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RESPONSE OF SALT STRESSED ROSEMARY PLANTS TO ANTISTRESS AGENTS

Hanan M.H. Ali* and Mona G. Attia**

*Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.
**Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

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Prof. Dr. E.S. Nofal, Kafr El-Sheikh Univ.

Prof. Dr. I.M.A. Haridy, Hort. Res. Inst., ARC.

ABSTRACT: A pot experiment was carried out during two successive seasons (2014 and 2015) at Salinity and Alkalinity Soil Research Laboratory in Alexandria. The goal of this work was to study the response of rosemary (*Rosmarinus officinalis* L.) plants irrigated with saline water at 0, 2000 and 4000 ppm NaCl to the application of antistress agents (salicylic acid "SA" at 0 and 0.2 mM and diatomaceous earth "DDM" at 0 and 6 g/10 kg soil). The effect of these antistress agents on the vegetative growth, volatile oil percentage and its chemical composition were recorded. In general results indicated that irrigation with saline water and antistress agents (DDM and SA each of them solely) had a significant effect on vegetative growth expressed as plant height, number of branches/plant, herb fresh and air dry weights and volatile oil percentage as compared to control in most treatments in both cuts of the two seasons. Moreover the plants received diatomaceous earth (DDM) at 6 g/10 kg soil combined with irrigation with non saline water (tap water) recorded the highest values of vegetative growth and volatile oil percentage in both seasons at the two cuts. Also the highest camphor content was achieved with this treatment. Proline content was decreased with increasing the rate of diatomaceous earth followed by salicylic acid, this refers to the response of rosemary plants grown under salt stress to the application of antistress agents (DDM and SA).

Key words: Rosemary, *Rosmarinus officinalis* L., saline water, salicylic acid, vegetative growth, volatile oil percentage, volatile oil composition.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) Lamiaceae, is an evergreen plant typical of Mediterranean region. Rosemary has long been considered an important plant for its essential oil used in perfumes and medicine (Miguel *et al.,* 2007). The plant was reported to possess several medicinal properties like carminative, stomachic, nervine spasmodic and stimulant. The leaves were also reported to possess antioxidant properties and used for culinary purposes (Singh and Guleria, 2013). The volatile oil exhibit some medicinal purposes such as anti-inflammatory, antiseptic, antispasmodic and anti-diabetic (Juhas *et al.*, 2009; Abu Al-Basal, 2010 and Beninca *et al.,* 2011).

Million hectares of agricultural areas as well as for the newly reclaimed lands is affected by varying degrees of salinity or sodicity. The major constraints for plant growth is the excessive uptake of mainly cl^- and Na^+ as well as nutrients. To ensure food security and sustainable economy, there is dire need to find ways to improve salinity tolerance of various cultivated crops. Various chemical, physical and biological strategies are adopted for economic crop production under salt stress. Of all these strategies, exogenous application of nutrients has gained a considerable ground as a shotgun approach to ameliorate the adverse effects of salt stress (Tahir *et al.,* 2006). Mineral nutrient application that ameliorated the adverse effect of salt stress were either essential as K, Ca, N… etc (Akram *et al.,* 2007), or non-essential as Si (Tahir *et al*., 2006 and Hanafy *et al*., 2008). Recent investigations showed Si efficiencies in mitigating salinity in various plants for instance the shoot and root growth was severely inhibited in rice grown at 100 mM NaCl was significantly ameliorated by Si addition at 0.89 mM (Ahmad *et al.,* 1992). Moreover Si supply to rose imposed under different salinity levels significantly enhanced their vegetative growth, improved the overall plant appearance and resulted in a higher number of marketable flowers or plant (Ulmer, 2010). Si was also able to enhance the fresh and dry weights, plant height, girth, internode length, number of tillers and plants, number of fruits or plant mean fruit weight (Ashraf, 2008 on sugar cane; Savvas *et al*., 2009 on *Zucchini alteviates* and Hashemi *et al.,* 2010 on *Brassica napus* L.)

Diatomites (DDM) is a natural occurring sedimentary rock primarily composed of fossilized remains of fresh water diatoms. It is chemically composed of Sio_2 (86 to 89%) in a soluble form available to plants and small amount of trace elements. It is considered as a complete, long lasting, recyclable and environmentally friendly soil enhancer by improving the physical structure of soil, aerating the plant's root zone, minimizing leaching and runoff thus increasing soil, water retention and reducing watering subsequently, diatomite promotes stronger, healthier, higher–yielding plants that mature quickly and acquire self resistance against abiotic and biotic stresses (Kruger, 2006; Jessen, 2007; Abdalla, 2009 and Abdalla, 2011 a).

Antioxidants, are designing of chemicals, when added in small quantities to a materials, react rapidly with the free radical intermediates of an autoxidation chain and stop their progressing. It has been reported that plants with high level of antioxidants, whether constitutive or induced have greater resistance to such oxidative damage (Mullineaux and Creissen, 1997). The primary components of this antioxidant system include carotenoids, ascorbate, glutathione, vitamin E (α–tocopherols) flavonoids, phenolic acids other phenols, alkaloids, polyamines and miscellaneous compounds. Number of studies indicated that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of antioxidative systems (Mekersie *et al.,* 1996).

Salicylic acid (SA) is a phenolic compound of hormonal nature produced by plants and plays an important role in response to abiotic stresses and pathogen attack (Noreen *et al*., 2009 and He *et al.,* 2002). SA has also been studied for its effects on various physiological processes related to growth and development of plants under normal conditions. Among these effects are the induction of flowering in herbaceous species (Hegazi and El-Shravi, 2007). Stimulation of root developments, stomatal closure and transpiration (Singh and Guleria, 2013). The effect of SA as an endogenous regulator of flowering was demonstrated in number of plant species belonging to different families (Hayath *et al.,* 2007). In addition to regulate flowering time, reproductive developments and links defense response (Martinez *et al.,* 2004). Exgenous application of SA at 1 and 2 mM enhanced shoot, root and total plant dry weight under no salt stress in (*Calendula officinalis*, L), besides providing an early flowering and high number of floral buds per plant (Bayat *et al.,*2012).

The goal of the present work was to investigate the influence of diatomites (DDM) and salicylic acid (SA) on the growth, yield and volatile oil of rosemary (*Rosmarinus officinalis* L.) plants under salt stress.

MATERIALS AND METHODS

A pot experiment was conducted at Experimental Farm of Salinity and Alkalinity Soil Research Laboratory, Alexandria, Egypt, in two seasons of 2014 and 2015.

1. Plant materials and procedures:

Small rosemary plants obtained from cuttings were cultivated in plastic pots (30 cm in diameter each pot was contained 10 kg soil) on 28th March, 2014 and 2015.

2. Chemical fertilization:

The chemical fertilizers (NPK) were ammonium sulphate (20.6% N), calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O). NPK fertilizers were added at the recommended level in four doses, the 1st was for all phosphorous amount which was added during soil preparation, the rest (NK) were applied in three equal doses the 1st was applied 45 days after planting, the second was added 30 days after the first one and the third was applied 10 days after the first cut.

3. Layout of the experiment:

The experiment layout was designed in split plot included nine treatments each treatment was replicated three times and every replicate consisted of nine pots (1 plant/pot). Irrigation with saline water occupied the main plots, while diatomite and salicylic acid treatments (antistress agents) were arranged in subplots. The analysis of variance (ANOVA) was conducted and the means of the treatments were compared using L.S.D. at 5% the statistical analysis according to (Snedecor and Cochran, 1980).

4. Treatments:

a. Irrigation with saline water was done at 0, 2000, and 4000 ppm NaCl.

b. Diatomite (DDM) is a natural diatomaceous earth originated from fossilized remains of fresh water cell wall impregnated with silica. It contains some elements as shown in Table (1).

Table 1. Major elements in diatomaceous earth according to (Abdalla, 2011 b).

Major elements	%
SiO_2	89.00
Al_2O_3	5.95
Fe_2O_3	0.88
CaO	0.10
K_2O	0.63
MgO	0.20
Na_2O	0.32
TiO_2	0.29
H_2O	3.00

Diatomite (DDM) was added to pots at the rates of 0, 6 g/10 kg soil. The application of diatomite was divided into four doses, the first and second were after 30 and 45 days from planting. The third one was applied after the first cut and the fourth after 15 days from the third doses.

c. Salicylic acid (SA) was sprayed at 0 and 0.2 mM. The application of SA was divided into four doses, the first spray was conducted two weeks after planting, the second was done 21 days after the first one, the third was applied one week after the 1st cut and the fourth was added three weeks after the third dose.

The plants were harvested twice, the first cut was on 25th July and the second one on 8th October in both seasons.

5. Data recorded:

The following data were recorded

1. Plant height (cm) and number of branches/plant.
2. Herb fresh and dry weights (air dried)
3. Volatile oil percentage was determined in dry herb (air dried) according to (British Pharmacopeia, 1963).
4. Volatile oil constituents, oil samples were taken from the oil obtained in the first cut of the second season and were analyzed using gas liquid chromatography (GLC) to determine their constituents according to (Bunzen *et al.,*1969 and Hoftman, 1967).
5. Proline content in dry leaves was determined according to (Bates *et al.,*1973).

RESULTS AND DISCUSSION

1. Vegetative growth

a. Plant height and number of branches/plant:

Data in Table (2) indicated that irrigation with saline water had a significant effect on plant height and number of branches/plant in both seasons. It was clear that the tallest plants in the first and second seasons at the two cuts were irrigated with non saline water (tap water) giving 48.48 and 48.34 cm at first and second cuts in the first season respectively. The same trend was observed in the two cuts of the second season. The shortest plants were those irrigated with saline water at 4000 ppm NaCl as shown in Table (2).

As for the effect of antistress agents (DDM and SA) on plant height, the application of antistress agents was found to have a significant effect in this respect. The addition of DDM had a significant effect on plant height of rosemary plants in the two seasons except for the first cut of the first season *ie*. rosemary plants showed no significant effect on plant height due to DDM at the first cut only. Tallest plants were those received DDM at 6 g/10 kg soil giving 43.57 and 40.78 cm at the first and second cuts respectively. In the second season the recorded data were 50.92 and 45.85 cm at the first and second cuts respectively. Control plants were the shortest plants giving 41.46 cm for the first cut and 38.87 cm for the second one in the first season, while in the second season the value were 48.05 and 44.15 cm at first and second cuts respectively. These results are in agreement with those reported by (Tahir *et al.,* 2006; Ulmer, 2010 and Abdalla, 2011 b). Diatomaceous earth (DDM) being chemically of Sio_2 (82-89%), Si application can balance nutrient element in plant tissue through the suppression of Al, Mn and Na and by mediating the uptake of others, namely P, Mg, K, Fe, Cu and Zn. Tuna *et al.* (2008) found that the concentrations of Ca and K in wheat plants depressed under salinity but increased markedly in shoots and roots after Si treatment. Na uptake was higher in plants grown under salinity, however Si application significantly reduced Na and Cl uptake, resulting in a significant increase in K:Na selectivity ratio in shoots (Tahir *et al.,* 2006 and Ulmer, 2010). Also the efficacy of salicylic acid (SA) as antistress substance has been discussed in many investigations. Salicylic acid (SA) has been shown to be an essential signal molecule involved in both local defense and induction of systemic resistance response of plants after salt stress. SA a plant phenolic, is now considered as a hormone like endogenous regulator (Wasti *et al.,* 2012). The addition of SA to broad bean plant significantly correct the negative effects of sea water irrigation on growth parameters (Azooz *et al.,* 2011). Also Syeed *et al.* (2011) on *Brassica juncea* L. reported that application of 0.5 mM SA alleviated the negative effects of 50 mM NaCl maximally, but 1.0 mM SA proved inhibitory.

Regarding the interaction between irrigation with saline water and antistress agents (DDM and SA) treatments on plant height, it was clear that there were insignificant differences on plant height at the two cuts in both seasons compared with control plants (grown at normal conditions) as shown in Table (2). These results mean that antistress agents gave positive effect on growth parameters (in term of plant height and branches), they reduced the harmful effect of salinity.

The same trend was observed in number of branches/plant the highest number of branches was recorded when the plants were irrigated with non-saline water (tap water) giving 18.30 and 18.01 branches/plant in the first and second cuts of the first season respectively. Also in the second seasons, the values were 19.08 and 18.80 branches/plant at the first and second cuts respectively.

Regarding the effect of antistress agents on number of branches data presented in Table (2) showed that the highest values of the first season were obtained when

Table 2. Effect of irrigation with saline water, diatomaceous earth and salicylic acid treatments on plant height (cm) and number of branches/plant of *Rosmarinus officinalis* L. plants in 2014 and 2015 seasons for two cuts.

Saline water / ANS	1st season								2nd season							
	1st cut				2nd cut				1st cut				2nd cut			
	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean
Plant height (cm)																
Control	47.59	44.43	32.37	41.46	47.99	41.27	27.34	38.87	54.16	51.42	38.56	48.05	49.35	45.82	37.27	44.15
DDM (6 g/10 kg soil)	49.35	45.13	36.24	43.57	49.45	43.63	32.25	40.78	57.91	54.85	40.00	50.92	50.91	47.39	39.24	45.85
SA (0.2 mM)	48.49	44.80	35.64	42.78	47.59	41.42	31.43	40.15	56.19	51.81	39.28	49.09	50.72	46.52	38.05	45.10
Mean	48.48	44.78	34.55		48.34	42.11	30.34		56.09	52.69	39.28		50.33	46.58	38.19	
LSD at 5% Irrigation	2.30				4.05				2.36				3.28			
LSD at 5% ANS	NS				1.73				1.50				1.78			
LSD at 5% Interaction	NS				NS				NS				NS			
Number of branches/plant																
Control	16.65	11.61	5.60	11.29	16.34	10.75	5.08	10.72	17.90	12.45	7.60	12.65	17.61	11.86	7.19	12.22
DDM(6 g/10 kg soil)	19.72	15.19	9.84	14.92	19.43	14.48	9.43	14.45	20.26	15.98	10.64	15.63	19.81	15.58	10.15	15.18
SA(0.2 mM)	18.53	12.87	8.31	13.24	18.25	12.58	7.91	12.91	19.08	14.10	9.14	14.11	18.99	13.90	8.99	13.99
Mean	18.30	13.22	7.92		18.01	12.60	7.47		19.08	14.18	9.13		18.80	13.81	8.78	
LSD at 5% Irrigation	0.76				0.60				0.50				0.52			
LSD at 5% ANS	0.71				0.61				0.51				0.54			
LSD at 5% Interaction	NS				NS				NS				NS			

S_0 = NaCl 0 ppm — **DDM = diatomaceous earth**
S1= NaCl 2000 ppm — **SA = salicylic acid**
S2= NaCl 4000 ppm — **ANS = antistress agents (DDM and SA)**

rosemary plants treated with DDM at 6 g/10 kg soil giving 14.92 and 14.45 branches/plant at the first and second cuts and 15.63 and 15.18 in the second season at the first and second cuts respectively. The lowest values 11.29 and 10.72 branches/plant was obtained from untreated plants (control) in the first season at the two cuts. In the second season the values were 12.65 and 12.22 branches/plant in the first and second cuts respectively.

Concerning the effect of interaction on number of branches/plant the results showed insignificant differences in both seasons at the two cuts.

b. Herb fresh and dry weights of aerial parts:

Data presented in Table (3) showed that, irrigation with saline water had a significant effect on herb fresh and dry weights in the two seasons. Increasing the concentration of NaCl in water significantly decreased herb fresh and dry weights of aerial parts in both seasons in the two cuts. The lowest fresh and dry weights/plant were recorded when the rosemary plants were irrigated with saline water at 4000 ppm NaCl . These results may be due to salt stress is known to induce oxidative damage to plant cells from reactive oxygen species this can lead to a reduction in plant yield (Azevedo *et al.,* 2006). The reactive oxygen species this can damage membranes, photosynthetic pigments proteins, DNA and lipids (Fahmy *et al.,*1998) for stress protection, plants have developed enzymatic and non enzymatic scavenging mechanisms for the reactive oxygen species (Demiral and Turkan, 2005). These scavenging mechanisms, such as the production of catalase to reduce hydrogen peroxide (Herandez *et al.,* 2000) enable the plant to maintain growth under stress condition.

As for the effect of antistress agents (DDM and SA) on the fresh and dry weights of rosemary plants, it was clear that diatomaceous earth (DDM) was effective in this concern than the salicylic acid (SA). The highest values recorded when rosemary plants were treated with DDM at the rate 6 g/10 kg soil giving 122.77, 120.33 g fresh weight and 54.45, 53.31 g dry weight/plant in the first and second cuts, respectively. The same trend was observed in the second season for the two cuts, the values were (128.28, 124.74 g fresh and 56.76, 55.20 g dry weight/plant). These results may be due to high silica uptake which improved resistance and increased plant growth rate and yield (Marschner, 1986; Pirorr, 1986 and Belanger, 1995). Lowest fresh and dry weights were obtained from untreated plants (control).

Concerning the interaction effects between irrigation with saline water treatments and antistress agents (DDM and SA). Data in Table (3) showed that highest values (161.54, 161.40 g/plant fresh weight and 71.85, 71.18 g dry weight/plant) recorded when the plants were irrigated with non saline water (tap water) and treated with DDM at 6 g/10 kg soil. The same trend was observed in the second season.

These results are in agreement with (Marschner, 1986; Pirorr, 1986; Belanger *et al.,* 1995 and Abdalla *et al.,* 2011 b) reported that, the application of diatomaceous earth (DDM) has been shown to correct the negative effects of salinity on plant growth. Also (Shabani *et al.,* 2009 and Simaei *et al*., 2011) stated that spraying salicylic acid (SA) improved plant resistance and decreased the deleterious effects induced by NaCl salinity.

The increment in herb weight may be due to effective role of both DDM and SA in increasing photosynthesis consequently more metabolic activities which led to an increment in plant growth.

2. Volatile oil percentage:

Data in Table (4) showed the effect of irrigation with saline water, diatomaceous earth (DDM), salicylic acid (SA) and the interaction between them on volatile oil of rosemary plants. It was found that, volatile oil percentage showed a decreased tendency

Table 3. Effect of irrigation with saline water, diatomaceous earth and salicylic acid treatments on herb fresh and dry weights/plant (g) of *Rosmarinus officinalis* L. plants in 2014 and 2015 seasons for two cuts.

Saline water / ANS	1st season								2nd season							
	1st cut				2nd cut				1st cut				2nd cut			
	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean
Herb fresh weight/plant (g)																
Control	137.06	93.35	49.32	93.24	135.61	88.24	45.69	89.85	142.32	106.55	53.55	100.81	138.45	102.48	51.07	97.33
DDM (6 g/10 kg soil)	161.54	129.73	77.04	122.77	160.40	125.48	75.10	120.33	164.32	135.61	84.91	128.28	162.57	130.67	80.98	124.74
SA (0.2 mM)	150.22	114.61	61.56	108.80	149.02	109.46	56.58	105.02	158.36	121.01	69.98	116.45	155.07	119.34	65.28	113.23
Mean	149.61	112.56	62.64		148.34	107.73	59.12		155.00	121.06	69.48		152.03	117.50	65.78	
LSD at 5% Irrigation	2.62				3.46				0.95				1.83			
LSD at 5% ANS	1.70				2.45				1.70				1.45			
LSD at 5% Interaction	NS				4.25				2.55				2.44			
Herb dry weight/plant (g)																
Control	60.64	45.85	21.82	42.60	60.00	39.05	20.22	39.76	62.97	47.15	23.69	44.60	61.26	45.35	22.60	43.07
DDM(6 g/10 kg soil)	71.85	57.40	34.09	54.45	71.18	55.52	33.23	53.31	72.71	60.00	37.57	56.76	71.94	57.82	35.83	55.20
SA(0.2 mM)	66.47	50.72	39.27	52.15	65.94	48.43	25.03	46.47	70.07	53.54	34.09	52.57	68.70	52.80	28.89	50.13
Mean	66.32	51.32	31.73		65.71	47.67	26.16		68.58	53.56	31.78		67.30	51.99	29.11	
LSD at 5% Irrigation	7.05				1.09				0.65				0.81			
LSD at 5% ANS	9.35				1.54				0.65				0.64			
LSD at 5% Interaction	NS				1.88				1.13				1.08			

S_0 = NaCl 0 ppm **DDM = diatomaceous earth**
S_1 = NaCl 2000 ppm **SA = salicylic acid**
S_2 = NaCl 4000 ppm **ANS = antistress agents (DDM and SA)**

Table 4. Effect of irrigation with saline water, diatomaceous earth and salicylic acid treatments on volatile oil percentage of *Rosmarinus officinalis* L. plants in 2014 and 2015 seasons for two cuts.

	Volatile oil percentage (dry herb)															
Saline water	1st season								2nd season							
	1st cut				2nd cut				1st cut				2nd cut			
ANS	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean	S_0	S_1	S_2	Mean
Control	0.389	0.352	0.354	0.321	0.384	0.349	0.319	0.351	0.382	0.346	0.317	0.348	0.378	0.344	0.315	0.346
DDM (6 g/10 kg soil)	0.432	0.375	0.383	0.342	0.428	0.373	0.338	0.380	0.425	0.369	0.335	0.376	0.422	0.366	0.331	0.373
SA (0.2 mM)	0.418	0.364	0.370	0.328	0.411	0.360	0.327	0.366	0.407	0.358	0.324	0.363	0.403	0.354	0.322	0.360
Mean	0.413	0.364	0.330		0.408	0.361	0.328		0.405	0.358	0.325		0.401	0.355	0.323	
LSD at 5% Irrigation	0.008				0.008				0.009				0.007			
LSD at 5% ANS	0.003				0.004				0.004				0.003			
LSD at 5% Interaction	0.007				0.007				0.008				0.007			

S_0 = NaCl 0 ppm **DDM = diatomaceous earth**
S_1 = NaCl 2000 ppm **SA = salicylic acid**
S_2 = NaCl 4000ppm **ANS = antistress agents (DDM and SA)**

with increasing NaCl concentration *ie*. increasing the concentration of NaCl from 0 up to 4000 ppm resulted in significant decreases in volatile oil percentage in both cuts in the two seasons. These results may be explained through the findings of (Khalaga *et al.,* 2009) on *Vicia faba* concluded that, salinity affects the plant at all growth stages but this effect differs according to growth stages and species. The major effect of salinity on plant growth has been attributed to osmotic inhibition of water availability, the toxic effect of ion and nutritional imbalance caused by such ions.

Regarding the effects of DDM and SA, it was clear that all treatments significantly increased volatile oil percentage as compared to control. The highest volatile oil percentage recorded when rosemary plants were treated with DDM at 6 g/10 kg soil giving (0.342 and 0.380%) in the two cuts of the first season, respectively. The same trend was observed in the second season for the two cuts. The values were (0.376 and 0.373%). Also, it was noticed that, SA affected positively on the volatile oil content in comparison with control.

As for interaction between irrigation with saline water and antistress agents the results showed significant differences in volatile oil percentage in the two cuts in both seasons. The highest volatile oil percentage was obtained from plants irrigated with tap water interacted with DDM at 6 g/10 kg soil giving 0.432 and 0.428% in the first season and 0.425 and 0.422% in the second season in both cuts. The increment in volatile oil percentage may be due to that diatomaceous earth (DDM) correct the negative effect of salinity on chlorophyll content and on photosynthetic activity. DDM promote salt tolerance in plants by enhancing the activity of antioxidant enzymes which in turn decreases the permeability of plasma membrane and in the same time increases its integrity, stability and functioning (Savvas *et al.,* 2009). Also salicylic acid (SA) alleviates the negative effects of NaCl. It participates in the regulation of many physiological processes in plant body, maintains water homeostasis and triggers defense mechanism (Syeed *et al.,* 2011 and Danish *et al.,* 2012).

3. Volatile oil constituents:

GLC analysis were carried out on the essential oil of rosemary plants of 3 treatments in the first cut of the first season, S0+DDM at 6 g/10 kg soil, S2 at 4000 ppm NaCl and S2+ DDM at 6 g/10 kg soil . Data were recorded in Table (5) and Figures (1-3) indicated that, camphor content was influenced under these treatments. It showed an increase in case of S0 with DDM at 6 g/10 kg soil (34.35%). While it was decreased in case of S2 and S2+DDM treatments. The opposite trend was recorded in case of β pinene which reached 12.56 and 12.38% in S2 and S2+DDM treatments respectively while it was 6.23% in S0+DDM treatment. Also, 1, 8-cineole showed the same trend of β pinene. As for Borneol, this compound recorded the highest value in the essential oil of S0+DDM treatment.

4. Proline content:

Data in Table (6) indicated that proline content significantly increased with increasing NaCl concentration in water. The highest proline value (1499.67 µg/g dry leaves) was obtained from plants which were irrigated with saline water at 4000 ppm NaCl, followed by plants irrigated with saline water at 2000 ppm NaCl compared to those received non saline water (tap water) giving (191.67 µg/g dry leaves). These results are in agreement with (Mansour, 2000 and Abraham *et al.,* 2003) they mentioned that proline accumulate in plants subjected to salt stress.

Regarding antistress agents (DDM and SA), they had significant effect on proline content. Applied DDM at (6g/10 kg soil) gave the lowest proline content in this concern the value was (333.33 µg/g dry leaves), followed by foliar spray with SA (0.2 mM**)** giving (824.33 µg/g dry leaves). These results may be due to the beneficial role of Silicon (Si) which represent 89.00% of DDM for the plant growth and

Table 5. Effect of irrigation with saline water, diatomaceous earth (DDM) treatments on volatile oil constituents (%) of *Rosmarinus officinalis,* L. plants in the 1st Season (1st cut).

Treatments / Volatile oil components	S_0+DDM	S_2	S_2 + DDM
β–Pinene	6.23	12.56	12.38
Camphene	1.34	2.84	2.31
Limonene	1.81	3.14	2.99
1,8- Cineole	10.25	16.88	16.09
Camphor	34.35	23.23	29.79
α-Terpinode	2.99	2.05	2.26
Borneol	7.11	1.34	1.58
Bornyl acetate	7.52	10.59	4.62
Eugenol	7.91	7.35	5.00
β-Caryophyllene	3.10	3.06	4.64
Unkown	17.39	16.96	18.34

S_0 = NaCl 0 ppm **DDM = 6 g/ 10 kg soil**
S_1 = NaCl 2000 ppm
S_2 = NaCl 4000 ppm

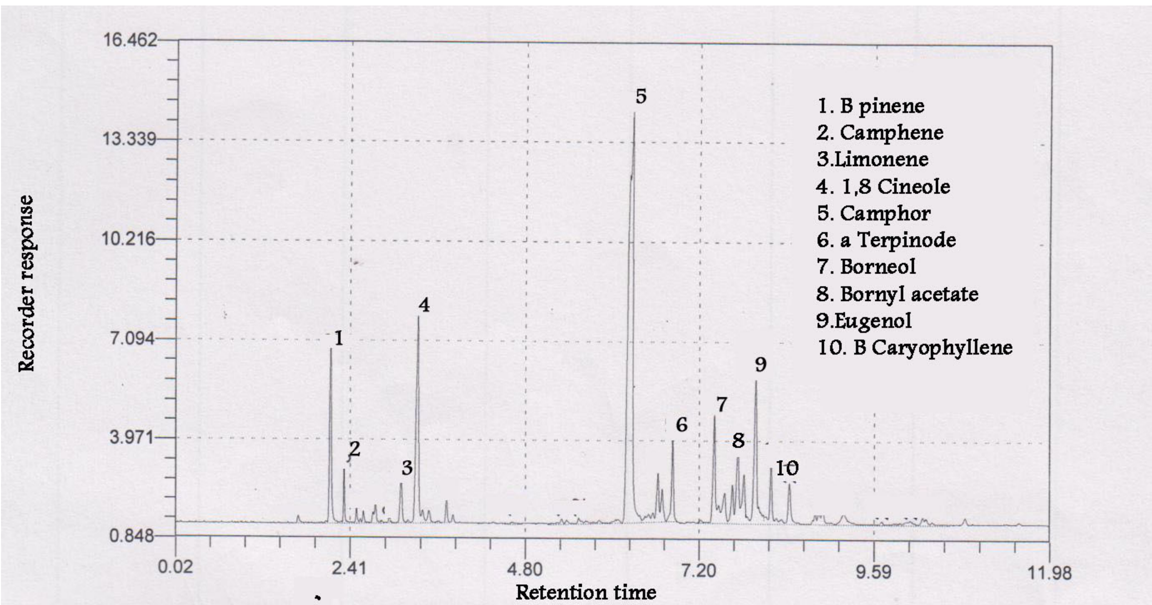

Fig. 1. Chromatogram of rosemary volatile oil under S_0 + DDM at 6 g/10 kg soil.

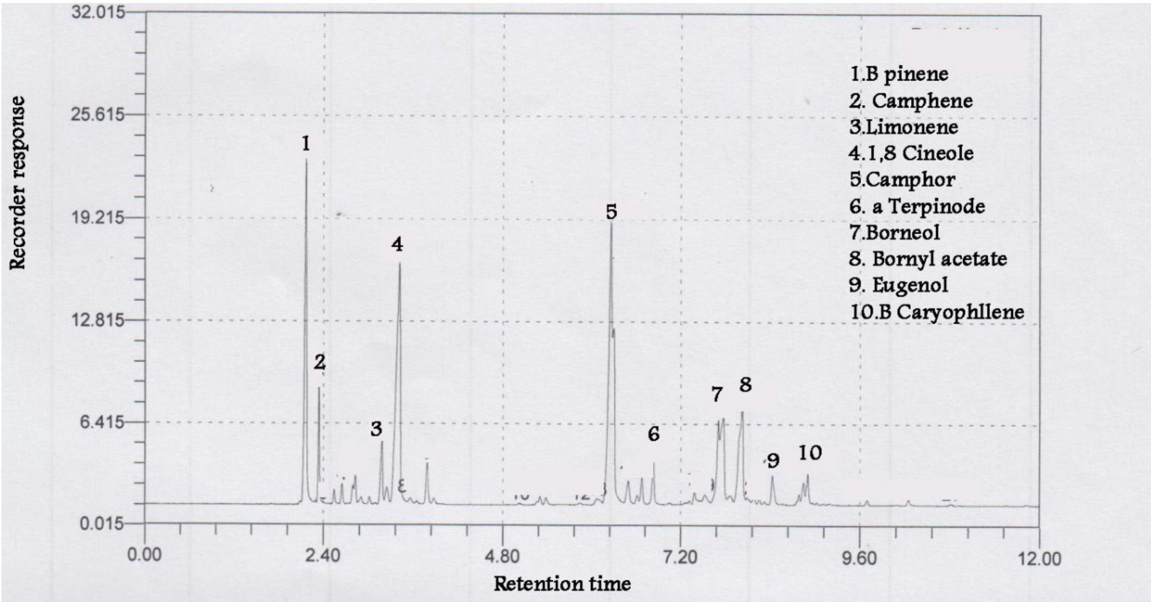

Fig. 2. Chromatogram of rosemary volatile oil under S_2 at 4000 ppm NaCl.

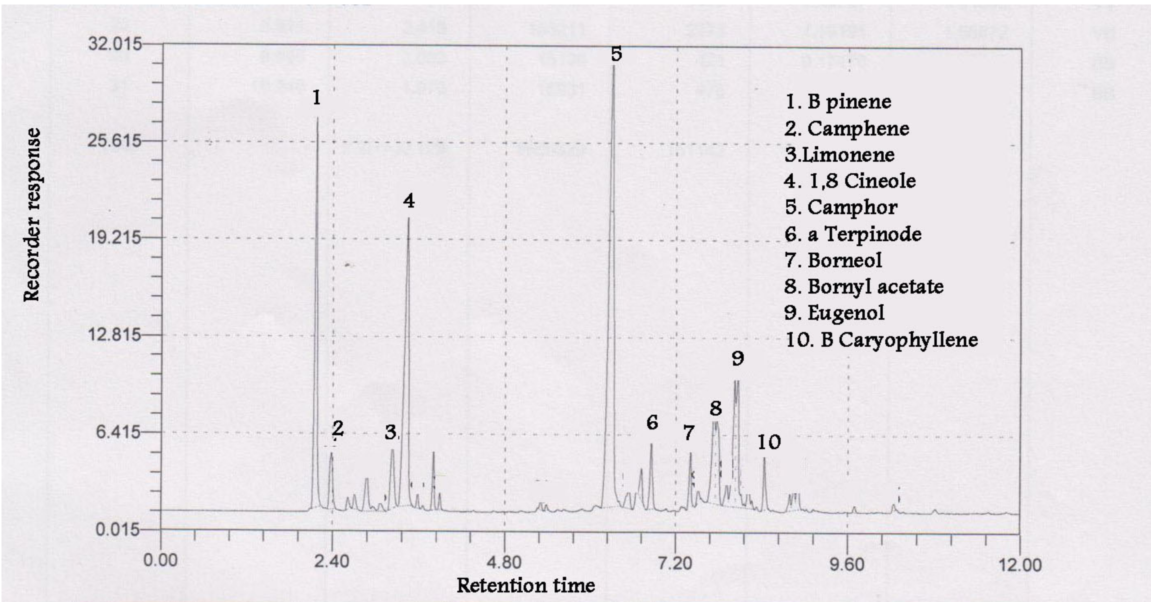

Fig. 3. Chromatogram of rosemary volatile oil under S_2 + DDM at 6 g/10 kg soil.

Table 6. Effect of irrigation with saline water, diatomaceous earth and salicylic acid treatments on proline content (µg/g dry leaves) of *Rosmarinus officinalis,* L. plants in the 1st season (1st cut).

Saline water / ANS	S_0	S_1	S_2	Mean
Control	250.00	400.00	2025.00	891.67
DDM at (6 g/10 kg soil)	150.00	300.00	550.00	333.33
SA at (0.2 mM)	175.00	374.00	1924.00	824.33
Mean	191.67	358.00	1499.67	
LSD at 5% Irrigation	6.61			
LSD at 5% ANS	6.40			
LSD at 5% Interaction	10.53			

S_0 = Na Cl 0 ppm **ANS = anti stress agent (DDM and SA)**
S1= Na Cl 2000 ppm
S2= Na Cl 4000 ppm

photosynthetic activity. According to the literature and under the salt stress conditions, Si enhanced K^+:Na^+ ratio against the toxic effect of Na^+ (Rama and Hussein, 2014). Also, the exogenous application of SA improved plant tolerance to salt stress (Borsoni *et al.*, 2001).

As for the effect of the interaction between saline water and antistress agents (DDM and SA), the results showed a significant difference in proline content. The lowest proline content (150.00 µg/g dry leaves) achieved when rosemary plants were irrigated with non saline water (tap water) and received DDM at (6 g/10 kg soil) compared to those irrigated with saline water at 4000 ppm NaCl and untreated with antistress agents (control) which gave (2025.00 µg/g dry leaves).

CONCLUSION

It could be concluded that the application of antistress agents (DDM and SA) correct the negative effect of salinity on rosemary plants. The application of DDM at (6 g/10 kg soil) was the most effective treatment. It gave the highest vegetative growth, the highest yield of fresh and dry herb as well as oil percentage and the highest main constituents in volatile oil.

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استجابة نباتات حصا لبان المتأثرة بالملوحة لمضادات الاجهادات

حنان محمد حرب على[*] و مني جميل عطية[**]

[*]معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.

[**]معمل بحوث الأراضي الملحية والقلوية بالإسكندرية، مركز البحوث الزراعية، الجيزة، مصر.

أجريت هذه الدراسة خلال موسمين متتالين (٢٠١٤/ ٢٠١٥) فى مزرعة معمل بحوث الاراضى الملحية والقلوية بالاسكندرية بهدف دراسة مدى استجابة نباتات حصا لبان المروية بالماء المالح بتركيز صفر، ٢٠٠٠ و٤٠٠٠ جزء فى المليون كلوريد صوديوم للمعاملة بمضادات الاجهادات (السالسيلك بتركيز صفر و ٢ملى مولر والصخور الدياتومية بتركيز صفر و ٦ جرام/١٠ كجم تربة و دراسة تاثيرها أيضاً على النمو الخضرى والمحصول الطازج والجاف وكذلك نسبة الزيت الطيار والتركيب الكيماوى للزيت. وكانت النتائج كالتالي: أدت مضادات الاجهادات إلى زيادة النمو الخضرى (إرتفاع النبات و عدد الافرع والوزن الطازج و الجاف للنبات) و نسبة الزيت الطيار زيادة معنوية مقارنة بالكنترول فى معظم المعاملات فى كلا الموسمين. وتم الحصول على افضل النتائج من التفاعل بين اضافة الصخور الدياتومية بتركيز ٦جرام /١٠ كجم تربة مع الرى بماء الصنبور حيث اعطت أعلى نمو خضرى وكذلك أعلى نسبة للزيت الطيار وذلك فى

كلا الحشتين للموسمين . وقد حققت هذه المعاملة ايضا أعلى نسبة للمكونات الرئيسية في الزيت. وفيما يتعلق بمحتوى البرولين فقد ادت إضافة مضادات الإجهادات (السالسيلك و الصخور الدياتومية) إلى تقليل محتوى البرولين في النبات. وهذه النتائج تشير إلى إستجابة نباتات حصا لبان النامية تحت ظروف الملوحة لمضادات الاجهادات.