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Effect of salicylic acid on growth, photosynthetic pigments and essential oil components of Shara (*Plectranthus tenuiflorus*) plants grown under drought stress conditions

Rewaa S. Jalal[1], Sameera O Bafeel[1] and Ansary E. Moftah[2*]

[1]Faculty of Science King Abdulaziz University-Jeddah, KSA
[2]*College of Agric and Veterinary Medicine, Qassim University, KSA

Abstract

Water is one of the most important environmental factors that regulate plant growth and development. The water deficit risticts the normal growth and development of plants, hence, several chemical materials have been used to reduce the harmful effects of water deficit. Salicylic acid (SA) is an important signal molecule modulating plant response to water stress. The current research was conducted to investigate the impact of salicylic acid (SA) at 0.5 mM on growth and biochemical parameters, and essential oil contents in Shara (*Plectranthus tenuiflorus*) under different levels of drought treatments. Dry weight, relative growth rate (RGR), photosynthetic pigments and oil content (%) were reduced at all stages of growth under severe water stress. Further, drought decreased the concentrations of thymol, carvacrol and terpens in plant leaves. On the contrary, SA at 0.05 mM spray reduced the harsh influences of water deficit resulted in improved growth and increased photosynthetic pigments as well as essential oil constituents. It seems that SA was able to enhance the tolerant ability of the plant to drought stress.

Keywords: Drought stress, salicylic acid (SA), shara plant, tolerance ability.

INTRODUCTION

Shara (*Plectranthus tenuiflorus*) plant, family *Lamiaceae*, is one of the medicinal herbs that have a promising economical future in the Kingdom of Saudi Arabia and is being cultivated as a source of essential oils, food or food flavorings (khorshid *et al*., 2011). The components of the essential oil of *P. tenuiflorus* have an antimicrobial effect and are used in folk medicine, as pain balms, for headaches, sores, burns, dermatitis, acute edematous, stomachache, against nausea, scorpion stings and as purgative (Rahman *et al*., 2004).

The subject of oil components of Shara plants still unclear and needs further studies. In an early study, Mwangi *et al*. (1993) found that the essential oil of *P. tenuiflorus* from Kenya contained α-terpinene (10.2%), p-cymene (10.9%) and carvacrol (14.3%) as the major components, while Al-Yahya (2004) found that the essential oil of *P. tenuiflorus* grown in Saudi Arabia contained Δ^3-Carene (58%) as a major component. On the other hand, Khorshid *et al*. (2011) has reported that the principle component of the extracted oil from plant harvested in Saudi Arabia was found to be Thymol (85.3%).

Drought is a regular and common feature in Saudi Arabia. Severe water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient

*Corresponding Author E-mail: ansary80@yahoo.com

metabolism and growth promoters (Farooq *et al*., 2008). The most obvious effect of water stress is growth reduction (Amin *et al*., 2009). Thus, a common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al*., 2009). Drought stress causes closing stoma and reducing leaf area (Premachandra *et al*., 1995); consequently decreasing photosynthetic pigments and activity (Tasgin *et al*., 2006). Moreover, Salicylic acid (SA) is phenolic in nature, which regulates various physiological processes in plants (Shakirova *et al*., 2003) and plays a key role in alleviating the deleterious effect of drought stress (Bideshki and Arvin, 2010). It improves plant growth even under stress (Khodary, 2004), stomatal regulation and photosynthesis (Khan *et al*., 2003) and ion uptake and transport (Gunes *et al*., 2005). The results of early studies suggested that exogenously applied SA could induce drought resistance in wheat, bean and tomato (Waseem *et al*., 2006). Information on the effect of drought stress on shara plants are very limited, therefore, the objective of the present work was to determine the effect of SA on growth parameters, photosynthetic pigments and essential oil components of *P. tenuiflorus* plants grown under water stress conditions.

MATERIAL AND METHODS

The present study was carried out at the King Abdulaziz University Experimental Station, Jeddah; Saudi Arabia during summer seasons (June - September) of 2010 and 2011 which is usually characterized by sunny, hot, dry days and warm nights. The average daily maximum temperature was 37.6^{o}C, and the daily minimum temperature during the experiment ranged from 15.6 to 28.5^{o}C. There was no rain during the experimental periods (as recorded by the Meteorological Station of Jeddah area, KSA).

Cuttings of *P. tenuiflorus* stems were grown in plastic pots (25 cm diameter) filled with soil mixed of sand and peat moss (3:1). The soil pH in the pots was adjusted to 6.8 -7.2 for normal plant growth.

Plants were grown outdoors under natural conditions in a shade-free location. Plants were watered to field capacity for about one month until roots were established and shoot system was in a reasonable size, having about 15 leaves. Soil surfaces in the pots were covered with opaque white polyethylene film to minimize evaporation.

A completely randomized design with 8 treatments and 10 replicates in this experiment. Plants of uniform height (one plant per pot) were selected and located in lines with a spacing of 2 m between lines and 1 m between pots to avoid mutual shading.

Three weeks after transplanting, all seedlings were fertilized 3 times, at 10 day intervals, with the complete water-soluble fertilizer “Sangral” compound fertilizer (20N-20P-20K, plus micronutrients) at the rate of 2 g/kg soil.

For the first 4 weeks, pots were weighed every other day, and all plants were watered to field capacity (FC), supplying an amount of water equal to transpiration losses. Later, pots were divided into two groups one of them was sprayed with 0.5 mM SA as recommended by Németh *et al*. (2008) and the other group was left untreated. Each group was, in turn, divided into 4 sub-groups which received an amount of water equal to 100% (control), 80, 60 or 40% of the field capacity and allowed to grow for 4 more weeks, during which plants were sprayed 3 times with SA. The field capacity was determined gravimetrically and found to be 16%.

The following growth parameters were recorded one week after the SA treatment:

Dry weights of shoots and roots

Dry weights of plant shoots and roots were determined after drying the fresh plant organs in electrical oven at 70^{o}C until constant weight. Weights were recorded at three growth stages, seedling stage (stage I), mature stage (stage II) and harvest stage (stage III). The crop was harvested at physiological maturity, soon after noticing the ageing symptoms.

Relative growth rate (RGR)

Relative growth rate (RGR) was estimated as per Hunt (1990) using the equation: RGR (mg/g/day) = (Ln W_2 – Ln W_1)/(t_2-t_1); where, W_1 and W_2 the dry weight of first and second sample, respectively. t_1 and t_2 the time at the first and the second sample, respectively.

Number of opened and closed Stomata

The number of opened and closed stomata was determined using film of clear nail polish as described by Brodkin (2000). Briefly, mark three leaves on a plant and apply a thick patch (at least one square centimeter) of clear nail polish on the underside of the leaf surface being studied. Thereafter, the nail polish was allowed to dry completely followed by taping clear cellophane tape to the dried nail polish patch. This was gently peeled from the leaf surface by pulling a corner of the tape off the leaf. This peeled impression was observed under light microscope using microscope slide. The leaf impressions were examined under a light microscope at 400X. All the

stomata in the microscopic field were counted and the number was recorded. From the average number/400X microscopic field, number of stomata/mm^2 was calculated.

Photosynthetic pigments

Chlorophyll a, b and carotenoids were extracted from the middle fresh leaves with 80% acetone and the extraction was determined spectrophotometrically according to Wettestein method (1957). Briefly, 2 g of fresh leaves of each sample is divided into two equal parts. Part one (1 g) was ground with a small amount of acetone (85%) until the mixture became homogeneous, then the extract was filtered and the filtrate was collected in a flask and completed to 100 ml with acetone (85%). The second part (1 g) was dried at 70°C for 72 hours and the dry weight was registered. Three replicates for each sample were taken and the absorbance of the extracts was measured using Spectrophotometer (Model 48100 (HACH Company, Colorado, USA)) at wave lengths of 663, 644 and 440 nanometers (nm) to estimate chlorophyll (a), chlorophyll (b) and beta carotene, respectively. The pigment concentrations were calculated according to the following equations using acetone 85% as a blank.

Chlorophyll (a) = 10. 3 A_{663} - 0.918 A_{644} = ……µg / ml

Chlorophyll (b) = 19.7 A_{644} - 3.870 A_{663} = ……µg / ml

Carotenoids = 4.2 A_{440}– [0.0264 Chl.(a) + 0.426 Chl.(b)] = …. µg / ml

The concentration of the pigment was calculated on the basis of mg pigment/g dry weight.

Essential oil

Essential oil was extracted from harvested fresh plant material using a laboratory scale steam distillation unit as described by Dunford and Vazquez (2005). The oil fraction was recovered from the water phase using petroleum ether. Oil samples were analyzed by (GC) system equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE). A Perkin Elmer PE-1 capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) was used for separation of the oil components.

Essential oil standards (thymol, carvacrol and terpinene) were (Sigma- Aldrich, St. Louis, MO). The helium carrier gas flow rate was 26 cm/s. The injector temperature was maintained at 250°C. A temperature program with total run time of 25 min was used. The column temperature, after an initial isothermal period of 1 min at 55°C, was increased to 95°C at a rate of 3°C min^{-1}, and maintained at this temperature for 1 min. Then the column temperature was further increased to 220°C at a ramp rate of 20°C min^{-1} and maintained at this temperature for 3.42 min. The detector conditions were: 250°C, H_2 flow 40 mL/min, air flow 400 ml min^{-1} and make-up gas (He) 30 ml min^{-1}. Oil samples were injected by an autosampler. Peak areas were calculated and results were presented in (%) concentration.

Statistical analysis

The experiment was arranged in a completely randomized design and data were statistically analyzed and ANOVA was tested according to Snedecor and Cochran (1980) with the aid of SPSS9 (1990) computer program for statistics. Differences among treatment means were tested with Duncan test.

RESULTS

Dry weight of shoots and roots

Dry weight of shoots and roots (Table1) as well as total dry weight decreased significantly under water stress condition as compared with unstressed condition (Figure 1). The lowest water regime (40% FC) reduced the shoot dry weights by about 50%, 45% and 57% at the stage I, II and III, respectively, as compared with control (100% FC). The corresponding reductions in dry weights of roots were about 20, 35 and 40%, respectively.

Total dry weight (Figure 1) of SA-untreated plants decreased significantly by water stress particularly at 40% FC at which the dry weight reduced by about 32, 40 and 50% at Stage I, II and III, respectively. The corresponding reduction in SA-treated plants was about 18, 25 and 27%, respectively. It was clear also that SA treatments increased significantly the total dry weight of water stressed or unstressed plants, compared with control. The most increase in shoot and root dry weights was opserevd with SA-treated plants at 80% FC, at which shoot dry weight increased by about 37, 18 and 29% at stage I, II and III, respectively, as compared with control. The corresponding increase in root dry weights were about 81, 68 and 58%, respectively.

Relative growth rate (RGR)

Data in Table (2) illustrated that RGR was in a high value at the early growth stages (between stage I and II) and lowered at the latest stages (between stage II and III). This trend was expected because at early stages plant

Table 1. Effect of drought and salicylic acid (SA) on dry weight (g) of *P. tenuiflorus* shoot and root.

FC	Stage I	Stage II	Stage III
Shoot (g/plant)			
100% (cont.)	5.7 b	15.9 b	31.2 b
80%	5.8 b	14.7 bc	26.3 c
60%	4.3 c	10.1 ef	18.5 e
40%	3.8 c	8.8 f	13.3 f
100% + SA	6.4 b	16.8 b	37.8 a
80% + SA	7.8 a	18.8 a	40.2 a
60% + SA	6.1 b	12.9 cd	28.6 c
40% + SA	4.3 c	11.5 de	22.4 d
Root (g/plant)			
100% (cont.)	0.77 ab	1.9 ab	4.8 bc
80%	1.3 ab	2.1 ab	6.1 ab
60%	1.1 ab	2.3 ab	5.2 bc
40%	0.60 c	1.2 c	2.9 d
100% + SA	1.2 ab	2.5 ab	5.2 bc
80% + SA	1.4 a	3.2 a	7.6 a
60% + SA	1.1 ab	2.1 ab	6.1 ab
40% + SA	0.97 b	1.8 b	4.2 cd

Means in the same column that have the same letter are not significantly different at $P < 0.05$

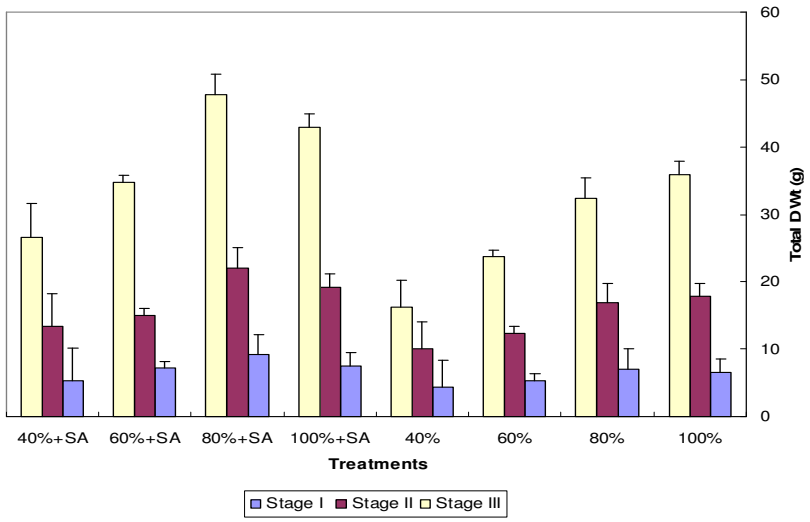

Figure 1. Effect of drought and salicylic acid (SA) on total dry weight (g) of *P. tenuiflorus* plant. Vertical bars indicate ± standard error

Table 2. Effect of drought and salicylic acid (SA) on Relative Growth Rate (RGR) (mg dwt.g^{-1}dwt.day^{-1}) of *P. tenuiflorus* plants during different stages of growth.

FC	RGR1	RGR2
100% (cont.)	2.14 c	0.081 a
80%	2.54 a	0.072 c
60%	2.27 b	0.070 c
40%	2.09 d	0.062 d
100%+SA	2.67 a	0.083 a
80%+SA	2.77 a	0.078 b
60%+SA	2.43 b	0.075 bc
40%+SA	2.35 b	0.077 b

RGR1 in the period of stage I to stage II; RGR2 in the period of stage II to stage III.Means in the same column that have the same letter are not significantly different at $P < 0.05$

Table 3. Effect of drought and salicylic acid (SA) on number of opened and closed stoma in upper and lower surfaces of *P. tenuiflorus* leaves.

FC	Upper surface		Lower surface	
	open	closed	Open	closed
100% (cont.)	12 b	4 d	30 b	20 c
80%	10 bc	8 bc	25 c	8 d
60%	11 bc	10 b	16 cd	21 c
40%	6 d	18 a	8 e	35 a
100% + SA	14 b	6 c	35 a	10 d
80% + SA	17 a	5 cd	30 b	12 cd
60% + SA	14 b	12 b	20 d	17 c
40% + SA	5 d	12 b	15 cd	28 b

cells are more active for division and elongation than later stages.

Water stress decreased RGR as it reduced the growth values. It is clear that drought was more effective in reducing RGR at later growth stages, particularly at 40% FC, (RGR2 = -20%) than early stages (RGR1= -3%). On the other hand SA treatment increased RGR either for water stressed or unstressed plants. This increase was due to the positive effect of SA on the accumulation of dry matter of plant.

Number of Stomata

It is clear that drought stress decreased the number of opened stomata and increased the number of closed ones (Table 3). The most effective water stress was the 40% FC at which the closed stomata was about 75% and the opened ones was 25% in the upper leaf surface. The corresponding values in the lower surface were about 80% and about 20%, respectively. In SA- treatment plants, the number of closed stomata at 40% FC was about 70% in the upper leaf surface and 65% in the lower one.

Photosynthetic pigments

Data recorded in Table 4 and Figure 2 shows that, generally chlorophyll (chl) content decreased significantly at stage III while carotenoids (Table 5) increase with plant age. Drought stress alone (especially at 40% FC) decreased chlorophyll a, b, total chl and carotenoides as compared with control plants (100% FC) particularly at

Table 4. Effect of drought and salicylic acid (SA) on chl a and b in *P. tenuiflorus* plants

FC	Stage I	Stage II	Stage III
Chl (a)			
100% (cont.)	2.30 a	3.09 b	1.00 b
80%	2.25 a	4.02 a	1.16 b
60%	1.01 b	3.29 b	0.72 c
40%	0.85 c	2.37 c	0.65 c
100%+SA	2.38 a	3.85 a	1.35 a
80%+SA	1.99 a	3.58 ab	1.38 a
60%+SA	1.32 b	3.42 bc	1.50 a
40%+SA	1.03 b	3.72 ab	1.39 a
Chl (b)			
100% (cont.)	0.31 c	1.38 b	0.28 c
80%	0.39 bc	1.72 a	1.29 a
60%	0.53 b	1.25 b	0.86 b
40%	0.23 d	1.04 c	0.21 c
100% + SA	0.38 bc	1.53 a	0.95 b
80% + SA	1.06 a	1.64 a	1.85 a
60% + SA	0.64 b	1.53 a	1.43 a
40% + SA	0.32 c	1.39 b	0.71 b

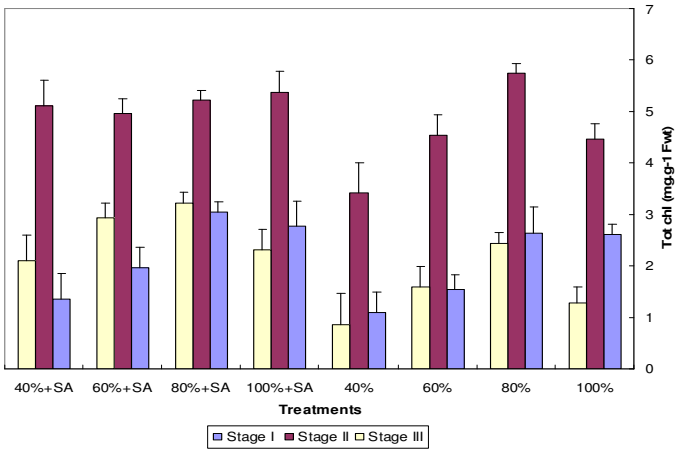

Figure 2. Effect of drought and salicylic acid (SA) on total chlorophyll in *P. tenuiflorus* plants. Vertical bars indicate ± standard error

Table 5. Effect of drought and salicylic acid (SA) on carotenoids in *P. tenuiflorus* plants.

FC	Stage I	Stage II	Stage III
100%	0.45 bc	0.61 b	1.26 c
80%	0.55 b	0.70 a	1.61 a
60%	0.35 cd	0.48 cd	1.39 ba
40%	0.37 cd	0.52 c	1.11 c
100% + SA	0.45 bc	0.46 d	1.60 a
80% + SA	0.65 a	0.71 a	1.48 b
60% + SA	0.36 cd	0.65 a	1.48 b
40% + SA	0.35 cd	0.48 cd	1.49 b

Table 6. Effect of drought and salicylic acid (SA) on essential oil composition (%) of *P. tenuiflorus* leaves.

Field capacity	Oil%	Thymol%	Carvacrol%	Terpens%
100%	2.2 e	38.63 e	2.11 de	5.02 c
80%	3.8 c	45.47 c	4.05 b	6.33 b
60%	2.6 d	43.59 cd	2.50 cd	5.23 c
40%	1.8 f	32.68 f	0.99 f	2.22 d
100% + SA	2.8 cd	48.98 b	4.21 b	6.49 b
80% + SA	4.5 a	56.83 a	5.89 a	9.76 a
60% + SA	3.6 b	50.77 b	2.93 c	5.99 bc
40% + SA	2.6 d	40.64 de	1.79 ef	3.08 d

the last stages of growth.

On the other side, SA treatment had a positive effect on all the photosynthetic pigments either in stressed or unstressed plants. The effect of SA was more pronounced at more severe water stress (40% FC) than at mild stress (80% FC).

It seems that the increase in total chlorophyll was attributed to the increase in chl a more than the increase in chl b. The positive effect of SA on carotenoid content was more observed at the last stage of growth as compared with the other two stages.

Essential oil composition

Totally, 17 constituents were identified by GC–MS for the Shara essential oil. Thymol content was the dominant constituent of the essential oil for all samples tested, ranging from 32.68% at 40% FC without SA treatment to 56.83% at 100% FC with SA treatment (Table 6). As shown in the table, the other major constituents were terpens (ranging from 3.08 to 9.76%) and carvacrol (ranging from 0.99 to 5.89%). Other constituents such as α-pinene), α-terpinene, α-thujene, germacrene, caryophyllene, terpineol. Linalool, limonene, borneol, α-terpineol, bornyl acetate, carvacrol acetate, elemene, cadinene and caryophyllene oxide were present in amount less the 1% (data not shown).

The recorded data indicated clearly that the essential oil, thymol, carvacrol and terpens concentrations were negatively affected by water stress treatments. It is obvious that the irrigation regime of 80% FC gave the highest values of all contents while the 40% FC treatment gave the lowest values. In this regard oil concentration increased at 80% FC by about 70% while thymole increased by about 17%, and carvacrol increased by about 90% and terpens by about 75% of control treatments (100% FC). The 40% FC treatment, on the other hand, decreased the concentration of essential oil, thymol, carvacrol and terpens by about 18, 15, 50 and 55%, respectively.

Data showed also that SA increased concentrations of essential oil, thymol, carvacrol and terpens at 100% FC by about 27, 27, 90 and 30%, respectively, as compared to SA untreated plants at the same FC. Moreover, SA enhanced the formation of all compounds in water stressed plants. At 40% FC the SA treatment increased the essential oil, thymol, carvacrol and terpens by about 40, 25, 80 and 40%, respectively, as compared with SA untreated plants at 40% FC.

DISCUSSION

In general there is a substantial reduction in the the plant growth by water stress. It may be, because of the lower turgor pressure of cells under water deficit resulting in a lower cell expansion rate (Amin *et al*., 2009).These results are corresponding with findings of Pattangul and Madore (1999) who reported that decreased leaf area, vegetative growth and RGR in drought conditions due to decreased division and expansion of cells. Similarly, Bideshki and Arvin (2010) found that drought reduced the garlic shoot height by 20%, root fresh weight by 47%, root dry weight by 60%, leaf area by 29% and whole plant fresh weight by 37%, over well watered plants. It is well established that water deficit results in a decline in metabolic activity of plant cells, which in turn might inhibit their growth (Amin *et al*., 2009). Moreover, drought-induced growth reduction has been attributed to inhibition of photochemical processes and to the increased production of reactive oxygen species (ROS) in the chloroplast was reported by (Sultana *et al*., 2000)

In the present study, 0.5 mM SA spray reduced the drought stress damages on growth parameters of Shara plants and was expressed in terms of increased shoot and root dry weights Table (1). Similarly, in other studies SA has Increased the dry weight and RGR in the

stressed barley (El-Tayeb, 2005) and stressed maize (Khodary, 2004) and other plants (Bideshki and Arvin, 2010). The increase in growth parameters of water stressed plants in response to SA may be related to the induction of antioxidant responses that protect the plant from damage (Daneshmand *et al.,* 2010). Further it was also worthy to note that SA sets the cell expansion and division (Zhang *et al*., 2002), regulates many physiological processes (Zhang *et al*., 2004) and causes plants adaptation to environmental stress (Shah *et al*., 2002).

There is an ample evidence for the negative effects of water stress on stomatalo movement (Lawson, 2009; Reynolds-Henne *et al*., 2010). Stomatal closure in response to drought stress restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation as well as decreasing water loss from the leaves (Chaves, 2002). It seems that the reduction of water status leads to the reduction of cell turgor and turgor pressure which results in closing of the stomata (Yazdanpanah *et al*., 2011). SA spray had positive effects on photosynthetic pigments and exhibited higher values of pigment content than those of untreated ones. The results are in agreement with the findings of El-Tayeb, 2005 who reported an increase in photosynthetic pigments, under abiotic stress conditionsupon SA spray. This may be due to the fact that SA protects photosynthetic apparatus through increasing the ability of cell antioxidation and new proteins synthesis (Avancini *et al*., 2003 and . Daneshmand *et al.,* 2010) further in detail,reported Chl *a, b* and carotenoids in stressed barly plants and significant increase in them with SA spray in both stress and non stress treatments. Similarly, in bean plants, foliar spray with SA, increased Chl *a, b* and carotenoids under normal field conditions was reported by (Doganlar *et al*., 2010).

The decrease in chlorophyll content of drought stressed plants may be attributed to increasing the production of active oxygen radicals that cause peroxidation and degradation of these pigments (Siddiqi *et al*., 2009). It has been reported that the accumulation of active oxygen types that produce during drought stress, damage to many cell compounds like fat, protein and photosynthetic pigments (Jiang and Hunag, 2001).

The major components of the oil in *P. tenuiflorus* were isolated early by Mwangi *et al*. (1993) who reported carvacrol (14.3%) α-terpinene (10.2%) and *p*-cymene (10.9%). On the other hand, Mwangi *et al*. (1993) who recorded major componants of the essential oil of *P. tenuiflorus* as α-terpinene (10.2%), p-cymene (10.9%) and carvacrol (14.3%). However later, Alsufyani (2006) reported that the principle component of the oil produced from plant harvested in Saudi Arabia was found to be Thymol (85.3%). In the present study, water stress decreased levels of essential oil and its components, while adequate water supply increased the essential oil percentage. An early study showed that the essential oil content of plant leaves and its components were strongly influenced by abiotic stress, especially water/salt stress (Simon *et al*., 1992) inadequate mineral nutrition (Stutte, 2006).The transformation rate of p-cymene to thymol or carvacrol can be affected by water stress conditions (Aziz *et al.,* 2008). Thus, it might be claimed that the formation and accumulation of essential oil is directly dependent on balanced growth and development of the plants producing oils. Therefore, the decrease in oil production under water stress might be due to the decrease in plant anabolism (Said-Al Ahl and Hussein, 2010). SA spray was found to increase essential oil components, this might be due to maintaining the balanced growth of plants. It had been reported that SA protects plants from Oxygen radical effects , membrane and pigment disintegration. It is evident from the reports of Rowshan *et al*. (2010) and Lee and Yang (2005) who reported increased terpens in *Chrysanthemum* and essential oil in *Carum copticum* and *Satureja hortensis* (Marandi *et al*., 2011) with SA spray**.**

CONCLUSION

Salicylic acid stimulates vegetative growth, RGR, chlorophyll and increases the percentage of essential oil components of Shara (*P. tenuiflorus*).even under drought stress conditions where as severe water stress suppress plant growth, pigments and essential oil contents.

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