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Acute and chronic toxicity of *Ziziphora clinopodioides* and *Ferula gummosa* essential oils against *Plodia interpunctella* (Lepidoptera: Pyralidae)

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Abstract
Growing concerns of chemical pesticides lead the researchers around the world to find new avenues of insect pest control. Plant essential oils could be considered as one of the ecofriendly alternative for synthetic pesticides. In the present research, lethal and sublethal toxicity of *Ziziphora clinopodioides* Lam. and *Ferula gummosa* L. oils were studied against *Plodia interpunctella* (Hübner). Results of bioassays showed that the estimated LC_{50} values of *Z. clinopodioides* and *F. gummosa* oils were 96.98 and 49.82 µl/l air against one-day-old eggs and 40.68 and 38.91 µl/l air for four-day-old eggs, respectively. LC_{50} values of *Z. clinopodioides* and *F. gummosa* oils were 10.12 and 7.49 µl/l air on 1st instar larvae and 34.11 and 12.66 µl/l air on 5th instar larvae, respectively. Moreover, *Z. clinopodioides* oil (LC_{50} = 25.77 µl/l air) was significantly more toxic than *F. gummosa* oil (LC_{50} = 28.72 µl/l air) to the adult moths. Based on the estimated LT_{50} values, it was determined that persistency of *Z. clinopodioides* and *F. gummosa* oils were 6.31 and 7.56 days, respectively. Increasing the oils concentration resulted in a significant increase in oviposition deterrenc. Also, hematology assays indicated that total hemocyte population of one-day-old 5th instar larvae was decreased as the oils concentration and exposure time increased. Collectively, results of the present study demonstrated the potent fumigant toxicity of *Z. clinopodioides* and *F. gummosa* oils against *P. interpunctella.* Also, tests on different growth and developmental stages of the studied moth showed clearly differential susceptibility levels of this pest against the oils.
Keywords: Essential oil, Bioassay, Oviposition deterrence, Hematology assay

سمیت حاد و مزمن اسانس کاکوتی چندساله *Ziziphora clinopodioides* و آنغوزه *Ferula gummosa* علیه شب‌پره هندی *Plodia interpunctella* (Lepidopters: Pyralidae)

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چکیده
تشدید نگرانی‌های حاصل از مصرف آفت‌کش‌های شیمیایی باعث شده تا پژوهشگران دنیا به دنبال راه کارهای جدید برای کنترل حشرات آفت باشند. اسانس‌های گیاهی می‌توانند به عنوان یکی از ایمن‌ترین جایگزین‌های آفتکش‌های شیمیایی در نظر گرفته شوند. در پژوهش حاضر، سمیت کشندگی و زیرکشندگی اسانس کاکوتی چندساله و آنغوزه روی شب‌پره هندی مطالعه شد. نتایج زیست‌سنجی‌ها نشان داد که مقادیر برآوردشده LC_{50} اسانس کاکوتی و آنغوزه علیه تخم‌های یک‌روزه به ترتیب ۹۶/۹۸ و ٤۹/۸۲ میکرولیتر بر لیتر هوا و روی تخم‌های چهارروزه به ترتیب ٤۰/۸٦ و ۳۸/۹۱ میکرولیتر بر لیتر هوا بود. مقادیر LC_{50} اسانس کاکوتی و آنغوزه روی لاروهای سن اول به ترتیب ۱۰/۱۲ و ۷/٤۹ میکرولیتر بر لیتر هوا و روی لاروهای سن پنجم به ترتیب ۳٤/۱۱ و ۱۲/٦٦ میکرولیتر بر لیتر هوا برآورد شد. همچنین، اسانس کاکوتی با LC_{50} معادل

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۲۵/۷۷ میکرولیتر بر لیتر هوا در مقایسه با اسانس آنغوزه با LC_{50} معادل ۲۸/۷۲ میکرولیتر بر لیتر هوا از سمیت بیشتری علیه حشرات کامل این شب‌پره برخوردار است. بر اساس میزان LT_{50} برآوردشده، میزان دوام اسانس کاکوتی و آنغوزه علیه حشرات بالغ به ترتیب ٦/٣١ و ٧/٥٦ روز بود. افزایش غلظت اسانس‌ها منجر به افزایش معنی‌دار میزان بازدارندگی تخم‌ریزی شد. همچنین، نتایج آزمایش‌های خون‌شناسی نشان داد که با افزایش غلظت اسانس و زمان اسانس‌دهی تعداد کل سلول‌های خونی لاروهای یک روزه سن پنجم به طور معنی‌داری کاهش یافت. به‌طور کلی، نتایج مطالعه حاضر نشان داد که اسانس کاکوتی و آنغوزه از سمیت تنفسی قابل توجهی علیه شب‌پره هندی برخوردار هستند. همچنین میزان حساسیت مراحل مختلف رشد و نمو این حشره به اسانس‌های مورد آزمایش، اختلاف معنی‌داری با یکدیگر دارد.

واژگان کلیدی: اسانس، زیست‌سنجی، بازدارندگی تخم‌ریزی، آزمایش خون‌شناسی

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Introduction

Insect pests often attack to a wide range of stored products which inflict great damage to farmers (Haque *et al*., 2000; Koul *et al*., 2008). In addition to negative impacts on quantity and quality of stored food crops, insects also affect the nutritive value, viability and marketability of such crops (Rajendran & Sriranjini, 2008). The Indian meal moth, *Plodia interpunctella* (Hübner), belonging to Pyralidae family is a cosmopolitan insect pest of stored products and processed food commodities. It can infest a variety of dry food stuffs and is perhaps the most economically important insect pest of processed food (Sauer & Shelton, 2002; Shojaaddini *et al*., 2005; Mohandass *et al*., 2007).

The use of agrochemicals has increased many folds over the past decades in agriculture sector, particularly storage systems which have undoubtedly resulted in a wide variety of serious health implications to our ecosystem. Despite their efficacy as the most common and effective tools to control stored-product pests (Huang & Subramanyam, 2005), chemical pesticides act like a double-edged sword causing negative effects on the environment, human health, non-target organisms, etc. (Isman, 2000; Lamiri *et al*., 2001). Therefore, it is a priority to find substances such as naturally derived compounds with less harmful consequences (Kordali *et al*., 2006; Regnault-Roger *et al*., 2012). Over the recent years, essential oils as biopesticides have received a specific attention by scientists all over the world and many scientific reports have shown their potential to apply in stored-product pest control programs. Essential oils which are rich in monoterpenes act in different routes such as insect killer (Ghasemi *et al*., 2014; Kheirkhah *et al*., 2015; Heidari *et al*., 2017; Naseri *et al*., 2017), repellant (Liu & Ho, 1999; Wang *et al*., 2006), oviposition deterrent (Kheirkhah *et al*., 2015; Heidari *et al*., 2017), antifeedant (Huang *et al*., 2000), etc., against various insect pests.

Hemocytes, especially plasmatocytes (PLs) and granulocytes (GRs) play vital roles in the cellular immune system of insects against biotic and abiotic stressors (Gupta, 1979; Gillespie *et al*., 1997; Lavine & Strand, 2002; Ghasemi *et al*., 2013; Ghasemi *et al*., 2014). It has been reported that chemical and non-chemical pesticides could affect some

hematological features of insect pests. Regarding this, Pandey *et al*. (2012) indicated that treating *Papilio demoleus* L. larvae with leaf extracts of *Eucalyptus globulus* Labill., *Ageratum conyzoides* L., and clove extract of *Allium sativum* L. caused a significant reduction in total hemocyte count (THC). In another study by Ghasemi *et al*. (2014), it was proved that topical application of *Callistemon viminalis* Gaertn. and *Ferula gummosa* oils resulted in a drastic reduction of total hemocyte count of *Ephestia kuehniella* Zeller larvae. In a recently published paper (Sadeghi *et al*., 2017), it is shown that the treatment of *Sesamia cretica* Lederer larvae by the essential oil of *Ferula ovina* Boiss decreased the numbers of total and differential hemocyte counts.

Ziziphora clinopodioides Lam. with the Persian name of "kakuti-e-kuhi" (Lamiaceae) and *Ferula gummosa* L. (Apiaceae) with the Persian name of "Barijeh" are the valuable plants widely used by Iranians because of their medicinal properties (Sonboli *et al*., 2006; Ghafari *et al*., 2006; Behravan *et al*., 2007; Murat & Pinar, 2009; Mahboudi, 2016). It has already been found that essential oils from these two plant species possess insecticidal activity against *E. kuehniella*, a very close species to *P. interpunctella* (Ghasemi *et al*., 2014; Kheirkhah *et al*., 2015). Nevertheless, no report is available on their insecticidal activity against *P. interpunctella*.

In this study, we aimed at assessing the fumigant toxicity of essential oils from *Z. clinopodioides* and *F. gummosa* on different growth and developmental stages of *P. interpunctella*. Changes in oviposition rate and hemocyte population of the insects treated with sublethal concentrations of the oils are also evaluated and discussed.

Materials and methods

Insect colony

Adults of *P. interpunctella* were obtained from an insectarium and reared on an artificial diet consists of 200 g wheat bran, 40 g powdered yeast, 50 ml glycerol, and 50 ml honey within plastic containers (Sait *et al*., 1997). The cultures were kept in a growth chamber set at 27 ± 1 °C and 65 ± 5% R.H. in darkness.

Plant samples

Leaves of *Z. clinopodioides* and roots of *F. gummosa* were collected from their natural habitats in Shirvan, North Khorasan province, Iran, during 2015-2016. The plant material was dried in the shade with proper ventilation and then maintained at -20 °C until required.

Extraction of the essential oils

Extraction of essential oil from the plant materials was carried out using a modified Clevenger-type apparatus at the following condition: 50 g of air-dried material, 600 ml distilled water, and 4 h distillation. The yield of the essential oil from *Z. clinopodioides* and *F. gummosa* was 2.5 ± 0.2% and 1.8 ± 0.15% (v/w based on dry weight), respectively. Anhydrous sodium sulfate was used to remove water after extraction. The resulting oil was poured into sealed glass containers and stored in refrigerator at 4 °C.

Fumigant toxicity on the eggs

To determine the fumigant toxicity of the oils, twenty-one day-old and four-day-old *P. interpunctella* eggs were separately put in a glass petri dish (3 cm diameter and 1 cm height). For better observation of the white eggs, a black paper was installed on the bottom of dishes. The eggs were treated with random concentrations of the oils. After preliminary dose-setting tests, the final concentrations of the oil causing 5–95% egg mortality were obtained based on logarithmic distance (Robertson *et al*., 2007). The final concentrations of the oils were infused on the filter paper (Whatman No. 1, cut into 2 cm diameter pieces) and then were attached to the lid of dishes. Oils were applied as pure using microapplicator. The lids of dishes were sealed tightly with parafilm. Control eggs received no oil. Each concentration was replicated four times. The eggs were fumigated for 24 h and then after were kept in a clean glass Petri dish until complete hatch of untreated eggs (almost 4 days). After that, number of hatched eggs in treated dishes was counted using a stereomicroscope. Eggs were considered hatched when 1st instar larvae were observed.

Fumigant toxicity on the larvae

Like the previous experiment, twenty 1st and 5th instar larvae were separately placed into 280 ml dark glass bottles with screw lids and then were treated with final concentrations of tested oils for 24 h. At the end of each trial, treated larvae were gently stimulated with a paint brush and when no movement was observed, insects were considered dead.

Fumigant toxicity on the adults

In the case of adult moths, twenty newly emerged individuals of mixed-sex were released into a 280 ml dark glass bottles with screw lids and then were treated with calculated concentrations of the oils. Number of dead and alive insects in each vial was counted 24 h after commencement of exposure to the oils. When no leg or antennal movements were observed, insects were considered dead.

All toxicology tests were carried out at 27 ± 1 °C and 65 ± 5% R.H. in darkness. Percentage insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott, 1925).

Durability of oils' toxicity

In this experiment, one-day-old adult moths of both sexes were treated with the concentration of the oils causing 90% mortality (LC_{90}) in dark glass vials of 280 ml in different time intervals. To do so, the estimated concentration of the oils were infused on the filter paper (Whatman No. 1, cut into 2 cm diameter pieces) and then were attached to the lid of 280 ml dark glass vials. The lids of vials were sealed tightly with parafilm. Then, 20 adult moths were released into each vial 1, 3, 5, 7, 9, 11, 13, 15, and 17 days after commencement of exposure to the oils. The lids of vials were sealed again tightly using parafilm and insect mortality was recorded after 24 h. Control moths received no oil. Each concentration was replicated four times.

Oviposition deterrence tests

In this experiment, newly emerged adult moths of both sex were selected for each concentration and transferred to dark glass vials (280 ml) using an aspirator. Male and female moths were separately fumigated with the estimated concentrations of tested oils (LC_{10}, LC_{30}, and LC_{50}) for 24 h. Cap of the vials were screwed and sealed tightly with Parafilm. No oil was applied in control vials. Then, two pairs (2 males and 2 females) of treated alive insects were transferred to a clean single-use glass containing 5 g of mung bean grains. Insects were maintained in a germinator set at 27 ± 1 °C and 65 ± 5% R.H. in darkness. Four replications were used for each concentration. The number of laid eggs per female was recorded after 96 h. Oviposition deterrence was calculated with the following formula:

$$\%\text{Oviposition deterrence} = \left[1 - \left(\frac{NEt}{NEc}\right)\right] \times 100$$

Where, NE_t is the number of eggs in treatment and NE_c is the number of eggs in control.

Hematology assays

To count total hemocytes of the one-day-old 5th instar larvae treated with the estimated concentrations of the oils, the hemolymph sample was obtained from the severed proleg of insects using a microapplicator and immediately diluted in Tyson buffer (NaCl 2.72 mM; Na_2SO_4 8.96 mM; glycerol 43.68 mM; methyl violate 0.061 mM). Cell counting was conducted 24 and 48 h post treatment using a standard hemocytometer. The cells were counted by a light microscope and number of total hemocytes per cubic millimeter (mm^3) was calculated according to the following formula (Jones, 1962):

$$\frac{\text{Hemocytes in x } 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Where, dilution = 50 times, depth factor of the chamber = 10 (constant) and No. of squares counted = 5.

Data analyses

The LC values and 95% confidence limits were calculated from probit regressions (Finney, 1971) using the POLO-PC computer program (LeOra Software). Data from oviposition deterrence, durability of oils' toxicity, and hematology assays were analyzed using the SPSS program version 21.0 for analysis of variance (ANOVA). All data were tested for normality with Kolmogorov–Smirnov test. If needed, data were transformed to meet statistical assumptions. Differences were considered significant at $P<0.05$ using Tukey's test.

Results

Fumigant toxicity tests

Results obtained from the fumigant toxicity of *Z. clinopodioides* and *F. gummosa* oils on *P. interpunctella* eggs are presented in Table 1. The estimated LC_{50} values of *Z. clinopodioides* and *F. gummosa* oils were 96.98 and 49.82 µl/l air against one-day-old eggs and 40.68 and 38.91 µl/l air for four-day-old eggs, respectively. It was also shown that susceptibility of the eggs to the oils increased as age of embryo was increased from one-day-old to four-day-old. According to the estimated relative median potency (RMP), four-day-old eggs were 2.26 and 1.27 times more sensitive than one-day-old eggs to *Z. clinopodioides* and *F. gummosa* oils, respectively.

LC values of tested oils against *P. interpunctella* larvae are presented in Table 2. The estimated LC_{50} values of *Z. clinopodioides* and *F. gummosa* oils were 10.12 and 7.49 µl/l air on 1st instar larvae and 34.11 and 12.66 µl/l air on 5th instar larvae, respectively. It was also shown that 5th instar larvae were more tolerant than 1st instar larvae to the oils. According to the estimated RMP, 1st instar larvae were 3.35 and 1.63 times more sensitive than5th instar larvae to *Z. clinopodioides* and *F. gummosa* oils, respectively.

Moreover, data from the fumigant toxicity of *Z. clinopodioides* and *F. gummosa* oils on adult moths are indicated in Table 3. *Z. clinopodioides* oil (LC_{50} = 25.77 µl/l air) was significantly more toxic than *F. gummosa* oil (LC_{50} = 28.72 µl/l air) to the adult moths.

Table 1. LC values of *Ziziphora clinopodioides* and *Ferula gummosa*oils against *Plodia interpunctella* eggs after 24 h fumigation

Oils	Growth stage	n	LC values (µl.l^{-1} air)[a]				Pearson Goodness-of-Fit test			RMP (95% CL)[b]
			LC_{10}	LC_{30}	LC_{50}	LC_{90}	Slope±SE	X^2 (df)	*P*-value	
Z.clinopodioides	1-day-old eggs	560	61.16 (54.23-66.81)	80.31 (74.67-85.04)	96.98 (92.23-101.56)	153.78 (143.14-169.17)	6.40±0.55	3.35 (4)	0.500	2.26 (1.57-3.95)
	4-day-old eggs	560	18.12 (14.96-20.95)	29.30 (26.04-32.26)	40.86 (37.48-44.63)	92.11 (81.07-108.79)	3.63±0.29	2.49(4)	0.645	
F. gummosa	1-day-old eggs	560	21.96 (18.21-25.29)	35.63 (31.83-39.10)	49.82 (45.81-54.09)	113.04 (98.87-134.86)	3.60±0.29	0.49 (4)	0.974	1.27 (1.13-1.46)
	4-day-old eggs	560	19.47 (16.80-21.78)	29.31 (26.83-31.59)	38.91 (36.29-41.79)	77.75 (68.97-91.10)	4.26±0.35	1.46 (4)	0.833	

[a]LC values are expressed with their 95% confidence limits (CL)
[b]Relative median potency = LC_{50} of the oil against one-day-old eggs divided by LC_{50} of that oil on four-day-old eggs

Table 2. LC values of *Ziziphora clinopodioides* and *Ferula gummosa* oils against *Plodia interpunctella* larvae after 24 h fumigation

Oils	Growth stage	n	LC values (µl.l^{-1} air)[a]				Pearson Goodness-of-Fit test			RMP (95% CL)[b]
			LC_{10}	LC_{30}	LC_{50}	LC_{90}	Slope ± SE	X^2 (df)	*P*-value	
Z.clinopodioides	1st instar larvae	560	2.13 (1.51-2.75)	5.35 (4.37-6.34)	10.12 (8.66-11.86)	48.08 (36.78-68.66)	1.89 ± 0.15	2.34 (4)	0.673	3.35 (1.76-5.50)
	5th instar larvae	560	15.61 (13.12-17.81)	24.77 (22.28-27.07)	34.11 (31.46-36.96)	74.56 (65.61-88.11)	3.77 ± 0.30	5.30 (4)	0.258	
F. gummosa	1st instar larvae	560	3.20 (2.66-3.68)	5.29 (4.76-5.79)	7.49 (6.89-8.18)	17.53 (15.03-21.57)	3.47 ± 0.30	1.12 (4)	0.891	1.63 (1.24-2.32)
	5th instar larvae	560	3.47 (2.59-4.30)	7.45 (6.31-8.56)	12.66 (11.15-14.43)	46.20 (36.66-63.22)	2.28 ± 0.20	3.89 (4)	0.420	

[a]LC values are expressed with their 95% confidence limits (CL)
[b]Relative median potency = LC_{50} of the oil against fifth instar larvae divided by LC_{50} of that oil on first instar larvae

Table 3. LC values of *Ziziphora clinopodioides* and *Ferula gummosa* oils against *Plodia interpunctella* adults after 24 h fumigation

		LC values (µl.l^{-1} air)[a]				Pearson Goodness-of-Fit test			RMP (95% CL)[b]
Oils	n	LC_{10}	LC_{30}	LC_{50}	LC_{90}	Slope ± SE	X^2 (df)	*P*-value	
Z.clinopodioides	560	10.25 (8.40-11.88)	17.97 (16.12-19.70)	25.77 (23.64-28.24)	62.15 (52.74-77.80)	3.35 ± 0.29	3.07 (4)	0.546	1.11 (0.93-1.34)
F. gummosa	560	12.12 (5.73-6.31)	20.18 (14.79-24.65)	28.72 (23.39-35.81)	68.04 (50.19-127.17)	3.42 ± 0.29	10.56 (4)	0.032	

[a]LC values are expressed with their 95% confidence limits (CL)
[b]Relative median potency = LC_{50} of *F. gummosa* divided by LC_{50} of *Z.clinopodioides*

Table 4. LT values of *Ziziphora clinopodioides* and *Ferula gummosa* oils at LC_{90} against *Plodia interpunctella* adults

		LT values (day)[a]		Pearson Goodness-of-Fit test		
Oils	n	LT_{50}	LT_{90}	Slope ± SE	X^2 (df)	*P*-value
Z.clinopodioides	560	6.31 (5.72-6.88)	1.25 (0.7-2.17)	-0.20 ± 0.01	2.01 (6)	0.847
F. gummosa	640	7.56 (6.85-8.25)	0.83 (0.07-2.00)	-0.15 ± 0.01	2.47 (7)	0.871

[a]LT values are expressed with their 95% confidence limits (CL)

Durability of oils' toxicity

Results clearly indicated that the oils' toxicity decreased by time and after 15 and 17 days no mortality was observed in case of *Z. clinopodioides* and *F. gummosa* oils, respectively (Fig. 1). Based on the findings presented in Table 4, the estimated LT_{50} values of *Z. clinopodioides* and *F. gummosa* oils at LC_{90} were 6.31 and 7.56 days, respectively.

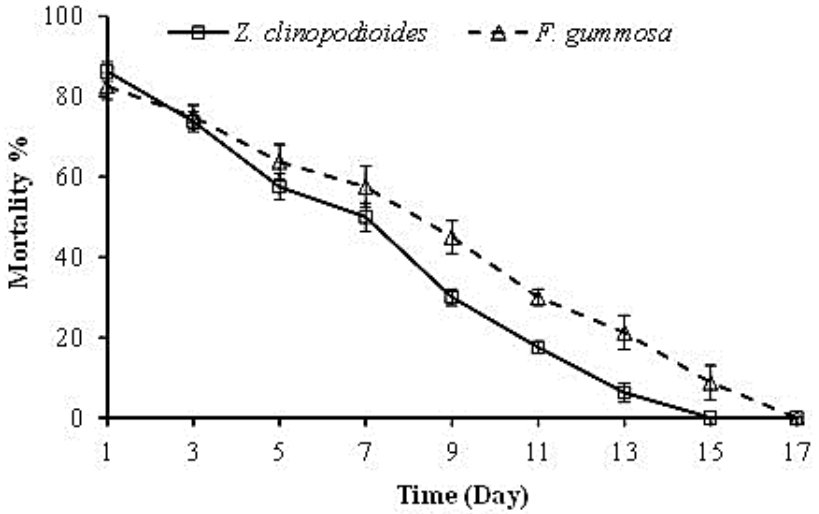

Fig. 1. Durability of *Ziziphora clinopodioides* and *Ferula gummosa* oils toxicity against *Plodia interpunctella* adults

Oviposition deterrence

Oviposition deterrence activity of different concentrations of the oils on the females of *P. interpunctella* is shown in Fig. 2. The results indicated that oviposition rate of treated female moths decreased as concentration of the oils was increased from LC_{10} to LC_{50} (Fig. 2). On the other hand, the oil's ovipositional deterrency significantly increased with increased concentrations of the oils (F = 13.26; df = 3; P< 0.000). In case of *Z. clinopodioides* oil, treating female moths with concentrations of LC_{10}, LC_{30}, and LC_{50} resulted in a 6.7, 50.42, and 84.02% decline in the oviposition rate, respectively. Also, treating the moths with concentrations of *F. gummosa* oil caused a 1.30, 29.25, and 54.01% decline in the oviposition rate, respectively (Fig. 2).

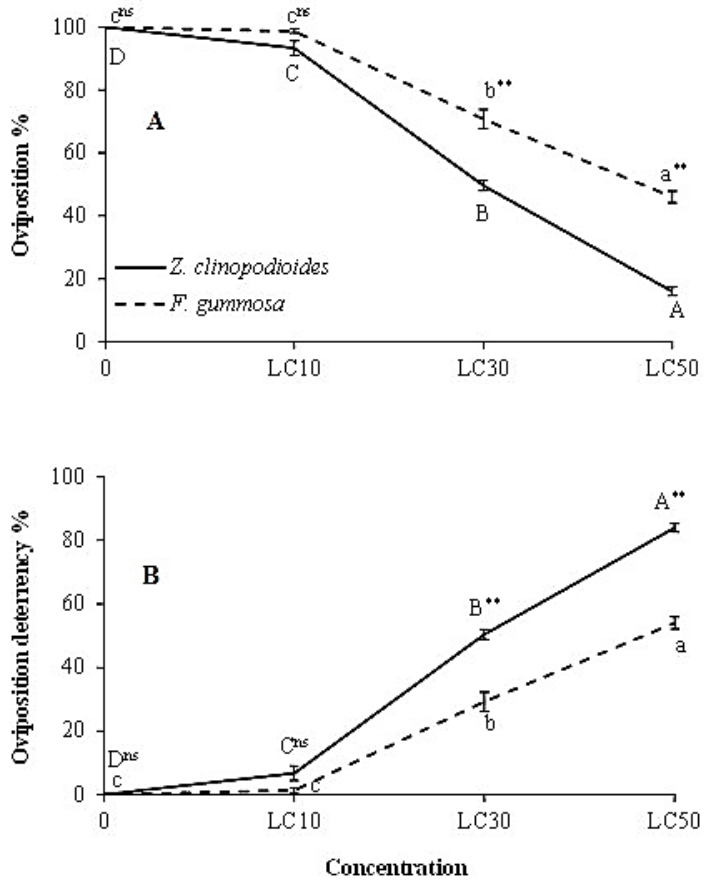

Fig. 2. Effect of sublethal concentrations of *Ziziphora clinopodioides* and *Ferula gummosa* oils on oviposition rate (A) and percentage oviposition deterrence (B) of *Plodia interpunctella* female mothsafter 24 h fumigation (the mean ± SE). Means followed by different letters for each oil (larg letters for *Z. clinopodioides* and amall ones for *F. gummosa* oil) indicate significant differences at $P < 0.05$, Tukey's test. Means were compared pairwise for each concentration between *Z. clinopodioides* oil and *F. gummosa* oil by Student's t-test. Statistically significant differences are denoted with * ($P < 0.05$), ** ($P < 0.01$), and *ns* (no significant difference).

Total hemocyte count

Our findings proved a significant change in THC of one-day-old 5th instar larvae following treatment with lethal and sublethal concentrations of the oils (Fig. 3). It was found that total hemocyte population in treated larvae was drastically reduced with increase in concentration of the oils and exposure time ($F = 12.31$; df = 3; $P < 0.000$). Compared to naive larvae (25000 cell/mm^3), THC declined to 16,800 and 5,600 cell/mm^3 48 h after treating the larvae with the highest concentrations (LC_{50}) of *Z. clinopodioides* and *F. gummosa* oil, respectively (Fig. 3).

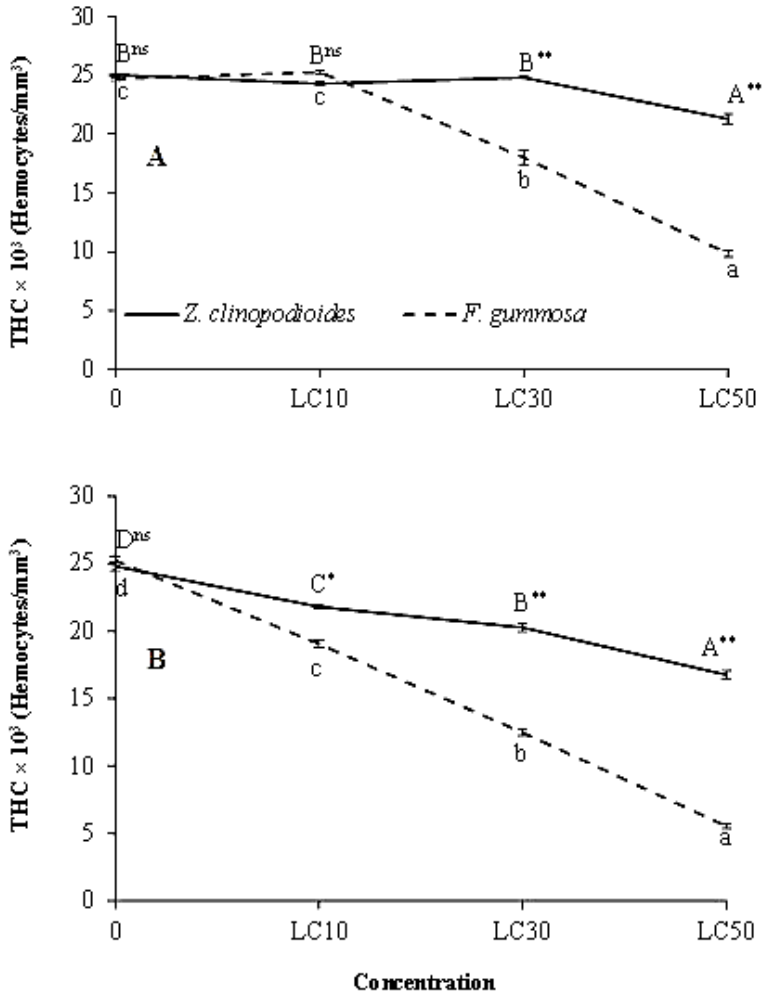

Fig. 3. Effect of sublethal concentrations of *Ziziphora clinopodioides* and *Ferula gummosa* oils on total hemocyte count of *Plodia interpunctella*fifth instar larvae after 24 h (A) and 48 h (B) fumigation (the mean ± SE). Means followed by different letters for each oil (larg letters for *Z. clinopodioides* and amall ones for *F. gummosa* oil) indicate significant differences at $P < 0.05$, Tukey's test. Means were compared pairwise for each concentration between *Z. clinopodioides* oil and *F. gummosa* oil by Student's t-test. Statistically significant differences are denoted with * ($P < 0.05$), ** ($P < 0.01$), and *ns* (no significant difference).

Discussion

Results of toxicity tests for the essential oil of *Z. clinopodioides* and *F. gummosa* showed a remarkable difference in susceptibility against eggs, larvae, and adults of *P. interpunctella*. Moreover, it was indicated that the tested oils could cause a negative impact on oviposition rate of adult female moths as well as total number of larval circulating hemocyte.

In the most previous studies, essential oils were assessed for their toxicity against mobile developmental stages (larvae and adults) of insect pests and less attention has been paid on immobile stages including eggs and pupa. Regarding this, Sarac & Tunc (1995) reported that

insects' larvae and adults are more susceptible than the eggs to essential oils. They proposed that this susceptibility could be linked to the higher respiration rate of insects in their active stages. Our bioassays results clearly indicated that *P. interpunctella* eggs were more tolerant than the larvae and adults against the toxic vapors of *Z. clinopodioides* and *F. gummosa* oils. These findings were in agreement with those reported in case of *Callosobruchus maculatus* (Saeidi *et al*., 2014), and *Tribolium confusum* du Val. (Sarac & Tunc, 1995). Some researcher, on the other hand, proved that insects' embryo is as susceptible as larvae (Negahban & Moharramipour, 2007) or even less tolerant to essential oils (Sarac & Tunc, 1995) which are not consistent with the results of present paper.

Based on the estimated LC_{50}, *F. gummosa* oil was more toxic than *Z. clinopodioides* oil against *P. interpunctella* eggs. Also, we found that four-day-old eggs were more susceptible than one-day-old ones to the both oils. Similar results were obtained by the previous scholars who studied different species of stored-products pests and showed that insect eggs turn to be more susceptible as they get old (Papachristos & Stamopoulos, 2002; Sahaf & Moharramipour, 2008; Saeidi *et al*., 2014). Since the most possible target of essential oil neurotoxicity is the octopaminergic system in insects' brain (Enan, 2001), it sounds that susceptibility of older eggs rather than younger ones to tested oils could be linked to higher growth and development of the target system (the central nervous system or CNS).

Bioassays indicated that *P. interpunctella* larvae are more susceptible to the oils rather than eggs and adults. It was also found that the oils are more toxic to 1^{st} instar larvae than 5^{th} instar ones. In other words, the larvae susceptibility to the oils significantly decreased, as the age rises which are in agreement with previous research results (Park *et al*., 2003; Papachristos & Stamopoulos, 2002; Wang *et al.*, 2006; Taghizadeh Sarokolai *et al*., 2015). As mentioned above, plant essential oils are highly volatile, containing lipophilic molecules with fumigant action which penetrate into insects' body mainly through cuticle layer of tracheal system and eventually affect CNS (Enan, 2001). Thus, increasing the hardness of the cuticle in older larvae may be a reason for their tolerance to toxic molecules of essential oils (Gouamene-Lamine *et al*., 2003; Lee *et al*., 2003; Gokce *et al*., 2006). Besides, factors including body size, body weight, and amount of fat body could play major role in tolerance of high instar larvae against toxic compounds (Matsumura, 1985).

Several studies have examined chronic effects of essential oils on the behavioral, biological, and physiological characteristics of different insect species. We also found that apart from their toxicity, sublethal concentrations of the studied oils negatively influenced some physiological features of *P. interpunctella*, such as oviposition rate of female moths and the total hemocyte population of fifth instar larvae.

It is generally believed that the obtained total energy of an animal must be allocated in such a way that balances the maintenance, growth, reproduction, and survival (Kunz &

Orrell, 2004). Reproduction and immune functions are energy-consuming physiological processes of insects. From another point of view, insects take the advantages of detoxifying enzymes once they are exposed to insecticides. Nevertheless, the detoxification processes need a lot of metabolic energy and are energetically expensive (Rand *et al*., 2015). Since, the amount of energy that an animal can devote to physiological activities is limited (Kunz & Orrell, 2004), therefore, it sounds like that low capability of our oil-treated insects in reproduction and hemocyte propagation could probably attributed to the lack of required energy. Moreover, Abbo *et al*., (2017) observed a significant reduction in the titer of vitellogenin (Vg), a storage protein synthesized by the fat body that regulates insects development and behavior and often are linked to energy homeostasis, in honey bees exposed to imidacloprid. They suggested that significant decline in the titer of Vg in bees exposed to imidacloprid could be linked to increased energetic cost for mounting detoxification during the pesticide exposure. Regarding the importance of Vg in reproduction process and immunity, it seems that significant reduction in oviposition rate and hemocyte number of the oils-treated insects in the present study could be attributed to decline in the titer of this storage protein.

Conclusions

This study is the first report to evaluate fumigant toxicity as well as physiological effects of sub-lethal concentrations of *Z. clinopodioides* and *F. gummosa* oils against *P. interpunctella.* Altogether, our findings suggest that studied oils are effective and promising candidates in the control of *P. interpunctella* as fumigant insecticides. However, further researches are needed to evaluate their efficacy in practical scale of storage systems.

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