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Ameliorative Effect of GA_3 as Foliar Spray Treatment on Performances of Salt-Stressed Damsisa (*Ambrosia maritima* L.) Plants

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Abstract

A pot experiment was carried out to investigate the effect of foliar spray with GA_3 on growth and chemical composition of damsisa plants *(Ambrosia maritima* L) grown under saline conditions. Results revealed that increasing the concentration of salts reduced, plant height, number of leaves, number of branches, stem diameter, root length, as well as the fresh and dry weight of leaves and roots. Under saline conditions chlorophyll, a. b and total carotenoids, total carbohydrates, total indols, N, P and K were markedly reduced. However, total free amino acids, proline, total and reducing sugars, total and free phenols, Na and total sesquiterpene lactones content in leaves were increased. Treatment with GA_3 at 100 ppm nullified the harmful effect of salinity and increased sail tolerance by increasing synthesis of different metabolites (sugars, free amino acids, proline and phenols) and enhancing biochemical and physiological processes and consequently increase the biosynthesis and accumulation of the major active constituent (sesquiterpene lactones) which have an insecticidal activity against bilharzias.

Keywords: *Saline, GA_3, Damsisa (Ambrosia maritime L.), Growth, Chemical contents*

1. Introduction

Ambrosia maritima L. (Asteraceae) is a widely available weed in the Mediterranean region and African countries, particularly Egypt and Sudan, where it locally known as Demsissa and it grows abundantly near water catchments and on the banks of the Nile River (Saeed *et al*., 2015). It contains important sesquiterpene lactones, such as neoambrosin, ambrosin, and damsin, which have molluscidal and cytotoxic activities (Saeed *et al*., 2015). In addition, this plant contains several phytochemicals, such as coumarins, flavonoids, sterols and tannins, and exhibits considerable antioxidant activity (Dirar *et al*., 2014). Despite the common use of Damsissa in traditional medicine, to date, there are no reports on its use as a natural preservative of food products.

Salinity stress is being one of the great challenges in agriculture and has been the subject of intensive research. Saline soil, especially in dry lands, causes great losses to agriculture by reduction the vegetative growth, yield and its components of various crops and also causes productive land to be with draw from agriculture (Nimir *et al*., 2017; Hemida *et al*., 2017; Rady *et al*., 2018).

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Salinity is a major problem of irrigated agriculture in the arid and semi-arid regions. The reduction in growth of many crop plants by salinity may result from salt effects on dry matter allocation, ion relations, water status, physiological processes, biochemical reactions and/or a combination of such factors (Epstein *et al.*, 1980; Maggio *et al*., 2010; Nimir *et al*., 2017).

Many investigators showed that salinity conditions are known to have different adverse and impaired effects on growth, yield and its components of many plants (Ghallab and Seif El-Yazal, 2006; Hossain *et al*., 2015; Nimir *et al*., 2017; Hemida *et al*., 2017; Rady *et al*., 2018; Seif El-Yazal *et al*., 2020).

However, the information on the effect of salinity stress on damsisa plants is not available. Therefore, several methods have been tested for improving salt tolerance of plants. GA_3 was recommended as an effective agent for increasing salt and drought tolerance of plants, El-Ekhtyar *et al*. (2014), Bassiouni *et al*. (2016), Ullah *et al*. (2018), Rathinavelu *et al*. (2018) and Rady *et al*. (2021).

The aim of the present study is to increase salt tolerance of the tested plants by treating with GA_3.

2. Materials and Methods

This experiment was carried out during the two successive seasons 1999/2000 and 2000/2001 in the Experimental Area of Faculty of Agric., Fayoum Cairo Univ. Egypt. Seeds of damsisa *(Ambrosia maritima* L.) secured from the Experimental Farm of the Pharmaceutical Sciences Department. National Research Center, were sown in prepared seed beds on September 10th for both studied seasons. Uniform seedlings 10-15cm length was transplanted after 50 days in 30 cm diameter pots filled with 5 kg of air dried clay soil. Some physical and chemical characteristics of the used soil before salinization were determined according to Wilde *et al.* (1985) as shown in Table 1.

The experiment was performed to investigate the effect of GA_3 at 100 ppm on salt tolerance through its effect on growth and chemical composition of damsisa plants *(Ambrosia maritima* L).

Table 1: Physical and Chemical Characters of the Used Soil Before Salinization

Property			
Physical		**Soluble Cations (meq. L^{-1})**	
Clay %	16.0	Ca^{-2}	4.16
Silt %	18.4	Mg^{-2}	1.52
Sand %	65.6	Na^{-}	3.24
Soil Tecture	Sandy loam	K^{-}	0.14
Chemical		**Soluble Anions (meq. L^{-1})**	
pH	7.7	CO^{-3}	-
ECe (dsm^{-1})	1.57	HCO^{-3}	0.58
Organic Matter %	0.44	Cl^{-}	3.60
		SO^{-4}	**2.13**

The presented experiment contained 14 treatments. Each treatment was replicated four times. Each replicate contained one pot. One plant per pots. The pots were distributed in a randomized block design, results were statistically analyzed using the LSD at probability level of 5% for comparisons (Gomez and Gomez, 1983). The soil was artificially salinized, using sodium chloride (NaCl) salts. The different treatments are summarized in Table 2.

One dose of the above mentioned salts were mixed with the soil before transplanting. The plants were sprayed twice (30 and 50 days after transplanting) with GA_3 100 ppm. The plants were fertilized with urea (46% N), potassium sulphate (15.5% P_20_2) at the rates of 3.3 and 5 g/pot divided into two equal doses, the first after one month, the second after 3 months from planting. Plant height (cm), number of leaves/plant, root length (cm), number of branches/plant, stem diameter (cm), herb fresh weight /plant (g), herb dry weight/plant (g) and flowering time at 25% of flowering (days) were recorded after (167)days from planting.

Table 2: The Different Used Treatments

Salinity Treatment		Salinity + GA_3 Treatment	
1	Control	2	100 ppm GA_3
3	2g/pot NaCl	4	2g/pot NaCl+100 ppm GA_3
5	4g/ pot NaCl	6	4g/ pot NaCl+100 ppm GA_3
7	6g/ pot NaCl	8	6g/ pot NaCl+100 ppm GA_3
9	8g/ pot NaCl	10	8g/ pot NaCl+100 ppm GA_3
11	10g/ pot NaCl	12	10g/ pot NaCl+100 ppm GA_3
13	12g/ pot NaCl	14	12g/ pot NaCl+100 ppm GA_3

2.1. Chemical Constituents

For each treatment, two samples were collected in the morning after (126 days) and (167 days) after transplanting. The concentration of (chlorophyll a, b, total and caroteniods mg/g.) was determined in fresh leaves according to Wellburn and Lichtenthaler (1984). Total carbohydrates (mg/g D.W) were determined colorimetric according to the method described by Dubois *et al*. (1956). Total and reducing sugars (mg/g F.W. was determined according to A.O.A.C. (1995). Proline concentration (mg/g F.W.) was determined according to Bates *et al*. (1973). Total free amino acids (mg/g) were determined according to Jayarman (1981). Total indals (mg/g) were determined according Larson *et al*.(1962).Total phenol (mg/g) were determined according to Snel and Snel (1953). Free phenols (mg/g) were determined according to A.O.A.C. (1995). Nitrogen and phosphorus (%) were determined according to A.O.A.C. (1995). Sodium and potassium (%) were determined by Flame-Photometer Parkin -Elmer model 52 with acetylene burner according to Page *et al*. (1982).

Total sesquiterpene lactones concentration (mg/g) were determined calorimetrically in dry herb according to the method described by El-Sawy *et al*. (1987), the method depends on measuring the color produced by the effect of Baljet's reagent on the sesquiterpene lactones (ambrosin and damsin) which give orange color, the resulting orange colour is measured colorimetric ally (Spectronic Bauch and Lomb) At 495 nm.

The values presented in the results obtained in this investigation is the mean of the two seasons under the study.

3. Results

3.1. Growth Characters

Results in Table 3 revealed in the two successive seasons that increasing the concentration of salts in soil reduced plant height, number of leaves, number of branches and stem diameter in the treated plants. Similar result was detected in root

Table 3: Effect of GA_3 Foliar Spray on Some Growth Character of Damsisa Plants (*Ambrosia maritime* L.) Grown Under Salinity Stress

Treatments		Plant Height (cm)		No. of Leaves		No. of Branches		Root Length		Stem Diameter (cm)	
Salinity Levels	Spray Treatment	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	H_2o	36.5	33.33	47	43.67	46	40	10.27	9.5	1.33	1.23
Zero ppm	GA_3	58	53.17	45.04	43.67	44	39.33	10.77	10	1.23	1.1
2000 ppm	H_2o	35.17	29.5	39	37	35.67	34.67	6.33	6.83	1.33	1.2
2000 ppm	GA_3	54	51.67	43.33	41.67	41	35.67	7.17	7	1	0.9
4000 ppm	H_2o	34.5	33.5	39.33	38	37.33	35	6	6.33	1.3	1.13
4000 ppm	GA_3	46.17	44.33	44	38	37.33	37.65	9.5	8.8	1.2	1.1

Table 3 (Cont.)

Treatments		Plant Height (cm)		No. of Leaves		No. of Branches		Root Length		Stem Diameter (cm)	
Salinity Spray	Levels Treatment	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
6000 ppm	H_2o	35.67	30.5	39.33	37.04	36	34.33	7.33	7	1	0.93
6000 ppm	GA_3	50.33	47	43.33	30.67	37	37.33	9.5	9	0.9	0.83
8000 ppm	H_2o	34.33	31.67	39.33	37.33	37.33	35.67	7.67	6.5	1.2	1.1
8000 ppm	GA_3	46.5	43.5	42.33	41.33	36.67	34.67	9.33	9.17	1	0.93
10000ppm	H_2o	35.67	30.83	38.67	37.33	34	33	7	6	1.1	1
10000ppm	GA_3	42.33	41	40	38.33	38.67	33.33	7.67	6.17	1	0.9
12000ppm	H_2o	28.67	29	39	35	26.67	27	5.83	6	1.1	1
12000ppm	GA_3	43.33	39	40.33	39	37	32	7.67	6.17	1	0.9
$L.S.D_{0.05}$	%	4.25	4.89	2.68	2.2	4.89	4.36	1.34	1.76	0.24	0.09

length. Spraying with GA_3 increased plant height, number of leaves number of branches, stem diameter and root length of the plant irrespective of salt concentration. Effect of GA_3 was pronounced in low levels of salt treatment. The favoring effect of GA3 in counteracting the negative effect of salinity on growth characters might be due to that GA3 induced salt tolerance by supporting synthesis of the different metabolites (sugars, nucleic acids, proteins and proline) as well as increasing nutrients uptake by the plant roots.

3.2. Flowering Date

All soil salinity level significantly delayed flowering date in both seasons comparing to untreated plants, except the low salinized soil treatment (4000 ppm) which showed insignificant effect in the first seasons Table 4. GA_3 application decreased the flowering date (days) especially at the low concentration of salts whereas slight effect was obtained at the high salt concentration.

Table 4: Effect of GA_3 Foliar Spray on Flowering Period, Fresh and Dry Weight of Damsisa Plants (*Ambrosia maritime* L.) Grown Under Salinity Stress

Treatments		Flowering Date (Days)		Herb Fresh Weight (g)		Herb Dry Weight (g)		Root Fresh Weight (g)	
Salinity Levels	Spray Treatment	1999	2000	1999	2000	1999	2000	1999	2000
Control	H_2o	155	156	124.06	106.46	41.1	35.25	48.02	45.83
Zero ppm	GA_3	153	163	114.3	108.3	42.56	39.95	43.5	43
2000 ppm	H_2o	161	171	105.62	97.66	33.79	32.06	31.27	29.17
2000 ppm	GA_3	153	165	119.61	106.32	37.75	34.12	42.86	39.75
4000 ppm	H_2o	159	169	105.62	90.4	36.94	33.84	30.77	31.6
4000 ppm	GA_3	156	168	111.75	95.17	39.39	34.12	35.49	31.83
6000 ppm	H_2o	160	172	86.07	70.39	28.35	31.27	33.27	32.77
6000 ppm	GA_3	158	170	105.62	97.83	36.94	33.84	37.17	37
8000 ppm	H_2o	163	174	90.87	84.6	32.19	29.97	29.03	27.63

Table 4 (Cont.)

Treatments		Flowering Date (Days)		Herb Fresh Weight (g)		Herb Dry Weight (g)		Root Fresh Weight (g)	
Salinity Levels	Spray Treatment	1999	2000	1999	2000	1999	2000	1999	2000
8000 ppm	GA_3	154	166	99.23	88.83	37.52	33.84	36.49	34.67
10000ppm	H_2o	160	172	91.83	79.13	29.74	27.88	27.03	26.33
10000ppm	GA_3	160	171	99.73	88.83	35.67	31.27	36.7	31.99
12000ppm	H_2o	160	172	66.4	69.71	24.04	22.44	24.4	29.17
12000ppm	GA_3	155	164	84.65	80.57	27.25	25.63	33.83	36.13
$L.S.D_{0.05}$	%	4.69	4.30	20.18	19.79	7.12	6.79	6.65	6.86

3.3. Fresh and Dry Weight in Herb and Roots

Data in Table 4 clearly shows that increasing the concentration of salts reduced fresh and dry matter accumulation in the treated plants in both the two growing seasons.GA_3 application increased fresh and dry matter contents of various plant parts irrespective of salt concentration.

Concerning the distribution of fresh and dry matter content between the different plant organs as affected by salt treatment as well as GA_3 application, the result in Table 4 reported that, at high salt concentration, percentage of fresh and dry weight in roots and shoots decreased markedly. Moreover, GA_3 application stimulated the fresh and dry matter accumulation in roots and shoots especially plant leaves.

3.4. Total Chlorophylls

Data in Table 5 shows that leaf plastid pigments concentration (chlorophyll a, b, total and carotenoids) of damsisa plant leaves collected in the first sample was greater than that in the second one. In addition, leaf plastid pigments concentration was decreased significantly with increasing salinity levels. Treating with GA_3 reduced the adverse effect of salinity on chlorophyll content especially at the low concentration of salts whereas slight effect was obtained at the high salt concentration.

Table 5: Effect of GA_3 Foliar Spray on Chemical Constituents of Damsisa Plants (*Ambrosia maritime* L.) Grown Under Salinity Stress

Treatments		Chlorophyll A mg/g F.W.		Chlorophyll B mg/g F.W.		Total Chlorophylls mg/g F.W.		Carotenoids mg/g F.W.		Total Carbohydrates mg/g D.W.	
Salinity Levels	Spray Treatment	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	H_2o	3.3	2.05	2.85	1.26	5.88	3.31	0.33	0.18	189.58	164.39
Zero ppm	GA_3	2.8	2.13	2.88	1.29	5.67	3.42	0.34	0.23	190.83	173.22
2000 ppm	H_2o	2.92	1.97	2.23	1.11	5.15	3.08	0.24	0.13	167.5	156.17
2000 ppm	GA_3	3.06	1.98	2.24	1.11	5.32	3.09	0.32	0.19	175.83	170.55
4000 ppm	H_2o	2.51	1.5	1.47	0.89	4.25	2.39	0.32	0.15	166.67	120.55
4000 ppm	GA_3	3.16	1.66	1.86	0.99	5.02	2.66	0.34	0.17	169.39	143.5
6000 ppm	H_2o	2.92	1.41	2.24	0.87	5.16	2.28	0.33	0.17	175	145.55
6000 ppm	GA_3	3.04	1.55	2.52	0.98	5.56	2.55	0.34	0.19	176.45	156.17
8000 ppm	H_2o	2.85	1.46	2.38	0.9	5.23	2.37	0.31	0.12	143.5	148.28

Table 5 (Cont.)

Treatments		Chlorophyll A mg/g F.W.		Chlorophyll B mg/g F.W.		Total Chlorophylls mg/g F.W.		Carotenoids mg/g F.W.		Total Carbohydrates mg/g D.W.	
Salinity Levels	Spray Treatment	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
8000 ppm	GA_3	3.02	1.53	2.41	0.9	5.44	2.43	0.34	0.13	187.61	150
10000ppm	H_2o	2.92	1.38	2.16	0.97	5.08	2.35	0.27	0.13	177.61	139.39
10000ppm	GA_3	3.02	1.45	2.24	1.02	5.26	2.47	0.31	0.19	189.39	142.55
12000ppm	H_2o	3.82	1.44	2.19	0.86	5.01	2.3	0.32	0.13	163.33	156.17
12000ppm	GA_3	3.04	1.55	2.28	1.04	5.32	2.59	0.32	0.2	177.61	166.55
$L.S.D_{0.05}$	%	0.09	0.05	0.07	0.05	0.06	0.05	0.05	0.01	3.16	2.86

3.5. Carbohydrates

Data in Tables 5 and 6 shows that total carbohydrates concentration in the plant leaves collected in the first sample was greater than that in the second one. On the other hand, the data show a marked decrease in total and reducing sugars concentration in the plant leaves collected in the first sample than that in the second one. In addition the obtained data also

Table 6: Effect of ga3 Foliar Spray on Chemical Constituents of Damsisa Plants (*Ambrosia maritime* l.) Grown Under Salinity Stress

Treatments		Total Sugars mg/g F.W.		Reducing Sugars mg/g F.W.		Proline mg/g F.W.		Total Free Amino Acids mg/g F.W.		Total Indoles mg/g F.W.	
Salinity Levels	Spray Treatment	First Level	Second Level	First Level	Second Level	First Level	Second Level	First Level	Second Level	First Level	Second Level
Control	H_2o	5.35	4.23	1.84	2.05	17.46	18.89	2.37	4.84	1.76	2.24
Zero ppm	GA_3	7.44	6.33	2.9	5.22	19.4	20.44	2.46	4.84	2.25	2.48
2000 ppm	H_2o	6.06	9.02	2.35	5.22	20.42	21.78	2.46	5.28	1.73	2.19
2000 ppm	GA_3	8.32	6.61	3.72	3.24	22.86	21.45	4.39	5.16	1.81	2.25
4000 ppm	H_2o	6.73	11.33	3.12	2.34	19.39	21.63	2.97	5.55	1.61	1.48
4000 ppm	GA_3	6.99	6.96	1.75	3.74	22.13	22.55	3.63	6.11	1.64	2.2
6000 ppm	H_2o	6.12	4.35	2.84	2.47	20.43	20.89	3.35	5.12	1.51	1.86
6000 ppm	GA_3	7.59	10.36	3.72	5.31	20.06	22.55	3.78	5.75	1.74	2.02
8000 ppm	H_2o	5.96	6.96	2.04	2.88	20.07	19.37	4.81	9.89	1.61	2
8000 ppm	GA_3	6.89	9.82	4.74	4.21	20.12	20.89	5.79	10.74	1.71	2.12
10000ppm	H_2o	5.88	9.66	3.07	3.98	19.05	21.26	6.01	9.62	1.24	1.34
10000ppm	GA_3	6.75	7.29	2.63	2.95	19.39	19.46	6.46	10.26	1.97	1.56
12000ppm	H_2o	5.72	4.89	1.91	2.88	18.5	20.31	4.43	8.26	0.62	1.8
12000ppm	GA_3	7.1	10.78	2.93	4.17	20.33	22.44	6.93	9.48	0.93	1.84
$L.S.D_{0.05}$	%	0.62	0.59	0.39	0.4	1.78	1.01	0.38	0.61	0.33	0.32

show that total carbohydrates were decreased significantly in damsisa plant leaves with increasing salt level. Whereas the data also shown a marked increase in total and reducing sugars for plant leaves grown under high saline conditions. GA_3 favored the accumulation of higher amounts of sugars in leaves of all treatments. The best effect was obtained when the plants were grown under the normal conditions. The favoring effect of GA_3 on carbohydrate accumulation may be due to its enhancing effect on chlorophyll synthesis and consequently the photosynthetic activity.

3.6. Free Proline, Total Free Amino Acids and Total Indols

Results in Table 6 showed that proline was significantly increased in leaves of damsisa plants grown under salinity. Similar effect was detected in total free amino acids. In contrast total indoles were significantly decreased in leaves of damsisa plants grown under salinity. On the other hand, the data also show that proline, total free amino acids and total indoles content in the plant leaves collected in the second sample was greater than in the first one.GA_3 application resulted in increase in the free amino acid, proline and indoles contents of leaves irrespective to salt treatments, especially in the second sample amid at the low concentration of salts.

3.7. Phenolic Compounds

Data in Table 7 showed clearly that total and free phenol concentrations of damsisa plant leaves generally increased in the leaves collected in the second sample than that in the first one. In addition the results showed that total and free phenols were significantly increased in leaves of damsisa plants grown under high level of salinity. GA_3 application decreased total and free phenols concentrations of plant leaves irrespective of salt concentration..

3.8. Sodium, Nitrogen, Phosphorus, Potassium Concentration

The data in Table 7 shows that Na, N, P and K were greater in the leaves of second sample than in the first one. The obtained data also elucidate that Na was increased with increasing salinity levels in leaves and reverse was true for N,

Table 7: Effect of GA_3 Foliar Spray on Chemical Constituents of Damsisa Plants (*Ambrosia maritime* L.) Grown Under Salinity Stress

Treatments		Total Phenols mg/g F.W.		Free Phenols mg/g F.W.		Na %		N %		P %		K %	
Salinity Levels	Spray Treatment	First Level	Second Level	First Level	Second Level	First Level	Second Level	First Level	Second Level	First Level	Second Level	First	Second
Control	H_2o	1.08	3.03	0.29	1.28	1.02	0.81	3.75	3.98	0.18	0.18	2.52	4.36
Zero ppm	GA_3	1.03	2.8	0.22	1.25	0.61	0.84	4.01	4.12	0.19	0.18	2.84	4.44
2000 ppm	H_2o	1.74	4.76	0.35	2.25	1.81	1.64	3.66	3.59	0.12	0.15	2.02	3.21
2000 ppm	GA_3	1.13	4.19	0.29	2.02	1.01	1.23	3.89	3.78	0.14	0.17	2.07	4.15
4000 ppm	H_2o	2.34	5.33	0.3	2.6	1.25	1.23	3.25	3.43	0.12	0.13	2.12	3.44
4000 ppm	GA_3	2.19	4.88	0.3	2.03	1.02	1.13	3.33	3.48	0.13	0.14	2.29	4.19
6000 ppm	H_2o	1.85	5.05	0.57	2.59	1.23	1.81	2.89	3.11	0.1	0.15	2.15	4.16
6000 ppm	GA_3	0.99	3.32	0.29	2.09	0.81	0.81	2.99	3.21	0.12	0.17	2.52	4.21
8000 ppm	H_2o	1.2	5.87	0.39	2.19	1.23	1.3	2.56	2.99	0.12	0.12	2.25	4.1
8000 ppm	GA_3	0.66	4.72	0.36	2.23	1.23	1.23	2.66	2.75	0.15	0.13	2.29	4.13
10000ppm	H_2o	1.12	5.51	1.02	2.62	1.23	1.72	2.3	2.78	0.13	0.14	2.12	4.34
10000ppm	GA_3	1.02	4.57	0.78	2.03	0.61	1.64	2.41	2.81	0.14	0.15	2.29	4.19
12000ppm	H_2o	1.09	5.03	0.59	2.95	2.86	1.84	2.01	2.78	0.13	0.15	2.05	4.13
12000ppm	GA_3	1.06	4.52	0.55	2.65	0.84	1.43	2.11	2.89	0.14	0.18	2.07	4.36
$L.S.D_{0.05}$	%	0.28	0.28	0.04	0.46	0.18	0.11	0.08	0.05	0.03	0.01	0.07	0.27

P and K concentration as compared with un salinized plants. GA_3 increased markedly N, P and K content of the leaves irrespective of the concentration of salt and reverse was true for Na concentration.

3.9. Sesquiterpene Lactones mg/g D.W.

Data in Table 8 shows that sesquiterpene lactones in dry herb as affected by salinity and GA_3 treatment. The results indicated that a significant increase in sesquiterpene lactones occurred with increasing the level of salts. Spraying with GA_3 resulted in an increase in the sesquiterpene lactones concentration of leaves irrespective to salt treatments, especially in the second seasons and at the high concentration of salts.

Table 8: Effect of GA_3 Foliar Spray on Sesquiterpene Lactones of Damsisa Plants (*Ambrosia maritime* L.) Grown Under Salinity Stress

Treatments		Sesquiterpene Lactones (mg/g. D.W.)	
Salinity levels	**Spray treatments**	**1999**	**2000**
Control	H_2o	8.43	11.06
Zero ppm	GA_3	8.63	12.35
2000 ppm	H_2o	8.79	12.52
2000 ppm	GA_3	8.85	12.92
4000 ppm	H_2o	8.11	13.25
4000 ppm	GA_3	8.13	13.26
6000 ppm	H_2o	8.83	13.85
6000 ppm	GA_3	8.82	14
8000 ppm	H_2o	8.85	14.01
8000 ppm	GA_3	10.13	14.75
10000ppm	H_2o	10.03	14.2
10000ppm	GA_3	10.45	15.15
12000ppm	H_2o	10.63	14.8
12000ppm	GA_3	10.79	15.12
$L.S.D_{0.05}$	%	0.93	1.08

4. Discussion

It is clear from the present data that increasing NaCl concentration decreased the plant height, number of leaves, number of branches, stem diameter, root length, herb fresh weight /plant, herb dry weight/plant and this may be attributed to our results with the reduction in total indoles in leaves of damsisa plants grown under saline condition. In this respect, O'Leary and Prisco (1970) reported that the adverse distributed hormonal balance in the leaves, and less synthesis of cytokinins in the roots of salt stressed plants and as a consequence less hormone would be delivered to the shoots. In addition, Townsend (1980) suggested that the reduction in growth under the stress of salinity might be due to osmotic inhibitions of water absorption, toxicity at one or more specific and the combination of the two factors. However, Zhaoliang *et al.* (1995) concluded that the reduction in plant height and number of branches could be attributed to the reduction in auxins and gibberellins content that reflect on cell division and cell elongation. Also Gomez *et al.* (1998) found that salt stress increased levels of abscisic acid (ABA) in roots and leaves, and increased CL^- accumulation.

The data also show that GA_3 increased seedling height, stem diameter, leaf area, number of branches, and root length. In this concern, EL-Dessouki (2001) reported that GA_3 alleviated the adverse effect of salinity on sour orange and citrus

seedlings. In addition, EL-Desouky and Atawia (1998) reported on sour orange that the biological activities of endogenous phytohormones (cytokinins, gibberellins and auxins) were significantly reduced by excess salinity (5000 ppm). In this connection, Awad and Boutros (1987) reported that spraying the seedling with 100 or 200 ppm GA_3 decreased the retardation effect of salinity on total growth, at least, by 34%. They attributed this effect to the influence of GA_3 in increasing the osmotic pressure of the cell sap and consequently in improving the water economy of the seedling through decreasing transpiration and increasing water absorption and also enhanced the total uptake of potassium than sodium. Moreover, data also show that salinity delayed flowering date this result might be due to the effect of salinity on decreasing net photosynthesis and delayed plant growth and flowering (Abdalla *et al*., 1989). In this respect Nir and Lavee (1993) reported that the induction of flowering could be correlated with natural rise in gibberellin which cause or promote flower formation in plants by either facilitating the formation of flowering hormone in the leaves or expressing it in the growing buds. Gibberellins also are a primarily responsible for bolting which may be essential for the formation of the floral stimulus in leaves. The depressive effect of salinity on fresh and dry matter accumulation could be explained on the basis of the harmful effects of salinity on the growth due to inhibition of chlorophyll synthesis (Muller and Santarius, 1978), the reduction in carbon fixation in photosynthesis process and were less efficient in metabolizing their dry matter comparing to non-salinized ones (Rizk, 1993 and El-Desouky *et al*., 2000), and decreased photosynthesis (Khan *et al*., 1997). Such effects was also recorded by Eisa and Ibrahim (1989) who indicated that, NaCI accumulated in the leaf cells and affected lipid-synthesizing enzymes such as galactosyl transferase and cylase which are attached to the chloroplast envelop. However, the reduction in chlorophyll a, b, total and carotenoids linked with increasing levels of salinity might be attributed to (I) die suppress of the specific enzyme which is responsible for the synthesis of photosynthetic pigments (Strognova *et al.* (1970), (ii) the destruction of chlorophyll, Afria *et al*. (1998) or (iii) the decrease in the absorption of minerals needed for chlorophyll biosynthesis. i.e. iron and manganese, Salama *et al*. (1992).The data also show that GA_3 application enhanced the synthesis of chlorophyll. In this respect, EL-Dessouki (2001) indicated that GA_3 application stimulated certain physiological processes and enhanced the synthesis of chlorophyll in cotton; pea and citrus plants.

The increase in soluble sugars observed under salt stress might be as a result of the activity of the hydrolytic enzymes. In this respect, Munns and Weir (1981) found that salts weakened sink strength rather than from an active mechanism of osmotic adaptation. Moreover, GA_3 favored the accumulation of higher amounts of sugars in leaves of all treatments. These results might be attributed to increasing free and combined sugars or by increasing some organic products synthesized in cell sap to adapt them to saline condition or this increase may be due to the reduced respiration at high salinity level which might accumulate sugars (Munns *et al*., 1982).

The decrease in carbohydrate content might results from the production of relatively high energy by increasing respiration to overcome the relatively low availability of water and nutritional elements in saline medium (Moursi *et al.*, 1976). The increase in free proline and total free amino acids under salt stress might be as a result of an adaptive mechanism for osmoregulation in plants cells. In this respect, Gadallah *et al.* (2001) reported that the accumulation of free proline in salt stressed plants may be attributed to an adaptive mechanism for osmoregulation in plants cells to cope with salinity problems. Moreover, this increase in proline and total free amino acids may be interpreted that NaCI salinity stimulate the conversion of protein into amino acids (Shadded, 1990). In this regard Handa *et al*. (1985) suggested that proline accumulation considered an adaptive response to stress. Also, Hussein *et al*. (1984) attributed such increase partially due to the higher degradation rats of proteins in plants under salt stress conditions. The decrease in total indoles under salt stress conditions was agree with Ali and Salem (1985) which reported that salt condition reduced the amount of IAA in peach seedlings. This effect may be attributed to that phenolic compounds constitute a part of cellular solutes and provide reducing environment that could be an adaptive mechanism for scavenging oxygen free radicals during stress. The positive effect of GA_3 on the content of free amino acids, proline and indoles in leaves may be interpreted on the basis of their effect on retardation of senescence delaying chlorophyll degradation and stimulation of protein in biosynthesis as well as activation of DNA and RNA synthesis (Robert and Francis, 1985). In addition Younis *et al*. (1991) stated that gibberellic acid treatment partially or completely eliminated the salinity -induced changes in protein nitrogen, total soluble nitrogen and proline and also nullified the accumulation of sodium ions and reduced the ABA content in the plant shoots. They attributed the effect of GA_3 in increasing the salt tolerance of plants at the metabolic level to its capacity to restore the synthesis of various metabolites and or to inhibit that of others. The data also show that total and free phenol increased under salt stress conditions. In this respect, El-Sallami and Makary (2001) stated that salinity stress was accompanied by an increase in the content of phenolic compounds up to toxic concentration. Moreover, Stark and Karwowska (1978), working on bean plants and suggested that physiological changes occurring in plants under salt caused by hormonal imbalance. The obtained data also elucidate that Na was increased with increasing salinity levels in leaves and reverse was true for N, P and K concentration as compared with un salinized plants. In this

concern, Mengel and Kirkby (1979) reported that, such reduction might be due to the decrease in mineral uptake by plants with increasing salinity levels which might affected time activity of the different enzymes systems and the different metabolic processes inside the plant. Also, Ismail (1996) found that the high Na concentration as a result of salt stressed caused damage in the plant metabolic results from high cytoplasmic Na/K ratio. High Na concentration may interfere with K acquisition. Moreover, Hassan *et al*. (1970) reported that phosphorus is not highly mobile in the soil, the depression in P under salinity conditions may be related to a reduction in root growth caused by salinity and an associated decrease in the surface area of roots in contact with P in the soil. In addition, possible decreases in Co_2 in the soil with increasing salinity may have reduced the uptake of P by the plant. The data also elucidate that under GA_3 treatments Na was decreased in leaves and reverse was true for N, P and K concentration as compared with control. In this connection Awad and Boutros (1987) found that the application of GA_3, especially at the higher rate enhanced the total uptake of potassium than sodium causing higher K/ Na ratio in the different organs of the seedlings. In addition, Amzallag *et al*. (1992) suggested that application of GA_3 had the same effect as the addition of nitrogen, phosphorus or potassium supplementation on plant growth. They reported that the limitation of growth at high sodium chloride levels was due to growth regulator imbalance rather than to mineral deficiency and that the change in plant nutrient concentration, resulting from N, P or K supplementation, acted as a signal for a change in growth regulator balance; inducing the synthesis of endogenous gibberellic acid or cytokinin. The Data also show that there was an increase in sesquiterpene lactones occurred with increasing the level of salts. Spraying with GA_3 resulted in an increase in the sesquiterpene lactones concentration of leaves irrespective to salt treatments. These findings are in accordance with those reported by Amer (1994) which found that some volatile oil constituents were increased and others were decreased or disappeared with increasing salinity level. In this connection Sidky and E1-Mergawi (1997) on damsisa show that increasing the rate of K fertilization from 40 to 80 Kg K_2O/feddan produced plants containing a low concentration of sesquiterpene lactones leaves. The reduction in K concentration under salinity was previously reported by Stark and Kozinska (1980) on faba bean. This reduction in K was due to substitution mechanisms and/or antagonistic state between Na and K (Medani, 1988).

5. Conclusion

GA_3 at 100 ppm nullified the harmful effect of salinity and increased sail tolerance by increasing synthesis of different metabolites (sugars, free amino acids, proline and phenols) and enhancing biochemical and physiological processes and consequently increase the biosynthesis and accumulation of the major active constituent (sesquiterpene lactones) which have an insecticidal activity against bilharzias.

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