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Effect of essential oils from *Callistemon viminalis* and *Ferula gummosa* on toxicity and on the hemocyte profile of *Ephestia kuehniella* (Lep.: Pyralidae)

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This study was carried out to evaluate toxicological and haematological effects of essential oils from *Callistemon viminalis* and *Ferula gummosa* against *Ephestia kuehniella* larvae through fumigant and topical exposure. The results indicated that fumigant LC_{10}, LC_{30} and LC_{50} values were 8.42, 15.86 and 24.60 µl/l air for *C. viminalis* oil and 38.51, 57.75 and 76.44 µl/l air for *F. gummosa*, respectively. Also, topical LD values were measured to be 4.28, 9.64 and 16.91 µg insect^{-1} for *C. viminalis* oil and 1.28, 3.39 and 6.65 µg insect^{-1} for *F. gummosa* oil, respectively. Haematological observations showed that in comparison to fumigation, topical application of tested oils caused a drastic reduction in total hemocyte count of treated larvae in a dose-dependent manner at all time intervals. Also, it is shown that plasmatocytes and granulocytes were the most sensitive hemocytes of *E. kuehniella* larvae to the tested oils after topical application. So, it is concluded that topical application of essential oils of *C. viminalis* and *F. gummosa* provided effective control of *E. kuehniella* causing negative effects on its immune cells.

Keywords: *Callistemon viminalis*; *Ferula gummosa*; *Ephestia kuehniella*; fumigant and topical exposure; total hemocyte count; different hemocyte count

1. Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zell., is considered as a serious cosmopolitan pest of stored products, particularly flour (Brindley 1930). It is also used to rear parasitoids and predators for biological control programmes against some insect pests (Paust et al. 2008).

Control of stored product pests primarily depends on using of dangerous chemical pesticides such as Phosphin (Mueller 1990) and methyl bromide. Although effective, the intensive utilisation of these chemical substances causes unfavourable problems such as contamination of stored products, human health and increased probability of pest resistance (Meaklim 1998; Haque et al. 2000). For these reasons, the more recent approaches for controlling these pests were by the use of relatively

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safe natural products, especially plant essential oils and extracts. These substances are used increasingly because they are generally inexpensive and have fewer health hazards to both quality of stored products and human health (Isman 2000).

Till now, many investigations have been carried out to assess the bioactivity of different essential oils against various stages of *E. kuehniella*. For example, Karaborku et al. (2011) indicated that Marjoram (*Origanum majorana* L.) and lemon (*Citrus limon* L.) oils were the most effective of all the essential oils tested against Mediterranean flour moth. Also, Bachrouch et al. (2010), studying composition and insecticidal activity of essential oil from *Pistacia lentiscus* L., found that this oil was more toxic to *E. kuehniella* than *Ectomyelois ceratoniae* Zeller. It was also proved that adults of *E. kuehniella* were more sensitive to the essential oil from *Elettaria cardamomum* L. than the Coleoptera (Abbasipour et al. 2011). These results show that among different essential oils studied, some of them have considerable insecticidal potential against *E. kuehniella*.

Insect's cellular immune response consists of several types of circulating hemocytes including prohemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), oenocytoids (OEs) and spherulocytes (SPs) (Gupta 1979; Lavine & Strand 2002). Insect hemocytes have important functions on the immune system, metabolism and detoxification, and also play a crucial role in the defence of xenobiotics or microbial infection (Gupta 1979). Hemogram profile, i.e. total hemocyte count (THC), differential hemocyte count (DHC), hemolymph volume, mitotic index (MI) and cytological features of hemocytes, is a very important index to evaluate insect's immunological status. Phagocytosis, nodulation, encapsulation and other related defence mechanisms are primarily depending on the availability of circulatory immune cells particularly PLs and GRs. In this case, Sharma et al. (2008) showed that the number and proportion of different hemocytes were beneficial for insects to develop environmental fitness. Changes in insect's hemogram have been associated with some factors including ecdysis, sex, mating, temperature, starvation, pathogens, parasitoids and so on (Gupta 1979).

Essential oils and plant extracts as nonpolluting and eco-friendly pesticides are also found to have significant effects on the insect's hemocytes. Sharma et al. (2003) evaluated the effect of oral treatment of Neem gold (0.15% azadirachtin) on hemogram and ultrastructure of hemocytes of *Spodoptera litura* Fab. They found a dose-dependent increase in THC after 24 and in contrast, a dose-dependent decrease in the THC after 48 and 72 h of treatment. In another assay, larvae treated with leaf extracts of *Eucalyptus globulus* Labill. and *Ageratum conyzoides* L. and clove extract of *Allium sativum* L. showed a significant reduction in THC as well as great deal of variation in relative percentage of hemocytes compared to their control (Pandey et al. 2012).

Although there have been lots of investigation on the toxicity of essential oils on various developmental stages of *E. kuehniella*, no information is available on their influence on its hemocytes. Similarly, there is no report so far on comparison of insecticidal activity between topical and fumigant application of *Callistemon viminalis* Gaertn. and *Ferula gummosa* L. oils on *E. kuehniella* larvae. So, the present study is the first attempt to evaluate the fumigant and topical toxicity of essential oils from *C. viminalis* and *F. gummosa* oils and also to assess the effects of sublethal concentrations of subjected oils on the hemocytes of *E. kuehniella*.

2. Materials and methods

2.1. Insect culture

Eggs of *E. kuehniella* were provided by the Insectarium of Scientific and Industrial Research Organisation of Iran and reared in plastic jars half filled with mixture of equal parts of wheat flour and bran. Powdered yeast (5 g) was also added to the diet. The rearing conditions were 26 ± 1 °C, 75% RH in darkness.

2.2. Essential oils

Leaves of *C. viminalis* and roots of *F. gummosa* were collected from their natural habitats in Iran. The plant material was dried in the shade with proper ventilation and then maintained at −24 °C until required. When needed, the plant material was hydrodistillated to extract their essential oils.

Extraction of essential oil from plants was carried out using a modified clevenger-type apparatus (Negahban et al. 2007) at the following conditions: 40 g of air-dried material, 500 ml distilled water and 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. The resulting oil was placed in a sealed glass tubes and stored in refrigerator at 4 °C.

2.3. Bioassays

2.3.1. Fumigation

For fumigant toxicity tests, 10 larvae of *E. kuehniella* were placed in each glass container of 100 ml volume. After preliminary dose-setting experiments, the final concentrations of the oils causing 5–95% mortality were obtained based on logarithmic distance (Robertson et al. 2007). The calculated concentrations of each oil was infused on the filter paper pieces of 2 cm in diameter and then were attached to the caps of glass vials. Oils were applied as pure using microapplicator. The caps of vials were sealed tightly with Parafilm. Control filter papers received no oil.

2.3.2. Topical application

In a separate experiment to determine the topical toxicity values of the oils, 10 larvae of *E. kuehniella* were topically treated with 1 µl of different dosage of tested oils in Petri dishes (diameter 9 cm). Oils were diluted in acetone and applied on the mesosternum of larvae using Hamilton syringe (Burkard Co., England). Controls received 1 µl of acetone alone. After a preliminary dose-setting trial, logarithmic series of dilutions were offered to identify the effective range for 5–95% mortality (Robertson et al. 2007). For more accuracy, larvae weighting 20 ± 1 mg were used for bioassay.

In both assays, all treatments and controls were kept at 26 ± 1 °C and 70% RH in darkness. Each oil concentration and dosage was replicated three times. At the end of treatment for 24 h, larvae were transferred to a clean dish and live and dead tested insects were counted. Larvae were considered dead when no movement was observed when probed by a fine brush after 24 h.

2.4. Haematology assays

To count total hemocytes of the larvae treated with sublethal concentrations of *C. viminalis* and *F. gummosa* oils (both fumigant and topical), the hemolymph sample

was obtained from the severed proleg of insects using a microapplicator and immediately diluted in Tyson buffer. Cell counting was conducted at intervals of 6, 12, 24 and 48 h post oil-treating using a standard hemocytometer. The cells were counted using a light microscope and number of total hemocytes per cubic millimetre (mm^3) was calculated using the formula of 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.

For DHCs, drops of hemolymph were obtained from the prologe of treated and control larvae using a fine scissors and the smear was prepared on a clean glass slide. The air-dried smears were stained in the diluted Giemsa's stain for 25 min and subsequently rinsed in very dilute lithium carbonate for 30 s for red staining structures. DHC was performed by classifying 200 cells per smear (Arnold & Hinks 1976).

2.5. Statistical analysis

The LC and LD values and 95% confidence limits were calculated from probit regressions using the POLO-PC computer programme (LeOra Software). Data from haematology assays were analysed using the SPSS programme version 16.0 for analysis of variance (ANOVA) and the means were grouped using Tukey's test ($\alpha = 0.05$).

3. Results

3.1. Fumigant assays

The fumigant toxicity and sublethal concentrations of *C. viminalis* and *F. gummosa* oils on *E. kuehniella* are depicted in Table 1. The results show that calculated LC values for *C. viminalis* (based on overlap in 95% CL) were significantly higher than that for *F. gummosa*. Based on the results of parallelism test, regression lines of two subjected oils were not parallel ($\chi^2 = 6.46$, $df = 1$, $p = 0.011$). Estimated relative toxicity (based on LC_{50} values) indicated that essential oil of *C. viminalis* was 3.11-folds more toxic to *E. kuehniella* larvae than *F. gummosa* oil (Table 1).

3.2. Topical assays

The studied oils were also tested *in vivo* through topical application. Based on probit analysis, LD_{10}, LD_{30} and LD_{50} values were calculated as 4.28, 9.64 and 16.91 μg insect^{-1} for *C. viminalis* oil and 1.28, 3.39 and 6.65 μg insect^{-1} for *F. gummosa* oil, respectively (Table 2). According to the estimated relative toxicity (based on LC_{50} value), topical toxicity of *F. gummosa* oil was 2.56 times of that for *C. viminalis* oil. Regression lines of the subjected oils were parallel ($\chi^2 = 1.36$, d$f = 1$, $p = 0.243$).

3.3. Effects on THC and DHC

In the present study, total and differential number of circulating hemocyte of two-day-old fifth larval instars of *E. kuehniella* were studied after treatment with sublethal concentrations of the subjected oils both as fumigant and topical application.

Table 1. LC values of essential oils from *C. viminalis* and *F. gummosa* for *E. kuehniella*, and their relative toxicity (RT) applied as fumigant.

Essential oils	*n*	LC values (95% CL)[a]			Slope ± SE	X^2 (d*f*)	RT (95% CL)[b]
		LC_{10}	LC_{30}	LC_{50}			
C. viminalis	280	8.42 (06.00–10.63)	15.86 (12.97–18.65)	24.60 (21.05–28.91)	2.75 ± 0.31	1.45 (4)	0.32 (0.19–0.46)
F. gummosa	280	38.51 (30.43–45.13)	57.75 (50.17–64.32)	76.44 (68.99–84.53)	4.30 ± 0.50	2.27 (4)	

[a]LC values are expressed as µl/l air of pure essential oil with their 95% confidence limits (CL).
[b]Relative toxicity = LC_{50} of *C. viminalis* divided by LC_{50} of *F. gummosa*.

Table 2. LD values of essential oils from *C. viminalis* and *F. gummosa* for *E. kuehniella*, and their relative toxicity (RT) applied as topical.

Essential oils	*n*	LD values (95% CL)[a]			Slope ± SE	X^2 (d*f*)	RT (95% CL)[b]
		LD_{10}	LD_{30}	LD_{50}			
C. viminalis	280	4.28 (2.78–5.76)	9.64 (7.43–11.89)	16.91 (13.80–20.85)	2.14 ± 0.23	0.78 (4)	2.56 (1.74–4.13)
F. gummosa	280	1.28 (0.75–1.84)	3.39 (2.47–4.36)	6.65 (5.22–8.51)	1.79 ± 0.20	1.96 (4)	

[a]LC values are expressed as µg insect^{-1} with their 95% confidence limits (CL).
[b]Relative toxicity = LD_{50} of *C. viminalis* divided by LD_{50} of *F. gummosa*.

Table 3. Effect of *C. viminalis* and *F. gummosa* oils on THC in *E. kuehniella*, at different time interval applied as fumigant.

Essential oil	Concentration (µl/l air)	Duration after treatment			
		6 h (THC/mm^3)	12 h (THC/mm^3)	24 h (THC/mm^3)	48 h (THC/mm^3)
C. viminalis	0	30,140 ± 698.99^{ab}	31,090 ± 486.92^{a}	31,470 ± 470.53^{a}	34,260 ± 582.75^{a}
	08.42	31,050 ± 661.43^{ab}	30,700 ± 859.65^{a}	32,100 ± 762.72^{a}	33,500 ± 176.06^{a}
	15.86	32,400 ± 813.01^{a}	31,800 ± 1044.50^{a}	30,950 ± 886.98^{a}	32,800 ± 861.97^{a}
	24.60	28,400 ± 503.73^{b}	26,850 ± 467.70^{b}	25,300 ± 558.79^{b}	22,270 ± 967.29^{b}
F. gummosa	0	29,440 ± 534.18^{ab}	31,340 ± 815.84^{a}	31,260 ± 577.58^{a}	35,700 ± 752.32^{a}
	38.51	29,870 ± 180.00^{a}	30,670 ± 517.59^{a}	32,418 ± 235.42^{a}	32,680 ± 571.09^{b}
	57.75	28,950 ± 934.61^{ab}	29,880 ± 430.58^{a}	29,430 ± 308.05^{b}	29940352.98^{c}
	76.44	27,400 ± 293.25^{b}	26,200 ± 339.11^{b}	25,700 ± 520.57^{c}	23,180 ± 478.95^{d}

Means with the same letters in each column are not significantly different at $p < 0.05$, Tukey's test.
Values are expressed as mean ± SE ($n = 10$).

Table 4. Effect of *C. viminalis* and *F. gummosa* oils on THC in *E. kuehniella*, at different time interval applied as topical.

Essential oil	Concentration (µg/insect)	Duration after treatment			
		6 h (THC/mm^3)	12 h (THC/mm^3)	24 h (THC/mm^3)	48 h (THC/mm^3)
C. viminalis	0	28,460 ± 300.99^{a}	27,650 ± 660.68^{a}	31,390 ± 660.75^{a}	34,160 ± 471.80^{a}
	4.28	26,580 ± 424.73^{b}	26,440 ± 347.27^{a}	24,810 ± 502.09^{b}	25,200 ± 228.03^{b}
	9.64	22,520 ± 399.24^{c}	21,580 ± 584.29^{b}	20,140 ± 222.71^{c}	18,240 ± 413.03^{c}
	16.91	16,740 ± 488.46^{d}	17,120 ± 533.29^{c}	14,960 ± 368.23^{d}	13,624 ± 582.38^{d}
F. gummosa	0	27,840 ± 487.18^{a}	31,680 ± 446.54^{a}	32,400 ± 254.95^{a}	34,960 ± 382.88^{a}
	1.28	25,520 ± 324.65^{b}	25,580 ± 354.11^{b}	23,180 ± 312.08^{b}	21,680 ± 521.91^{b}
	3.39	19,730 ± 438.63^{c}	18,340 ± 675.72^{c}	17,660 ± 233.66^{c}	16,020 ± 354.11^{c}
	6.65	11,300 ± 670.07^{d}	9700 ± 763.54^{d}	9060 ± 411.82^{d}	8520 ± 339.70^{d}

Means with the same letters in each column are not significantly different at $p < 0.05$, Tukey's test.
Values are expressed as mean ± SE ($n = 10$).

Table 5. Differential hemocyte count (mean ± SE) in the oil-treated larvae of *E. kuehniella*, 24 h after fumigation.

Essential oil	Concentration (µl/l air)	% Hemocyte type (Mean ± SE)					
		PR	PL	GR	OE	SP	MI
C. viminalis	0	13.10 ± 0.50^{a}	49.20 ± 1.21^{a}	27.50 ± 1.00^{a}	4.80 ± 0.40^{a}	5.40 ± 0.33^{a}	3.50 ± 0.22^{a}
	08.42	14.30 ± 0.58^{a}	48.40 ± 0.88^{a}	26.50 ± 0.50^{a}	5.20 ± 0.33^{a}	5.60 ± 0.33^{a}	2.70 ± 0.30^{ab}
	15.86	11.01 ± 0.57^{b}	47.76 ± 1.18^{a}	29.61 ± 0.67^{a}	5.21 ± 0.40^{a}	6.41 ± 0.50^{a}	2.30 ± 0.37^{ab}
	24.60	08.94 ± 0.33^{b}	51.54 ± 2.17^{a}	28.14 ± 1.03^{a}	5.64 ± 0.48^{a}	5.74 ± 0.43^{a}	1.80 ± 0.43^{b}
F. gummosa	0	12.50 ± 0.54^{a}	52.50 ± 0.90^{a}	25.10 ± 0.60^{a}	06.40 ± 0.57^{a}	03.50 ± 0.44^{a}	4.20 ± 0.58^{a}
	38.51	10.82 ± 0.46^{a}	50.82 ± 0.99^{a}	26.72 ± 1.21^{a}	08.62 ± 0.64^{a}	03.02 ± 0.31^{a}	1.20 ± 0.25^{b}
	57.75	11.22 ± 0.75^{a}	50.22 ± 0.96^{a}	26.32 ± 1.15^{a}	08.22 ± 0.64^{a}	04.02 ± 0.31^{a}	0.00 ± 0.00^{b}
	76.44	07.26 ± 0.48^{b}	49.96 ± 1.39^{a}	28.46 ± 0.91^{a}	09.06 ± 0.87^{a}	05.26 ± 0.62^{a}	0.70 ± 0.37^{b}

Means with the same letters in each column are not significantly different at $p < 0.05$, Tukey's test ($n = 5$).
(PR: Prohemocyte; PL: Plasmatocyte; GR: Granulocyte; OE: Oenocytoid; SP: Spherulocyte; MI: Mitotic index).

Table. 6. Differential hemocyte count (mean ± SE) in the oil-treated larvae of *E. kuehniella*, 24 h after topical application.

Essential oil	Concentration (µg/insect)	% Hemocyte type (Mean ± SE)					
		PR	PL	GR	OE	SP	MI
C. viminalis	0	12.20 ± 0.64^{a}	50.80 ± 0.93^{a}	28.10 ± 0.90^{c}	3.60 ± 0.50^{b}	5.30 ± 0.40^{b}	4.70 ± 0.76^{a}
	4.28	9.60 ± 0.57^{b}	46.20 ± 0.80^{a}	31.50 ± 1.58^{c}	5.70 ± 0.80^{b}	7.00 ± 0.57^{ab}	2.50 ± 0.27^{ab}
	9.64	6.10 ± 0.57^{c}	37.60 ± 2.11^{b}	42.30 ± 0.91^{b}	5.90 ± 0.55^{b}	8.10 ± 0.74^{a}	1.70 ± 0.71^{b}
	16.91	3.10 ± 0.65^{d}	29.60 ± 1.12^{c}	49.70 ± 1.99^{a}	10.10 ± 1.13^{a}	7.50 ± 0.74^{ab}	1.10 ± 0.45^{b}
F. gummosa	0	14.90 ± 1.02^{a}	45.20 ± 1.37^{a}	30.50 ± 1.25^{d}	5.10 ± 0.40^{c}	4.30 ± 0.25^{b}	4.60 ± 0.53^{a}
	1.28	12.30 ± 0.98^{ab}	35.00 ± 1.32^{b}	37.00 ± 0.75^{c}	8.10 ± 0.64^{b}	7.60 ± 0.79^{ab}	1.00 ± 0.15^{b}
	3.39	8.60 ± 0.84^{b}	32.00 ± 1.21^{b}	42.90 ± 1.19^{b}	9.00 ± 0.75^{b}	7.50 ± 1.03^{ab}	0.00 ± 0.00^{b}
	6.65	2.60 ± 0.79^{c}	20.50 ± 1.26^{c}	54.60 ± 1.50^{a}	13.10 ± 0.79^{a}	9.20 ± 1.38^{a}	0.00 ± 0.00^{b}

Means with the same letters in each column are not significantly different at $p < 0.05$, Tukey's test ($n = 5$).
(PR: Prohemocyte; PL: Plasmatocyte; GR: Granulocyte; OE: Oenocytoid; SP: Spherulocyte; MI: Mitotic index).

The results in Table 3 show that the two studied oils caused different effects on THC after fumigant application. In case of *C. viminalis* oil, total count of hemocytes did not significantly differ from that for controls in all concentrations and intervals except LC_{50}. In contrast, THC was significantly reduced with increasing the concentration and exposure time of *F. gummosa* oil. It is also found that total number of circulating hemocytes in topically treated larvae was drastically reduced with increase in concentration of both oils (Table 4). Compared to nontreated larvae (2860 and 27,840 cell/mm^3), THC reached to 13,624 and 8520 cell/mm^3 48 h after treating the larvae with *C. viminalis* and *F. gummosa* oil, respectively. We also observed that THC in the early exposure with acetone was a bit lower than that for the rest of the intervals (Table 4).

No considerable changes have been found in proportion of circulating hemocytes except PRs and MI after treatment with sublethal concentrations of the tested oils as fumigant (Table 5). Based on the results presented in Table 6, a decrease in PRs and PLs and a significant increase in GRs, EOs and SPs have been observed along with the concentration increase in topical delivery of the oils. Per cent of mitotically dividing hemocytes was significantly decreased as concentration of both oils increased. No cell structural abnormality was observed in hemocytes of treated larvae.

4. Discussion

In general, the results of present study showed the high insecticidal efficiency of subjected oils against *E. kuehniella* either in fumigant or topical application. It is widely accepted that numerous factors including the kind of the oil used, the concentration and exposure time can all affect the effectiveness of a treatment. Chemical constitutes also has a noticeable influence on the efficiency of compounds used. The chemical composition of the essential oils tested in this work was identified by Hamzavi (2011) and Ghasemi (2010). 1,8-cineole (41.3%) and *β*-pinene (87.3%) were, respectively, the largely predominant specific component for *C. viminalis* and *F. gummosa* essential oils. These two active ingredients are among the most toxic chemical compounds identified in plant's oils and their biological activities have been proved by different researchers. Ghasemi et al. (2011) reported that among tested materials, *F. gummosa* oil (LC_{50} = 2.68 µl/l air) had high fumigant toxicity for honey bees. Hamzavi (2011) showed that essential oil of *C. viminalis* (LC_{50} = 50 µl/l air) caused high mortality on 20-day-old larvae of *Tribolium confusum* Duval. after fumigation. However, there is no data on the topical toxicity of essential oils, but the results of present study showed good insecticidal properties of the subjected oils.

According to the parallelism test, regression lines of two examined oils were not parallel for *E. kuehniella* afte fumigation. Nonparallel lines may indicate that detoxification enzymes differ qualitatively or that totally different enzymes occur in the organisms (Robertson and Rappaport 1979). In contrast, regression lines of subjected oils were parallel for the larvae in topical application. It probably means that *E. kuehniella* larvae have qualitatively similar, but quantitatively different, levels of enzymes necessary for detoxification of oil molecules penetrated into the hemolymph.

Data on the comparison of topical and fumigant activity of essential oils against insect pests are scarce. The relative toxicity of *C. viminalis* to *F. gummosa* was estimated to be 0.32 and 2.56 for fumigant and topical application, respectively. These results proved that essential oil of *C. viminalis* was more toxic to the Mediterranean flour moth than *F. gummosa* oil through fumigation, while the reverse was true in case

of topical application of the oils. In a parallel study, Rice and Coats (1994) studying topical vs. fumigant toxicity for a group of terpens revealed different levels of effectiveness for the same terpenes. These authors also showed that thymol and carvacrol were more topically toxic than saturated alcohols, but saturated alcohols were better fumigants against houseflies than phenols. Certainly, different application methods of a given compound follow various responses in an insect. For this reason, we used hemolymph as a physiological medium to evaluate the effect of different delivery methods of the tested oils on hemocyte of *E. kuehniella* larvae.

There is no information available on the comparative effectiveness of fumigant and topical applications of essential oils on insect's hemagram, so far. The results of present study obviously showed that THC was drastically declined after topical application of the oils than fumigation. In other words, in comparison to fumigation, topical application of the tested oils could functionally reduce the hemocytic immune ability of the subjected larvae. According to the report of Enan (2001), present knowledge of the site of action of essential oils and terpens as fumigant is incomplete, but they may interfere with the acethylcholine esterase (AChE) and octopamine receptors in insects. In contrast, essential oils used topically could directly penetrate *E. kuehniella* larvae hemolymph through the cuticle (Matsumura 1985). Given these two different mechanisms of action, application of essential oils as topical can quickly affect the insect hemocytes than that as fumigant. So, the strong immune-suppressive activity of topical application of tested oils on *E. kuehniella* larvae could be attributed to their site of actions. The drastic reduction in THC in *E. kuehniella* larvae following the oils application is similar to the reports of Pandey et al. (2008 and 2012) in *Danaus chrysippus* L. and *Papilio demoleus* L., respectively. The marked reduction in THC may be linked to the toxic effects of present oils on hemocytes or to their inhibitory influences on the release of PRs from hemapoetic organs. It is also found that number of mitotically dividing hemocytes decreased with increasing concentration and exposure time of the oils used as topical. Despite the low participation of mitotically dividing hemocytes in cell production, the significant decline in MI could be another reason for reduction of THC.

The results of some studies revealed that different oils cause much variation in the proportion of hemocytes. For example, it is shown that sweet flag rhizome oil led to a decrease in the proportion of PLs and SPs while the proportion of GRs increased in *S. litura* larvae (Sharma et al. 2003, 2008).

Our results indicated that the per cent of PRs declined after treatment of larvae with the oils both as fumigant and topical. In a parallel study, Pandey et al. (2012) pointed out that these changes in PRs population may be attributed to some factors including inhibition of their mitotic division, their conversion to other type of cell or to the inhibition of activity of hematopoeitic organs responsible for their production. It is also found that PLs and GRs were the most sensitive hemocytes of *E. kuehniella* to the tested oils after topical application. These two cells are the main hemocytes in immune responses toward the foreign materials within the hemolymph (Strand 2008). Sharma et al. (2003) found the same variation of PLs and GRs in Neem gold-treated larvae of the tobacco armyworm, *S. litura*. It is also believed that OEs play crucial roles in phenoloxidase (PO) cascade when an immune challenge occurs (Beckage 2008; Strand 2008). Therefore, the significant increase in their population could be led to stimulation of immune system of the oil-treated larvae to the secretion of PO.

5. Conclusion

It is generally concluded that topical application of essential oils from *C. viminalis* and *F. gummosa* have enough insecticidal activity against *E. kuehniella* causing negative effects on its hemocytes.

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