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EFFECT OF PACLOBUTRAZOL ON CHAMOMILE PLANTS GROWN UNDER NACL SALINITY

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ABSTRACT

Both paclobutrazol (PACLO) and the high levels of NaCl (2000 and 4000 ppm) caused a significant decreased in plant height. In addition, the high levels of NaCl decreased number of branches as well as shoot fresh and dry weights. While, the lower level of NaCl (1000ppm)and PACLO led to an increase in this respect.

Concerning the photosynthetic pigments it was found that the increase in soil salinity level led to a decreased in all photosynthetic pigments, while the lower level (1000ppm) and PACLO caused an increase in this respect.

On the other hand, plants growing under salinity conditions showed a decrease in the essential oil percentage as well as its yield per plant. While, Spraying chamomile plants with PACLO caused an increase in this respect.

As for the receptacle structure data show that, the low level of salinity (1000ppm) and PACLO levels increased the receptacle diameter due to the increase in ground tissue thickness and number of vascular bundles. In addition to an increase in number and diameter of oil ducts. On the other hand , the high salinity levels caused a decreased in this respect.

Generally, the application of PACLO, partially overcame the depressing effect of the high level of NaCl salinity in most of the previous parameters. However, PACLO at 50 ppm proved to be more effective in this respect.

INTRODUCTION

Chamomilla recutita (L.) Rausch. is one of the medicinal plants that have maintained a firm place in medical therapy which used as endogenous drug and a source of blue essential oil (Wallis, 1967).

Soil salinity is a major problem that negatively influenced plant production. Plants vary in the degree of response to excess soluble salts in their media and in the means by which they regulate their tissue salt content. Chamomile had no attention concerning salt tolerance. Stress-tolerant plants often grow more slowly than intolerant plants, therefore, growth retardants may include adaptive mechanisms or at least cause intolerant plants to mimic the behavior of tolerate plants during environmental stress. For example, Dawh (1989) found that cycocel, a gibberellin biosynthesis inhibitor, decreased Chamomile growth and increased salt tolerance.

Paclobutrazol (PACP) is a trizole-type plant growth regulator with a wide spectrum of biological activity. In addition, triazol acts as "multiplant-protectants" (Fletcher & Hofstra, 1985). Similar to the other growth retardents triazoles have the ability to protect plants against several types of stress. Triazoles have been reported to ameliorate injury induced by drought, low and high temperature and air pollutant (Lee *et al.*, 1985).

A few studies have been undertaken to alleviate the effects of these growth regulators on growth and metabolism under saline stress. Izumi *et al.*

(1988) stated that uniconazole, a triazole growth retardants, has been reported to increase ethylene production and active forms of cytokinins and decrease gibberellin biosynthesis.

El-Desouky & Atawia (1998) stated that, the biological activities of the endogenous phytohormones (cytokinins, gibberellins and auxins) were reduced by excess salinity but PACLO treatment reversed these effects.

It was found that PACLO reduced the effect of salt stress and PACLO-treated plants had better growth under salt stress than untreate ones (Banon *et al.*, 2003; Ozmen *et al.*, 2003; El-Kheir *et al.*, 2001; El-Desouky & Atawia, 1998; Dawh *et al.*, 1998 and Abou El-Khashab, 1997).

This investigation was conducted to confirm the effect of PACLO on chamomile plant growth and how PACLO reduces the effects of salt stress on certain morphological and physiological characters as well as yield and its components in chamomile plant.

MATERIALS AND METHODS

Two pot experiments were conducted during the two successive seasons of 2000/2001 and 2001/2002 in the experimental Farm, Faculty of Agriculture, Mansoura University.

Fruits of chamomile were sown in beds on October 15th in the nursery and uniform seedlings (10 cm length) were transplanted on December 15th in 25 cm pot diameter filled with 4.5 Kg loamy clay soil. The physical and chemical properties of the soil used were recorded in Table 1 (Piper, 1950). Then, nitrogen, phosphorus and potassium fertilizers were added at the recommended rates. Fifteen days later the plants were irrigated with tap water for control or 1000/2000 and 4000 ppm NaCl solution in salinity treatments.

Table (1): The chemical and physical analysis of the soil.

Soil content	%
Mechanical analysis:	
Coarse sand	1.72
Fine sand	27.80
Silt	31.20
Clay	38.50
Chemical analysis:	
Organic matter	1.36
Available nitrogen (ppm)	40.25
EC (mmhos/cm) at 25°C.	4.81
PH (soil reaction)	8.33

The plants were sprayed twice with paclobutrazol, [(2RS, 3RS.)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl] at 0, 25, 50 and 100 ppm after one and two months from adding salinity.

Plant samples were taken one month later (April 1st) at full flowering stage and following data were recorded : plant height, number of branches, as well as shoot fresh and dry weights. Samples were collected from the 5th leaf from plant tip to determine photosynthetic pigments (chlorophyll a, b and carotenoides) which extracted with methanol and determined as mg/g fresh weight according to Mackinny (1941). Also samples were taken from the receptacle for anatomical investigation from salinity and PACLO treatments. The receptacle sample were fixed in formalin acetic alcohol (FAA), dehydrated in alcohol series and embedded in paraffin wax (52°C m.p). After paraffin wax infiltration, sections at 15 µ thick were prepared by a rotary microtome, stained in crystal violet and erythrosin combination and mounted in Canda balsam (Gerlach, 1977). The sections were examined microscopically.

Four collection of capitula were obtained, numbered, weighted freshly (g/plant) and air dried then weighted (g/plant). The dried capitula were used for determination of oil percentage according to Chandra *et al.* (1968). Oil yield/ plant was calculated from oil percentage based on flower yield/plant.

The experimental design was factorial experiment with three replicates in complete randomized block design, each replicate contained 3 pots. The data (average of the two seasons) were statistically analysed according to Gomez & Gomez (1984).

RESULTS AND DISCUSSION

1) Growth parameters:

Tables (2 and 3) show that NaCL salinity at the high levels (2000 and 4000 ppm) caused a significant reduction in chamomile growth expressed as: plant height, number of branches/plant as well as shoot fresh and dry weights and the high level (4000 ppm) was more effective while the low level of salinity (1000 ppm) led to an increase in this respect.

Table (2): Effect of NaCl salinity and PACLO as well as their Interaction on plant height (cm) and number of branches per plant.

Salinity	Plant height (Cm) PACLO				Salinity average	No of branches / plant PACLO				Salinity average
	0	25	50	100		0	25	50	100	
0	44.5	41.0	38.0	36.0	39.88	9.5	12.0	13.0	12.0	11.63
1000	45.0	42.0	41.0	33.0	40.25	11.6	11.9	12.5	11.8	11.95
2000	40.0	39.0	36.0	31.5	36.63	8.5	10.0	11.0	11.0	10.13
4000	34.5	34.0	30.5	29.5	32.13	7.0	8.0	8.0	6.0	7.25
PACLo average	41.0	39.0	36.38	32.5		9.15	10.48	11.13	10.20	
	S	P	S x P				S	P	S x P	
LSD 5%	1.330	1.329	1.1884				0.9110	0.911	0.8145	

Concerning the effect of PACLO, data presented in the same Table show that PACLO decreased significantly plant height. While, number of branches as well as shoot fresh and dry weights were increased. PACLO at 50 ppm was more effective in this respect.

As for the interaction treatments, data in the same Table showed an additive effects of salinity on decreasing plant height. Moreover, the application of PACLO interacted with low and moderate salinity levels (1000 and 2000 ppm) increased number of branches/plant as well as shoot fresh and dry weights compared with the control. Similar results were obtained by El-Kheir *et al.* (2001) and Dawh *et al.* (1998).

The inhibitory effect of salinity on plant height may be due to the decrease in cell size and number of cells per unit area (Strogonov, 1964) as well as suppression of meristimatic activity (Nieman & Leon, 1959). Nieman (1965) and Kurth *et al.* (1986) attributed the inhibition effects of salinity to its effects on cell division, cell elongation and/or inhibited apical growth as well as hormone unbalance. (Strogonov *et al.*, 1970) concluded that absorption of chloride salts from saline media and their accumulation up to atoxic level may affect the metabolic activity of plant tissues and cause the appearance of some intermediate toxic compounds consequently a decrease in plant growth. Moreover, the depressing effect of salinity on plant growth seems to be due to the high respiration rate (Burchett et al., 1989) and disturbance in various metabolic processes such as net photosynthesis (Robinson *et al.*, 1983; Yeo *et al.*, 1985), ionic balance (Torres-Schumann *et al.*, 1989), hormone balance (Kavikishor, 1989) and water absorption (Pessarakli *et al.*, 1989).

Table (3): Effect of NaCl salinity and PACLO as well as their interaction on shoot fresh and dry weights (g/plant).

Salinity	Shoot fresh weight (g/plant) PACLO 0	25	50	100	Salinity average	Shoot dry weight (g/plant) PACLO 0	25	50	100	Salinity average
0	28.70	34.89	39.61	32.64	33.96	10.10	12.29	13.25	10.75	11.60
1000	32.52	34.57	35.41	35.45	34.49	11.17	12.28	13.35	13.75	12.62
2000	24.53	25.30	26.49	25.78	25.52	8.50	9.64	9.92	9.55	9.40
4000	20.40	22.48	23.68	18.54	21.28	7.50	8.48	8.99	8.79	8.49
PACLO average	26.54	29.31	31.30	28.10		9.32	10.67	11.37	10.71	
	S	P	S x P				S	P	S x P	
LSD 5%	0.9751	0.9751	0.9334				0.4910	0.4910	0.4043	

The reduction in plant height due to PACLO application may be attributed to a reduction in internode length (Wood, 1984) and to the inhibition of GA_3 biosynthesis by triazoles (Buchenauer and Rohner, 1981). Wang *et al.* (1998) reported that the reduction in plant height by PACLO may be attributed to the reduction in synthesis and action of auxin in plant through enhancing the ctivity of IAA oxidase as well as reducing the rate of transformation of tryptophan into IAA and reducing the distribution of IAA in the plant. While, the stimulating effect of PACLO on number of brancges may be due to the inhibition of terminal bud growth and stimulation of lateral shoot development (Davis *et al.*, 1988). Moreover, this stimulating effect of PACLO on plant growth may be due to the increase in photosynthesis (Sankhla *et al.*, 1985), root diameter (Davis *et al.*, 1988) and their interfering with gibberellin biosynthesis (Wang *et al.*, 1985) as well as shifting in the balance of the important plant hormones including cytokinins (Iszumi *et al.*, 1988) gibberellins and auxins (El-Desouky & Atawia, 1998) and ABA, which induced plant-water balance (Wareing and Phillips, 1970).

Concerning to the interaction between salinity and PACLO, Abo El-Khashab *et al.* (1997) showed that salinity reduced growth by 60%, but only by 30% for PACLO-treated plants. PACLO-treated plants had less defoliation and fewer leaves/plant and had higher rates of leaf gas exchange than non-treated plants. They showed also that PACLO application to Salinized plants reduced Na^+ and Cl^- contents in leaves, roots and stems. They suggested that PACLo promoted salt stress avoidance by reducing the uptake and accumulation of harmful Na^+ and Cl^- ions in plant tissues.

2) Photosynthetic pigments:

The effects of NaCl salinity, PACLO and their interaction on chlorophyll a, b and carotenoides are presented in Table (4).

Table (4): Effect of NaCl salinity and PACLO as well as their interaction on chlorophyll a, chlorophyll b and carotenoides (mg/g fresh weight).

Salinity	Chlorophyll a (mg/g fresh weight)				Salinity average	Chlorophyll b (mg/g fresh weight)				Salinity average	Carotenoides (mg/g fresh weight)				Salinity average
	PACLO					PACLO					PACLO				
	0	25	50	100		0	25	50	100		0	25	50	100	
0	1.481	1.824	1.874	1.811	1.748	1.090	1.300	1.240	1.140	1.193	0.483	0.485	0.503	0.514	0.491
1000	1.725	1.946	1.851	1.831	1.838	1.228	1.268	1.286	1.271	1.263	0.485	0.501	0.524	0.510	0.505
2000	1.098	1.425	1.390	1.022	1.224	0.968	1.075	1.137	1.175	1.088	0.371	0.408	0.428	0.399	0.401
4000	0.946	1.178	1.170	1.081	1.094	0.838	0.928	0.908	0.728	0.850	0.301	0.373	0.390	0.389	0.363
PACLo average	1.313	1.593	1.571	1.436		1.031	1.142	1.142	1.079		0.406	0.441	0.461	0.453	
	S	P	S x P				S	P	S x P		S	P	S x P		
LSD 5%	0.0601	0.0601	0.0079				0.1341	0.1341	0.01788		0.0418	0.0418	0.00172		

Data in Table (4) reveal that the high salinity levels (2000 and 4000 ppm) decreased chlorophyll a, b and carotenoides while, the lower level (1000 ppm) caused an increase in this respect. On the other hand, PACLO treatments increased the above mentioned parameters.

Concerning the interaction, the results presented in the same Table proved that PACLO enhanced the photosynthetic pigments in chamomile plants mainly at NaCl level of 1000 ppm. Obviously the highest level of salinity combined with PACLO was more effective in decreasing photosynthetic pigments.

The decrease in chlorophyll under salinity may be due to the inhibitory effects of Cl^- on the activity of Fe-containing enzymes, cytochrome oxidase, which in turn, decrease the rate of chlorophyll biosynthesis (El-Nimr, 1986). On the other hand, these enzymes under Cl^- salinity may affect chloroplast structure (Strogonov *et al.*, 1970). El-Banna (1985) stated the high salinity level distributed chloroplast structure, number and size. In addition, Salama *et al.* (1994) stated that salinity caused swelling of membranes in chloroplasts of sensitive plants, which affect their chlorophyll content.

The increase in photosynthetic pigments by PACLO application may be due to enhanced chlorophyll biosynthesis or is simply a "concentrating effect" due to reduced leaf expansion (Sankhla *et al.*, 1985). Izumi *et al.* (1988) and El-Desouky and Atawia (1998) added that PACLO increased cytokinins content which delay senescence of leaves by decreasing chlorophylase enzyme activity (Sabater and Rodriguez, 1978) or increasing prochlrophyllide content and activity of chlorophyll synthesis (Chen, 1990).

Generally, PACLO partially overcome the depressing effects of salinity on photosynthetic pigments. These results are in agreement with the results of Ozmen *et al.* (2003) on barley and El-Kheir *et al.* (2001) on sunflower. Banon *et al.* (2003) found that PACLO increased chlorophyll content in plant leaves in both saline and non saline conditions. They suggested that PACLO reduced saline stress symptoms and mortality in stressed saline ions in the medium.

3) Capitula yield/plant:

Data presented in Table (5) show a significant reduction in flowering yield / plant expressed as number of capitula as well as its fresh and dry weights under the high salinity levels while, the low level showed an increase in this respect. Dawh (1989) and Arun Prasad *et al.* (1997) stated that chamomile flower yield was decreased with increase in salinity.

As for PACLO effect on capitula yield, data in Table (5) indicate that PACLO treatments significantly increased capitula yield/plant, however, PACLO at 50 ppm appeared to be more effective.

With regard to the effects of the interaction between NaCl salinity and PACLO, the same table shows that PACLO enhanced capitula yield under the low salinity level.

It could be concluded that the reduction in capitula yield/plant under NaCl salinity may be due to the decrease in the flower productivity attributed to the decrease in the number of branches/plant (Table 2) under salinity levels and this led consequently to a decrease in flower buds. Also, it may be attributed to the disturbance in carbohydrate metabolism (Wignarajah *et al.*, 1975b); Protein synthesis (El-Gaaly, 1982) and mineral uptake (Ragheb et al., 1993). In this investigation the deleterious effects of salinity on capitula yield may be due to its depressing effects on growth (Table 2&3) and photosynthetic pigments (Table 4).

Table (5): Effect of NaCl salinity and PACLO as well as their interaction on numbers of capitula, as well as flower fresh and dry weights of chamomile.

Salinity	No of capitula / plant				Salinity average	Flower fresh weight (g/plant)				Salinity average	Flower dry weight (g/plant)				Salinity average
	PACLO					PACLO					PACLO				
	0	25	50	100		0	25	50	100		0	25	50	100	
0	578.65	580.51	592.57	588.63	585.09	40.56	43.07	45.55	48.24	44.38	16.64	17.84	18.80	18.20	17.87
1000	587.36	589.41	592.32	601.43	593.88	42.65	44.43	48.60	48.52	46.06	18.97	18.52	18.65	19.51	18.91
2000	400.68	462.32	521.72	492.13	469.21	38.68	43.58	40.50	44.35	41.77	14.40	15.98	17.48	15.62	15.87
4000	380.61	388.44	435.25	401.17	401.37	32.65	38.78	40.35	39.30	37.27	13.24	13.53	14.58	13.77	13.78
PACLo average	486.83	505.17	596.72	520.84		38.64	41.71	43.75	45.10		15.81	16.47	17.38	16.63	
	S	P	SxP				S	P	SxP		S	P	SxP		
LSD 5%	14.598	14.598	209.234				1.1725	1.1725	1.3480		0.7814	0.7614	0.5692		

The stimulative effect of PACLO on capitula yield may be due to the increase of branching carrying flower-heads (Table 2) and to enhancing photosynthetic pigments formation of chamomile (Table 4). The role of PACLO in overcoming the depressing effect of salinity on chamomile flower yield may be due to enhancing photosynthetic pigments (Table 4) and the increase in ethylene production and cytokinin content (Izumi et al., 1988).

4) The anatomical structure of the receptacle:

As previously mentioned, only the receptacle of salinity and PACLO treatments only were choosen in addition to the control for anatomical studies of the receptacle.

Data in Table (6) reveal that the low level of salinity (1000 ppm) increased number of oil ducts and the receptacle diameter.

Table (6): Effect of NaCl salinity and PACLO treatments on chamomile receptacle structure.

Treatments	Receptacle diameter		Ground tissue thickness		Number of vascular bundles		Number of oil ducts	
Control	292	100%	40	100%.	38	100%	15	100%
Salinity								
1000	440	+150.7	45	+112.5	40	+111.1	18	+120.0
2000	230	-78.8	35	-87.5	26	+72.2	11	-73.3
4000	160	-54.8	22	-55.0	18	-50.0	6	-40.0
PACLO								
25	499	+170.9	46.	+115.0	43	+119.4	17	+113.3
50	580	+198.6	50	+125.0	52	+144.4	28	+186.7
100	485	+166.1	44	+110.0	48	+133.3	22	+147.7

%: Growth percents over or under control

This increase due to the increase in number and size of vascular bundles as well as thickness of the ground tissue Fig. 1 and Table (6). On the other hand, the high levels of salinity (2000 and 4000 ppm) decreased all these parameters.

Concerning the effect of PACLO, data in the same Table show that PACLO at all levels increased number of oil ducts and its diameter (Fig. 1b and c). Moreover, PACLO caused an increase in the receptacle diameter which may be due to the increase in number and size of the vascular bundles as well as ground tissue thickness. PACLO at 50 ppm was more effective in this respect.

It could be concluded that the low level of salinity and PACLO at 50 ppm caused an increase in the number of oil ducts and its diameter and this consequently will be reflect on the oil yield.

5) Essential oil percentage and yield:

Data presented in Table (7) reveal that all salinity levels decreased significantly both oil percentage and oil yield/plant. The high levels of salinity (2000 and 4000 ppm) showed a pronounced decrease in this respect.

With regard to the effects of PACLO, the same Table show that, the oil percentage and oil yield were enhanced with PACLO application. PACLO at 50 ppm was more effective in this respect.

Concerning the interaction between salinity levels and PACLO, date in Table (7) reveal that PACLO overcome the harmful effect of salinity on essential oil percentage and its yield in chamomile flowers. PACLO at 50 ppm with the low level of salinity (1000 ppm) was more effective in increasing oil % and its yield/plant. Similar findings were reported by Dawh (1989) and Dawh et al., (1998).

Table (7): Effect of NaCl salinity and PACLO as well as their interaction on chamomile oil % and its yield.

Salinity	Oil %				Salinity average	Oil yield / plant				Salinity average
	PACLO					PACLO				
	0	25	50	100		0	25	50	100	
0	0.34	0.40	0.44	0.42	0.40	0.057	0.071	0.083	0.045	0.064
1000	0.32	0.37	0.40	0.39	00.37	0.061	0.069	0.075	0.078	0.070
2000	0.24	0.34	0.38	0.36	0.33	0.035	0.054	0.066	0.056	0.053
4000	0.20	0.29	0.34	0.31	0.29	0.027	0.039	0.050	0.043	0.040
PACLo average	0.28	0.35	0.39	0.37		0.045	0.058	0.069	0.055	
	S	P	SxP			S	P	SxP		
LSD 5%	0.0231	0.0231	0.00523			0.0048	0.0048	0.000023		

The reduction in oil percentage under NaCl salinity may be due to its conversion to carbohydrates which are the essential metabolites in respiration (Yang *et al.*, 1990a). Moreover, the reduction in oil yield with salinity was due to both reduction in oil percentage (Table 7) and capitula yield (Table 5), as well as number of oil ducts in the receptacle (Table 6).

Generally, it could be concluded that spraying PACLO at 50 ppm overcome the harmful effect of salinity on growth and oil yield of chamomile plants.

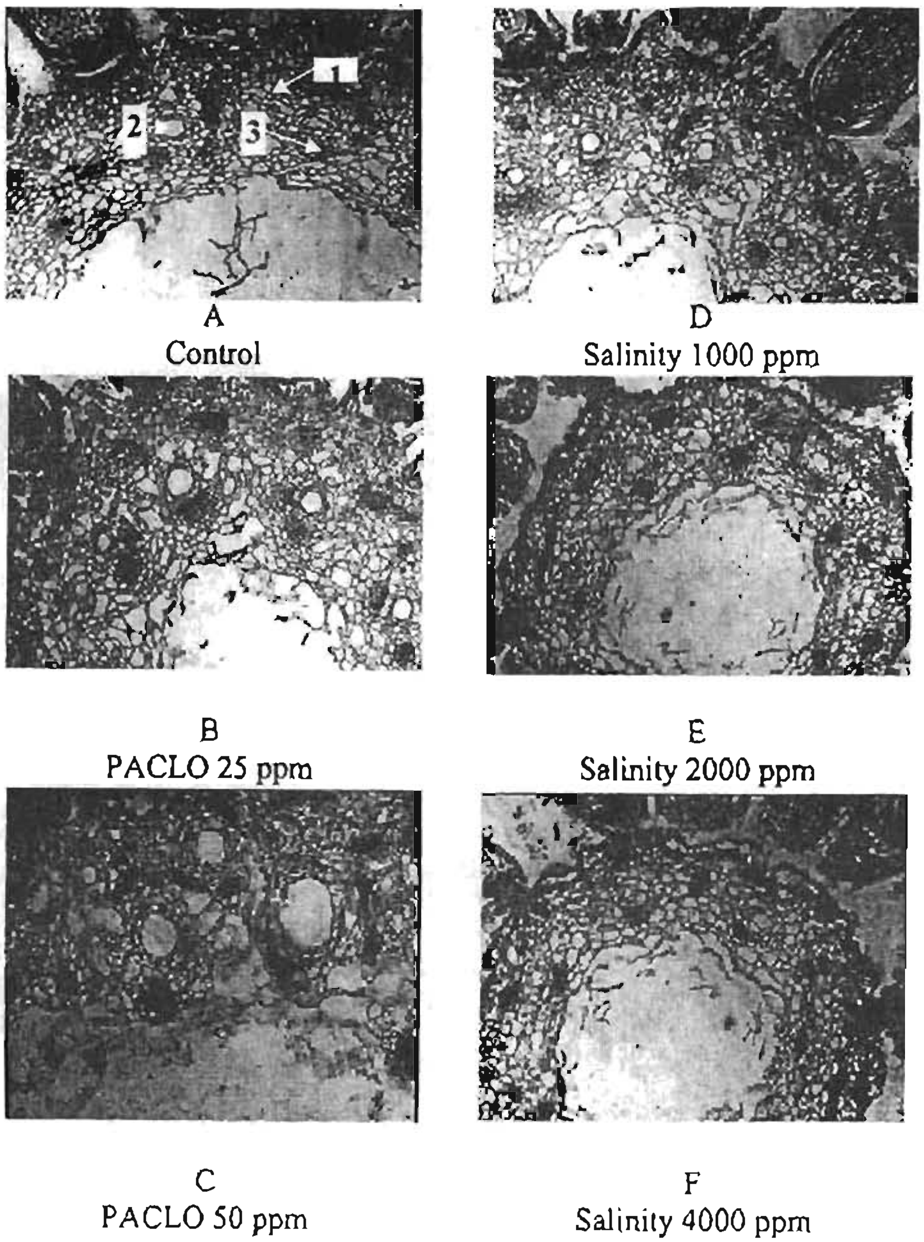

Fig. (1): Effects of NaCl salinity and PACLO treatments on chamomile receptacle structure. (100 x).
1. Ground tissue; 2. Oil duct; 3. Vascular bundles.

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تأثير الباكلوبيوترازول على نباتات البابونج النامية تحت ظروف ملوحة كلوريد الصوديوم

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– أدي كلا من الباكلوبيوترازول بتركيزاته المختلفة (٢٥، ٥٠، ١٠٠ جزء في المليون) والمستويات العالية من ملوحة كلوريد الصوديوم (٢٠٠٠، ٤٠٠٠ جزء في المليون) إلـــي نقــص معنوي في طول النبات، بالإضافة إلي ذلك أدت المستويات العالية من الملوحة إلي نقص في عدد الفروع الجانبية وكذلك الأوزان الغضة والجافة للمجموع الخضري، بينما أدي المستوي المنخفض من الملوحة والباكلوبيوترازول إلي زيادة في هذه الصفات مقارنة بالكنترول.

– وبالنسبة لصبغات البناء الضوئي لقد أدت زيادة ملوحة التربة إلـــي نقـــص محتـــوي صبغات البناء الضوئي في حين سبب المستوي المنخفض (١٠٠٠جـــزء فـــي المليـــون) وكـــذلك الباكلوبيوترازول إلي زيادة في هذا الشأن

– ومن ناحية أخري فإن النباتات النامية تحت ظروف ملحية أظهرت نقصا في النســـبة المئوية للزيت الطيار ومحصول النبات من الزيت بينما رش نباتات البابونج بالباكلوبيوترازول أدت إلي زيادة في هذا المجال.

– وفيم يتعلق بالتركيب الداخلي لتخت النورة الهامة فقد أدي التركيـــز المـــنخفض مـــن الملوحة (١٠٠٠جزء في المليون) وكذا الباكلوبيوترازول بتركيزاته المختلفة إلي زيادة فـــي قطـــر التخت بسبب الزيادة في سمك النسيج الأساسي وعدد الحزم الوعائية بالإضافة إلي زيادة في عـــدد القنوات الزيتية. وعلي الجانب الأخر فقد أدت التركيزات العالية من الملوحـــة إلـــي نقـــص هـــذه الصفات.

– عموما المعاملة بالباكلوبيوترازول أدت وبصـورة جزئية إلي التغلب علي الأثر الضـار للتركيزات العالية من ملوحة كلوريد الصوديوم علي معظم الصفات السابقة، وعلي أية حـــال فقـــد وجد أن الباكلوبيوترازول بتركيز ٥٠ جزء في المليون أنه أكثر فاعلية في هذا المجال.