1. Introduction

Hyssop (Hyssopus officinalis L.) belongs to the family Lamiaceae family and is a native plant from southern Europe and Near East to the region surrounding the Caspian Sea and cultivated in central and south European countries, including Russia, Spain, France, Yugoslavia, Netherlands, Hungary and Italy (Mitić & Đorđević, 2000). This plant grows on dry...
banks and among rocks and ruins with a height ranging from 50 to 120 cm (Le Strange, 1977; K?nemann, 1999). The leaves are mainly used as an aromatic condiment (Baytop, 1997). H. officinalis has been known as a culinary and medicinal herb for hundreds of years. Hyssop oil finds its greatest use in flavoring preparations for alcoholic beverages, meat products and seasonings. It is used in tea blends for cough relief, antispasmodic effects, and relieving catarrh (Khazaieet al., 2008).

H. officinalis var. angustifolius (Persian name: “Zoofa”) is grown and cultivated in some parts of Iran. The aerial parts of hyssop are used in Iranian folk medicine for their asthma, bronchitis, ulcers and wounds, carminative, antiseptic and antimicrobial (Zargari 1990; Ghasemi Pirbalouti, 2013). The essential oil and extracts isolated from H. officinalis have been shown to have biological and pharmacological activities, including antibacterial (Michalczyk et al., 2012), antifungal (Fraternelle et al., 2004), anti-oxidant (Fern?ndez-Lpez et al., 2003; Kizil et al., 2010), sedative (Churl et al., 2005), spasmylytic (Mazzanti et al., 1998), anti-viral (Herrmann Jr & Kucera, 1967; Kreiset al., 1990), cytotoxic (Renziniet al., 1999), insecticidal (Pavela, 2004), and antiplatelet (Tognolini et al., 2006). Kazaziet al. (2007) reported the main components of the extracts under different SFE conditions from H. officinalis cultivated in Iran were sabinene (4.2–17.1%), iso-pinocamphene (0.9–16.5%) and pinocamphene (0.7–13.6%).

There are several commercially available chemical compounds that could be used as elicitors to modify plant secondary metabolites and subsequently the bioactivity of medicinal plants. The most well-known chemical elicitors include salicylic acid (Métraux et al., 1990; Van Wees et al., 2000), jasmonic acid and its derivatives (Yazakiet al., 1997; Shararenet al., 1998; Vazquez-Flota and De Luca, 1998; Palazoniet al., 2003; Arimuraet al., 2005; Zheljazkov and Astatkie, 2012). Jasmonic acid and its volatile methyl ester, MJ, collectively termed jasmonates, are regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development (Creelmanet al., 1997).

The hypothesis of this study was that JA would have bioactivity or influence plant growth and development; hence, the JA could be used to promote or to limit plant growth. Few studies have been done to investigate the effects of JA foliar application on the accumulation of secondary metabolites in medical plants in agricultural systems. Therefore, this study was done to evaluate the effect of various concentrations of JA on essential oil content and its composition of hyssop (H. officinalis) cultivated in greenhouse conditions.

2. Materials and Methods

2.1. Chemicals

Alkan standard solution C5-C24 and jasmonic acid (JA) were purchased from Sigma–Aldrich Co. (Steineheim, Germany). Acetone and anhydrous sodium sulphate were bought from Merck Co. (Darmstadt, Germany).

2.2. Plant material and field site description

H. officinalis seeds were obtained from the Pakan Seed Company, Isfahan, Iran. In the third week of February 2012, seeds were sown in each plot (0.5m x 0.5m) with plant density of 12 (plants/m2). This experiment was conducted under plastic greenhouse conditions at Research Field, I.A.U., Shahrekord Branch, ChaharmahalvaBakhtiari province, southwestern Iran (latitude 32° 20’ N, longitude 50° 51’ E, altitude 2071 m above sea level). Plants were grown in a greenhouse in the relative humidity of 60-65% and, daily and nightly temperatures of 27±2 °C and 20±3 °C, respectively. No inorganic fertilizer and systemic pesticide were used during the experiment. For the first month (established phase), a watering level equivalent to 60-65% of soil field capacity was applied once a day. For the subsequent months, the plots were irrigated when 50% of soil available water was depleted (irrigation intervals varied from 2 or 3 days). The aerial parts of hyssop were collected from each pot before flowering stage (10 June 2012), all of which were dried at 40 °C in the dark until it reached to a constant weight. Oil yield based matter dried (v/w) and chemical composition of essential oils were measured.

2.3. Treatments

Experimental treatments included (I) water foliar application (control), (II) water + acetone foliar application (as a solvent), (III-V) 50, 100 and 200 JA µL. Treatments were foliar-sprayed once at ten days before harvesting.

2.4. Essential oil isolation
Fifty gram dried plant material were powered and subjected to hydro-distillation for 3 h using a Clevenger- type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulphate and kept in amber vials at 4±1 °C prior to use.

2.5. GC/MS analysis

The essential oils were analyzed using an Agilent 7890a gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60 °C for 4 min initially, and then, raised at the rate of 4 °C/min to 260 °C. Injector and detector temperatures were set at 290 and 300 °C, respectively. Helium was used as carrier gas at a flow rate of 2 mL/min, and 0.1 µL samples were injected manually in the split mode. Peaks area percentages were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200 °C. Retention indices were calculated for all components using a homologous series of n-alkanes (C9-C24) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system (Adams, 2007; McLafferty, 2009).

2.6. Statistical analysis

The data was statistically analyzed using a CRD by the program SPSS (17.0). Means of the characteristics were compared by Duncan’s multiple range test at P<0.05 level.

3. Results and Discussion

The color of extracted oil was yellow. The oil yields of studied treatments ranged between 0.77 to 0.86% (v/w), based on dry weight. Statistical analysis indicated that there was no significant difference between treatments for oil yield (Table 1). There also was no significant difference between treatments for total dry matter of hyssop.

The chemical constituents identified by GC-MS, are presented in Table 1. Totally, thirty eight components were identified representing more than 85-99% of the oil composition. The analysis of essential oils detected major compounds, viz. cis-3-pinanone, trans-3-pinanone, β-pinene, β-phellandrene, trans-caryophyllene, myrtenol, germacrene D, elemol, bicyclogermacrene, α-pinene, β-myrcene, sabinene and pinocarvone (Table 1 and Fig 1-3). In this study, the essential oils obtained from hyssop contained oxygenated monoterpenes, monoterpen hydrocarbons and sesquiterpenes. Similarly, Ozer et al. (2005) reported that the major constituents in the essential oil obtained by hydro-distillation from the aerial parts of H. officinalis L. subsp. angustifolius (Bieb.) collected from Turkey were pinocarvone (36.3%), pinocamphone (19.6%), β-pinene (10.6%), 1,8-cineole (7.2%), and isopinocamphone (5.3%). The results of earlier studies indicated that the major volatile constituents obtained from the aerial parts of H. officinalisiwere sabinene, isopinocamphone, pinocamphone, camphor and β-pinene, α and β-phellandrene, germacrene D, myrtenol and pinocamphene (Garget et al., 1999; Mitić&Dorđević, 2000; Chalchat et al., 2001; Fraternale et al., 2004; Khazaie et al., 2008).

The result of analysis of variance indicated that different levels of the foliar application of JA had a significant impact on the main constituents in the essential oils of hyssop (Table 1). Percentages cis-3-pinanone, trans-3-pinanone, β-pinene, β-phellandrene, myrtenol, and α-pinene amounts in oils of H. officinalis under foliar application 200 µL JA decreased (Fig 1-3). Decreased amount of these constitutes in plants grown under JA (200 µL) foliar application might be attributed to stress condition, which would activate the synthesis of secondary metabolites. On the other hand, content of sesquiterpenes group including trans-caryophyllene, germacrene D, elemol and bicyclogermacrene increased under JA 200 µL treatment (Table 1). The results of present study revealed that the foliar application of JD 200 µL caused significantly increased sesquiterpenes in comparison with monoterpenes.

JA and MJ are known as transducers of elicitor signal transduction that results in the biosynthesis of plant secondary metabolites. They act as inducers of
phytoalexins (Zhao et al., 2005). Accumulation of isoflavonoids, as phytoalexins, was enhanced in vitro cultures of *Pueraria lobata* (Willd.) Ohwi (Thiem and Krawczyk, 2010). Ashrafiet al. (2012) reported that the foliar application of JA (100 µL) have increased thymol and carvacrol contents in essential oil obtained from *Thymus daenensis* aerial parts. JA and its derivatives are known to stimulate production of secondary metabolites in plants (Sanzet et al., 2000). JAs have been shown to regulate the synthesis of various secondary metabolites including caffe-o-ylputrescine in tomato leaves (Chen et al., 2006), anthocyanins (Zhao et al., 2005; Uppalapati et al., 2005) and defense-related volatiles (Thaler et al., 2002; Ament et al., 2004). JA induces the production of ajmalicine and catharanthine in *Catharanthus roseus* (Vazquez-Flota and De Luca, 1998), rosmanarinic acid and shikonin in *Lithospermum erythrorhizon* (Yazakiet al., 1997), scopoletin and scopolin in *Nicotiana tabacum* (Sharan et al., 1998) and taxol and paclitaxel in *Taxus* sp. cell suspensions (Palaznet et al., 2003). Induction of plant secondary metabolite accumulation by the JA signaling pathway is not limited to certain types of metabolites, but includes a wide variety of plant secondary products including terpenoids, flavonoids, alkaloids, and phenylpropanoids plus many other types of secondary metabolites in most plants (Zhao et al., 2005). Therefore, the JA signaling pathway is generally regarded as an integral signal for biosynthesis of many plant secondary products (Zhao et al., 2005). Increasing evidence indicates that JA-induced changes in secondary metabolism constitute a ubiquitous plant defense response (Gundlach et al., 1992; Memelink et al., 2001; Goossens et al., 2003; Zhao et al., 2005).

JA has also been implicated as the signal molecule responsible for increased synthesis of nicotine, an insecticidal compound produced by *Nicotiana sylvestris* upon leaf wounding (Zheng et al., 1997). JAs are produced and accumulated in plants, but exogenous application of JA and MJ can elicit secondary metabolite accumulation in defense response induction (Thiem and Krawczyk 2010).

4. Conclusion

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors and other industrial materials. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Jasmonic and salicylic acids compounds have long been observed to be transducers or elicitor signals for the production of plant secondary metabolites. JA (200 µL) could be used as foliar application in *H. of?cinalis* for increasing phenolic components including carvacrol and thymol. Phenolic components production in lemon balm could be partially changed by supplementation of different elicitors.

![Fig 1. Effect of foliar application of JA on β-pinene content](image)

**Table 1.** Effect of JA foliar application on some of main components contents in *H. of?cinalis* oil

<table>
<thead>
<tr>
<th>Treatments</th>
<th>β-phellandrene (%)</th>
<th>β-Myrcene (%)</th>
<th>Pinocarvone (%)</th>
<th>Myrtenol (%)</th>
<th>β-Caryophyllene (%)</th>
<th>Germacrene D (%)</th>
<th>Bicyclo-germacrene (%)</th>
<th>Eltemol (%)</th>
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<tr>
<td>JA50</td>
<td>2.36</td>
<td>2.29</td>
<td>4.16</td>
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<td>3.11</td>
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<td>2.44</td>
<td>2.01</td>
<td>3.77</td>
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<td>2.73</td>
<td>0.00</td>
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<tr>
<td>JA200</td>
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<td>0.99</td>
<td>2.01</td>
<td>0.73</td>
<td>5.82</td>
<td>13.19</td>
<td>10.17</td>
<td>17.26</td>
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<td>0.00</td>
<td>2.95</td>
<td>1.45</td>
<td>3.10</td>
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<tr>
<td>Acetone</td>
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<td>3.17</td>
<td>1.03</td>
<td>0.00</td>
<td>0.00</td>
<td>2.98</td>
</tr>
</tbody>
</table>
Fig 2. Effect of foliar application of JA on trans-3-pinanone content

Fig 3. Effect of foliar application of JA on cis-3-pinanone content

References


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biosynthesis in *Catharanthus roseus*. 


