Acta Sci. Pol. Hortorum Cultus, 22(5) 2023, 89–97

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2023.4964

ORIGINAL PAPER

Received: 15.10.2022
Accepted: 30.08.2023
Issue published: 30.10.2023

FOLIAR APPLICATION OF SALICYLIC ACID AND PROLINE TO MITIGATE WATER DEFICIT IMPACT ON PURPLE CONEFLOWER (*Echinacea purpurea* (L.) Moench.)

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ABSTRACT

The effects of foliar spraying of salicylic acid and proline on *Echinacea purpurea* under different soil moistures were investigated in the field conditions in the Southwest of Iran (2017–2019). The experiment treatments were the foliar application of salicylic acid (SA) and proline (both at 1 mM concentration) and 2 irrigation frequencies (6 and 10 every day based on 75–80% and 40–45% field capacity, respectively). The field experiment was set as a complete randomized block design with 3 replications. The volatile oils were analyzed using GC-FID and GC-MS. Germacrene D, *p*-cymene, *β*-caryophyllene, *α*-pinene, and *β*-bisabolene were detected as the main constituents. Deficit irrigation decreased the growth parameters of the plants; however, it improved the contents of the volatile oil and the major compounds of volatile oil. In addition, the maximum values of the volatile oil content and the main constituents of volatile oil were extracted from the plants under SA × water deficit treatment. In conclusion, the foliar spraying of SA under water deficit conditions was an applicable strategy to maintain and stabilize the growth and yield of *E. purpurea*.

Key words: germacrene D, GC-MS, growth parameters reduced irrigation, volatile oil

INTRODUCTION

Purple coneflower (*Echinacea purpurea* (L.) Moench.), as a member of the family *Asteraceae*, is an essential medicinal herb in the world [Mehrpooya et al. 2021]. One of the most critical properties of purple coneflower is to increase the immune system's strength against pathogens [Ahmadi et al. 2022]. *Echinacea purpurea* contains medicinally vital components, including polysaccharides, flavonoids, caffeic acid derivatives, volatile oils, alkylamides, polyacetylenes, and other chemicals [Cozzolino et al. 2006]. Germacrene D, spathulenol, *β*-caryophyllene, and *α*-humulene were detected as the main constituents in the volatile oil extracted from purple coneflower [Mehrpooya et al. 2021].

Results of some studies indicated that *E. purpurea* can be a tolerant plant to a range of environmental stresses such as water deficit and salinity [Darvizheh et al. 2019, Ahmadi et al. 2022]. Water shortage is a critical factor limiting crop production in arid and semi-arid zones [Ghasemi Pirbalouti et al. 2014]. Almost all aspects of a plant's growth and development, such as respiration, photosynthesis, nutrient metabolism, ion

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uptake, and growth promoters, are negatively impacted by drought stress [Darvizheh et al. 2019]. In addition, lack of sufficient water influences the metabolic pathways responsible for accumulating plant secondary products [Bohnert et al. 1995, Alavi Samany et al. 2022]. Although medicinal and aromatic plants may produce higher concentrations of secondary products in response to environmental stresses, particularly salinity, and drought [Selmar and Kleinwachter 2013, Babaei et al. 2021, Maghsoudi et al. 2023], the total extractable ingredients are often reduced due to decreased total biomass. Exogenous application of plant growth regulators such as salicylic acid (SA) may promote drought tolerance of plants while improving the biosynthesis of the secondary metabolites [Darvizheh et al. 2019]. Salicylic acid is a phenolic compound that plays a crucial role in regulating the antioxidant activity, biochemical pathways, and other plant metabolism by inhibiting or activating the enzymatic antioxidant system and thus controlling reactive oxygen species (ROS) production in plants under water deficit stress [Babaei et al. 2021, Maghsoudi et al. 2023]. Results of previous studies [Danesh-Shahraki et al. 2023, Shaykh Samani et al. 2023] showed that the foliar application of SA increased the essential oil content in some herbs under water deficit conditions. Another approach to mitigate the damage caused by drought stress is applying exogenous proline. Proline is an amino acid that regulates cellular osmotic potential in stressful environments [Cheplick et al. 2018]. Generally, many reports have shown that the exogenous application of SA and proline improved the active substances of various species of medicinal and aromatic plants under adverse environmental conditions [Khorasani et al. 2023, Danesh-Shahraki et al. 2023, Güneri and Dalkılıç 2023].

The effects of foliar application of SA and proline on the growth, accumulation of essential oil, and chemical composition profile of the volatile oil from *E. purpurea* are not well-documented. We hypothesize that applying SA and proline can promote plant growth and stimulate purple coneflower to produce more secondary metabolites under water deficit stress. This study aimed to assess the influence of the foliar application of SA and proline on the growth parameters, quantity, and quality of volatile oil from *E. purpurea* under different irrigation conditions.

MATERIALS AND METHODS

Experimental site description. This study was conducted from 2017 to 2019 at the Experimental Farm, Research Center of Agricultural and Natural Resources of Chaharmahal and Bakhtiari Province in Southwestern Iran (latitude 20°32' N, longitude 50°51' E, altitude 2061 m). Based on the Köppen climate classification, the climate of the study area is classified as cold, semi-arid, and semi-humid (Tab. 1).

Plant material and soil analysis. Seeds of *Echinacea purpurea* (L.) Moench. were sterilized and sown in 8 cm plastic pots on 26 March 2017. The pots were kept in a glasshouse with 25°/15°C (day/night) air temperatures, 65–70% humidity, and 12/12 hours (light/dark). The pots were filled with a combination of farm soil (Tab. 2), sand, and peat. After about 45 days from sowing on 10 May 2017, the seedlings with 4–6 true leaves and 10–12 cm tall were transferred to the experimental field. The dimensions of each experimental plot were 6.0×2.4 m, and the distance between replicates was 2 m. The cultivation density was 20 cm between plants and 60 cm between the rows (~6 plant m^{-2}). Soil samples were taken before the experiment from three random parts of each plot from 0 to 30 cm depth (Tab. 2) – cultivation in the research field after 1-year uncultivated fallow was done. In winter 2016, the field soil was plowed using a moldboard plow (up to a depth of 30 cm). Along with plowing, cow manure was used at the rate of 10-ton ha^{-1} according to the advice of the soil nutrition expert. No inorganic fertilizer or systemic pesticide was used during the experiment, and weeds were controlled manually.

Treatments and experimental design. Experimental treatments were arranged as a factorial in a randomized complete block design (RCBD) with 3 replications (18 plots). The experiment treatments were plant irrigation regimes and foliar application of SA and proline. Irrigation frequencies include 6 intervals at 75–80% field capacity (optimum irrigation) and 10 intervals at 40–45% field capacity (reduced irrigation). Irrigation of each plot was performed using the soil moisture curve – permanent wilting point (PWP) and field capacity (FC) were –1500 and –33 kPa, respectively. Daily, soil moisture was measured using a TDR device (PMS-714, Lutron, Taiwan) following

Table 1. Meteorological parameters of the study region during growth (from March to October) of the studied plant

Parameters	Year	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Average temperature (°C)	2017-2018	9.0	12.8	20.0	24.3	24.5	22.3	15.8	8.8	5.3	3.2	2.9	2.1
	2018-2019	8.6	14.2	19.5	27.3	24.1	20.9	16.8	7.4	3.0	1.7	1.3	7.1
Average rainfall (mm)	2017-2018	117.4	6.5	13.0	–	–	2.4	5.8	53.8	44.8	90.5	25.6	32.9
	2018-2019	74.2	32.2	5.6	–	7.1	–	1.0	25.2	35.8	16.1	16.0	52.6
Relative humidity (%)	2017-2018	52.5	44.5	38.5	25.0	27.5	29.5	38.5	54.0	54.0	55.0	54.0	54.5
	2018-2019	55.5	47.0	38.5	27.0	28.5	28.0	35.5	47.0	64.5	59.0	50.5	51.0

Table 2. Some physicochemical properties of the soil of the study area

Depth (cm)	Soil texture	pH	EC (dS m^{-1})	Organic Carbon (%)	N (%)	P	K	Zn	Mn	Fe	Cu	B
						(mg kg^{-1})						
0–30	silty clay loam	7.84	1.03	0.35	0.03	2.2	167.0	0.49	7.26	2.58	1.15	1.14

Table 3. The simple impacts of the experimental factors on the growth and phytochemical traits of *Echinacea purpurea*

Treatments		Characteristics									
		plant height (cm)	plant fresh weight (g m^{-2})	plant dry weight (g m^{-2})	proline content (µmol g^{-1})	essential oil content (*v/w* %)	germacrene D (%)	*α*-pinene (%)	*β*-caryophyllene (%)	*p*-cymene (%)	β-bisabolene (%)
IRR[§]	optimum irrigation	32.53 ±11.12¥	1295.8 ±0.56	413.72 ± 0.45	13.83 ±2.43 b*	0.063 ±0.06 b	52.35 ±0.60 b	3.49 ±0.82 b	6.37 ±0.26 b	8.34 ± 0.25 b	3.24± 0.33 b
	deficit irrigation	32.97 ±14.66	1364.1 ±0.42	475.06 ± 0.56	37.11 ±5.34 a	0.071 ±0.08 a	52.66 ±0.84 b	3.84 ±0.40 a	6.86 ±0.64 a	8.81 ± 0.76 a	3.82 ± 0.63 a
ANOVA		n.s	n.s	n.s	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
FA	non-spraying	30.71 ±12.61	1224.7 ±0.56	377.50 ±0.44	36.75 ±5.85 a	0.061 ±0.05 b	52.13 ±0.42 b	3.32 ±0.94 b	6.25 ±0.46 c	8.28 ±0.26 b	3.22 ±0.39 b
	spraying-SA	33.87 ±10.31	1392.3 ±0.32	498.00 ±0.57	15.83 ±6.61 c	0.075 ±0.09 a	52.95 ±0.87 a	4.17 ±0.70 a	7.10 ±0.23 a	8.92 ±0.30 a	3.90 ±0.74 a
	spraying-PR	33.67 ±14.24	1315. 4 ±0.50	457.67 ±0.52	23.83 ±4.39 b	0.069 ±0.08 b	52.44 ±0.64 b	3.51 ±0.98 b	6.50 ±0.51 b	8.52 ±0.46 b	3.48 ±0.31 b
ANOVA		n.s	n.s	n.s	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.05$

* The averages with at least a common alphabet are not statistically significant at the 5% level
[§] IRR – irrigation treatment, FA – foliar application, SA – salicylic acid, PR – proline
¥ Values are an average of two years

the manufacturer's protocol [Jafari et al. 2019]. The irrigation water was applied through in-line drippers (2 plant/emitters with a discharge of 4 L h^{-1}).

The foliar application treatment included plants without spraying (control), with foliar application of SA (at 1 mM), and foliar application of proline (at 1 mM). The SA and proline were diluted in distilled water. The solutions were sprayed to the whole aboveground parts of plants at three stages of growth, including: before flowering, 25% flowering, and 50% flowering at dew point (~150 mL $plant^{-1}$) with a hand sprayer for both years [Ghasemi Pirbalouti et al. 2019]. The SA and proline were purchased from Sigma-Aldrich Co. (Steineheim, Germany).

Morpho-physiological measurements. Each year, at the end of the vegetative growth period, 10 plants from each plot were randomly collected, and then, the plant height, fresh and dry weights, and proline content were measured. Three replicates from a single plant at individual tests measured relative fresh and dry weights and dry weights. The proline content was determined using the method of Bates et al. [1973]. For this purpose, 10 mL of 3% sulfosalicylic acid was added to 0.5 g of plant fresh matter. After 24 h, the solution was centrifuged at 15,000 rpm for 10 min at 4°C. Then, 2 mL of ninhydrin acid and 2 mL of glacial acetic acid were mixed with 2 mL of the filtered extract. At the same time, the standard curve of proline was drawn. The samples were heated in a hot water bath for 1 h and then placed in an ice bath. Then, the proline content was measured by a spectrophotometer (Perkin-Elmer Lambda UV/Vis, Perkin-Elmer, USA) at 520 nm.

Extraction and analysis of volatile oil. The aerial parts (including leaves, stems, and flowers) were hand-harvested at the flowering of *E. purpurea* and then were dried in the shade at room temperature (25°C ±4°C) for ten days. The samples were ground to a fine powder using a micro hammer cutter mill and passed through a sieve. The volatile oil was extracted from 100 g of powdered tissue by hydro-distillation method using the Clevenger-type (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran) with 500 mL water for 3 h according to the British Pharmacopoeia [Ghasemi Pirbalouti et al. 2017]. The obtained volatile oil was dried over anhydrous sodium sulfate and then stored at 4°C until analyzed. The chemical compositions of the *E. purpurea* volatile oil were determined by GC-FID and GC-MS. The samples were analyzed using an Agilent Technologies 7890 gas chromatograph coupled to an Agilent 5975 C mass selective detector (MSD) and quadrupole EI mass analyzer (Agilent Technologies, Palo Alto, CA, USA). An HP–5MS 5% column (coated with methyl silicone) (30 m × 0.25 mm, 0.25 μm film thicknesses) was used as the stationary phase. Helium (99.999% pure) was used as the carrier gas at 0.8 mL min^{-1} flow rate. The temperature was programmed from 60°C to 280°C at a 4°C min^{-1} ramp rate. The injector and the GC-MS interface temperatures were maintained at 290°C and 300°C, respectively. Mass spectra were recorded at 70 eV. The mass range was from 50–550 *m/z*. The ion source and the detector temperatures were maintained at 250°C and 150°C, respectively. Split injection was conducted with a ratio split of 1:100. The volatile oil samples of 0.1 μL were injected neat. Chemical constituents of the volatile oils were identified by comparison of their RI relative to C_5-C_{24} *n*-alkanes obtained on a nonpolar HP–5MS column by comparison of the RI, provided in the literature, by comparison of the mass spectra with those recorded by Willey (ChemStation data system). The individual constituents were identified by retention indices and compared to constituents known from the literature [Adams 2007]. The peak area percentages were computed from the HP-5MS column without FID response factors.

Statistical analysis. Simple and interaction effects of irrigation frequency and foliar application on the growth and phytochemical traits were statistically analyzed based on the GLM procedure of the SAS statistical package (SAS/STAT® v. 9.2. SAS Institute Inc., Cary, NC). In addition, the significance of differences among treatment means was tested using Duncan's multiple range tests (at $p \leq 0.05$ level). The characteristics of plants were presented as the mean with standard deviation (SD).

RESULTS

Morpho-physiological traits. As shown in Table 3, the experimental treatments, including the foliar application and irrigation frequency, had no significant effects on the growth parameters such as plant height, dry and fresh weights (dry matter and biomass yields); however, the simple effects of the foliar application and irrigation ($p \leq 0.01$) on the proline content were significant (Tab. 3). According to the comparison of the means, the amount of proline in water deficit stress treatment (37.11 μmol g^{-1}) was higher than the amount of proline (13.83 μmol g^{-1}) under optimum irrigation (Tab. 3). For the foliar application treatment, the proline content (36.75 μmol g^{-1}) in the control plants was higher than the foliar applications of SA and proline (Tab. 3).

The combined effects of irrigation frequency × foliar application did not significantly affect growth parameters such as plant height, biomass, and dry matter (Tab. 3). However, the interaction effect of irrigation and foliar application on the proline content was significant. The highest value of the concentration of proline (57.83 μmol g^{-1}) was obtained from the plants under non-foliar spraying (control) and water deficit conditions (Tab. 4).

Phytochemical traits. The simple effects of the foliar application and irrigation on volatile oil content ($p \leq 0.01$) were significant (Tab. 3). The highest value of the volatile oil content (71 μL/100 g dry matter) was obtained from the plants that received reduced irrigation (Tab. 3). In addition, the foliar-spraying of SA improved the volatile oil content of *E. purpurea* compared to no foliar application (Tab. 3). According to the average of the two experimental years, the maximum value of the volatile oil content (73.7 μL/100 g dry matter) was obtained from the *E. purpurea* plants under water deficit × foliar-spraying of SA treatment (Fig. 1).

The results from GC-FID and GC-MS analysis detected 5 significant constituents in the volatile oils under the experimental treatments (Fig. 2). The dominant compounds were germacrene D (52–54%), *p*-cymene (8–9.5%), *α*-pinene (3–5%), *β*-caryophyllene (6–8%), and *β*-bisabolene (3–4.5%). In general, sesquiterpene hydrocarbons and monoterpene hydrocarbons were the main class of chemical components in the volatile oil (Fig. 2). The simple impact of the experimental treatments significantly affected the amounts of the major constituents. The highest percentages of germacrene D, *p*-cymene, *α*-pinene, *β*-caryophyllene, and *β*-bisabolene were obtained from the *E. purpurea* plants sprayed by SA (Tab. 3). In addition, the deficit water treatment increased these main constituents compared to optimum irrigation (Tab. 3). The results of the interaction effects indicated that the maximum percentage of germacrene D, as the abundant compound, was detected in the volatile oil from the plants treated by water deficit × foliar-spraying of SA (53.68%) (Tab. 4). The highest concentrations of *p*-cymene,

Table 4. The interaction effect of proline and salicylic acid foliar application and irrigation regimes on the phytochemical traits of *Echinacea purpurea*

Treatments		Characteristics					
irrigation	foliar application	proline content (μmol g^{-1})	germacrene D (%)	*α*-pinene (%)	*β*-caryophyllene (%)	*p*-cymene (%)	β-bisabolene (%)
Optimal	control	15.67 ±2.94 d*¥	52.02 ±0.46 d	3.25 ±0.35 d	6.12 ±0.51 d	8.11 ±0.47 d	3.00 ±0.46 d
	salicylic acid	13.17 ±1.83 e	52.21 ±0.59 c	3.68 ±0.80 b	6.52 ±0.56 b	8.45 ±0.46 c	3.21 ±0.57 c
	proline	12.67 ±1.21 f	52.81 ±0.49 b	3.54 ±0.51 c	6.48 ±0.58 b	8.45 ±0.31 c	3.51 ±0.76 b
Deficit	control	57.83 ±9.86 a	52.23 ±0.39 c	3.39 ±0.21 d	6.38 ±0.65 c	8.46 ±0.72 c	3.45 ±0.70 b
	salicylic acid	18.50 ±1.51 c	53.68 ±0.16 a	4.65 ±0.63 a	7.68 ±0.71 a	9.39 ±0.61 a	4.58 ±0.49 a
	proline	35.00 ±4.14 b	52.07 ±0.66 d	3.48 ±0.30 c	6.52 ±0.28 b	8.59 ±0.61 b	3.44 ±0.51 b
ANOVA		$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

*The averages with at least a common alphabet are not statistically significant at the 5% level
¥ Values are an average of two years

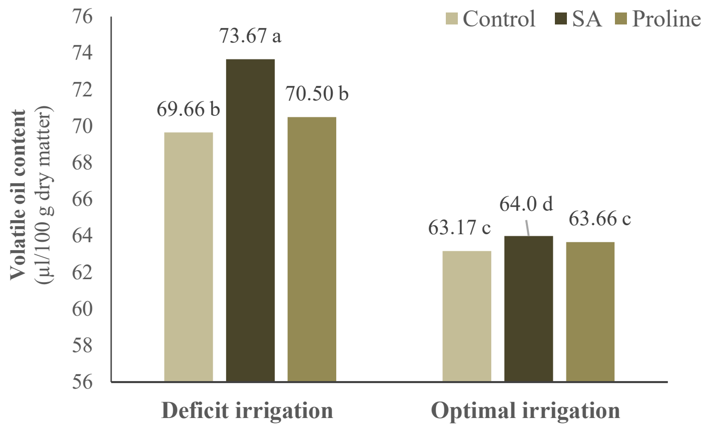

Fig. 1. Effect of the foliar application of salicylic acid and proline under two irrigation regimes on the volatile oil content of *E. purpurea* L. Values are an average of two years. The averages in columns with at least a common alphabet are not statistically significant at the 5% level

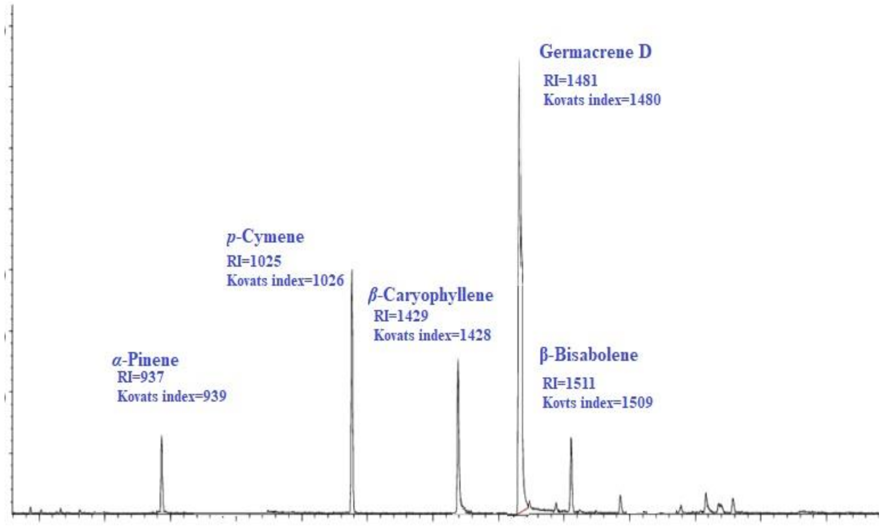

Fig. 2. Total ion chromatogram of GC-MS of the volatile oil from *E. purpurea*

α-pinene, *β*-caryophyllene, and *β*-bisabolene were also related to water deficit condition × foliar application of SA (Tab. 4).

DISCUSSION

The genetic factor, environmental conditions, and their interaction effects significantly impact plants' growth and phytochemical characteristics. Plant height as the main trait of morphological characteristic varies under genetic factors and environmental conditions, especially soil moisture availability [Carvalho et al. 2018]; however, in the present study, the effect of experimental treatments on plant height was not significant. Probably, *E. purpurea* under water deficit conditions maintained the stomatal conductance by maintaining the turgor pressure of the stomatal protection cells, which can keep its growth rate, photosynthesis, and morphological characteristics unchanged significantly [Ghasemi Pirbalouti et al. 2014]. Despite no significant differences between treatments regarding dry matter yield, an increase in biomass (23%) was observed in the plants treated by SA under water deficit conditions. Darvizheh et al. [2019] suggested that SA application could help reduce the adverse effects of water deficit stress and is considered a key in providing tolerance to water deficit. Under these conditions, the plant's transpiration rate is reduced, the amount of water required by the leaves is preserved, and division and cell growth are more favorable than in the case of non-application of SA [Mousavi et al. 2021]. The application of SA can activate antioxidant enzymes and be involved in the biosynthesis and accumulation of secondary metabolites, thus limiting the adverse effects of water deficit stress on biological performance [Zhao et al. 2005]. In contrast to our results, Sartip and Sirousmehr [2017] reported that the fresh and dry weights of cumin decreased under water deficit stress; however, under the application of SA, the rate of reduced fresh and dry weights was slower. In the present study, in water deficit conditions, the proline accumulation rate was the highest in control plants and the lowest with SA application. It could be due to water stress mitigation by the foliar applications of SA. The amount of alleviation effect on the drought stress by applying SA was higher than the spraying of proline. The foliar application of SA by improving the antioxidant enzyme activities probably clears ROS caused by environmental stresses [Momeni et al. 2020]. The exogenous application of growth regulators like SA is an approach to improve plant efficiency and mitigate the damages of various stresses [Darvizheh et al. 2019].

The volatile oil content under deficit irrigation conditions increased compared to optimal irrigation. The results of many studies also confirmed that the formation and accumulation of volatile oils in herbs under water deficiency conditions tend to increase because, in cases of stress, more secondary metabolites are biosynthesized to prevent intracellular oxidation [Ghasemi Pirbalouti et al. 2017, Khorasaninejad et al. 2018]. Additionally, the use of SA enhanced the content of the *E. purpurea* volatile oil; however, Darvizheh et al. [2019] found that the foliar application of SA reduced the volatile oil content in the leaves and flowers of purple coneflower despite irrigation regimes. The volatile oil of aromatic plants is located in specialized structures named glandular trichomes [Hazzoumi et al. 2019, Es-sbihi et al. 2020]. It was hypothesized that the increase in volatile oil yield under SA treatment could be related to its beneficial effect on the number of glands [Idrees et al. 2011]. Generally, the SA enhances the biosynthesis and accumulation of secondary metabolites, thereby increasing plant drought tolerance [Zhao et al. 2005].

In the current experiment, 5 major constituents were detected in the volatile oils where germacrene D formed more than 50% of the volatile oil composition. This compound is a class of sesquiterpene hydrocarbons and has anticancer, antiseptic, and insect-repellent properties [Mehrpooya et al. 2021]. This study's results agree with Mehrpooya et al. [2021], who underlined that germacrene D in the *E. purpurea* volatile oils was the dominant compound. For the experimental treatments' interactive effects, the significant constituents' highest concentrations were extracted from plants treated by water deficit conditions × foliar-spraying of SA. It seems that increasing the concentrations of these constituents by SA treatment aimed to regulate plant adaptation to water deficit conditions and is a kind of defense mechanism to maintain the vital activities of plants under stress. The increase in the quantity and quality of the volatile oil in the plants treated with SA may be due to its role in enhancing the overall growth of aerial parts [Janda and Ruelland 2015]. Drought stress, by altering the synthesis of the secondary metabolites through metabolic pathways and changes in the activity of enzymes and metabolism of the herbs, resulted in changes in the composition of the volatile oils [Ghasemi Pirbalouti et al. 2017]. Regarding the chemical composition of volatile oil, we observed qualitative and quantitative variations depending on the treatments. These modifications result in the appearance or disappearance of some major constituents and changes in the contents of pre-existing compounds. However, treating plants with SA increased the levels of sesquiterpene hydrocarbons affected by water deficit stress.

CONCLUSIONS

The results of the current study suggested that reduced irrigation had a positive effect on the phytochemical traits of *E. purpurea*, including the volatile oil, proline, and the percentages of the major constituents of the *E. purpurea* volatile oil, especially germacrene D. However, water deficit stress had negative impacts on the growth parameters. Therefore, the foliar application of SA could be practical for the stability of the quantity and quality of volatile oil under deficit irrigation conditions, which can be used to produce the medically active substance. Also, the foliar application of SA could be exploited in the sustainable production of *E. purpurea* under water deficit conditions, especially in arid and semi-arid climates.

REFERENCES

Adams, R.P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corp., Carol Stream, 456, 544–545.

Ahmadi, F., Samadi, A., Sepehr, E., Rahimi, A., Shabala, S. (2022). Morphological, phytochemical, and essential oil changes induced by different nitrogen supply forms and salinity stress in *Echinacea purpurea* L. Biocatal. Agric. Biotechnol., 43, 102396. https://doi.org/10.1016/j.bcab.2022.102396

Alavi Samany, S.M., Ghasemi Pirbalouti, A., Malekpoor, F. (2022). Phytochemical and morpho-physiological changes of hyssop in response to chitosan-spraying under different levels of irrigation. Ind. Crops Prod., 176, 114330. https://doi.org/10.1016/j.indcrop.2021.114330

Babaei, K., Moghaddam, M., Farhadi, N., Ghasemi Pirbalouti, A. (2021). Morphological, physiological and phytochemical responses of Mexican marigold (*Tagetes minuta* L.) to drought stress. Sci. Hortic., 284, 110116. https://doi.org/10.1016/j.scienta.2021.110116

Bates, L.S., Waldern, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil., 39, 205–207. https://doi.org/10.1007/BF00018060

Bohnert, H.J., Nelson, D.E., Jensen, R.G. (1995). Adaptations to environmental stresses. Plant Cell., 7(7), 1099–1111. https://doi.org/10.1105/tpc.7.7.1099

Carvalho, M.E.A., Castro, P.R.C., Gaziola, S.A., Azevedo, R.A. (2018). Is seaweed extract an elicitor compound? Changing proline content in drought-stressed bean plants. Comun. Scientiae, 9(2), 292–297. https://doi.org/10.14295/CS.v9i2.2134

Cheplick, S., Sarkar, D., Bhowmik, P.C., Shetty, K. (2018). Improved resilience and metabolic response of transplanted blackberry plugs using chitosan oligosaccharide elicitor treatment. Plant Sci., 98(3), 717–731. https://doi.org/10.1139/CJPS-2017-0055

Cozzolino, R., Malvagna, P., Spina, E., Giori, A., Fuzzati, N., Anelli, A., Garozzo, D., Impallomeni, G. (2006). Structural analysis of the polysaccharides from *Echinacea angustifolia* radix. Carbohyd. Polym., 65(3), 263–272. https://doi.org/10.1016/j.carbpol.2006.01.012

Danesh-Shahraki, H., Pirbalouti, A.G., Rajabzadeh, F., Kachouei, M.A. (2023). Water deficit stress mitigation by the foliar spraying of salicylic acid and proline on the volatile oils and growth features of hyssop (*Hyssopus officinalis* L.). J. Essent. Oil-Bear. Plants., 26(1), 115–129. https://doi.org/10.1080/0972060X.2022.2160279

Darvizheh, H., Zahedi, M., Abbaszadeh, B., Razmjoo, J. (2019). Changes in some antioxidant enzymes and physiological indices of purple coneflower (*Echinacea purpurea* L.) in response to water deficit and foliar application of salicylic acid and spermine under field condition. Sci. Hortic., 247, 390–399. https://doi.org/10.1016/j.scienta.2018.12.037

Es-sbihi, F.Z., Hazzoumi, Z., Benhima, R., Amrani Joutei, K. (2020). Effects of salicylic acid on growth, mineral nutrition, glandular hairs distribution and essential oil composition in *Salvia officinalis* L. grown under copper stress. Environ. Sustain., 3, 199–208. https://doi.org/10.1007/s42398-020-00109-x

Ghasemi Pirbalouti, A., Rahmani Samani, M., Hashemi, M., Zeinali, H. (2014). Salicylic acid affects growth, essential oil and chemical compositions of thyme (*Thymus daenensis* Celak.) under reduced irrigation. Plant Growth Regul., 72, 289–301. https://doi.org/10.1007/s10725-013-9860-1

Ghasemi Pirbalouti, A., Malekpoor, F., Salimi, A., Golparvar, A., Hamedi, B. (2017). Effects of foliar of the application chitosan and reduced irrigation on essential oil yield, total phenol content and antioxidant activity of extracts from green and purple basil. Acta Sci. Pol. Hortorum Cultus, 16(6), 177–186. https://doi.org/10.24326/asphc.2017.6.16

Ghasemi Pirbalouti, A., Nekoei, M., Rahimmalek, M., Malekpoor, F. (2019). Chemical composition and yield of essential oil from lemon balm (*Melissa officinalis* L.) under foliar applications of jasmonic and salicylic acids. Biocatal. Agric. Biotechnol., 19, 1–5. https://doi.org/10.1016/j.bcab.2019.101144

Güneri, M., Dalkılıç, Z. (2023). Effects of salicylic acid application on germination, growth and development of rough lemon (*Citrus jambhiri* Lush.) under salt stress. Acta Sci. Pol. Hortorum Cultus, 22, 13–26. https://doi.org/10.24326/asphc.2023.4798

Hazzoumi, Z., Moustakime, Y., Joutei, K.A. (2019). Essential oil and glandular hairs: diversity and roles. In: Essential oils, El-Shemy, H.A. (ed.). IntechOpen. https://doi.org/10.5772/intechopen.86571

Jafari, S., Garmdareh, S.E.H., Azadegan, B. (2019). Effects of drought stress on morphological, physiological, and biochemical characteristics of stock plant (*Matthio-*

la incana L.). Sci. Hortic., 253, 128–133. https://doi.org/10.1016/j.scienta.2019.04.033

Idrees, M., Naeem, M., Aftab, T., Khan, M.M.A. (2011). Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. Acta Physiol. Plant., 33, 987–999. https://doi.org/10.1007/s11738-010-0631-6

Janda, M., Ruelland, E. (2015). Magical mystery tour: salicylic acid signaling. Environ. Exp. Bot., 114, 117–128. https://doi.org/10.1016/j.envexpbot.2014.07.003

Khorasani, H., Rajabzadeh, F., Mozafari, H., Ghasemi Pirbalouti, A. (2023). Water deficit stress impairment of morphophysiological and phytochemical traits of Stevia (*Stevia rebaudiana* Bertoni) buffered by humic acid application. S. Afr. J. Bot., 154, 365–371. https://doi.org/10.1016/j.sajb.2023.01.030

Khorasaninejad, S., Álizadeh Ahmadabadi, A., Hemmati, K. (2018). The effect of humic acid on leaf morphophysiological and phytochemical properties of *Echinacea purpurea* L. under water deficit stress. Sci. Hortic., 239, 314–323. https://doi.org/10.1016/j.scienta.2018.03.015

Maghsoudi, E., Abbaspour, H., Ghasemi Pirbalouti, A., Saeidi-Sar, S. (2023). Influence of the foliar applications of paclobutrazol and 24-epibrassinolide on the quantitative and qualitative traits of sage (*Salvia officinalis* L.) volatile oil under different soil moisture conditions. J. Plant Growth Regul., 42, 5495–5506. https://doi.org/10.1007/s00344-023-10931-2

Mehrpooya, Z., Abdoli, M., Talebian, M.R. (2021). Effect of salicylic acid and yeast extract on caffeic acid derivatives production in *Echinacea purpurea* L. J. Medicinal Plants., 20(78), 36–47. https://doi.org/10.52547/jmp.20.78.36

Momeni, M., Ghasemi Pirbalouti, A., Mousavi, A., Badi, H.N. (2020). Effect of foliar applications of salicylic acid and chitosan on the essential oil of *Thymbra spicata* L. under different soil moisture conditions. J. Essent. Oil-Bear. Plants., 23(5), 1142–1153. https://doi.org/10.1080/0972060X.2020.1801519

Mousavi, A., Masoumeh, N., Ghilizadeh, A., Rahemi Karizaki, A. (2021). The Effect of elicitors on some physiological characteristics, essential oil percentage and yield in hyssop (*Hyssopus officinalis* L.) under different irrigation regimes. Env. Stresses Crop Sci., 14(2), 415–424. In Persian. https://doi.org/10.22077/escs.2020.2851.1740

Sartip, H., Sirousmehr, A.R. (2017). [Evaluation of salicylic acid effects on growth, yield and some biochemical characteristics of cumin (*Cuminum cyminum* L.) under three irrigation regimes]. Env. Stresses Crop Sci., 10(4), 547–558. In Persian. https://doi.org/10.22077/escs.2017.21.1007

Selmar, D., Kleinwachter, M. (2013). Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. Plant Cell Physiol., 54(6), 817–826. https://doi.org/10.1093/pcp/pct054

Shaykh Samani, Á., Ghasemi Pirbalouti, A., Yadegari, M., Rajabzadeh, F. (2023). [Evaluation of the foliar application effect of salicylic acid on the morpho-physiological and phytochemical traits of the essential oil from *Satureja bachtiarica* Bunge. under deficit irrigation conditions]. Eco-phytochem. J. Med. Plants., 10(4), 95–108. In Persian. https://doi.org/10.30495/ejmp.2022.1949355.1673

Zhao, J., Davis, L.C., Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol. Adv., 23(4), 283–333. https://doi.org/10.1016/j.biotechadv.2005.01.003