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Response of *Thymus vulgaris* L. to Salt Stress and Alar (B_9) in Newly Reclaimed Soil

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Abstract: Soil salinity is consider a sever problem in agriculture, as it results in a noticeable reduction in the productivity of crops, so two pot experiments were conducted to investigate the effect of salinity and Alar (B_9) on plant growth, essential oil yield and its main constituents. Application of NaCl at 1500 ppm decreased plant height, number of branches and fresh and dry mass (g plant^{-1}) of Thymus vulgaris L. On the other side, oil percentage increased as the salinity concentration increased. The highest essential oil percent was obtained when applying 4500 ppm NaCl. Alar at 100 ppm decreased all parameters comparing to untreated plants. Thymus plants grown under 4500 ppm NaCl and 100ppm Alar gave the highest relative percentage of thymol (60.1%). Oxygenated compounds recorded the highest value with 4500 ppm NaCl and 100 ppm Alar while the maximum non-oxygenated ones resulted from 0 NaCl and 100 ppm Alar.

Key words: *Thymus vulgaris* L., Salinity, Alar (B_9), Essential Oil, GLC, Medicinal and Aromatic Plants.

INTRODUCTION

Thyme (*Thymus vulgaris* L.) belongs to the Lamiaceae family and is an aromatic and medicinal plant of increasing economic importance. Thyme volatile phenolic oil has been reported to be among the top 10 essential oils, showing antibacterial, antimycotic, intoxicative, natural food preservative and mammalian age delaying properties.[1,2] Schwarz *et al*[3] reported that the main essential oil components of *Thymus vulgaris* L. were thymol, carvacrol and ρ-cymene. Thymol showed strong effect on common respiratory tract[4].

Soil salinity is a sever problem in agriculture as it results in a noticeable reduction in the productivity of crops. Lack of fresh water for irrigation together with poor drainage in cultivated soils resulted in the accumulation of salts. According to a report of the world's irrigated land, about 20 to 27% may be salt affected[5].

In saline environments NaC1 is usually the most injurious and predominant salt but also other salts including Ca^{+2}, Mg^{+2} and $SO4^{-4}$ may be present(6). Ashraf and Mc-Neily[7] stated that the extent of plant growth depression under saline conditions varies with salt composition, the physiological stage of plant when it exposed to salinity and the plant species. The biosynthesis of secondary metabolites although controlled genetically, is affected strongly by environmental factors.[8]

Kandil and Eleiwa[9] showed that many attemps have been made to overcome the adverse effect of salinity, one of them is application of growth regulators, particularly growth retardants to plant under saline condition. Daminozide (trade name Alar) is a plant growth regulator, a chemical sprayed on the fruit to regulate their growth, make their harvest easier, and enhance their color. Spraying Alar incrase fresh and dry weights of stems and roots and also resulted in height control of some plants by reducing both peducle and internodal elongation[10,11].

The main aim of this work is to study the effect of salinity and Alar (B_9) on growth, yield and oil content of *Thymus vulgaris L.*

MATERIALS AND METHODS

Two pot experiments were carried out during two successive seasons of 2005 and 2006 in the greenhouse of National Research Centre, Dokki, Giza, Egypt to study the effect of salinity and Alar (B_9) on growth, yield and essential oil of thyme. Seeds of thyme were provided from "SEKEM" company and cultivated in nursery on 2nd of November, for both seasons. Thymus seedlings were transplanted on 1st of March into the pots (30 cm in diameter), with 3 replicates for each treatment. Each replicate includes 6 pots. All normal agricultural practices were performed as usual with thyme cultivation. Physical and chemical properties of the soil were as follow:

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Clay	Silt	Sand	$C_{org.}$	$M_{org.}$[1]	pH	EC (Sm^{-1})	N^2	P^3	K^3
			(%)					(ppm)	
14.53	28.17	56	0.17	0.22	8.11	2.5	460	36.2	38.7

1 – organic matter, 2 – total, 3 – available.

Salt stress treatments were applied using NaCl in four doses: 0,1500, 3000 and 4500 ppm and Alar (B_9) substance as a source of salinity resistance in concentrations of 0,100 and 200 ppm added with each NaCl treatment. These treatments were applied after one month from transplanting. Factorial arrangement of the combination between treatments was designed.

Two cuts were taken during the growing seasons (the first one on July and the second one on October). The following parameters were recorded at each cut: plant height (cm), Number of branches per plant, fresh and dry mass of herb, survival plants and essential oil percentage.

Data were subjected to statistical analysis according to the methods of Sendecor and Cochran[12] using L.S.D at the level of 5%.

The percentage of the volatile oil of air dried herb for each treatment was determined by hydro-distillation according to Guenther[13]. The resulted essential oil was collected, dehydrated over anhydrous sodium sulfate and kept in refrigerator till Gas Liquid Chromatography (GLC) analysis.

GLC analysis of volatile oil of each treatment was performed separately with a Hewlett-Packard model 5890. A fused silica capillary column (Carbowax 20M measuring 20m x 0.32 mm internal diameter, film thickness of 0.17µm) was used. The temperature program adopted was maintained at 75°C for 5 min. with an increase of 4°C min^{-1} until 220°C (10min). The carrier gas was Helium and the working flow rate was 1.0ml/min, detector was 9144 HP. The identification of the compounds was achieved by matching their retention times with those of authentic samples injected under the same conditions.

RESULTS AND DISCUSSION

1- Effect of Salt Stress: Data reported in Tables (1) and (2) showed the effect of salt (NaCl) and/ or Alar (B_9) on growth and oil percentage of thyme plants during 2005 and 2006, respectively. NaCl application significantly affected growth parameters, survival percent and essential oil percentage in the first and second cut in both seasons. It is clear that increasing NaCl concentration up to 4500 ppm significantly decreased plant height, number of branches and fresh and dry mass of herb (g $plant^{-1}$).

In this connection, salt stress may directly or indirectly inhibit cell division and/or cell elongation in growing tissues of roots, stems and leaves[14]. Hang and Cox[15] stated that salinity reduces plant height and dry mass of *Catharanthus roseus* and *Tagets erecta*, it also reduced the dry matter of roots gerbera plants[16,17]. Salts also induced water stress, reduction of chloroplast stomatal volume and generation of reactive oxygen species (ROS) which play an important role in inhibiting photosynthesis[18].

On the other hand, application of 1500 ppm of NaCl significantly increased essential oil percent in both cuttings in both seasons. This may be attributed to the function of secondary metabolites i.e essential oils as self defence components against stress conditions. In other words, the stress conditions resulted from salt application accelerated the biosynthesis of essential oils.

Tabatabaie and Nazari[19] reported that the concentration of oil of Lemon Verbena progressively increased as the NaCl salinity increased. Badawy et al[20] found that 2500 ppm NaCl resulted in the highest oil percent in *Majorana hortensis*, while oil yield decreased as salinity level increased.

It is clear that increasing salinity gradually increased total oxygenated compounds from 49.9% (control) to 65.9% (4500 ppm NaCl). On the other hand, non-oxygenated compounds gradually decreased from 43.7% to 33.6% for the same order. This may be attributed to the effect of salinity stress on the enzymatic systems that control the oxidation of terpenic hydrocarbons to oxygenated ones[21].

2- Effect of Alar (B_9): Application of Alar (B_9) significantly affected all studied parameters except survival percentage in both cuts of both seasons (Tables 1 & 2). Generally, application of 200ppm alar increased all vegetative characters comparing with other treatments. On the other hand, B_9 treatments decreased essential oil percentage compared with control.

El-Sheibany *et al*[22] used Alar in different concentrations to produce shorter chrysanthemum plants (Local Variety). Treated plants were significantly shorter in stem length, internodes length associating with increased in stem diameter.

Abou-Zeid[23] reported that all Alar concentrations (250, 500 or 1000ppm) markedly increased the volatile oil percentage of *Ocimum gratissimum* with its eugenol content in comparison to control. Eid and Rofaeel[24] mentioned that spraying geranium with Alar at 1000 and 2000 ppm increased its essential oil contents.

Table 1: Effect of salinity and Alar (B_9) on growth and yield of *Thymus vulgaris* season 2005

Characters / Treatment		Plant height (cm)		No. of branches plant^{-1}		Fresh mass of herb g plant^{-1}		Dry mass of herb g plant^{-1}		% Survival		Essential Oil percentage (v/w)	
Concentration (ppm)		1st. cut	2nd.cut	1st. cut	2nd.cut	1st. cut	2nd.Cut	1st. cut	2nd.cut	1st. cut	2nd.cut	1st. cut	2nd.cut
(NaCl)	(B_9)												
0	0	33.7	29.8	12.8	13.3	35.6	38.4	11.9	12.9	100	100	1.7	1.6
	100	28.8	25.5	10.7	12.5	30.7	34.2	10.2	11.3	100	100	1.4	1.4
	200	28.2	25.3	10.2	11.0	28.6	30.8	9.5	10.2	100	100	1.6	1.4
Mean value of 0 NaCl		30.2	26.9	11.2	12.3	31.6	34.5	10.5	11.5	100	100	1.6	1.5
1500	0	25.5	22.5	8.7	9.3	27.1	26.8	8.5	8.6	100	100	1.9	1.9
	100	25.7	22.5	9.2	10.8	27.6	29.1	8.6	9.3	100	100	1.8	1.6
	200	27.0	23.5	10.3	11.5	28.3	31.6	8.9	9.9	100	100	1.8	1.7
Mean value of 1500		26.1	22.8	9.4	10.5	27.7	29.2	8.7	9.3	100	100	1.8	1.7
3000	0	21.8	18.8	8.2	9.8	23.5	28.8	7.9	9.6	75	75	2.2	2.3
	100	24.2	19.8	9.3	11.8	25.5	27.8	8.6	9.5	91	91	2.0	2.2
	200	25.3	21.3	10.2	12.0	26.0	31.3	8.8	10.5	91	91	2.0	2.2
Mean value of 3000		23.8	20.0	9.2	11.2	25.0	29.3	8.4	9.9	85.7	85.7	2.1	2.2
4500	0	19.5	17.0	6.5	7.0	17.9	20.3	5.4	6.2	58	58	2.3	2.3
	100	22.5	17.8	7.3	8.0	20.6	21.3	6.2	6.9	75	75	2.2	2.1
	200	23.2	20.5	8.2	12.0	22.4	24.2	6.7	7.3	75	75	2.3	2.3
Mean value of 4500		21.7	18.4	7.3	9.0	20.3	21.9	6.1	6.8	69.3	69.3	2.3	2.2
Mean	0	25.1	22.0	9.1	9.9	26.0	28.6	8.4	9.3	83.2	83.2	2.0	2.0
value	100	25.3	21.4	9.1	10.8	20.1	28.1	8.4	9.3	91.5	91.5	1.8	1.8
of B_9	200	25.9	25.6	9.7	11.6	26.3	29.4	8.5	9.5	91.5	91.5	1.9	1.9
L.S.D at 5% of: NaCl		1.5	1.1	0.9	1.3	2.2	3.1	0.9	0.7	5.1	5.1	0.1	0.1
	B_9	N.S.	1.1	0.5	1.3	2.1	0.8	N.S.	0.1	4.3	4.3	0.1	0.1
Interaction		2.1	1.4	1.4	1.4	2.9	3.6	1.0	0.9	6.4	6.4	0.1	0.1

Table 2: Effect of salinity and Alar (B9) on growth and yield of *Thymus vulgaris* season 2006

Characters / Treatment		Plant height (cm)		No. of branches plant^{-1}		Fresh mass of herb g plant^{-1}		Dry mass of herb g plant^{-1}		% Survival		Essential Oil percentage (v/w)	
Concentration (ppm)		1st. cut	2nd.cut	1st. cut	2nd.cut	1st. cut	2nd.Cut	1st. cut	2nd.cut	1st. cut	2nd.cut	1st. cut	2nd.cut
(NaCl)	(B_9)												
0	0	32.7	35.8	12.6	17.8	35.9	41.6	11.9	13.9	100	100	1.8	1.6
	100	25.5	27.8	10.9	14.8	30.5	35.6	10.2	11.9	100	100	1.5	1.5
	200	27.5	26.8	9.3	13.3	28.6	33.8	9.5	11.3	100	100	1.7	1.7
Mean value of 0 NaCl		28.6	30.1	10.9	15.3	31.7	37.0	10.5	12.4	100	100	1.7	1.6
1500	0	24.7	24.3	8.8	12.8	26.8	29.7	8.4	9.9	100	100	2.3	2.2
	100	20.5	25.3	9.2	15.5	27.9	32.5	8.7	10.8	100	100	2.2	1.9
	200	25.7	25.3	9.8	15.0	28.3	33.9	8.8	11.3	100	100	2.2	2.1

Table 2: Continue

Mean value of 1500		23.6	24.9	9.3	14.4	27.7	32.3	8.6	10.7	100	100	2.2	2.1
3000	0	22.5	20.3	8.0	11.0	23.8	31.3	8.1	10.4	83	83	2.6	2.5
	100	25.2	22.0	9.3	14.0	25.6	32.8	8.7	10.6	91	91	2.3	2.2
	200	26.2	24.3	10.7	13.8	26.8	35.8	9.1	11.8	91	91	2.3	2.2
Mean value of 3000		24.6	22.2	9.3	12.9	25.4	33.3	8.6	10.9	88.3	88.3	2.4	2.3
4500	0	19.3	18.8	6.3	9.3	17.9	23.0	5.4	7.7	41	41	2.7	2.6
	100	22.2	19.8	7.7	11.0	21.1	25.3	6.4	8.4	83	75	2.3	2.2
	200	24.5	21.3	8.0	13.0	23.1	27.3	6.9	9.1	83	75	2.7	2.6
Mean value of 4500		22.0	19.9	7.3	11.1	20.7	25.2	6.2	8.4	69.0	63.7	2.6	2.5
Mean	0	24.8	24.4	8.9	12.7	26.1	31.4	8.4	10.5	81.0	81.0	2.3	2.2
value	100	23.3	23.7	9.2	13.8	26.2	31.6	8.5	10.4	93.5	91.5	2.1	1.9
of B_9	200	25.0	24.4	9.4	13.7	26.7	32.7	8.5	10.9	93.5	91.5	2.2	2.1
L.S.D at 5% of: NaCl		1.7	2.1	0.9	0.7	2.3	2.9	0.9	0.9	4.2	4.5	0.1	0.1
	B_9	1.5	0.5	0.4	0.7	0.4	0.9	N.S.	0.3	4.1	3.9	0.1	0.1
	Interaction	3.1	2.4	0.9	0.8	2.7	3.1	0.0	0.9	7.2	7.1	0.1	0.1

Table 3: Effect of salinity (NaCl) and Alar (B_9) on essential oil composition of *Thymus vulgaris* L.

Compounds	0 NaCl (ppm) Alar (ppm)			1500 NaCl (ppm) Alar (ppm)			3000 NaCl (ppm) Alar (ppm)			4500 NaCl (ppm) Alar (ppm)		
	0	100	200	0	100	200	0	100	200	0	100	200
α-Pinene	2.3	2.8	2.6	2.9	2.6	3.1	2.9	2.5	1.8	1.1	2.1	1.6
Myrecen	2.7	3.7	3.2	3.1	3.8	2.9	2.7	3.2	3.4	2.7	2.1	2.9
ρ-cymene	34.5	34.4	30.1	31.1	30.6	27.3	28.6	27.1	26.6	26.5	25.9	25.1
γ-terpinene	1.9	2.6	3.3	4.1	4.2	3.6	4.0	4.4	4.5	1.2	1.1	1.8
Linalool	2.6	2.5	2.5	2.8	2.8	3.7	2.9	3.4	3.5	3.1	3.2	3.2
Comphor	0.4	0.5	0.5	0.6	0.5	0.8	0.4	0.4	0.4	0.9	0.7	1.1
Boroneol	3.8	4.3	4.4	3.4	4.0	4.8	4.7	3.9	4.8	4.0	2.6	3.3
Terpineol	1.8	1.9	2.2	1.8	2.9	2.5	2.2	1.9	1.8	1.1	0.9	0.7
Thymol	39.8	40.9	43.1	43.6	44.8	47.9	48.2	47.2	50.4	56.4	60.1	56.9
Carvacrol	1.1	1.1	1.2	1.0	0.8	0.3	0.3	0.2	0.4	0.3	0.2	0.2
β-bourbonene	0.3	0.4	0.4	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Traces
Trans-caryo-phyllene	0.7	1.3	1.8	0.6	0.4	0.4	0.4	0.3	0.4	0.2	0.2	0.2
Cadinene	0.9	0.8	0.7	0.9	1.1	1.7	1.3	1.6	0.1	0.6	0.3	0.4
Germacrene-D	0.4	0.3	0.7	0.7	0.3	0.3	0.2	0.4	0.3	0.2	0.1	0.2
Caryophyllen Oxide	0.4	0.3	0.5	0.1	0.2	0.1	0.1	0.7	0.0	0.1	0.0	0.1
Non-oxygenated	43.7	46.3	43.4	43.6	43.1	39.4	40.2	39.6	37.2	33.6	31.9	32.2
Oxygenated	49.9	51.5	54.2	53.3	56.0	60.1	58.8	57.7	61.4	65.9	67.6	65.5
Total ident. compounds	93.6	97.8	97.2	96.9	99.1	99.5	99.0	96.3	98.6	99.5	99.5	97.7

Proportions of citronellol and geraniol, the main oil constituents were considerably increased by 200ppm Alar. Among monoterpenic alcohols, Boroneol (2.6-4.8%) and Linalool (2.3-3.7%) were the most dominant, while Trans-caryouphyllene (0.2-1.8%), Cadinene (0.1-1.7%) and Germacrene-D (0.1 – 0.7%) were the most abundant sesquiterpenes.

Increasing Alar increased total oxygenated compound comparing to non-treated plants with all treatments of NaCl. The opposite was true with non-oxygenated compounds. These findings are in agreement with Ruddat *et al*[25].

3- Effect of Interaction: Plant height recorded the highest values with the control treatment of both NaCl and Alar for both seasons. The same trend was observed for number of branches per plant and fresh and dry mass of herb. It is clear that the injurious effects of salinity on plant growth characters was observed with adding the 1st. dose of NaCl (1500ppm). Alar (B_9) application overcome these effects through retarding the growth and increase salt tolerance in thyme plants.

The interaction between salinity and Alar application was significantly in all parameters in both cuts of 2005 and 2006 seasons. The maximum essential oil percent was observed with plants treated with 4500 ppm NaCl and 0 ppm B_9 in the first growing seasons. The minimum percent was recorded with plants treated with 100ppm B_9 with no saline treatment in both cuts of the two successive seasons.

Data in Table (3) showed the effect of salt stress (NaCl) and Alar (B_9) the main components of the on essential oil of *Thymus vulgaris* L.GLC analysis indicated that thymol is the major compound in the essential oil of all treatments and ranged from 60.1 to 39.8% in 4500 ppm NaCl with 100ppm Alar and control treatment, respectively. The second main compound in all treatments was found to be ρ -cymene. It showed the opposite trend of thymol in which it ranged from 34.5 (in control treatment) to 25.1% in the treatment of 4500ppm NaCl with 200 ppm Alar. It is clear that increasing salt concentration increased the biosynthesis of thymol, while the opposite was true with ρ-cymene. It is well known that ρ-cymene transforms to Thymol or carvacrol and the environmental conditions affect the rate of transformation[26,27].

Increasing alar concentration increased the biosynthesis of thymol in all treatments of NaCl. On the other hand and in most cases, increasing Alar concentration decreased ρ-cymene content.

Conclusion: Applying 4500 ppm NaCl and 100 ppm alar (B_9) resulted in the highest essential oil percentage and the best relative percentage of Thymol (the major compound).

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