EVALUATION OF THE INSECTICIDAL ACTIVITIES OF THREE EUCALYPTUS SPECIES CULTIVATED IN IRAN, AGAINST *HYPHANTRIA CUNEA* DRURY (LEPIDOPTERA: ARCTIIDAE)

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Abstract: In the current study, the larvicidal activity of leaf essential oils from three eucalyptus species (*Eucalyptus largiflorens* Meull, *Eucalyptus oleosa* Meull, and *Eucalyptus spathulata* Hook) against American white moth, *Hyphantria cunea* Drury 1773 (Lepidoptera: Arctiidae), was investigated. Mortality was recorded daily for three days after treatment. Leaf disc bioassays revealed that all three oils had strong insecticidal activity on the experimental insects insofar as 50% lethal concentrations (LC_{50}) for *E. oleosa*, *E. spathulata,* and *E. largiflorens* at 24 h exposure time were 0.36, 0.61, and 1.24%, respectively. The time needed to kill 50% (LT_{50}) values were calculated as 9.09 h with *E. largiflorens*, 11.03 h with *E. oleosa*, and 13.03 h with *E. spathulata* at the highest concentrations (2.5% for *E. largiflorens*, 2% for *E. oleosa*, and 2.5% for *E. spathulata*). Based on probit analysis, an increase in the susceptibility of the insect was associated with an increase in the different concentrations of all oils and the increase in the time of exposure. The results of this study show that leaf essential oils of *E. largiflorens*, *E. oleosa,* and *E. spathulata* might be considered as a potent source for the production of fine natural larvicides.

Key words: essential oil, *Eucalyptus largiflorens*, *Eucalyptus oleosa*, *Eucalyptus spathulata*, *Hyphantria cunea*, larvicidal

INTRODUCTION

The fall webworm or American white moth, *Hyphantria cunea* Drury 1773 (Lepidoptera: Arctiidae), is native to North America but spread into central Europe and Asia in the 1940s (Ito and Miyashita 1968). This polyphagous defoliator has caused great damage to forests, urban ornamental trees, and agricultural crops in China because of its wide host range (Su *et al*. 2008). Young larvae feed upon the upper and lower leaf surfaces, leaving the veins. Larger larvae feed on the whole leaves and build impressive silk webs that sometimes enclose entire branches (Yarmand *et al*. 2009). The feeding activity of this pest leaves the trees without leaves. Photosynthesis is significantly decreased, causing low quality of the fruits and several deficiencies in wood processing. An outbreak of fall webworms can completely defoliate host trees. Several consecutive defoliations can cause dieback in the crown, and may contribute to the death of weak, declining trees (El-Sayed *et al*. 2005). As many as 175 different plant species within 49 families and 108 genera have been known to serve as hosts to the fall webworm (Yang *et al*. 2006).

The management of *H. cunea* has been typically carried out by chemical insecticides. But, chemical insecticides are associated with environmental contamination, high levels of resistance and damage to non-target organisms (Elbert and Nauen 2000; Roditakis *et al*. 2005). To protect crops in modern agriculture and an increasingly regulated world, natural plant-based insecticides can be a feasible plant pest management method and an attractive alternative to synthetic chemical insecticides. Botanicals reputedly pose little threat to the environment, non-target organisms or to human health (Isman 2006). Plant essential oils may be an alternative source for *H. cunea* control because these are secondary metabolites and a source of bioactive chemicals that plants produce for defense against herbivory and disease (Suthisut *et al*. 2011). Essential oils are the steam-distillable fraction of plant tissues. These oils are often responsible for a plants distinctive scent or taste. Essential oils have a rather complex composition, with component compounds generally consisting of low-molecular-weight monoterpenes (10-carbon) and related phenols (Toloza *et al*. 2006). The main characteristics of the essential oils are that they are easily extractable, ecofriendly, biodegradable, possess low or no toxicity against mammals, and are very effective against a wide spectra of insect pests (Lucia *et al*. 2012).

Although previous reports focused on the potential effectiveness of selected essential oils and their compo-

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nents as insecticides (Tripathi *et al*. 2009; Ebadollahi *et al*. 2010a; Ebadollahi 2011; Regnault-Roger *et al*. 2012; Ebadollahi 2013), we know of only two data sources written on the susceptibility of *Hyphantria* to plant essential oils (Zibaee *et al*. 2010; 2011). In these two studies, the biological effects of the essential oils isolated from *Artemisia annua* L. (Asterasea) and *Lavandula stoechas* L. (Lamiacaea) against *H. cunea* were demonstrated.

The genus *Eucalyptus* has leaves that contain aromatic oils with a characteristic odour whose recovery by steam distillation produces essential oils (Denny 2002). *Eucalyptus* has been prized as a rich source of essential oil. The oil has been used commercially in food, flavoring, and perfumery, and in the pharmaceutical industries (Batish *et al*. 2008; Singh *et al*. 2009). In addition, the essential oils of the *Eucalyptus* species possesses important biological activities including analgesic, antibacterial, antiinflammatory, antimalarial, antiseptic, diaphoretic, disinfectant, and antioxidant properties (Cimanga *et al*. 2002; Elaissi *et al*. 2012).

Iran has rich flora containing essential oils useful for medicinal purposes. Iranian plants show potential utilization in insect pest management programs due to their availability, efficiency, and because they are safe for the environment and non-target organisms (Ebadollahi 2011). As part of our ongoing research on natural insecticides from the flora of Iran, three *Eucalyptus* species including *E. largiflorens* Meull, *E. oleosa* Muell, and *E. spathulata* Hook were collected and tested on *H. cunea* larvae. This approach will allow us to identify natural and safer agents for the development of biorational insecticides to manage *H. cunea*.

MATERIAL AND METHODS

Plant materials

The leaves of *E. largiflorens*, *E. oleosa,* and *E. spathulata* were collected in May 2013 from Kashan, Isfahan province, Iran. A voucher specimen of the plants with the voucher specimen numbers of KBG 1493 for *E. oleosa*, KBG 1344 for *E. largiflorens,* and KBG 1462 for *E. spathulata* were deposited at the herbarium of Kashan botanical garden. The leaves were dried in the shade (at room temperature).

Extraction of essential oils

Essential oils were isolated by hydrodistillation of 50 g of dried plant material in 500 ml of distilled water for 12 h using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4–5°C prior to the bioassay. The yield of the extraction was calculated based on the dry weight of the each sample.

Insect

Hyphantria cunea larvae were collected from sycamore in Guilan University, Rasht, Iran. They were reared on mulberry leaves in the laboratory, in cages 30 × 30 × 40 cm. The insects were kept in the lab at the temperature of 27±2°C, and 60±5% relative humidity (RH) with 14:10 (L:D) photoperiod. The 3rd instar larvae were used to the experiments.

Bioassay

Different concentrations were prepared to evaluate insect mortality after an initial dose-setting experiment. Bioassay experiments were performed by five concentrations of essential oils (0.4, 0.63, 1, 1.58, and 2.5% from *E. largiflorens*, 0.1, 0.21, 0.45, 0.95, and 2% from *E. oleosa* and 0.2, 0.38, 0.61, 1.33, and 2.5% from *E. spathulata* in acetone as solvent) on 7 cm in diameter mulberry leaf discs in 9 cm in diameter petri dishes. Two fresh mulberry leaf discs were dipped in 20 ml of each concentration for 20 s. After the acetone evaporated (2 min), ten larvae were placed on treated leaf discs in each petri dish. The control insects were kept under the same conditions without any essential oil. Each concentration was replicated four times. The number of dead and live insects for each essential oil was counted at the end of 24, 48, and 72 h exposure periods.

Statistical analysis

Experiments were arranged in a completely randomized design and the means were separated by Tukey's test at the 5% level. The lethal concentration (LC_{50} and LC_{90}) and lethal time (LT_{50} and LT_{90}) values with their fiducial limits were calculated by probit analysis with SPSS software.

RESULTS AND DISCUSSION

The essential oils isolated by hydro-distillation from the leaves of three *Eucalyptus* species were found to be light yellowish for *E. oleosa* and *E. spathulata,* to terra cotta for *E. largiflorens* liquids. The obtained oil yields for *E. largiflorens*, *E. oleosa,* and *E. spathulata* were 4.5%, 6.7%, and 1.7% (w/w), respectively.

There are several reports showing the insecticidal activity of *Eucalyptus* essential oils and their main components, in different species (Lucia *et al*. 2007; Batish *et al*. 2008; Toloza *et al*. 2008; Toloza *et al*. 2010; Ebadollahi *et al*. 2010b) but the present study is the first to show that essential oils from *E. spathulata*, *E. largiflorens,* and *E. oleosa* can function as insecticide against *H. cunea*. On the other hand, to the best of our knowledge, there is no published report on the essential oil activities of *Eucalyptus* against *H. cunea*. Figure 1 displays the mortality percentages of five different essential oil concentrations used three times on *H. cunea* larvae. A comparison of the means showed that there were significant differences in the mortality of *H. cunea* exposed to different essential oil concentrations for 24, 48, and 72 h (Fig. 1). All essential oils revealed strong toxicity against the *H. cunea* larvae at several concentrations and exposure times. The lethal concentration of 50% (LC_{50}) for *E. oleosa*, *E. spathulata,* and *E. largiflorens* at 24 h exposure time were 0.36, 0.61, and 1.24%, respectively. Therefore, *E. oleosa* was most efficient and *H. cunea* larvae were most susceptible (Table 1). The time needed to kill 50% of the population (LT_{50} values) were calculated as 9.09 h with *E. largiflorens*, 11.03 h with *E. oleosa,* and 13.03 h with *E. spathulata* at the highest concentrations (2.5% for *E. largiflorens*, 2% for *E. oleosa* and 2.5% for *E. spathulata*). Generally, LT_{50} values decreased when the essential oil concentration increased (Table 2). Based on probit analysis, the susceptibility of the insect increased with exposure

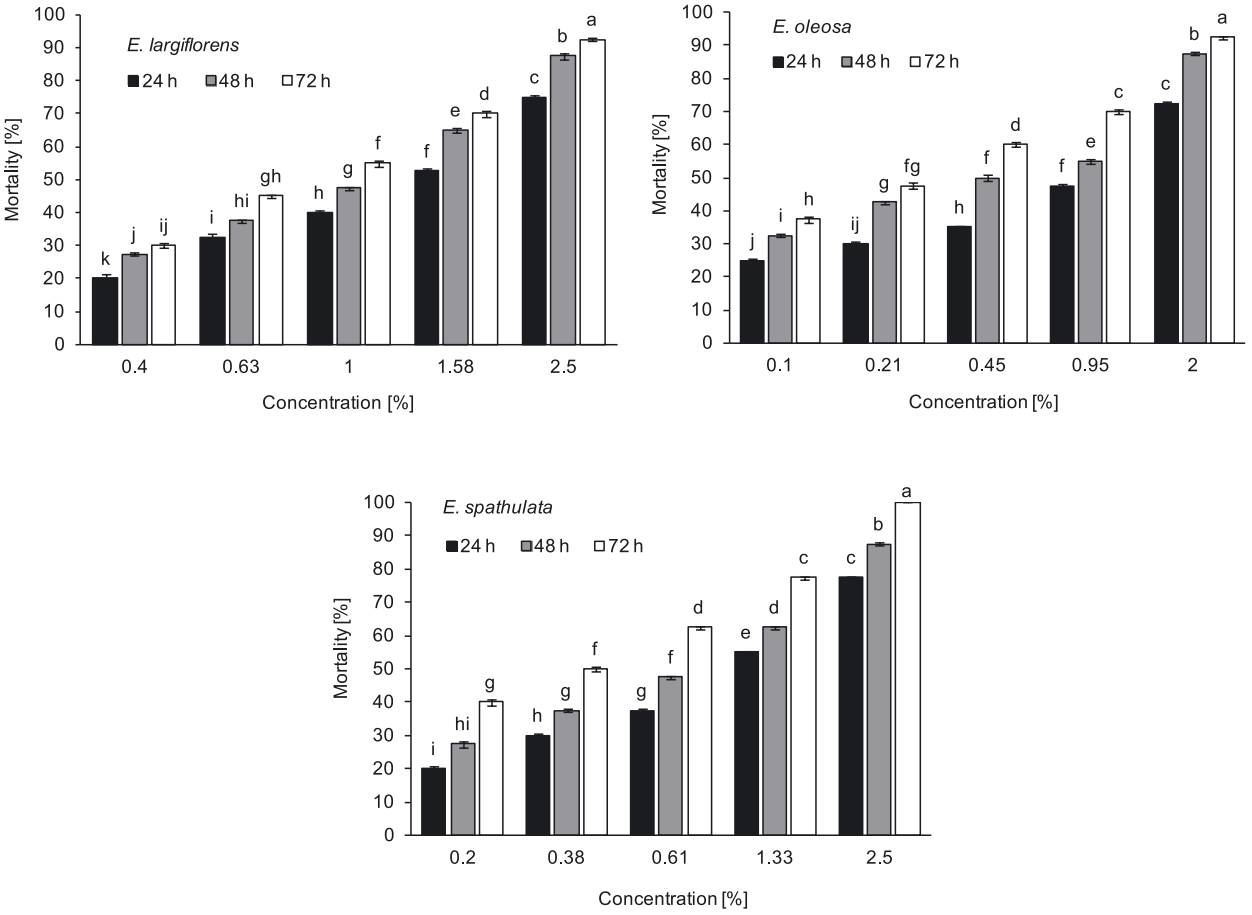

Fig. 1. Mean mortality of *H. cunea* larvae exposed to different concentrations of the essential oils from *E. largiflorens*, *E. oleosa*, and *E. spathulata*. Different letters on top of columns are significant differences according to Tukey's test at p = 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error of the mean (±); very small values are not represented

Table 1. Results of probit analysis for LC_{50} values from the toxicity of essential oils of *E. largiflorens*, *E. oleosa*, and *E. spathulata* against 3rd instar larvae of *H. cunea* at three different times

Essential oil	Time [h]	LC_{50}[a] [%]	Slope	Intercept	Chi-square [df = 3]	Sig
E. largiflorens	24	1.24 (0.98–1.68)	1.77	4.83	1.09	0.78*
	48	0.90 (0.71–1.11)	2.05	5.09	2.16	0.54*
	72	0.76 (0.59–0.92)	2.16	5.26	2.42	0.49*
E. oleosa	24	0.77 (0.49–1.51)	0.92	5.10	2.72	0.44*
	48	0.36 (0.22–0.54)	1.01	5.45	5.30	0.15*
	72	0.23 (0.13–0.33)	1.17	5.74	2.66	0.45*
E. spathulata	24	0.91 (0.67–1.31)	1.41	5.06	0.95	0.81*
	48	0.61 (0.44–0.82)	1.47	5.32	2.13	0.54*
	72	0.35 (0.08–0.61)	1.75	5.81	5.55	0.14**

[a]95% lower and upper fiducial limits are shown in parenthesis

*since the significance level is greater than 0.15, no heterogeneity factor is used in the calculation of confidence limits

**since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits

Table 2. Results of probit analysis for LT_{50} values from the toxicity of essential oils of *E. largiflorens*, *E. oleosa*, and *E. spathulata* against 3rd instar larvae of *H. cunea* at the three high concentrations

Essential oil	Concentration [%]	LT_{50}[a] [h]	Slope	Intercept	Chi-square [df = 1]	Sig
E. largiflorens	1.00	39.47 (19.23–51.17)	1.50	2.59	1.45	0.23*
	1.58	20.37 (13.26–39.12)	0.98	3.72	0.01	0.90*
	2.50	9.09 (0.00–19.19)	1.98	3.47	0.01	0.98*
E. oleosa	0.45	41.31 (27.16–57.27)	1.83	2.04	0.57	0.45*
	0.95	29.61 (11.05–46.78)	1.15	3.30	0.54	0.46*
	2.00	11.03 (0.08–20.43)	1.78	3.14	0.04	0.95*
E. spathulata	0.61	47.51 (14.15–66.85)	1.16	3.05	0.18	0.67*
	1.33	20.62 (6.59–33.73)	1.22	3.39	0.64	0.42*
	2.50	13.03 (0.06–23.59)	2.56	2.14	2.87	0.09**

[a]95% lower and upper fiducial limits are shown in parenthesis

*since the significance level is greater than 0.15, no heterogeneity factor is used in the calculation of confidence limits

**since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits

time and essential oil concentrations. On the other hand, there was an increased susceptibility of the insect associated with an increase in the different concentrations of all oils and time of exposures (Table 1 and 2). For example, the LC_{50} value for *E. spathulata* essential oil decreased from 0.91% at 24 h exposure time to 0.61, and 0.35% after 48 and 72 h, respectively (Table 1).

The chemical composition of the essential oils of *E. largiflorens*, *E. oleosa,* and *E. spathulata* has been evaluated in many studies. For example, twenty-six compounds were characterized in the oil of *E. largiflorens* with 1,8-cineole (37.5%), p-cymene (17.4%) and neoisoverbenol (9.1%) as the main components (Sefidkon *et al*. 2007). In the study of Rahimi-Nasrabadi, Nazarian *et al*. (2013), 1,8-cineole (23.1%), cryptone (15.1%), 4-allyloxyimino-2-carene (11.2%), and 4-terpineol (9.6%) were found to be the main constituents of the *E. largiflorens* essential oil. Twenty-one compounds were identified in the oil of *E. spathulata* with 1,8-cineole (72.5%) and α-pinene (12.7%) as the main components (Sefidkon *et al*. 2007). Zhang *et al*. (2012) found that the *E. spathulata* oil contained 60 compounds, predominantly 1,8-cineole (52.9%) and α-pinene (31.0%). The main components identified in *E. oleosa* were 1,8-cineole (89.4%), β-pinene (1.2%), and α-pinene (1%) (Jaimand *et al*. 2009). Essential oils obtained by hydrodistillation from different plant parts (stems, adult leaves, immature flowers, and fruits) of *E. oleosa* were screened for their chemical composition by Naceur Ben-Marzoug *et al*. 2011. According to GC-FID (gas chromatography-flame ionization detection) and GC-MS (gas chromatography-mass spectrometry), the principal compound of the stem, immature flowers, and the fruit oils was 1,8-cineole, representing 31.5%, 47.0% and 29.1%, respectively. Spathulenol (16.1%) and γ-eudesmol (15.0%) were the two principal compounds in the oil of the adult leaves. The principal differences in the four essential oils were related to 1,8-cineole, spathulenol, and γ-eudesmol: the highest percentage of 1,8-cineole (47.0%) was detected in essential oil from immature flowers, whereas it was lowest in adult leaves (8.7%). In the study of Rahimi-Nasrabadi, Pourmortazavi *et al*. (2013) on essential oil isolated from *E. oleosa* in Iran, the major constituents were 1,8-cineole (45.1%), α-pinene (14.5%), and α-terpineol (4.3%).

The results showed that 1,8-cineole was the main component of the essential oils of all the *Eucalyptus* species, but the main constituents of the previously mentioned essential oils were different. These differences might have been derived both from harvest time and local, climatic and seasonal factors, or it may be hypothesized that these samples belong to a different chemotype (Rahimi-Nasrabadi, Nazarian *et al*. 2013). The high amounts of 1,8-cineole in the oils of *E. largiflorens*, *E. oleosa,* and *E. spathulata* is remarkable. More than 300 species of *Eucalyptus* contain volatile oils in their leaves. Out of these 300 species, fewer than 20 are known for their high content of 1,8-cineole (more than 70%). These 20 have been commercially used for the production of essential oils in the pharmaceutical and cosmetic industries (Pino *et al*. 2002). Furthermore, previous studies have shown that, in general, the toxicity of plant essential oils against insect pests is related to their major components such as 1,8-cineole (Choi *et al*. 2006; Toloza *et al*. 2006; Alzogaray *et al*. 2011; Akhtar *et al*. 2012).

The toxicities of essential oil's main components and their relationship, have been investigated by many researches. Regression analysis of component concentrations of different *Eucalyptus* species with toxicity parameters obtained in *Aedes aegypti* Linnaeus larvae and adults showed significant correlations. Relationships were revealed between larvae mortality and the concentration of 1,8-cineole and p-cymene. This indicated that *Eucalyptus* species with a higher 1,8-cineole concentration and lower p-cymene concentration have less effect on *A. aegypti* larvae (Lucia *et al*. 2008). Toloza *et al*. (2008) determined the fumigant activity of essential oils from *Eucalyptus* and its main components on *Pediculus humanus* Linnaeus capitis De Geer, and a simple regression analysis revealed a significant correlation between KT_{50} data and the percentage of 1,8-cineole in these essential oils. Furthermore, a significant correlation was observed between the content of 1,8-cineole in *Eucalyptus* essential oils and the corresponding KT_{50} data in *A. aegypti* adults (Lucia *et al*. 2009). A similar correlation, between KT_{50} data produced by *Eucalyptus* essential oils and the percentage of 1,8-cineole, was found by Juan *et al*. (2011) in *Haematobia irritans* (Linnaeus) and Alzogaray *et al*. (2011) in *Blattella germanica*

(Linnaeus). Essential oils are complex mixtures of various molecules. Their biological effects might be either the result of a synergism of all the molecules or could reflect only those of the main molecules. In that sense, for biological purposes, it could be more informative to study the entire oil rather than some of its components because the concept of synergism seems to be important.

The mechanisms of essential oil toxicity have not been fully identified. However, rapid action of essential oils or its constituents against insect pests is indicative of neurotoxic actions. Treating the insects with essential oils or pure compounds may cause symptoms that indicate neurotoxic activity. These symptoms include hyperactivity, seizures, and tremors followed by knock down. Such symptoms are very similar to those produced by the insecticides pyrethroids (Isman 2006). Enan (2001) suggested that toxicity of the essential oil constituents is related to the octopaminergic nervous system of insects. Octopamine is a neurotransmitter, neurohormone, and circulating neurohormone-neuromodulator. Disruption of octopamine results in a total breakdown of the nervous system in insects. The lack of octopamine receptors in vertebrates provides the mammalian selectivity of essential oils as insecticides. Several reports indicate that essential oils cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity (Kostyukovsky *et al*. 2002; Houghton *et al*. 2006; Abd El-Galeil *et al*. 2009). However, some activity on the hormone and pheromone system and on the cytochrome P450 monooxygenase enzyme has also been seen (Tsao and Coats 1995; De-Oliveira *et al*. 1997). These reports in the literature show that there are varied target sites where the mode of action of the essential oils are located.

Results of this and earlier studies indicate that essential oils including *E. largiflorens*, *E. oleosa,* and *E. spathulata* are a source of biologically active vapors which may potentially prove to be efficient insecticides. Furthermore, the evaluated essential oils in the present study are used as pharmaceutical agents, and are thus considered to reducing the harmful effect of conventional insecticides on humans and the environment. Large quantities of plant material must be processed to obtain sufficient quantities of essential oils for commercial-scale tests. Breeding these plants in great quantities then becomes necessary. For the practical application of the essential oils as insecticides, further studies on the development of formulations are necessary to improve efficacy and stability, and to reduce cost.

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