms involved and the interaction between salinity and AMF remains largely unknown.

Phytohormones suggested playing important roles in stress responses and adaptation (Sharma et al., 2005). They have also been shown to influence salinity tolerance through modulating several physiological processes and biochemical mechanisms (Fatma et al., 2013). Among the phytohormones, gibberellins have been the main focus of some plant scientists to alleviate the negative effects of salinity (Basalah and Mohammad, 1999). Limited information is available about the effect of GA_3 on the growth of plants under salinity and few reports showed the ability of GA_3 to alleviate the negative effects of salinity (Misratia et al., 2013). The exogenous application of gibberellins produces some benefit in alleviating the adverse effects of salt stress and also improves growth, development and seed yields and yield quality (Javid et al., 2011). GA_3 treatment not only alleviated the inhibitory effect of salt stress, but also increased the crop yield of two wheat cultivars. Moreover, carbohydrate and proline content increased significantly by salinity stress in the different plant organs in most cases (Shaddad et al., 2013). Iqbal and Ashraf (2013) reported that it is likely that GA_3 interacts with polyamines to modulate the accumulation and partitioning of ions in plant tissues under salt stress. GA_3 that primarily affect cell enlargement and growth must also coordinately interact with ABA under stress and possibly other stress metabolites, including antioxidants and ROS scavengers (Achar et al., 2006). It has been reported that foliar application of GA_3 improved water use efficiency and chlorophyll content (Misratia et al., 2013). To date, although the importance of chamomile plant, the effects of salt stress on its growth, yield and volatile oil content little are not well investigated. Moreover, there was no enough information concerning the alleviation of negative effects of salt stress on this plant by AMF or GA_3 . There is a compiling need to study the biochemical and physiological changes that may occur in this plant due to salt stress. Therefore, the aim of this study was to investigate the effects of salinity on the growth and volatile oil content of chamomile plant and to understand the different mechanisms by which AMF symbiosis and GA_3 can protect the chamomile plant against salt stress. Some biochemical and physiological changes on chamomile plant as a result of AMF and GA_3 application were also investigated.

2. Materials and Methods

2.1 Plant Material

Pot experiment was conducted to investigate the alleviation of salt stress by AMF and GA_3 on chamomile plant. This study was carried out at Faculty of Science - Taif University - Saudi Arabia during 2012/2013 and 2013/2014 seasons. Chamomile seeds were sown in the nursery at September 1st in both seasons and 45 days later, homogenous seedlings were transplanted into (30 x 20 cm) pots containing sandy soil. The physical properties of soil used in this study were (sand, 81.30 %, silt 6.80 % and clay 11.90 %) and chemical properties were (pH, 8.12, EC, 2.21 $ds\ m^{-1}$, OM, 0.13 %, Total $CaCO_3$, 0.87 %, Na^+ , 3.56 ($meqL^{-1}$), Ca^{+2} , 43.26 ($meqL^{-1}$), SO_4^{-2} , 48.46 ($meqL^{-1}$), HCO_3 , 2.12 ($meqL^{-1}$), Cl^- , 0.67 ($meqL^{-1}$), total N^+ , PO_4^{-3} , K^+ were 0.16, 0.037 and 0.041 %, respectively).

2.2. Experimental treatments

2.2.1. Salinity treatment

Salinity treatments were 0, 3 and 6 $dS\ m^{-1}$ NaCl. Plants subjected to saline irrigation water after 14 days from transplanting. Irrigation started with 3 $dS\ m^{-1}$ saline water and was increased by 1 $dS\ m^{-1}$ every other day until reaching the exact salinity level to prevent shock to plants. The plants were alternatively irrigated every 3 days with saline and tap water for two months. The pots were flushed out with saline water to ensure homogeneity of salinity and to prevent the induction of salt build up.

2.2.2. Gibberillic acid treatment

The levels of GA_3 used in this experiment were 0, 75 and 150 $mg\ L^{-1}$. GA_3 was applied as foliar spray and were started one week after salinity treatments. Spraying with GA_3 was repeated every two weeks in the early morning.

2.2.3. Mycorrhizae treatment

The mycorrhizal fungi were isolated from the experimental farm of Biology Department, Faculty of Science, Taif University, Taif region, Saudi Arabia. Then, AMF were grown on roots of basil (*Ocimum basilicum* L.) in pot culture containing loam:sand (1:1) medium. AMF inocula was placed 3 cm below the surface of the soil (before transplanting) to produce mycorrhizal plants according to Asrar and Elhindi (2011). The control soil has a similar culture, however will not be inoculated with AMF. The treatments were arranged in RCBD (split plot) contained 12 treatments with four replicates each. Salinity treatments were randomly distributed in the main plots, while GA_3 and AMF treatments were in the sub plots.

2.3 Growth and yield characters

Plant height (cm), number of branches/plant and flower dry yield/plant were recorded in this experiment.

2.4 Volatile oil percentage and yield per plant

Flower volatile oil percentage was determined by water distillation method using a cleverger-type apparatus

described in British Pharmacopoeia (1963). Then, the oil yield/plant was calculated.

2.5 Relative water content (RWC)

Herb RWC was determined and calculated from the following relationship:

$(W_{\text{fresh}} - W_{\text{dry}}) / (W_{\text{turgid}} - W_{\text{dry}}) \times 100$, where W_{fresh} is the sample fresh weight, W_{turgid} is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley, 1950).

2.6 Chlorophyll Content

Randomly samples of fresh leaves were taken from the middle part of stem for chlorophyll determination. Chlorophyll content was determined according to Sadasivam and Manickam (1992) by using spectrophotometer (Pharmacia, LKB-Novaspec II and calculated as mg g^{-1} FW).

2.7 Total Soluble Sugars

Total soluble sugars were determined in leaf samples according to the method of Dubois et al., (1956).

2.8 Proline Determination

The free proline content was determined as described by Bates et al. (1973). Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3 % sulfosalicylic acid at 4 °C. Then, the obtained extract was filtered with Whatman No. 2. Mixture of 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid was mixed in a test tube and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as $\mu\text{mol g}^{-1}$ FW.

2.9 Membrane Permeability

Membrane permeability of the excised leaves was measured at the end of the experiment (Yan et al., 1996), fresh part from the middle of leaves was weighted into a glass beaker containing reverse osmosis water. The beakers were immersed at $30 \pm 1^\circ\text{C}$ for 3 h, and then the conductivity of the solution was measured with a conductivity meter. The conductivity was measured again after boiling the samples for 2 min when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated by using the formula, $\text{EC \%} = (C1/C2) \times 100$, since C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

2.10 Antioxidant Enzyme Activity

To obtain the enzyme extract for antioxidant enzymes determination, the method previously described by Hassan and Mahfouz (2012) was used. The resulting supernatant

was used as an enzyme extract to determine superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities. Soluble protein contents of the enzyme extract were assayed according to the method of Bradford (1976).

SOD (Ec 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). SOD activity was expressed as SOD units $\text{min}^{-1} \text{mg}^{-1}$ protein. One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50 % as described by Giannopolitis and Ries (1977) by measuring the absorbance at 560 nm by a spectrophotometer.

CAT (Ec 1.11.1.6) activity was spectrophotometrically estimated by method of Clairbone (1985), following the disappearance of H_2O_2 at 240 nm. The level of enzyme activity was expressed as $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein. POX (Ec 1.11.1.7) activity was tested according to Shanon et al. (1966). Sodium acetate buffer (0.1M) and 0.5 % guaiacol was added to the enzyme extract. The reaction was started with 0.1 % H_2O_2 . The rate of change in absorbance was spectrophotometrically measured at 470 nm and the level of enzyme activity was expressed as $\mu \text{mol min}^{-1} \text{mg}^{-1}$ protein.

2.11 Leaf Mineral Content

The wet digestion procedure for dried sample (0.5 g) was performed to determine nutrient content according to Jackson (1978). Nitrogen percentage in leaves was determined in the digestion using the micro-Kjeldahl method (Black et al., 1965). Phosphorus, potassium and sodium percentages were determined as described by Jackson (1978).

2.12 Statistical Analysis

The results of two experiments were pooled ($n = 8$) and analyzed using MSTAT program, USA. Analysis of variance (ANOVA) was performed and means were separate using LSD test at a significance level of 0.05.

3. Results

3.1. Plant Height

Data presented in Table (1) clearly indicate that the plant height of chamomile was negatively affected by salinity treatments. Increasing salinity levels gradually decreased the plant height. The reduction in plant height as a result of salinity was alleviated when GA_3 or AMF was applied. Using GA_3 at 150 ppm was better than 75 ppm in this respect under any salinity level. Among all treatments applied, the tallest chamomile plants were recorded by the treatment of GA_3 at 150 ppm with or without salinity treatments.

3.2 Branch Number

The effects of salinity, GA_3 and AMF on the number of branches per chamomile plant are tabulated in Table (1). It could be noticed that there is a gradual decrease in branch number with increasing salinity levels and the lowest values

in this concern were obtained by the highest salinity level (6 dSm⁻¹). On the other hand, applying GA₃ at 75 or 150 ppm enhanced the branch number and the same promotion effect was observed when AMF was used. Although GA₃ and AMF treatments alleviated the negative effects of salinity on branching, the treatment of GA₃ at 150 ppm was superior.

3.3 Relative Water Content (RWC)

GA₃ or AMF treatments significantly increased RWC compared with the control in salinity treated or untreated plants (Table 1). Increasing salinity level from 0 to 6 dSm⁻¹ resulted in a gradual decrease in RWC. However, when salinity treatments combined with GA₃ or AMF treatments, the reduction in RWC was retarded. The highest RWC was obtained by applying 150 mg L⁻¹ GA₃ or AMF treatments separately or under any salinity level without significant differences between them.

Table 1: Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on plant height, branch number/plant and relative water content (RWC) of chamomile herb.

Treatments		Plant height (cm)	Branch number/plant	RWC (%)
Salinity	GA ₃ and AMF			
0	0	39.22	9.29	76.33
	75 mgL ⁻¹	42.56	10.00	80.00
	150 mgL ⁻¹	44.92	10.67	83.33
	AMF	42.96	9.33	82.33
3 dSm ⁻¹	0	33.57	7.36	67.67
	75 mgL ⁻¹	34.17	8.30	76.00
	150 mgL ⁻¹	36.35	9.31	79.67
	AMF	35.87	9.06	80.33
6 dSm ⁻¹	0	25.00	5.37	61.00
	75 mgL ⁻¹	30.57	6.51	73.33
	150 mgL ⁻¹	29.56	7.71	77.00
	AMF	32.58	7.25	76.00
LSD 0.05		1.86	0.69	2.24

3.4 Dry Flower Yield

The dry flower yield of chamomile plant was significantly affected by salinity and salinity alleviators treatments. While salinity treatments significantly decreased the dry flower yield, GA₃ or AMF significantly increased it when applied solely or minimized the reduction occurred as a result of salinity (Table 2). The most effective treatments in this concern were 150 mg L⁻¹ GA₃ or AMF. The reduction in flower yield was 33.14 and 53.85 % when salinity levels were applied at 3 and 6 dSm⁻¹, respectively. However, this reduction was 11.83 and 10.06 % when 150 mg L⁻¹ GA₃ and AMF were combined with the highest salinity level, respectively.

3.5 Volatile Oil Percentage

The volatile oil percentage was positively affected by salinity treatments since a gradual increase was observed with increasing salinity levels (Table 2). A promotion effect on volatile oil was also observed when GA₃ or AMF were used. The highest volatile oil percentages (0.96 and 0.97 %) were recorded by 150 mg L⁻¹ GA₃ and AMF treatments, respectively without significant differences. However, the

lowest volatile oil percentage (0.66 %) was obtained by untreated control.

Table 2: Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on dry flower yield, volatile oil and oil yield/ml plant of chamomile leaves

Treatments		Dry flower yield (g/pot)	Volatile oil (%)	Oil yield/ml plant
Salinity	GA ₃ and AMF			
0	0	56.31	0.66	0.37
	75 mgL ⁻¹	65.35	0.70	0.45
	150 mgL ⁻¹	68.32	0.73	0.49
	AMF	69.00	0.72	0.50
3 dSm ⁻¹	0	37.67	0.71	0.27
	75 mgL ⁻¹	51.36	0.77	0.39
	150 mgL ⁻¹	58.47	0.81	0.48
	AMF	60.67	0.83	0.50
6 dSm ⁻¹	0	26.04	0.73	0.19
	75 mgL ⁻¹	45.03	0.84	0.38
	150 mgL ⁻¹	49.64	0.96	0.47
	AMF	50.67	0.97	0.49
LSD 0.05		2.38	0.08	0.05

3.6 Volatile oil yield

Data presented in Table (2) show that the volatile oil yield/plant was significantly decreased as a result of increasing salinity level from 0 to 6 dSm⁻¹ however it was significantly increased by using GA₃ or AMF treatments compared with the control. When GA₃ or AMF treatments were applied under salinity levels the decrease in volatile oil yield was retarded. The volatile oil yield obtained by the combined treatments of 150 mg L⁻¹ GA₃ or AMF under any salinity level was not significantly different from that recorded by both treatments without salinity.

3.7 Chlorophyll content

The resulted obtained in this study show that the chlorophyll content of chamomile leaves were decreased with increasing salinity levels (Table 3). The highest chlorophyll content was obtained by salinity untreated and GA₃ or AMF treated plants. The highest salinity level recorded the lowest values in this respect. However, the chlorophyll degradation occurred by salinity was prevented by using GA₃ or AMF treatments since both of them minimized the degradation level and maintained higher chlorophyll content even under salinity compared with the salinity treated control.

3.8 Total soluble sugar (TSS)

The total TSS in chamomile herb was significantly improved by salinity application. GA₃ and AMF also increased TSS compared with the control (Table 3). The TSS was 7.45, 8.81 and 9.73 % when salinity was applied at 0, 3 and 6 dSm⁻¹. The promotion effect was clearly observed when salinity levels were combined with GA₃ or AMF treatments. The greatest TSS (13.58 and 13.80 %) was recorded by the highest salinity level combined with 150 mg L⁻¹ GA₃ or AMF, respectively.

3.9 Proline content

Increasing salinity level from 0 to 6 dS m⁻¹ led to a gradual increase in proline accumulation in chamomile herb. The proline content was (1.82, 1.94 and 2.13 μmol g⁻¹ FW) at 0, 3 and 6 dS m⁻¹ salinity treatments (Table 3). Plants not treated with salinity did not show significant differences with the others treated with GA₃ or AMF solely. However, proline accumulation was more pronounced when salinity was combined with GA₃ or AMF treatments and the greatest values were recorded by 6 dS m⁻¹ salinity level combined with 150 mg L⁻¹ GA₃ or AMF.

Table 3: Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on chlorophyll content, total soluble sugar (TSS), proline content and membrane stability index (MSI) of chamomile leaves

Treatments		Chlorophyll content (mg g ⁻¹ FW)	TSS (%)	Proline (μmol g ⁻¹ FW)	MSI (%)
Salinity	GA ₃ and AMF				
0	0	0.96	7.45	1.82	78.47
	75 mgL ⁻¹	1.02	8.72	1.83	81.67
	150 mgL ⁻¹	1.19	11.50	1.85	82.17
	AMF	1.10	11.72	1.85	82.33
3 dSm ⁻¹	0	0.82	8.81	1.94	72.13
	75 mgL ⁻¹	0.95	10.30	2.01	78.33
	150 mgL ⁻¹	1.02	11.87	2.15	81.37
	AMF	0.99	12.04	2.18	82.67
6 dSm ⁻¹	0	1.05	9.73	2.13	68.33
	75 mgL ⁻¹	0.84	11.42	2.24	74.33
	150 mgL ⁻¹	0.96	13.58	2.36	77.67
	AMF	0.94	13.80	2.38	80.33
LSD 0.05		0.13	0.74	0.14	2.72

3.10 Membrane stability index (MSI)

In salinity untreated plants, application of GA₃ or AMF significantly increased MSI compared with the control. Meanwhile, MSI was significantly reduced with increasing salinity level from 0 to 6 dS m⁻¹ (Table 3). The reduction in MSI by salinity was prevented when salinity treatments were combined with GA₃ or AMF treatments.

3.11 Antioxidant enzyme activities

It could be noticed from Table (4) that the activities of CAT, SOD and POX enzymes were gradually increased with increasing salinity level. The activities of the previous enzymes were greater when salinity treatments combined with GA₃ or AMF. Without salinity treatment, chamomile plants treated with GA₃ or AMF did not show any differences in this respect. The highest antioxidant enzyme activities were obtained by 6 dS m⁻¹ salinity level combined with 150 mg L⁻¹ GA₃ or AMF treatments.

Table 4: Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) of chamomile leaves

Treatments		Antioxidant enzyme activities		
Salinity	GA ₃ and AMF	CAT μmol min ⁻¹ mg ⁻¹ protein	SOD units min ⁻¹ mg ⁻¹ protein	POX μmol min ⁻¹ mg ⁻¹ protein
0	0	0.95	0.72	17.24
	75 mgL ⁻¹	0.96	0.73	18.44
	150 mgL ⁻¹	0.97	0.74	19.35
	AMF	0.97	0.73	19.84
3 dSm ⁻¹	0	0.98	0.75	18.47
	75 mgL ⁻¹	1.12	0.77	20.40
	150 mgL ⁻¹	1.26	0.80	23.63
	AMF	1.28	0.81	25.72
6 dSm ⁻¹	0	1.13	0.84	22.44
	75 mgL ⁻¹	1.34	0.87	26.24
	150 mgL ⁻¹	1.45	0.89	31.82
	AMF	1.49	0.88	33.70
LSD 0.05		0.06	0.03	1.54

3.12 Nutrient elements

Data in Table (5) indicate that N, P and K percentages were gradually decreased with increasing salinity levels. However, this decrease was retarded when salinity combined with GA₃ or AMF treatments. An opposite trend was observed concerning Na percentage since it gradually increased with increasing salinity levels. Applying GA₃ or AMF under any salinity level significantly reduced Na percentage and the best treatments in this concern were 150 mg L⁻¹ GA₃ or AMF treatments. GA₃ or AMF treatments also reduced Na:K ratio compared with the control under 3 or 6 dS m⁻¹ salinity levels.

Table 5: Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on nutrient elements of chamomile leaves

Treatments		Nutrient elements (%)				
Salinity	GA ₃ and AMF	N	P	K	Na	Na:K
0	0	1.93	0.33	2.03	3.10	1.53
	75 mgL ⁻¹	2.01	0.37	2.06	2.96	1.44
	150 mgL ⁻¹	2.10	0.38	2.14	2.92	1.36
	AMF	2.02	0.46	2.18	2.85	1.30
3 dSm ⁻¹	0	1.60	0.28	1.86	5.27	2.86
	75 mgL ⁻¹	1.78	0.31	1.89	3.91	2.07
	150 mgL ⁻¹	1.92	0.34	1.95	3.73	1.91
	AMF	1.90	0.39	2.02	3.18	1.58
6 dSm ⁻¹	0	1.47	0.20	1.52	8.32	5.48
	75 mgL ⁻¹	1.62	0.25	1.69	5.24	3.10
	150 mgL ⁻¹	1.72	0.28	1.81	4.45	2.46
	AMF	1.67	0.36	1.89	4.19	2.21
LSD 0.05		0.07	0.04	0.12	0.67	0.16

4. Discussion

The results of this study showed that the growth and flower yield of chamomile plant were negatively affected by salinity treatments. The plant height, branch number and dry flower yield were gradually decreased with increasing salinity levels. The reduction of growth is a common

indicator of salt stress because of inadequate water uptake (Borsani et al., 2003) and hence, RWC was significantly decreased in relation to salinity in this study and resulted in limited water availability for the cell extension process (Tuna et al., 2008). Qaderi et al. (2006) reported that inhibition of shoot growth has been considered a whole plant adaptation to salt stress. These results support the others obtained by (Dadkhah, 2010; Shores et al., 2011). In this regard, Caia et al. (2014) reported that under salt stress the uptake of water and some mineral nutrients were restricted and hence plant growth and development were inhibited, as well as a series of metabolic functions.

The decrease in chlorophyll content in relation to salinity may be due to a reduction in the uptake of minerals i.e. Mg needed for chlorophyll biosynthesis or membrane deterioration (Sheng et al., 2008). Our results are in agreement with many authors who revealed that, the total chlorophyll content of leaves was reduced by increasing salinity level (Tuna et al., 2008, Shores et al., 2011, Celik and Atak, 2012). The obtained results showed a significant increase of total soluble sugars as result of salinity. This increment may be occurred to regulate the osmotic potential under salinity treatments (Teixeira and Pereira, 2007) or to sustain metabolism, prolong energy supply and for better recovery after stress relieve (Slama et al., 2007).

Proline may play a role in stress adaptation within the cell (Gilbert et al., 1998). Therefore, we observed an increase in proline content due to salinity and this increment is considered a physiological response of plants under salt stress. Proline plays a protective function against salinity stress in plants (Verbruggen and Hermans, 2008). Moreover, Silva-Ortega et al. (2008) reported that proline functions as an osmolyte for the intracellular osmotic adjustment and its accumulation plays a critical role in protecting photosynthetic activity under salt stress. Such proline accumulation as a result of salt stress is well documented (Celik and Atak 2012; Ashfaque et al., 2014). Salinity treatment also significantly increased membrane permeability compared with the control. These results could be explained through the negative effects of salinity on Ca level since Ca is required to improve membrane stability (Shores et al. 2011). Similar trend has been observed in other plants (Tuna et al. 2008; Shores et al. 2011).

Our results show that the antioxidant enzymes activities (CAT, SOD and POX) were increased by salt stress compared with the control. There is evidence that salt stress can induce oxidative stress (Malik et al., 2011) due to generation of reactive oxygen species (ROS) which are harmful to plant growth because of their detrimental effects on the sub cellular components and metabolism of the plant, leading to the oxidative destruction of cells and finally cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (Mishra and Choudhuri, 1999). As a result of ROS production, plant cell has to activate the antioxidant defense system including enzymatic antioxidant to scavenge ROS (Sairam et al., 2005). It was reported that that high peroxidase activity is correlated with the reduction of plant growth and this increment may play an important role as defense against salt stress (Agarwal and Pandey, 2004). The increment in antioxidant enzyme

activity under salt stress has been reported in several plants (Bernstein et al., 2010).

Applying salinity treatments decreased N, P and K, contents however Na was increased. Decreasing N under salinity treatment has been previously reported (Tarighaleslami et al., 2012). Moreover, reduction of P uptake in saline soils was attributed to precipitation of H_2PO_4 with Ca^{2+} ions in soil and of K and Ca to a competition with Na (Marschner, 1995). The reduction of K percentage could be explained through the competition exists between Na^+ and K^+ leading to a reduced level of internal K^+ at high external NaCl concentration (Botella et al., 1997). Increasing Na absorption under salinity in this study is agreeing with Turan et al. (2007). Moreover, the accumulation of NaCl disturbed the homeostasis not only Na^+ but also of essential cations such as K^+ (Roussos et al., 2007) and hence a decrease in K^+ was observed. This can explain why the Na: K ratio was increased in our study in salinity treated plants and hence, it reflects in reducing the growth as appeared in our results. In a recent study of Caia et al. (2014) they concluded that salinity treatment enhances the accumulation of leaf Na^+ and Cl^- ions, thereby reducing plant growth rate and hence minimizing the ion uptake by the roots and ion accumulation in the shoots are important mechanisms of salt tolerance.

The obtained results showed the beneficial effect of AMF in alleviation of the negative effects of salinity. Improved growth and productivity of chamomile plant under salinity by AMF treatment may be attributed to enhanced uptake of immobile nutrients such as phosphorus, zinc and copper and inducing plant hormones production (Sharma, 2003). Increasing volatile oil percentage and yield in relation to AMF treatment are in accordance with the results of Kapoor et al. (2004) and Farkoosh et al. (2011). AMF improve plant growth and nutrient uptake through building a symbiosis with the majority creating hyphal networks that extend the plant root system (Smith and Read, 2008). Asrar and Elhindi (2011) reported that AMF affects host plant positively on growth, pigments, and phosphorous content, flower quality and thereby alleviates the stress. Moreover, AMF plants contained lower levels of ABA in compared with untreated plants, suggesting that they were less stressed than non-colonized plants (Aroca et al., 2013). The growth and yield of different plants were enhanced as a result of mycorrhization under salt stress (Giri et al., 2003; Sannazzaro et al., 2007; Colla et al., 2008; Asrar and Elhindi, 2011).

AMF treatment increased RWC of chamomile herb. Sheng et al. (2008) reported that plants inoculated with AMF maintain relatively higher water content compared with uninoculated plants. Inoculation with AMF often results in increased nutrient uptake, accumulation of an osmoregulator, an increase in photosynthetic rate and water use efficiency, suggesting that salt stress alleviation by AMF results from a combination of nutritional, biochemical and physiological effects (Evelin et al., 2009). In addition, our results show that AMF may promote salinity tolerance by enhancing nutrient acquisition and maintenance of the Na^+ : K^+ ratio. Similar trend has been previously reported (Sharifi et al., 2007; Smith and Read, 2008; Asrar and Elhandi, 2011).

AMF have been shown to increase transport of nutrients (absorption and/or translocation) by AMF (Sharifi et al., 2007). Moreover, increasing nutrients in relation to AMF treatment could be explained through the extra radical AMF hyphae spread around the roots and hence provide a surface area by which the AMF absorbs elements especially phosphorus (P) and transfers them to the plant (Smith and Read, 2008). Consequently, AMF treatment reduces the negative effects of salinity, improves the growth rate and increases antioxidant enzyme activities (Garg and Manchanda, 2008).

The results also indicate that AMF treatment support a higher chlorophyll concentration and these results may be occurred by improving Mg^{2+} (Giri et al., 2003). Higher chlorophyll content in leaves of mycorrhizal plants under saline conditions has been observed by various authors (Colla et al., 2008; Sheng et al., 2008). Our results support the others of Giri and Mukerji (2004) who mentioned that salt interferes less with chlorophyll synthesis in mycorrhizal than non-mycorrhizal plants. In addition, consistent with our results, AMF can improve plant physiological processes such as adjusting the osmotic balance and composition of carbohydrates as well as accumulation of osmolytes (Ruiz-Lozano, 2003). The increase in total carbohydrates is found to be positively correlated with mycorrhization of the host plant has been reported (Porcel and Ruiz-Lozano, 2004). The positive correlation between sugar content and mycorrhization is due to the sink effect of the fungus demanding sugars from the shoot tissues or to hydrolysis of starch to sugars in inoculated plants with mycorrhiza (Nemec, 1981).

We observed an increase in proline content as a result of using AMF. Proline accumulation has been found to increase when the plant is colonized by AMF (Sharifi et al., 2007). Accumulation of proline is one of the most frequently reported modifications induced by salt stress in plants (Sannazzaro et al., 2007). Increasing the antioxidant enzyme activities in AMF treated plants suggests that this increment is a possible mechanism for salt stress tolerance. Several studies reported that AMF treatment helps plants to alleviate salt stress by enhancing the activities of antioxidant enzymes (Ghorbanli et al., 2004). The positive effects of AMF inoculation may result in improving integrity and stability of the membrane since the membrane permeability was reduced as a result of AMF application. Plants treated with AMF maintained a higher electrolyte concentration than the non-mycorrhizal and hence retained membrane stability (Garg and Manchanda, 2008).

In this study, GA_3 treatment had a beneficial effect on growth, yield as well as physiological and chemical parameters investigated. Moreover, the negative effects of salinity on chamomile were alleviated when GA_3 was applied. The promotion effect of GA_3 on chamomile plant may be due to the role of GA_3 in leaf expansion and stem elongation (Magome et al., 2004). Alleviation of negative effects of salinity by using GA_3 is in agreement with Misratia et al. (2013) who mentioned that GA_3 in salt stressed plants showed an increased photosynthetic capacity—a vital factor for higher dry matter synthesis. Applying GA_3 under salinity treatment was found to restore normal

chlorophyll levels. These results may be due to the GA_3 -generated enhancement of ultra structural morphogenesis of plastids coupled with retention of chlorophyll and delay of senescence caused by the hormone treatment (Arteca, 1997). Improving chlorophyll content and TSS by GA_3 treatment may be achieved through osmoregulation which in turn increased RWC using the organic solutes (saccharides and proteins), which in turn increased the photosynthetic area of plant. The same trend has been reported by Shaddad et al. (2013).

As our data indicated, salinity alleviation by GA_3 may occur through its effect on proline metabolism via regulating N accumulation (Iqbal et al., 2014). Tuna et al. (2008) reported that foliar application of GA_3 increased proline content which counteracted some of the adverse effects of salinity by maintaining membrane permeability and increasing macro and micronutrient levels. This enhanced accumulation of proline may represent a major biochemical adaptation in plants osmotic adjustment (Siddiqui et al., 2008). GA_3 also increased the antioxidant enzyme activities which may consider a mechanism for salinity alleviation. Foliar application of GA_3 led to induce maintenance of K^+ , hence altered ion homeostasis as the obtained data showed. Similar trend has been reported (Iqbal and Ashraf, 2010). Moreover, Iqbal and Ashraf (2010) reported that hormonal homeostasis under salt stress might be the possible mechanism of GA_3 -induced plant salt tolerance since salinity perturbs the hormonal balance in plants. Several reports have indicated that GA_3 application on crops produced some benefit in alleviating the adverse effects of salt stress (Xiong and Zhu, 2002).

As a conclusion, salinity treatments negatively affected the growth and flower yield of chamomile plants. Under salinity treatments RWC and chlorophyll were decreased. However, TSS, proline, membrane permeability and antioxidant enzyme activities were increased. On the other hand, AMF or GA_3 treatments alleviated the negative effects of salinity on the previously mentioned parameters. AMF or GA_3 treatment increased proline content and antioxidant enzymes activities (SOD, CAT and POX) as well as prevented ion homeostasis which may consider possible mechanisms for salinity alleviation in chamomile.

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