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STRUCTURAL AND PHYSIOLOGICAL RESPONSES OF ROSEMARY (*Rosemarinus officinalis*, L.) PLANTS UNDER SALINITY CONDITIONS

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ABSTRACT

A pot experiments were carried out during two successive seasons of 2003/2004 and 2004/2005 at the Experimental Station of Medicinal and Aromatic Plants, Fac. of Agric., Mansoura Univ., to study the effect of different salinity level of diluted sea water (2.5, 5 and 10%), which were equivalent to the application of 825, 1650 and 3300 ppm, respectively on growth, oil content, oil components and chemical composition of rosemary (*Rosemarinus officinalis*, L.). Whereas control plants were irrigated with tap water.

The results showed that increasing salinity levels reduced all growth traits (plant height, number of branches/plant, herbage fresh and dry weights) compared to the control. All saline water treatments decreased the content of chlorophylls a and b, oil percentage and essential oil yield/plant, as well as K percentage and K: Na ratio in herb, during the cuts harvested, in both seasons. Reducing, non-reducing and total soluble sugars (%), free proline content (mole/g F.W.) and Na and Cl percentages in herb showed opposite trend. Gas liquid chromatography analysis of samples revealed seventh identified compounds. Salinity had a clear effect on the chemical composition of the extracted rosemary oil, since some of the major constituents increased such as α-Terpinene, Cineole, Linalool and Borneol. While, P-cymene and Thymol decreased with the higher salinity levels.

Anatomically, salinity decreased stem diameter, cortex thickness, phloem, xylem and pith tissues thickness. Width of cambial zone were also decreased. Moreover, salinity decreased leaf thickness, palisade and spongy tissues thickness, midvein vascular bundle dimension as well as xylem and phloem tissues thickness.

INTRODUCTION

Rosemary (*Rosemarinus officinalis*, L.) is one of the most important aromatic and medicinal species of the family of *Lamiaceae* in all Mediterranean basins. It grows as a small shrub with perennial foliage and small leaves and is an important herb cultivated or imported and sold in many markets all over the world (Robbins and Greenhhalgh, 1979). The external uses of rosemary are similarly numerous. Rosemary tea is recommended for treatment of eye diseases. Young shoots and leaves are used in aromatic baths after childbirth. Bronchitis related coughing is relieved by smoking cigarettes of dried rosemary leaves, and by inhaling the stem of decoction of rosemary. In addition, the decoction of rosemary alone or with thyme is used as a rub to reduce fever (Boukef, 1986). Rosemary oil has been widely used for countries as an ingredient mainly in cosmetics, soaps, perfumes as well as in the flavoring and conservation of food products and in the pharmaceutical industry. In addition, rosemary oil is used in the preparation of ointments to soothe rheumatism, sprains, wounds and eczemas and is mixed with other drugs as an antiseptic and insecticide (Arnold *et al.*, 1997).

In Egypt, increasing aromatic plant production is dependent upon irrigated agricultural because the rainfall is insufficient to grow crops. Unfortunately, the quantity of good quality of water supplies available to agriculture, is limited. Therefore, saline water ought to be used in these causes for irrigation.

Salinity is known to retrial plant growth through its influence on several facts of plant behavior like osmotic adjustment, ion uptake, protein and nucleic acid synthesis, photosynthesis, enzyme activities and hormonal balance (El-Kheir *et al.*, 2001); Lead to a reduction in the content of other nutrients and inhibited protein synthesis. These changes were associated with the modification in the internal stucture of plant organs (Santos *et al.*, 2001).

At higher salinities, it was known that higher salinity induce low energy case, inactivate the ATP dependent ion transporter H-ATP-ase system, impair membrane integrity and selectivity (low K/Na), sharp increase in Na^+ and Cl^- uptake (Drew and Lauchli, 1985 and Montero *et al.*, 1998). In addition, free proline accumulation in plants was found with salt stress osmo regulation of solutes in many plants (Morgan, 1990) It has been found to be accumulated in plants subjected to the severe condition of both drought and salt stress. Proline in a plant under salt stress could act as both a nitrogen reserve can be easily converted to glutamate which takes part in the synthesis of other essential amine acids and in osmo regulation. There is a negative correlation between proline content and salt tolerance (Ashraf, 1994).

As a result, the stressed plants became severely susceptible to osmotic/turgor effects, specific salt ions effects, nutritional imbalance and disorders and energy lack. Thereby impaired chlorophyll, carbohydrate and protein metabolism, in turn serious growth and yield decline with salinity (Maiti *et al.*, 2002). Many authors studied the effect of different levels of saline water irrigation on the various plant growth traits and obtained negative responses, i.e. Aziz and Youssef (2001) on rosemary, Massoud (2002) on thyme, El-Hindi and El-Ghamry (2005) on cherrygold and El-Hindi (2006) on pot marigold plants.

The present study was conducted to test the suitability of rosemary plants for cultivation under conditions of saline irrigation water to determine their tolerance and to evaluate changes in physical and chemical composition.

MATERIALS AND METHODS

Pot experiments were carried out at the Experimental Station of Medicinal and Aromatic Plants, Fac. of Agric., Mansoura Univ. during the two successive seasons of 2003/2004 and 2004/2005.

Uniform terminal cuttings (thirty day old) of *Rosemarinus officinalis*, L. about 10 – 12 cm length bearing 10 – 12 leaves, were planted in the nursery under shaded conditions for rooting. After three months, on October, 15th in both seasons the rooted cuttings were individually transplanted in clay pots (30 cm in diameter) filled with loamy clay soil. The physical and chemical characteristics of the used soil were determined according to Chapman and Pratt (1978) and presented in Table (1).

Table 1: Soil physical and chemical characters in two seasons.

Properties	2003/2004	2004/2005
Sandy %	35.5	35.0
Silt %	21.0	22.0
Clay %	43.5	43.0
Texture	Clay loam	Clay loam
pH (1 : 2.5 suspension)	8.2	8.5
EC (1 : 2.5 extracts) dSm^{-1}	2.3	2.5
Calcium carbonate %	23.0	25.0
Organic matter %	1.2	1.0
Total nitrogen mg/100 g soil	42.0	40.0
P mg/100 g	1.8	1.7
K mg/100 g	23.0	25.0
Ca mg/100 g	504.0	500.0
Mg mg/100 g	40.0	42.0
Fe ppm	5.0	4.5
Mn ppm	6.0	5.5
Zn ppm	1.0	0.9
Cu ppm	3.0	3.2

The pots were held under natural environmental conditions and all pots were irrigated with tap water for 15 days, during which, the success of transplants took place, then the irrigation with saline water started using sea water, obtained from Mediterranean sea, Gamasa region, Dakahlia Governorate (EC = 51.56, dSm^{-1} = 32998.4 ppm about 33000 ppm). Four levels of salinity at the rate of 0.0, 2.5, 5 and 10% sea water which were equivalent to the application of 825, 1650 and 3300 ppm, respectively. While, the control were irrigated with tap water. The pots were weighted regularly every 3 days intervals to determine the water amounts required to raise the soil moisture to 70% of field capacity. The chemical analysis of sea water shown in (Table 2). Plants were fertilized with 2.5 g ammonium sulfate (20.6% N), 3.59 calcium super phosphate (15.5% P_2O_5) and 1.25 g potassium sulfate (48% K_2O) per pot after 3 weeks from transplanting. The lay out of the experiment was in complete randomized block design with three replicates, each replicate contained fifteen pots.

Table 2: Sea water analysis (ppm).

Na	Mg	K	Ca	S	CL	Br
10770	1290	380	412	905	19400	65

All the plants received normal agricultural practices, whenever they were needed. Two cuts were harvested, the first at April, 1st, and the second three months later. The data recorded for each cut included:

Growth parameters

The following parameters were recorded: plant height (cm), number of branches/plant and both fresh and dry weights of herb/plant (g).

Biochemical determinations:

1- Total soluble sugars, reducing sugar and non-reducing sugar percentage were assessed according to A.O.A.C. (1990).
2- Free proline content (µmole proline/100 g fresh weight) in herb was determined according to Bates *et al.* (1973) using a calorimetric method at 520 nm.
3- Chlorophyll a and b (mg/g fresh weight) were determined using the method of Moran (1982).
4- Sodium, potassium and chloride contents: Sodium, potassium and chloride were extracted from dried herb material according to Chaudhary *et al.* (1996). Sodium and potassium contents in the aqueous extracts were measured with Flam Photometer. Meanwhile, chloride was determined by titration with 0.001 N $AgNO_3$ using potassium chloride as indicator.

Essential oil extraction and determination:

1- Essential oil percentage in the dried herb of each treatment was determined by hydro-distillation according to the Egyptian Pharmacopoeia (1984). Qualitative and quantitative determinations of the different main constituents of rosemary oils obtained from the first cut of each treatment had been carried out in parallel with authentic samples of different oil components by Gas Liquid Chromatographic (GLC) technique.
2- Oil yield (ml/plant): was calculated in proportion to the herb fresh weight/plant.

Anatomical studies:

For the anatomical studies, specimens (10 mm) were taken from the middle part of the 3rd internode of one year old stem and from the middle part (5 x 5 mm) of the leaf of rosemary plants. Samples were fixed in FAA, 10% alcohol solution, dehydrated in ethyl alcohol series, cleared with xylene and embedded in paraffin wax (55 – 58°C – m.p). Cross sections (12 – 15 µm) thich were prepared by a rotary microtome, stained with saffranin-light green combination, cleared in clove oil and mounted in Canada balsam (Gerlach, 1977). Sections were examined microscopically.

Statistical analysis:

A randomized complete block design with four replicates was used. Data were subjected to the statistical analysis according to Steel and Torrie (1980). The treatment means were compared using the least significant difference (LSD) test at the 0.05 level.

RESULTS AND DISCUSSIONS

Growth parameters:

Data presented in Table (3) show that salinity at all concentrations (2.5, 5 and 10%) caused a significant reduction in rosemary plant growth expressed as plant height (cm), number of branches per plant and both fresh and dry weights (g/plant). The great reduction in these parameters was observed under highest salinity level (10%).

The depressing effect of salinity on plant growth may be a result of an inhibition of cell division, cell elongation and internal hormonal imbalance (Younis *et al.*, 2003). Moreover, the retardation in plant growth caused by salinity may be attributed mainly to the osmotic stress, which reduced the availability and uptake of water and essential nutrients (Neumann, 1997) as well as, the excessive accumulation of both toxic ions, i.e. Na^{+} and intermediate compounds such as reactive oxygen species which cause oxidative damage to DNA, lipids and proteins and consequently a decrease in plant growth (Rodriguez *et al.*, 2004).

These results were in harmony with those reported by Khalil (2002) on rosemary, Ghallab and El-Ghadban (2003) on marjoram, El-Hindi and El-Ghamry (2005) on cherrygold and El-Hindi (2006) on pot marigold plants.

Table 3: Effect of salinity levels on some growth parameters of rosemary plants during the two successive seasons of 2003/2004 and 2004/2005.

Growth parameters	Plant height (cm)		No. of branches/plant		Fresh weight g/plant		Dry weight g/plant	
	First season							
Salinity levels / Cuts	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	70.62	59.65	24.18	17.39	344.72	225.57	125.85	115.46
2.5%	68.29	58.54	22.06	16.51	315.67	217.43	122.11	109.50
5.0%	60.56	53.26	18.61	13.95	236.34	199.75	107.07	101.19
10.0%	56.69	51.55	13.23	12.32	199.72	186.56	101.41	97.49
L.S.D 5%	2.04	2.35	1.64	1.16	8.27	8.91	4.62	5.93
	Second season							
0.0	71.57	60.53	26.67	21.04	360.72	266.25	145.65	128.18
2.5%	69.62	55.71	23.05	17.23	344.17	237.57	129.78	122.82
5.0%	61.87	55.41	21.13	15.04	274.94	212.87	114.51	105.93
10.0%	58.63	52.89	14.88	13.12	224.29	200.35	105.62	98.36
L.S.D 5%	2.38	2.59	1.14	1.32	10.59	13.70	2.58	3.73

Biochemical determinations:

1- Reducing, non-reducing and total soluble sugars:

Data in Table (4) reveal that, reducing, non-reducing and total soluble sugars of rosemary herb were increased significantly with increasing salinity level. In both seasons, plants irrigated with tap water (control) gave the lowest values. While, raising the salt concentration resulted in a gradual increase in these parameters, since the highest salt concentration (10% sea water) gave the highest values. It has been suggested that sugar accumulation in plants exhibited by rosemary may indicate a strategy to cope with the enhancement in the solute and osmotic potential of the media. This accumulation has been attributed to an impaired carbohydrate utilization (Munns and Termaat, 1986) and reduced respiration rate at high salinity level. An increase of total carbohydrate content in the plant organs was observed in NaCL treatment. This was due to an increase in glucose concentration not only in the leaves but also in the roots, associated to the presence of high concentration of CL^- in the plant tissues (Fernandez-Ballester *et al.*, 1998), which would be indicative of an important role of ion i.e. CL in any step of synthesis and/or degradation of carbohydrates. The glucose accumulation could be explained by decreasing starch synthesis and by starch degradation. In addition, Stoop and Pharr (1994) stated that the increase in the soluble sugars i.e. glucose caused by a decreased demand for carbon due to stunting of plants under salinity stress. These findings are in agreement with those obtained by Hussein *et al.* (2000) on canola plants.

2- Proline content:

Data presented in Table (4) show that, in most cases, the proline content in the herb of rosemary plants was increased steadily with raising the salt concentration. Corresponding results presented in Table (4) show that plants irrigated with tap water had the lowest mean of proline contents (5.06 and 4.83 moles/g fresh matter for two cuts in the 1st season, respectively). While, proline content averaged 5.73 and 5.59 for two cuts in the 2nd season, respectively. On the other hand, those receiving the highest salt concentration (10% sea water) gave the highest mean values (11.32 and 11.54 moles/g fresh matter for two cuts in the 1st season, respectively). Whereas, this trait averaged 10.49 and 11.47 moles/g for two cuts in the 2nd season, respectively. The increase in the proline content under saline conditions may lead to the conclusion that proline plays an important role in plant tolerance to salinity. This role was explained by Greenway and Munns (1980) who mentioned that proline can be considered as stabilizer of osmotic pressure within the plant cell. Also, Marcum and Murdoch (1994) concluded that proline can make a substantial contribution to cytoplasmic osmotic adjustment. Moreover, Taiz and Zeiger (1998) mentioned that proline accumulates in the cytoplasm under conditions of stress, in order to maintain water potential equilibrium within the cells. Other functions of proline accumulation have also been proposed to include; proline act as a storage compound for energy and reduced carbon and nitrogen needs (Stewart and Lee, 1974); Provides as a radial for detoxification (Smironff and Cumbes, 1989); It improves the stability of some cytoplasmic and mitochondrial enzymes (Nash *et al.*, 1982), and increases the solution of protein (Paleg *et al.*, 1984). Proline accumulation due to salinity conditions was observed, also, by several investigators on different plants as Zidan and Al-Zahrani (1994) on sweet basil, Meawad and Faiad (1999) on *Nigella sativa* and Khalil (2002) on rosemary plants.

Increasing of total soluble carbohydrates in Table (4) may by correlated to proline accumulation, in this concern Larher *et al.* (1993) confirmed the strong correlation between levels of non-structural carbohydrates such as sucrose and induction of proline accumulation. Many authors indicated that the importance of soluble carbohydrates in stimulation of the proline accumulation, through, a) Inhibition of the degradation enzyme of proline (Heineke *et al.*, 1992), meanwhile stimulated the synthesis enzyme of proline, in this concern, Hare and Cress (1996), found that mRNA transcript encoding P5CR was increased in phloem tissue in response to water deprivation. b) High sucrose levels in phloem may be responsible for constitutively elevated levels of proline synthesis in this tissue. The well documented increases in sucrose synthesis in response to osmotic stress provide further evidence in favour of a role for the accumulation of non-structural carbohydrates in the stimulation of proline biosynthesis Quick *et al.* (1989). However, this effect was mediated through the supply of carbon skeleton for proline synthesis (Larher *et al.*, 1993).

Table 4: Effect of salinity on soluble sugars (%) and proline (mole/g F.W.) in the herb of rosemary plants during the two seasons of 2003/2004 and 2004/2005.

Growth parameters	Reducing		Non-reducing		Total		Free proline	
	First season							
Cuts / Salinity levels	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	1.15	1.24	9.84	10.72	10.99	11.95	5.06	5.73
2.5%	1.27	1.29	10.67	11.38	11.94	12.68	8.48	9.07
5.0%	1.35	1.35	10.92	11.54	12.26	12.89	9.70	10.21
10.0%	1.40	1.43	11.72	12.26	13.12	13.69	11.32	11.54
L.S.D 5%	0.04	0.05	1.09	0.98	1.07	0.97	0.65	0.56
	Second season							
0.0	0.99	1.11	8.91	10.22	9.90	11.33	4.83	5.59
2.5%	1.25	1.26	9.85	10.98	11.10	12.24	7.97	8.87
5.0%	1.31	1.29	10.31	11.33	11.62	12.62	9.65	9.97
10.0%	1.36	1.38	10.92	11.82	12.28	13.20	10.49	11.47
L.S.D 5%	0.09	0.05	0.82	0.65	0.81	0.63	0.57	0.61

3- Chlorophyll content:

Data presented in Table (5) show that chlorophylls a and b were reduced in response to increasing the salinity levels in irrigation water. In both seasons, plants irrigated with tap water (control) gave the highest values of these parameters. While, raising the salt concentration resulted in a gradual reduction, since the highest salt concentration (10% sea water) gave the lowest values for chlorophyll a and b. Since, it had a parallel trend to the other growth parameters in response to salinity.

The decrease in photosynthetic pigments under salinity may be due to one or more of the following processes: The inhibitory effect of chloride on the activity of Fe containing enzymes cytochrome oxidase which, in turn, may decrease the rate of chlorophyll biosynthesis and increases chlorophyll degradation (Santos *et al.*, 2001) and causes disruption of chloroplasts by oxidative stress which causes a decrease in chlorophyll content (Rahman *et al.*, 2000). The former results are in harmony with those found by Salama (2004) on chamomile and El-Hindi (2006) on pot marigold plants.

4- Ion content:

Corresponding data in Table (5) show that salinity at all levels (2.5, 5 and 10%) increased gradually Na^+ and CL^- concentrations. These increases were accompanied by a corresponding decline in K^+ concentration and K^+/Na^+ ratio.

Excessive amount of Na^+ and CL^- under saline conditions resulted in less K^+/Na^+ ratio which impaires the selectivity of the root membrane resulted in the passive accumulation of Na^+ in the root and shoot systems (Khan *et al.*, 1997). In addition, the increase in the Na and CL contents as a result of raising the salt concentration, since the accumulation of these elements at high concentrations may interfere with the mechanisms responsible for the closure of stomata, thus resulting in an increase in the rate of transpiration from the plant. This may eventually lead to plant wilting or death (Meidner and Mansfield, 1968).

Table 5: Effect of salinity on the contents of chlorophyll a and b (m/g fresh weight), K, Na, Cl (%) and K: Na ratio in the herb of rosemary plants in the two seasons of 2003/2004 and 2004/2005.

Growth parameters	K%		Na%		K : Na		CL%		Chlorophyll a		Chlorophyll b	
	First season											
Salinity levels \ Cuts	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	1.16	1.23	2.20	2.25	0.55	0.56	2.22	2.30	1.71	1.57	0.46	0.47
2.5%	1.14	1.17	2.52	2.66	0.46	0.48	2.63	2.70	1.66	1.57	0.35	0.40
5.0%	1.03	1.09	2.68	2.89	0.40	0.45	2.86	2.89	1.29	1.21	0.25	0.28
10.0%	0.92	1.02	2.86	2.98	0.29	0.32	2.99	3.07	1.12	1.04	0.22	0.23
L.S.D 5%	0.05	0.08	0.08	0.08	0.02	0.02	0.05	0.10	0.16	0.14	0.06	0.05
	Second season											
0.0	1.15	1.18	2.11	2.19	0.44	0.54	2.17	2.23	1.88	1.65	0.35	0.44
2.5%	1.13	1.14	2.46	2.59	0.41	0.46	2.53	2.64	1.78	1.65	0.30	0.34
5.0%	1.01	1.07	2.61	2.80	0.36	0.39	2.76	2.82	1.36	1.36	0.23	0.30
10.0%	0.90	0.96	2.78	2.93	0.28	0.28	2.95	3.00	1.21	1.14	0.18	0.25
L.S.D 5%	0.02	0.04	0.05	0.07	0.04	0.03	0.05	0.06	0.14	0.09	0.04	0.02

The reduction in internal potassium concentration could be related to: the antagonism between K^+ and Na^+ actions, which increased considerably as salinity increased (Rahman *et al.*, 2002). This antagonism may be due to the direct competition between sodium and potassium on the absorption sites of root, an increase in potassium efflux into the root media due to a disrupt in membrane integrity and an interference with its uptake or its transport caused by Na^+ accumulation (Carmer *et al.*, 1985). Increases in the Na and CL contents with increasing the salinity level have been reported by El-Mahrouk *et al.* (1992) on *Dodonaea viscosa* and El-Khateeb (1994) on *Murraya exotica* and El-Shakhs *et al.* (2002) on the two ornamental shrubs.

Essential oil extraction and determination:

1- Essential oil content:

The effect of different treatments of salinity on the essential oil percentage (ml/100 g V.W. herb) and oil yield/plant in the herbage of rosemary plants in Table (6) reveal that all salinity levels decreased significantly both oil percentage and oil yield with increasing salinity level. The highest level of salinity (10%) showed a pronounced decrease in this respect, in both seasons. Data in (Table 6) reveal that rosemary's oil percentage and yield/plant (ml) were in harmony with growth parameters due to salinity levels. Data presented in the same table show that the lowest oil percentages were (0.11 and 0.16%) for the two cuts in the first season, respectively, when (10% seawater) was applied. The same trend was observed in the second season. The reduction in oil percentage under salinity may be due to its conversion to carbohydrates, which are the essential metabolites in respiration (Yang *et al.*, 1990). Moreover, the reduction in oil yield with higher levels of salinity was due to reduction in oil percentage. In addition, this might be due to inhibition of invertase enzyme activity, photosynthesis and photosynthates correlated with increases in the oxidative enzymes and inhibition of anabolism (Shehata, 1999) and (Rafla, 2001). These results are in accordance with those found by Khalil (2002) on rosemary and Salama (2004) on chamomile plants.

Table 6: Effect of salinity levels on the contents of oil (%) and oil yield (mL/plant) in the herb of rosemary plants during the two seasons of 2003/2004 and 2004/2005.

Treatments	Oil (%)		Oil yield/plant	
	First season			
Salinity levels \ Cuts	1st	2nd	1st	2nd
0.0	0.34	0.44	0.42	0.33
2.5%	0.25	0.33	0.30	0.23
5.0%	0.16	0.23	0.16	0.13
10.0%	0.11	0.16	0.11	0.09
L.S.D 5%	0.03	0.03	0.03	0.01
	Second season			
0.0	0.29	0.34	0.63	0.43
2.5%	0.22	0.29	0.42	0.36
5.0%	0.13	0.21	0.26	0.22
10.0%	0.10	0.14	0.16	0.13
L.S.D 5%	0.02	0.03	0.04	0.02

2- Essential oil constituents:

The gas liquid chromatography determination of the distilled oil obtained from herb of rosemary plant is documented in Fig. (1) which indicated that the highest contents of the principle components increased such as α-Terpinene, Cineole, Linalool and Borneol. On the other hand, the P-cymene and Thymole decreased with the salinity levels. This finding strongly confirms the previous conclusion drawn about the essential oil percentage and oil yield (Table 6). These results are in accordance with those obtained by both Aziz and Youssef (2001) and Khalil (2002) on rosemary plants.

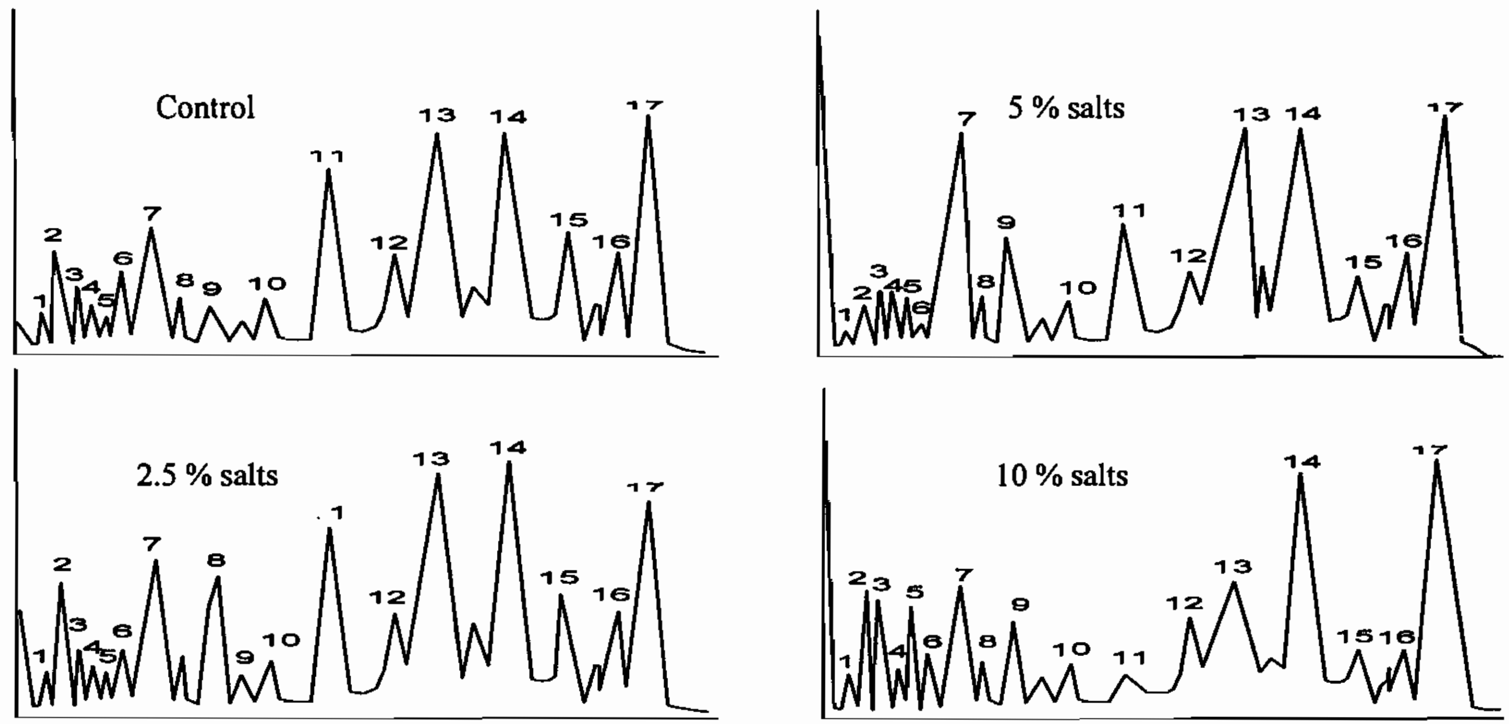


Fig.(1) : G.L.C of the esential oil of dry rosemary herbs as affected by different salinity levels in 2004/2005 season.

1- α-pinene. 2- Camphene. 3- β-pinene. 4- Sabinene. 5- Myrcene. 6- α-phylandrene. 7- α-Terpinene. 8- Limonene. 9- Caryophlene. 10- Y-Terpinene. 11- P-Cymene. 12- Camphor. 13- Cineole. 14- Linalool. 15- Citronellol. 16- Thymol. 17- Borneol.

Anatomical structure:

A- Stem structure:

The stem anatomy of rosemary plant resembles, in general, the internal structure description of other dicotyledonous plant (Fahn, 1990). The most striking anatomical features in the stems irrigated with the different levels of sea-water are early formation the secondary growth and development of phloem fibers Fig. (2-B) as well as an increase the number of xylem vessels (Fig. 2-C), although the xylem tissue thickness decreased (Table 7). The increase of xylem vessels under saline condition may help the plant in obtaining its requirements from water as well as leading to improve the plant tolerance against the high level of salinity.

It can be concluded from Table (7) and Figs. (2-B and C) sea-water salinity decreased stem diameter, cortex tissue thickness, phloem, xylem and pith tissues thickness. Width of cambial zone was also decreased. The reduction in stem diameter under higher salinity levels may be due to the decrease in cell division as a result of nuclear degradation of meristimatic cells (Katsuhara and Kawaski, 1996).

Table 7: Measurements (µm) of some anatomical characters of rosemary stem as affected by sea-water salinity levels (Average of 5 sections).

Parameters / sea-water salinity levels	Stem diameter	Cortex tissue thickness	Phloem tissue thickness	Cambial zone width	Xylem tissue thickness	No. of xylem vessels	Pith diameter
Control	2065	300	170	120	600	15	875
2.5%	1780	240	155	85	450	18	850
5%	1320	150	95	45	320	20	710
10%	1073	130	55	38	200	23	650

B- Leaf blade structure:

The leaf blade internal structure of rosemary plant is similar to other dicotyledonous plants (Fahn, 1990). It consists of upper and lower epidermis, the upper epidermis covered with thick cuticle layer. Mesophyll tissue composed of palisade and spongy tissues, midvein consists of open collateral vascular bundle (Fig. 3-A).

Data in Table (8) and illustrated in Figs. (2-B and C) show that salinity at all concentrations used decreased leaf thickness in the midvein region, palisade and spongy tissues thickness, midvein vascular bundle dimension as well as both xylem and phloem tissues thickness were also decreased. Highest salinity level (10%) was most effective in this respect (Fig. 3-C).

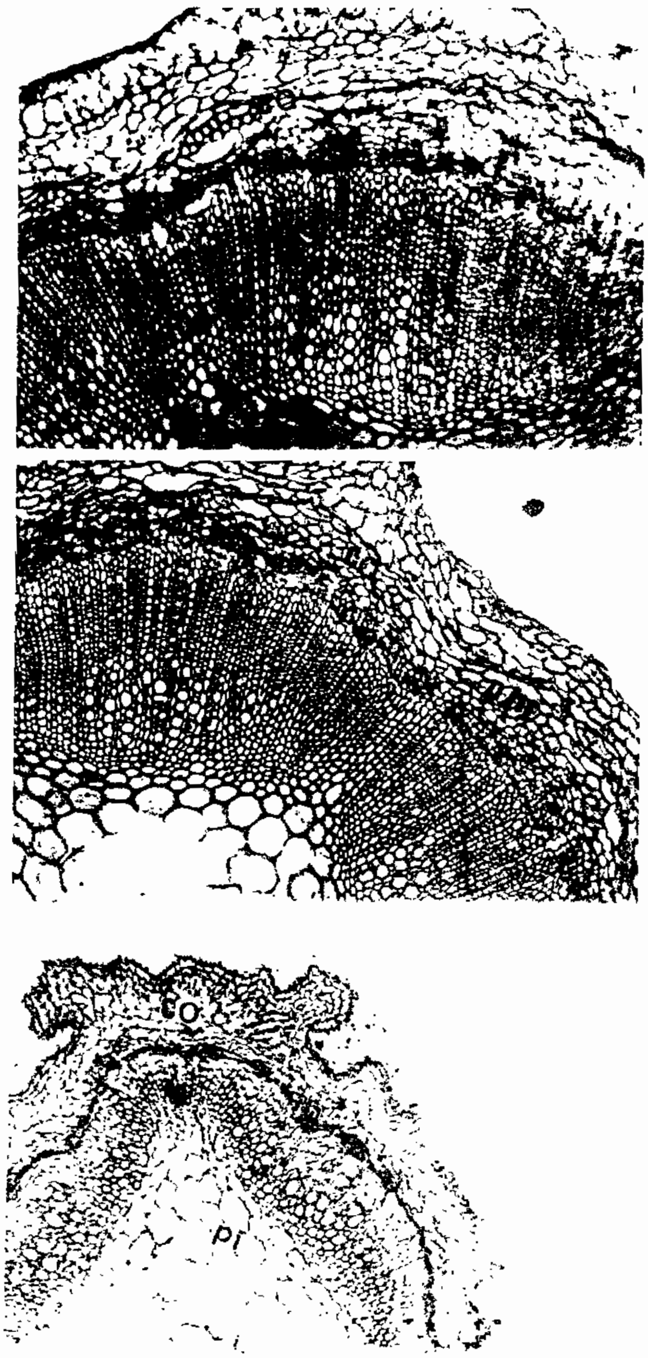
The decrease in leaf thickness due to salinity higher levels may be attributed to decreasing mesophyll tissue thickness (Table 8) and midrib vascular bundle dimension due to inhibition of cambial cell division and differentiation their derivatives. Wignarahjah *et al.* (1975a) reported that the decrease in Kidenybean leaflet thickness under salinity may be due to inhibition of cell division and cell expansion as well as reduction in the palisade parenchyma layer thickness.

A

B

C

Fig. (2): Cross sections in the stem of rosemary plants irrigated with tap water (A), 2.5% sea water (B) and 10% sea water (C). (Obj X 10.Oc. X 15). Co: cortex; ph: phloem, X: Xylem, Pi: pith, phF: phloem Fibers.



A

B

C

Fig. (3): Cross sections in the leaves of rosemary plants irrigated with tap water (A), 2.5% sea water (B) and 10% sea water (C). (Obj X 10.Oc. X 15). VB: vascular bundle; pl: palisade tissue, sp: spongy tissue.

Table 8: Measurements of some anatomical characters (μm) of rosemary leaf as affected by sea water salinity levels (Average of 5 sections).

Parameters / sea-water salinity levels	Leaf thickness in the midvein region	Palisade tissue thickness	Spongy tissue thickness	Midrib. V.B dimension		Xylem tissue thickness	Phloem tissue thickness
				Length	Width		
Control	850	250	100	250	300	190	55
2.5%	800	200	80	230	260	172	50
5%	730	180	65	220	250	160	42
10%	700	150	50	190	210	145	36

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الاستجابات التركيبية والفسيولوجية لنبات الحصالبان تحت ظروف الملوحة.

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أجريت تجربة أصص خلال موسمى ٢٠٠٣/٢٠٠٤، ٢٠٠٤/٢٠٠٥ بمزرعة النباتات الطبية والعطرية بكلية الزراعة – جامعة المنصورة بهدف دراسة تأثير مستويات الملوحة المختلفة لماء البحر المخفف على النمو ومحصول العشب وإنتاجية الزيت الطيار والتركيب الكيماوى لنبات الحصا لبان والنباتات النامية تم ريها بماء البحر المالح المخفف بمعدل (٢,٥، ٥، ١٠%) والتى تعادل ٨٢٥، ١٦٥٠، ٣٣٠٠ جزء فى المليون على التوالى، بينما نباتات الكنترول تم ريها بماء الصنبور. وقد أوضحت النتائج المتحصل عليها مايلى:-

- زيادة معدلات الملوحة أدت إلى نقص صفات النمو (إرتفاع النبات، عدد الأفرع على النبات، الوزن الطازج والجاف للعشب).
- وجد أن جميع معاملات الرى الملحى أدت إلى نقص محتوى كلورفيل أ، ب، النبسة المئوية ومحصول الزيت. وكذلك النسبة المئوية للبوتاسيوم، والنسبة بين البوتاسيوم والصوديوم فى المجموع الخضرى وذلك فى الحشتين المأخوذتين خلال الموسم. أما محتوى البرولين الحر، السكريات المختزلة وغير المختزلة والسكريات الكلية، والنسبة المئوية لكل من الصوديوم والكلوريد فى المجموع الخضرى قد أخذت إتجاه معاكس.
- أظهر التحليل الكروماتوجرافى لعينات الزيت سبعة عشرة مركباً، حيث أدت زيادة تركيز الملوحة إلى زيادة بعض المركبات الرئيسية مثل ألفاتربينين، سينول الينالول، وبورانيول. بينما كان تأثيرها سلبياً على البعض الآخر مثل بيتاسيسين واليثمول.
- تشريحيا أدت المعاملة بالملوحة إلى نقص قطر الساق، سمك أنسجة القشرة واللحاء والخشب والنخاع وكذلك عرض منطقة الكامبيوم الوعائى. علاوة على ذلك أدت الملوحة إلى نقص سمك الورقة وكل من النسيج العمادى والأسفنجى وأبعاد الحزم الوعائية الممثلة للعرق الوسطى بالإضافة إلى سمك نسيجى اللحاء والخشب.