INSECTICIDAL EFFECT OF SIX NATIVE MEDICINAL PLANTS ESSENTIAL OIL ON INDIAN MEAL MOTH, *PLODIA INTERPUNCTELLA* HÜBNER (LEP.: PYRALIDAE)

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ABSTRACT: The essential oils are considered powerful source of natural derivatives useful against stored product pests; they are as new control strategies. In this research it was investigated fumigant toxicity of six essential oils from *Melissa officinalis* L., *Mentha piperata* L., *Petroselinum sativum* Hoffmann, *Lavandula angustifolia* Mill., *Ziziphora clinopodioides* Lam., and *Artemisia dracunculus* L., on first instar larvae and eggs, ovipositional deterrency of *Plodia interpunctella* Hübner. LC_{50} values were determined. Ovicidal activity was assessed in Petri dishes on one-day old eggs that exposures three concentrations as 3, 12 and 24 micro liter essential oil per liter air. For studying effect of essential oils on oviposition deterrence one pair of new emergence adult were exposured by 2 µl essential oil. LC_{50s} were between 5 to 10 µl/l and *Z. clinopodioides* had most ovicidal activity. Increasing concentrations increased activity of essential oils. The least oviposited eggs have been observed in *L. angustifolia*, *M. piperata* and *Z. clinopodioides*. These results showed that *L. angustifolia*, and *Z. clinopodioides* had more ovicide and egg deterrence effect for this pest. The essential oils investigated in this study are used as pharmaceuticals and in flavoring. They are considered because they are less harmful to humans than most synthetic insecticides.

KEY WORDS: Essential oil, *Plodia interpunctella,* Insecticidal effect.

The Indian meal moth (IMM), *Plodia interpunctella* Hübner (Lep.: Pyralidae) is a major economic insect pest of stored products. The insect is a pest of grain, grain-based crops, and more than 20 different nuts, fruits, and candies from the agricultural system (Hamlin et al., 1931; Johnson et al., 1992). It prefers to feed on broken grains and more especially on milled cereal, dried fruits and almonds, pistachios and walnuts and groundnuts. It is found in warehouses (Perez-Mendoza & Aguilera-Pena, 2004). The larvae spin a silky web inside and on top of the food surface and feed in it. it contains larval excreta and gives an unpleasant odor to the infested food (Phillips et al., 2000). The loss of chemical insecticides and resistance to synthetic insecticides, new laws and interpretations of those laws, economic costs of pesticide regulations, consumer preferences and expectations, have important consequences for the management of IMM and other stored-product insects. The impending loss of the fumigant methyl bromide through compliance with the Montreal Protocol (Anonymous, 2004) will undoubtedly further affect management programs for IMM, accelerating the demand for new control strategies (Zettler, 1973; Phillips et al., 2000; Lee et al.

2001). There is therefore the need to develop safer alternatives to conventional fumigants. Several natural products, including essential oils from many species of spices, herbs and medicinal plants, are known to have a range of useful biological properties against insects (Regnault-Roger, 1997; Shaaya et al. 1997; Obeng-Ofori & Reichmuth, 1997; Isman, 2000; Tripathi et al., 2002; Mahfuz, 2007). The essential oils are useful to control stored product pests and their insecticidal activities are considerable (Lee et al., 2001).

Although many studies have demonstrated the fumigant activity of various essential oils and their constituents on adults and larval stages of stored grain insects, relatively little attention has been given to the egg stage(Obeng-Ofori & Reichmuth, 1997; Tripathi et al., 2002). Essential oils have a low toxicity to vertebrate, high volatility, and toxicity to stored-grain insect pests. Most of these oils are environmentally non-persistent and non-toxic to humans (with some exceptions), while being effective against several pest species. (Regnault-Roger, 1997; Shaaya et al. 1997; Isman, 2000; Tripathi et al., 2002; Mahfuz, 2007).

The biologically active materials derived from plant sources can act as larvicide, ovicide and oviposition deterrence. In recent years, essential oils have received much attention as potentially useful bioactive compounds against insects. Essential oils of *Myrtus communis* L., *Origanum syriacum* L., *Lavendula stoechas* L. have been documented for larvicidal activities towards *Culex pipens molestus* (Pushpanathan et al., 2006; Raja & William, 2008).Very little consideration has been given to the egg stage and only recently have some authors described toxicity of essential oils against eggs of stored product insects (Obeng-Ofori & Reichmuth, 1997) and they should have the ability to kill all stages of insects. (Yunc et al., 2000; Ngamo et al., 2007; Regnault-Roger, 1997).

In the present work, larvicidal and Ovicidal activity in addition to oviposition detterency of essential oils from six medicinal plants have been studied against IMM.

MATERIAL AND METHODS

Plant materials included *Lavandula angustifolia* Mill. (Leaves), *Artemisia dracunculus* L. (leaves), *Ziziphora clinopodioides* Lam. (leaves), *Melissa officinalis* L. (leaves), *Petroselinum sativum* Hoffmann (seeds) and *Mentha piperata* L. (leaves), were collected in Markazi province of Iran, from Agricultural Research Center. Plants were dried in dark, cut in pieces and hydrodistilled. The extraction of essential oils was carried out in a Clevenger-type.

The IMM was reared in growth chamber sets at 28±1 ^{0}C, 60±5% r.h., photoperiod of 11:13 (L: D) h. Larvae were reared in an artificial diet containing: wheat bran (800 g), brewers yeast (160 g), honey (200 ml), Glycerin (200 ml), Methyl-parabon (1 g), Choloramphenicol (1 g) (Sait et al., 1997). Adults after immergence were transferred to plastic funnel that covered with a net cloth for gathering eggs.

The experiment was carried out in four replications with first instar larvae. For each replication, essential oil solutions were poured in the inner surface of petri dish's door. After 20 minutes, ten larvae with one gram were placed in petri dishes and protected with parafilm. The mortality was recorded after 24 h. Data were analyzed by Probit analysis using the SAS 6.12.

To assess ovicidal activity of essential oils, each petri dish has divided to 80 cells by tangle-foot glue and has placed a one-day old egg in each cell. The eggs were treated with three concentrations of essential oils (3, 12 and 24 μl/l air) in three replications. The larval emergences were determined after 4 days. Percent of egg hatching was calculated by formula (Bruce et al., 2004).

$$\%EH = \frac{100 \times A}{B}$$

%EH= percent of egg hatching
A= number of hatched eggs
B= number of all eggs

Mortality in treatments was corrected by Abbott's formula (Abbott, 1925).

$$\%Mortality = \frac{(T - C) \times 100}{100 - C}$$

T=Mortality in treatment C= Mortality in control

Oviposition deterrency was studied with one pair of new adults treated with 2 µl essential oils. After four days, fecundity of moths was recorded and in all treatments were calculated percent of inhibitory of egg releasing comparison with control by formula (Chaubey, 2008).

$$\%I = 100 - \frac{Nt \times 100}{Nc}$$

I= Inhibitory of egg releasing Nt= Number of eggs in treatment
Nc= Number of eggs in control

All data was analyzed as completed randomized design SAS 6.12 (ANOVA). All means have been compared by Turkey's test at 95% probability.

RESULTS

Larvicidal activity of volatile oils against *P. interpunctella*: The results of larvicidal effects showed that all essential oils have toxicant effect on first instar larvae of Indian meal moth. LC_{50} of essential oils has been showed in table 1. Most and least LC_{50} of essential oils were observed in lemon balm and lavender about 5.57 and 9.11 µl/l, respectively. All essential oils were very toxicant for first instar larvae of *P. interpunctella* and LC_{50} of them were less than 10 µl/l.

Ovicidal activity of volatile oils against *P. interpunctella*: The results of this study revealed that the essential oils exhibited significant ovicidal activity against *P. interpunctella*. There are significant differences between essential oils at 1% ($F=33.12$; $df= 5, 54$; $P<0.01$). Also there are significant differences between concentrations at 1% ($F=322.16$; $df= 2, 54$; $P<0.01$).

The results showed that by increasing concentration of essential oils, percent of egg mortality increases. Eggs mortality has been recorded in 3, 12 and 24 µl/l concentrations about 29, 42 and 75.38 percent, respectively. This experiment also showed there are significant differences between egg mortality that induced by essential oils. *Ziziphora clinopodioides* and *L. angustifolia* had more ovicidal effect about 68.73 and 53.78 percent, respectively. Between other essential oils there are no significant differences, but they have Ovicidal effect about 40-46% (Table 2).

This experiment also shows there is significant difference between egg mortality induced by essential oils with different concentrations (Table 2). Essential oils showed variable toxicity to eggs of *P. interpunctella*. *L. angustifolia***,** *Z. clinopodioides* and *A. dracunculus* essential oils at 24 micro liters per liter concentration had most ovicidal effect about 85.98, 84.24 and 82.75, respectively.

The lowest ovicidal effect has been induced by *A. dracunculus, P. sativum* and *M. officinalis* at 3 µl/l. It is observed that in all concentrations *Z. clinopodioides* had most Ovicidal effect.

Effect of essential oils on oviposition of *P. interpunctella* adults: The results of analyses of variance effect of essential oils on oviposition of *P. interpunctella* adults showed that there was significant difference at 1% between percent of egg released in different treatments (essential oils) (F=30.97; df= 5, 18; P<0.01). It is observed the least inhibitory of egg released in *A. dracunculus* treatment. Releasing eggs were inhibited about 36.17%. There were not any significant differences between other essential oils and egg releasing in adults were inhibited in *P. sativum, M. officinalis, L. angustifolia*, *M. piperata* and *Z. clinopodioides* about 82.17, 96.01, 97.50, 98.83 and 99.67 percent, respectively (Figure 1). These results showed except *A. dracunculus* all essential oils had high deterrence of egg oviposition effect on Indian meal adult as fumigant pesticide.

DISCUSSION

In this study the efficacy of the oils followed in the order Parsley, Lavender, Tarragon, *Z. clinopodioides,* Lemmon balm, and Peppermint. The LD50 values in case of 24 h after treatment were 9.46, 9.21, 7.21, 6.48, 5.57, and 8.09 on first instar larvae of Indian meal moth, respectively. Regnault-Roger (1997) found LC_{50} of Lavender and Peppermint on *Acanthoscelides obtectus* was 12.6 and 22.4. The differences between our results with another researches is about the differences between insects. Mirkazemi (2007) showed LC_{50} of five essential oils on *Tribolium confusum* and *Callosobruchus maculatus.* LC_{50} of *A. dracunculus* and *L. verra* has been achieved about 26.24 and 50.33 on *C. maculatus* and 9.07 and 19.44 on *T. confusum*, respectively. In our results LC_{50} were less than it, it may be the cause of different of insects in two experiments. First instar larvae of *P. interpunctella* are more susceptible to essential oils.

The results of Raja & William (2008) revealed that the essential oils exhibited significant ovicidal activity against the cowpea beetle. Among the treatment categories citrodora oil showed 88.43% protection followed by citronella oil 58.01% protection. The oils tested showed moderate effect on the eggs of *C. maculatus*. Bioactive compounds of plant origin having insecticidal and ovicidal properties are being used as grain protection agents against beetles in storage (Raja & William, 2008).

Risha et al., (1990) have reported oil vapors have strong latent effect toxic to adults and eggs of *C. pahseoli.* Singh et al., (1995) reported plant volatile oils have strong odor that will block the tracheal respiration by *S. oryzae* in stored sorghum, which ultimately kill the insect (Raja & William, 2008). The highest mortality in the egg chorion and embryo died due to decrease of oxygen for respiration (Raja & William, 2008).

The difference observed among the mortality due to these oils is due to their active volatiles mostly monoterpenes which are very active on insects. The amount of eggs laid by treated adults is significantly lower than control. Some essential oils are able to halt the egg incubation or to kill the emerging larvae. The present work gives evidence on the negative impact of the application of these essential oils on adult's oviposition. Lethal concentrations of essential oil need important quantity of material for their extraction (Ngamo et al., 2007). Many environmental factors affect the breakdown of essential oils, most importantly, temperature and light. Limited residual toxicity is an important advantage for these pesticides (Isman, 2000).

The essential oils investigated in this study are used as pharmaceuticals and in flavoring and are therefore considered less harmful to humans than most conventional insecticides and they can use as safe fumigants for controlling *P. interpunctella*.

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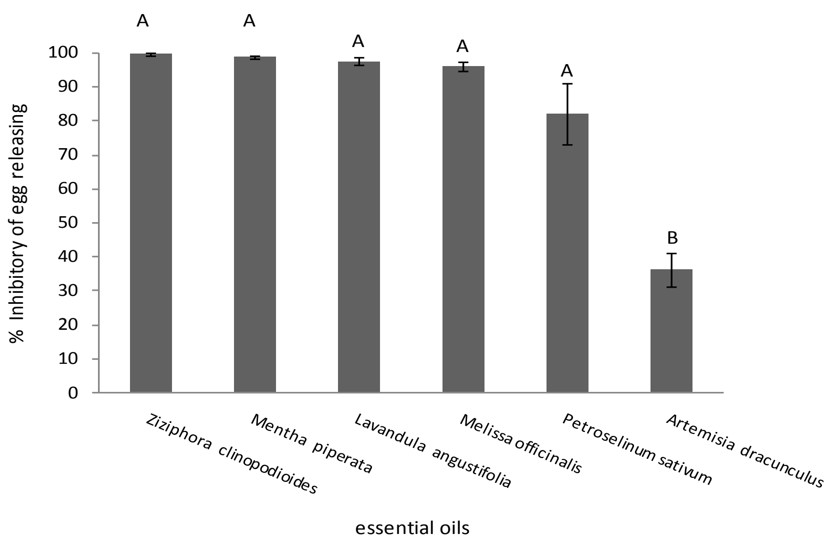

Figure 1. Effect of fumigation of essential oils on oviposition of *Plodia interpunctella* (vertical bars indicate standard error of means and the same letters on each column bar indicate no significant difference using Tukey's test at 5% level).

Table 1. LC_{50} of six essential oils on first instar larvae of *Plodia interpunctella* adults.

Essential oil	χ^2 (df=3, n=240)	Slope±SE	LC_{50} (µl/l)	95% fiducial limits (µl/l)	
				lower	upper
Artemisia dracunculus	2.57	0.84 ± 0.21	7.29	3.79	12.16
Lavandula angustifolia	3.62	2.43 ± 0.56	9.11	3.44	13.45
Melissa officinalis	1.60	1.46 ± 0.27	5.57	3.70	7.60
Mentha piperata	0.58	1.53 ± 0.29	8.09	5.40	11.25
Petroselinum sativum	1.99	0.99 ± 0.24	9.46	5.42	14.58
Ziziphora clinopodioides	3.75	1.59 ± 0.32	6.50	2.48	11.00

Table 2. Effect of essential oils on percentage of eggs mortality of *Plodia interpunctella* at different concentrations.

plants	Concentration (µl/l air)			Mean ± SE
	3	12	24	
Artemisia dracunculus	15.68 ± 5.22^{b}	40.37 ± 3.16^{BC}	82.75 ± 3.49^{a}	46.27 ± 8.62 ^{BC}
Lavandula angustifolia	31.43 ± 4.77^{b}	43.95 ± 0.98^{B}	85.98 ± 2.51^{a}	53.78 ± 7.23 ^{B}
Melissa officinalis	23.15 ± 1.75^{b}	37.05 ± 2.22^{BC}	64.15 ± 1.76^{b}	41.45 ± 5.23 ^{C}
Mentha piperata	28.36 ± 4.40^{b}	33.45 ± 1.82^{C}	61.12 ± 5.85^{b}	40.98 ± 4.90^{C}
Petroselinum sativum	16.34 ± 1.97^{b}	36.40 ± 1.61^{BC}	74.06 ± 4.41^{ab}	42.27 ± 7.38 ^{C}
Ziziphora clinopodioides	59.06 ± 1.19^{a}	62.88 ± 2.53^{A}	84.24 ± 3.20^{a}	46.27 ± 3.57 ^{A}

Means ± SE by the same letters in each column are not significantly different using Tukey's test at 5% level