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Phytochemical response of hyssop (*Hyssopusofficinalis* L.) to foliar application of jasmonic acid

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ABSTRACT

Background & Aim: Jasmonic acid (JA) is regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development.Effect of foliar application of JA on the essential oil and oil chemical components of hyssop (*Hyssopusofficinalis* L.) were investigated.

Experimental: This study conducted in a CRD with three replications and in experimental greenhouse, I.A.U., Shahrekord Branch, Iran at 2012.Experimental treatments included (I) water foliar application (control), (II) water + aceton foliar application (as a solvent), (III-V) 50, 100 and 200 JA µL. The essential oils obtained by hydro-distillation were analyzed by Gas Chromatography-Mass Spectrometry.

Results & Discussion: The results of GC-MS showed that the major components of the oil were *cis*-3-pinanone (10-39%), *trans*-3-pinanone (4-28%) and *β*-pinene (27-34%). The results of analysis of variance of the experiment showed that different levels of the foliar application of JA do have significant impacts on chemical constitutes in the essential oil.Finally, foliar application of 200 µL JA on some of secondary metabolite production in *H. officinalis* oil could be partially changed.

Recommended applications/industries:

Abbreviation: JA: Jasmonic Acid, MJ: Methyl Jasmonate, GC-MS: Gas Chromatography-Mass Spectrometry µL: Micro liter, M: Molar; ANOVA: Analysis of Variance, CRD: Completely Randomized Design.

1. Introduction

Hyssop (*Hyssopusofficinalis*L.) belongs to the family Lamiaceae familyand 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, Netherland, 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 (Khazaie*et al.*, 2008).

H. officinalisvar. 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; GhasemiPirbalouti, 2009). The essential oil and extracts isolated from *H. officinalis* have been shown to have biological and pharmacological activities,includinganti-bacterial (Michalczyk*et al.*, 2012), anti-fungal (Fraternale*et al.*, 2004), anti-oxidant (Fern?ndez-L?pez*et al.*, 2003; Kizil *et al.*, 2010), sedative (Churl *et al.*, 2005), spasmolytic (Mazzanti*et al.*, 1998), anti-viral (Herrmann Jr&Kucera, 1967; Kreis*et al.*, 1990), cytotoxic (Renzini*et 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 derivates (Yazaki*et al.*, 1997; Sharan*et al.*, 1998; Vazquez-Flota and De Luca, 1998; Palazon*et al.*, 2003; Arimura*et 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 (Creelman*et 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 C_5-C_{24} and jasmonic acid (JA) were purchased from Sigma–Aldrich Co. (Steineheim, Germany). Acetone and anhydrous sodium sulphatewere 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/m^2). 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 areal 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 + aceton 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 7890,a 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 wascoupled 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 (C_5-C_{24}) 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 containedoxygenated monoterpenes, monoterpene hydrocarbons and sesquiterpenes. Similarly, Ozeret al. (2005) reported that the major constituents in the essential oil obtained by hydro-distillation from the aerial parts of*H.of?cinalis* 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 the major volatile constituents obtained from the aerial parts of *H. officinalis*were sabinene, *iso*-pinocamphene, pinocamphone, camphor and β-pinene, α and β-phellandrene, germacrene D, myrtenol and pinocamphene (Garg*et al.*, 1999; Mitić&Đorđević, 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 JAhad a significant impact on the main constitutes 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.of?cinalis* 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 JA 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 vitrocultures of *Puerarialobata*(Willd.) Ohwi(Thiem and Krawczyk, 2010).Ashrafiet al.(2012) reported that the foliar application of JA (100 µL) have increased thymol and carvacrolcontents in essential oil obtained from *Thymus daenensis* aerial parts. JA and its derivatives are known to stimulate production of secondary metabolites in plants (Sanzet *al.*, 2000). JAs have been shown to regulate the synthesis of various secondary metabolites including caffe-oylputrescine in tomato leaves (Chen *et al.*, 2006), anthocyanins(Zhao *et al.*, 2005; Uppalapatiet *al.*, 2005) and defense-related volatiles (Thaler*et al.*, 2002; Ament *et al.*, 2004). JA induces the production of ajmalicine and catharanthine in *Catharanthusroseus* (Vazquez-Flota and De Luca, 1998), rosmarinic acid and shikonin in *Lithospermumerythrorhizon* (Yazaki*et al.*, 1997), scopoletin and scopolin in *Nicotianatabacum* (Sharan*et al.*, 1998) and taxol and paclitaxel in *Taxus* sp. cell suspensions (Palazon*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; Goossenset *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 *Nicotianasylvestris* 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 of 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 includingcarvacrol and thymol. Phenolic components production in lemon balm could be partially changed by supplementation of different elicitors.

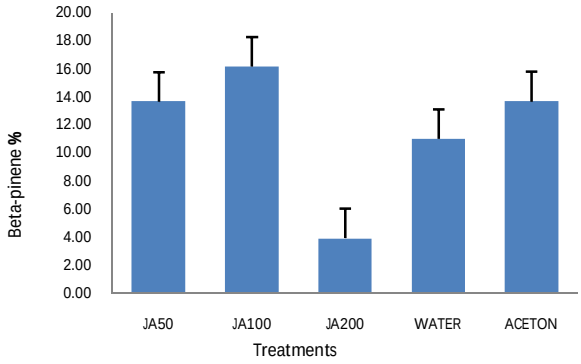

Fig 1. Effect of foliar application of JA on β-pinene content

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

Treatments	β-phellandrene	β-Myrcene	Pinocarvone	Myrtenol	β-Caryophyllene	Germacrene D	Bicyclo-germacrene	Elemol
JA50	2.36	2.29	4.16	3.82	1.49	3.11	0.00	0.00
JA100	2.54	2.44	2.01	3.77	1.57	3.24	2.73	0.00
JA200	0.56	0.99	2.01	0.73	5.82	13.19	10.17	17.26
Water	6.10	1.95	0.00	2.95	1.45	3.10	2.60	0.00
Acetone	0.00	1.15	0.00	3.17	1.03	0.00	0.00	2.98

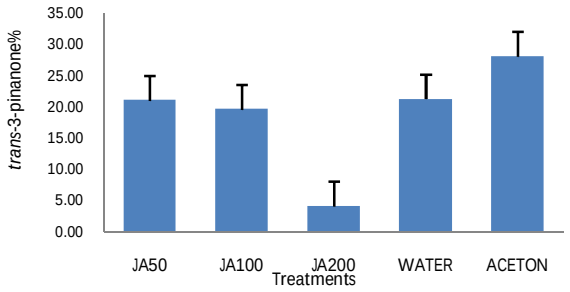

Fig 2. Effect of foliar application of JA on *trans*-3-pinanonecontent

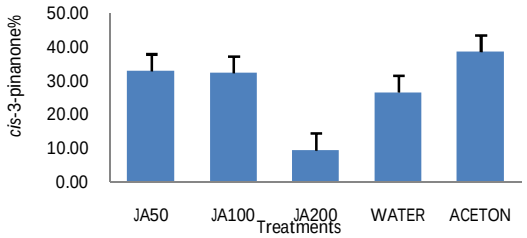

Fig 3. Effect of foliar application of JA on *cis*-3-pinanonecontent

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