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RESEARCH ARTICLE

Exogenous salicylic acid improves photosynthetic pigments and morphological traits of four medicinal plants in an aeroponic system

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ABSTRACT: Salicylic acid (SA) is a phenolic phytohormone that acts as a key regulator of the signaling network in plants under biotic and abiotic stresses. SA exerts stimulatory effects on various physiological processes to plant growth and development. In this research, valerian, chicory, withania, and purple coneflower plants were transferred into an aeroponic system where the effects of SA were investigated on some plant characteristics under greenhouse conditions. The plants were foliar sprayed with SA (0, 50, 100, and 150 mg l^{-1}) at 20, 40, and 60 days after transplanting (DAT). Results showed that the highest chlorophyll a+b, carotenoid, plant height, root length, root volume, number of leaves per plant, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight were obtained from 150 mg l^{-1} SA in all the studied medicinal plants. The results revealed that the exogenous application of SA in the aeroponic system increased the root fresh weight, root dry weight, shoot fresh weight and shoot dry weight of chicory, withania, valerian, and purple coneflower plants. Also, the studied medicinal herbs grew well in the aeroponic system.

KEYWORDS: Chicory, Purple coneflower, Soilless culture, Valerian, Withania

INTRODUCTION

A wide range of prokaryotic and eukaryotic organisms, including plants, produces salicylic acid (SA). Studies over the last two decades have shown that SA has important regulatory functions in plants [12]. SA and other salicylates are known to affect various physiological, biochemical and molecular processes in plants [21] including thermogenesis [42], seed germination [39], seedling establishment [4], cell growth [50], respiration [36], stomatal responses [31, 25], senescence [40, 41], thermotolerance [14] and nodulation [48]. Also, the effect of SA as an endogenous regulator of flowering has been demonstrated in several plant species from different families [22]. In addition to regulating the flowering time, SA also links defense responses and reproductive development [32]. Exogenous application of SA enhanced shoot, root, and total plant dry weight under no salt stress in *C. officinalis*. It provided early flowering and a higher number of floral buds per plant [8].

In addition to investigating the regulator effects on plant growth and development, their effects on the accumulation of secondary metabolites have also been studied in medicinal plants. Specifically, regarding the exogenous application of SA, it may also induce many defense genes that encode particular enzymes of a secondary metabolic pathway to form bioactive compounds such as phenolics [3].

In growth-controlled environments, the aeroponic culture technique may also be used instead of the soil-less culture method. The underground organs are placed in a dark chamber, and a solution of mineral nutrients is added to it with a misting device. Aeroponic systems optimize root aeration, which significantly enhances yield compared to

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classical hydroponics [47]. Recirculation of nutrient solution, limited usage of water, and good monitoring of nutrients and pH are among the other benefits. This technique has been applied to produce different horticultural and ornamental species [10, 34]. Using controlled environments for medicinal herb and root plants can improve the quality, purity, consistency, bioactivity, and biomass production of the raw material.

Valeriana officinalis L., commonly called valerian, is a perennial herb that belongs to the family valerianaceae [52]. Valerians contain over 150 chemical constituents, many of which are physiologically active [38]. The roots and rhizomes of *V. officinalis* are highly varied in the composition of these constituents, such as valerenic acids, valepotriates, baldrinal and homobaldrinal, alkaloids, amino acids such as arginine, glutamine, tyrosine, phenolic acids, and flavonoids [17]. Valerians have sedative, anxiolytic, antidepressant, antihypertensive, antioxidant, and antispasmodic applications in traditional medicine. The pharmacological effects of valerians have primarily been attributed to valepotriates (iridoid esters), ballerinas, volatile oils, monoterpenes, and sesquiterpenes constituents [52].

Withania coagulans Dunal is well known for its ethnopharmacological activities [24]. *W. coagulans*, which is commonly found in Iran, Pakistan, Afghanistan, and East India, is used in folk medicine. The fruits of the plant have a milk-coagulating characteristic [6]. The fruits, leaves, and roots of *W. coagulans* are used as a treatment for many disorders. It is a rich source of esterases, free amino acids, fatty oils, essential oils, and withanolides. Withanolides are steroidal lactones, several of which possess significant pharmacological activities [33].

Echinacea purpureais one of the most important medicinal plants used in the pharmaceutical industry of developed countries and is a traditional North American perennial medicinal herb that has gained international popularity because of its nutraceutical and medicinal properties [26]. Cichoric acid (a major compound in *E. purpurea*) has shown phagocytic and antihyaluronidase activity [9], an inhibitory effect on HIV-1 integrase and replication [28], and immunostimulatory properties [13].

Chicory (*Cichorium intybus* L.) is a member of the family Asteraceae. The species originates from the Mediterranean region [51]. Historically, chicories were popularized in many countries [44, 7, 49]. Chicory is an important medicinal plant that accumulates specific organic compounds, such as storage polysaccharide inulin, sesquiterpene lactones, coumarins, phenolic acids, and flavonoids [7, 45].

The present study aimed to examine the effects of foliar application of salicylic acid (SA) on valerian's growth and quantitative and qualitative characteristics, withania, purple coneflower, and chicory in an aeroponic system.

MATERIALS AND METHODS

The plants of chicory, valerian, withania, and purple coneflower were obtained from seed culture in pots for one month in a greenhouse. Then, the uniform plants (approximately 20 cm height) were transferred into an aeroponic system in a controlled greenhouse.

Aeroponic system

An aeroponic system (phytorhizotron) was used for the present study [35]. The phytorhizotron consisted of two compartments: the upper compartment was supplied with photoperiod control, and the lower compartment was kept in darkness. The plants were cultured on the upper compartment's board with a spacing of 13 13 cm, and about one-third of the stems' length was placed inside the lower compartment. The shoots grew in the upper compartment, and the roots were developed in the lower compartment in darkness. The lower compartment was a closed container (100 100 120 cm), which had a removable front panel for monitoring and harvesting. The plant roots were periodically sprayed (every 20 min. for 20 sec.) with the nutrient solution using twelve fog nozzles per m^2. The nutrient solution was renewed weekly. The remaining nutrient solution flowed back into a collecting tank and was recirculated.

Nutrient solution and growing conditions

The components of the applied nutrient solution are shown in Table 1. The solution's electrical conductivity (EC) was adjusted at 1.6 ± 0.2 dS m^{-1}. The initial pH was adjusted at 5.8 ± 0.2, but then, it was not controlled. The plants grew in a greenhouse under a 16-h photoperiod.

Table1. Concentrations of nutrients used in the aeroponic system (mg l^{-1})

Elements	Concentration (mg l^{-1})	Elements	Concentration (mg l^{-1})
K	200	Fe	1
N	190	Mn	0.5
Ca	150	B	0.5
S	70	Zn	0.15
Mg	45	Cu	0.1
P	35	Mo	0.05

Table 2. Means comparison for the effect of different SA concentrations on morpho-physiological traits of valerian in aeroponic system

Treatment	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)
Control (0)	18.5 ^{d}	91.5 ^{d}	10.2 ^{d}	64.2 ^{d}	90.4 ^{d}
50 mg l^{-1}	20.3 ^{c}	105.4 ^{c}	12.2 ^{c}	70.1 ^{c}	112.7 ^{c}
100 mg l^{-1}	24.2 ^{b}	127.2 ^{b}	15.4 ^{b}	82.5 ^{b}	123.6 ^{b}
150 mg l^{-1}	28.3 ^{a}	138.5 ^{a}	18.5 ^{a}	91.3 ^{a}	130.6 ^{a}

Treatment	Root length (cm)	Leaf number	Root volume (cm^3 $plant^{-1}$)	Carotenoid (mg g^{-1})	Chlorophyll a + b (mg g^{-1})
Control (0)	41.3 ^{d}	22.3 ^{c}	18.2 ^{d}	1.39 ^{c}	2.68 ^{c}
50 mg l^{-1}	45.6 ^{cd}	24.7 ^{c}	21.5 ^{c}	1.45 ^{c}	3.21 ^{b}
100 mg l^{-1}	52.3 ^{b}	38.4 ^{b}	27.2 ^{b}	1.82 ^{b}	3.49 ^{b}
150 mg l^{-1}	67.5 ^{a}	45.7 ^{a}	38.4 ^{a}	2.01 ^{a}	4.13 ^{a}

Treatments

The effect of foliar application of salicylic acid (0, 50, 100, and 150 mg l^{-1}) was assessed on the growth and development of chicories, valerians, withania, and purple coneflowers. The control plants were treated with distilled water. In all treatments, Tween 20 was applied at 0.05 ml l^{-1} as a wetting agent. The plants were foliar sprayed with SA 20, 40, and 60 days after transplanting (DAT) in the aeroponic system. The experiment was carried out according to a completely randomized design (CRD) layout with five replications. Each replication consisted of three plants.

The plants were harvested six months after transplanting. The plant height, root length, number of leaves, volume of root, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, chlorophyll a, chlorophyll b, and carotenoids were recorded for the plants. The photosynthetic pigments of the leaves were determined by Lichtenthaler [27].

Data Analysis

Primary statistical analyses such as normality test (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test) were conducted. After analyzing variance (ANOVA), the means of treatment combinations were compared using Duncan's Multi Range Test (DMRT). All the above statistical analyses were carried out using SPSS version 21.

RESULTS

The effect of SA on photosynthetic pigments and some growth characteristics of valerians in the aeroponic system.

According to the analysis of variance, the effect of SA was highly significant ($P < 0.01$) on all the recorded traits. Means comparison (Table 2) indicated that 150 mg l^{-1} SA resulted in the highest plant height (130.6 cm), root length (67.5 cm), number of leaves per plant (45.7), root volume per plant (38.4 cm^3 $plant^{-1}$), root fresh weight (91.3 g $plant^{-1}$), root dry weight (18.5 g $plant^{-1}$), shoot fresh weight (138.5 g $plant^{-1}$), shoot dry weight (28.3 g $plant^{-1}$), chlorophyll a + b (4.13 mg g^{-1}) and carotenoid (2.01 mg g^{-1}). The effect of SA on photosynthetic pigments and some growth characteristics of chicories in the aeroponic system.

According to the analysis of variance, the effect of SA was highly significant ($P < 0.01$) on all the traits. Means comparison (Table 3) revealed that 150 mg l^{-1} SA was related to the highest plant height (213.2 cm), root length (116.1 cm), number of leaves per plant (81.4), root volume per plant (48.7 cm^3 $plant^{-1}$), root fresh weight (292.4 g $plant^{-1}$), root dry weight (43.9 g $plant^{-1}$), shoot fresh weight (395.7 g $plant^{-1}$), shoot dry weight (79.3 g $plant^{-1}$), chlorophyll a + b (4.32 mg g^{-1}) and carotenoid (1.55 mg g^{-1}).

The effect of SA on photosynthetic pigments and some growth characteristics of purple coneflowers in the aeroponic system. The analysis of variance revealed that the effect of SA was highly significant ($P < 0.01$) on all traits. Means comparison (Table 4) indicated that the plants treated with 150 mg l^{-1} SA exhibited the highest plant height (98.2 cm), root length (55.3 cm), number of leaves per plant (29.2), root volume per plant (35.1 cm^3 $plant^{-1}$), root fresh weight (51.8 g $plant^{-1}$), root dry weight (11.24 g $plant^{-1}$), shoot fresh weight (81.2 g $plant^{-1}$), shoot dry weight (16.8 g $plant^{-1}$), chlorophyll a + b (2.3 mg g^{-1}) and carotenoid (1.44 mg g^{-1}).

Table 3. Means comparison for the effect of different SA concentrations on morpho-physiological traits of chicory in aeroponic system

Treatment	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)
Control (0)	48.6 d	243.2 d	29.02 d	193.5 d	110.6 d
50 mg l^{-1}	63.4 c	317.2 c	31.6 c	211.3 c	127.7 c
100 mg l^{-1}	70.1 b	350.5 b	38.6 b	257.7 b	167.4 b
150 mg l^{-1}	79.3 a	395.7 a	43.9 a	292.4 a	213.2 a

Treatment	Root length (cm)	Leaf number	Root volume (cm^3 $plant^{-1}$)	Carotenoid (mg g^{-1})	Chlorophyll a + b (mg g^{-1})
Control (0)	68.37 c	47.1 d	25.4 d	1.14 d	2.95 d
50 mg l^{-1}	93.75 b	56.3 c	27.3 c	1.29 c	3.14 c
100 mg l^{-1}	109.4 b	60.7 b	30.1 b	1.37 b	3.43 b
150 mg l^{-1}	116.1 a	81.4 a	48.7 a	1.55 a	4.32 a

Table 4. Means comparison for the effect of different SA concentrations on morpho-physiological traits of purple coneflower in aeroponic system

Treatment	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)
Control (0)	9.2 d	42.3 d	6.18 c	29.3 c	75.2 c
50 mg l^{-1}	12.2 c	60.5 c	8.12 b	38.3 b	78.3 b
100 mg l^{-1}	15.3 b	72.4 b	9.53 b	44.3 b	82.4 b
150 mg l^{-1}	16.8 a	81.2 a	11.24 a	51.8 a	98.2 a

Treatment	Root length (cm)	Leaf number	Root volume (cm^3 $plant^{-1}$)	Carotenoid (mg g^{-1})	Chlorophyll a+ b (mg g^{-1})
Control (0)	34.5 c	18.2 d	21.2 d	1.08 d	1.46 d
50 mg l^{-1}	48.2 b	22.3 c	25.12 c	1.17 c	1.62 c
100 mg l^{-1}	50.8 b	25.7 b	29.7 b	1.31 b	1.86 b
150 mg l^{-1}	55.3 a	29.2 a	35.1 a	1.44 a	2.3 a

The effect of SA on photosynthetic pigments and some growth characteristics of withania in the aeroponic system.

According to the analysis of variance, the effect of SA was highly significant ($P < 0.01$) on all the studied traits. Means comparison (Table 5) showed that the plants treated with 150 mg l^{-1} SA had the highest plant height (92.3 cm), root length (54.3 cm), number of leaves per plant (37.4), root volume per plant (9.54 cm^3 $plant^{-1}$), root fresh weight (17.8 g $plant^{-1}$), root dry weight (5.01 g $plant^{-1}$), shoot fresh weight (35.5 g $plant^{-1}$), shoot dry weight (8.93 g $plant^{-1}$), chlorophyll a + b (2.64 mg g^{-1}) and carotenoid (1.83 mg g^{-1}).

DISCUSSION

The study results showed an overall increase in morphological traits and photosynthetic pigments with the foliar application of SA in all the studied medicinal plants. As reported in other studies, plant height was one of the traits showing an increase in response to the foliar application. In a study, the plant height of garlic was increased in the SA-treated plants, which agrees with our experiment [11]. The foliar application of SA probably increases growth through carbon fixation, metabolite synthesis, and the conservation of water status in plant tissues [20].

The foliar application of SA increased other growth characteristics of medicinal plants under study, such as fresh and dry weights of the roots and shoots. Manaa *et al.* (2014) indicated that the higher concentration of SA resulted in better vegetative growth, including the fresh and dry weights of the plants [29]. In addition, the results of this study agreed with a study on eggplant by Mandal [30]. It has been reported that the application of SA increases the dry matter produced by the cucumbers [37]. Moreover, a study on basil and marjoram showed that the

Table 5. Means comparison for the effect of different SA concentrations on morpho-physiological traits of withania in aeroponic system

Treatment	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)
Control (0)	4.6 ^{c}	21.3 ^{d}	2.86 ^{c}	9.13 ^{c}	45.3 ^{d}
50 mg l^{-1}	4.93 ^{c}	25.4 ^{c}	3.24 ^{b}	12.9 ^{b}	55.2 ^{c}
100 mg l^{-1}	6.3 ^{b}	29.2 ^{b}	3.86 ^{b}	14.2 ^{b}	73.5 ^{b}
150 mg l^{-1}	8.93 ^{a}	35.5 ^{a}	5.01 ^{a}	17.8 ^{a}	92.3 ^{a}

Treatment	Root length (cm)	Leaf number	Root volume (cm^3 $plant^{-1}$)	Carotenoid (mg g^{-1})	Chlorophyll a + b (mg g^{-1})
Control (0)	21.5 ^{d}	21.2 ^{d}	6.12 ^{b}	1.11 ^{c}	1.55 ^{d}
50 mg l^{-1}	27.8 ^{c}	28.5 ^{c}	6.59 ^{b}	1.32 ^{b}	1.85 ^{c}
100 mg l^{-1}	37.2 ^{b}	33.1 ^{b}	7.21 ^{b}	1.49 ^{b}	2.2 ^{b}
150 mg l^{-1}	54.3 ^{a}	37.4 ^{a}	9.54 ^{a}	1.83 ^{a}	2.64 ^{a}

SA application enhanced these plants' fresh and dry weights [1].

The foliar application of SA increased the number of leaves in plants grown in the aeroponic systems, an observation consistent with other studies such as Eraslan et al. [18] and Du et al. [16]. Angooti and Nourafcan [5] experimented on basil (*Ocimum basilicum* L.) and reported a significant increase in the number and dry weight of the leaves due to the foliar application of SA.

The study results showed that the application of SA to the plants produced in the aeroponic system increased the chlorophyll contents of the plants. These results are in agreement with the observations of Abreu and Munn-Bosch [2]. This can be attributed to the fact that SA prevents chlorophyll decomposition by hindering the activities of chlorophyll oxidase enzymes and, hence, increases photosynthesis. The results of the experiment by Zhou et al. [54] on soybean indicated that the application of phenolic compounds, such as SA, increased photosynthesis due to the enhanced activities of photosynthetic enzymes.

Overall, the findings showed that the application of SA can be effective from germination until senescence, contributing to more efficient photosynthesis and final products. Previous studies report the undeniable effect of SA and other salicylate derivatives on many physiological and morphological plant processes. The amounts of these changes are diverse and may be stimulatory in some reactions, accelerating in some other, and inhibitory in others [43]. Previous research has shown that the use of SA in crops enhances yield and yield components [53] due to the effect of SA on the physiological processes of plants.

Salicylic acid is a hormonal substance that plays a key role in regulating different physiological processes, such as growth, plant development, absorption of ions, and enhancement of chlorophyll. The yield enhancement caused by foliar application of SA may be due to its effect on photosynthesis, which enhances the quantitative and qualitative traits of the products [46]. Salicylic acid increases the rate of photosynthesis, CO_2, water use efficiency, and yield [19]. It seems that SA can increase photosynthesis through an increase in the chlorophyll content of leaves at the beginning of the senescence process, thereby enhancing growth and yield [15]. Foliar application of SA improves yield through an increase in cell division and growth [23]. On the other hand, the positive effect of SA on the increase in growth and yield can be due to its effect on plant hormones; by altering the balance between auxin, cytokinin, and abscisic acid, SA increases growth and yield.

The results showed the positive effect of SA on the traits studied. Furthermore, the results revealed that aeroponic systems could be used to study the effect of SA, especially its effect on the roots because all the stages of root growth and the direct effect of the hormone on them can be easily observed in a controlled environment. This study showed that the studied medicinal herbs grew well in the aeroponic system, producing medicinal herbs. Medicinal herb production in aeroponic systems has several advantages. The most important of which are a considerable economy in the water and nutrients, more root growth due to better oxygen availability, higher absorption levels of nutrient solutions, more growth periods caused by faster growth and maturity, the possibility of controlling the temperature around the roots, the absence of weeds and, consequently, optimal plant growth, which reduces herbicide consumption. Higher availability of CO_2 for photosynthesis and the production of healthy roots free from soil-borne pathogens can also be mentioned. Given water scarcity and the need for more efficient water use, it is necessary to use this system for economically important plants, such as potatoes with virus-free microtubers, medicinal herbs, saffron, etc.

CONCLUSION

According to the results of the study, it can be concluded that the application of SA has significant effects on photosynthetic pigments, plant height, root length, number of leaves per plant, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight in all plants including chicory, withania, valerian and purple coneflower plants. Also, it should be noted that this positive effect was linear so that the highest concentration (150 mg l^{-1}) performed the best.

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تاثیر کاربرد خارجی اسید سالیسیلیک بر رنگدانه‌های فتوسنتزی و ویژگی‌های مورفولوژیکی چهار گیاه دارویی در سیستم هواکشت

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چکیده

اسید سالیسیلیک یک فیتوهورمون فنلی است که به عنوان تنظیم کننده اصلی شبکه پیام رسانی در گیاهان تحت تنش‌های زیستی و غیرزیستی عمل می‌کند. اسید سالیسیلیک اثرات تحریک کننده‌ای روی فرایندهای مختلف فیزیولوژیکی در رشد و نمو گیاه دارد. در این مطالعه،گیاهان سنبل الطیب، کاسنی، سرخارگل و پنیرباد به سیستم هواکشت منتقل شدند و سپس اثر اسید سالیسیلیک بر برخی صفات این گیاهان در شرایط گلخانه مورد بررسی قرار گرفت. گیاهان با اسید سالیسیلیک (۰، ۵۰، ۱۰۰ و ۱۵۰ میلی‌گرم در لیتر)، ۲۰، ۴۰ و ۶۰ روز پس از انتقال به سیستم هواکشت، محلول‌پاشی شدند. نتایج نشان داد که بیشترین کلروفیل a+b، کاروتنوئید، طول گیاه، طول ریشه، حجم ریشه، تعداد برگ در گیاه، وزن تر ریشه، وزن خشک ریشه، وزن تر اندام هوایی، وزن خشک اندام هوایی در تیمار با ۱۵۰ میلی‌گرم در لیتر اسید سالیسیلیک در همه گیاهان مورد مطالعه بدست آمد. نتایج دلالت بر این داشت که کاربرد خارجی اسید سالیسیلیک وزن تر و خشک ریشه و اندام هوایی گیاهان سنبل الطیب، کاسنی، سرخارگل و پنیرباد را در سیستم هواکشت افزایش داده است. همچنین این گیاهان دارویی بخوبی در سیستم هواکشت رشد کردند.

کلمات کلیدی: پنیرباد، سرخارگل، سنبل الطیب، کاسنی، کشت بدون خاک