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Effect of Salicylic Acid on the Growth, Metabolic Activities and Oil Content of Basil and Marjoram

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ABSTRACT

The response of sweet basil (*Ocimum basilicum* L.) and marjoram (*Majorana hortensis*) plants to foliar application of salicylic acid (SA) at 10^{-5}, 10^{-4} and 10^{-3} M was determined in pot experiments conducted during 2004 - 2005. SA increased plant height, number of (branches, nodes & leaves) per plant, leaf area, fresh and dry weight of herbs, total carbohydrates, crude protein, total amino acids, free proline, photosynthetic pigments as well as microelement content and uptake up to 10^{-4} M relative to un-treated controls and decreased thereafter both in basil and marjoram. All SA treatments enhanced putrescine, spermidine as well as total polyamines contents, while reduced the level of spermine in both plants. Oil percentage and yield per plant for three cuttings also increased about two fold on a fresh weight basis with SA application at 10^{-4} M in case of basil and 10^{-3} M in marjoram relative to un-treated controls. GC/MS revealed that common components of *Ocimum basilicum* essential oil under all treatments were linalool (46.63 - 43.32%), methyl eugenol (13.83 - 5.68%), 1, 8 - cineol (13.20 - 4.43%), eugenol (12.64 - 7.16%) and α-cadinol (9.59 - 4.46%). SA at 10^{-4} M increased the production of top quantity and quality of basil oil to the fragrance and food industries by increasing the percentage of eugenol and antioxidant activity in the herb. On the other hand, the marjoram essential oil contains *cis*-sabinene hydrate(37.50 - 14.27%), terpinen- 4-ol (24.33 - 13.99%), p-cymene (18.21 - 2.29%), sabinene (17.69 - 4.11%), γ-terpinene (10.64 - 4.77) in addition to α-terpineol (5.52 - 3.96%), trans-sabinene hydrate (5.45 - 8.19%), α-terpinene (2.41 - 0.00%) and β-caryophyllene (3.82 - 1.76%). Moreover, SA at 10^{-5} M and 10^{-3} M improved oil quality by increasing the level of sabinene accompanied by a decrease in the proportion of *cis*-sabinene hydrate relative to controls. The data suggest that in both species, SA treatment especially at 10^{-4} M may have higher adaptive capacity to stress, originating from promoting polyamines synthesis and better osmotic adjustment.

Key Words: *O. basilicum; M. hortensis*; Salicylic acid; Vegetative growth; Free proline; Polyamines; Essential oils

INTRODUCTION

Aromatic plants represent a renewable source of flavoring substances, which can be used in the food, perfumery and pharmaceutical industries. The family lamiaceae includes large number of volatile oil plants and the most important members are sweet basil (*Ocimum basilicum*) and marjoram (*Majorana hortensis*). Sweet basil is one of the most widespread spices in the world and its dried leaves are used commonly for flavoring many food products. Salicylate content of spices was bio-available, and may contribute to the low cancer incidence in rural India (Paterson *et al*., 2006). Basil contains a wide range of essential oils rich in phenolic compounds (Simon *et al*., 1990; Phippen & Simon, 2000) and polyphenols such as flavonoids and anthocyanins (Phippen & Simon, 1998). The volatile basil oil is used in pharmaceutical and perfume industry. On the other hand, marjoram is used world wide as a spice and crude drug. The quality of the product is usually determined by chemical analysis, whereas in food industry applications sensory tests are also practiced. Marjoram essential oil components mainly revealed differences between plant species. In addition, basil essential oil and sweet marjoram water extracts possess high antioxidant activity (Triantaphyllou *et al*., 2001; Juliani & Simon, 2002). Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of disease due to the presence of antioxidant components such as phenolics (Rice-Evans *et al.,* 1996; Scalbert & Wuilliamson, 2000).

The chemical composition of basil and marjoram oils is very variable. The chemovarieties and the environmental conditions caused to this fact. The major components of basil oils from different origins were found to be linalool, eugenol, estragole and methyl cinnamate (Akgül, 1989; Juliani & Simon, 2002). Contrarity, several workers reported that essential oil components of marjoram were terpinen-4-ol, gamma-terpinene, trans-sabinene hydrate, linalool, thujanol, terpinolene and thymol (El-Ghorab *et al*., 2004).

Salicylic acid (SA) naturally occurs in plants in very low amounts. It has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack (Alvarez, 2000). After a pathogen attack, SA levels often increases and induces the expression of pathogenesis-related proteins and initiates the development of systemic acquired resistance and hypersensitive response (Grüner *et*

al., 2003; Kachroo *et al*., 2005). SA, jasmonic acid (JA), and ethylene-dependent signaling pathways regulates plant responses to both abiotic and biotic stress factors. In *Arabidopsis* ecotype Cvi-0, SA and JA signaling have a role in influencing O_3-induced cell death and an antagonistic relationship between JA- and SA-signaling pathways in controlling the magnitude of O_3-induced cell death was demonstrated (Mulpuri *et al*., 2000). During O_3 exposure, transgenic plants with a phenotype of reduced O_3-induced ethylene production accumulated less SA than did wild-type plants. Ethylene promotes SA accumulation by regulating the expression of the chorismate mutase and phenylalanine ammonia-lyase genes in O_3-exposed tobacco (Ogawa *et al.*, 2005). Young maize plants exhibited increased cold tolerance upon treatment with SA or aspirin (Janda *et al*., 1999). Exogenously added SA also increased the heat tolerance of mustard (Dat *et al*., 2000). Moreover, SA treatments at 0.5 mm strongly or completely suppressed the Cd-induced up-regulation of the antioxidant enzyme activities of barley (Metwally *et al*., 2003). SA has a direct physiological effect through the alteration of antioxidant enzyme activities. Certain enzymes were activated by SA treatment, while others like catalase, were inhibited. Catalase seems to be a key enzyme in SA-induced stress tolerance, as it was inhibited by binding in several plant species (Chen *et al*., 1993; Conrath *et al*., 1995). In grapevine (*Vitis amurensis*), SA-responsive *cis*-acting elements and the W-boxes are important for the SA induction of the *VCH 3* promoter, which might have a potential use in plant genetic engineering (Hai-Yan *et al*., 2006). SA induces flowering, increase flower life, retards senescence and increases cell metabolic rate. The sustained level of SA may be a prerequisite for the synthesis of auxin and/or cytokinin (Metwally *et al*., 2003), yet the influence of these new plant growth hormone on essential oil production and secondary metabolism has received little attention.

The present study was undertaken to examine the effects of foliar application of SA on growth, chemical composition, essential oil production and composition as well as nutrient uptake of *O. basilicum* and *M. hortensis*, another common essential oil producing species.

MATERIAL AND METHODS

A pot experiment was conducted at the experimental farm of Helwan University, Cairo, Egypt during 2004/2005. Seeds of *Ocimum baslicum* and *Majorana hortensis* (kindly provided by Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt) were sown in beds on 1st February. Uniform 45 days old seedlings were transplanted per earthenware pot (40 cm in diameter) of 15 kg of sandy loam soil. The pots were arranged in complete randomized blocks design with four treatments, four replicates per treatment and each replicate represented by 2 plants. Physical and chemical properties of the soil used in the experiment were evaluated according to Jackson (1973): The soil type was sandy loam with pH of 8.7, organic matter 0.35%, electrical conductivity 0.46 mhos cm^{-1}. The soil analysis, 2.96% containing $CaCO_3$, available 4.46, 23.46, 169 and .32.2 mg 100^{-1} g soil of P, K, Mg and Na, respectively and also available 7.2, 9.4, 2.80 and 4.82 ppm soil of Fe, Mn, Cu and Zn, respectively.

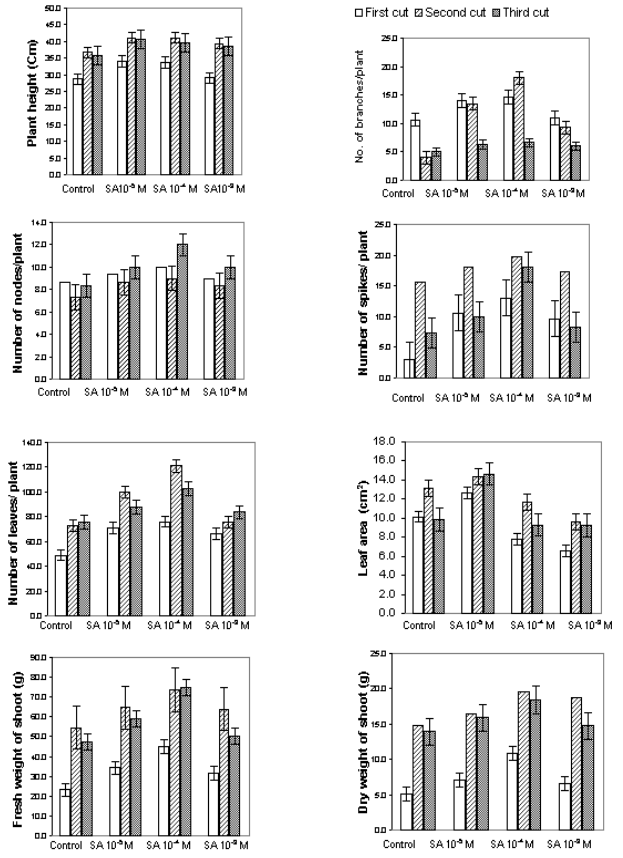
Fertilization was carried out for each pot at proportion of 1 g ammonium nitrate (33.5% N), 2 g calcium superphosphate (15.5% P_2O_5) and 1 g potassium sulphate (48% K_2O). These fertilizers were applied in two doses at 60 and 75 days after planting and repeated after the first and second cuts. Irrigation was regularly done at intervals according to weather conditions to keep the moisture content of the soil to field capacity. Plants were foliarly sprayed twice with SA (10^{-5}, 10^{-4} & 10^{-3} M), the first spray was done after 75 days after sowing, while the second spray was applied one week later after the first one. Treatments were repeated again 2 weeks after the first and second cuts. Control plants were sprayed with distilled water and the volume of the spraying solution was maintained just to cover completely the plant's foliage till drip. Three cuts were taken 2, 4 and 6 month after transplantation.

At full bloom, the plant herbage was cut 10 cm above the soil surface and data on plant height, number of branches, nodes, leaves and spikes per plant as well as fresh and dry weight of herb for plant growth parameter for the 3 cuts was recorded. Plant samples were dried in an oven with drift fan at 70°C until constant dry weight was obtained. Representative fresh samples were taken from each treatment for the determination of essential oil content and oil constituents.

Quantitative determination of *O. baslicum* and *M. hortensis* essential oil from the fresh samples was achieved by hydro-distillation at first, second and third cuts. Essential oils from the first cut were separated and analyzed qualitatively by GC/MS at National Research Centre, Dokki, Cairo. The GC analysis was carried out using Varian-3400 GC equipped with a DB-5 fused silica capillary column. Mass spectrometer was a Varian–Finnigan SSQ 7000.

Total carbohydrates were determined in the dried samples of first cut, using phenol sulphuric method (Dubois *et al.*, 1956). Total nitrogen was determined using the modified Micro-Kjeldahl method according to AOAC (1980). Macro and microelements were determined after wet digestion. Phosphorus was determined by using vanadate-molybdate method (Jackson, 1973). Potassium was measured by flame photomtere, Eppendorff. Microelements were estimated by using Inductively Coupled Spectrometry Plasma (ICP) Model Ultima 2-Jobin Yvon. The method of Müting and Kaiser (1963) as modified by EL-Araby (1987) was used for estimation of total amino acids. Free proline was determined according to the method described by Bates *et al*. (1973). Photosynthetic pigments of fresh leaves were determined and calculated according to Wettstein (1957).

Fig. 1. Effect of foliar spray with salicylic acid on the vegetative growth of *Ocimum basilicum* L plants. Vertical bars represent LSD at 5%



Fresh herb of basil and marjoram developed from the third cut were used for the extraction of polyamines (Flores & Galston, 1982 as adopted by Deabes, 2000) and HPLC was used for measurement of individual polyamines (Smith *et al*., 1985). Data were subjected to analysis of variance and the values of least significant differences (LSD at 5% level) were calculated to compare the treatments means (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

Growth criteria. Salicylic acid (SA) increased growth in terms of plant height, number of branches, spikes and leaves per plant, leaf area and fresh and dry weights of herb in both species during three cutting (Fig. 1 & 2). This promoting effect was at maximal at 10^{-4} M SA for all growth parameters except plant height and leaf area, showing maximum values at 10^{-5} M. Similarly, treatment of pepper seeds with SA and sulfosalicylic acid (both at 10^{-4} M) was effective in inducing highest leaf number, a tallest seedling and more plant fresh and dry weight, compared to lower (10^{-3} M) and higher (10^{-5} M), which showed a negative effect on seedling final fresh and dry weight (Mendoza *et al*., 2002). Kord and Hathout (1992) found that foliar application of salicylaldehyde at 10^{-5} M stimulated different morphological and growth criteria of tomato plants but inhibitory effects were observed at 10^{-3} M. Spraying mungbean with SA, significantly enhanced the total seed yield (Jaiwal & Bhambie, 1989). Moreover, the increase in plant height was due to increase in number of internodes, while the increase in the fresh and dry weights of basil and marjoram at low concentration might be attributed to an increase in number of branches and leaves as well as leaf area, leading to increased photosynthetic activity. Hamada (1998) found that soaking of wheat grains in 100 ppm aspirin showed a marked increase in the fresh and dry matter gain in shoots and roots through the regulation of some photosynthetic reactions. A significant increase in leaf area per plant in basil and marjoram was also noticed at 10^{-5} M of SA but markedly decreased with 10^{-4} and 10^{-3} M, especially at 1st and 2nd cuttings. Large leaf size of some types of basil with industrial values coupled with the high content of essential oils has potential use in herbal tea trade (Simon, 1992).

Oil content. Foliar spray of both basil and marjoram with SA increased essential oil yield per plant for the three cuts compared with their corresponding controls and also increased oil percentage gradually specially with the second cut in basil but showing the opposite response marjoram under control condition (Fig. 3). Similarly, Bottcher *et al.* (1999) found that *M. hortensis* herbs (1st cut) contained 22% more essential oils than physiologically younger plants (2nd cut). The highest mean values of essential oil percentage of the three cuts when pooled together increased significantly with about (26.52% in basil & 36.06% in marjoram), while on a per plant basis oil yield increased by 90.33% and 100.09% relative to control by SA application at 10^{-4} M in basil and 10^{-3} M in marjoram, respectively. The increment in oil yield might be due to the increase in vegetative growth, nutrients uptake or changes in leaf oil gland population and monoterpins biosynthesis. In accordance, total N and P uptake as well as essential oil yield of *Coriandrum sativum* increased with increasing rates of N and P application up to 60 and 45 kg ha^{-1}, respectively (Tiwari & Banafar, 1995).

The mean values of oil percentage and yield per plant of the three cuts when pooled together increased 5.49 and 26.66%, respectively over control plants by SA application at 10^{-3} M. Ram *et al*. (1997) reported that SA application (100 ppm) had no effect on the herbage and essential oil yields in *Pelargonium graveolens*, *Mentha arvensis* and *Cymbopogon martini.* They concluded that either the synthesis of essential oil constituents occurs constitutively, without the intervention of SA or the amount of SA required for the induction of synthesis of essential oil constituents are already available in these plants.

Main components of the essential oil. Basil essential oil appeared to be complex and riche in flavour notes and have higher proportions of linalool representing 46.63% in the oil, but intermediate proportions of methyl eugenol

Fig. 2. Effect of foliar spray with salicylic acid on the vegetative growth of *Origanium hortensis* plants. Vertical bars represent LSD at 5%

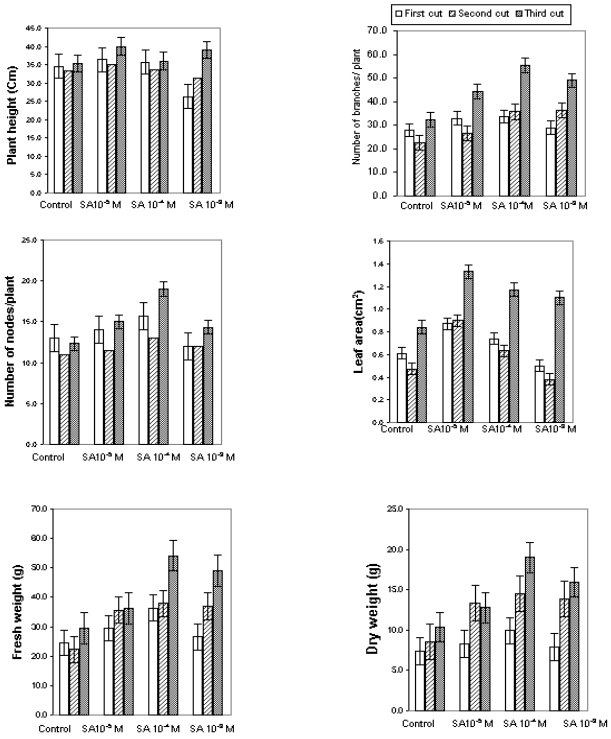
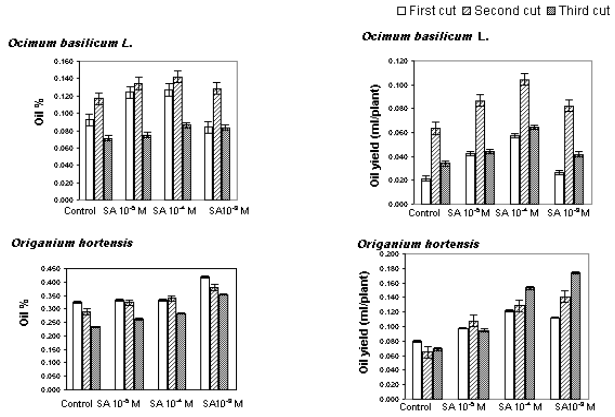


Fig. 3. Effect of foliar spray with salicylic acid on the oil content of *Ocimum basilicum* L and *Origanium hortensis* plants. Vertical bars represent LSD at 5%



(13.83%), 1, 8-cineol (13.20%), eugenol (7.16%) and α-cadinol (4.46%). Juliani and Simon (2002) found that the major oil components of basil was linalool, eugenol and germcrene D accompanied by lesser amount of α-pinene, camphene, sabinene, β-pinene, myrcene, 1, 8 -cineol, *cis*-ocimene, terpinolene, camphore, borneol, 4 -terpinol, α-terpineol, bornyl acetate, α-cubebene, α-copaene, β-elemene, methyleugenol, β-caryophyllene, α-humulene, α-amorphene, biclogermacrene, guaiene and cadinene. Our results indicated that most percentage of the oil was oxygenated compounds with much higher quantities of linalool and 1, 8 -cineol (Table I & II), but also other compounds are nearly similar to my findings. The oil of Egypt basil can be classified as Linalool Rich Type (Nacar & Tansi, 2000). However, there was a pronounced difference in the essential oil of basil content due to SA application. Common components of essential oil in all treatments were linalool (46.63 - 43.32%), methyl eugenol (13.83 - 5.68%), 1, 8 -cineol (13.20 - 4.43%), eugenol (12.64 - 7.16%) and α-cadinol (9.59 - 4.46%). In addition, SA application increased the eugenol level from 7.13% in control to 12.64% at maximal yield with SA at 10^{-3} M and was accompanied by an increase in 1, 8 -cineol content (from 13.20 to 15.31%), all other components exhibited a slight change. In this regard, the relative percentage of eugenol was correlated with the antioxidant activity of basil essential oil in two assays (Juliani & Simon, 2002). Therefore, a high percentage of eugenol in the SA treated herb is of particular interest in basil, which could constitute new sources of antioxidant phenolics in diet.

On the other hand, the proportions of terpinen-4-ol, *cis*-sabinene hydrate, p-cymene and γ-terpinene of control marjoram essential oil are closer to those of marjoram grown on Reunion Island (Vera & Ming, 1999). Under SA application, marjoram exhibited the same main components. Possible changes in the proportions of the major components were monitored under the influence of SA; the essential oil was rich in *cis*-sabinene hydrate (37.50 - 14.27%), terpinen-4-ol (24.33 - 13.99%), p-cymene (18.21 - 2.29%), sabinene (17.69 - 9.79%), γ-terpinene (10.64 - 4.77), α-terpineol (5.52 - 3.96%), trans-sabinene hydrate (8.19 - 5.45%), α-terpinene (2.41 - 0.00%) and β-caryophyllene (3.82 - 1.76%). SA at 10^{-5} M and 10^{-3} M produced an increase in the level of sabinene, which was accompanied by a decrease in the proportion of *cis*-sabinene hydrate relative to controls (Table II). Similar changes were evident in *M. hortensis* by salt stress (El-Keltawi & Croteau, 1987). On the other hand, the levels of numerous other monoterpenes including α-pinene, α-myrcene, α-terpinene, p-cymene, γ-terpinene as well as of the sesquiterpenes caryophyllene and α-humulene were increased. The level of *cis*-sabinene hydrate was concomitantly decreased relative to controls at 10^{-5} M and 10^{-3} M SA. At, 10^{-4} M the proportion of *cis*-sabinene hydrate, γ-terpinene and terpinen-4-ol markedly increased with only a minor influence on α-terpinenes. The Egyptian marjoram oil is rich in the main component, terpinen-4-ol (Refaat *et al*., 1990).

Carbohydrate, protein, total amino acids and free proline. The effect of foliar application of SA on the total carbohydrate content was accompanied by similar effects on crude proteins, total free amino acids and proline content of *O. basilicum* and *M. hortensis* at first cut (Table III & Fig. 4). Data revealed that maximum values of total carbohydrates and crude proteins of both species were obtained by foliar application of 10^{-4} M SA. In other

Table I. Effect of foliar spray with salicylic acid on the composition of essential oil of *Ocimum basilicum* L plants

Oil components (%)	Control	Salicylic acid		
		10^{-5} M	10^{-4} M	10^{-3} M
α-Pinene	0.45	0.46	0.05	0.58
Camphene	0.04	0.08	-	-
Sabinene	1.46	1.47	0.21	2.01
α-myrcene	1.30	1.50	0.26	1.70
Limonen	0.05	-	-	-
1,8-cineol	13.20	14.44	4.43	15.31
Ocimene	1.16	1.73	0.54	1.38
γ-terpinene	0.37	0.33	0.29	0.29
Linalool	46.63	45.62	46.63	43.32
Camphor	0.76	0.60	0.26	0.47
4-terpineol	0.48	0.69	0.46	-
Borneol	-	-	-	0.44
α-terpineol	0.79	1.18	0.98	1.44
Endobornyl actate	0.97	1.45	1.63	1.46
Methyl cinnamate	1.60	1.50	1.78	0.67
β-caryophyllene	0.01	-	-	-
α-copaene	0.01	0.01	0.07	0.01
Eugenol	7.16	8.83	9.69	12.64
Methyl eugenol	13.83	8.91	10.70	5.68
α-bergamotene	-	-	1.78	-
α-cubebene	0.01	0.07	0.11	0.02
α-humulene	0.83	0.84	0.95	1.05
δ -muurolene	-	0.09	-	0.01
Germacrene D	0.96	0.40	2.64	0.78
Bicycloge- rmacrene	0.59	0.68	1.59	0.74
α -guaiene	0.23	0.43	-	0.50
δ-cadinene	1.66	2.27	2.22	2.55
α-muurolene	0.04	0.08	0.05	0.09
Nerollidol	0.13	0.12	1.19	0.13
Spathulenol	0.06	0.05	0.16	0.03
Iso-spathulenol	0.04	0.04	0.11	0.04
Carotol	0.50	0.61	1.18	0.71
α-cadinol	4.46	4.94	9.59	5.21
α-eudesmol	-	0.35	0.65	0.25
α-bisabolol	-	0.05	0.09	0.08
Farnesol	-	0.03	-	0.04
Unidentified	0.22	0.15	0.71	0.37

studies, the highest increase in the level of the total soluble carbohydrate and proteins of wheat and tomato plants was obtained with 10^{-4} M and 10^{-5} M salicylaldehyde, respectively (Mohamed *et al*., 1989; Kord & Hathout, 1992). A similar trend of total free amino acid and proline content was noted in basil and marjoram as a result of using 10^{-4} M SA. At high concentration, free proline protects membranes and proteins against the adverse effects of inorganic ions. Temperature extremes can act as an enzyme-stabilizing agent under salinity (Samaras *et al*., 1995; Demir & Koccacaliskan, 2001) and reduce peroxidative damage to the lipid membranes due to salt dependent oxidative stress (Jain *et al*., 2001). Present data suggest that in both species, SA has higher adaptive capacity to stress, originating from better osmotic adjustment.

Photosynthetic pigments. Foliar spray of SA increased photosynthetic pigments in both species under study. Here again SA applied at 10^{-4} M was the most effective (Table III & Fig. 4). These results are in agreement with those obtained by Khurana and Makeshwari (1978), who found that in *Spirodela Polyrrhiza* SA at 10^{-5} M stimulated total chlorophyll synthesis whereas 10^{-3} M has a reverse effect. Stomatal index and stomatal density of pepper seeds were negatively affected by treatment with SA and SSA (10^{-3} M). On the other hand, SA and SSA at 10^{-4} and 10^{-5} M increased the stomatal index and its density on abaxial side, showing the opposite response in the adaxial side (Mendoza *et al*., 2002).

Macro and microelements. SA treatment increased the content of N, P, K, Mn, Fe, Zn, Na and Cu, in *O. basilicum* and *M. hortensis* (Table IV). The maximum mean values of all macro and micronutrients content of both species were obtained as a result of SA at 10^{-4} M and 10^{-5} M. Sarangthem and Singh (2003) found that the level of N, proteins and nitrate reductase activity were increased in *Phaseolus vulgari*s by foliar application of SA at 0.1%. In this study, increased nutrient content seemed to be involved in stress-tolerance mechanism and played an important role in enhancing the activity of enzymes responsible for drought resistance (Wu *et al.,* 1999; Cherki *et al*., 2002). SA applied at 10^{-3} M showed a variable pattern of nutrients. The uptake of all macro and micro-element of herbs were slightly increased except P (in case of basil) and Zn, Na and Cu content (in case of marjoam). Similarly, in tomato, significant increase in the uptake of elements was obtained with 10^{-4} M and 10^{-5} M salicylaldehyde (Kord & Hathout, 1992). Application of SA might improve physiological performance in terms of production of photosynthates, total

Table II. Effect of foliar spray with salicylic acid on the composition of essential oil of *Origanum marjorana* plants

Oil components (%)	Control	Salicylic acid		
		10^{-5} M	10^{-4} M	10^{-3} M
α-pinene	1.59	1.92	0.96	2.34
Sabinene	9.79	14.78	4.11	17.69
α- myrcene	1.87	2.75	0.88	2.51
Phellandrene	0.02	-	0.05	-
α-terpinene	2.41	-	2.61	0.38
p-cymene	6.28	17.68	2.29	18.21
Γ-terpinene	4.77	9.68	5.21	10.64
trans-sabinene hydrate	6.72	8.19	6.21	5.45
cis-sabinene hydrate	37.50	14.83	40.63	14.27
cis-2-p-menthen-1-ol	0.67	0.39	1.47	0.50
Terpinen-4-ol	15.71	16.60	24.33	13.99
α-terpineol	4.80	3.96	5.52	5.42
Linalyl acetate	1.55	1.40	0.29	1.07
Endobornyl actate	0.07	0.03	0.21	0.11
α-terpinyl propionate	0.22	0.21	0.14	0.18
Nerolidol	0.01	0.26	0.07	0.42
Bicycloelemene	0.31	0.17	0.26	0.1 7
Farnesyl acetate	0.07	-	0.04	-
β-caryophyllene	2.63	3.82	1.76	2.96
Aromadendrene	1.15	0.55	0.98	-
α-humulene	0.19	0.24	0.15	0.60
Leden	0.91	0.91	0.71	0.81
α-cadinene	0.06	0.03	0.15	0.05
Spathulenol	0.25	0.35	0.12	0.48
α -elemene	0.11	0.18	-	0.35
Unidentified compounds	0.34	1.07	0.85	1.40

Table III. Effect of foliar spray with salicylic acid on chemical constituents of *Ocimum basilicum* L. and *Origanium hortensis* plants ± standard deviation

Ocimum basilicum	Carbohydrates	Crude proteins	Free amino acids	Free proline	Photosynthetic pigments (mg/g dry wt)				
Treatments	%	%	Mg/g dry wt	mg/g dry wt	Ch.a	Ch.b	Ch.a+b	Carotenoids	Total pigments
Control	19.25±0.52	19.00±0.25	4.8±0.03	6.84±0.10	2.90±0.07	2.01±0.02	4.91±0.07	1.21±0.07	6.12±0.14
SA (10^{-5} M)	21.69±0.26	25.15±0.16	5.48±0.35	14.03±0.36	3.32±0.05	2.12±0.01	5.43±0.04	1.46 ± 0.05	6.89±0.06
SA (10^{-4} M)	22.98±0.28	27.49±0.26	9.30±0.11	19.37±0.45	3.74±0.03	2.32±0.05	6.05±0.04	1.74 ± 0.06	7.79±0.06
SA (10^{-3} M)	18.33±0.44	22.02±0.16	6.06±0.47	18.99±0.43	3.05±0.11	1.35±0.10	4.39±0.20	1.45 ± 0.07	5.85±0.14
L.S.D. at 5%	0..90	0.45	0.60	0.57	0.10	0.13	0.23	0.15	0.23
Origanium hortensis									
Control	20.19±0.52	10.00±0.13	2.09±0.10	3.75±0.08	1.96±0.08	1.04±0.10	2.99±0.07	2.45±0.05	5.44±0.09
SA (10^{-5} M)	22.39±0.21	11.25±0.19	2.35±0.07	5.19±0.02	2.66±0.04	1.29±0.07	3.95±0.02	2.62±0.02	6.57±0.03
SA (10^{-4} M)	24.94±0.13	12.50 0.50	2.65±0.03	5.41±0.03	3.18±0.06	1.55±0.06	4.73±0.08	2.84±0.03	7.57±0.07
SA (10^{-3} M)	23.73±0.39	10.00±013	2.45±0.01	3.76±0.03	1.62±0.03	0.72±0.03	2.34±0.04	2.22±0.02	4.56±0.04
L.S.D. at 5%	0.65	0.62	0.15	0.11	0.13	0.16	0.13	0.07	0.13

Table IV. Effect of foliar spray with salicylic acid on macro and micronutrients content of *Ocimum basilicum* L. and *Origanium hortensis* plants

Ocimum basilicum	Macronutrients content (%)			Micronutrients content (ppm)				
Treatments	N	P	K	Mn	Fe	Zn	Na	Cu
Control	3.04	0.29	3.50	122.50	888.00	78.00	16.79	31.50
Salicylic acid 10^{-5} M	4.02	0.41	3.61	148.50	1063.00	96.00	18.86	32.00
Salicylic acid 10^{-4} M	4.40	0.45	3.68	154.50	1098.00	235.00	20.70	33.00
Salicylic acid 10^{-3} M	3.52	0.18	3.13	130.00	904.50	93.00	14.03	30.00
Origanium hortensis								
Control	1.60	0.34	2.08	84.00	1399.00	61.00	14.95	20.50
Salicylic acid 10^{-5} M	1.80	0.49	2.32	85.00	1735.00	67.00	18.86	21.50
Salicylic acid 10^{-4} M	2.00	0.59	2.39	103.00	1770.00	73.00	35.88	47.50
Salicylic acid 10^{-3} M	1.60	0.31	2.22	91.00	1576.00	49.50	13.34	17.50

Table V. Effect of foliar spray with salicylic acid on macro and micronutrients uptake of *Ocimum basilicum* L. and *Origanium hortensis* plants

Ocimum basilicum	Macronutrients uptaket (mg plant^{-1})			Micronutrients uptaket (mg plant^{-1})				
Treatments	N	P	K	Mn	Fe	Zn	Na	Cu
Control	154.43	14.53	177.85	62.23	451.10	39.62	8.53	16.26
Salicylic acid 10^{-5} M	286.63	29.45	257.18	105.88	757.92	68.45	13.45	22.82
Salicylic acid 10^{-4} M	479.60	48.61	400.79	168.41	1196.82	256.15	22.56	35.97
Salicylic acid 10^{-3} M	234.08	12.10	208.28	86.45	601.49	61.85	9.33	19.95
Origanium hortensis								
Control	118.56	24.82	153.83	62.24	1036.66	45.20	11.08	15.19
Salicylic acid 10^{-5} M	148.86	40.69	192.11	70.30	1434.85	55.41	15.60	17.78
Salicylic acid 10^{-4} M	198.80	58.15	237.86	102.38	1759.38	72.56	35.66	47.22
Salicylic acid 10^{-3} M	126.88	24.82	175.81	91.00	1576.00	49.50	13.34	17.50

oil and dry matter yield, which can be related to increased nutrient uptake by SA-treated plants (Abad-Farooq & Misra, 1983; Cheol *et al*., 2001).

Polyamines. In control basil plants spermidine (Spd) was dominant followed by putrescine (Put), while spermine (Spm) was undetectable (Table IV & Fig. 5). Contrarily, control marjoram not only showed greater total polyamines (TPAs) but also differential pattern of individual polyamines, spermine being the main, followed by spermidine and putrescine. In both species, endogenous putrescine (Put), spermidine (Spd) as well as total polyamines (TPAs) contents were gradually increased with the increase of SA concentration up to 10^{-4} M relative to their controls. In an earlier study, the application of polyamines and SA at 400 mg L^{-1} to *Citrus reticulate* increased endogenous PA and SA concentrations, leading to an improvement in fruit quality and prolonged storage life. Moreover, SA, Spm and Spd treatments were better than a Put (Zheng & Zhang, 2004). Polyamines are considered anti-senescence in nature and regulate the action of the pro-senescence ethylene (Cassol & Mattoo, 2002). Spermine was the main polyamine in marjoram under control condition, which was un-detectable in basil. Furthermore, at 10^{-5} M of SA, a marked decrease in spermine (reduced to approximately 63.9% of the control) was observed in marjoram plants, whereas it was un-detectable in basil. Again, SA at 10^{-4} M displayed the maximum values of spermine in both species, relative to controls. Polyamines accumulation under conditions of water-deficit stress in drought-tolerant alfalfa may be a general strategy for

Fig. 4. Effect of foliar spray with salicylic acid on chemical constituents of *Ocimum basilicum* L. and *Origanium hortensis* plants. Vertical bars represent ± standard deviation.

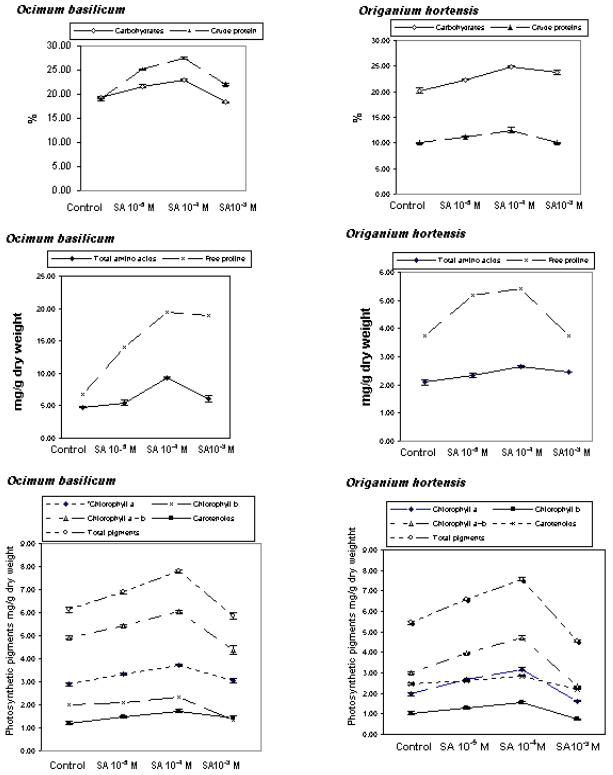
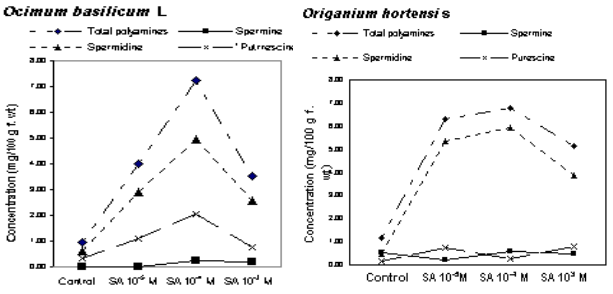


Fig. 5. HPLC analysis for endogenous concentrations of polyamines of *Ocimum basilicum* L and *Origanium hortensis* plants sprayed with salicylic acid.



improvement. Consequently, SA treatment may activate polyamine oxidase and improve abiotic stress tolerances in basil and marjoram plants.

CONCLUSION

SA applied on basil and marjoram stimulated the growth and oil yield by enhancing photosynthesis and nutrient uptake. Basil may be a new source of antioxidant phenolics in the diet due to the greater production of eugenol by SA. All SA treatments enhanced total free amino acid, free proline, Put, Spd as well as total polyamines TPAs, whilst differently affecting the level of spermine. However, further investigations are required to elucidate the possible role of SA on plant growth regulating activity.

Table VI. HPLC analysis for endogenous concentrations of polyamines of *Ocimum basilicum* L. and *Origanium hortensis* plants sprayed with salicylic acid

Ocimum basilicum	Polyamines levels (mg 100^{-1} g F.Wt)			
Treatments	**Put**	**Spm**	**Spm**	**Total**
Control	0.332	0.625	N.S	0.957
SA (10^{-5}M)	1.077	2.923	N.S	4.000
SA (10^{-4}M)	2.071	4.951	0.219	7.241
SA (10^{-3}M)	0.744	2.556	0.204	3.504
Origanium hortensis				
Control	0.142	0.493	0.535	1.170
SA (10^{-5}M)	0.746	5.375	0.193	6.314
SA (10^{-4}M)	0.278	5.944	0.585	6.807
SA (10^{-3}M)	0.772	3.854	0.489	5.115

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