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Salt Stress Alleviation of Chamomile Plant by Mycorrhizal Fungi and Salicylic Acid

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A B S T R A C T

This experiment was carried out to study the impact of arbuscular mycorrhizal fungi (AMF) inoculation and/or salicylic acid (SA) treatments on salt stress mitigation on chamomile plant. Salinity levels used in this study were 0, 150 and 300 mM NaCl and SA was used at 0, 0.2 and 0.4 mM. Under salt stress condition, the plant height, main branch number and relative water content (RWC) were significantly reduced compared to the control. Otherwise, the volatile oil percentage was improved while the volatile oil yield was reduced under salinity treatments. Salinity also decreased the chlorophyll content, N, P, K, percentages and membrane stability index (MSI) however; total soluble sugars (TSS) and proline content were increased relative to the control. On the other hand, SA or AMF treatments mitigated the abovementioned adverse effects of salinity. The accumulation of proline and maintaining the membrane stability as a result of SA or AMF treatments are suggested to play important roles in chamomile defense against salinity. To mitigate the adverse effects of salinity on chamomile plant, treatment of SA or AMF inoculation treatment was recommended.

Introduction

Chamomile (*Matricaria chamomilla*, L*)* plant, belonging to Asteraceae family, has been cultivated in arid and semi-arid regions (Renuka, 1992). Chamomile medicinal compounds make it one of the highest consuming medicinal plants that have been largely recognized (Farkoosh *et al.*, 2011). The main constituents of chamomile volatile oil are chamazulen and bisabolol that are used widely in pharmaceutical and flavoring industries (Glambosi and Holm, 1991). Chamomile volatile oil has been reported to be used as a carminative, antiseptic, sedative and anti-inflammatory (Avallone *et al.*, 2000). Salinity is the major problem in different counties in Arab lands (Ruiz-Lozano *et al.*, 2001) and hence the sustainable production in many areas is at risk due to soil salinization (Rengasamy, 2006). The adverse effects of salinity not only observed on the growth and development but also decrease the productivity (Giri *et al.*, 2003).

Salt stress negatively affected the vegetative growth characteristics and dry weight of several plants (Shoresh *et al.*, 2011; Asrar and Elhindi, 2011). Dadkhah (2010) found that the vegetative growth characters and flower yield of chamomile were decreased due to salinity however volatile oil was increased at the same salinity level. Under salt stress condition, RWC and chlorophyll content were

decreased (Tuna *et al.,* 2008) however; total soluble sugars, proline content, membrane permeability and MDA were increased (Shoresh *et al.,* 2011; Celik and Atak, 2012; Hassan *et al.,* 2017).

Several strategies have been adopted to mitigate the adverse effects of salinity and efforts are made to explore the mechanisms of salinity tolerance. Arbuscular myccorrhizal fungi (AMF) have been reported as one of the most widespread strategies to improve the tolerance of environmental stresses (Brachmann and Parniske, 2006). AMF inoculation improved the growth and volatile oil content of fennel (Kapoor *et al.,* 2004) and chamomile (Farkoosh *et al.,* 2011, Ali and Hassan, 2014). AMF application also improved the yield of various plants (Giri *et al.,* 2003; Sannazzaro *et al.,* 2007; Colla *et al.,* 2008). AMF application positively affects the host plant on photosynthetic pigments, phosphorous content and flower quality and hence mitigates the stress (Asrar and Elhindi, 2011). AMF inoculation maintained the RWC (Sheng *et al.,* 2008), improved the chlorophyll content (Giri *et al.,* 2003; Colla *et al.,* 2008) and increased the accumulation of proline (Sharifi *et al.,* 2007) compared with the control.

Salicylic acid (SA) is considered as a plant growth regulator, that plays an important role in regulating the photosynthesis and improves the plant growth and development under salinity (Esan *et al.,* 2017) therefore, it alleviates the adverse effects of environmental stresses (Bideshki *et al.,* 2010). SA application has been reported to induce the salt stress tolerance (Jayakannan *et al.,* 2015). The growth, yield and volatile oil components of rosemary plants were significantly increased due to SA foliar application relative to the control (Hassan *et al.,* 2017). To date, there was no enough information about the mitigation of negative effects of salinity on chamomile plant using AMF or SA. It is very important to investigate the physiological and biochemical processes of this plant under salt stress. Therefore, this study aimed to assess the different mechanisms by which AMF symbiosis and SA can protect the chamomile plant against salinity.

Materials and Methods

Plant material

This pot experiment was carried out at the experimental farm of Faculty of Agriculture, Menoufia University during 2014/2015 and 2015/2016 seasons. Chamomile seeds were sown at September 1st in the nursery in both seasons and after 45 days; seedlings were transplanted into (30 x 20 cm) pots containing sandy soil. The soil was analyzed and the physical properties were (sand, 80.20 %, silt 6.90 % and clay 12.90 %). The chemical properties of soil were (OM, 0.12 %, pH, 8.06, Total $CaCO_3$, EC, 2.11 dsm^{-1}, 0.77 %, Na^{+}, 3.22 ($meqL^{-1}$), SO_4^{-2}, 44.52 ($meqL^{-1}$), Ca^{+2}, 42.17 ($meqL^{-1}$), Cl^{-}, 0.57 ($meqL^{-1}$), HCO_3, 2.08 ($meqL^{-1}$), total N^{+}, PO_4^{-3}, K^{+} were 0.15, 0.032 and 0.039 %, respectively).

Salinity treatment

Salinity treatments were 0, 150 and 300 mM NaCl. Plants subjected to saline irrigation water after 21 days from transplanting. To prevent shock to plants, salinity started with 50 mM saline water and was increased by 50 mM every other day until reaching the required salinity level.

Plants were irrigated alternatively every 3 days with saline and tap water for two months using 0.5 L irrigation water per pot. Every two weeks the pots were flushed out with saline water to prevent the induction of salt build up and to ensure homogeneity of salinity.

Mycorrhizae and SA treatments

The mycorrhizal fungi were isolated from the experimental farm of Faculty of Agriculture, Shibin El-Kom, Menofiya University. In pot culture medium containing loam:sand (1:1), AMF were grown on roots of basil (*Ocimum basilicum* L.). Then, AMF inocula was put below the surface of the soil by 3 cm (before transplanting) to produce mycorrhizal pants as reported by Asrar and Elhindi (2011). Otherwise, control soil not inoculated with AMF but has a similar culture. Salicylic acid (SA) was dissolved in 100 mL dimethyl sulfoxide and 0, 0.2 and 0.4 mM were prepared using distilled water containing 0.02 % Tween 20. SA was applied as foliar spray and the application was started one week after salinity treatment. Foliar spraying with SA was weekly applied in the early morning. Control plants were sprayed with distilled water containing 0.02 % Tween 20 only. The applied treatments were arranged in split plot design with four replicates each. In the main plots, salinity treatments were randomly distributed while AMF and SA treatments were in the sub plots.

Growth and yield evaluation

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

Volatile oil percentage and yield per plant

Water distillation method was used for volatile oil extraction and determine the oil percentage in flowers using a clevenger-type apparatus described in British Pharmacopea (1963) using the following equation :Volatile oil percentage = oil volume in the graduated tube / fresh weight of sample x 100. Finally, the oil yield/plant was calculated in relation to the dry flower yield.

Relative water content (RWC)

Herb RWC was assessed using the following relationship according to Weatherley (1950): $(W_{fresh}-W_{dry})$ / $(W_{turgid}-W_{dry})$ x 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.

Chlorophyll content

The chlorophyll content of leaf samples were determined by the method of Metzner *et al.*, (1965). Leaf discs (0.2 g) were homogenized in 50 mL acetone (80 %). A cheese cloth was used for slurry straining and the extract was centrifuged at 15000 *g* for 10 min. The optical density of the acetone extract was spectrophotometrically observed at 663 nm for chlorophyll (a) and 645 nm for chlorophyll (b) and were expressed in mg g^{-1} fresh weight.

Total soluble sugars

Total soluble sugars were evaluated in leaf samples using the method of Dubois *et al.*, (1956).

Proline determination

The proline content was assessed as reported by Bates *et al.*, (1973). Frozen leaf sample (0.5 g) was homogenized in 10 mL of 3 % sulfosalicylic acid at 4 °C. 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 absorbance at 520 nm was spectrophotometrically observed with toluene as the blank. The proline content was

calculated based on a standard curve and was expressed as µmol g^{-1} FW.

Membrane stability index (MSI)

MSI was assessed by the method of Sairam *et al.,* (1997). Briefly, 2 leaf samples (0.2 g) each were taken and put in 20 mL of double distilled water in two different 50 mL flasks. The first one was kept at 40 °C for 30 min while the second one was kept at 100 °C in boiling water bath for 15 min. The electric conductivity of the first (C_1) and second (C_2) samples was investigated with a conductivity meter. The ions leakage was expressed as the membrane stability index according to the following formula, MSI = [1- (C_1/C_2)] X 100.

Leaf mineral content

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

Statistical analysis

The results of this study were 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.

Results and Discussion

Plant height

The plant height of chamomile was significantly decreased due to salinity treatments. Increasing the level of salinity further decreased the plant height in both seasons. However, application of SA or AMF alleviated the reduction in plant height occurred by both salinity levels and SA treatment at 0.4 mM was superior to 0.2 mM or AMF treatments. Under higher salinity level, there were no significant differences between SA and AMF treatments in alleviation the plant height reduction.

Branch number

From data presented in Table (1) it could be noticed that the branch number was gradually decreased with increasing salinity level and the lowest branch number was obtained by 300 mM NaCl treatment. Meanwhile, SA or AMF application enhanced the branch number of chamomile grown under salinity more so with SA at 0.4 mM or AMF inoculation in the two experimental seasons.

Relative water content (RWC)

The RWC was significantly increased as a result of SA or AMF treatments compared with the control. However, it was decreased when plants grown under salinity in both seasons (Table 1). Otherwise, the reduction in RWC due to salinity was retarded by applying SA or AMF treatments. In this concern, using SA at 0.4 mM or AMF was superior to SA at 0.2 mM in both seasons.

Dry flower yield

The flower yield of chamomile plant was significantly reduced due to salinity treatment compared with the control. The flower yield was reduced by 57.42 and 56.25 % when 300 mM NaCl was used in both seasons, respectively. While the application of SA or AMF improved the flower yield whether applied solely or under salt stress condition (Table 2). Both SA and AMF successfully mitigated the adverse effects of salinity on flower yield more so with 0.4 mM SA or AMF treatments in both seasons. At 150 mM salinity level, SA or AMF treatments

completely alleviated the reduction of flower yield caused by salinity, however under 300 mM the reduction in flower yield was 4.21 and 3.91 % in the first season and was 4.14 and 4.78 % in the second one when SA at 0.4 mM or AMF treatments were applied, respectively.

Volatile oil percentage and yield

The volatile oil percentage was enhanced when plants grown under salinity compared with non-stressed plants and the highest salinity level produced higher volatile oil percentage in both seasons (Table 2). Additionally, SA or AMF treatments significantly improved the volatile oil percentage relative to the control in both seasons (Table 2). When chamomile plants grown under 300 mM salinity level and treated with SA at 0.4 mM or AMF treatments the highest percentage of volatile oil was recorded.

On the other hand, the volatile oil yield/plant was significantly decreased due to increasing salinity level from 150 to 300 mM. However, SA or AMF applications significantly increased the oil yield relative to the control. Furthermore, the reduction in oil yield due to salinity was retarded when SA or AMF treatments were applied (Table 2). Chamomile plants grown under 150 or 300 mM salinity levels and applied with SA at 0.4 mM or AMF treatments the highest volatile oil yield was recorded.

Chlorophyll content

Increasing salinity levels decreased the chlorophyll content of chamomile leaves compared with the non-stressed plants in both seasons (Table 3). SA or AMF treatments improved the chlorophyll content when applied solely without salt stress and their applications under stress condition retarded the reduction observed in chlorophyll due to salinity in both experimental seasons and maintained higher chlorophyll content even under salinity.

Total soluble sugar (TSS)

It is very clear from data presented in Table (2) that TSS in chamomile herb was significantly enhanced when plants grown under any salinity level and the increase in salinity level, the increase in TSS content. Also, SA or AMF increased TSS compared with the control in both seasons. The highest TSS percentages were observed when plants were grown under 300 mM of salinity and treated with 0.4 mM SA or AMF inoculation.

Proline content

The proline accumulation in chamomile herb was increased with increasing salinity level from 150 mM to 300 mM in both seasons. Under non-stress condition, there were no significant differences among SA or AMF treatments and control (Table 4). Higher proline accumulation was observed when plants grown under salinity and treated with SA or AMF in both seasons..

Membrane stability index (MSI)

It is obvious from data in Table (4) that in non-stressed plants, SA or AMF applications significantly improved MSI compared with the control. Meanwhile, MSI was significantly reduced with increasing salinity level from 150 to 300 mM in both seasons. Otherwise, SA or AMF treatments prevented the reduction in MSI caused by salinity.

Nutrient elements

The percentages of N, P and K were significantly decreased due to salinity treatments and this reduction was gradual with the increase in salinity level in both seasons (Table 5).

Table.1 Effects of arbuscular mycorrhizal fungi (AMF) and salicylic acid (SA) on plant height, branch number/plant and relative water content (RWC) of chamomile plant grown under salt stress

Treatments		2014/2015 season			2015/2016 season		
Salinity	SA and AMF	Plant height (cm)	Branch number/plant	RWC (%)	Plant height (cm)	Branch number/plant	RWC (%)
0	0	39.65	8.33	79.87	38.48	8.47	80.28
	0.2 mM	42.19	9.57	81.65	42.36	9.62	81.89
	0.4 mM	46.22	10.46	84.32	45.17	10.67	84.66
	AMF	43.53	9.62	83.74	43.88	9.84	83.94
150 mM	0	33.66	6.24	70.47	32.45	6.47	71.18
	0.2 mM	35.47	7.82	75.88	35.17	7.95	75.33
	0.4 mM	37.75	8.45	78.92	37.45	9.33	79.68
	AMF	35.41	8.16	80.77	35.64	8.56	81.67
300 mM	0	25.27	5.74	63.55	24.68	5.87	65.36
	0.2 mM	29.67	6.63	70.76	29.47	6.77	71.48
	0.4 mM	29.86	7.49	75.86	29.88	7.89	75.64
	AMF	30.72	7.33	76.33	30.15	7.43	76.53
LSD 0.05		1.85	0.67	2.34	1.79	0.63	2.27

Table.2 Effects of arbuscular mycorrhizal fungi (AMF) and salicylic acid (SA) on dry flower yield, volatile oil percentage and oil yield / plant of chamomile grown under salt stress

Treatments		2014/2015 season			2015/2016 season		
Salinity	SA and AMF	Dry flower yield (g/plant)	Volatile oil (%)	Oil yield (mL/ plant)	Dry flower yield (g/plant)	Volatile oil (%)	Oil yield (mL/ plant)
0	0	49.83	0.59	0.29	50.67	0.58	0.29
	0.2 mM	55.27	0.68	0.38	56.37	0.69	0.39
	0.4 mM	62.94	0.70	0.44	63.75	0.71	0.45
	AMF	63.56	0.69	0.44	64.11	0.70	0.45
150 mM	0	39.94	0.63	0.25	40.73	0.64	0.26
	0.2 mM	47.52	0.72	0.34	48.15	0.71	0.34
	0.4 mM	52.18	0.73	0.38	52.88	0.74	0.39
	AMF	53.49	0.72	0.39	53.19	0.73	0.39
300 mM	0	21.22	0.68	0.14	22.17	0.66	0.15
	0.2 mM	44.68	0.79	0.35	44.39	0.76	0.34
	0.4 mM	47.73	0.81	0.39	48.57	0.82	0.40
	AMF	47.88	0.80	0.38	48.25	0.81	0.39
LSD 0.05		1.89	0.08	0.04	1.83	0.07	0.05

Table.3 Effects of arbuscular mycorrhizal fungi (AMF) and salicylic acid (SA) on chlorophyll content and total soluble sugar (TSS) of chamomile grown under salt stress

Treatments		2014/2015 season		2015/2016 season	
Salinity	SA and AMF	Chlorophyll content (mg g^{-1} FW)	TSS (%)	Chlorophyll content (mg g^{-1} FW)	TSS (%)
0	0	0.95	8.39	0.92	8.27
	0.2 mM	1.01	8.66	0.99	8.39
	0.4 mM	1.15	10.76	1.12	10.68
	AMF	1.09	10.91	1.11	10.84
150 mM	0	0.83	8.81	0.84	9.11
	0.2 mM	0.94	10.30	0.96	11.23
	0.4 mM	1.03	11.87	1.01	12.17
	AMF	0.98	12.04	0.98	11.98
300 mM	0	0.78	9.73	0.80	9.88
	0.2 mM	0.82	11.42	0.82	11.36
	0.4 mM	0.97	13.58	0.94	13.67
	AMF	0.96	13.80	0.95	13.58
LSD 0.05		0.13	0.74	0.12	0.72

Table.4 Effects of arbuscular mycorrhizal fungi (AMF) and salicylic acid (SA) on proline content and membrane stability index (MSI) of chamomile grown under salt stress

Treatments		2014/2015 season		2015/2016 season	
Salinity	SA and AMF	Proline (µmol g^{-1} FW)	MSI (%)	Proline (µmol g^{-1} FW)	MSI (%)
0	0	1.80	79.58	1.81	78.33
	0.2 mM	1.82	82.67	1.84	82.24
	0.4 mM	1.81	83.53	1.83	82.17
	AMF	1.83	83.16	1.84	82.87
150 mM	0	1.87	71.67	1.91	72.25
	0.2 mM	1.98	78.68	2.04	78.62
	0.4 mM	2.23	80.94	2.17	81.12
	AMF	2.19	82.11	2.16	82.67
300 mM	0	1.99	69.27	2.11	68.70
	0.2 mM	2.20	75.13	2.22	75.55
	0.4 mM	2.19	77.84	2.29	76.89
	AMF	2.21	77.89	2.27	78.12
LSD 0.05		0.13	2.67	0.12	2.69

Table.5 Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on nutrient elements of chamomile grown under salt stress

Treatments		2014/2015 season			2015/2016 season		
Salinity	SA and AMF	Nutrient elements (%)			Nutrient elements (%)		
		N	P	K	N	P	K
0	0	1.88	0.32	1.97	1.87	0.34	1.92
	0.2 mM	1.94	0.36	2.09	1.96	0.37	2.06
	0.4 mM	2.09	0.37	2.25	2.06	0.36	2.21
	AMF	2.04	0.47	2.21	2.07	0.45	2.19
150 mM	0	1.61	0.26	1.81	1.63	0.28	1.78
	0.2 mM	1.73	0.35	1.88	1.70	0.33	1.86
	0.4 mM	1.90	0.36	1.97	1.87	0.34	1.94
	AMF	1.91	0.40	2.03	1.89	0.39	1.93
300 mM	0	1.52	0.22	1.56	1.55	0.23	1.57
	0.2 mM	1.67	0.27	1.72	1.64	0.27	1.74
	0.4 mM	1.74	0.29	1.74	1.73	0.29	1.77
	AMF	1.72	0.35	1.86	1.74	0.36	1.84
LSD 0.05		0.06	0.03	0.13			

Application with SA or AMF increased the percentages of N, P and K compared with the control when applied in non-stress condition. Additionally, under salt stress condition, SA or AMF treatments retarded the reduction in nutrient elements investigated in both seasons. AMF inoculation recorded higher P percentage compared to SA treatment.

In this study, the growth characters and flower yield of chamomile were adversely affected by salinity treatments. The growth reduction is considered a common indicator of salt stress due to inadequate water uptake (Borsani *et al.,* 2003) therefore, RWC also was significantly decreased and resulted in limited water availability for the cell extension process (Tuna *et al.,* 2008). The inhibition of shoot growth has been considered a whole plant adaptation to salt stress (Qaderi *et al.,* 2006). These results support the others obtained by (Dadkhah, 2010; Shoresh *et al.,* 2011; Ali and Hassan, 2014).

The chlorophyll reduction due to salinity treatment could be ascribed to a reduction in the minerals uptake i.e. Mg that needed for chlorophyll biosynthesis (Sheng *et al.,* 2008). These results are in accordance with the findings of Tuna *et al.,* (2008), Shoresh *et al.,* (2011) and Celik and Atak (2012) who reported that the chlorophyll content was reduced by increasing salinity level. The obtained results also showed a significant increase in TSS due to salinity application. This increment in TSS is a plant mechanism to regulate the osmotic potential under salt stress or to sustain metabolism, prolong energy supply and for better recovery after stress relieve (Slama *et al.,* 2007).

Proline plays a protective function against salinity stress in plants (Ali and Hassan,

2014). Therefore, an increase in proline content was observed under salinity. Additionally, proline functions as an osmolyte for the intracellular osmotic adjustment and plays a critical role under salt stress in protecting photosynthetic activity (Silva-Ortega *et al.,* 2008). Proline accumulation due to salt stress is well documented (Celik and Atak 2012; Hassan *et al.,* 2017). Therefore, the membrane stability was reduced as a result of salt stress which ascribed to the reduction of calcium under stress condition that leads to membrane damage (Shoresh *et al.,* 2011). Similar results have been observed (Tuna *et al.,* 2008; Shoresh *et al.,* 2011). In this study, salinity treatments decreased N, P and K, contents. Decreasing N under salt stress has been reported (Ali and Hassan, 2017). The reduction of P uptake in salt stressed plants was ascribed 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 under salinity may be due to the competition exists between Na^+ and K^+ leading to a reduced level of internal K^+ at high external NaCl concentration (Botella *et al.,* 1997).

In this experiment, AMF application had beneficial effect in salt stress mitigation. The improvement in chamomile growth and productivity under salinity due to AMF treatment may be ascribed to enhanced uptake of immobile nutrients such as phosphorus, zinc and copper and inducing plant hormones production (Sharma, 2003). Smith and Read 2(008) reported that AMF improves the growth and nutrient uptake through building a symbiosis with the majority creating hyphal networks that extend the plant root system. Asrar and Elhindi (2011) found that AMF positively affects the growth, pigments, and phosphorous content, flower quality of host plant and therefore alleviates the stress. The enhancement of growth and yield of several plants has been reported due to AMF application under salt stress (Giri *et al.,* 2003; Sannazzaro *et al.,* 2007; Colla *et al.,* 2008; Asrar and Elhindi, 2011). Moreover, AMF treatment enhanced the volatile oil content which in accordance with the results of Kapoor *et al.,* (2004) and Farkoosh *et al.,* (2011).

Increasing nutrients due to AMF treatment could be ascribed to the extra radical hyphae spread around the plant roots and hence provide a surface area by which the AMF absorbs elements especially phosphorus and transfers them to the plant (Smith and Read, 2008). AMF have been also shown to enhance transport of nutrients (Sharifi *et al.,* 2007). The present study results also show that AMF application maintained higher chlorophyll content in chamomile leaves relative to the untreated plants which may be ascribed to the improvement of Mg as reported by Giri *et al.,* (2003). The same trend under salinity has been observed (Colla *et al.,* 2008; Sheng *et al.,* 2008; Ali and Hassan, 2017).

AMF can improve plant physiological processes such as composition of carbohydrates as well as accumulation of osmolytes (Ruiz-Lozano, 2003) which is consisting with our results. Ali and Hassan (2017) reported that the increment in carbohydrates is positively correlated with mycorrhization of the host plant. We observed an increase in proline content as a result of using AMF. Proline has been found to improve by AMF inoculation (Sharifi *et al.,* 2007). Proline accumulation is one of the most frequently observed modifications motivated by salt stress (Sannazzaro *et al.,* 2007). Therefore, membrane maintenance was observed in chamomile plants treated with AMF which in accordance with Ali and Hassan (2014).

SA treatment alleviated the deleterious effects of salinity on growth and yield of chamomile

plant. SA is considered as an important signal molecule for modulating plant responses to environmental stress and therefore mitigated the deleterious effects of some environmental stresses (Hela *et al.,* 2009). Increasing RWC due to SA application may be ascribed to the fact that foliar application with SA can induce the leaf resistance and lower transpiration rates (Karlidag *et al.,* 2009) or the function of SA in accumulation of compatible osmolytes in salt stressed plants (Kabiri *et al.,* 2014). SA treatment enhanced the volatile oil percentage and yield of chamomile which in accordance of the findings of Gharib (2006) on marjoram and basil. This increase in volatile oil may be ascribed to the changes in population of leaf oil glands, carbohydrates content and the impact of SA on metabolism and enzyme activities that responsible for mono or sesqueterpene-biosynthesis (Gharib, 2006).

SA application improved the total chlorophyll content and mitigated the adverse effects of salinity. It has been reported that SA promotes the protective reactions involving the photosynthetic pigments and maintaining membrane integrity [46]. Similar results have been reported (Singh *et al.,* 2014; Hassan *et al.,* 2017). In current study, SA maintained the membrane stability and therefore ameliorated the adverse effects of salinity. Najafian *et al.,* (2009) found that the amount of ion leakage was reduced in salt stressed seedlings of rosemary showing that SA application has facilitated the membrane functions maintenance under salinity. The protective effect of SA in membrane integrity and ion uptake regulation has been also reported (Gunes *et al.,* 2007; Hassan *et al.,* 2017). SA treatment accumulated more proline than the control and this accumulation has been correlated with stress tolerance and is considered among the plant strategies adapted to cope up with salinity (Misra and Gupta, 2005). Such proline accumulation has been previously reported due to SA treatment under salinity (Hassan *et al.,* 2017). The uptake of N, P and K was stimulated due to SA treatment in our study. These results are in agreement with Grattan and Grieve (2009) who reported that SA application altered the miniral uptake and mitigated the deleterious effects of salinity and this alteration is considered a mechanism for the alleviation of salt stress (Grattan and Grieve, 1999). Similar trend has been observed by Hassan *et al.,* (2017) on rosemary.

As a conclusion, salinity treatment had several adverse effects on chamomile growth and flower yield. Salinity treatment decreased RWC and chlorophyll content. However, TSS, proline and membrane stability were increased. Otherwise, AMF or SA treatments alleviated the adverse effects of salinity on the previously mentioned characters. Improving proline accumulation and prevented ion homeostasis and hence maintained the membrane integrity due to AMF or SA treatments is suggested to be possible mechanisms for salinity tolerance in chamomile.

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