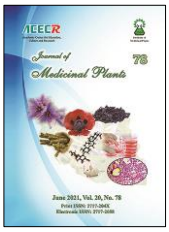
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Research Article

Effect of salicylic acid and yeast extract on caffeic acid derivatives production in *Echinacea purpurea* L.

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ABSTRACT

Background: Purple coneflower, *Echinacea purpurea* (L.) Moench, has long been used in herbal remedies to prevent and treat a wide range of diseases like common cold, simple cough, pulmonary infections, dermatologic disorder and chronic diseases due to immunodeficiency. **Objective**: The present study was conducted to investigate the effects of salicylic acid and yeast extract foliar application on caffeic acid derivatives production in coneflower. **Methods:** Two factorial experiments were performed individually under greenhouse conditions during the years 2017-2018. In the first experiment, the effect of salicylic acid (0, 80, 160 and 320 mg/L) and each at four exposure times (24, 48, 72 and 96 hours) was studied. Also, in the second experiment, the effect of yeast extract (0, 0.75, 1 and 1.5 g/L) and each at four exposure times was investigated. **Results:** The results indicated that main effects and interactions of different concentrations of salicylic acid and yeast extract at different exposure times on caffeic acid derivatives content were significant ($P < 0.01$). The highest cichoric acid, caftaric acid and echinacoside production was obtained after 96 hours of treatment at 160 mg/L salicylic acid treated plants. The highest cichoric acid production 7.150 mg/g DW was obtained after 96 hours of treatment at 1.5 g/L yeast extract treated plants, this was 3.58-fold higher compared to respective control. The lowest level of caffeic acid derivatives production was observed in control plants. **Conclusion:** In conclusion, salicylic acid and yeast extract foliar application have a positive effect on caffeic acid derivatives production in *Echinacea purpurea* aerial parts.

1. Introduction

Purple coneflower, *Echinacea purpurea* (L.) Moench, is a perennial herbaceous medicinal plant of *Asteraceae* family. Echinacea species are currently one of the best-selling herbs in North America and have gained great attention because of their increasing medicinal value [1]. The potential active compounds in coneflower are caffeic acid derivatives (CADs), alkamides, polysaccharides and glycoproteins that show

Abbreviations: CADs, Caffeic Acid Derivatives; DW, Dry Weight; HPLC, High Performance Liquid Chromatography; SE, Standard Error; C.V., Coefficient of Variation

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various medical effects such as antioxidative, antibacterial, antifungal properties and are used for treating common cold, respiratory and urinary diseases [2]. Several caffeic acid derivatives such as cichoric acid, chlorogenic acid, caftaric acid and caffeic acid have been identified in Echinacea species. Cichoric acid is thought to be important based on the results of immunological and pharmacological studies [3]. Cichoric acid has shown phagocytic, anti-hyaluronidase, antiviral activity and inhibited HIV-1 integrase and replication and used as immune stimulant compound [4]. Regular chlorogenic acid consumption can reduce the risks of hypertension, heart failure, atherosclerosis, myocardial infarction, and other factors associated with cardiovascular risk, such as obesity and type 2 diabetes [5]. Secondary metabolites are a group of organic substances that are produced by plants in response to environmental stress and as a part of their defense mechanism. These bioactive compounds show diverse biological activities and are used as pharmaceuticals, agrochemicals, fragrances, colors, flavors, and food additives in daily human life [6]. The quality of medicinal plants used for the production of pharmacologically valuable compounds is usually assessed by the content of biologically active compounds [7]. Secondary metabolites are often produced at low levels (less than 1 % dry weight), and their production depends significantly on the physiological and developmental stage of the plant [8]. Several methods to enhance the production of secondary metabolites are currently being studied. In particular, using biotic or abiotic elicitor could be an appropriate way to increase the production of valuable secondary metabolites in medicinal plants [9], we see that currently it is implemented extensively owing to its low cost and simplicity of usage [10]. Salicylic acid is a hormone-like substance that plays an important role in the regulation of plant growth and development [11]. Exogenous application of salicylic acid is presented as a highly potent, cost effective, eco-friendly and quick strategy to enhance the synthesis and accumulation of secondary metabolites in plants [12]. The effect of exogenous salicylic acid depends on several factors such as the species and developmental stage of the plant, the mode of application, concentration of salicylic acid and its endogenous level in the given plant [13]. In several studies, the effect of salicylic acid on production of many bioactive compounds in medicinal plants was confirmed [7, 14, 15, and 16]. One of the biotic elicitors that can result in the improvement of secondary metabolites content is yeast extract [17, 18]. Like other elicitors, yeast extract concentration is an important factor with significant impact on biosynthesis of valuable metabolites and its optimal level may be different for each plant species [18]. The stimulating influence of yeast extract on secondary metabolites was confirmed in several studies [17-20]. Another factor effecting on the cellular response and deciding on the action of the yeast extract may be time exposure. Few studies have been carried out to investigate the effects of salicylic acid and yeast extract foliar application on the accumulation of secondary metabolites in *E. purpurea*. The effect of various treatments like salicylic acid on field-grown plants of *E. purpurea* has been reported earlier [7]. Therefore, in order to economically produce secondary metabolites, it is necessary to use elicitors optimally in medicinal plants. The present study was conducted to investigate the effects of foliar spraying with salicylic acid and yeast extract on enhancement of valuable secondary metabolites production in the aerial parts of *E. purpurea*.

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2. Materials and Methods

2.1. Experimental field

The present study was conducted during December 2017 to November 2018 at the greenhouse of the Parks and Green Space Organization, Malayer Municipality (latitude: 34° 19´ N, longitude: 48° 51´ E, altitude: 1725 m above the sea level), located in the west of Iran and southeast of Hamadan province.

2.2. Plant materials, experimental set up and treatments

The seeds of purple coneflower, *Echinacea purpurea* (L.) Moench, were purchased from Pakanbazr Company (Isfahan, Iran) (http://www.pakanbazr.com/). For cultivation, 84 clay pots with a height of 25 cm in diameter (radius) of 20 cm were used. All pots were containing a 1:1:1 uniform mixture of field soil, rotten leaf soil and sand. 10 seeds of Echinacea were sown in each clay pot in superficially depth of 0.5 to 1 cm and a thin layer of rotten manure was poured on them and irrigation was carried out immediately. The pots were placed in the greenhouse under natural light; the average greenhouse temperature was 25 °C, while the relative humidity varied between 50 and 55 %. After establishing the seedlings and thinning the extra plants, their number reached 5 plants. The plants were irrigated as needed. In this study, two different factorial experiments were performed with a completely randomized design and each experiment with 16 treatments and 3 replications. In the first experiment, the effect of 16 treatments including four levels of salicylic acid foliar application (0, 80, 160 and 320 mg/L) and each at four exposure times (24, 48, 72 and 96 hours) was studied on caffeic acid derivatives production in *E. purpurea.* In the second experiment, the effect of 16 treatments including four levels of yeast extract (0, 0.75, 1 and 1.5 g/L) and each at four exposure times (24, 48, 72 and 96 hours) on caffeic acid derivatives production in *E. purpurea* was investigated. Yeast extract was dissolved in distilled water. Stock solution of salicylic acid was made by dissolving weighed quantity in minimum quantity of ethanol and final volume made by distilled water. Foliar application of salicylic acid and yeast extract on coneflower aerial parts was performed at one stage at the beginning of flowering. Distilled water was also used for foliar application of control plants. For each treatment, three pots having five seedlings per replicate were used. Spraying was done completely on aerial parts of the plants. To evaluate the phytochemical properties of *E. purpurea*, the sampling was performed 24, 48, 72 and 96 hours after foliar application of the elicitors. The samples were dried in a shadow with proper ventilation and normal room temperature (25 - 30 °C) for 7 days. Each sample was placed in a plastic bag separately and their lids were closed. The characteristics such as amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside were measured using high-performance liquid chromatography (HPLC).

2.3. Extraction and determination of caffeic acid derivatives by HPLC

Extraction and quantification of caffeic acid derivatives, e.g., caftaric acid, chlorogenic acid, echinacoside, cichoric acid and cynarin from aerial parts of *E. purpurea* were performed and calculated on the basis of the HPLC assay described by Brown [21]. Aerial parts samples were ground to a fine powder using a porcelain mortar, homogenized in 50 ml conical tubes with 25 ml extraction solvent (60 % aqueous methanol solution). Each sample tube was then mixed with a wrist action shaker for 30 min at room temperature. The extract were centrifuged at

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5000 rpm for 5 min. The supernatants were collected and filtered through 0.2 µm filters for HPLC analysis. High-performance liquid chromatography analysis was performed on Unicam-Crystal 200 HPLC system. Analytical column was Cosmosil 5C18-AR-II, 150 × 4.6 mm id. Column temperature adjusted at 25 °C and the target caffeic acid derivatives were successfully detected at wavelength of 330 nm. Mobile phase A; 0.1 % phosphoric acid in water (filtered through 0.2 µm nylon filter). Mobile phase B; acetonitrile. Injection volume of each sample was 5 µl. The flow rate was 1.5 ml/min. Each analysis was carried out in triplicate. Retention time of reference standards was as follows: caftaric acid (Rt = 4.15 min), chlorogenic acid (Rt = 4.65 min), cynarin (Rt = 7.50 min), echinacoside (Rt = 7.92 min), and cichoric acid (Rt = 13.00 min). The peak identification of the analytes was done by the accurate retention time of each individual standard. The linearity for each analyte was evaluated using five-point standard calibration curves. Coefficient of determination values for the regression curves were calculated for each quantified caffeic acid derivative ($r^2 \geq 99.5$ %). Contents of various caffeic acid derivatives were automatically calculated on the basis of the relevant calibration curve of each caftaric acid, chlorogenic acid, echinacoside, cichoric acid and cynarin and the concentration of the injected sample was determined by the device and its print was recorded below the curve. Concentrations of caffeic acid derivatives were measured on a dry weight basis (mg/g).

2.4. Statistical analysis

Analysis of data was carried out using the SPSS (Version 18.0) software. The experiments were performed in factorial format based on a completely randomized design with three replications per treatment. The mean values were compared using Duncan's multiple range test at $P < 0.01$ significant level. The values are presented as mean ± standard error (SE) of three replications.

3. Results

Caffeic acid derivatives produced in aerial parts of *E. purpurea*, were analyzed and quantified by HPLC (Fig. 1). Effect of elicitors on caffeic acid derivatives production in foliar application on aerial parts of *E. purpurea* was shown in Table 1 - 4. In the first experiment, the results of analysis of variance (ANOVA) showed that the effect of different concentrations of salicylic acid and different exposure times and their interaction on production of caffeic acid derivatives in coneflower was highly significant at 1 % probability level (Table 1).

Considering the significant interaction effect of salicylic acid and exposure times, it can be concluded that the two factors did not act independently. Therefore, the mean comparison of the main effects is not sufficiently valid and only the mean comparison of the interaction effects was examined (Table 2). Salicylic acid significantly altered the amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside. As shown, the highest cichoric acid production (5.256 mg/g DW) was obtained after 96 hours of treatment at 160 mg/L salicylic acid treated plants, which was 2.63-fold higher compared to respective control. This was followed by 320 mg/L salicylic acid for 96 hours exposure time. The lowest level of cichoric acid production was observed in control. The highest chlorogenic acid contents (1.057 mg/g DW) were

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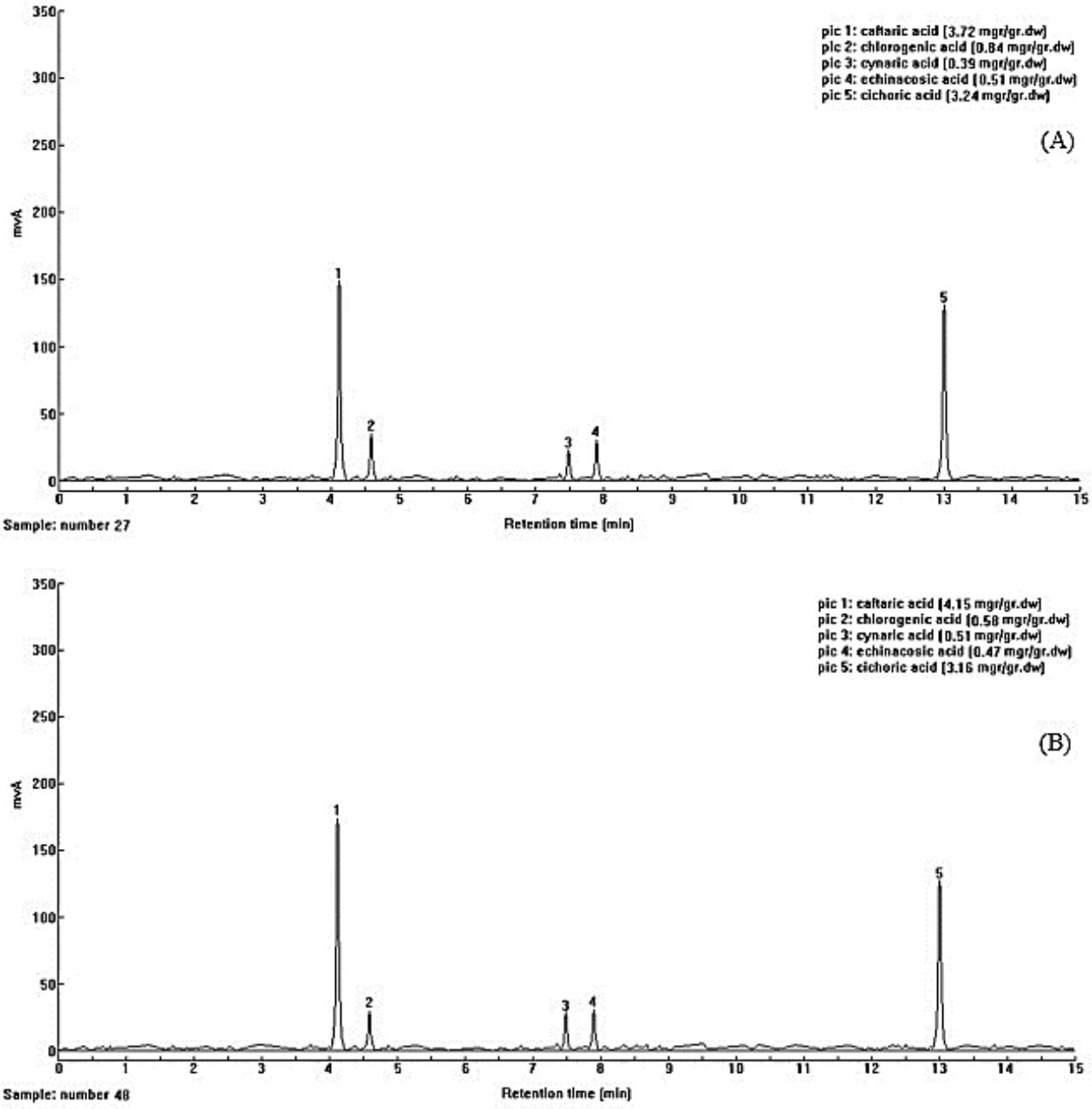


Fig. 1. HPLC chromatogram of elicitation by (A) 1.5 g/L yeast extract for 48 hours exposure time (B) 160 mg/L salicylic acid for 72 hours exposure time in *E. purpurea*. The compounds indicated are (1) caftaric acid, (2) chlorogenic acid, (3) cynarin, (4) echinacoside, (5) cichoric acid.

approximately 2.16 times greater than control level, and were obtained in the plants treated with 320 mg/L salicylic acid and 96 hours exposure time. The lowest level of chlorogenic acid production was observed in control. The highest amount of caftaric acid content (6.690 mg/g DW) was produced in the treatment 160 mg/L salicylic acid for 96 hours. Elicitation by 160 mg/L salicylic acid for 96 hours exposure time increased caftaric acid production (2.84-fold)

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Table 1. Analysis of variance for the effect of salicylic acid and exposure time on caffeic acid derivatives production in *E. purpurea*

Source of variation (S.O.V)	df	Mean of Squares				
		Cichoric acid	Chlorogenic acid	Caftaric acid	Echinacoside	Cynarin
Salicylic acid (SA)	3	5.490**	0.403**	24.034**	0.185**	0.354**
Exposure times (ET)	3	7.432**	0.075**	7.866**	0.261**	0.035**
SA×ET	9	0.932**	0.016**	1.214**	0.021**	0.020**
Error	32	0.014	0.002	0.022	0.020	0.002
C.V. (%)		4.2	6.7	3.5	10.7	21.6

** Significant at $P < 0.01$

Table 2. Mean comparison for interaction effects of salicylic acid and exposure time on caffeic acid derivatives production in *E. purpurea*

Salicylic acid (mg/L)	Exposure time (hours)	Secondary metabolites (mg/g DW)				
		Cichoric acid	Chlorogenic acid	Caftaric acid	Echinacoside	Cynarin
0	24	1.883 ± 0.052^{ghi}	0.410 ± 0.025^{hi}	2.170 ± 0.051^{j}	0.233 ± 0.026^{gh}	0 ± 0^{e}
	48	1.783 ± 0.066^{i}	0.360 ± 0.026^{i}	2.287 ± 0.043^{j}	0.203 ± 0.019^{h}	0 ± 0^{e}
	72	1.830 ± 0.049^{hi}	0.443 ± 0.026^{hi}	2.117 ± 0.061^{j}	0.280 ± 0.026^{fgh}	0 ± 0^{e}
	96	1.997 ± 0.048^{ghi}	0.490 ± 0.032^{gh}	2.353 ± 0.043^{j}	0.310 ± 0.021^{fg}	0 ± 0^{e}
80	24	1.963 ± 0.056^{ghi}	0.570 ± 0.021^{fg}	3.030 ± 0.067^{i}	0.257 ± 0.021^{gh}	0 ± 0^{e}
	48	2.447 ± 0.041^{f}	0.590 ± 0.023^{fg}	4.080 ± 0.092^{gh}	0.327 ± 0.015^{fg}	0 ± 0^{e}
	72	3.080 ± 0.057^{e}	0.627 ± 0.024^{ef}	4.340 ± 0.102^{fg}	0.447 ± 0.019^{de}	0.323 ± 0.094^{abcd}
	96	3.847 ± 0.085^{c}	0.740 ± 0.026^{bcde}	4.600 ± 0.095^{ef}	0.590 ± 0.026^{c}	0.250 ± 0.015^{cd}
160	24	2.110 ± 0.056^{gh}	0.687 ± 0.032^{def}	3.157 ± 0.095^{i}	0.300 ± 0.017^{fgh}	0.240 ± 0.025^{d}
	48	2.590 ± 0.074^{f}	0.723 ± 0.020^{cde}	4.887 ± 0.086^{e}	0.430 ± 0.026^{de}	0.290 ± 0.017^{abcd}
	72	3.817 ± 0.071^{c}	0.687 ± 0.032^{ef}	6.100 ± 0.111^{b}	0.600 ± 0.035^{bc}	0.363 ± 0.018^{abc}
	96	5.256 ± 0.134^{a}	0.820 ± 0.031^{bc}	6.690 ± 0.106^{a}	0.860 ± 0.031^{a}	0.260 ± 0.017^{bcd}
320	24	2.167 ± 0.074^{g}	0.750 ± 0.026^{bcd}	3.910 ± 0.070^{h}	0.330 ± 0.021^{fg}	0.390 ± 0.026^{a}
	48	2.723 ± 0.055^{f}	0.790 ± 0.025^{bcd}	5.263 ± 0.095^{d}	0.380 ± 0.021^{ef}	0.377 ± 0.022^{ab}
	72	3.387 ± 0.055^{d}	0.847 ± 0.032^{b}	5.657 ± 0.077^{c}	0.507 ± 0.022^{cd}	0.400 ± 0.025^{a}
	96	4.187 ± 0.075^{b}	1.057 ± 0.038^{a}	6.137 ± 0.123^{b}	0.690 ± 0.021^{b}	0.410 ± 0.021^{a}

Means followed by similar letter (s) in each column are not significantly different by Duncan's multiple range test at $P < 0.01$
The values are mean ± standard error (SE) of three replicates.

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Table 3. Analysis of variance for the effect of yeast extract and exposure time on caffeic acid derivatives production in *E. purpurea*

Source of variation (S.O.V)	df	Mean of Squares				
		Cichoric acid	Chlorogenic acid	Caftaric acid	Echinacoside	Cynarin
Yeast extract (YE)	3	14.880**	1.364**	8.421**	0.389**	0.520**
Exposure times (ET)	3	18.935**	0.662**	5.295**	0.452**	0.075**
YE×ET	9	2.375**	0.096**	0.685**	0.046**	0.012**
Error	32	0.021	0.003	0.036	0.002	0.001
C.V. (%)		4.2	7.2	5.7	27.9	10.9

** Significant at $P < 0.01$

Table 4. Mean comparison for interaction effects of yeast extract and exposure time on caffeic acid derivatives production in *E. purpurea*

Yeast extract (g/L)	Exposure time (hours)	Secondary metabolites (mg/g DW)				
		Cichoric acid	Chlorogenic acid	Caftaric acid	Echinacoside	Cynarin
0	24	1.883 ± 0.052^{ijk}	0.410 ± 0.025^{h}	2.170 ± 0.051^{gh}	0.233 ± 0.026^{gh}	0 ± 0^{h}
	48	1.783 ± 0.066^{k}	0.360 ± 0.026^{h}	2.287 ± 0.043^{gh}	0.203 ± 0.019^{h}	0±0^{h}
	72	1.830 ± 0.049^{jk}	0.443 ± 0.026^{h}	2.117 ± 0.061^{h}	0.280 ± 0.026^{fgh}	0 ± 0^{h}
	96	1.997 ± 0.048^{ijk}	0.490 ± 0.032^{gh}	2.353 ± 0.043^{gh}	0.310 ± 0.021^{fgh}	0 ± 0^{h}
0.75	24	2.020 ± 0.047^{ijk}	0.370 ± 0.026^{h}	2.220 ± 0.047^{gh}	0.283 ± 0.023^{fgh}	0.183 ± 0.01^{g}
	48	2.707 ± 0.075^{h}	0.470 ± 0.021^{gh}	2.890 ± 0.048^{ef}	0.390 ± 0.032^{ef}	0.287 ± 0.020^{ef}
	72	3.670 ± 0.082^{f}	0.580 ± 0.021^{fg}	3.407 ± 0.050^{d}	0.540 ± 0.035^{de}	0.333 ± 0.023^{def}
	96	4.830 ± 0.091^{d}	0.720 ± 0.031^{de}	3.970 ± 0.076^{bc}	0.757 ± 0.035^{b}	0.353 ± 0.014^{cde}
1	24	2.143 ± 0.045^{ij}	0.597 ± 0.032^{efg}	2.630 ± 0.066^{fg}	0.347 ± 0.019^{fg}	0.260 ± 0.021^{fg}
	48	3.127 ± 0.097^{g}	0.770 ± 0.029^{d}	3.297 ± 0.071^{de}	0.480 ± 0.025^{de}	0.390 ± 0.025^{cd}
	72	4.417 ± 0.086^{e}	0.963 ± 0.035^{c}	3.953 ± 0.080^{bc}	0.780 ± 0.032^{b}	0.410 ± 0.006^{bcd}
	96	5.793 ± 0.122^{b}	1.287 ± 0.055^{b}	4.280 ± 0.068^{b}	0.860 ± 0.021^{b}	0.490 ± 0.025^{b}
1.5	24	2.197 ± 0.052^{i}	0.680 ± 0.025^{def}	2.960 ± 0.061^{ef}	0.370 ± 0.025^{ef}	0.300 ± 0.025^{ef}
	48	3.383 ± 0.074^{fg}	0.900 ± 0.032^{c}	3.560 ± 0.081^{cd}	0.547 ± 0.020^{cd}	0.423 ± 0.020^{bc}
	72	5.180 ± 0.091^{c}	1.370 ± 0.035^{b}	5.050 ± 0.108^{a}	0.623 ± 0.022^{c}	0.570 ± 0.032^{a}
	96	7.150 ± 0.157^{a}	1.680 ± 0.044^{a}	5.373 ± 0.035^{a}	1.087 ± 0.050^{a}	0.630 ± 0.036^{a}

Means followed by similar letter (s) in each column are not significantly different by Duncan's multiple range test at $P < 0.01$
The values are mean ± standard error (SE) of three replicates.

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higher than the control plants. This was followed 320 mg/L salicylic acid for 96 hours (6.137 mg/g DW) exposure time. The lowest level of caftaric acid production was observed in control. The results showed that elicitation with salicylic acid at 320 mg/L for 96 hours increased the accumulation of echinacoside (2.23-fold) higher than the control plants in *E. purpurea* aerial parts. This happens while at other concentration, a higher production of echinacoside with 160 mg/L concentration for 96 hours exposure time (0.86 mg/g DW) was also elicited. The highest amount of cynarin content (0.41 mg/g DW) was produced in the treatment 320 mg/L salicylic acid for 96 hours. The results of a HPLC analysis of the aerial parts of the plant showed that the concentrations of salicylic acid and different exposure times can significantly influence the contents of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside. In the second experiment, the results of analysis of variance (ANOVA) for amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside in *E. purpurea* aerial parts showed that between different concentrations of yeast extract stimulant and four exposure times and their interaction, there was a significant difference ($P < 0.01$) (Table 1). Considering the significant interaction effect of yeast extract and exposure times, only the mean comparison of the interaction effects was examined (Table 4). Yeast extract significantly altered the amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside. As shown, the highest cichoric acid production 7.150 mg/g DW was obtained after 96 hours of treatment at 1.5 g/L yeast extract treated plants. This was 3.58-fold higher compared to respective control and was followed 1.5 g/L yeast extract for 96 hours (5.793 mg/g DW) exposure time. The lowest level of cichoric acid production was observed in control. The highest chlorogenic acid contents (1.680 mg/g DW) were approximately 3.41 times greater than control level, and were obtained in the plants treated with 1.5 g/L yeast extract and 96 hours exposure time. The lowest level of chlorogenic acid production was observed in control. In yeast extract treated plants, 96 hours of elicitor (1.5 g/L yeast extract) treatment resulted in highest caftaric acid content (5.373 mg/g DW). Elicitation by 1.5 g/L yeast extract for 96 hours exposure time increased caftaric acid production (2.28-fold) higher than the control plants. This was followed 1.5 g/L yeast extract for 72 hours (5.050 mg/g DW) exposure time. The lowest level of caftaric acid production was observed in control. The results showed that elicitation with yeast extract at 1.5 g/L for 96 hours the accumulation of echinacoside (3.51-fold) higher than the control plants in *E. purpurea* aerial parts. The highest amount of cynarin content (0.63 mg/g DW) was produced in the treatment 1.5 g/L yeast extract for 96 hours.

4. Discussion

Elicitation is one the most effective techniques currently being used for improving production of bioactive secondary metabolites. Elicitors are compounds that stimulate any type of plant defense and promote secondary metabolism [22]. In general, the basis of elicitation is the activation of a plant's defence response which up-regulates the production of many bioactive compounds of commercial and industrial value. Despite the fact that the majority of medicinal plants are currently grown in field conditions, however, very few studies has focused on elicitation of plants grown on soil to improve the production of pharmaceutically active substances [7]. Enhancing the accumulation of active compounds by adding

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elicitors to the liquid medium has been studied extensively in various plants [19]. Our experimental results show that caffeic acid derivatives in *E. purpurea* aerial parts can be stimulated by both biotic and abiotic elicitors. Salicylic acid significantly altered the amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside. Caftaric acid and cichoric acid were the major caffeic acid derivatives produced by the treated plants. Elicitation by 160 mg/L salicylic acid for 96 hours exposure time increased caftaric acid and cichoric acid production (2.84 and 2.63-fold respectively) higher than the control plants. In agreement with the observation, the same effect of salicylic acid on field-grown plants of *E. purpurea* has been reported earlier. In that study, the cichoric acid content was significantly increased by salicylic acid application, which elicits a 1.71 and 1.58-fold increase in cichoric and caftaric acid in *E. purpurea* aerial parts, and a 2.37-fold increase of cichoric acid in the roots when applied as a foliar spray to field-grown plants [7]. Also, foliar treatment of lemon balm (*Melissa officinalis*) plants with salicylic acid considerably enhanced the monoterpene oxygenated and sesquiterpenes secondary metabolites [14]. The lowest levels of caffeic acid derivatives production was observed in control plants that distilled water was used for foliar application. Generally, natural phytohormone such as salicylic acid, is involved in the signal transduction cascades of plant defense responses [15, 16]. In particular, caffeic acid derivatives, and also phenolics and flavonoids appear to be most responsive to elicitor induction, likely through phenylalanine ammonia lyase (PAL) up-regulation and defense response [7]. The results showed that by increasing salicylic acid concentrations from 0 to 320 mg/L, the amount of caffeic acid derivatives increased. It is suggested that increased endogenous levels of salicylic acid in treated plants can trigger cell signaling responses that regulates the expression of defense genes encoding enzymes related to phenylpropanoids production pathway [23]. The increase in the activity of key enzymes in this metabolic pathway such as phenylalanine ammonia lyase and chalcone synthase, is as a result of the salicylic acid application [24, 25]. As inferred, the enhanced accumulation of caffeic acid derivatives production in the elicited *E. purpurea* might be ascribed to the activation of genes related to caffeic acid derivatives biosynthesis. The results showed that by increasing exposure times from 24 to 96 hours in most concentrations (80, 160 and 320 mg/L) of salicylic acid, the amount of caffeic acid derivatives was increased. Longer time of elicitation (72 and 96 hours) was required to enhance the production of caffeic acid derivatives and 96 hours was the optimum exposure time for foliar application of this elicitor on aerial parts of *E. purpurea*. It has been reported that 3 days of elicitation elevated the production of examined metabolites [18, 20]. The results of a HPLC analysis of the aerial parts of the plant showed that yeast extract had significantly altered the amount of caffeic acid derivatives. The results showed that elicitation with yeast extract at 1.5 g/L for 96 hours the accumulation of caffeic acid derivatives was higher than the control plants in *E. purpurea* aerial parts. Also, by increasing exposure times from 24 to 96 hours in yeast extract concentrations (0.5, 1 and 1.5 g/L), the amount of cichoric acid, chlorogenic acid, caftaric acid, cynarin and echinacoside increased. This study evaluated the effect of different concentrations of yeast extract on the production of caffeic acid derivatives in aerial parts of *E. purpurea* in greenhouse depending on the exposure time of

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elicitor. The majority of biotic elicitors are recognized by specific receptors bound to the cell membrane. This stimulus is then transferred to the cell by a signal transduction system, producing changes that ultimately lead to the formation of phytoalexins [22]. Elicitors such as yeast extract stimulate the production of phenolics in Echinacea, presumably by mimicking a pathogenic fungal infection [26].

5. Conclusion

In conclusion, the results of present study demonstrated that by optimizing the concentrations of the elicitors and the exposure time of elicitation, it is possible to produce the desired secondary metabolites of *E. purpurea* in greenhouse conditions. Both biotic and abiotic elicitors increase caffeic acid derivatives production in *E. purpurea* aerial parts. Elicitors were effective at high concentration. Caftaric acid and cichoric acid were the major caffeic acid derivatives produced by the treated plants. Elicitation by 160 and 320 mg/L salicylic acid and 1.5 g/L yeast extract for 96 hours exposure times were the optimum treatments for caffeic acid derivatives production. Longer time of elicitation (72 and 96 hours) was required to enhance the production of caffeic acid derivatives in foliar application of these elicitors on *E. purpurea* aerial parts.

Author contributions

Zh. M. carried out the experiments and contributed in data gathering. M. A. supervised the research and wrote the manuscript. MR. T. was advisor in the present research and assisted in performance of experiments.

Conflict of Interest

The authors declare that there is no conflict of interest.

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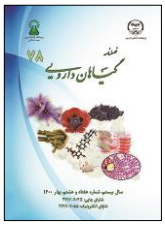
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مقاله تحقیقاتی

تاثیر سالیسیلیک اسید و عصاره مخمر بر تولید مشتقات کافئیک اسید در سرخارگل

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اطلاعات مقاله

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عصاره مخمر

چکیده

مقدمه: گیاه سرخارگل (*Echinacea purpurea* L.)، مدت زیادی است که در درمان‌های گیاهی برای پیشگیری و درمان طیف وسیعی از بیماری‌ها مانند سرماخوردگی، سرفه، عفونت‌های ریوی، التهابات پوستی و بیماری‌های ناشی از نقص ایمنی استفاده می‌شود. **هدف:** این پژوهش، با هدف بررسی اثرات محلول پاشی برگی با سالیسیلیک اسید و عصاره مخمر بر تولید مشتقات کافئیک اسید در سرخارگل اجرا شد. **روش بررسی:** این تحقیق در شرایط گلخانه‌ای طی سال‌های ۱۳۹۷-۱۳۹۶ به صورت دو آزمایش فاکتوریل مجزا انجام شد. در آزمایش اول، اثر سالیسیلیک اسید (۰، ۸۰، ۱۶۰ و ۳۲۰ میلی‌گرم در لیتر) و هر کدام در چهار زمان برداشت (۲۴، ۴۸، ۷۲ و ۹۶ ساعت) بررسی شد. همچنین، در آزمایش دوم، اثر عصاره مخمر (۰، ۰/۷۵، ۱ و ۱/۵ گرم در لیتر) و هر کدام در چهار زمان مذکور بررسی شد. **نتایج:** نتایج نشان داد برای مقدار تولید مشتقات کافئیک اسید، بین غلظت‌های مختلف محرک‌ها، زمان‌های برداشت و اثر متقابل آنها اختلاف معنی‌داری وجود دارد ($P < ۰/۰۱$). بیشترین مقدار شیکوریک اسید، کافتاریک اسید و اکیناکوزید، در تیمار ۱۶۰ میلی‌گرم در لیتر سالیسیلیک اسید به مدت ۹۶ ساعت به دست آمد. بیشترین میزان شیکوریک اسید (۷/۱۵۰ میلی‌گرم بر گرم ماده خشک) در تیمار ۱/۵ گرم در لیتر عصاره مخمر و مدت زمان ۹۶ ساعت به دست آمد که نسبت به شاهد ۳/۵۸ برابر بیشتر بود. کمترین تولید مشتقات کافئیک اسید در گیاهان شاهد مشاهده شد. **نتیجه‌گیری:** محلول پاشی با سالیسیلیک اسید و عصاره مخمر، تأثیر مثبتی بر تولید مشتقات کافئیک اسید در سرخارگل دارد.

مخففها: CADs، مشتقات کافئیک اسید؛ DW، وزن خشک؛ HPLC، کروماتوگرافی مایع با کارایی بالا؛ SE، خطای استاندارد؛ C.V.، ضریب تغییرات

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