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Short Communication

Chemical components and insecticidal effects of *Lavandula angustifolia* and *Origanum vulgare* essential oils on the growth different stages of *Habrobracon hebetor* Say (Hymenoptera: Braconidae)

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Abstract

Habrobracon hebetor Say is an ectoparasitoid of larval stage of various lepidopteran pests. In this study, fumigant toxicity of *Lavandula angustifolia* and *Origanum vulgare* essential oils on the different growth stages of *H. hebetor* were assessed at 26 ± 2 °C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Essential oils were extracted from the plant samples using a Clevenger-type apparatus where the plant material is subjected to hydrodistillation. The chemical constituents of essential oils were detected by Gas Chromatography-Mass spectrometry (GC-MS). Glass vials containing 250 ml were used for the experiments. Chemical analysis by GCMS displayed 1,8-Cineol (45.31%) and Camphor (15.78%) for *L. angustifolia* and Pulegone (37.83%) and 1,8-Cineol (17.02%) for *O. vulgare* as major constituents. LC_{50} values of *L. angustifolia* and *O. vulgare* essential oils were 0.06 and 0.08 µl for larval stage, 0.29 and 1.39 µl for pupal stage, 0.23 and 0.91 µl for female adults, and 0.17 and 0.23 µl for male adults, respectively. Fumigant bioassays revealed that *L. angustifolia* oils were more toxic than *O. vulgare* oils against all stages of *H. hebetor*. This research indicates that *O. vulgare* essential oils have less toxicity on *H. hebetor* and recommends as a compatible botanical compound with this biocontrol agent in integrated pest management programs.

Keywords: Habrobracon hebetor, bioassay, GC-MS, essential oils, Lavandula angustifolia, Origanum vulgare.

1. Introduction

The repeated and intense use of synthetic insecticides for several decades has raised long-term human health and environmental concerns, mainly due to their slow degradation in the environment and toxic residues in the products, and the evolution of resistance to pesticides in pest populations (Isman, 2006). These effects have increased the need for effective and biodegradable pesticides and created a significant market opportunity for alternative products (Isman, 2000; Isman *et al.,* 2011). Botanical insecticides have the advantages of reducing risk to non-target organisms due to their rapid degradation in the environment and providing novel and multiple mode of actions that reduce the probability of developing resistance in pest populations (Isman, 2006; Rajendran and Sriranjini, 2008).

Essential oils (EO) are volatile mono- and sesquiterpenoids that interfere with basic metabolic, biochemical, physiological, and behavioral functions in insects and have been demonstrated to possess contact, fumigant, inhalation and ingestion toxicity. They also have antifeedant activity, capacity to delay development, adult emergence and fertility, deterrent effects on oviposition and arresting and repellent action (Tripathi *et al.,* 2009). Also, the biological and insecticidal activities of essential oils are influenced by their chemical composition that needs to be identified.

Biological control using natural enemies is receiving greater attention as an environmentally friendly management option for controlling pests in crops (Scholler *et al.,* 2006). *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is a gregarious larval ectoparasitoid of several species of pyralid and nuctoid moths (Dweck *et al.,* 2008). In Iran, mass rearing of *H. hebetor* is performed on Mediterranean flour moth, *Anagasta kuehniella* (Zeller). For the success of the IPM, contemporaneous use of biological control agents and chemical compounds are recommended (Hull and Beers, 1985). Therefore, the effects of compounds on biological control agents should also be evaluated.

The available information about the effects of plant essential oils on the ectoparasitoid wasp, *H. hebetor* is very limited. Asadi *et al.,* (2018a) evaluated the effects of *Rosmarinus officinalis* L. and *Salvia officinalis* L. (Lamiaceae) essential oils on *Habrobracon hebetor* Say (Hymenoptera: Braconidae) in *Ephestia kuehniella* Zeller (Lep.: Pyralidae) larvae and their bioassay results showed that LC_{50} values for *R. officinalis* and S. *officinalis*

essential oils are 4.15 and 18.36 µl/l air, respectively. *R. officinalis* essential oils showed high acute toxicity on the female wasps of *H. hebetor* compared with *S. officinalis* essential oils.

The objective of the current study is to evaluate the effects of the *Lavandula angustifolia* and *Origanum vulgare* essential oils on the different growth stages of *H. hebetor* to enable selection of soft pesticides to protect beneficial insects and thereby improve the IPM.

2. Materials and methods

2.1. Rearing of insects

Colony of *H. hebetor* parasitoid wasps was obtained from the Department of Plant Protection, University of Mohaghegh Ardabili, Iran. Then, the parasitoid wasps were reared under laboratory conditions in growth chamber that was set at 26 ± 2 ℃, 60 ± 5% RH and a photoperiod of 16:8 (L:D) h, on fifth-instar larvae of flour moth (*A. kuehniella*), that was reared on flour in a growth chamber at above mentioned environmental conditions. Moreover, a honey solution (10%) was applied as food source for feeding of the adult parasitoids (Mahdavi *et al.*, 2011).

2.2. Plant material and extraction of essential oils

L. angustifolia and *O. vulgare* were collected from Zanjan province, Iran. The essential oils were obtained by hydro-distillation in a Clevenger type apparatus. The extraction condition was as follows: 50 g of dry plant (in powder form); 500 ml of distilled water, and 3 h distillation. The obtained oils were dried over anhydrous sodium sulphate to extract the oils. Extracted oils were stored in a refrigerator at 4 ^{o}C for required studies.

2.3. Chromatographic analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the essential oils of *L. angustifolia* and *O. vulgare*. GC-MS analysis was carried out on a GC 6890 N (Agilent, USA) equipped with a split injector and MS 5973 N mass selective detector system. Chromatographic separation was carried out in an HP5ms capillary column (30 m × 0.25 mm, 0.25 µm in film thickness). Helium (99.99%) was used as the carrier gas with a flow rate of 1 ml min^{-1}. The injector temperature was set at 150℃, the column temperature program started at 10 ℃ for 3 min, increased by 10℃ min^{-1} to 120 ℃, by 10℃ min^{-1} to 150℃, and by 7℃ min^{-1} to 240℃, and was maintained for 5 min. Identification of spectra was carried out by studying their fragmentation and comparing with standard spectra present in the library of the instrument (Adams 2001).

2.4. Fumigant toxicity of essential oils

Acute toxicity bioassay test on the immature (larval and pupal) and mature (male and female) stages were carried out by using the fumigant method (Mahdavi *et al.*, 2018). Preliminary dose-setting experiments were carried out to determine 20 and 80% mortality ranges. Glass containers (250 ml) were used as a fumigation chamber. Twenty individuals of selected developmental stages of insects were placed in the glass vials. Distilled water was used in control treatments. Each concentration of the essential oils was bioassayed in four replications. Different growth stages were exposed to the treatments for 24 h. Mortality was recorded 24 h after treatment in all stages, except pupal stage. Mortality were recorded in the pupal stage after the pupal period.

2.5. Data analysis

Experiments were tested for lack of fit by using PROC GENMOD (Robertson *et al.*, 2007; SAS Institute, 2002), and data were analyzed using PROC PROBIT to compute (Lethal Concentration) LC_{10}, LC_{50} and LC_{90} values on a standard and log scale with associated 95% fiducial limit by SAS program (SAS Institute, 2002).

3. Results

3.1. Chemical components of essential oils

The GC-MS analysis results of isolated essential oils are shown in Tables 1 and 2. Twelve major compounds from *L. angustifolia* essential oils and eleven compounds from *O. vulgare* essential oils were detected. The constituents 1,8-Cineol (45.31%), Camphor (15.78) and Borneol (14.46%) from *L. angustifolia* and Pulegone (37.83%), 1,8-Cineol (17.02%) and Menthofuran (12.14%) from *O. vulgare* were detected as major constituents of each mentioned essential oils.

Table 1. Chemical analysis of essential oils of *L. angustifolia* by GC-MS

Components	**Retention time (min)**	**Rate (%)**
α- Pinene	10.86	1.48
o-Cymene	16.33	2.93
Limonene	16.79	1.51
1,8-Cineol	16.99	45.31
Linalool	20.67	1.07
Camphor	23.14	15.78
Borneol	24.59	14.46
Crypton	25.24	2.11
Isobornyl formate	26.99	1.42
Cumin aldehyde	27.91	1.91
Thymol	30.06	7.71
Carvacrol	33.43	0.81
Total		96.5

Table 2. Chemical analysis of essential oils of *O. vulgare* by GC-MS

Components	Retention time (min)	Rate (%)
α- Pinene	10.86	2.02
2-β-Pinene	12.95	2.99
1,8-Cineol	16.98	17.02
Menthofuran	22.43	12.14
Cis-iso-pulegone	23.01	9.38
Borneol	24.60	1.23
Neo-iso-dihydro carveol	25.18	2.24
Pulegone	26.33	37.83
2-Cyclohexane-1-van-1-decene	30.77	4.01
Caryophellene oxide	40.17	1.94
Total		90.8

3.2. Fumigant bioassay

The LC_{10}, LC_{50} and LC_{90} values for *L. angustifolia* and *O. vulgare* essential oils against the larvae, pupae,

males and females of *H. hebetor* are shown in table 3. LC_{50} values of *L. angustifolia* and *O. vulgare* essential oils at the larval stage of the *H. hebetor* were 0.06 and 0.08 µl, respectively. The LC_{50} values of *L. angustifolia* and *O. vulgare* essential oil at the pupal stage were 0.29 and 1.39 µl, respectively. For the female adult stage, these values were 0.23 and 0.91 µl, respectively. Moreover, the LC_{50} values of *L. angustifolia* and *O. vulgare* essential oils at the male adult stage were 0.17 and 0.23 µl, respectively (table 3). A statistically significant difference in the toxicity of *L. angustifolia* and *O. vulgare* essential oil treatments was found at the pupa and female adult growth stages of *H. hebetor*, as inferred by the lack of overlap in the LC_{50} confidence intervals (table 3).

Table 3. Toxicity of essential oils to different growth stages of the ectoparasitoid *Habrabracon hebetor*

Essential oil plants	Growth stages	Number	Slope ± SE	Lethal concentrations (µl)		
				LC10 (95% FL)	LC50 (95% FL)	LC90 (95% FL)
Lavandula angustifolia	Male adults	360	2.88 ± 0.4	0.06	0.17	0.46
				(0.04-0.08)	(0.14-0.19)	(0.36-0.71)
	Female adults	360	2.36 ± 0.34	0.07	0.23	0.79
				(0.04-0.09)	(0.19-0.27)	(0.58-1.33)
	Pupae	360	2.13 ± 0.3	0.07	0.29	1.16
				(0.04-0.1)	(0.24-0.35)	(0.82-2.05)
	Larvae	360	5.59 ± 0.79	0.005	0.06	0.77
				(0.002-0.009)	(0.04-0.08)	(0.41-2.26)
Origanum vulgare	Male adults	360	1.42 ± 0.2	0.03	0.23	1.86
				(0.01-0.05)	(0.18-0.31)	(1.11-4.4)
	Female adults	360	4.99 ± 0.75	0.5	0.91	1.64
				(0.39-0.58)	(0.84-0.98)	(1.41-2.12)
	Pupae	360	1.17 ± 0.17	0.82	1.39	2.35
				(0.66-0.93)	(1.30-1.49)	(2.06-2.95)
	Larvae	360	1.92 ± 0.28	0.02	0.08	0.35
				(0.008-0.024)	(0.06-0.09)	(0.24-0.68)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002)

4. Discussion

The essential oils of plants could be alternative sources for pest control because of their innate biodegradability, minimal effects on non-target organisms and on environment (Feldlaufer and Ulrich, 2015), and their different pesticide mode of action (El-Wakeil, 2013). In this study, the fumigant toxicity of *L. angustifolia* and *O. vulgare* essential oils on different growth stages (larvae, pupae, female adults and male adults) of *Habrobracon hebetor* was evaluated.

The results of the effects of plant essential oils on different growth stages of the parasitoid wasp showed that the male parasitoids are more susceptible than female insects. This difference in sensitivity may be due to differences in terms of size (weight) and the amount of fat in the body (Weaver *et al.,* 1995; Papachristos and Stamopoulos, 2002). Based on the values of LC_{50}, the larval and pupal stages were the most sensitive (with the least value of LC_{50}) and the most resistant (with the highest value of LC_{50} to the essential oils, respectively. Also, the results of our studies showed that lavender essential oils has a higher toxicity to growth stages of the parasitoid wasp compared to the *O. vulgare* essential oil. Asadi *et al.,* (2018b) investigated effects of *Allium sativum* L., *Rosmarinus officinalis* L., *Piper nigrum* L., *Salvia officinalis* L. and *Glycyrrhiza glabra* L. essential oils on *H. hebetor* Say (Hymenoptera: Braconidae) in its host. Their results showed that the acute toxicity of *R. officinalis* essential oils on the female wasps of *H. hebetor* was higher than the others. Also, *G. glabra* essential oils showed the lowest acute toxicity suggesting that *G. glabra* essential oils can be recommended with *H. hebetor* in integrated pest management. Other studies have also been conducted by researchers regarding the insecticidal effects of essential oils on different insect pests and natural enemies (González *et al.,* 2013; Hashemi *et al.,* 2014; Naghizadeh *et al.,* 2016; Mahdavi *et al.,* 2017).

Several studies have shown that the insecticidal effects of essential oils are associated with some chemical constituents existing in essential oils (Regnault-Roger *et al.,* 1993). The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant activity that might be of importance for controlling stored-product insects (Coats *et al.,* 1991; Konstantopoulou *et al.,* 1992; RegnaultRoger and Hamraoui, 1995; Ahn *et al.,* 1998). It is possible to understand the structure of essential oil constituents with the help of gas chromatography/mass spectrometry (GC/MS). GC/MS analysis showed that 1,8-Cineol (45.31%) and Camphor (15.78) in *L. angustifolia* and Pulegone (37.83%) and 1,8-Cineol (17.02%) in *O. vulgare* were detected as major constituents. These results are consistent with the results of Yazdani *et al.,* 2013 where the major constituents of the essential oils of *L. angustifolia* Mill were identified as Borneoll (8.57%)). The toxic effects of *L. angustifolia* and *O. vulgare* could be attributed to major constituents such as Camphor and 1,8-cineol.

Based on these laboratory results, it seems that *O. vulgare* essential oils are potentially more compatible with a chosen IPM approach. After laboratory studies, more attention should be devoted on storage environment experiments to obtain more applicable results under storage conditions.

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