Response of growth, productivity, and active constituents of *Hyssopus officinalis* to irrigation and salicylic acid foliar application

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Background
As hyssop was recently introduced for cultivation in Egypt, it is recommended to know its optimal agronomic management practices, especially the irrigation management.
Objective
This work demonstrates the effect of the number of irrigation times per week and foliar spraying of different concentrations of salicylic acid and the interaction between them on the hyssop planted in sandy soil and under the drip irrigation system.
Materials and methods
In the 2-year experiment, three irrigation treatments, that is, once, twice, and three times per week, were applied with three concentrations of salicylic acid (0, 100, and 200 ppm). The growth parameters, photosynthetic pigments, antioxidant activity, total phenolics, proline content, essential oil percentage, and yield along with the main constituents of the essential oil were studied in hyssop herb to find out the relationship between these characteristics and the applied treatments.
Results and conclusion
It was observed that increasing the number of irrigation times from once to twice and three times per week increased growth, yield, essential oil percentage (%), content (ml/plant), and yield (l/ha) significantly in both seasons, except essential oil (%) from plants irrigated twice per week in the first season, which increased insignificantly. The essential oil showed the main compounds as 3-pinanone, cis in most treatments, except plants irrigated twice and three times per week and sprayed with tap water, where the main component was trans-3-pinanone (38.70 and 32.94 %, respectively). 3-pinanone, cis relative percent ranged from 40.81 to 63.47% in plants irrigated once per week and from 16.78 to 58.49 % in plants irrigated at twice per week, and then from 30.17 to 47.56 % in plants irrigated three times per week. Oxygenated compounds increased under water deficit (once per week) and decreased with increasing salicylic acid concentration. It may be concluded that hyssop plants that were irrigated twice per week and sprayed with 100 ppm salicylic acid produced the highest yield of both herb and essential oil.

Keywords:
antioxidant activity, essential oil, GC-MS, *Hyssopus officinalis*, irrigation, salicylic acid, total phenolics

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Introduction

Hyssopus officinalis L. is an important culinary and medicinal plant that belongs to family Lamiaceae and is wildly cultivated in Europe and in the temperate regions of America and Asia [1]. Hyssop is a perennial evergreen plant with small, linear leaves and purplish-blue flowers [2]. It has been used in traditional folk medicine to treat respiratory diseases or to enhance digestion and appetite [1,3].

H. officinalis is rich in volatile oil, phenolics, flavonoids, marrubin, tannins, and vitamin C, and also it has many different pharmacological activities, for example, antioxidant, antimicrobial, antifungal, anti-inflammatory, antidiabetic, anticonvulsant, antiulcer, antispasmodic, antihemolytic, antileishmaniasis, anticatarrhal, muscle relaxant, antiasthmatic, and anti-HIV activities [4–6]. Lately, its essential oil has been well known for its biocidal (insecticidal, mosquito larvicidal, nematicidal, and bactericidal) activity [7,8]. Essential oil of hyssop is used extensively in food technology (industry of flavors and food additives) as well as a fragrance component in soaps, fragrances, and cosmetics [9,10].

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Venditti *et al*. [11] found linalool and methyl eugenol as the main components in essential oil of *H. officinalis*. However, Jop *et al*. [12] reported that monoterpene ketones (isopinocamphone and pinocamphone) are the main constituents followed by mononeterpene and sesquiterpene hydrocarbons [β-pinene, germacrene D, bicyclogermacrene, and (E)-β-caryophyllene] and oxygenated constituents, for example, myrtenyl methyl ether and elemol.

It is commonly recognized that in the near future, particularly in third-world countries, water will become scarce owing to the acceleration of climatic changes, the ensuing rise in drought, and the quick increase in population [13]. When there is a high demand for water, irrigation management increases agricultural production and income per unit of water utilized while decreasing irrigation requirements and improving water use efficiency [14].

Numerous chemical substances that are commercially available can be employed as elicitors to alter plant secondary metabolites, which in turn affects the bioactivity of plants, enables them to survive water stress, and improves yield and agronomic performance of various crops [15,16]. The role of salicylic acid in nutrient uptake, water relations, membrane stability, photosynthesis, growth stomatal regulation, and restriction of ethylene production has been linked to its capacity to improve plant development under abiotic stress circumstances [17–21].

As hyssop was recently introduced for cultivation in Egypt, it is recommended to know its optimal agronomic management practices, especially the irrigation management. It is important to determine the suitable irrigation intervals that produced the best plant growth and production. Therefore, this study aimed to investigate the effect of three irrigation treatments (once, twice, and three times a week) combined with three foliar application treatments with two levels of salicylic acid (100 and 200 ppm) and tap water (control)) on vegetative growth, yield, and active constituents of hyssop (*H. officinalis*) cultivated in Egypt under drip irrigation system.

Materials and methods

To investigate the effect of different irrigation treatments (number of irrigation times per week) as well as the effect of salicylic acid foliar application on productivity, essential oil and some active constituents of hyssop (*H. officinalis*) plant, a 2-year field experiment was conducted under a drip irrigation system during 2018/2019 and 2019/2020 at SEKEM Company Farm, Belbeis, Sharkia Governorate west of the Nile Delta, Egypt (30°35’15.65″ N and 31°30’7.20″ E).

Seeds of *H. officinalis* L. were brought from Pharmasaat-SEEDS and PLANTS Company (PHARMASAAT-Seeds and Plants of Medicinal and Spice Herbs, Canada). Seeds were cultivated at SEKEM experimental Farm, SEKEM Company, El-Adlya Belbeis, EL-Sharkiya Governorate, and a herbarium specimen was kept in the NRC Herbarium under the No. M524. Seeds were cultivated in trays on September 16 and 14, 2018 and 2019, respectively. The seedlings were transplanted in the permanent field after the seedlings reached the suitable size and has a good root system (after 40 days) on October 27 and 24, 2018 and 2019, respectively. The metrological data of the experimental farm region during the growing period are presented in Table 1.

The soil was prepared 2 weeks before transplantation and divided into rows of 1 m apart. The used soil’s physical and chemical properties were determined

Table 1 Monthly average of metrological data of the experimental area during 2018/2019 and 2019/2020 seasons

2018/2019 season					2019/2020 season				
	Air temperature °C					Air temperature °C			
Month	Maximum	Minimum	Average.	RH %	Month	Maximum	Minimum	Average	RH %
Sept. 2018	37	23	29	57	Sept. 2019	37	20	28	58
Oct. 2018	34	17	26	54	Oct. 2019	35	19	26	59
Nov. 2018	31	15	21	59	Nov. 2019	32	13	22	54
Dec. 2018	23	10	17	58	Dec. 2019	24	9	16	56
Jan. 2019	25	7	14	46	Jan. 2020	21	6	13	59
Feb. 2019	31	10	16	54	Feb. 2020	26	9	15	61
Mar. 2019	29	10	18	55	Mar. 2020	30	10	18	55
Apr. 2019	35	13	21	48	Apr. 2020	34	14	21	50
May 2019	45	16	27	50	May 2020	43	17	26	45

RH, relative humidity.

according to Jackson [22] and are shown in Table 2, from which it could be observed that the soil is sandy loam soil in texture.

During soil preparation, 36 m^3/ha of compost, 180 kg/ha of elemental sulfur (99.9% S), and 715 kg/ha of calcium superphosphate (15.5% P_2O_5) were added. All treatments received during plant growth period 950 kg/ha of ammonium sulfate (20.5% N) and 360 kg/ha of potassium sulfate (48.5%K_2O) divided into three doses. Plants were irrigated with drip irrigation system (4 L/h). The chemical analyses of the compost were performed according to Jackson [22], and the results are shown in Table 3.

The layout of the experiment was a split-plot design for nine treatments with three replicates. The treatments represent the combinations between three irrigation treatments (once, twice, and three times per week) in the main plots with two levels of salicylic acid (100 and 200 ppm) in addition to the control treatment (Tap water) in the subplots. Spraying salicylic acid was performed twice at the first week of January and the first week of March in both seasons.

Plant height and fresh and dry weights of herb (g/plant and ton/ha) of each replicate were recorded for five individual plants at the end of the experiment. Photosynthetic pigments in leaves (chlorophyll a, chlorophyll b, and total carotenoids (mg/g fresh leaves) were determined according to Moran [23]. Proline content (mg/g fresh leaves) was determined according to Bates *et al.* [24]. Essential oil percentage (%) was determined with hydrodistillation using a Clevenger-type apparatus according to Said-Al Ahl *et al.* [25] and Hassanein *et al.* [26].

Preparation of the dry herb extract was done as follows: half a gram of the dry powdered herb was mixed with 10 ml of 70% methanol and stored at room temperature; after 48 h, infusions were filtered

Table 2 The physical and chemical properties of the experimental soil during 2018/2019 and 2019/2020 seasons

	Physical properties						
	Very coarse sand (2–1 mm)	Coarse sand (1–0.5 mm)	Medium sand (0.5–0.25 mm)	Fine sand (0.25–0.1 mm)	Very fine sand (0.1–0.05 mm)	Silt+clay (0.5> mm)	Texture
2018/2019	12.73	55.70	0.29	20.04	8.77	2.38	Sandy
2019/2020	10.73	59.03	0.69	18.77	9.27	1.50	Sandy

	Chemical properties									
	pH (2.5 : 1)	EC (dS/m) (1 : 1)	Cations (meq/l)				Anions			
			Ca++	Mg++	Na+	K+	$CO3^-$	HCO_3^-	Cl^-	SO_4^-
2018/2019	8.04	0.55	2.0	0.5	2.64	0.4	–	1.2	3.5	1.84
2019/2020	8.07	0.82	3.0	0.5	4.92	0.5	–	2.0	3.7	3.22

Table 3 The chemical analysis of the used compost during 2018/2019 and 2019/2020 seasons

Characteristic	2018/2019	2019/2020
pH (1 : 10)	7.57	6.99
EC (dS/m) (1 : 10)	3.67	4.16
Organic matter (%)	26.5	39.4
Organic carbon (%)	15.4	22.9
Total nitrogen (%)	0.92	1.28
C/N ratio	17:1	18:1
Total phosphorus (%)	0.12	0.31
Total potassium (%)	1.42	0.56
Ash (%)	73.5	60.6

through Whatman No. 1 filter paper. Then, total phenolic content (mg/g dry herb) and antioxidant activity (%) by DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging were determined in the methanolic extract.

Total phenolic content (mg/g dry herb) was determined according to Singleton *et al.* [27]. The ability of the plant extract to scavenge DPPH free radicals [antioxidant activity by DPPH free radical scavenging (%)] was determined by the standard method [28], adopted with suitable modifications [29] using 15 μl of dry herb methanolic extract.

The GC-MS (Gas chromatography-Mass spectrometry) analysis of all essential oil samples was done using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center, as reported by Abd-ElGawad *et al.* [30] and Omer *et al.* [31].

The recorded data were analyzed as a split-plot design by analysis of variance using the General Linear Models procedure of CoStat [32]. Least significant difference test was applied at 0.05 probability level to compare the means of the treatments.

Results and discussions

Plant growth parameters and biomass yield

The results of *H. officinalis* plant height, number of branches, fresh and dry weights (g), and fresh and dry yields (ton/ha) as affected by irrigation, salicylic acid foliar-spray application, along with their interaction during two growing seasons (2018–2019 and 2019–2020) are shown in Table 4. All vegetative parameters responded significantly to irrigation, application of salicylic acid, as well as to their interactions in both seasons. It is clear that plant height and number of branches as well as fresh and dry weight and yield of herb increased significantly with increasing number of irrigation times from once per week to twice per week in both seasons, whereas their values were decreased by increasing irrigation times from twice to three per week. The highest values in both seasons resulted from irrigation twice per week, whereas irrigation once per week gave the lowest values of all parameters. Our results are in accordance with those of Ismail *et al.* [33] on *Tagetes lucida*, Ahmed [34] on ajwain, Pirbalouti *et al.* [16] on thyme, Khanam and Patra [35] on gladiolus plants, Herman *et al.* [13] on *Salvia hispanica*, and Fouad et al. [36] on chia (*S. hispanica*). All vegetative parameters responded significantly to salicylic acid foliar application in both seasons (Table 4). Application of salicylic acid (100 and 200 ppm) recorded greater values than the control in both season. Increasing the concentration from 100 to 200 ppm decreased the values significantly compared with spraying with 100 ppm. These results are in harmony with Pirbalouti *et al.* [16] on thyme, Zeid *et al.* [37] on ajwain (*Trachyspermum ammi*), Abd Allah *et al.* [38] on quinoa, and Fouad *et al.* [36] on chia (*S. hispanica*).

Irrigation twice per week interacted with salicylic acid at 100 ppm treatment gave the maximum values of plant height, number of branches, and herb fresh and dry weights and yields. The minimum values were recorded with irrigation once a week with tap water (control) foliar-spray treatment. The results agreed with those of Ullah *et al.* [39] on canola, Pirbalouti *et al.* [16] on thyme, Khatiby *et al.* [40] on sesame, and Fouad *et al.* [36] on chia (*S. hispanica*).

The pronounced effect of irrigation that was obtained from irrigation two times per week may be attributed to sufficient soil moisture being available within the root zone, which would lead to a greater proliferation of root biomass and thus increase the different physiological processes such as improvement of nutrient uptake and absorption of water, better plant growth, better photosynthesis rates and so excess of fresh and dry matter buildup, and increase in vegetative biomass growth [41–44]. It could also be attributed to increased cell elongation and division, which require more water supplies [45].

On the contrary, although we expected that irrigation three times per week will not lead to water stress, vegetative growth parameters such as production of fresh and dry matter were lower in this treatment. The reduction in vegetative growth parameters and yield under irrigation three times per week may be owing to exposure to excessive water, which led to similar symptoms to those suffered by plants under drought. Therefore, we can explain the reduction occurred in hyssop plant under irrigation three times per week by that plant adjusted to excessive water stress by easing up the overall growth, and stem elongation still happened, but plants did not fill-up. The growth decline which is strongly correlated to the stem limitation growth can be mainly explained by a slowdown of growth under root anoxia [13,46].

When there is more water than the optimum requirement, it is referred to as water logging. Water

Table 4 Effect of irrigation (times per week) and salicylic acid (ppm) on vegetative parameters of *Hyssopus officinalis* plants during 2018–2019 and 2019–2020 seasons

Treatment		Plant height (cm)		Number of branches		Herb fresh weight (g/plant)		Herb dry weight (g/plant)		Herb fresh yield (ton/ha)		Herb dry weight (ton/ha)	
Irrigation (times per week)	Salicylic acid (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Once	0	41.7	43.3	21.3	18.3	105.5	91.7	35.3	21.0	3.53	3.06	1.18	0.70
	100	52.0	54.7	22.0	24.7	160.0	207.4	45.9	47.9	5.35	6.92	1.53	1.60
	200	48.3	50.3	21.7	22.7	123.0	168.1	37.9	34.6	4.08	5.62	1.26	1.16
Twice	0	51.0	53.3	34.0	29.0	206.2	213.0	36.9	50.7	6.89	7.10	1.23	1.69
	100	62.3	64.3	43.0	39.7	333.6	296.6	58.4	65.5	11.10	9.88	1.94	2.18
	200	54.7	55.7	36.0	32.3	211.8	225.0	50.2	54.8	7.07	7.51	1.67	1.82
Three	0	54.7	52.7	24.0	23.7	185.2	173.2	31.7	39.0	6.20	5.77	1.06	1.30
	100	58.7	58.3	28.7	27.3	279.8	263.2	56.0	57.3	9.33	8.78	1.87	1.91
	200	54.3	54.7	26.0	26.0	221.0	198.6	37.9	41.1	7.37	6.63	1.26	1.37
LSD at 5%		3.791	3.333	3.445	2.671	34.111	27.059	6.263	5.722	1.122	0.903	0.211	0.191
Irrigation (times per week)													
Once		50.0	49.4	21.7	21.9	129.5	155.8	39.7	34.5	4.32	5.20	1.32	1.15
Twice		56.0	57.8	37.3	33.7	250.5	244.9	48.5	57.0	8.35	8.16	1.62	1.90
Three		55.9	55.2	26.2	25.7	228.7	211.7	41.9	45.8	7.63	7.06	1.40	1.52
LSD at 5%		3.526	1.469	2.085	1.563	32.528	23.627	4.668	5.196	1.057	0.796	0.158	0.174
Salicylic acid (ppm)													
0		49.1	49.8	26.4	23.7	165.6	159.3	34.7	36.9	5.54	5.31	1.15	1.23
100		57.7	59.1	30.9	30.6	257.8	255.7	53.4	56.9	8.60	8.53	1.78	1.90
200		52.4	53.6	27.9	27.0	185.2	197.2	42.0	43.5	6.17	6.58	1.40	1.45
LSD at 5%		2.188	1.996	2.064	1.600	20.433	16.209	3.751	3.428	0.672	0.541	0.126	0.114

LSD, least significant difference.

fills the pores of the soil in the water logged soils, so the concentration of oxygen in the soil decreases. Lack of oxygen reduces the growth and survival of plants growing in this soil. The flooding frequently induces stomatal closing mostly in plants [47–49].

The increment in vegetative growth as a result of salicylic acid application may be due to that low concentration of salicylic acid increased photosynthetic activity (which improved the plant growth, internodes number, development, and differentiation of cells and tissues of plants) and stimulate physiological processes that had an impact on the improvement of both active translocation of the photosynthetic products from source to sink and vegetative growth [50–52]. Additionally, SA (Salicylic acid) may have higher adaptive capacity to stress as it encourages the synthesis of polyamines and improves osmotic adjustment [51].

Exogenous SA increased transpiration and proline accumulation, and this may also be an efficient defense mechanism against the damaging effects of drought for the plant [53]. The growth characteristics decrement under salicylic acid high concentration could be harmful to plants, including deleterious effects on cellular metabolisms or inhibitory effects on growth [54].

Chemical constituents

Photosynthetic pigments and proline (mg/g)

Data in Table 5 showed significant differences in photosynthetic pigments and proline concentrations in *H. officinalis* leaves as a result of the different irrigation treatments, salicylic acid foliar application, and their interactions in the first and second seasons, except chlorophyll (b) concentration in both seasons and total carotenoids in the second season that did not respond significantly to the interaction between irrigation treatments and salicylic acid.

Photosynthetic pigment concentration (mg/g)

Increasing irrigation times from one time per week to two times per week resulted in a significant increase in chlorophyll a,b and total carotenoids (mg/g) in hyssop leaves, which indicate that drought stress decreased significantly photosynthetic pigment content. Plants irrigated at three times per week contained photosynthetic pigments lower than those irrigated twice per week and higher than those irrigated once per week (Table 5). These results are in harmony with Herman *et al.* [13] on chia, Ullah *et al.* [39] on canola, Pascale *et al.* [55] on gladiolus, and Elewa *et al.* [56] on quinoa.

Application of salicylic acid increased Chl. a,b and total carotenoids significantly in both seasons compared

Table 5 Effect of irrigation (times per week) and salicylic acid (ppm) on chlorophyll (a, b), total carotenoids and proline of *Hyssopus officinalis* fresh leaves during 2018–2019 and 2019–2020 seasons

Treatment		Chlorophyll (a) (mg/g)		Chlorophyll (b) (mg/g)		Total carotenoids (mg/g)		Proline (mg/g)	
Irrigation (times per week)	Salicylic acid (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Once	0	1.94	2.39	0.81	1.05	1.01	1.46	4.52	3.48
	100	2.38	3.23	1.18	1.45	1.56	2.08	3.50	3.23
	200	2.03	2.96	0.96	1.25	1.27	1.75	4.14	2.72
Twice	0	2.55	3.10	1.22	1.61	1.64	2.16	2.03	1.82
	100	3.26	3.70	1.76	2.23	2.66	2.94	1.34	2.24
	200	2.67	3.45	1.42	2.02	2.17	2.34	1.76	2.46
Three	0	2.26	2.94	1.03	1.44	1.29	1.82	3.80	3.74
	100	2.66	3.40	1.30	1.83	1.69	2.28	2.69	1.77
	200	2.44	3.08	1.12	1.64	1.42	2.06	3.64	3.05
LSD at 5%		0.128	0.186	NS	NS	0.207	NS	0.219	0.638
Irrigation (times per week)									
Once		2.12	2.86	0.98	1.25	1.28	1.76	4.06	3.14
Twice		2.82	3.41	1.47	1.95	2.16	2.48	1.71	2.17
Three		2.46	3.14	1.15	1.64	1.46	2.05	3.38	2.85
LSD at 5%		0.114	0.268	0.219	0.168	0.130	0.128	0.052	0.357
Salicylic acid (ppm)									
0		2.25	2.81	1.02	1.37	1.31	1.81	3.15	2.01
100		2.77	3.44	1.41	1.84	1.97	2.44	2.51	2.41
200		2.38	3.16	1.16	1.64	1.62	2.05	3.48	3.74
LSD at 5%		0.077	0.111	0.141	0.099	0.124	0.153	0.131	0.382

LSD, least significant difference.

with control. Increasing salicylic acid level from 0 to 100 and 200 ppm significantly increased photosynthetic pigments in both seasons. Hyssop leaves showed significant decrement in their content of photosynthetic pigments by raising salicylic acid application from 100 to 200 ppm. The obtained results were found to be agreed with Abd Allah *et al.* [38] on quinoa, Ullah *et al.* [39] on canola and Bakry *et al.* [52] on linseed.

The greatest values of chlorophyll a content and total carotenoids resulted from plants irrigated twice per week and sprayed with 100 ppm salicylic acid. On the contrary, irrigation once per week and spraying with tap water (control) produced the lowest values of chl. a and total carotenoids. The previous result is in harmony with Ullah *et al.* [39] on canola.

Chlorophyll concentration reduction is considered as a drought response mechanism to reduce chloroplast's absorption of light [57]. Lisar *et al.* [58] mentioned that under water stress, there are several co-factors that reduce a plant's photosynthesis, including quantitative and qualitative alterations in the pool of photosynthesizing pigments, poor CO_2 assimilation rates in photosynthetic leaves, and reduced CO_2 uptake due to stomatal closure. Moreover, as water becomes scarcer, leaf water content declines to levels that negatively affect metabolism and oxidation of pigments, resulting in damage to photosynthetic pigments and decreased pigment biosynthesis. This, in turn, results in metabolic limitations to photosynthesis [59,60].

Salicylic acid is an antioxidant concentrated in the chloroplast and protects the photosynthetic system when the plant is exposed to drought stress [61] by safeguarding the chloroplasts and preventing the harmful reactive oxygen radicals from degrading the chlorophyll [62]. Moreover, salicylic acid had stimulatory effects on photosynthetic pigments and rubisco activity attributed to that it enhanced the net photosynthetic rate, assimilation intercellular CO_2, transpiration, stomatal conductance, and water use efficiency [63,64].

Proline concentration (mg/g)

Contrary to all the previous results, proline took a completely opposite direction as a result of irrigation and foliar spraying and their interaction. It is clear that, increasing times of irrigation per week from once to twice and three times led to a decrease in proline concentration. In other words, water stress increased proline content in leaves. On the contrary, increasing irrigation interval from twice to three times per week increased proline content of leaves.

The same trend was observed with salicylic acid foliar application, as spraying plants with tap water (0 ppm salicylic acid) resulted in the highest concentration of proline compared with 100 and 200 ppm. Conversely, increasing the concentration from 100 to 200 ppm led to an increase in proline again.

The greatest values of proline resulted from plants irrigated once per week and did not spray with salicylic acid (sprayed with tap water) in both seasons. On the contrary, irrigation twice per week and spraying with 100 ppm of salicylic acid produced plants that contained the lowest values of proline content in both seasons.

These results agreed with Ullah *et al.* [39] on canola, Elewa *et al.* [56] on quinoa, Ahmed [65] on *T. lucida*, Mustafavi *et al.* [66] on valerian (*Valeriana officinalis*), and Ahmed [67] on chia (*S. hispanica*).

Proline increment may be attributed to the osmotic adjustment that occurs when plants are subjected to drought stress as plants maintain their water content by the piling up in their cytoplasm high amounts of osmotically active substances such as proline (known as compatible solutes) [68,69]. In addition, increased protein degradation under high drought stress may be lead to increased proline content [38].

It is evident that spraying plants under drought stress with high salicylic acid concentration increased osmolyte accumulation (like proline) to maintain leaf's relative water content [39].

Phenolic concentration (mg/g) and antioxidant activity (%)

Values shown in Table 6 point out that the different irrigation treatments and/or salicylic acid foliar spraying resulted in significant differences in both total phenolic compounds and antioxidant activity of *H. officinalis* herb.

The lower number of irrigation (times per week) produced significantly high phenolics concentration and antioxidant activity in the herb. Irrigation twice per week resulted in the lowest phenolics significantly compared with once or three times per week, and also plants irrigated three times per week were lower than plants irrigated once per week in their phenolic compounds and antioxidant activity in both seasons.

Table 6 Effect of irrigation (times per week) and salicylic acid (ppm) on essential oil production, total phenolics, and antioxidant activity of *Hyssopus officinalis* during 2018–2019 and 2019–2020 seasons

Treatment		Total phenlics (mg/g)		Antioxidant activity (%)		Oil percentage (%)		Oil content (ml/plant)		Oil yield (l/ha)	
Irrigation (times per week)	Salicylic acid (ppm)	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Once	0	88.3	95.6	83.7	85.0	0.30	1.00	0.105	0.209	3.50	6.98
	100	71.7	91.4	64.2	76.1	0.33	1.18	0.152	0.565	5.06	18.82
	200	78.0	111.3	76.1	88.2	0.20	0.72	0.077	0.250	2.56	8.32
Twice	0	73.2	83.1	68.2	72.7	0.32	1.27	0.119	0.639	3.97	21.29
	100	42.3	56.5	41.6	59.8	0.38	1.32	0.221	0.858	7.36	28.61
	200	69.2	71.4	54.4	65.2	0.25	1.23	0.126	0.672	4.19	22.40
Three	0	80.3	89.0	73.9	77.0	0.55	1.45	0.176	0.564	5.87	18.79
	100	66.8	76.5	60.4	68.6	0.37	1.23	0.210	0.703	6.99	23.44
	200	77.3	84.2	72.3	72.9	0.18	1.05	0.070	0.430	2.32	14.32
LSD at 5%		5.706	4.048	4.913	4.854	0.076	0.204	0.0391	0.0694	1.302	2.313
Irrigation (times per week)											
Once		79.3	99.4	74.7	83.1	0.28	0.97	0.111	0.341	3.71	11.37
Twice		61.6	70.3	54.7	65.9	0.32	1.27	0.155	0.723	5.17	24.10
Three		74.8	83.2	68.8	72.8	0.37	1.24	0.152	0.566	5.06	18.85
LSD at 5%		4.067	3.675	3.107	3.506	0.061	0.183	0.0322	0.0979	1.073	3.262
Salicylic acid (ppm)											
0		80.6	89.2	75.3	78.2	0.39	1.24	0.133	0.471	4.44	15.68
100		60.3	74.8	55.4	68.1	0.36	1.24	0.194	0.709	6.47	23.63
200		74.9	89.0	67.6	75.4	0.21	1.00	0.091	0.450	3.02	15.01
LSD at 5%		3.418	2.425	2.943	2.908	0.046	0.122	0.0234	0.0416	0.780	1.386

LSD, least significant difference.

It could be concluded that water stress significantly increased the hyssop herb phenolic content.

The two used levels of salicylic acid (100 and 200 ppm) significantly decreased phenolic concentration and antioxidant activity in both seasons when compared with tap water spraying, except 200 ppm, which resulted in insignificant decrease in the second season. Increasing salicylic acid concentration from 100 to 200 ppm led to a significant increment in total phenolics. It was observed that there was a decrease in phenolic content as a result of decreasing the concentration of salicylic acid from 200 to 100 ppm.

The interaction between irrigation at once per week and tap water (0 ppm salicylic acid) foliar-spray application resulted in the maximum value of phenolics and antioxidant activity in the first season. However the highest values in the second season resulted from plants irrigated once per week and sprayed with 100 ppm salicylic acid. The minimum values of both phenolics and antioxidant activity in both seasons were produced from the treatment of irrigation twice per week interacted with salicylic acid with 100 ppm concentration.

Total phenolics results are similar to those obtained by Abd Allah *et al.* [38] and Elewa *et al.* [38,56] on quinoa, Ahmed [67] on chia (*S. hispanica*), Khandaker *et al.* [70] on red amaranth, and Abd Elhamid *et al.* [71] on fenugreek.

In addition, antioxidant activity findings are in accordance with Khandaker *et al.* [70] on red amaranth, Abd Allah *et al.* [38] on quinoa, Mustafavi *et al.* [66] on valerian who stated that antioxidant enzymes activity in plant leaves increased with the increase of drought stress, and Ahmed [67] on chia (*S. hispanica*).

The increase in phenolics by inadequate irrigation could be a plant response as coping mechanism for drought stress [72], as phenols have a crucial role in regulation of plant metabolic processes, which in turn affects total plant growth [73]. Moreover, phenols serve as a substrate for many antioxidants enzymes, which reduces the effects of stress damage [74]. Owing to their ability to reduce membrane fluidity, phenolic compounds have additional mechanisms behind their antioxidative effects [75].

The increment in total phenolic content under the high concentration of salicylic acid (200 ppm) may be due to the phenolic nature of salicylic acid [76]. This rise in the phenolic content may be one way that salicylic acid helps to lessen the suppressive effects of drought, as phenols have perfect structural chemistry for free radical scavenging activity [52].

From our previous results, we can notice that antioxidant activity took the same trend of total phenolic concentration and it can be explained by a highly positive correlation between the content of total phenolics and the antioxidant activity as stated by Ahmed [67] and Abootalebian *et al.* [77]. Moreover, phenolics are widely spread and serve as a substrate for a variety of antioxidant enzymes, and also as mentioned before phenolics have idealistic structural chemistry for free radical scavenging activity, so they have the capability by a single-electron transfer to scavenge free radicals, hydroxyl, and superoxide radicals [52,74,78].

Increasing antioxidant activity under irrigation once per week may be attributed to the fact that plants under water stress have evolved numerous antioxidant defense mechanisms that rely on a series of antioxidant enzymes (including peroxidase, catalase and superoxide dismutase, as well as the low-molecular-weight antioxidants, for example, phenolics and ascorbic acid) to shield themselves from reactive oxygen species [79–81].

Salicylic acid is an antioxidant that prohibits reactive oxygen species activity. Salicylic acid regulates antioxidant enzymes activity and so increases plant resilience to abiotic stress, as SA is an endogenous phenolic growth regulator [82].

Essential oil

Essential oil percentage (%), content (ml/plant), and yield (L/ha)

Data in Table 6 show that the effect of irrigation treatments, salicylic foliar application, and their interaction on air dried herb base essential oil percentage (%), content (ml/plant), and yield (L/ha) was significant in both seasons.

It was observed that increasing the number of irrigation times from once to twice and three times per week increased essential oil percentage, content, and yield significantly in both seasons, except essential oil percentage from plants irrigated twice per week in the first season, which increased insignificantly. However, increasing the number of irrigation from twice to three times had insignificant effect on all parameters in the first season and significantly increased content and yield in the second season. This could be explained as increasing water amount increased the oil percentage and the opposite was true.

The previous results of irrigation agreed with those of Herman *et al.* [13] and Ahmed [13,67] on chia (*S. hispanica*), Ahmed [65] on *T. lucida,* and Hussain *et al.* [83] on sunflower.

Application of salicylic acid at 100 ppm insignificantly decreased essential oil percentage in the first season and did not affect it in the second season. Increasing salicylic acid concentration to 200 ppm significantly decreased the essential oil percentage compared with 100 ppm treatment in both seasons. Spraying salicylic acid with 100 ppm concentration resulted in the highest significant content and yield compared with the other two treatments in both seasons. Spraying with tap water increased essential oil content and yield significantly compared with 200 ppm salicylic acid in the first season and insignificantly in the second season. These findings are in agreement with Bakry *et al.* [52] on linseed, Ahmed [67] on chia (*S. hispanica*), and Ahmed *et al.* [84] on sunflower.

The uppermost essential oil percentage in both seasons was recorded in plants irrigated three times per week and sprayed without salicylic acid. However, irrigation twice per week united with 100 ppm salicylic acid led to the maximum oil content and yield in both seasons.

The decrease in oil percentage when plants were irrigated one time per week was explained by Chanirar *et al.* [85], who illustrated that shoot yield and oil % values were correlated; thus, when the shoot yield declines owing to drought stress, oil percentage also decreases. Based on the previous fact (there was a correlation between the shoot yield and oil percentage), we can explain the increase of essential oil percentage in plants irrigated twice per week. On the contrary, the increase of essential oil percentage under increasing number of irrigation per week (twice and three times per week) indicated that irrigation once a week is not sufficient for the plant to exercise its normal physiological activities, such as the production of secondary compounds, including volatile oils.

The high percentage of essential oil in the absence of salicylic acid may be attributed to the fact that the plant is suffering from stress and there is no assistant factor to help it withstand this stress and its harmful effects, so as we mentioned in the previous paragraph the plant was forced to produce essential oil. This stimulatory effect of salicylic acid at 100 ppm can be attributed to its effects on enzymatic activity and transfer of metabolites to the herb [38]. The increase in vegetative growth and nutrients uptake may be the cause of the increment in oil percentage [86]. The reduction in growth and yield parameters under the highest concentration of salicylic acid may be the result of decreased oil content under this concentration [87]. The increment and decrement of the essential oil content per plant and yield per ha as a result of irrigation intervals and salicylic acid foliar application could be explained through its effect on increasing or decreasing the corresponding plant weight and yield [88].

The main constituents of the essential oil

The main constituents of the essential oil as affected by different irrigation treatments and salicylic acid foliar application during 2019–2020 season are shown in Table 7.

About 30 compounds were identified and accounted for more than 99.7% of the separated compounds in all

Table 7 The relative percentage of the main constituents of the essential oil of *Hyssopus officinalis* as affected by irrigation treatments and salicylic acid application during 2019–2020 season

			Relative percentage (%)								
			Once per week			Twice per week			Three times per week		
RT	KI	Compounds	0 SA	100 SA	200 SA	0 SA	100 SA	200 SA	0 SA	100 SA	200 SA
3.55	914	3-Thujene	–	–	0.11	0.20	0.10	0.62	–	0.35	–
3.69	921	1R-α-Pinene	–	0.28	0.51	0.79	0.72	1.42	0.30	0.90	0.79
4.06	941	Camphene	–	–	–	–	–	0.18	–	–	–
4.58	965	Sabinene	0.13	1.34	1.98	2.24	1.99	4.17	1.59	3.08	2.02
4.70	971	(1S)-(–)-β-Pinene	3.09	10.96	13.14	16.13	18.59	13.54	11.36	19.41	13.81
4.96	982	β-Pinene	–	0.21	0.38	0.60	0.19	3.18	0.44	1.56	3.74
6.14	1.027	D-Limonene	–	–	–	–	–	–	–	–	0.64
6.26	1031	α-Phellandrene	0.51	3.56	6.40	6.34	1.06	12.84	6.77	5.29	13.54
6.70	1046	β-Ocimene	–	–	–	–	–	0.18	–	–	–
8.78	1108	β-Linalool	–	–	0.07	–	–	0.90	0.20	0.24	0.58
8.92	1112	3-Thujanone	0.06	0.08	0.09	–	–	–	–	–	–
10.15	1147	Pinocarveol	0.10	–	–	–	–	–	0.06	–	–
10.57	1158	6-Isopropenyl-3-methoxymetoxy –3-methyl-cyclohexene	5.28	7.03	4.76	3.82	2.50	5.72	3.24	4.47	3.43
10.99	1168	trans-3-Pinanone	–	0.75	22.90	38.70	1.51	19.35	32.94	1.09	0.78
11.06	1170	Pinocarvone	18.72	1.26	–	–	0.36	–	4.17	0.67	0.76
11.45	1179	endo-Borneol	0.11	0.18	0.07	–	0.18	–	0.06	–	–
11.66	1184	3-Pinanone, cis	56.07	63.47	40.81	16.78	58.49	25.17	30.17	45.94	47.56
12.43	1202	(–)-Myrtenol	2.70	0.59	1.24	1.03	3.81	1.80	2.88	3.66	0.84
19.99	1387	(–)-β-Bourbonene	0.69	1.02	0.71	0.87	1.02	1.16	0.84	1.74	0.52
20.93	1410	α-Gurjunene	0.21	0.18	0.20	0.33	0.11	0.23	0.08	0.24	–
21.51	1425	Caryophyllene	1.88	2.02	0.64	2.16	1.58	1.41	0.55	1.58	1.12
22.02	1438	β-copaene	0.04	–	–	0.07	–	–	–	–	–
22.56	1452	γ-Muurolene	–	–	–	–	–	–	–	–	–
23.09	1465	Humulene	1.08	1.18	0.34	0.90	0.85	1.01	0.21	0.85	–
23.24	1469	Alloaromadendrene	0.63	0.50	0.59	0.81	0.33	0.63	0.29	0.68	0.72
24.20	1491	Germacrene D	4.22	2.30	2.63	3.44	3.62	3.22	1.91	4.85	2.95
24.79	1506	Elixene	4.25	2.65	2.42	4.59	2.76	3.26	1.96	3.10	2.25
25.65	1529	γ-Cadinene	0.21	0.20	–	0.14	0.25	–	–	–	–
25.97	1538	trans-calamenene	–	–	–	–	–	–	–	0.29	–
27.44	1576	Elemol	–	–	–	–	–	–	–	–	2.18
30.77	1665	ç-Eudesmol	–	–	–	–	–	–	–	–	1.02
32.42	1710	Guaia-1(10),11-diene	–	–	–	–	–	–	–	–	0.76
Monoterpens			86.77	89.71	92.46	86.63	89.5	89.07	94.18	86.66	88.49
Sesquiterpens			13.21	10.05	7.53	13.31	10.52	10.92	5.84	13.33	11.52
Total oxygenated compounds			83.04	73.36	69.94	60.33	66.85	52.94	73.72	56.07	57.15
Oxygenated monoterpens			83.04	73.36	69.94	60.33	66.85	52.94	73.72	56.07	53.95
Oxygenated sesquiterpens			–	–	–	–	–	–	–	–	3.2
Total nonoxygenated compounds			16.94	26.4	30.05	39.61	33.17	47.05	26.3	43.92	42.86
Nonoxygenated monoterpenes			3.73	16.35	22.52	26.3	22.65	36.13	20.46	30.59	34.54
Nonoxygenated sesquiterpens			13.21	10.05	7.53	13.31	10.52	10.92	5.84	13.33	8.32
Total identified			99.98	99.76	99.99	99.94	100.0	99.99	100.0	99.99	100.0

treatments during 2019–2020 season. The major compound was identified as 3-pinanone, cis in most treatments, except plants irrigated twice and three times per week and sprayed with tap water, where the main component was trans-3-pinanone (38.70 and 32.94 %, respectively). 3-pinanone, cis relative percent ranged from 40.81 to 63.47 % in plants irrigated once per week and from 16.78 to 58.49 % in plants irrigated at twice per week, then from 30.17 to 47.56 % in plants irrigated three times per week. Generally, 3-pinanone, cis decreased with increasing number of irrigation times per week. Foliar application of 100 ppm salicylic acid almost gave the maximum percent of 3-pinanone, cis in all irrigation treatments, followed by control and then 200 ppm salicylic acid under irrigation once per week.

However, 200 ppm salicylic acid and then tap water gave better results in plants irrigated twice per week. There was almost no difference between 3-pinanone, cis values in plants sprayed with 100 and 200 ppm and irrigated three times per week. The second major compound was either (1S)-(–)-β-pinene or trans-3-pinanone in the different treatments, except plants irrigated once per week and sprayed with tap water, where the second major compound was pinocarvone.

The maximum percentage of 3-pinanone, cis (63.47 %) resulted from plants irrigated once per week and sprayed with 100 ppm salicylic acid, whereas the interaction of irrigation twice per week and 0 ppm salicylic acid foliar application led to the lowest value of 3-pinanone, cis (16.78%).

It is clear that oxygenated compounds increased under water deficit (once per week) and decreased with increasing salicylic acid concentration, which may be because this behavior is one of the plant defense mechanisms to protect itself from the harmful effects of stress by producing more oxygenated compounds as a tool against oxidative stress.

The results of GC-MS analyses and identification of the essential oil of hyssop plant are similar to those obtained by other studies [1,4,89,90].

Conclusion

It may be concluded that hyssop plants that were irrigated twice per week and sprayed with 100 ppm salicylic acid produced the highest yield of both herb and essential oil. Therefore, this leads us to recommend irrigating *H. officinalis* twice per week combined with foliar-spray application of salicylic acid at 100 ppm for the highest yield in Egypt under the conditions of used soil.

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Conflicts of interest

There are no conflicts of interest.

References

1 Fathiazad F, Mazandarani M, Hamedeyazdan SS. Phytochemical analysis and antioxidant activity of Hyssopus officinalis L. from Iran. Adv Pharm Bull 2011; 1:63–67.

2 Lawless J. The Encyclopedia of Essential Oils. London, UK: Thorsons 2002;110–111

3 Zajac M, Duda I, Skoczylas L, Tabaszewska M. Potential use of Hyssopus officinalis and Borago officinalis as curing ingredients in pork meat formulations. Animals 2020; 10:1–17.

4 Kizil S, Toncer O, Ipek A, Arslan N, Saglam S, Khawar KM. Blooming stages of Turkish hyssop (*Hyssopus officinalis* L.) affect essential oil composition. Acta Agric Scand B Soil Plant Sci 2008; 58:273–279.

5 Grzeszczuk M, Jadczak D. The estimation of biological value of some species of spice herbs. Acta Horticult 2009; 830:681–686.

6 MTahir M, Khushtar M, Fahad M, Rahman MdA. Phytochemistry and pharmacological profile of traditionally used medicinal plant Hyssop (Hyssopus officinalis L.). J Appl Pharm Sci 2018; 8:132–140.

7 Benelli G, Pavela R, Canale A, Cianfaglione K, Ciaschetti G, Conti F, *et al.* Acute larvicidal toxicity of five essential oils (Pinus nigra, Hyssopus officinalis, Satureja montana, Aloysia citrodora and Pelargonium graveolens) against the filariasis vector Culex quinquefasciatus: synergistic and antagonistic effects. Parasitol Int 2017; 66:166–171.

8 Ortiz de Elguea-Culebras G, Sánchez-Vioque R, Berruga MI, Herraiz-Peñalver D, González-Coloma A, Andrés MF, *et al.* Biocidal potential and chemical composition of industrial essential oils from Hyssopus officinalis, Lavandula x intermedia var. superand Santolina chamaecyparissus. Chem Biodivers 2018; 15:e1700313.

9 Fernández-López J, Sevilla L, Sayas-Barberá E, Navarro C, Marín F, Pérez-Alvarez JA. Evaluation of the antioxidant potential of Hyssop (*Hyssopus officinalis* L.) and Rosemary (*Rosmarinus officinalis* L.) extracts in cooked pork meat. J Food Sci 2003; 68:660–664.

10 Paun G, Litescu SC, Neagu E, Tache A, Lucian-Radu G. Evaluation of *Geranium* spp., *Helleborus* spp. and *Hyssopus* spp. polyphenolic extracts inhibitory activity against urease and α-chymotrypsin. J Enzyme Inhib Med Chem 2014; 29:28–34.

11 Venditti A, Bianco A, Frezza C, Conti F, Bini LM, Giuliani C, *et al.* Essential oil composition, polar compounds, glandular trichomes and biological activity of *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman from central Italy. Ind Crop Prod 2015; 77:353–363.

12 Jop B, Krajewska A, Wawrzyǹczak K, Polaszek K, Synowiec A. Phytotoxic effect of essential oil from hyssop (*Hyssopus officinalis* L.) against spring wheat and white mustard. Biol Life Sci Forum 2021; 3:1–5.

13 Herman S, Marco G, Cecilia B, Alfonso V, Luis M, Cristián V, *et al.* Effect of water availability on growth, water use efficiency and omega3 (ALA) content in two phenotypes of chia (*Salvia hispanica* L.) established in the arid Mediterranean zone of Chile. Agric Water Manag 2016; 173:67–75.

14 Capra A, Consoli S, Russo A, Scicolone B. Integrated agro-economic approach to deficit Irrigation on lettuce crops in Sicily (Italy). J Irrig Drain Eng 2008; 134:437–445.

15 El-Sherbeny MR, Da Silva JAT. Foliar treatment with proline and tyrosine affect the growth and yield of beetroot and some pigments in beetroot leaves. J Hortic Res 2013; 21:95–99.

16 Pirbalouti AG, Samani MR, Hashemi M, Zeinali H. Salicylic acid affects growth, essential oil and chemical compositions of thyme (*Thymus daenensis* Celak.) under reduced irrigation. Plant Growth Regul 2014; 72:289–301.

17 Glass ADM, Dunlop J. Influence of phenolic acids on ion uptake,IV/ depolarization of membrane potentials. Plant Physiol 1974; 54:855–858.

18 Srivastava MK, Dwivedi UN. Delayed ripening of banana fruit by salicylic acid, Plant Sci 2000; 158:87–96.

19 Khan W, Prithiviraj B, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. J Plant Physiol 2003; 160:485–492.

20 Stevens J, Senaratna T, Sivasithamparam K. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* ncv.Roma): associated changes in gas exchange, water relations and membrane stabilization. Plant Growth Regul 2006; 49:77–83.

21 Arfan M, Athar HR, Ashraf M. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress?. J Plant Physiol 2007; 164:685–694.

22 Jackson ML. Soil Chemical Analysis. Englewood Cliffs, NJ: Prentice-Hall Inc; 1973. 498.

23 Moran R. Formulae for determination of chlorophyllous pigments extracted with n,n-dimethylformamide. Plant Physiol 1982; 69:1376–1381.

24 Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies, Plant Soil 1973; 39:205–207.

25 Said-Al Ahl HAH, El Gendy AG, Omer EA. Effect of ascorbic acid, salicylic acid on coriander productivity and essential oil cultivated in two different locations. Adv Environ Biol 2014; 8:2236–2250.

26 Hassanein HD, El-Gendy AE-NG, Saleh IA, Hendawy SF, Elmissiry MM, Omer EA. Profiling of essential oil chemical composition of some Lamiaceae species extracted using conventional and microwave-assisted hydrodistillation extraction methods via chemometrics tools. Flavour Fragr J 2020; 35:329–340.

27 Singleton VL, Orthofer R, Lamuela-Raventos M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299:152–178.

28 Tekao T, Watanabe N, Yagi I, Sakata K. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 1994; 58:1780–1783.

29 Kumarasamy Y, Byres M, Cox PJ, Jasapars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother Res 2007; 21:615–621.

30 Abd-ElGawad A, El Gendy AN, Assaeed A, Al-Rowaily S, Omer EA, Dar BA, *et al.* Essential oil enriched with oxygenated constituents from invasive plant *Argemone ochroleuca* exhibited potent phytotoxic effects. Plants 2020; 9:1–13.

31 Omer EA, Aziz EE, Fouad R, Fouad H. Qualitative and quantitative properties of essential oil of *Mentha Pulegium* L. and *Mentha Suaveolens* Ehrh. affected by harvest date. Egypt J Chem 2022; 65:709–714.

32 Snedecor GW, Cochran WG. Statistical Methods. Ames, IA: Iowa State University Press 1967. 593.

33 Ismail RF, Kandeel AM, Ibrahim AK, Omer EA. Effect of planting date and plant spacing on growth, yield and essential oil of Mexican marigold (*Tagetes lucida* L.) cultivated in Egypt. J Appl Sci Res 2013; 9:330–340.

34 Ahmed ShAHH. Response of ajwain plant to some treatments of water stress [MSc Thesis]. Faculty of Agriculture, Cairo University, Egypt, 2014; 138p.

35 Khanam R, Patra SK. Effect of gravity drip irrigation on growth, yield and water use efficiency of gladiolus in lower gangetic plain of West Bengal, India. Int J Ecosyst 2015; 5:132–137.

36 Fouad R, Omer EA, Kandeel AM, Ibrahim AK, Hendawy SF. Effect of some irrigation levels and foliar-spray application with some chemical substances on growth and yield of *Salvia hispanica* in Egypt.Egyptian Universities Libraries Consortium, Arab Universities. J Agric Sci 2018; 26:971–984.

37 Zeid FA, Omer EA, Amin AY, Hanafy ShAH. Effect of putrescine and salicylic acid on ajwain plant (Trachyspermum ammi) at vegetative stage grown under drought stress. Int J Agric Sci Res 2014; 4:61–80.

38 Abd Allah MMS, El-Bassiouny HMS, Elewa TAE, El-Sebai TN. Effect of salicylic acid and benzoic acid on growth, yield and some biochemical aspects of quinoa plant grown in sandy soil. Int J Chemtech Res 2015; 8:216–225.

39 Ullah F, Bano A, Nosheen A. Effects of plant growth regulators on growth and oil quality of canola (*Brassica napus* L.) under drought stress. Pak J Bot 2012; 44:1873–1880.

40 Khatiby A, Vazin F, Hassanzadeh M, Shadmehri AAH. Effect of foliar application with salicylic acid on some morphological and physiological characteristics of sesame (*Sesamum* indicum L.) under drought stress. Cercet Agron Mold 2016; 4:35–42.

41 Singh M, Ganesha Rao RS, Ramesh S. Irrigation and nitrogen requirement of lemongrass (*Cymbopogon flexuosus* (Sleud) Wats) on a red sandy loam soil under semiarid tropical conditions. J Essential oil Res 1997 9:569–574.

42 Gaafer SA, Refai KM. Modeling water effects on growth and yield of melon (*Cucumis melo* cv. Reticulotus). Egypt J Appl Sci 2006; 21:682–693.

43 Rashidi M, Seyfi K. Effect of water stress on crop yield and yield components of cantaloupe. Int J Agric Biol 2007; 9:271–273.

44 Simsek M, Comlekcioglu N. Effects of different irrigation regimes and nitrogen levels on yield and quality of melon (*Cucumis melo* L.). Afr J Biol 2011; 10:10009–10018.

45 Hammad SAA. Physiological response of snap bean plant to water supply [MSc thesis]. Department of Agricultural Botany, Faculty of Agriculture, Minufiya University. 1991; 150p.

46 Amri A, El Ouni MH, Salem MB. Waterlogging affect the development, yield and components, chlorophyll content and chlorophyll fluorescence of six bread wheat genotypes (*Triticum aestivum* L.). Bulg J Agric Sci 2014; 20:647–657.

47 Akhtar I, Nazir N. Effect of waterlogging and drought stress in plants. *Int J Water Resour* Environ Sci 2013; 2:34–40.

48 Vasellati V, Oesterheld M, Medan D, Loreti J. Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. Ann Bot 2001; 88:355–360.

49 González JA, Gallardo M, Hilal M, Rosa M, Prado FE. Physiological responses of quinoa (Chenopodium quinoa Willd.) to drought and waterlogging stresses: dry matter partitioning. Bot Stud 2009; 50:35–42.

50 Öpik H, Rolfe SA. The Physiology of Flowering Plants. Cambridge: Cambridge University Press; 2005. 191.

51 Gharib FAE. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. Int J Agric Biol 2006; 8:485–492.

52 Bakry BA, El-Hariri DM, Sadak MS, El-Bassiouny HMS. Drought stress mtigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil. J Appl Sci Res 2012; 8:3503–3514.

53 Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhudinova RA, Fatkhutdinova DR. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci 2003; 164:317–322.

54 Nanjo T, Fujita M, Seki M, Kato M, Tabata S, Shinozaki K. Toxicity of free proline revealed in an arabidopsis T-DNA tagged mutant deficient in proline dehydrogenase. Plant Cell Physiol 2003; 44:541–548.

55 Pascale SD, Paradiso R, Barbieri G. Recovery of physiological parameters in gladiolus under water stress. Colture Protette 2001; 30:65–69.

56 Elewa TA, Sadak MSH, Saad AM. Proline treatment improreatment improves physiological responses in quinoa plants under drought stress. Biosci Res 2017; 14:21–33.

57 Pastenes C, Pimentel P, Lillo J. Leaf movements and photoinhibition in relation to water stress in field-grown beans. J Exp Bot 2005; 56:425–433.

58 Lisar SYS, Motafakkerazad R, Hossain MM, Rahman IMM. Water stress in plants: causes, effects and responses. In: Rahman IMM, Hasegawa H (editors). Water Stress. Rijeka, Croatia: InTech; 2012; 1:1–14.

59 Anjum SA, Xie X, Farooq M, Wang L, Xue L, Shahbaz M, *et al.* Effect of exogenous methyl jasmonate on growth, gas exchange and chlorophyll contents of soybean subjected to drought. Afr J Biotechnol 2011; 10:9640–9646.

60 Pandey HC, Baig MJ, Bhatt RK. Effect of moisture stress on chlorophyll accumulation and nitrate reductase activity at vegetative and flowering stage in Avena species. Agric Sci Res J 2012; 2:111–118.

61 Kranner I, Beckett RP, Wornik S, Zorn M, Pfeifhofer HW. Revival of a resurrection plant correlates with its antioxidant status. Plant J 2002; 31:13–24.

62 Aono M, Kubo A, Saji H, Tanaka K, Kondo N. Enhanced tolerance to photooxidative stress of transgenic (Nicoliana lahaci) with high chloroplastic glutathione reductase activity. Plant Cell Physiol 1993 34:129–135.

63 Szepesi A, Ciszar J, Bajkan S, Gemes K, Horvath F, Erdei L, *et al.* Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt and osmotic stress. Acta Biol Szeged 2005; 49:123–125.

64 Fariduddin Q, Hayat S, Ahmad A. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. Photosynthetica 2003; 41:281–284.

65 Ahmed RFI. Effect of some agricultural practices on growth, yield and active constituents of mexican marigold (*Tagetes lucida*) [MSc Thesis]. Faculty of Agriculture, Ain Shams University. 2013; 171p.

66 Mustafavi SH, Shekari F, Maleki HH. Influence of exogenous polyamines on antioxidant defence and essential oil production in valerian (Valeriana officinalis L.) plants under drought stress. Acta Agric Slov 2016; 107:81–91.

67 Ahmed RFI. Effect of some agricultural practices on growth, yield and active constituents of chia (Salvia hispanica) [PhD Thesis]. Faculty of Agriculture, Ain Shams University. 2018; 233p.

68 Harinasut P, Srisunak S, Pitukchaisopol S, Charoensataporn R. Mechanisms of adaptations to increasing salinity of mulberry: proline content and ascorbate peroxidase activity in leaves of multiple shoots, Sci Asia 2000; 26:207–211.

69 Jagesh K, Tiwari AD, Munshi RK, Raghu N, Pandey AJS, Bhat AKS. Effect of salt stress on cucumber: Na+/ K+ ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiol Plant 2010; 32:103–114.

70 Khandaker L, Akond AM, Oba S. Foliar application of salicylic acid improved the growth, yield and leaf's bioactive compounds in red amaranth (Amaranthus tricolor L.). Veg Crop Res Bull 2011; 74:77–86.

71 Abd Elhamid EM, Sadak MSH, Tawfik MM. Physiological response of fenugreek plant to the application of proline under different water regimes. Res J Pharm Biol Chem Sci 2016; 7:580–594.

72 English-Loeb G, Stout MJ, Duffey SS. Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. Oikos 1997; 79:456–468.

73 Lewis NG, Yamamoto E. Lignin: occurrence, biosynthesis and biodegradation. Ann Rev Plant Physiol 1990; 41:455–561.

74 Khattab H. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. Aust J Basic Appl Sci 2007; 1:323–334.

75 Gaballah MS, Ouda SA, Mandour MS, Rady MM. Predicting the role of antioxidants and irrigation sunflower grown under saline condition. Int J Nat Eng Sci 2007; 1:5–10.

76 Raskin I. Role of salicylic acid in plants. Ann Rev Plant Physiol Plant Mol Biol 1992; 43:439–463.

77 Abootalebian M, Keramat J, Kadivar M, Ahmadi F, Abdinian M. Comparison of total phenolic and antioxidant activity of different *Mentha spicata* and *M. longifolia* accessions. Ann Agric Sci 2016; 61:175–179.

78 Deng WG, Fang XW, Wu JL. Flavonoids function as antioxidants: by scavenging reactive oxygen species or by chelating iron. Radiat Phys Chem 1997; 50:271–276.

79 Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Ann Rev Plant Physiol Plant Mol Biol 1998; 49:249–279.

80 Asada K. The water-water cycle in chloroplasts, scavenging of active oxygens and dissipation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 1999; 50:601–639.

81 Yang DM, Wang QS, Ke LQ, Jiang JM, Ying TJ. Antioxidant activities of various extracts of lotus (Nelumbo nuficera Gaertn) rhizome. Asia Pac J Clin Nutr 2007; 16:158–163.

82 Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. Environ Exp Bot 2010; 68:14–25.

83 Hussain M, Malik MA, Farooq M, Khan MB, Akram M, Saleem MF. Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. J Agron Crop Sci 2009; 195:98–109.

84 Ahmed F, Baloch DM, Hassan MJ, Ahmed N. Role of plant growth regulators in improving oil quantity and quality of sunflower hybrids in drought stress. Biologia (Pakistan) 2013; 59:315–322.

85 Chanirar NJ, Malavia DD, Baldha NM. Effect of irrigation, nitrogen and phosphorus on the productivity of sunflower. Indian J Agron 1989; 34: 399–401.

86 Bukhsh MAHA, Malik AU, Ishaque M, Sadiq SH. Performance of sunflower in response to exogenously applied salicylic acid under varying irrigation regimes. J Animal Plant Sci 2009; 19:130–134.

87 Khalil SE, El-Noemani AA. Effect of irrigation intervals and exogenous proline application in improving tolerance of garden cress plant (*Lepidium sativum* L.) to water stress. J Appl Sci Res 2012; 8:157–167.

88 El-Mekawy MAM. Effect of irrigation intervals and biofertilization on growth and oil yield of *Thymus cspitatus* L under EL-Arish conditions. Agric Res Kafrelsheikh Univ 2009; 35:1050–1068.

89 Hold KM, Sirisoma NS, Sparks SE, Casida JE. Metabolism and mode of action of cis- and trans-3-pinanones (the active ingredients of hyssop oil). Xenobiotica 2002; 32:251–265.

90 De Martino L, De Feo V, Nazzaro F. Chemical composition and *in vitro* antimicrobial and mutagenic activities of seven Lamiaceae essential oils. Molecules 2009; 14:4213–4230.