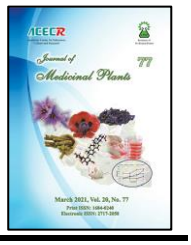
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Research Article

Improving biochemical traits, oleo-gum yield and compositions of asafoetida (*Ferula assa-foetida* L.) essential oil using 24-epibrassinolide in Kerman natural habitats (Iran)

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ABSTRACT

Background: Asafoetida (*Ferula assa-foetida* L.) is an endangered endemic plant which is widely used in food, cosmetics and pharmaceutical industries. It is necessary to enhance the quantity and quality of its secondary metabolites in order to meet requirements of the industries. **Objective:** This study investigated the impact of 24-epibrassinolide (EBL) on biochemical traits, essential oil yield and chemical compositions of *Ferula assa-foetida* in its natural habitat. **Methods:** We foliar sprayed the EBL at the concentrations of 0, 0.1, 0.5 and 1 µM with three times during a week at fully matured leaves phase. One month after treatments application, leaf sampling and gum gathering of the plants was done and the mentioned parameters were measured. **Results:** ANOVA results indicated that 1 µM EBL showed the best results for chlorophyll a, b, total chlorophyll, carotenoids, reduced sugar and protein contents (17.77, 13.08, 31.57, 7.50, 40.45 and 0.34 mg/g FW). The main components of the essential oil are *α*-pinene (8.82 %), *β*-pinene (11.57 %) and myrcene (1.12 %) that showed a significant increase at 1 µM of EBL. Although, EBL at 0.5 µM was proved to be the best treatment for leaf total phenol content (79.37 mg/g FW) and sulfurous compounds such as: *(E)*-1-propenyl sec-butyl disulfide (51.48 %), bis (1-methyl propyl) disulfide (0.9 %) and *n*-propyl sec-butyl disulfide (0.41 %). The highest amount of flavonoids (146.18; 162.56 mg/g FW) and essential oil yield (7.91 %; 8.16 %) were obtained at 0.5 and 1 µM EBL concentrations, respectively compared to the control (6 %). **Conclusion:** Our results indicated the promising and positive effects of EBL, as an environmental friendly strategy, to improve oleo-gum quantity and quality.

Abbreviations: EBL, 24-Epibrassinolide; FW, Fresh Weight; PAL, Phenylalanine Ammonia Lyase; PVPP, Polyvinylpolypyrrolidone; PMSF, Phenylmethylsulfonyl Fluoride; EC, Electrical Conductivity; ANOVA, Analysis of Variace

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1. Introduction

Medicinal and aromatic plants produce important secondary metabolites which are widely used in food, cosmetics and pharmaceutical industries [1, 2]. These compounds play an important role in plants survival under environmental conditions. It is vital to enhance the quality and quantity of plant biochemical traits in order to meet the market and industry requirements [3].

Asafoetida oleo-gum is obtained from different species of *Ferula* which is used in pharmaceutical, perfume, and other industries. Ferula is perennial plants genus belongs to Apiaceae family with 185 species [4]. Asafoetida (*Ferula assa-foetida* L.) is an endangered native herbaceous perennial plant that grows in arid rangelands of Iran, some parts of India and Afghanistan [5, 6]. Its fresh leaves are used by indigenous people in traditional foods [7]. The most important pharmacological uses of the oleo-gum obtained from this plant are for digestive and nervous disease treatments, anti-fungal, antioxidant activities and anti-tumor properties [8-10]. Asafoetida major components are resin (40-64 %), gum (25 %) and essential oil (10-17 %) [5]. Asafoetida essential oil has high commercial value and the main components consist of α-pinene, β-pinene, myrcene and sulfurous compounds [1, 11]. Finding methods to increase economical profit from this plant have significant role in sustainable development of rural communities. Plant growth regulators have been used to manipulate medicinal plants growth, essential oil compositions and yield under natural conditions, biotic or abiotic stresses [3, 12-15]. It is well documented that exogenous application of phytohormones such as brassinosteroides is helpful to improve the productivity of cereals, vegetables, oil seed and medicinal plants [3, 15].

Brassinosteroids are cost-effective and novel group of phytohormones which can function as growth regulators [3, 16, 17]. Among 70 types of identified brassinosteroids, brassinolide, 24-epibrassinolide (EBL) and 28-homobrassinolide are proved to have economic effects on plant growth, productivity, metabolism and high stability under field conditions [3]. They can protect plants from different biotic and abiotic stresses such as heat, drought, salt, heavy metals and fungi and virus diseases [18-22]. They are a type of steroid hormones which involved in a wide range of developmental, physiological and biochemical process [3, 23-26]. BRs application on different plants has been extensively studied recently. Exogenous application of BRs as an environmental friendly strategy reduced the phytotoxic effects of herbicides and insecticides [27, 28]. Application of 28-homobrassinolide improved *Satureja khuzestanica* essential oil yield and chemical compounds such as carvacrol and para-cymene [13]. Application of EBL (24-epibrassinolide) at 0.5 ppm significantly increased the essential oil yield and compositions of peppermint [29].

Although many studies have confirmed the potential of brassinosteroids to improve crops and several medicinal plants performance, there is no report about the effect of brassinsteroids on *Ferula assa-foetida* under natural conditions. According to the fact that *Ferula assa-foetida* is one of the most valuable and economically important plants which is widely used in different industries and in order to enhance its oleo-gum quality, the aim of this study was to investigate the effects of exogenous EBL application on biochemical traits, essential oil yield and chemical compositions of asafoetida oleo-gum under natural conditions.

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2. Materials and methods

2.1. Plant materials, growth conditions and treatments

The present study was conducted in the natural habitat of asafoetida in Kerman province. The area is located at 30º 34' to 30º 39' N and 55º 00' to 55º 07' E with average annual precipitation of 176 mm. The average altitude is 2620 m above sea level and the average annual temperature is 14.8 ºC. Soil texture was sandy loam. Foliar spray was performed at concentrations of 0, 0.1, 0.5 and 1 µM. For this purpose, several uniform asafoetida plants with the same ages (5-6 years old) were randomly chosen for each treatment. Their locations were determined by GPS and marked. All climatic and topographic (slope, aspect and height) conditions were relatively the same.

To make 24-epibrassinolide (EBL) solution, EBL powder (Sigma-Aldrich, Saint Louis, USA) was gently mixed with 96 % ethanol until completely dissolved, and then distilled water was added to make desirable concentrations. EBL treatment was applied by spraying aerial parts of the plants at fully matured leaves stage. This process replicated three times every other days in a week. One month after application of EBL, leaf samples were collected and the plants were prepared for gum gathering. Collected oleo-gums were used to extract the essential oil.

2.2. Biochemical parameters

2.2.1. Determination of chlorophyll and carotenoids contents

Chlorophyll a, b, total and carotenoid content were determined according to Lichtenthaler method [30]. Fresh leaves were homogenized in 3 ml of 70 % acetone. Then centrifuged at 12000 × g for 15 min. Absorbance was observed at 662, 645 and 470 nm to estimate chlorophyll a, b and carotenoid contents using a spectrophotometer (Cary 50, Germany), respectively. 70 % acetone was taken as blank. Total chlorophyll content was determined by adding the contents of chlorophyll a and b.

2.2.2. Total phenol contents

Total phenol content was determined according to Swain and Hillis method [31]. Leaves powder (0.5 g) was extracted in 80 % methanol and centrifuged at 10000 × g for 10 min at 4 ºC. Total phenol content in the supernatant was determined using Folin-Ciocalteu reagent. Its absorbance was measured at 725 nm against a blank control. The amount of total phenol content was calculated according to the standard graph of Gallic acid at different concentrations.

2.2.3. Total flavonoid determination

The total flavonoid content was determined using a modified spectrometrically method. In brief, 1 g of fresh leaves was weighed and grounded with 10 ml of 95 % methanol and then was filtered with Whatman filter. An aliquot of 1 ml of solution was mixed with 0.6 ml of 5 % sodium nitrate and vortexed well and kept in room temperature for 6 min. 0.4 ml of 10 % aluminum chloride was added and placed for further 6 min. 0.5 ml NaOH 1 M was added. The samples absorbance was measured at 510 nm with UV-Vis spectrophotometer (Cary 50, Germany) versus blank control. Four replications were used for each treatment.

2.2.4. Electrolyte leakage measurement

Electrolyte leakage was determined using electrical conductivity meter. Samples were washed by deionized water and dried by filter paper. Fresh leaves (1 g) were soaked in deionized water (20 ml) and incubated at 25 ºC. Electrical conductivity (EC_1) of the solution was determined after 24 h. Samples were autoclaved (120 ºC) for 20 min to kill the tissues completely

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and release all electrolytes. After cooling the samples at 25 ºC, final electrical conductivity (EC_2) was recorded. Then, the electrolyte leakage was determined by the following formula: Electrolyte leakage = EC1/EC2 × 100.

2.2.5. Reduced sugar content determination

Reducing sugar content (mg/g FW) was estimated according to Nelson method [32]. Absorbance was measured at 620 nm using spectrophotometer.

2.2.6. Determination of phenylalanine ammonia lyase (PAL Enzyme) activity

Leaf powder (300 mg) was pulverized in cold mortar containing 50 mM Tris-HCl (pH = 8.8) and 15 mM beta-mercaptoethanol. The mixture was centrifuged at 5000 × g for 30 min and the supernatant solution was used for PAL assays. In this method, PAL catalyzes and converts phenylalanine to cinamic acid. An aliquot of 1 ml of extraction buffer, 0.5 ml phenylalanine (10 mM) and 1 ml enzyme extraction were mixed and maintained at 37 ºC for 60 min. The reaction was terminated by increasing 500 µl of trichloroacetic acid (10 %). Cinamic acid concentration was estimated at 290 nm absorbance using spectrometer (UV-VIS, Cary 50, Germany). One unit of PAL activity was defined as 1 µmol of produced cinamic acid in a minute.

2.2.7. Protein content

Leaf powder (0.5 g) was ground in 50 mM phosphate buffer (pH = 7) containing 1 mM EDTA, 2 % (w/v) polyvinylpolypyrrolidone (PVPP) and 2 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged at 13000 × g for 20 min. Protein content was estimated according to Lowry [33]. To calculate the amount of protein in the samples, a graph (absorbance versus concentration) for standard solutions of protein was depicted and used.

2.3. Essential oil preparation

The essential oil of asafoetida oleo-gum was extracted by hydro-distillation method for 4 h, using Clevenger apparatus. The essential oil was dried using sodium sulfate and stored at 4 ºC until analyzed. The yield of essential oil was calculated based on dry oleo-gum weight.

2.4. Identification of essential oil components

The essential oil was analyzed using model 6890 gas chromatography coupled with an Agilent model 5973-N mass spectrometry which equipped with an HP-5MS capillary column and phenyl methyl siloxane phase (30 m × 0.25 mm i.d. × film thickness 0.25 µm). The ionization energy was 70 eV. Temperature was programmed from 60 to 246 ºC with a rate of 3 ºC/min. Injector and detector were maintained at 250 ºC. Samples (1 µl) were injected with a split ratio of 1:50. Helium was used as a carrier gas with a flow rate of 1.5 ml/min. Retention indices were determined using retention times of *n*-alkanes (C_8-C_{25}) which were injected after the essential oils under the same chromatographic conditions. The components of the essential oil were identified by comparing different parameters such as retention times, Kovats retention index and their mass spectera with those of a computer library, authentic compounds, Adams library and data published in the literature [34].

2.5. Statistical analysis

The experiments were arranged in completely randomized design and sampling was done randomly with five replications for each parameter. The collected data was analyzed by ANOVA test. Mean values were compared using

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Duncan's multiple range test at $P < 0.05$. All analyses were conducted by SPSS and EXCEL software.

3. Results

The results showed that EBL treatment had significant effects on some biochemical parameters in *Ferula assa-foetida* leaves (Table 1).

EBL at concentration of 1 µM, significantly showed the maximum level of Chlorophyll a (17.77 mg/g FW) and b (13.08 mg/g FW), and total (31.57 mg/g FW) and carotenoids content (7.50 mg/g FW). While the minimum amounts of these parameters were observed in control (3.65, 2.55, 6.20 and 2.44 mg/g FW, respectively) and at 0.1 µM concentration (5.2, 3.39, 8.59 and 2.57 mg/g FW). There was no significant difference between these treatments in terms of chlorophyll and carotenoid contents (Fig. 1. a, b, c, d).

The results indicated that EBL treatment had a significantly increased the total phenol content in asafoetida leaves. The highest amount of total phenol was observed at 0.5 µm concentration (79.37 mg/g FW). EBL at 0.1 µm concentration (73.33 mg/g FW) was in the second level. There was no significant difference between 1 µm EBL concentration with control. The control treatment had the least amount of total phenol (63.91 mg/g FW) (Fig. 1.e).

EBL application markedly increased flavonoid levels compared to the control. There was no significant difference among different concentrations of EBL in terms of flavonoid content. The amounts of flavonoids were 64.25, 151.26, 146.18 and 162.56 mg/g FW in control, 0.1, 0.5 and 1 µM treatments, respectively (Fig1.f). EBL treatment reduced the electrolyte leakage in asafoetida leaves, and the control treatment had the highest electrolyte leakage (77.87 %). The lowest electrolyte leakage was observed at 1 µM concentration (34.44 %) (Fig. 1.g).

Reduced sugar content increased significantly concomitant with EBL concentrations. The highest amount of reduced sugar was obtained from the plants treated with 1 µM EBL (40.45 mg/g FW). Control had the least reduced sugar content (13.05 mg/g FW, Fig. 1.h).

PAL enzyme contents significantly decreased after EBR application (Fig. 1.i). The highest and the least amount of PAL enzyme was observed in control (0.19 U mg^{-1} protein) and 0.5 µM EBL concentration (0.07 U mg^{-1} protein), respectively.

The highest level of protein content was obtained at 1 µM concentration (0.34 mg/g FW), while no significant difference was observed among control, 0.1 and 0.5 µM EBL concentrations (Fig. 1.j).

EBL application caused a significant increase and improvement in essential oil contents at 1 µM (8.16 %) and 0.5 µM (7.91 %) concentrations. There was no significant difference between these concentrations. Control (6 %) and 0.1 µM EBL (6.10 %) treatments had the least amounts of essential oil (Fig. 2).

Table 1. Analysis of variance according to the experiment for measured biochemical parameters

Source	df	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid	Phenol	Flavonoid	Electrolyte leakage	Reduced sugar	PAL Enzyme	Protein	Essential oil (%)
EBL	3	123.62**	68.65**	375.3**	16.71**	129.17**	6091.72**	1016.36**	10.57**	0.007**	0.0019*	4.07**
Error	16	1.25	1.06	26.34	0.27	8.79	319.91	26.69	1.02	0.0002	0.0003	0.02
CV%	-	11.87	15.88	13.17	12.25	4.15	13.64	8.78	2.48	12.45	6.48	1.94

*: ($P < 0.05$), **: ($P < 0.01$)

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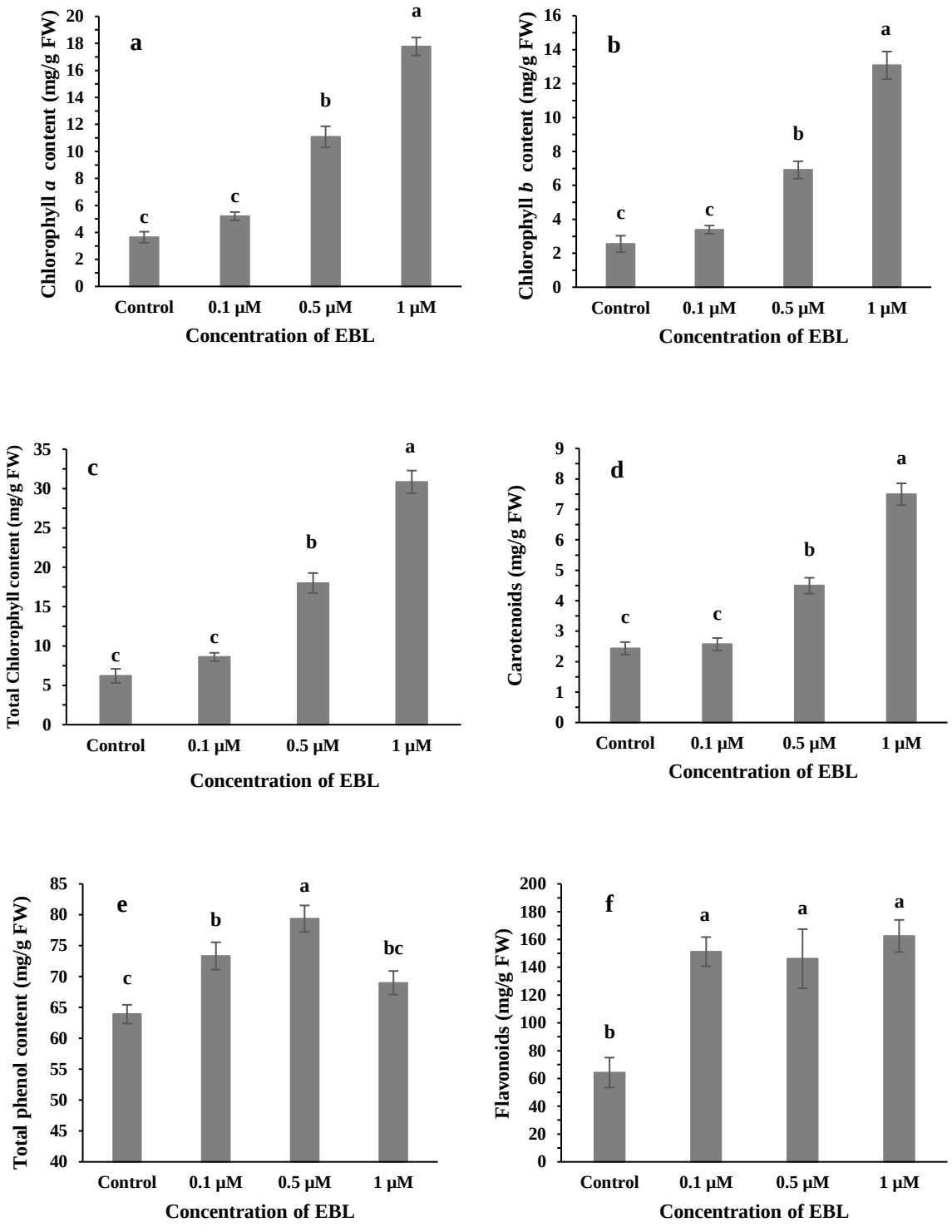


Fig. 1. Effects of EBL on Chlorophyll a (a), Chlorophyll b (b), Total Chlorophyll (c), Carotenoids (d), Total phenol content (e), Flavonoids (f), Electrolyte leakage (g), Reduced sugar content (h), PAL enzyme (i) and Protein (j) in *Ferula assa-foetida* leaves. Columns with different letters are significantly different.

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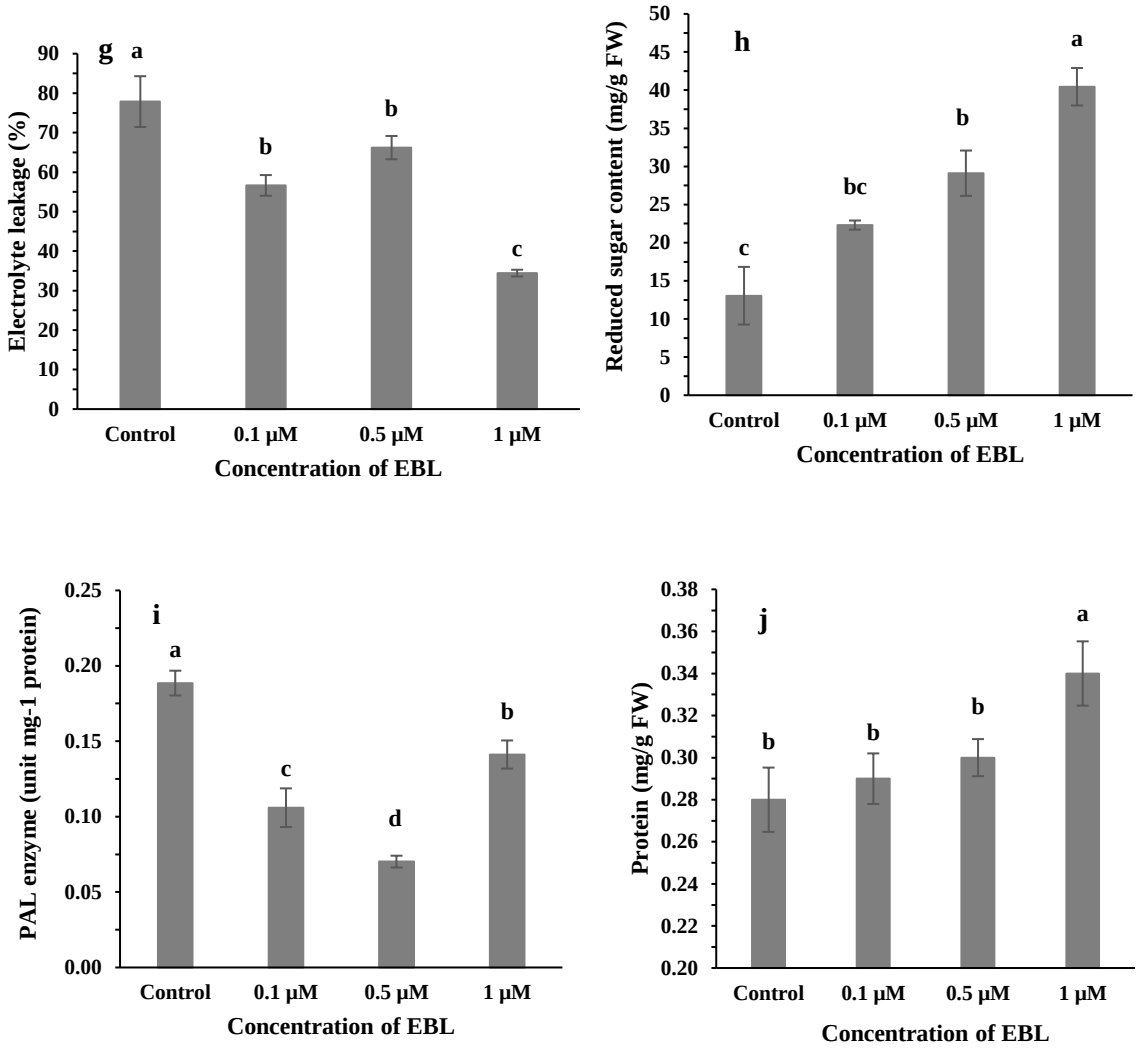


Fig. 1. Effects of EBL on Chlorophyll a (a), Chlorophyll b (b), Total Chlorophyll (c), Carotenoids (d), Total phenol content (e), Flavonoids (f), Electrolyte leakage (g), Reduced sugar content (h), PAL enzyme (i) and Protein (j) in *Ferula assa-foetida* leaves. Columns with different letters are significantly different. (Continued)

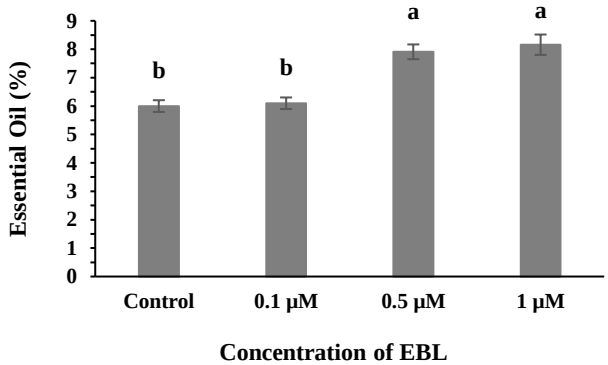


Fig. 2. Effects of EBL on essential oil content in *Ferula assa-foetida* gum

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In response to EBL treatment, there was a progressive enhancement in the content and yield of the main components as compared to the control (Table 2). EBL treatment at 1 µm concentration proved the best and significantly elevated the contents of *α*-pinene, *β*-pinene and myrcene by 8.82 %, 11.57 % and 1.12 %, respectively compared to other treatments. Although, *(Z)-β*-ocimene (9.34 %), *(E)-β*-ocimene (8.49 %) compounds and sulfurous compounds, the most important compound in asafoetida, such as *(E)*-1-propenyl sec-butyl disulfide (51.48 %), bis (1-methyl propyl) disulfide (0.9 %) and *n*-propyl sec-butyl disulfide (0.41 %) had the highest amount at 0.5 µm EBL concentration.

Other components with low percentages such as *α*-phellandrene (2.76 %), 2,3,4-trimethylthiophene (1.85 %), 2-ethyl-3,5-dimethylthiophene (0.41 %), *allo*-ocimene (0.27 %), *α*-guaiene (2.2 %), *α*-humulene (1.46 %), *cis*-cadina-1,4-diene (0.46 %), *γ*-cadinene (0.84 %), and *δ*-cadinene (1.19 %) also showed significant increase at 0.5 µm EBL (Table 2).

Table 2. Effects of EBL on essential oil compounds (similar identified compounds in different concentrations) in *Ferula assa-foetida* gum.

	Chemical compounds	RT	KI	Concentration of EBL			
				Control	0.1 µM	0.5 µM	1 µM
1	*α*-Pinene	6.74	909	2.11 ± 0.08^{c}	3.22 ± 0.07^{c}	5.71 ± 0.12^{b}	8.82 ± 0.32^{a}
2	*β*-Pinene	8.08	953	5.87 ± 0.21^{d}	6.90 ± 0.14^{c}	10.54 ± 1.1^{b}	11.57 ± 0.61^{a}
3	Myrcene	8.45	966	0.84 ± 0.08^{b}	0.71 ± 0.02^{b}	0.96 ± 0.03^{a}	1.12 ± 0.03^{a}
4	*α*-Phellandrene	8.96	982	1.87 ± 0.02^{c}	2.05 ± 0.04^{c}	2.76 ± 0.06^{a}	2.36 ± 0.04^{b}
5	2,3,4-Trimethylthiophene	9.44	998	1.17 ± 0.02^{c}	1.71 ± 0.05^{b}	1.85 ± 0.1^{a}	1.74 ± 0.03^{b}
6	*(Z)-β*-Ocimene	10.14	1017	8.75 ± 0.18^{b}	7.64 ± 0.4^{c}	9.34 ± 0.26^{a}	8.65 ± 0.13^{b}
7	*(E)-β*-Ocimene	10.52	1027	5.75 ± 0.08^{c}	7.37 ± 0.08^{b}	8.49 ± 0.08^{a}	7.38 ± 0.08^{b}
8	2-Ethyl-3,5-dimethylthiophene	13.49	1105	0.18 ± 0.01^{c}	0.31 ± 0.02^{b}	0.41 ± 0.03^{a}	0.32 ± 0.02^{b}
9	*allo*-Ocimene	13.77	1112	0.28 ± 0.04^{a}	0.24 ± 0.03^{b}	0.27 ± 0.03^{a}	0.25 ± 0.05^{b}
10	*neo-allo*-Ocimene	14.36	1126	0.30 ± 0.03^{a}	0.26 ± 0.02^{b}	0.24 ± 0.03^{b}	0.25 ± 0.03^{b}
11	*n*-Propyl sec-butyl disulfide	15.16	1145	0.18 ± 0.02^{c}	0.27 ± 0.05^{b}	0.41 ± 0.08^{a}	0.32 ± 0.06^{b}
12	*(E)*-1-Propenyl sec-butyl disulfide	15.92	1164	50.28 ± 2.12^{c}	51.10 ± 3.11^{b}	51.48 ± 2.2^{a}	49.75 ± 2.14^{d}
13	Bis(1-methylpropyl) disulfide	17.20	1195	0.41 ± 0.03^{c}	0.79 ± 0.38^{b}	0.90 ± 0.06^{a}	0.49 ± 0.02^{c}
14	*α*-Guaiene	26.74	1424	1.89 ± 0.18^{b}	0.94 ± 0.08^{c}	2.20 ± 0.12^{a}	0.84 ± 0.03^{d}
15	*α*-Humulene	27.21	1436	0.60 ± 0.04^{c}	1.08 ± 0.03^{b}	1.46 ± 0.06^{a}	0.57 ± 0.02^{c}
16	*cis*-Cadina-1,4-diene	28.82	1477	0.38 ± 0.04^{b}	0.21 ± 0.02^{c}	0.46 ± 0.05^{a}	0.22 ± 0.03^{c}
17	*γ*-Cadinene	29.62	1497	0.76 ± 0.07^{b}	0.30 ± 0.03^{d}	0.84 ± 0.08^{a}	0.26 ± 0.03^{c}
18	*δ*-Cadinene	29.94	1505	0.91 ± 0.07^{b}	0.32 ± 0.03^{c}	1.19 ± 0.13^{a}	0.25 ± 0.02^{c}

Columns with different letters are significantly different.

4. Discussion

Exogenous application of EBL substantially improved the biochemical parameters, essential oil yield and chemical compositions in asafoetida. EBL application resulted in enhancement of chlorophyll a, b and total, There are several reports of increasing chlorophyll levels after brassinosteroids application in plants such as *Chlorella vulgaris* [35], *Glycine max* [36], *Pelargonium graveolens* [37], *Coleus forskoblii* [15], *satureja khuzestanica* [13], *Jasminum sambac* [29], *Mentha piperita* [3].

Foliar spray of EBL on cucumber significantly enhanced photosynthesis components including net CO_2 assimilation, rubisco activity and other enzymes involve in photosynthesis substances production such as sucrose phosphate synthase, sucrose synthase, and acid invertase activities. The most effective concentration was 0.1 µM EBL which improved Calvin cycle capacity [38]. The effect of EBL at 2 µM on broccoli revealed that chlorophyll content was increased significantly, chloroplast ultrastructure was maintained much better

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compared to the control. Also, using the EBR was preserved the integrity of grana thylakoids in the chloroplast. The expression of chlorophyll genes degradation such as *BoACS4* and *BoACO3* with 2 µM of EBL reduced markedly. It was showed that EBR at low concentration could protect chlorophyll [39].

Carotenoid content was also increased after EBL application. Carotenoids are pigment with bioactivities in photosynthesis. The result of a study showed that brassinosteroids application caused increasing chlorophyll and carotenoids content in *Mentha arvensis* [40]. It was announced that elevated photosynthesis is parallel with enhancement of chlorophyll and carotenoids content in leaves [40].

Phenolic compounds contain hydroxyl groups that destroy free radicals. These important secondary metabolites release hydrogen atoms from their hydroxyl groups, scavange the radicals to produce stable phenoxyl radicals. Therefore, they have an important role in antioxidant activity [14]. Brassinosteroids application has negative effects on generating of free radicals [40, 41].

The results of this study showed that foliar application of EBL led to a significant enhancement in total phenol content. The highest level of total phenol was observed at 0.5 µm EBL concentration. Enhancement of the total phenol and flavonoid contents after brassinosteroids application was reported in several plants such as tomato [42], sweet basil [43] and *Tinospora cordifolia* [14].

EBL treatment decreased electrolyte leakage significantly in leaves in comparison with control. All EBL treatments improved the integrity of cell walls. In a research, it was found that EBL application reduced the electrolyte leakage in *Raphanus sativus* seedlings [24].

Asafoetida plants treated with 1 µm of EBL contained elevated levels of reduced sugar content compared to control and other treatments. In the present study, higher level of reduced sugar content might be attributed to the higher photosynthesis because of elevated chlorophyll level. The same results were reported in terms of increasing carbohydrates levels like reducing sugar after brassinosteroids application in Coleus [15].

An increase in protein content was observed after EBL application. Brassinosteroids plays a major role in DNA and protein synthesis [23]. It has been reported that brassinosteroids affect transcription and translation of proteins [44-46]. Similar results were observed by the researchers who demonstrated that brassinosteroids application has positive effect on protein activation [40].

It was showed that exogenous application of EBL improved biochemical traits of *Ferula assa-foetida* and showed that treated plants were more robust compared to the control [47]. Totally, BRs induce cell division and elongation by acidification of the cell membrane through H^+-ATPases and trigger other enzymes that improve assimilation of photosynthetic compounds in treated plants [48]. BRs can increase the most important enzymes that increase photosynthesis such as rubisco enzyme activity and the expression of related genes [49]. It was reported that BR increases the sensitivity of auxin receptors, which has the combined effect with BR and enhance the impact of EBL [50].

EBL application influenced not only essential oil content but also its chemical compositions at 0.5 and 1 µm concentrations. There was an increase in the essential oil yield and the main components such as α-pinene, β-pinene, myrcene and sulfurous compounds, depending on doses of different concentrations. EBL application at

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maximum level of concentration (1 µm) increased the main components such as *α*-pinene, *β*-pinene and myrcene. Most of chemical compositions including sulfurous compounds increased in 0.5 µm concentration of EBL. *α*-Pinene, *β*-pinene and myrcene substances are widely used in synthesizing artificial and aromatic compounds, cosmetics, flavorings, disinfectants and pesticides. These compounds have anti-microbial, anti-inflammatory and antibiotics properties [51, 52]. Thus these are the most demanded compounds by food, pharmaceutical and cosmetic industries. Sulfurous compounds like *n*-propyl sec-butyl disulfide, *(E)*-1-propenyl sec-butyl disulfide and bis (1-methyl propyl) disulfide are the key ingredients which reveal the quality of asafoetida gum [7]. In this study, EBL increased these compounds significantly and as a result elevated its quality. In contrast with the results of this study, it was found a decrease in *α*-pinene, *β*-pinene and myrcene contents in the presence of EBL in peppermint essential oil. These differences can be due to the growth regulator concentrations, time of application and plant species as mentioned in a study [3].

Brassinosteroids can effect on growth and metabolism and might indirectly cause increasing in essential oil yield. The other reason for enhancing essential oil can be due to inherent genetic potentiality of asafoetida plant which is activated under the effect of EBL. It was also mentioned the same reason in several studies [3, 16, 30]. It was reported that application of EBL increased the essential oil content in lavender [53]. Differences in essential oil content and the amount of chemical compositions may also be due to the influence of EBL on enzymes of the biochemical pathways as reported in a research [3]. There are several reports on the effect of brassinosteroids on secondary metabolites content in different kinds of plants include an increased shikonin content in *Onosma paniculatum* [54], artemisin levels in *Artemisia annua* [55], carvacrol and para-cymene contents in *Satureja khuzestanica* [13], menthol, menthone amount in *Mentha piperita* and *Mentha arvensis* essential oil [3, 40] and an increase in geraniol content and decrease in citronellol content in geranium [37].

There are a few studies regarding the effects of brassinosteroids application on the essential oil yield and the main components of medicinal and aromatic plants. A positive effect of EBL on asafoetida essential oil yield and its ingredients (like *α*-pinene, *β*-pinene, myrcene and sulfurous compounds) and even biochemical parameters of *Ferula assa-foetida* leaves has been reported for the first time under natural condition in this research. Further studies are needed to accurately ascertain the role of BRs in asafoetida plant.

5. Conclusion

According to the results, foliar application of EBL growth promoter at the appropriate dose can act efficiently in *Ferula assa-foetida*. Plants treated with 1 µm showed better results for chlorophylls, carotenoids, reduced sugar, protein contents and several main components like *α*-pinene, *β*-pinene and myrcene. EBL concentration at 0.5 µm proved to be the best treatment for leaf total phenol content and sulfurous compounds in asafoetida essential oil. EBL concentrations of 1 µm and 0.5 µm were the best treatments for flavonoids and essential oil contents. The results demonstrated the favorable and positive effect of EBL on leaf biochemical traits, essential oil yield and the main chemical composition of asafoetida gum which is very important economically. The results can be useful to apply in modern agriculture in order to enhance the quality of production.

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Author contributions

The first author performed the experiment, collected data, analyzed and interpreted biochemical data. He is also the corresponding author. The second author participated in statistically analyzing and interpreting phytochemical data and prepared the manuscript. The third author designed and performed the experiment, collected the data and edited the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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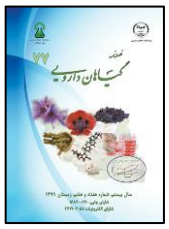
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مقاله تحقیقاتی

بهبود ویژگی‌های بیوشیمیایی، تولید اسانس و ترکیبات شیمیایی شیرابه آنقوزه (*Ferula assa-foetida* L.) با استفاده از ۲۴- اپی‌براسینولید در رویشگاه‌های طبیعی استان کرمان (ایران)

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اطلاعات مقاله

گل‌واژگان:
متابولیت‌های ثانویه
خصوصیات بیوشیمیایی
ترکیبات شیمیایی
آنقوزه
رویشگاه طبیعی

چکیده

مقدمه: گیاه آنقوزه (*Ferula assa-foetida* L.) گیاهی بومی و در معرض انقراض بوده که به طور وسیعی در صنایع غذایی، آرایشی و دارویی مورد استفاده می‌باشد. به دلیل افزایش تقاضا در صنایع مختلف، بالا بردن کمیت و کیفیت متابولیت‌های ثانویه این گیاه ضرورت دارد. **هدف:** در این مطالعه تأثیر محلول‌پاشی ۲۴- اپی براسینولید (EBL) بر ویژگی‌های بیوشیمیایی، تولید اسانس و ترکیبات شیمیایی شیرابه آنقوزه در رویشگاه طبیعی آن مورد بررسی قرار گرفت. **روش بررسی:** محلول‌پاشی EBL بر اندام هوایی گیاه با غلظت‌های ۰، ۰/۱، ۰/۵ و ۱ میکرومولار با فاصله سه بار در طول یک هفته در زمان برگدهی کامل انجام شد. پس از یک ماه، نمونه‌برداری از برگ‌ها و بهره‌برداری از شیرابه انجام و پارامترهای مورد نظر اندازه‌گیری گردید. **نتایج:** نتایج آنالیز واریانس حاکی از این بود که EBL در غلظت ۱ میکرومولار بهترین نتیجه را در مورد کلروفیل a، b، کلروفیل کل، کاروتنوئید، قندهای محلول و میزان پروتئین (به ترتیب ۱۷/۷۷، ۱۳/۰۸، ۳۱/۵۷، ۷/۵۰، ۴۰/۴۵ و ۰/۳۴ میلی‌گرم بر گرم وزن تر) نشان داد. مهم‌ترین ترکیبات اسانس شامل آلفا- پینن (۸/۸۲ درصد)، بتا- پینن (۱۱/۵۷ درصد) و میرسن (۱/۱۲ درصد) نیز در غلظت ۱ میکرومولار EBL افزایش معنی‌داری نشان دادند. اما غلظت ۰/۵ میکرومولار EBL بهترین تیمار برای محتوای فنل کل برگ (۷۹/۳۷ میلی‌گرم بر گرم وزن تر) و ترکیبات گوگردی مانند *(E)*-۱- پروپنیل سکو- بوتیل دی سولفید (۵۱/۴۸ درصد)، بیس (۱- متیل پروپیل) دی سولفید (۰/۹ درصد) و *n*- پروپیل سکو- بوتیل دی سولفید (۰/۴۱ درصد) بود. بیشترین مقدار فلاونوئیدها (۱۴۶/۱۸ و ۱۶۲/۵۶ میلی‌گرم بر گرم وزن تر) و بازدهی اسانس (۷/۹۱ درصد و ۸/۱۶ درصد) به ترتیب در غلظت‌های ۰/۵ و ۱ میکرومولار در مقایسه با شاهد (۶ درصد) به دست آمد. **نتیجه‌گیری:** نتایج نشان‌دهنده اثرات امیدبخش و مثبت EBL، به عنوان یک استراتژی دوستدار محیط زیست، برای بهبود کمیت و کیفیت صمغ آنقوزه بود.

مخففها: EBL، ۲۴- اپی براسینولید؛ FW، وزن تر؛ PAL، فنیل آلانین آمونیا لیاز؛ PVPP، پلی‌وینیل پلی‌پیرولیدون؛ PMSF، فنیل متیل سولفونیل فلوراید؛ EC، هدایت الکتریکی؛ ANOVA، آنالیز واریانس

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