

Insecticidal Activity of Four Plant Essential Oils against Two Stored Product Beetles

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Abstract

The insecticidal properties of essential oils prepared using water distillation method from aerial parts of *Mentha piperita*, *Mentha pulegium*, *Zataria multiflora* and *Thymus daenensis* were investigated on adult of *Bruchus lentis* and *Callosobruchus maculatus*. Essential oils were used in five concentrations and five replications. Mortality rate of insects were recorded after 3, 6, 24, 48 and 72 hours. Results indicated that the longer exposure of insect to essential oil and the higher concentration of essential oil, increased mortality of the two species in all treatments. There was significant difference among mortality effect of the essential oils. The LC_{50} value of *M. piperita* after 24 hours for *B. lentis* and *C. maculatus* was 14.62 and 13.70 μ l/l air respectively, while the values of LC_{50} were 92.32 and 95.80 for *M. pulegium*, 58.43 and 99.94 for *Z. multiflora* and 63.97 and 65.55 μ l/l air for *T. daenensis*, respectively. The least LT_{50} recorded was in *T. daenensis* essential oil with 26.21 and 24.15 hours against *B. lentis* and *C. maculatus*, respectively.

Keywords: Fumigation toxicity; The seed lentil beetle; The cowpea weevil; Essential oil; Yasouj

Introduction

In some rural areas of Iran that use traditional storages, damage caused by stored product insects can be as high as 80% [1]. Fumigation plays a major role in insect pest elimination in stored products [2]. Chemical control of stored products 'pests with current chemical pesticides may cause special problems on stored products [3].

These problems have highlighted the need for the development of new types of selective insect-control alternatives with fumigant action [4]. It is believed that essential oils have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability [5]. They do not leave residues toxic to the environment and have medicinal properties for humans [6]. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites [7]. Because of the intensity of plant-insect interactions, the plants have well developed defense mechanisms against pests and are excellent sources of new insecticidal substances. Their components and quality vary with geographical distribution, time of harvest, growing conditions and method of extraction [8]. Among others effects of essential oils on stored-product insect pests have been reported on extensively [9-14].

Nowadays, many studies have been done for evaluation of susceptibility of stored product insect pests specially *Callosobruchus maculatus* to plant essential oils. For example, the antifeedant activities of *Citrus reticulata* Blanco, *Citrus limon* L. and *Citrus aurantium* L. essential oils against eggs, larvae and adults of *C. maculatus* were studied [15]. The results showed that the effect of different concentrations of the essential oil vapors on egg hatchability as well as larval and adult mortality was found to be significant. *C. reticulata* and *C. aurantium* oils were more toxic on egg hatchability than *C. limon* extract and caused higher mortality on larvae as well. In the study of [15], the essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis* against *C. maculatus* was evaluated. The results revealed that *E. globulus* oils were more effective than *E. camaldulensis* oils, by significantly decreasing the RGR, RCR and ECI. Both of plant essential oils, with the same activity, increased FDI as the oil concentration was increased, showing high feeding deterrence activity against *C. maculatus*.

In the present study, also for importance of insecticidal activity in the integrated pest management, this effect was investigated by essential

oils of *M. piperita*, *M. pulegium*, *Z. multiflora* and *T. daenensis* grown in Iran against adults of *B. lentis* and *C. maculatus* two important storage pests.

Materials and Methods

Plant materials

The aerial parts (stem and leaves) *M. piperita*, *M. pulegium*, *Z. multiflora* and *T. daenensis* were collected at the ripening stage in the Dena protected area, Sisakht city, of the province Kohgiluyeh va Boyerahmad Ahmad (Iran), in July 2015. Plant taxonomists in the Department of Biology at Urmia University (Iran) confirmed the taxonomic identification of the plant species. The voucher specimens have been deposited at the herbarium of the Department of Natural Resources in, Kohgiluyeh va Boyerahmad Agricultural and Natural Resources Research and Education Center, Yasouj, Iran.

Insect rearing

Rearing of *Bruchus lentis*: The culture medium was the whole lentil grains sterilized at 60°C for 60-90 minutes. Ten jars of 300 mg were used. Each jar was filled with 250 g lentil grains and 30 beetles were added to each jar. The jars were then covered with muslin cloth, tied with rubber bands to avoid the escape of beetles. Beetles were left in the culture medium for 3 days for egg laying and then were removed with the help of sieves and fine camel hair brushes. The lentil grains containing eggs were placed again in the same jars and put in the incubator for incubation at 30 ± 2°C and 65% RH to get the homogenous (same age) population. Relative humidity was maintained inside the incubator by placing an open tray filled with saturated solution of $NaNO_2$.

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Rearing of *Callosobruchus maculatus*: A culture of the *C. maculatus* was established on the seeds of unshelled brown cowpea, *Vigna unguiculata* L. in one litter wide-mouthed glass jars under laboratory conditions. Parent adults were obtained from laboratory stock culture maintained at the Entomology Department, University of Urmia, Iran. The culture was maintained in the dark (similar to storage conditions) in a growth chamber set at $27 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity.

All experiments were carried out under the same environmental conditions. The 1-2 days old adults of *C. maculatus* were used in bioassay tests.

Essential oil supply

Plant materials of *M. piperita*, *M. pulegium*, *Z. multiflora* and *T. daenensis* were air dried in the shade at room temperature ($26\text{--}28^\circ\text{C}$) for 20 days and stored in darkness until distillation. The essential oils were isolated from dried plant samples by hydro-distillation using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 3 hours distillation. The essential oils were dried over anhydrous sodium sulfate and stored in glass tubes at $+4^\circ\text{C}$ in refrigerator.

Bioassay

Fumigant toxicity of essential oils: In fumigant toxicity assays, filter papers (What man No. 1, cut into 4×5 cm paper strip) were impregnated with different concentrations using a micro sampler. Twenty adults (3-5 days old) of *B. lentis* and *C. maculatus* were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical center of glass bottles, which were then sealed with air-tight lids. The experimental concentrations of essential oils on *B. lentis* and *C. maculatus* adults were determined by preliminary tests. Experimental concentrations for *M. piperita* were 10, 11, 12, 13 and 14 $\mu\text{l/l}$ air of oil and for *Z. multiflora* and *T. daenensis* were 40, 50, 60, 70 and 80 $\mu\text{l/l}$ air of oils and for *M. pulegium* were 60, 70, 80, 90 and 100 $\mu\text{l/l}$ air of oil. In the control bottles, only acetone was applied on the filter papers. Each experiment was replicated five times for each concentration. In all cases, the exposure times were 3, 6, 24, 48 and 72 hours. Treated insects were incubated at $27 \pm 1^\circ\text{C}$. After this time, the number of dead adults was counted. Those adults that did not move when lightly probed or shaken in light and mild heat were considered dead. Mortality in the control was not observed in any experiment.

Statistical analysis

The mortality data were corrected using Abbott's formula [16] for the mortalities in the controls, and then subjected to probit analyses using SPSS (version 16.0) software to estimate LC_{50} value. The percentage mortality value for different exposure times were subjected to analysis of variance (one-way ANOVA) using the SPSS (version 16.0) software statistical program. Data were transformed using arcsine \sqrt{x} to meet normality, before ANOVA. Comparison of means was done through Tukey's (HSD) test at 0.05.

Results

Among investigated essential oils, *M. piperita* essential oil has more fumigant toxicity than others on both species. With increasing concentrations of *M. piperita* essential oil, the mortality of mentioned species was increased so that the highest concentration (14 $\mu\text{l/l}$ air) caused 100% mortality on both adult insects after 72 hours ($F=3.01$ $P < 0.01$) (Table 1), but *T. daenensis* essential oil in 80 $\mu\text{l/l}$ air concentration caused 100% mortality on both adult insects ($F=2.23$ $P < 0.01$), (Table 2). The essential oil of *Z. multiflora* in 80 $\mu\text{l/l}$ air concentration caused 100 and 83% mortality respectively on both adult insects after 72 hours ($F=42.51$ $P < 0.01$), (Table 3). *M. pulegium* essential oil in 100 $\mu\text{l/l}$ air concentration caused 85 and 89% mortality respectively on both adult insects after 72 hours and had the lowest insecticidal effect in comparison to other essential oils ($F = 6.92$ $P < 0.01$), (Table 4). Calculated LC_{50} indicated that the LC_{50} of *M. piperita* essential oil on *B. lentis* and *C. maculatus* was 14.62 $\mu\text{l/l}$ and 13.70 $\mu\text{l/l}$ air concentrations respectively while the LC_{50} of *T. daenensis* essential oil for these insects was 63.97 and 65.55 $\mu\text{l/l}$ air concentration respectively. The LC_{50} of *Z. multiflora* essential oil was 58.43 and 99.94 $\mu\text{l/l}$ air concentration respectively.

The highest calculated LC_{50} belonged to *M. pulegium* essential oil with 92.32 and 95.80 $\mu\text{l/l}$ air on *B. lentis* and *C. maculatus*, respectively (Table 5). Based on LC_{50} and lower and upper confidence interval 95% the LC_{50} of *M. piperita* essential oil on *B. lentis* and *C. maculatus* with 14.62 and 13.70 $\mu\text{l/l}$ air had significantly different with other essential oils. The findings indicated that there wasn't any significantly different between the effects of *T. daenensis* and *M. pulegium* essential oils based on LC_{50} of the adults of *B. lentis*. But the essential oil of *Z. multiflora* was significantly different with *T. daenensis* and *M. pulegium* essential oils. Also, there wasn't any significantly different between effects of *M. pulegium*, *Z. multiflora* and *T. daenensis* essential oils on *C. maculatus* and they had overlap based on LC_{50} and confidence interval 95%. There

Means of mortality% \pm S. E						
Insect	Con($\mu\text{l/l}$ air)	Time (Hour)				
		3	6	24	48	72
<i>B. lentis</i>	10	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	27 \pm 2.53 ^{ml}	37 \pm 2.32 ^{kl}	59 \pm 3.00 ^{efg}
	11	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	27 \pm 2.51 ^{ml}	43 \pm 3.20 ^{hijk}	75 \pm 4.23 ^{bcd}
	12	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	31 \pm 3.69 ^{klm}	43 \pm 6.04 ^{hijk}	89 \pm 6.20 ^{ab}
	13	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	41 \pm 11.25 ^{hijkl}	45 \pm 7.00 ^{hig}	89 \pm 5.00 ^{ab}
	14	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	55 \pm 3.00 ^{gh}	71 \pm 4.45 ^{cde}	100 \pm 0.00 ^a
<i>C. maculatus</i>	10	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	21 \pm 5.10 ^m	31 \pm 5.42 ^{klm}	45 \pm 4.22 ^{ghij}
	11	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	29 \pm 3.20 ^{klm}	39 \pm 4.25 ^{ijkl}	65 \pm 2.17 ^{def}
	12	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	35 \pm 6.13 ^{klm}	53 \pm 6.56 ^{ghij}	85 \pm 3.00 ^{bc}
	13	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	53 \pm 4.21 ^{ghi}	67 \pm 3.00 ^{def}	89 \pm 2.27 ^{ab}
	14	0.00 \pm 0.00 ⁿ	0.00 \pm 0.00 ⁿ	53 \pm 5.21 ^{ghi}	77 \pm 3.14 ^{bcd}	100 \pm 0.00 ^a

ⁿMean in the same column followed by the same letters are not significantly different as determined by the Tukey's-test.

Table 1: Average mortality caused by *Mentha piperita* essential oil on *B. lentis* and *C. maculatus* at concentrations and different times.

wasn't any significantly difference between the effects of all studied essential oils on mortality of both *B. lentis* and *C. maculatus* adults (Tables 1-4).

The results showed that in 3 and 6 hours after applying of all concentrations of essential oils the mortality of both adult insects was zero and mortality was started for 24 hours after applying essential oils and reached after 72 hours to maximum after applying essential oils. In all studied essential oils, there was significantly difference between mortalities occurred for each concentration in 24, 48 and 72 hours after applying essential oils. Calculated LT_{50} for essential oils revealed that *T. daenensis* essential oil had the least LT_{50} for both species so that it was 26.21 and 24.15 hours for *B. lentis* and *C. maculatus* respectively. The

LT_{50} of *M. piperita* essential oil on *B. lentis* and *C. maculatus* was 29.66 and 29.43 hours respectively. The LT_{50} of *Z. multiflora* essential oil on *B. lentis* and *C. maculatus* was 28.45 and 39.62 hours respectively and the LT_{50} of *M. pulegium* essential oil on *B. lentis* and *C. maculatus* was 33.27 and 30.32 hours was calculated respectively (Table 6).

Discussion

According to the results with increasing in essential oil concentration, the adult mortality rate of both species was increased, which are in agreement with reports of other researcher [7,4,12,17], but in a similar experiment conducted by [18], the *B. lentis* sensitivity to *Achillea wilhelmsii* and *Allium sativum* essential oil is less than *T.*

Means of mortality% ± S. E						
		Time (Hour)				
Insect	Con(µl/l air)	3	6	24	48	72
<i>B. lentis</i>	40	0.00 ± 0.001	0.00 ± 0.001	35 ± 2.20 ^{hijk}	39 ± 4.16 ^{ghij}	45 ± 7.00 ^{ghi}
	50	0.00 ± 0.001	0.00 ± 0.001	35 ± 2.20 ^{hijk}	41 ± 7.21 ^{ghij}	55 ± 3.25 ^{defg}
	60	0.00 ± 0.001	0.00 ± 0.001	35 ± 2.12 ^{hijk}	53 ± 3.25 ^{efgh}	73 ± 4.18 ^{bcd}
	70	0.00 ± 0.001	0.00 ± 0.001	55 ± 11.32 ^{defg}	83 ± 4.20 ^{bc}	87 ± 5.00 ^{abc}
	80	0.00 ± 0.001	0.00 ± 0.001	63 ± 4.53 ^{def}	85 ± 5.52 ^{abc}	100 ± 0.00 ^a
<i>C. maculatus</i>	40	0.00 ± 0.001	0.00 ± 0.001	21 ± 3.60 ^m	39 ± 3.00 ^{ghij}	47 ± 6.20 ^{ghi}
	50	0.00 ± 0.001	0.00 ± 0.001	29 ± 3.12 ^{klm}	45 ± 4.12 ^{fghi}	55 ± 2.41 ^{defg}
	60	0.00 ± 0.001	0.00 ± 0.001	35 ± 4.41 ^{klm}	47 ± 6.23 ^{fghi}	69 ± 3.15 ^{cde}
	70	0.00 ± 0.001	0.00 ± 0.001	53 ± 2.24 ^{fghi}	81 ± 5.34 ^{bc}	87 ± 4.20 ^{abc}
	80	0.00 ± 0.001	0.00 ± 0.001	53 ± 3.28 ^{fghi}	91 ± 6.25 ^{ab}	100 ± 0.00 ^a

*Mean in the same column followed by the same letters are not significantly different as determined by the Tukey's-test.

Table 2: Average mortality caused by *Thymus daenensis* essential oil on *B. lentis* and *C. maculatus* at concentrations and different times.

Means of mortality% ± S. E						
		Time (Hour)				
Insect	Con (µl/l air)	3	6	24	48	72
<i>B. lentis</i>	40	0.00 ± 0.00k	0.00 ± 0.00k	27 ± 9.27 ⁱ	41 ± 9.27 ^{fghi}	59 ± 4.00 ^{cdef}
	50	0.00 ± 0.00k	0.00 ± 0.00k	41 ± 3.74 ^{fghi}	53 ± 6.32 ^{defg}	61 ± 2.15 ^{cde}
	60	0.00 ± 0.00k	0.00 ± 0.00k	47 ± 8.72 ^{efg}	69 ± 5.10 ^{bcd}	81 ± 4.45 ^{ab}
	70	0.00 ± 0.00k	0.00 ± 0.00k	57 ± 8.12 ^{cdef}	69 ± 8.72 ^{bcd}	89 ± 3.12 ^a
	80	0.00 ± 0.00k	0.00 ± 0.00k	63 ± 5.48 ^{defg}	71 ± 5.83 ^{bc}	100 ± 5.07 ^a
<i>C. maculatus</i>	40	0.00 ± 0.00k	0.00 ± 0.00k	21 ± 3.74 ^k	35 ± 3.74 ^{ghij}	51 ± 3.00 ^{fg}
	50	0.00 ± 0.00k	0.00 ± 0.00k	23 ± 4.47 ⁱ	41 ± 4.90 ^{fghi}	53 ± 2.34 ^{cdefg}
	60	0.00 ± 0.00k	0.00 ± 0.00k	29 ± 2.45 ^{hij}	49 ± 4.00 ^{efg}	69 ± 3.23 ^{bcd}
	70	0.00 ± 0.00k	0.00 ± 0.00k	35 ± 5.83 ^{ghij}	51 ± 3.74 ^{defg}	71 ± 4.22 ^{bc}
	80	0.00 ± 0.00k	0.00 ± 0.00k	45 ± 3.74 ^{efgh}	57 ± 5.10 ^{cdef}	83 ± 5.11 ^{ab}

*Mean in the same column followed by the same letters are not significantly different as determined by the Tukey's-test.

Table 3: Average mortality caused by *Zataria multiflora* essential oil on *B. lentis* and *C. maculatus* at concentrations and different times.

Means of mortality% ± S. E						
		Time (Hour)				
insect	Con(µl/lir)	3	6	24	48	72
<i>B. lentis</i>	60	0.00 ± 0.00j	0.00 ± 0.00j	33 ± 0.00 ^{hi}	39 ± 3.32 ^{ghi}	47 ± 3.00 ^{gh}
	70	0.00 ± 0.00j	0.00 ± 0.00j	39 ± 4.20 ^{ghi}	45 ± 2.14 ^{fgh}	59 ± 3.13 ^{cdef}
	80	0.00 ± 0.00j	0.00 ± 0.00j	45 ± 2.54 ^{fgh}	55 ± 4.17 ^{fgh}	67 ± 3.00 ^{cde}
	90	0.00 ± 0.00j	0.00 ± 0.00j	53 ± 6.21 ^{efg}	71 ± 4.12 ^{abcde}	75 ± 3.10 ^{abc}
	100	0.00 ± 0.00j	0.00 ± 0.00j	57 ± 3.12 ^{cdefg}	71 ± 6.23 ^{abcde}	85 ± 2.15 ^{ab}
<i>C. maculatus</i>	60	0.00 ± 0.00j	0.00 ± 0.00j	21 ± 2.34 ⁱ	33 ± 4.15 ^{hi}	41 ± 5.17 ^{gh}
	70	0.00 ± 0.00j	0.00 ± 0.00j	29 ± 5.14 ^{hi}	41 ± 5.11 ^{fgh}	45 ± 3.25 ^{gh}
	80	0.00 ± 0.00j	0.00 ± 0.00j	29 ± 3.20 ^{hi}	43 ± 11.17 ^{fgh}	57 ± 7.12 ^{cdefg}
	90	0.00 ± 0.00j	0.00 ± 0.00j	53 ± 6.04 ^{efg}	69 ± 4.23 ^{bcde}	75 ± 6.15 ^{abc}
	100	0.00 ± 0.00j	0.00 ± 0.00j	59 ± 4.00 ^{cdef}	73 ± 7.31 ^{abcd}	89 ± 3.23 ^a

*Mean in the same column followed by the same letters are not significantly different as determined by the Tukey's-test.

Table 4: Average mortality caused by *Mentha pulegium* essential oil on *B. lentis* and *C. maculatus* at concentrations and different times.

Essential oil	Insect	N	X^2 (df = 3)	Slope \pm S. E	LC ₅₀ (μ l/lair)	95% (Confidence limit)	
						Lower	Upper
<i>Mentha piperita</i>	<i>B. lentis</i>	300	2.12	6.92 \pm 1.54	14.62	12.25	19.75
	<i>C. maculatus</i>	300	1.05	8.09 \pm 1.56	13.70	11.82	14.82
<i>Mentha pulegium</i>	<i>B. lentis</i>	300	0.01	5.09 \pm 0.96	92.32	76.75	161.16
	<i>C. maculatus</i>	300	1.30	6.69 \pm 0.99	95.80	84.81	116.30
<i>Zataria multiflora</i>	<i>B. lentis</i>	300	0.16	5.58 \pm 0.96	58.43	49.99	71.54
	<i>C. maculatus</i>	300	0.78	4.98 \pm 0.99	99.94	73.75	442.75
<i>Thymus daenensis</i>	<i>B. lentis</i>	300	2.40	5.65 \pm 0.97	63.97	57.50	82.96
	<i>C. maculatus</i>	300	4.06	6.77 \pm 0.99	65.55	59.83	78.23

Table 5: Calculated values LC₅₀ of essential oils from *M. piperita*, *M. pulegium*, *Z. multiflora* and *T. daenensis* on *B. lentis* and *C. maculatus* after 24 h.

Essential oil	Insect	N	X^2 (df = 3)	Slope \pm S. E	LT ₅₀ (Hour)	95% (Confidence limit)	
						Lower	Upper
<i>Mentha piperita</i>	<i>B. lentis</i>	200	12.02	5.67 \pm 0.65	29.66	8.60	50.17
	<i>C. maculatus</i>	200	7.65	5.94 \pm 0.55	29.43	15.42	42.63
<i>Mentha pulegium</i>	<i>B. lentis</i>	200	7.48	4.62 \pm 0.35	33.27	19.66	54.98
	<i>C. maculatus</i>	200	6.11	4.88 \pm 0.23	30.32	26.92	36.84
<i>Zataria multiflora</i>	<i>B. lentis</i>	200	9.99	5.13 \pm 0.26	28.45	13.43	47.53
	<i>C. maculatus</i>	200	5.24	4.71 \pm 0.14	39.62	34.49	47.45
<i>Thymus daenensis</i>	<i>B. lentis</i>	200	6.07	5.99 \pm 0.23	26.21	22.21	29.89
	<i>C. maculatus</i>	200	4.41	6.24 \pm 0.33	24.15	21.36	28.62

Table 6: Calculated values LT₅₀ of essential oils from *M. piperita*, *M. pulegium*, *Z. multiflora* and *T. daenensis* on *B. lentis* and *C. maculatus*.

daenensis essential oil, which is evaluated in present study, so that, the LC₅₀ of *A. wilhelmsii* essential oil on this insect 122.1 μ l/l air was determined while in this study, LC₅₀ of essential oil on mentioned insect 63.97 μ l/l air was evaluated.

According to several surveys, the main active ingredients of *M. piperita* essential oil are menthone and menthol [19] and thymol and 1, 8-cinirole were reported as the main active ingredients of *T. daenensis* [20].

Based on the present results with increasing the concentrations of essential oils, the mortality of adults of both species was increased, that these results are in agreement with other similar reports [21]. Studies carried out by [22] showed that the sensitivity of *Tribolium castaneum* to *Artemisia aucheri* essential oil less than *Artemisia haussknechtii* essential oil. The LC₅₀ of *A. aucheri* essential oil was reported 122.1 μ l/l air by [22], while in present study; the LC₅₀ of *T. daenensis* essential oil on this mentioned species was 63.97 μ l/l air. More researchers stated that the major insecticidal properties of *T. daenensis* essential oil related to Thymol ingredient and it seems that the more toxicity of *M. piperita* on *B. lentis* in the present study is related to menthone. In another study *A. wilhelmsii* essential oil at a concentration of 1 μ l/l caused 80% mortality of adult females of *Tribolium confusum* 48 hours after application [17], but in the present work, the concentration of 80 μ l/l of *Z. multiflora* caused such a mortality for mentioned insect. This different result may be due to differences in plant active ingredient so that there are reports that show the active ingredient of a plant species is varied in terms of quantitative and qualitative in different geographical conditions [23].

Reference [17] showed that the LC₅₀ of *M. piperita* essential oil on *T. castaneum* was 573.94 μ l/l air that in comparison to *T. daenensis* essential oil that was evaluated in this study had lower fumigant toxicity on *B. lentis*. Also, [17] Calmasur et al. reported the LC₅₀ of *Mentha longifolia* essential oil on *T. castaneum* 0.56 μ l/l air, that according to this results the toxicity of essential oils studied are more than our results in the present study [24-26].

This difference may be related to more thymol in *Thymus* than other species of this genus thymol was demonstrated as an effective insecticide by several researchers. The results of present study revealed that among four essential oils that were surveyed on two species, *M. piperita* essential oil had high fumigant toxicity for both species and due to the low risk of essential ingredients of this plant for human health and the environment can be used in pest management programs.

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