Age-dependent responses in cellular mechanisms and essential oil production in sweet Ferula assa-foetida under prolonged drought stress

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ABSTRACT

The effect of environmental conditions at different developmental ages on the vegetative growth phase was investigated in sweet Ferula assa-foetida in order to identify the defensive mechanisms and essential oil production. Younger plants had more potential for producing the essential oil. Sulfur and sesquiterpenoid compounds were detected as the main groups of components. Monoterpene production was limited to camphene that particularly appeared in older plants. Thiourea as the main sulfur-containing component in fresh tissues helped plants to tolerate abiotic stresses due to scavenging activity of reactive oxygen species (ROS). Three isoforms of SOD and one isoform of catalase were identified in the plants subjected to environmental stresses. The significant enhancement in protein level and reduction in H₂O₂ level during the period of growth with strong induction in antioxidant enzymes’ activities in older plants indicated that they have more functional defensive mechanisms in comparing to younger plants.

Introduction

Ferula assa-foetida belongs to the Umbelliferae family. It is the herbaceous, monoecious, and perennial plant which is commonly known as asafetida. F. assa-foetida grows in the central and southern mountains of Iran, Afghanistan, and Kashmir (Iranshahy and Iranshahi 2011). It has two types which are bitter and sweet F. assa-foetida (IranHerbalPharmacopeia 2002).

Plant growth and development is adversely affected by nature’s wrath in the form of various abiotic and biotic stresses simultaneously (Zandalinas et al. 2017). They affect the geographical distribution of plants in nature and prevent them from reaching their full genetic potential, and also they limit crop productivity worldwide (Mahajan and Tuteja 2005; Zhu 2016). The adverse effects of these abiotic stresses are exacerbated by climate change (Zhu 2016). In addition, under field conditions, stress does not generally come in isolation. Among the environmental stresses, drought influences plant growth and productivity adversely (González-Villagra et al. 2018). High temperature and low humidity are the most important signs for characterizing drought and heat stresses in nature (Rao et al. 1996).

Response to abiotic stresses is a complicated mechanism at the whole plant and cellular levels (Mahajan and Tuteja 2005; Ashraf and Foolad 2007; Zandalinas et al. 2017). Under environmental stresses, electrons that have a high-energy state are transferred to molecular oxygen (O₂) to form reactive oxygen species (ROS) (Mittler 2002; Karabal et al. 2003) such as superoxide ions (O₂⁻), and hydrogen peroxide (H₂O₂). These toxic molecules can damage lipids, proteins and DNA. In order to avoid the harmful effects of these reactive molecules, plants have evolved an efficient scavenging system. Superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and polyphenol oxidase (PPO) are three kinds of ROS scavenging enzymes (Rice-Evans et al. 1997; Karabal et al. 2003). The other biochemical response to abiotic stresses in plants is the overproduction of different types of compatible organic solutes with a low molecular weight such as proline which can accumulate to high concentrations without damaging cellular macromolecules (Sofó et al. 2004).

On the other hand, Plants are the source of an enormous number of compounds, known as secondary metabolites, with medicinal or pharmaceutical applications (Reddy et al. 2004). Secondary metabolites play major defensive, attractive, biological, and ecological roles in the interaction between plants and their environment (Figueiredo et al. 2008; Tohidi et al. 2017). Figueiredo et al. (2008) suggested that physiological variations (i.e. organ development, seasonal variation, etc.), environmental conditions (such as climate, pollution, diseases, and pests), geographic variation, genetic factors and evolution influence the production of secondary metabolites of plants. Sulfur compounds are considered as the main components in sweet F. assa-foetida. Sulfur-containing products constitute a formidable wall of defense against a wide range of pathogens and pests (Nwachukwu et al. 2012). In addition, it has been shown that thiourea as the main sulfur component in sweet F. assa-foetida can act as a ROS scavenger and improve the antioxidant defense system in plants (Kelner et al. 1990).

In this study, we investigated the impact of environmental conditions and developmental ages on some physiological processes such as antioxidant mechanisms and essential oil production in sweet F. assa-foetida.

Material and methods

Plant materials, study site, and environmental conditions

In order to study on sweet F. assa-foetida, we collected fresh materials of this plant from Isfahan province, Khur and
Biyabanak, Iran on 19 April 2015. Our experiment focused on plants with a range of ages between 3 and 8 years old subjected to environmental stresses such as low rate of precipitation and relative humidity, a high temperature which leads to drought and oxidative stresses (Rao et al. 1996). Figures 1 and 2 show the average temperature, evaporation and relative humidity from September 2014 until February 2015 which affected F. assa-foetida growth and development. In addition, the amount of precipitation in the last 5 years (2011–2015) was reported in Figure 2. Expert’s plant taxonomists at the University of Tehran taxonomically identified this plant. Researchers in Natural Resources office of Isfahan and Tehran could estimate the approximate plants’ ages according to the top of the roots and the number of leaves that extracted from the above of the rhizomes and other factors like the length of the rhizomes. The samples put in a frozen pot and closed door in order to avoid evaporation and changing biochemical compounds. After transporting them to the University of Tehran, the aerial parts (A) of plants were separated from underground (U) surfaces and all of them kept in −70°C degree for future analyses.

Measurement of the content of biochemical compounds and activities of antioxidant enzymes

Protein and proline contents
All samples were prepared for soluble protein and enzyme analyses by homogenizing fresh tissue (2 g) in 2 mL of 1 M Tris–HCl buffer (pH = 6.8). The homogenate was centrifuged at 13,000 × g at 4°C for 20 min (Remi Compufuge, CPR-24). All operations were performed at 4°C. The supernatant was used for the content of soluble protein and enzyme activity assays. Soluble protein content was determined by the Bradford method using bovine serum albumin as a standard serum (Bradford 1976).

Proline was estimated by the method of Bates et al. (1973). Two g of fresh materials were homogenized in 10 mL 3% (w/v) sulfosalicylic acid. Then it was assayed by the acid ninhydrin method. The absorbance was measured at 520 nm with Shimadzu, UT-160 spectrophotometer.

Hydrogen peroxide and malondialdehyde content
Lipid peroxidation was measured by the amount of malondialdehyde (MDA), a product of unsaturated fatty acid peroxidation. MDA concentration was estimated by the method of Heath and Packer (1968). Samples (2 g) were homogenized in 3 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 g for 15 min. Two ml of 0.5% thiobarbituric acid (TBA) in 20% TCA were added to 1.0 ml aliquot of the supernatant. The mixture was heated at 95°C for 30 min on the water bath and then cooled in an ice bath. After centrifugation at 10,000 g (Remi Compufuge, CPR-24) for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value of non-specific absorption for each sample at 600 nm was also recorded and then subtracted it from the absorbance amount recorded at 532 nm in Shimadzu, UT-160 spectrophotometer. The concentration of MDA was calculated using an extinction coefficient of 155 mM cm⁻¹.

The content of H₂O₂ was determined according to the method of Yordanov et al. (2000). The fresh materials (2 g) were homogenized in 1 mL of 0.1% (w/v) trichloroacetic acid (TCA) on ice and centrifuged at 12,000 × g for 15 min (Remi Compufuge, CPR-24). One ml of potassium phosphate buffer and 1 ml potassium iodide (KI) were added to 0.5 ml aliquot of the supernatant. The absorbance of the supernatant was recorded at 390 nm with the same device for spectrophotometry analyses. H₂O₂ content was calculated by using a standard curve.

Antioxidant enzyme activities
For estimating enzymes activities, the fresh materials (2 g) were homogenized at 4 °C with 1 M Tris–HCl (pH 6.8) containing 0.3 M sucrose. The homogenates were centrifuged at

Figure 1. Average air temperatures (Av. Temperature), Average relative humidity (Av. RH), the rate of evaporation at the field site during the experimental period. The spots with different color refer to collecting time in our experiment.

Figure 2. The rate of precipitation during the last 5 years including the experimental period.
13,000 × g for 30 min at 4°C (Remi Compufuge, CPR-24). Supernatants were kept at −70°C and used for the determination of enzyme activities.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to the method of Beauchamp and Fridovich (1971), measuring inhibition in the photochemical reduction of nitroblue tetrzoalium (NBT) at 560 nm. Every reaction mixture contains 50 mM potassium phosphate buffer (pH 7.8) with 0.1 mM ethylenediaminetetraacetic acid (EDTA), 75 μM NBT, 13 mM methionine, 2 μM riboflavin, and 100 μL protein extract. Reactions were carried out for 12 min at the light intensity of 300-μmol photons m⁻² s⁻¹. The non-irradiated reaction mixture served as control and was deducted from absorption at 560 nm. One unit of SOD was defined as an amount of enzyme which caused 50% inhibition of NBT reduction under the assay condition. The results were expressed as units per milligram of protein.

SOD isoforms were examined in 10% acrylamide gel using the methods of Laemmli (1970). Gels were incubated in 0.2M Tris–HCl (pH 8.0) containing 4% riboflavin, 4% EDTA, and 20% NBT for 40 min in the dark place with the room temperature. Gels were exposed to white light until white bands appeared in a violet background. For SOD isoform identification, assays were performed in the presence of selective inhibitors. Cu/Zn-SOD isoform was inhibited by KCN (3 mM) and H₂O₂ (5 mM). Fe-SOD isoform was inhibited by H₂O₂ (5 mM). Mn-SOD was not inhibited by KCN or H₂O₂ (Lee et al. 2001).

APX (L-Ascorbate: H₂O₂ oxidoreductase, EC 1.11.1.11) was defined from the reduction in the absorbance at 290 nm (Nakano and Asada 1981). The reaction mixture contained 25 mM potassium phosphate (pH 7.0), 0.5 mM ascorbate, 2 mM H₂O₂, 0.1 mM EDTA, and enzyme extract.

Polyphenol oxidase (PPO; E.C. 1.14.18.1) was estimated by the following method of Raymond et al. (1993) at 40°C. The reaction mixture contained 2.5 mL of 200 mM potassium phosphate buffer (pH 7), 200 μL pyrogallol 20 mM, and 20 μL enzyme extract. The increment of absorbance was recorded at 430 nm. The PPO activity was defined as units per milligram protein. All of the spectrophotometry analyses were recorded by Shimadzu, UT-160 spectrophotometer.

Staining for CAT was performed using the method of Woodbury et al. (1971). The gel (10%) was soaked in 5 mM H₂O₂ for 10 min. The gels were washed with distilled water and CAT isoforms were detected by incubating the gels in 2% (w/v) ferric chloride and 2% (w/v) [K₃Fe(CN)₆]₄ until yellow bands on the dark green background appeared.

Essential oil extraction and GC-MS analysis

In order to determine the composition of the essential oil, Clevenger distillers with 1000 ml round, heating flasks were used. A total of 100–150 g of sliced frozen A. and U. parts of plants and 10 g of mass resin were boiled in distilled water in the flasks heated for 3 h from the start of boiling. The volume of the oil phase from each distillation was placed into the glass bottles, sealed with paraffin, and stored at 4°C in the dark place until the analyzing time with Gas chromatography–Mass spectrometry (GC-MS). Essential oils extracted by the hydro-distillation method were analyzed by gas chromatography using a GC 7890N, AGILENT and MS 5975C, MODEE, Injection Technique: Split (50:1). Injector and detector Temperature were set at 260°C and 230°C, respectively, HP-1MS Capillary column (30 m _ 0.25 mm, film thickness 0.25 mm). The column temperature was kept respectively at 60°C for 4 min, 3°C for 1 min, 100°C for 2 min, then 4°C to 250°C for 5 min as the final temperature. The flow rate of helium was 1 ml/min.

Statistical analysis

Each experiment was repeated three times. The obtained data were statistically analyzed for the mean ± SD, the difference during the period of growth was analyzed by one-way ANOVA using SPSS (Version 23) and the means were compared by Duncan’s test at the 0.05 level of confidence.

Results

There are some evidences to verify that sweet F. assa-foetida was threatened by environmental conditions during our experiment in April 2015. Water deficit and heat stress were characterized by high temperature and evaporation with low relative humidity in our experimental area that affected plant growth and development for years (Figure 1) (Rao et al. 1996). The other evidence was the rate of precipitation that was decreasing during previous years (2011–2015) especially in 2015 (Figure 2).

Protein and proline contents

In relation to the type of tissue, developmental ages, and environmental inhibitor factors the amount of total soluble protein in A. parts was much more than that in U. parts and also it was significantly increasing (Table 1, Figure 3(b)). Proline levels of A. parts were lower than that in U. parts in all of the samples at different ages except for 6-year-old plants (Table 1, Figure 3(a)). The higher amount of protein and proline contents was detected in 4-year-old plants, respectively in their A. and U. parts.

MDA and H₂O₂

The content of MDA and H₂O₂ as a non-radical ROS are given in Figure 3(c,d) and Table 1. Lipid peroxidation, measured as the content of MDA. The highest level of MDA content was determined in A3, U3, and A8. There was a decreasing amount of MDA for 4-year-old tissues but it had an enhancement again. Generally, except for 3-year-old plants as the younger samples, the amount of MDA was increasing during the period of growth. The content of H₂O₂ was significantly at a high level in A6 and it made this tissue more sensitive to oxidative stress. Generally, U. parts contained less amount of H₂O₂ in compare to A. parts.

Antioxidant enzymes

In plants subjected to environmental stress; CAT, SOD, and PPO activities were inhibited in younger plants. In contrast to the younger plants, the significant increment of these enzymes’ activities occurred in U8 (Figure 4 and Table 2). However, it seemed that PPO activity in A. parts was stimulated in comparison to CAT, SOD, and APX. Environmental factors caused a depression in the activity of PPO in U. parts, except for U8 (Figure 4(c)). The remarkable activities of these three antioxidant enzymes (PPO, APX, and SOD) were detected in U8 (Figure 4(a–c)). Polycrylicamide gel electrophoresis (PAGE) analysis of SOD and CAT activities revealed respectively three isoforms (Figure 5) and one
isoform (Figure 6) during different developmental ages. One Cu/Zn-SOD, one Mn-SOD and one Fe-SOD were detected. Among them, Cu/Zn-SOD was disappeared and damaged by environmental conditions. The most significant activity of CAT was observed in A. parts of plants while the activity of CAT was low in underground parts and the band of this isoform almost disappeared in U8.

**GC-MS analyses**

The results were obtained from GC-MS analyses of essential oil’s compositions are presented in Table 3. Constituents that were identified in essential oils mainly belonged to two types of compounds, sulfur and sesquiterpenoid components. There was only one kind of monoterpenes and it was camphene. Moreover, the GC-MS analyses of essential oil fraction of sweet F. assa-foetida demonstrated a wide spectrum of hydrocarbons, heterocyclic organic compounds, alicyclic hydrocarbons and their esters, which we classified them as ‘others’ (Figure 7).

In order to compare the essential oil compositions during the period of growth and developmental ages, we selected two different types of tissues (A. and U. parts) at different developmental ages (four and 8-year-old plants).

In A4 tissues, 0.54% camphene as the only monoterpene, 25.3% sulfur compounds, 30.61% sesquiterpenes, and sesquiterpenoid alcohol were detected out of 97.94% and 38 constituents. Thiourea N, N dimethyl (18.68%), cis propenyl sec-butyl disulfide (3.37%) were the main components of sulfur compound. Guaiol (4.09%), α-eudesmol (3.82%) and γ-eudesmol (21.59%) as the most abundant compounds belonged to sesquiterpenoid alcohol. Alicyclic hydrocarbons (16.79%) were the other types of chemical compositions reported in A4 essential oil (Table 3).

At the same age, in U4, 96.20% components in 35 constituents were identified. The main groups of compositions were sulfur (27.28%) and sesquiterpenoid compounds (39.57%) and there was no monoterpene. Thiourea N, N dimethyl (18.63%) was the main sulfur compound (Figure 9). Valencene (4.19%), β-selinene (5.10%), guaiol (8.51%), and

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**Table 3.** Environmental stress induced changes in total soluble protein (mg g⁻¹ F.W), proline (µg g⁻¹ F.W), H₂O₂ (nmol g⁻¹ F.W) and MDA (nmol g⁻¹ F.W) contents at different developmental ages (D.A) of sweet F. assa-foetida from aerial (A) and underground (U) parts of plants.

| D.A | Protein (mg g⁻¹ F.W) | Proline (µg g⁻¹ F.W) | H₂O₂ (nmol g⁻¹ F.W) | MDA (nmol g⁻¹ F.W) |
|-----|----------------------|----------------------|---------------------|---------------------|
|     | A        | U        | A        | U        | A        | U        | A        | U        |
| 3   | 0.74 ± 0.0705 c | 0.16 ± 0.0385 a   | 0.225 ± 0.1932 b  | 1.728 ± 0.2487 b  | 3.610 ± 0.2301 b | 3.041 ± 0.5488 b | 3.328 ± 0.6462 a | 4.515 ± 0.0774 a |
| 4   | 2.018 ± 0.3031 a | 0.225 ± 0.0987 a  | 0.404 ± 0.0752 b  | 2.496 ± 0.3966 a  | 2.794 ± 0.1703 c | 3.925 ± 0.4174 a | 1.238 ± 0.3128 b | 2.036 ± 0.1548 b |
| 6   | 1.188 ± 0.0369 b | 0.237 ± 0.0455 a  | 1.076 ± 0.2025 a  | 0.571 ± 0.1778 d  | 10.023 ± 0.8745 a | 2.257 ± 0.5318 b | 1.984 ± 0.2193 b | 2.292 ± 0.3345 b |
| 8   | 1.364 ± 0.269 b  | 0.041 ± 0.005 b   | 0.224 ± 0.0530 b  | 1.229 ± 0.1099 c  | 4.157 ± 0.0928 b | 0.742 ± 0.2023 c | 4.231 ± 0.9801 a | 2.498 ± 0.8493 b |

Notes: Data represent the means ± SD of three replicates. Different letters indicated significant (P < 0.05) differences based on Duncan’s range test at P ≤ 0.05.

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**Figure 3.** Effect of environmental factors on proline (a), total soluble protein (b) and H₂O₂ (c), and MDA contents (d) during the period of growth and development in aerial (A) and underground (U) parts of plant. Vertical bars indicate means ± SD based on three replicates. Different letters above columns indicated significant (P < 0.05) differences.
Figure 4. Effect of environmental factors on antioxidant enzyme activities: APX (a), SOD (b) and PPO (c) during the period of growth and development in aerial (A) and underground (U) parts of the plant. Vertical bars indicate means ± SD based on three replicates. Different letters above columns indicated significant ($P < 0.05$) differences.

Table 2. Environmental stress-induced changes in SOD, APX and PPO activities (U mg$^{-1}$ Protein) at different developmental ages (D.A) of sweet *F. assa-foetida* from aerial (A) and underground (U) parts of plants.

| D.A | SOD (U mg$^{-1}$ protein) | APX (U mg$^{-1}$ protein) | PPO (U mg$^{-1}$ protein) |
|-----|----------------------------|---------------------------|---------------------------|
|     | A                         | A                         | A                         | U                         |
| 3   | 1.9420 ± 0.54645 a         | 2.0884 ± 0.57270 b        | 0.0800 ± 0.01940 b        | 0.0743 ± 0.00058 b         |
|     | 1.1173 ± 0.07027 b         | 1.1535 ± 0.06149 b        | 0.1561 ± 0.01150 b        | 0.0460 ± 0.00964 c         |
| 6   | 1.4397 ± 0.14215 ab        | 0.8911 ± 0.24264 b        | 0.0760 ± 0.01125 b        | 0.0592 ± 0.01822 bc        |
|     | 13.4966 ± 2.76265 a        | 13.4966 ± 2.76265 a       | 0.0270 ± 0.01493 c        | 0.1073 ± 0.00931 a         |

Notes: Data represent the mean ± SD of three replicates. Different letters indicated significant ($P < 0.05$) differences based on Duncan’s range test at $P < 0.05$.

Figure 5. Changes in the isoform patterns of SOD activity bands in aerial (A) and underground (U) parts of plants during growth period and development in sweet *F. assa-foetida* in response to various environmental factors.

Figure 6. Pattern of CAT activity in aerial (A) and underground (U) parts of plants during the period of growth and development in sweet *F. assa-foetida* in response to various environmental factors.
Table 3. The essential oil compositions of sweet *F. assa-foetida* under multiple environmental factors during different period of growth and development from aerial (A), underground (U) parts of plants, and mass resin (M).

| Compounds                                      | Rt (min) | Percentage % |
|------------------------------------------------|----------|--------------|
| 1 Methyl sec-butyl disulfide                   |          |              |
| 2 Di-tert-butyl sulfide                        |          |              |
| 3 Propanethioic acid, 5-butyl ester            |          |              |
| 4 2,3,5-trimethylthiophene                     |          |              |
| 5 Butyric acid, thio- 5-sec-butyl ester        |          |              |
| 6 Butyric acid, 3-methylthio-, 5-sec-butyl ester|          |              |
| 7 Ethyl n-butyl disulfide                      |          |              |
| 8 1-phenylhexan-2-one                           |          |              |
| 9 Butyric acid, 3-, 2- methylpropyl ester      |          |              |
| 10 Triethylarson                                |          |              |
| 11 Disulfide, ethyl hexyl                       |          |              |
| 12 n-Propyl sec-butyl disulfide                 |          |              |
| 13 cis-propenyl sec butyl disulfide            |          |              |
| 14 Trans propenyl sec butyl disulfide          |          |              |
| 15 1,2-Dithiolane                               |          |              |
| 16 2,2-Dimethyldihydro-2H-furanone              |          |              |
| 17 sec-Butyl disulfide                          |          |              |
| 18 1,3-Dithiane                                 |          |              |
| 19 Nerylacetate                                 |          |              |
| 20 Aromadendrene                                |          |              |
| 21 Furanomycin                                 |          |              |
| 22 Camphene                                     |          |              |
| 23 Nerylacetate                                 |          |              |
| 24 Aromadendrene                                |          |              |
| 25 Furanomycin                                 |          |              |
| 26 Camphene                                     |          |              |
| 27 Disulfide, bis(1-methyl(propyl))             |          |              |
| 28 y Cadinene                                   |          |              |
| 29 Bicyclo[3.1.0]hexane, 6- isopropylidene-1-methyl-|          |              |
| 30 dihydromyrcene                               |          |              |
| 31 1-methylthio-2,2-bis                        |          |              |
| (methyldimethylenepropane)                      |          |              |
| 32 Thymine glycol                               |          |              |
| 33 2,5-Dimethyl-2-methylthio-1-propanol         |          |              |
| 34 2,2-Bis(methylthio)propane                   |          |              |
| 35 Disulfide, dibutyl                           |          |              |
| 36 Gymnomitrene                                 |          |              |
| 37 α Humulene                                   |          |              |
| 38 N-Methylthiacetamide                         |          |              |
| 39 Thiazole, tetrahydro-                       |          |              |
| 40 Trans β farnesene                            |          |              |
| 41 β Selinene                                   |          |              |
| 42 Dihydro β agarofuran                         |          |              |
| 43 α Farnesene                                  |          |              |
| 44 Trans y bisabolene                           |          |              |
| 45 cis α bisabolene                             |          |              |
| 46 β-Carene                                     |          |              |
| 47 α agarofuran                                 |          |              |
| 48 Thiacyclopanantan-3-ol                      |          |              |
| 49 Elemol                                       |          |              |
| 50 Hedycaryol                                   |          |              |
| 51 cis-8-ethylbicyclo[4.3.0]3-nonene            |          |              |
| 52 Thiourea, N,N-dimethyl                       |          |              |
| 53 Ethyliothione                                |          |              |
| 54 4-Thia-2,6-diazahexacyclo[5.4.2.0]           |          |              |
| 55 1'-2'-Hydroxy-3',4'-dimethylphenyl(ethanone) |          |              |
| 56 Thiophane, propyl-                           |          |              |
| 57 Carotol                                      |          |              |
| 58 Guaiol                                       |          |              |
| 59 α-Eudesmol                                   |          |              |
| 60 γ-Eudesmol                                   |          |              |

(Continued)
γ-Eudesmol (17.55%) were the main sesquiterpene and sesquiterpenoid alcohol compounds (Figure 8).

In A8 samples, 38.4% sulfur compounds, 1.92% monoterpene, 24.16% sesquiterpenes and sesquiterpenoid alcohols, and 31.6% of other types of compositions were identified (Figure 7). Cis propenyl sec-butyl disulfide (3.5%), thiochrome N, N dimethyl (1.63%), and carotol (1.1%), γ-Eudesmol (14.72%), valencene (4.07%), neoisolongifolene (6.28%) were the most important constituents. Alicyclic hydrocarbons, aldehydes derivatives (17.02%), and sulfur-containing decans (25.77%) were the other major compounds in A8 essential oil (Figures 8 and 9).

Total identified compounds of U8 essential oil were 92.59% in 38 constituents. The main fractions were 37.24% sulfur compounds, 2.21% monoterpene, and 30.92% sesquiterpene-sesquiterpenoids. Cis propenyl sec-butyl disulfide (4.5%), thiochrome N, N dimethyl (8.09%), ethylthiourea (20.35%) as the main sulfur compounds and carotol (11.71%), γ-Eudesmol (16.21%) from sesquiterpenoid alcohols group, and camphene (2.21%) as the only detected compound were identified in U8 essential oil (Figures 8 and 9).

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Extracting and analyzing essential oil from a mass resin obtained by incision method from the top of the roots indicated 34 constituents from 96.14% identified components. Sulfur compounds like thia cyclopentane-3-ol or Sulfolan-3-ol (7.74%), propyl thiophane (8.36%) and 1,2-dithiolane (36%) were the most important compounds. There was no significant amount of sesquiterpenes. In addition, monoterpenes were not detected (Figure 7).

The chemical profiles in this study were similar to the other reports, but remarkable differences occurred in the concentration of each constituent in plant tissues. In general, sulfur compounds were increasing during the period of growth, and the total amount of these components in 4-year-old
tissues were lower than these compounds in 8-year-old plants (Figure 7). The main sulfur-containing component in 4-year-old plants was N, N-dimethyl thiourea. In A8, the abundance of thioureas was decreasing, but the other kinds of components such as hydrocarbons containing sulfur elements in their structure appeared. 1,2-dithiolane was the important integrate of essential oil’s components in M. However, there was no 1,2-dithiolane in other samples. Synthesizing of monoterpene was limited in sweet F. assa-foetida. Our results reported only camphene in essential oil fraction. Despite monoterpene synthesizing, the presence of sesquiterpenes like β-selinene, valencene and sesquiterpenoid alcohol like eudesmol, guaiol and carotol in all essential oils profiles indicated a greater potential for stereochemical diversity of these type of compounds.

Discussion

According to our data from the Meteorological Organization and based on similar studies, there are several abiotic stresses in our experimental area, like drought, heat and oxidative stresses. The rate of precipitation is vital for F. assa-foetida due to its effect on growing season and photosynthesis rate. Therefore with elongation of the growth season, the plant has enough time for synthesis and accumulation of mass resin (Pirmoradei 2002; Moghaddam and Farhadi 2015). Abiotic stress can lead to increased production of ROS (Zagorchev et al. 2013; Das and Roychoudhury 2014) and affect the essential oil and secondary metabolites production (Linda et al. 2006; Figueiredo et al. 2008; Akula and Ravishankar 2011). Genetic factors, species and time harvesting (Gil et al. 2002; Mahmoud et al. 2018; Rampino et al. 2006), the length and severity of water loss periods (Bahe et al. 2002; Bettaieb et al. 2009), organ development, seasonal and geographic variation and evolution (Figueiredo et al. 2008) impact on essential oil production and its compositions as well.

Our results show that from the point of having a high potential for producing secondary metabolites, young plants (A4 and U4) have a more active mechanism (Table 3). The essential oils obtained from different developmental ages are rich in γ-eudesmol (especially in A4), guaiol, carotol, and thiourea. In U8, the other kind of sesquiterpenoids like carotol detected. By aging, the total amount of sesquiterpenoids was decreasing while the total amount of sulfur compounds was enhancing. All of these observations with the appearance of camphene as an only monoterpene in A4, A8, and U8 implicate that they can happen due to the effect of environmental conditions and developmental senescence on enzymes’ activities and metabolism (Burbott and David 1969). In many plant species, diterpenes and sesquiterpenes act as phytoalexins and sulfur-containing products constitute a formidable wall of defence against a wide range of pathogens and pests, and herbivores (Tomlin 2000; Cheng et al. 2007; Nwachukwu et al. 2012). γ-eudesmol as bicyclic sesquiterpenoid alkene alcohol found in all tissues and it was decreasing generally (Figure 8). It can display moderate activity against human diseases. α-farnesene as another compound can be effective against the bacteria that cause tooth decay (Ishnava et al. 2013). On the other hand, Thiourea as an important nitrogen-containing compound and non-physiological thiol was the main sulfur compound in sweet F. assa-foetida but it changed and replaced to 1,2-dithiolane in mass resin. Thiourea has been proved to be useful for stress tolerance and improve the yield of crops in plants like mustard (Sahu et al. 2005; Kocyigit-Kaymakcioglu et al. 2013; Pandey et al. 2013). In addition, it has known as ROS scavenger. Thiourea is capable of reacting with superoxide as well as with hydrogen peroxide or hydroxyl radicals (Kelner et al. 1990) and also it can control plant pathogens (Rodriguez-Fernandez et al. 2005). Thus, thiourea has this potential to be used as an effective bioregulator to impart abiotic stress.
tolerance under field conditions (Sahu and Singh 1995; Burman et al. 2004; Sahu et al. 2005, 2006). Due to containing a large amount of thiourea in fresh material of plants, using roots’ pieces and putting them on the waterway on the lands in agriculture can be an effective way of eliminating fungal diseases and pests, even more than using mass resin. Because of this, many farmers prefer to follow this method for years.

On the other hand, lipid peroxidation and the content of proline and protein generally were increasing under stressful conditions. Alexieva et al. (2001) indicated the same trend in pea and wheat under drought and UV-radiation. Proline is a typical plant stress response to environmental inhibitor factors (Zandalinas et al. 2017), but it is well-described in water and salt stresses (Alexieva et al. 2001). It causes a drop of the osmotic potential in the plant tissues, and consequently, it allows plant leaves to withstand to a greater evaporative demand without losing turgor (Hare and Cress 1997; Sofo et al. 2004). A lower amount of proline in A. parts of sweet F. assa-foetida can make them more vulnerable against abiotic stress. It has been reported that proline accumulated in A. thaliana plants in response to drought, but not in response to heat stress, or the combination of drought and heat. Consequently, proline was replaced by the other osmoprotectants such as sucrose in plants under several abiotic stresses (Rizhsky et al. 2004). This can explain why the amount of proline was not increasing as expected in A. parts of sweet F. assa-foetida. Similar results in purslane plants were reported by Jin et al. (2016). Changes in H$_2$O$_2$ and MDA contents indicated that water stress-induced oxidative damage in sweet F. assa-foetida. Our results showed a significant amount of MDA content in young and aged-senescence plants (Figure 3(d)). Moreover, increasing the level of protein content during the period of growth and the higher amount of MDA in younger plants (3 years old) showed that cellular mechanisms against environmental stress in older plants are more functional (Larcher 2003). In agreement with our results, González-Villagra et al. (2018) reported higher lipid peroxidation levels (around 60%) in young leaves of Aristotelia chilensis subjected to drought stress for 20 days. Also, Cechin et al. (2010) indicated the same results and higher amount of lipid peroxidation (about 30%) in young leaves in contrast to fully-expended leaves of Helianthus annuus affected by drought stress for 6 days. This could be attributed to the higher amount of chloroplasts compared to young leaves (Lepedus et al. 2011). PPO had the significant activity in the U. parts of sweet F. assa-foetida and U8 especially had strong antioxidant activities. In addition, in U8 as the remaining tissues of older plants in the next years for trying a new chance to enter the reproductive stage, thiourea can improve plants’ ability to being alive during hard field conditions such as drought and high temperature. With suppressing lipid peroxidation by synthesizing thiourea, sweet F. assa-foetida has another strong antioxidant mechanism to tolerate drought stress. According to our results, younger plants are more and early affected by drought stress and the older plants have stronger mechanisms to tolerate environmental stresses.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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