The effects of treatment with polyamines on dry matter, oil and flavonoid contents in salinity stressed chamomile and sweet marjoram

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ABSTRACT

The study was undertaken to determine a possible role of polyamines (putrescine, spermidine, spermine) as antioxidants in salt tolerance of *Chamomilla recutita* and *Origanum majorana*. Salinity generally induced variable changes in growth, contents of oil and flavonoids of both plants; foliar application of any polyamines counterbalanced the effects of salinity. In general, the degree of stimulation differed according to the type and concentration of the used additive and the type of the plant.

Keywords: *Chamomilla recutita*; *Origanum majorana*; NaCl; polyamines (putrescine, spermidine, spermine); oil; flavonoids

Salinity causes oxidative damage through generation of oxygen radicals such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals (Elstner 1982, Hernández et al. 1993, 1995), or inhibition of antioxidant systems in plants (Ali 2000).

However, some correlations were reported between lipid content and salinity in some plant species (Chavan et al. 1980, Heikal et al. 1980, Younis et al. 1987, Ali 2002).

Flavonoids were shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals; many of them play an important physiological and ecological role, being involved in resistance to different types of stress (Rice-Evans and Miller 1998, Ayaz et al. 2000, Ali and Abbas 2003).

Common polyamines (PAs), namely putrescine (put – diamine), spermidine (spd – triamine) and spermine (spm – tetramine), are biologically active compounds recognized as modulators of plant growth and development; they important for plant responses to environmental stress (Smith 1985, Evans and Malmberg 1989, Messiaen et al. 1997, Bouchereau et al. 1999, Ndayiragije and Lutts 2005).

Some studies indicate that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of antioxidative systems. However, the response of foliar application of antioxidants to salinity on medicinal plants has been poorly investigated. The aim of the present work is thus to study the response of foliar application of antioxidants to various levels of salinity. The interactive effect of salinity and foliar application of antioxidants (put, spd, spm) on growth, oil and flavonoids of *Chamomilla recutita* and *Origanum majorana* plants was studied.

MATERIAL AND METHODS

The plant materials used in this study were *Matricaria chamomilla* L. as compositae and *Origanum majorana* as aromatic labiatae; both of them are important medicinal plants grown in sandy areas in Egypt. Seeds were sown and grown in the nursery for 30 days. Uniform seedlings were transplanted in pots (30 cm in diameter) filled with 10 kg air-dry soil (clay/sand 2:1 v/v). Temperature in the greenhouse ranged from 15 to 25°C. In order to regulate the distribution of irrigation solution, a finely perforated plastic tube was inserted in each pot at the distance of 2 cm from the centre of the pot and ¾ way down in the soil. After next 7 days, the Hoagland's nutrient solution (Hewitt 1966) supplemented with different NaCl concentrations (0, 25, 50, 75, 100, 125, 150mM) was used for irrigation.

Antioxidants (put, spd, spm) were sprayed 4 times at 0.1mM. Untreated plants were sprayed with distilled water. The first spray was made 7 days after transplanting and repeated at 15-day intervals. The plants were sprayed with a manual pressure pump at an average of 10 cc. In order to prevent the accumulation of salts soil in each pot was leached every ten days with excessive amount of distilled water. The experiments were carried out in three replicates. At the end of the experimental period (60 days) plant growth was determined from the dry matter of different organs (roots, shoots and flowers).

Determination of oil content. The method adopted for solvent of the oil (the content of an oleaginous material) was described by Meara (1955) and applied by Younis et al. (1987).

Extraction and quantification of total flavonoids. Flavonoids of the plant materials were extracted with 80% methanol at 60°C, shaken for 20 min, and filtered. The filtrate was diluted at 1:3 and 100 μl of reactive solution (1% 2-aminoethyl diphenyl borate) was added (Hariri et al. 1991, Ali and Abbas 2003). Spectrometric measurements were done at the maximum wavelength of 404 nm. Extract absorption was compared with that of standard (luteolin) resulting in the calculation of total amount of flavonoids.

Statistical analysis. The experimental design was a random complete block with three replications. The data were analysed by the STATGRAPHICS (Statistical Graphics Corporation, Princeton USA) statistical package by the *t*-test and ANOVA functions to assess significant differences among means.

RESULT AND DISCUSSION

The accumulation of dry matter of roots, shoots and flowers of *Chamomilla recutita* was mostly enhanced with increasing salinization level (Figure 1, Table 1). This might indicate that *Chamomilla recutita* has an ability to exhibit variable responses to salinity stress. The same conclusion was recorded by Blacquiere and Lambers (1981) and Imamul-Hug and Larher (1984). In contrast, the values of dry matter of roots and shoots of *Origanum majorana* were generally decreased with increasing salinity (Figure 2, Table 2). The reduction was more obvious at higher salinity levels than at lower ones; as opposed to these results, the values of dry matter of flowers increased with increasing salinity. The inhibitory effect of water stress induced by salinity on growth parameters of *Origanum majorana* is in agreement with the results obtained by some authors using different plants (Hernández et al. 1995, Lin and Kao 1996, Ali 2002).

Foliar application of any polyamines (put, spd, spm) significantly increased dry matter of roots and shoots in *Chamomilla recutita*. Similarly, foliar application of any polyamines significantly increased dry matter of shoots and flowers in *Origanum majorana* (Figures 2–3, Tables 2–3); however, dry matter of roots in *Origanum majorana* decreased (Figure 2).

The characteristic effects of each polyamine on *Chamomilla recutita* or *Origanum majorana* could depend on the type of the amine, which is probably due to the variation in polyamine pools within the plant body (Chen and Galston 1985) or the alteration of endogenous polyamine pools as a result of exogenous polyamine application (Altamura et al. 1991, Bajaj and Rajam 1995, 1996, Ali 2000). The inhibitory effects of polyamines on *Origanum majorana* roots further substantiate the supposition that the effects of polyamines are tissue, organ and species-specific (Minocha and Minocha 1995).

Polyamines are involved in the regulation of growth and stress responses, and are also related to plant flowering and other reproductive activities, as demonstrated by changes in their concentration with the occurrence of these physiological events (Smith 1985, Evans and Malmberg 1989, Tiburcio et al. 1993, Galston et al. 1997, Ndayiragije and Lutts 2005). Moreover, putrescine applied together with NaCl effectively enhanced dry matter of shoots and flowers in *Chamomilla recutita* but reduced it in roots (Figures 1 and 3). Foliar application of either spermidine or spermine significantly decreased dry matter of roots, shoots and flowers when compared with the corresponding treatments with NaCl, however they remained higher than those of control plants.

Foliar spray of any polyamines together with NaCl effectively increased dry matter shoots, roots and flowers of *Origanum majorana* (Figures 2 and 3). These results are consistent with the studies of some authors who reported that exogenous application of putrescine can overcome the harmful effects of NaCl stress (Prakash and Prathapasenan 1988a, b, Krishnamurthy 1991, Ali 2000).

Increasing salinity gradually enhanced the oil contents in shoots and flowers of *Chamomilla recutita* and in roots and shoots of *Origanum majorana*, in contrast to flowers of *Origanum majorana* and roots of *Chamomilla recutita* (Figures 4

Table 1. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on dry matter (g/plant) of *Chamomilla recutita* plants

Polyamine	NaCl (mM)	Shoot	Root	Total
Control	0.0	0.083 ± 0.011	0.039 ± 0.007	0.122 ± 0.017
	25	0.146** ± 0.005	0.083* ± 0.009	0.229** ± 0.014
	50	0.195*** ± 0.008	0.105** ± 0.012	0.300** ± 0.019
	75	0.134* ± 0.008	0.109** ± 0.013	0.243** ± 0.020
	100	0.215** ± 0.025	0.115** ± 0.010	0.330** ± 0.028
	125	0.172 n.s. ± 0.038	0.153** ± 0.022	0.324* ± 0.055
	150	0.136* ± 0.016	0.137** ± 0.016	0.273** ± 0.017
Put	0.0	0.148 n.s. ± 0.026	0.043 n.s. ± 0.007	0.191 n.s. ± 0.033
	25	0.313*** ± 0.011	0.057 n.s. ± 0.011	0.370** ± 0.022
	50	0.249** ± 0.003	0.045** ± 0.004	0.294 n.s. ± 0.006
	75	0.228*** ± 0.008	0.089 n.s. ± 0.013	0.318* ± 0.020
	100	0.212 n.s. ± 0.006	0.071 n.s. ± 0.015	0.284 n.s. ± 0.019
	125	0.216 n.s. ± 0.012	0.087 n.s. ± 0.016	0.303 n.s. ± 0.028
	150	0.199 n.s. ± 0.023	0.040** ± 0.003	0.239 n.s. ± 0.025
Spd	0.0	0.127 * ± 0.007	0.123** ± 0.021	0.250** ± 0.027
	25	0.147 n.s. ± 0.001	0.086 n.s. ± 0.001	0.233 n.s. ± 0.001
	50	0.155** ± 0.003	0.086 n.s. ± 0.002	0.240 * ± 0.005
	75	0.139 n.s. ± 0.002	0.092 n.s. ± 0.007	0.231 n.s. ± 0.008
	100	0.162 n.s. ± 0.002	0.104 n.s. ± 0.006	0.266 n.s. ± 0.008
	125	0.254 n.s. ± 0.0014	0.101 n.s. ± 0.019	0.355 n.s. ± 0.033
	150	0.168 n.s. ± 0.004	0.096 n.s. ± 0.014	0.264 n.s. ± 0.018
Spm	0.0	0.155** ± 0.003	0.064 * ± 0.005	0.218** ± 0.003
	25	0.107** ± 0.003	0.067 n.s. ± 0.006	0.174* ± 0.008
	50	0.147** ± 0.005	0.063 * ± 0.001	0.210** ± 0.005
	75	0.126 n.s. ± 0.021	0.062* ± 0.007	0.188 n.s. ± 0.029
	100	0.130* ± 0.001	0.078* ± 0.003	0.208** ± 0.003
	125	0.169 n.s. ± 0.001	0.073* ± 0.002	0.242 n.s. ± 0.001
	150	0.150 n.s. ± 0.001	0.082* ± 0.002	0.233 n.s. ± 0.002
LSD 0.05 for put		0.039	0.035	0.065
LSD 0.01 for put		0.054	n.s.	0.092
LSD 0.001 for put		0.077	n.s.	0.130
LSD 0.05 for spd		0.022	0.031	0.049
LSD 0.01 for spd		0.030	0.042	0.069
LSD 0.001 for spd		0.043	n.s.	0.098
LSD 0.05 for spm		0.028	0.014	0.039
LSD 0.01 for spm		0.039	0.020	0.055
LSD 0.001 for spm		0.055	0.028	0.077

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$;***very highly significant at $P \leq 0.001$

Table 2. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on dry matter (g/plant) of *Origanum majorana* plants

Polyamine	NaCl (mM)	Shoot	Root	Total
Control	0.0	0.186 ± 0.020	0.144 ± 0.017	0.329 ± 0.033
	25	0.140 n.s. ± 0.003	0.092* ± 0.008	0.232* ± 0.008
	50	0.137* ± 0.004	0.089* ± 0.011	0.226* ± 0.016
	75	0.136 n.s. ± 0.016	0.078** ± 0.002	0.214* ± 0.017
	100	0.150 n.s. ± 0.019	0.079* ± 0.013	0.229 n.s. ± 0.030
	125	0.165 n.s. ± 0.023	0.086* ± 0.013	0.251 n.s. ± 0.032
	150	0.133 n.s. ± 0.026	0.107 n.s. ± 0.015	0.240 n.s. ± 0.023
Put	0.0	0.252 n.s. ± 0.035	0.131 n.s. ± 0.003	0.383 n.s. ± 0.036
	25	0.274* ± 0.039	0.146* ± 0.016	0.420** ± 0.031
	50	0.301** ± 0.032	0.198** ± 0.021	0.500** ± 0.047
	75	0.289* ± 0.046	0.138** ± 0.015	0.427* ± 0.055
	100	0.222* ± 0.019	0.136 n.s. ± 0.021	0.358* ± 0.018
	125	0.266* ± 0.026	0.127* ± 0.007	0.392* ± 0.032
	150	0.239** ± 0.009	0.139 n.s. ± 0.006	0.378** ± 0.014
Spd	0.0	0.359** ± 0.034	0.121 n.s. ± 0.013	0.480* ± 0.023
	25	0.324* ± 0.052	0.111 n.s. ± 0.020	0.435* ± 0.071
	50	0.278*** ± 0.013	0.108 n.s. ± 0.015	0.386*** ± 0.008
	75	0.297** ± 0.027	0.112* ± 0.011	0.409** ± 0.037
	100	0.235** ± 0.009	0.102 n.s. ± 0.010	0.337* ± 0.012
	125	0.205 n.s. ± 0.048	0.076 n.s. ± 0.005	0.281 n.s. ± 0.053
	150	0.237* ± 0.025	0.106 n.s. ± 0.023	0.343 n.s. ± 0.054
Spm	0.0	0.313 n.s. ± 0.054	0.117 n.s. ± 0.017	0.430 n.s. ± 0.071
	25	0.265*** ± 0.013	0.114 n.s. ± 0.023	0.379** ± 0.033
	50	0.276 n.s. ± 0.064	0.101 n.s. ± 0.010	0.377 n.s. ± 0.073
	75	0.192 n.s. ± 0.047	0.073 n.s. ± 0.011	0.265 n.s. ± 0.053
	100	0.228** ± 0.001	0.083 n.s. ± 0.011	0.311* ± 0.009
	125	0.276* ± 0.033	0.120 n.s. ± 0.010	0.396* ± 0.042
	150	0.159 n.s. ± 0.023	0.083 n.s. ± 0.002	0.242 n.s. ± 0.024
LSD 0.05 for put		0.071	n.s.	0.100
LSD 0.01 for put		n.s.	n.s.	n.s.
LSD 0.001 for put		n.s.	n.s	n.s.
LSD 0.05 for spd		n.s.	n.s.	n.s.
LSD 0.01 for spd		n.s.	n.s.	n.s.
LSD 0.001 for spd		n.s.	n.s.	n.s.
LSD 0.05 for spm		n.s.	0.044	n.s.
LSD 0.01 for spm		n.s.	n.s.	n.s.
LSD 0.001 for spm		n.s.	n.s.	n.s.

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ***very highly significant at $P \leq 0.001$

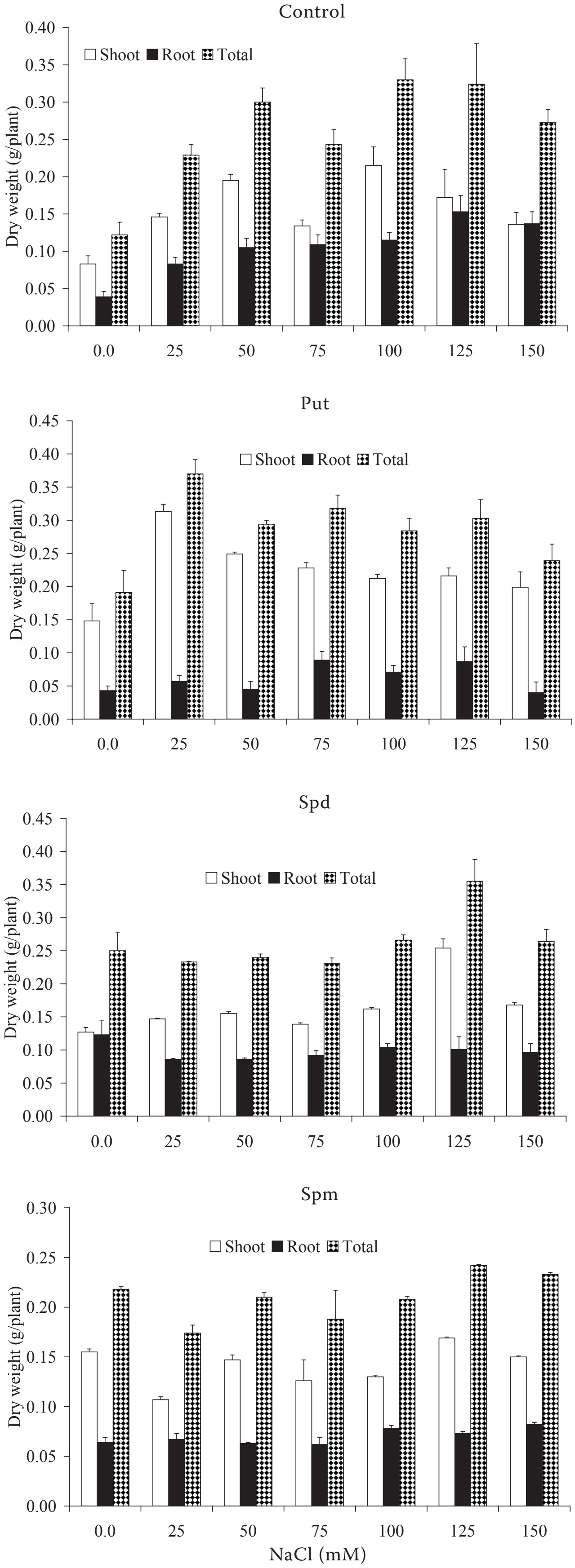

Figure 1. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on dry matter (g/plant) of *Chamomilla recutita* plants

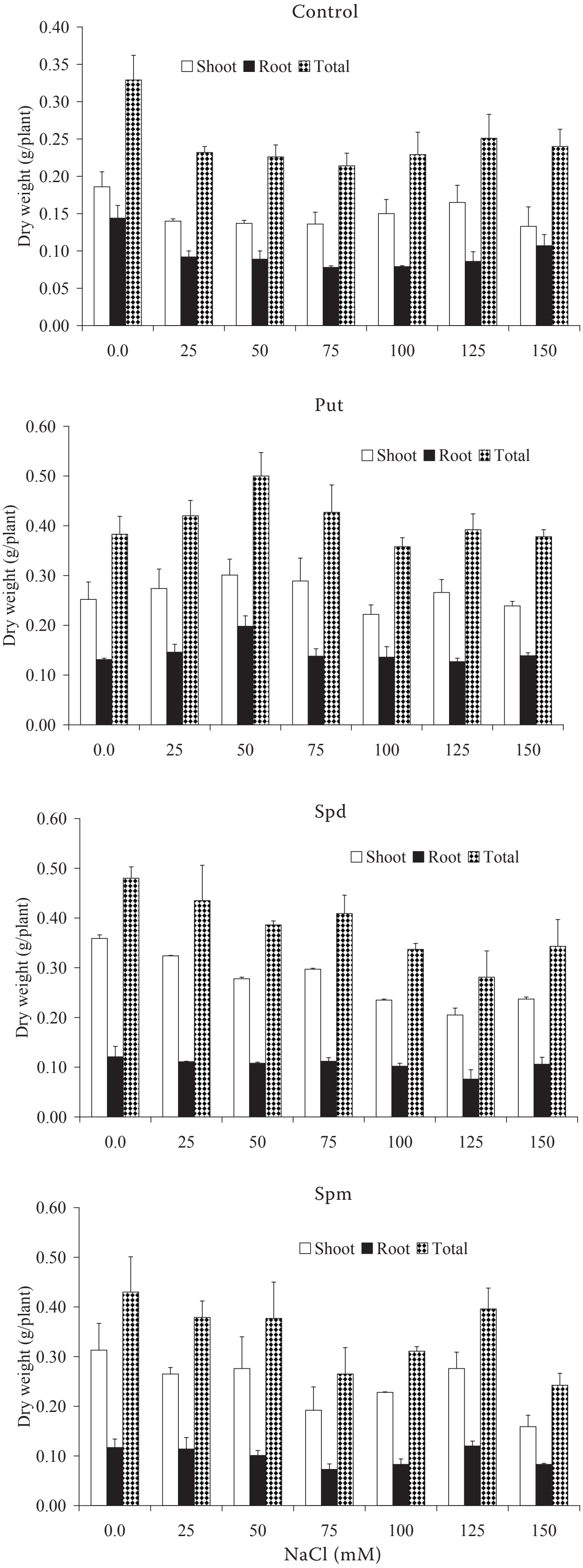

Figure 2. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on dry matter (g/plant) of *Origanum majorana* plants

Table 3. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on dry matter (mg/plant) of both plant flowers

Polyamine	NaCl (mM)	*Chamomilla recutita* flowers		*Origanum majorana* flowers	
		dry matter	(%)	dry matter	(%)
Control	0.0	35.23 ± 2.9	100	6.26 ± 0.6	100
	25	55.43** ± 1.2	157.34	4.51 n.s. ± 1.0	72.04
	50	50.36* ± 4.0	142.95	8.16 n.s. ± 1.2	130.35
	75	42.16 n.s. ± 1.2	119.67	7.33 n.s. ± 1.0	117.09
	100	38.66 n.s. ± 1.6	109.74	7.85 n.s. ± 1.7	125.40
	125	26.52 n.s. ± 3.5	75.28	10.33* ± 1.2	165.02
	150	11.23** ± 2.4	31.88	7.62 n.s. ± 0.9	121.73
Put	0.0	34.57 n.s. ± 2.4	98.13	6.29 n.s. ± 1.2	100.48
	25	49.52 n.s. ± 5.0	140.56	5.90 n.s. ± 0.6	94.25
	50	52.00 n.s. ± 2.3	147.60	8.71 n.s. ± 1.0	139.14
	75	51.25 n.s. ± 4.6	145.47	11.35* ± 0.6	181.31
	100	41.38 n.s. ± 4.0	117.46	11.26 n.s. ± 1.2	179.87
	125	33.04 n.s. ± 1.7	93.78	10.47 n.s. ± 1.2	167.25
	150	23.55** ± 1.6	66.85	9.42 n.s. ± 1.2	150.48
Spd	0.0	36.45 n.s. ± 1.7	103.46	13.15** ± 1.6	210.06
	25	40.40** ± 2.9	114.67	18.17*** ± 1.1	290.26
	50	46.38 n.s. ± 2.3	131.65	14.47** ± 0.4	231.15
	75	35.06** ± 1.1	99.52	14.41** ± 0.5	230.19
	100	27.50** ± 1.6	78.06	10.23 n.s. ± 0.6	163.42
	125	25.26 n.s. ± 2.3	71.70	9.73 n.s. ± 1.0	155.43
	150	28.52** ± 2.1	80.95	8.72 n.s. ± 0.6	139.30
Spm	0.0	33.48 n.s. ± 1.8	95.03	14.39*** ± 0.5	229.87
	25	38.11** ± 2.3	108.17	12.61** ± 1.3	201.44
	50	43.52 n.s. ± 1.6	123.53	17.55** ± 1.0	280.35
	75	35.33** ± 1.1	100.28	17.11*** ± 0.6	273.32
	100	30.13** ± 3.4	85.52	16.16** ± 0.5	258.15
	125	31.28 n.s. ± 0.6	88.79	11.27 n.s. ± 0.5	180.03
	150	31.63** ± 2.5	89.78	11.28** ± 0.1	180.19
LSD 0.05 for put		9.95		2.89	
LSD 0.01 for put		13.95		4.06	
LSD 0.001 for put		19.71		n.s.	
LSD 0.05 for spd		6.49		2.27	
LSD 0.01 for spd		9.09		3.18	
LSD 0.001 for spd		12.85		4.50	
LSD 0.05 for Spm		7.21		2.27	
LSD 0.01 for spm		n.s.		3.19	
LSD 0.001 for spm		n.s.		4.50	

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ***very highly significant at $P \leq 0.001$

Table 4. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on oil content (mg/g dm) of *Chamomilla recutita* plants

Poly-amine	NaCl (mM)	Root	(%)	Shoot	(%)	Flowers	(%)
Control	0.0	0.06 ± 0.012	100	0.06 ± 0.010	100	0.04 ± 0.0006	100
	25	0.07 n.s. ± 0.0058	116.67	0.09 n.s. ± 0.023	155.56	0.09*** ± 0.0058	225.00
	50	0.06 n.s. ± 0.0033	105.56	0.09 n.s. ± 0.018	144.44	0.08*** ± 0.0033	208.33
	75	0.05 n.s. ± 0.0067	88.89	0.08 n.s. ± 0.010	133.33	0.08** ± 0.0058	200.00
	100	0.05 n.s. ± 0.0013	88.89	0.07 n.s. ± 0.015	122.22	0.05*** ± 0.0058	125.00
	125	0.05 n.s. ± 0.0067	88.89	0.07 n.s. ± 0.0058	116.67	0.04 n.s. ± 0.0006	100.00
	150	0.05 n.s. ± 0.0058	83.33	0.06 n.s. ± 0.0058	100.00	0.03*** ± 0.0006	75.00
Put	0.0	0.08 n.s. ± 0.0067	127.78	0.11** ± 0.0067	188.89	0.04 n.s. ± 0.0006	100.00
	25	0.11** ± 0.0058	183.33	0.09 n.s. ± 0.0088	155.56	0.05*** ± 0.0006	125.00
	50	0.10** ± 0.0067	161.11	0.08 n.s. ± 0.0033	127.78	0.06** ± 0.0006	150.00
	75	0.09* ± 0.0088	144.44	0.08 n.s. ± 0.0033	127.78	0.08 n.s. ± 0.0058	200.00
	100	0.08 n.s. ± 0.0067	138.89	0.07 n.s. ± 0.010	116.67	0.05 n.s. ± 0.0033	133.33
	125	0.08* ± 0.0033	127.78	0.07 n.s. ± 0.0058	116.67	0.04 n.s. ± 0.0033	108.33
	150	0.07* ± 0.0067	122.22	0.06 n.s. ± 0.012	105.56	0.03 n.s. ± 0.0033	83.33
Spd	0.0	0.09 n.s. ± 0.010	150.00	0.10 n.s. ± 0.015	172.22	0.05** ± 0.0033	133.33
	25	0.09 n.s. ± 0.0088	144.44	0.08 n.s. ± 0.0058	133.33	0.07** ± 0.0033	166.67
	50	0.06 n.s. ± 0.0067	105.56	0.07 n.s. ± 0.013	122.22	0.08 n.s. ± 0.0033	191.67
	75	0.06 n.s. ± 0.071	100.00	0.07 n.s. ± 0.010	116.67	0.08 n.s. ± 0.0058	200.00
	100	0.06 n.s. ± 0.0058	100.00	0.07 n.s. ± 0.0007	111.11	0.07** ± 0.0033	183.33
	125	0.06 n.s. ± 0.010	100.00	0.07 n.s. ± 0.0033	111.11	0.05** ± 0.0033	133.33
	150	0.06 n.s. ± 0.0088	94.44	0.06 n.s. ± 0.0067	105.56	0.04** ± 0.0033	108.33
Spm	0.0	0.09 n.s. ± 0.0067	144.44	0.09 * ± 0.0058	150.00	0.06** ± 0.0033	141.67
	25	0.08 n.s. ± 0.0067	138.89	0.08 n.s. ± 0.0058	133.33	0.06*** ± 0.0033	141.67
	50	0.08 n.s. ± 0.0067	127.78	0.08 n.s. ± 0.0033	127.78	0.07 n.s. ± 0.0033	183.33
	75	0.07 n.s. ± 0.0088	122.22	0.08 n.s. ± 0.0088	127.78	0.09 n.s. ± 0.0058	225.00
	100	0.07 n.s. ± 0.0058	116.67	0.07 n.s. ± 0.0058	116.67	0.05 n.s. ± 0.0058	125.00
	125	0.06 n.s. ± 0.0067	105.56	0.06 n.s. ± 0.0058	100.00	0.04 n.s. ± 0.0033	108.33
	150	0.06 n.s. ± 0.010	100.00	0.06 n.s. ± 0.0033	94.44	0.03 n.s. ± 0.0033	83.33
LSD 0.05 for put		0.022		n.s.		0.005	
LSD 0.01 for put		0.030		n.s.		0.008	
LSD 0.001 for put		n.s.		n.s.		0.011	
LSD 0.05 for spd		n.s.		n.s.		0.011	
LSD 0.01 for spd		n.s.		n.s.		0.016	
LSD 0.001 for spd		n.s.		n.s.		0.022	
LSD 0.05 for spm		n.s.		n.s.		0.011	
LSD 0.01 for spm		n.s.		n.s.		0.015	
LSD 0.001 for spm		n.s.		n.s.		0.021	

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ***very highly significant at $P \leq 0.001$

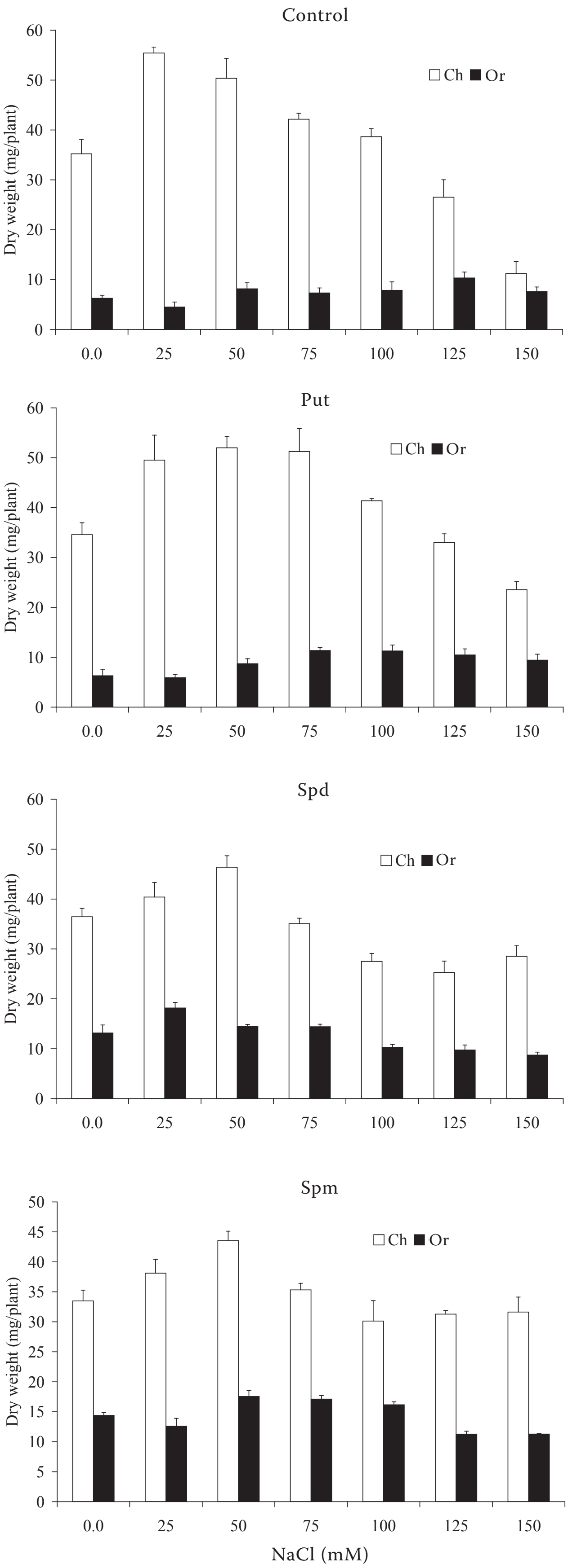

Figure 3. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on dry matter (mg/plant) of *Chamomilla recutita* and *Origanum majorana* flowers

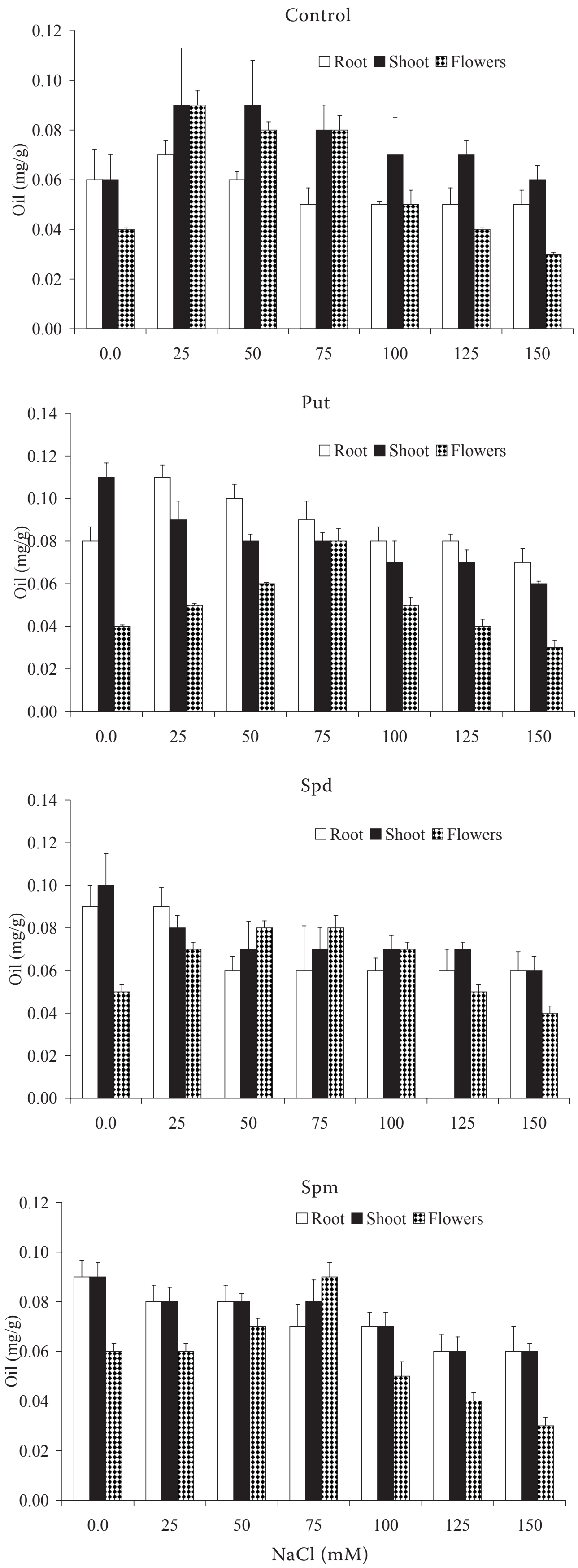

Figure 4. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on oil content (mg/g dw) of *Chamomilla recutita* plants

Table 5. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on oil content (mg/g dm) of *Origanum majorana* plants

Poly-amine	NaCl (mM)	Root	(%)	Shoot	(%)	Flowers	(%)
Control	0.0	0.128 ± 0.004	100	0.237 ± 0.006	100	0.059 ± 0.0027	100
	25	0.129 n.s. ± 0.002	100.78	0.369*** ± 0.003	155.70	0.038** ± 0.0023	64.41
	50	0.144 n.s. ± 0.016	112.50	0.331*** ± 0.004	139.66	0.045 n.s. ± 0.0069	76.27
	75	0.140 n.s. ± 0.005	109.38	0.324*** ± 0.007	136.71	0.053 n.s. ± 0.0035	89.83
	100	0.090* ± 0.011	70.31	0.321*** ± 0.008	135.44	0.025*** ± 0.0021	42.37
	125	0.072*** ± 0.004	56.25	0.316** ± 0.012	133.33	0.020*** ± 0.0039	33.90
	150	0.055*** ± 0.007	42.97	0.153* ± 0.021	64.56	0.032** ± 0.0062	54.24
Put	0.0	0.139 n.s. ± 0.012	108.59	0.283** ± 0.008	119.41	0.124** ± 0.0120	210.17
	25	0.170*** ± 0.002	132.81	0.354* ± 0.003	149.37	0.087** ± 0.0070	147.46
	50	0.206** ± 0.022	160.94	0.266** ± 0.008	112.24	0.084** ± 0.0061	142.37
	75	0.117** ± 0.003	91.41	0.251** ± 0.014	105.91	0.028** ± 0.0030	47.46
	100	0.063* ± 0.002	49.22	0.179*** ± 0.006	75.53	0.025 n.s. ± 0.0026	42.37
	125	0.052** ± 0.002	40.63	0.164*** ± 0.007	69.20	0.021 n.s. ± 0.0049	35.59
	150	0.037* ± 0.001	28.91	0.072* ± 0.007	30.38	0.011* ± 0.0006	18.64
Spd	0.0	0.146 n.s. ± 0.014	114.06	0.402*** ± 0.004	169.62	0.094** ± 0.0078	159.32
	25	0.161** ± 0.005	125.78	0.458*** ± 0.006	193.25	0.029 n.s. ± 0.0060	49.15
	50	0.215** ± 0.003	167.97	0.444*** ± 0.007	187.34	0.027 n.s. ± 0.0055	45.76
	75	0.163 n.s. ± 0.014	127.34	0.442*** ± 0.008	186.50	0.019** ± 0.0038	32.20
	100	0.144* ± 0.012	112.50	0.438** ± 0.020	184.81	0.018 n.s. ± 0.0064	30.51
	125	0.128** ± 0.007	100.00	0.353 n.s. ± 0.011	148.95	0.014 n.s. ± 0.0035	23.73
	150	0.126*** ± 0.003	98.44	0.449*** ± 0.007	189.45	0.026 n.s. ± 0.0068	44.07
Spm	0.0	0.248*** ± 0.009	193.75	0.509*** ± 0.002	214.77	0.081 n.s. ± 0.0094	137.29
	25	0.247*** ± 0.006	192.97	0.492*** ± 0.004	207.59	0.075** ± 0.0088	127.12
	50	0.242** ± 0.007	189.06	0.467*** ± 0.009	197.05	0.056 n.s. ± 0.0080	94.92
	75	0.240** ± 0.024	187.50	0.446** ± 0.027	188.19	0.047 n.s. ± 0.0084	79.66
	100	0.238*** ± 0.005	185.94	0.437*** ± 0.003	184.39	0.039 * ± 0.0053	66.10
	125	0.220*** ± 0.003	171.88	0.387** ± 0.007	163.29	0.022 n.s. ± 0.0050	37.29
	150	0.192*** ± 0.005	150.00	0.396*** ± 0.008	167.09	0.025 n.s. ± 0.0052	42.37
LSD 0.05 for put		0.007		0.022		0.013	
LSD 0.01 for put		0.009		0.030		0.018	
LSD 0.001 for put		0.013		0.043		0.026	
LSD 0.05 for spd		0.024		0.031		0.013	
LSD 0.01 for spd		0.034		0.043		0.019	
LSD 0.001 for spd		0.048		0.061		0.026	
LSD 0.05 for spm		0.033		0.039		0.021	
LSD 0.01 for spm		0.047		0.054		0.029	
LSD 0.001 for spm		0.066		0.077		0.041	

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ***very highly significant at $P \leq 0.001$

Table 6. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on flavonoids content (mg/g dm) of *Chamomilla recutita* plants

Poly-amine	NaCl (mM)	Root	(%)	Shoot	(%)	Flowers	(%)
	0.0	0.041 ± 0.0018	100	0.225 ± 0.0084	100	0.459 ± 0.0073	100
	25	0.054* ± 0.0030	131.97	0.228 n.s. ± 0.0012	101.18	0.545*** ± 0.0040	118.82
	50	0.060*** ± 0.0012	147.54	0.230 n.s. ± 0.0018	101.92	0.494** ± 0.0032	107.70
Control	75	0.052** ± 0.0023	128.69	0.197* ± 0.0012	87.28	0.445 n.s. ± 0.0033	97.09
	100	0.050** ± 0.0012	122.95	0.190** ± 0.0026	84.47	0.376*** ± 0.0026	81.98
	125	0.033* ± 0.0022	80.33	0.188** ± 0.0015	83.28	0.319*** ± 0.0012	69.62
	150	0.024** ± 0.0025	59.02	0.178** ± 0.0006	78.99	0.315*** ± 0.0018	68.60
	0.0	0.042 n.s. ± 0.0038	102.46	0.216 n.s. ± 0.0017	95.86	0.486* ± 0.0060	105.96
	25	0.058 n.s. ± 0.0017	142.62	0.257*** ± 0.0019	113.91	0.576** ± 0.0035	125.58
	50	0.068** ± 0.0018	168.03	0.283*** ± 0.0026	125.59	0.555*** ± 0.0039	120.93
Put	75	0.082*** ± 0.0018	200.82	0.244*** ± 0.0018	108.14	0.469** ± 0.0026	102.25
	100	0.066*** ± 0.0018	163.11	0.235*** ± 0.0009	104.44	0.412***± 0.0015	89.90
	125	0.037 n.s. ± 0.0019	90.16	0.214*** ± 0.0018	94.82	0.345*** ± 0.0023	75.29
	150	0.031 n.s. ± 0.0018	77.05	0.206*** ± 0.0015	91.42	0.301** ± 0.0017	65.63
	0.0	0.046* ± 0.0009	113.93	0.208 n.s. ± 0.0015	92.16	0.436* ± 0.0023	95.13
	25	0.047 n.s. ± 0.0045	114.75	0.193*** ± 0.0015	85.50	0.561* ± 0.0017	122.31
	50	0.049** ± 0.0012	120.49	0.183*** ± 0.0020	81.36	0.553*** ± 0.0026	120.64
Spd	75	0.036** ± 0.0012	87.70	0.206 n.s. ± 0.0039	91.27	0.482*** ± 0.0030	105.01
	100	0.026*** ± 0.0018	64.75	0.222*** ± 0.0015	98.52	0.424*** ± 0.0027	92.51
	125	0.023* ± 0.0015	56.56	0.212*** ± 0.0009	93.93	0.404*** ± 0.0018	88.15
	150	0.021 n.s. ± 0.0009	52.46	0.192* ± 0.0038	85.06	0.351*** ± 0.0029	76.53
	0.0	0.036 n.s. ± 0.0025	88.52	0.196* ± 0.0035	87.13	0.432* ± 0.0018	94.26
	25	0.039* ± 0.0035	95.08	0.179*** ± 0.0015	79.59	0.581*** ± 0.0026	126.60
	50	0.044* ± 0.0057	109.02	0.202*** ± 0.0024	89.50	0.564*** ± 0.0026	122.89
Spm	75	0.048 n.s. ± 0.0015	117.21	0.207** ± 0.0015	91.72	0.424** ± 0.0021	92.44
	100	0.057* ± 0.0020	139.34	0.196 n.s. ± 0.0024	86.83	0.411*** ± 0.0012	89.53
	125	0.040* ± 0.0012	97.54	0.186 n.s. ± 0.0015	82.69	0.373*** ± 0.0027	81.25
	150	0.023 n.s. ± 0.0012	56.56	0.180 n.s. ± 0.0012	79.88	0.326** ± 0.0015	71.00
LSD 0.05 for put		0.005		0.005		0.010	
LSD 0.01 for put		0.007		0.007		0.015	
LSD 0.001 for put		0.010		0.010		0.021	
LSD 0.05 for spd		0.007		0.008		0.008	
LSD 0.01 for spd		0.010		0.011		0.011	
LSD 0.001 for spd		0.014		0.016		0.016	
LSD 0.05 for spm		0.009		0.006		0.010	
LSD 0.01 for spm		0.013		0.008		0.014	
LSD 0.001 for spm		0.018		0.012		0.020	

n.s. not significant at $P > 0.05$; *significant at $P \le 0.05$; **highly significant at $P \le 0.01$; ***very highly significant at $P \le 0.001$

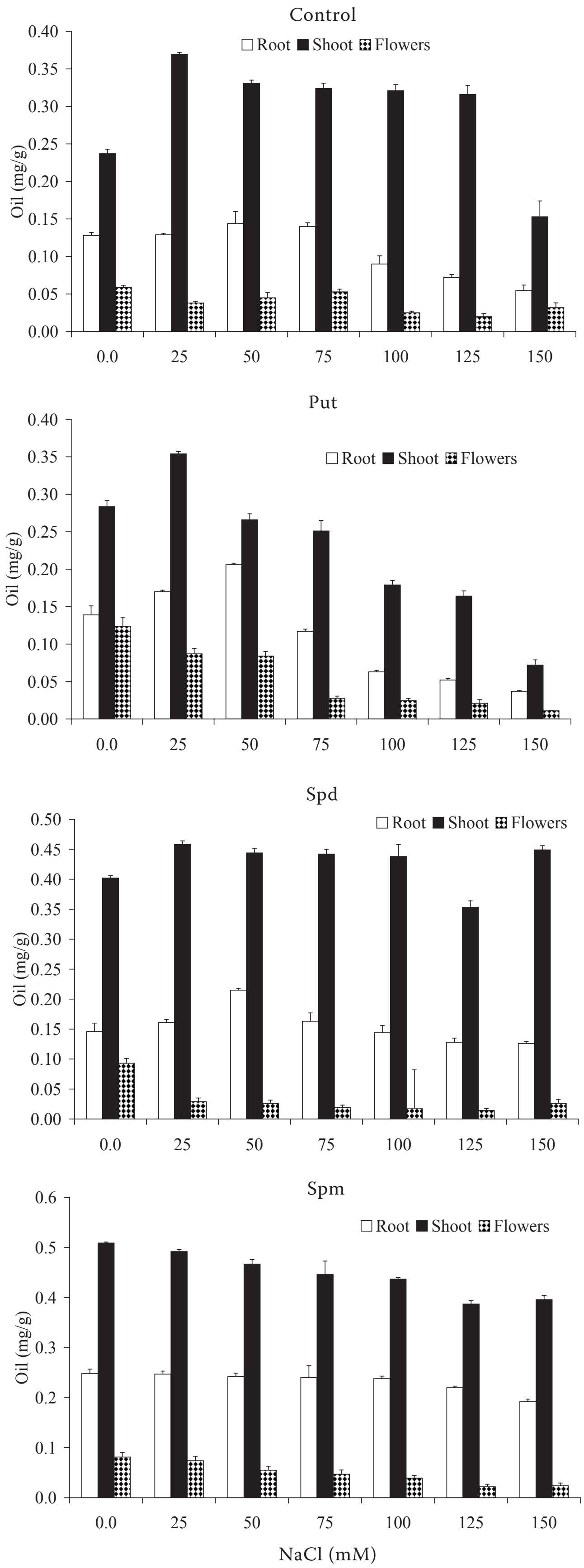

Figure 5. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on oil content (mg/g dw) of *Origanum majorana* plants

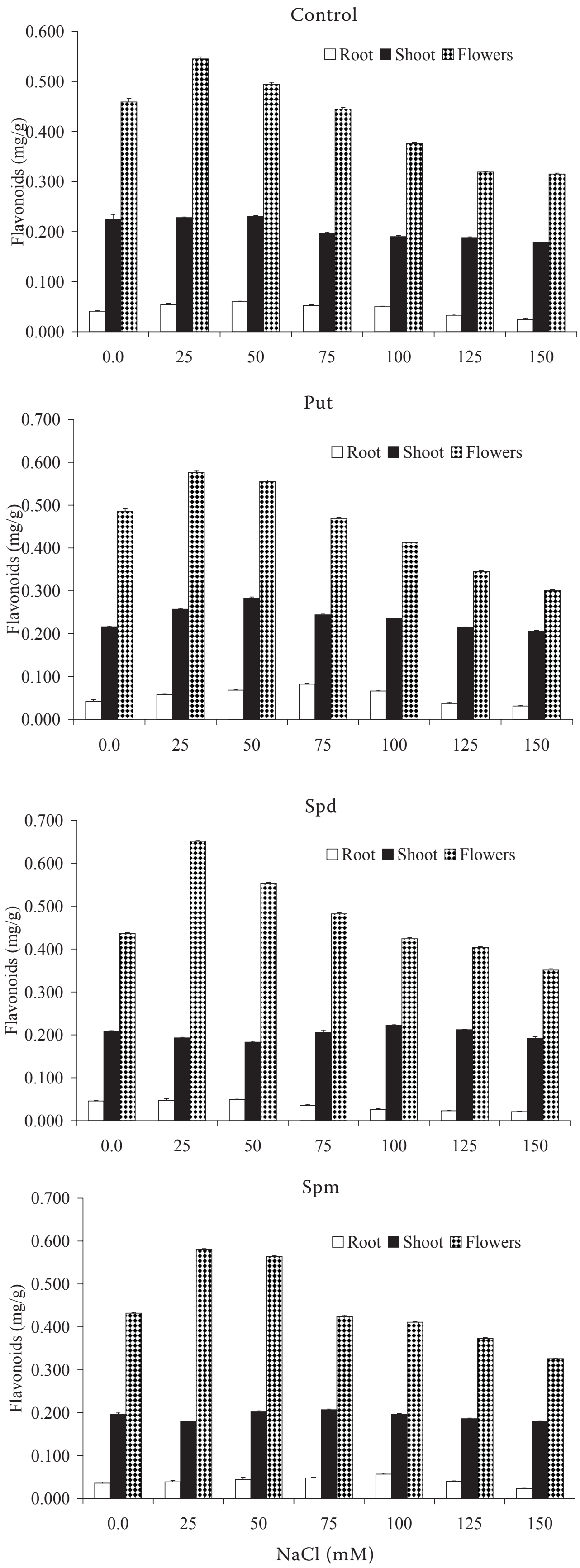

Figure 6. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on flavonoid content (mg/g dw) of *Chamomilla recutita* plants

Table 7. The effect of NaCl and treatment with 0.1mM polyamine (control, put, spd or spm) on flavonoids content (mg/g dm) of *Origanum majorana* plants

Polyamine	NaCl (mM)	Root	(%)	Shoot	(%)	Flowers	(%)
Control	0.0	0.590 ± 0.045	100	2.581 ± 0.18	100	1.108 ± 0.005	100
	25	0.412* ± 0.004	69.83	2.574 n.s. ± 0.14	99.73	1.310** ± 0.007	118.23
	50	0.344** ± 0.011	58.31	2.877 n.s. ± 0.058	111.47	1.511*** ± 0.011	136.37
	75	0.388** ± 0.023	65.76	2.762 n.s. ± 0.076	107.01	1.638*** ± 0.015	147.83
	100	0.508 n.s. ± 0.035	86.10	2.975 n.s. ± 0.074	115.27	1.968*** ± 0.009	177.62
	125	0.472 n.s. ± 0.041	80.00	2.322 n.s. ± 0.029	89.97	1.789*** ± 0.015	161.46
	150	0.293** ± 0.021	49.66	1.396** ± 0.24	54.09	1.598*** ± 0.013	144.22
Put	0.0	0.593 n.s. ± 0.034	100.51	3.021 n.s. ± 0.075	117.05	1.117 n.s. ± 0.023	100.81
	25	1.006*** ± 0.037	170.51	3.108* ± 0.11	120.42	2.139*** ± 0.011	193.05
	50	1.033*** ± 0.010	175.08	3.149* ± 0.067	122.01	2.817*** ± 0.013	254.24
	75	1.024*** ± 0.032	173.56	3.330** ± 0.031	129.02	3.200*** ± 0.012	288.81
	100	0.926*** ± 0.024	156.95	3.329** ± 0.036	128.98	3.286*** ± 0.026	296.57
	125	0.682** ± 0.004	115.59	3.356*** ± 0.017	130.03	2.322*** ± 0.014	209.57
	150	0.592*** ± 0.014	100.34	3.336*** ± 0.050	129.25	2.078*** ± 0.019	187.55
Spd	0.0	0.647 n.s. ± 0.010	109.66	2.778 n.s. ± 0.13	107.63	1.114 n.s. ± 0.015	100.54
	25	0.750** ± 0.058	127.12	2.791 n.s. ± 0.068	108.14	2.666*** ± 0.032	240.61
	50	0.817*** ± 0.029	138.47	2.799 n.s. ± 0.11	108.45	2.921*** ± 0.021	263.63
	75	0.605** ± 0.031	102.54	2.669 n.s. ± 0.036	103.41	3.181*** ± 0.016	287.09
	100	0.479 n.s. ± 0.042	81.19	2.530** ± 0.073	98.02	2.811*** ± 0.036	253.70
	125	0.471 n.s. ± 0.016	79.83	2.486 n.s. ± 0.10	96.32	2.422*** ± 0.043	218.59
	150	0.418* ± 0.026	70.85	2.377* ± 0.25	92.10	2.265*** ± 0.024	204.42
Spm	0.0	0.652 n.s. ± 0.021	110.51	2.820 n.s. ± 0.15	109.26	1.223 n.s. ± 0.051	110.38
	25	0.888*** ± 0.008	150.51	2.665 n.s. ± 0.15	103.25	3.295*** ± 0.033	297.38
	50	0.879*** ± 0.045	148.98	2.794 n.s. ± 0.17	108.25	3.303*** ± 0.024	298.10
	75	0.728** ± 0.051	123.39	2.729 n.s. ± 0.12	105.73	3.845*** ± 0.041	347.02
	100	0.592 n.s. ± 0.017	100.34	2.521 n.s. ± 0.19	97.68	2.731*** ± 0.038	246.48
	125	0.588* ± 0.015	99.66	2.263 n.s. ± 0.10	87.68	2.634*** ± 0.038	237.73
	150	0.457** ± 0.019	77.46	2.134* ± 0.10	82.68	2.313*** ± 0.008	208.75
LSD 0.05 for put		0.086		0.267		0.040	
LSD 0.01 for put		0.121		0.372		0.056	
LSD 0.001 for put		0.170		0.526		0.079	
LSD 0.05 for spd		0.121		n.s.		0.050	
LSD 0.01 for spd		0.169		n.s.		0.071	
LSD 0.001 for spd		0.240		n.s.		0.080	
LSD 0.05 for spm		0.079		0.389		0.092	
LSD 0.01 for spm		0.110		n.s.		0.130	
LSD 0.001 for spm		0.156		n.s.		0.183	

n.s. not significant at $P > 0.05$; *significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ***very highly significant at $P \leq 0.001$

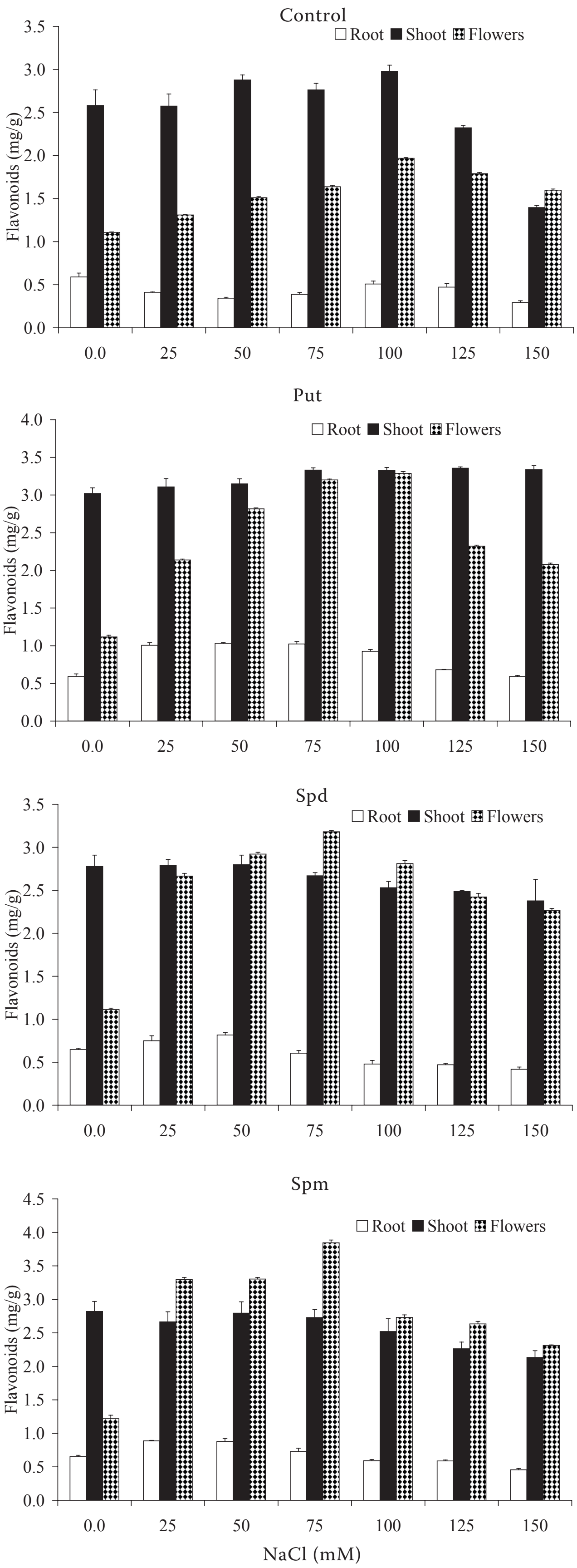

Figure 7. The effect of NaCl and treatment with 0.1mM polyamines (control, put, spd, spm) on flavonoid content (mg/g dw) of *Origanum majorana* plants

and 5, Tables 4 and 5). This is in accordance with the results obtained with cotton by Twersky and Felhendler (1973); they recorded a direct relation between the salinity of irrigation and oil concentrations. The observed salt tolerance of the oil-producing plants as well as the enhancement in their physiological activities by salinity may strengthen the supposition (Ahmed et al. 1977, 1979, Ali 2002) that these plants probably have their own mechanism to counterbalance the effect of salinity treatments by oil formation.

Generally, foliar application with any of the polyamines increased the oil content in roots, shoots and flowers of *Chamomilla recutita* and *Origanum majorana* (Figures 4 and 5). Also, foliar application with polyamines increased the oil content in roots, but decreased it in shoots and flowers of *Chamomilla recutita* when compared with the corresponding treatments with NaCl. However, in the case of *Origanum majorana* the oil content in roots increased at low NaCl level, spermidine or spermine increased the oil contents in roots and shoots when compared with the corresponding treatments with NaCl. Spermidine treatments decreased the oil content in flowers of *Origanum majorana*, whereas spermine increased it.

The flavonoid content of *Chamomilla recutita* roots and *Origanum majorana* shoots increased up to 100mM NaCl but decreased in roots of *Origanum majorana* plant (Figures 6 and 7, Tables 6 and 7). Furthermore, NaCl treatments were capable of acting as activators of flavonoid accumulation. The highest accumulation of flavonoids was at 100mM NaCl in the case of shoots and flowers of *Origanum majorana*, and at 50mM NaCl in roots of *Chamomilla recutita*. However, the flavonoid contents in roots, shoots and flowers of *Chamomilla recutita* were accumulated at lower salinization levels (25 and 50mM NaCl). It was observed that several classes of flavonoids show antioxidant activity towards a variety of easily oxidizable compounds, many of those play an important physiological and ecological role as they are involved in resistance to different types of stress (Rice-Evans and Miller 1998, Ayaz et al. 2000, Ali and Abbas 2003).

Foliar application with putrescine generally increased the level of flavonoids in different organs of both plants except for shoots of *Chamomilla recutita*. Treatments with spermidine increased the flavonoid content in shoots of *Chamomilla recutita* at moderate levels of salinity (from 75 to 125mM) and in different organs of *Origanum majorana* at lower levels of salinity (up to 75mM), but decreased it in roots and flowers of *Chamomilla recutita*. Treatments with spermine increased the flavonoid content in different organs of *Origanum majorana*, but decreased in shoots and flowers of *Chamomilla recutita*. Treatment of *Chamomilla recutita* and *Origanum majorana* plants with different levels of salinity with any of polyamines induced some changes in flavonoids synthesis. Putrescine generally increased the level of flavonoids in different organs of both plants. Similarly, spermidine increased the flavonoid contents in different organs of *Origanum majorana* plant and in shoots of *Chamomilla recutita*. Treatment with spermine increased the flavonoid contents in different organs of *Origanum majorana* and in roots of *Chamomilla recutita*, but variably changed in shoots and flowers of *Chamomilla recutita* plant.

To conclude, it was observed that salinity generally induced variable changes in the contents of oil and flavonoids in the plant parts of both *Chamomilla recutita* and *Origanum majorana*. The tendency of these changes leads to an assumption that the two medicinal plants tested in this investigation have some variable abilities not only to tolerate moderate salinization but also to grow well and to produce the same amount or even more oil and flavonoids than the control plants. Foliar application of any polyamines counterbalanced the adverse effects of salinity on growth and content of oils and flavonoids in *Chamomilla recutita* and *Origanum majorana* plants.

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