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Insecticidal potential of essential oil from the leaves of *Citrus aurantium* L. against *Oryzaephilus surinamensis* (F.), *Lasioderma serricorne* (L.) and *Sitophilus oryzae* (L.)

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Abstract
In search for alternatives of conventional pesticides, plant essential oils have been widely investigated. Essential oils from aromatic plants are recognized as proper alternatives to chemical fumigants. In this research fumigant toxicity, repellent activity and persistency of Citrus leaves *Citrus aurantium* L. essential oil were tested against three stored product pests, the sawtoothed grain beetle**,** *Oryzaephilus surinamensis* (F.)*,* cigarette beetle *Lasioderma serricorne* (L.) and the rice weevil, *Sitophilus oryzae* (L.). The essential oils were extracted from citrus dried leaves by Clevenger-type distiller. The Watman filter paper No: 1 was used for fumigant toxicity in 40 ml glass vial. Repellency effect of the essential oil was evaluated by comparing the number of adults in both treated and untreated portions. Results showed that the mortality increased with increasing concentration. The LC_{50} values were 64.94, 202.49 and 364.25 and the LC_{90} were 144.94, 659.29 and 663.05 µl/l air respectively. Toxicity of oil against *O. surinamensis* was higher than two others pest. The highest and lowest repellency effects of four concentrations (0.1, 0.2, 0.41 and 0.83 µl/cm^2) were 96.6% and 73.33% for *O. surinamensis* and *L. serricorne* respectively. Based on repellency percentage, average repellency is grades V, IV and IV respectively of the sextet classification. Persistency was different depending on concentration and species insects. The results have shown that the fumigant toxicity of *C. aurantium* on these stored pest were remarkable, therefore this oil could be a safer fumigant to control these pests than those currently used.

Keywords: Essential oil, fumigant toxicity, repellent activity, persistency

Introduction
Conservation of reserved food grain stocks is necessary to ensure a continuous supply at stable prices. Stored product pests are one of the important pests after harvesting in grain storage. These pests with feeding and contaminating with excrete can cause irreversible damage to the store products [6, 13]. Among these pests, coleopteran stored pests are the fiercest pests that are continually reproductive and extremely high generation in optimal conditions. In recent decades, human populations dramatically increase and the risks of food shortages are highly increased [9-7]. In this condition, several chemical insecticides have been used for protecting grain and other stored products from infestation to insects. Although effective, their repeated use has disrupted natural biological control systems and led to resurgence of these insects, resulted in the development of resistance, and had undesirable effects on no target organisms [16]. For this reason, alternative control means are necessary. Plants may provide potential alternatives to currently used insect control agents. Plant materials have several advantages over traditional pest control agents; such as specificity, biodegradability and low mammalian toxicity [11, 19]. Essential oils are potential sources of alternative compounds to currently used fumigants. Essential oils have low toxicity to warm-blooded animals, high volatility, and toxicity to stored-grain insect pests [14, 15].

The Sawtoothed grain beetle**,** *Oryzaephilus surinamensis* (L.), Cigarette beetle *Lasioderma serricorne* (F.) and rice Weevil, *Sitophilus oryzae* (L.) are three important store product pests. They can damage quantitatively and qualitatively to the stored products. The effect of different essential oils against these pests has been determined in various researches [8, 12, 17, 20].

The present study was undertaken to investigate the fumigant toxicity, repellent activity and persistency of *Citrus aurantiunium* L. essential oil against *O. surinamensis, L. serricorne* and *S. oryzae* in lab condition.

Material and methods

Insect rearing

L. serricorne and *O. surinamensis* were reared on mixture wheat and yeast (10:1) and *S. oryza* on rice in a crystalline plastic container. Insect rearing and all experimental procedures were carried out in a growth incubator at 27±3˚C and 65±5% RH.

Isolation of oil

Citrus leaves were collected from non-chemical sprayed citrus trees in Sari province, Sari, Iran during March to July 2014. The leaves were dried at room temperature (27±2) for five days. Dried materials were then subjected to hydro distillation with a Clevenger-type distiller. To extract essential oils by distillation, 60 g of air-dried plant material was put into water (1: 10 w/v) for 3 h. Then the oil was mixed with hexane as solvent and it was isolated with Separator funnel. Mix oil and hexane were dried over anhydrous sodium sulfate and then they were separated from the solvent using a Rotary evaporator. The oil was collected in dark glass containers with aluminum coating and kept in a refrigerator at 4 ˚C until their use.

Bioassay

Fumigant toxicity

The toxicity of the essential oil vapors against adults of *O. surinamensis*, *L. serricorne* and *S. oryzae* was examined using a modified fumigant toxicity assay as described by Huang *et al* [5]. 40 ml glass vials were used as fumigation chambers. Filter paper (Whatman, No. 1) 1×2 cm was treated with different concentration of essential oil by using micro pipette and attached inside of the glass vials. First, primary experiments were performed to determine the minimum and maximum concentration. After, five concentrations and also control were considered. Concentrations of essential oil used against, *O. surinamensis* were 25, 44.25, 79, 140.5 and 250 µl/l air, against *L. serricorne* were 125, 185.25 280.5 424.5 and 650 µl/l air and against *S. oryzae* were 325, 396, 487.25, 597.75 and 750 µl/l air. Somehow the filter paper was hung from the metal cap of the glass vials to prevent direct contact with adults. For control, non-treated filter paper was used. Five couples of insects up to 24 h old were released in each vial. Vial lid tightly closed and impenetrable with parafilm. Mortalities were evaluated 24 h post-treatment. All treatments were replicated four times. Insects were considered to be dead if appendages did not move when they were prodded with fine pins.

Persistency effect

Delayed preliminary test has been done to determine the concentrations of essential oil. Then, three concentrations were assessing for each pest. Similar fumigant toxicity experiment, glass vials were treated with various concentration of essential oil. Insects were released in vial 1, 2, 4, 12, 24 and 48 hrs post-treatments. Vial lid tightly closed and impenetrable with parafilm before and after transferring insects. The concentrations of essential oil against *O. surinamensis* were 175, 315 and 600 µl/l air, against *L. serricorne* 600, 950 and 1425 µl/l air and against *S. oryzae* were 500, 800 and 1280 µl/l air. 24 hours after the confinement, live and dead insects were counted. Three replicated set for this experiment.

Repellency effect

For repellency assay, four concentrations 0.1, 0.2, 0.41 and 0.83µl/cm² of essential oil were prepared by dissolving essential oil in 150 ml acetone. Whatman filter paper was cut into two halves of 80 mm discs and each oil solution was applied to a filter paper half as uniform as possible using a micropipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated halves were dried to evaporate the solvent completely. After that both treated and untreated halves were attached with cellophane tape and placed at the bottom in the Petri plate. Five couples of insects up to 24 h old were released at the center of the filter paper disc and then Petri plates were covered and kept in dark. Three replicates were set for each concentration of essential oil. Number of the insects on both the treated and untreated halves was recorded after one, two and three hours and repellency percentage computed as PR= $\frac{Nc-Nt}{Nc+Nt}\times 100$. Where, Nc; numbers of insects on the untreated and Nt; numbers of insects on the treated surface. Average repellency assigned to sextet group's 0-I-II-III-IV-V. Percent repellency in these categories is as follows :> 0.01-0.1(0), 0.1-20 (I), 20.1-40 (II), 40.1-60 (III), 60.1-80 (IV) and 80.1-100% (V) [18].

Statistical analysis

The mortality results were analyzed statistically using SPSS data processing software. Significant differences among the groups were determined using the unpaired Duncan-test. A value of $P<0.05$ was taken as an indication of statistical significance.

Results and Discussion

Fumigant toxicity

The LC_{50} values were 64.94, 202.49 and 364.25 µl/l air and the LC_{90} values were 144.94, 659.29 and 663.05 for *O. surinamensis*, *L. serricorne* and *S. oryzae* respectively at 24 hrs post-treatments (Table 1). The results has shown that *O. surinamensis*, was more sensitive and *S. oryzae* were less sensitive to this oil. Resistance of *L. serricorne* to essential oil has been investigated by many researchers. The LC_{50} value of *Agastache foeniculum* (Pursh) essential oil against *L. serricorne* and *O. surinamensis* were 21.56 and 18.78 µl/l air respectively [4]. Comparison of essential oil toxicity via LC_{50} and 95% confidence limit showed that the toxicity of *C. aurantium* essential oils against *O. surinamensis* was significantly higher than *L. serricorne*. The results have shown that with 3.75, 8.52 and 3.96 times of LC_{50}, 99% control of these pests has been obtained.

Table 1: Probit analysis of bioassay data *C. aurantium* essential oil against three stored product pests

Insect	Total	LC_{50} (µl/L air) (95% CL[a])	LC_{90} (µl/L air) (95% CL[a])	Slope	Chi Square (χ^2)	P-value
O. Surinamensis	240	64.94 (46.3-104.8)	144.94 (98.4-415.1)	4.05	7.32	0.6
L. serricorne	240	202.49 (161.1-241.3)	659.29 (498.8-1087.5)	2.5	3.88	0.2
S. oryzae	240	364.25(10.1-468.1)	663.05 (505.7-3503)	4.92	8.32	0.4

[a] CL denotes confidence limit

In all three species, mortality increased with increasing concentrations. Similar results were obtained by Manzoomi *et al* [10]. They showed with increasing Concentrations of *Lavandula officinalis* Mill. essential oil from 24 to 61µl/l, the mortality of *Callosobruchus maculatus* (f.) was increased from 3.75 to 95% however, in our study with increasing the concentration from 25 to 250 µl/l air, the mortality of *O. surinamensis* was increased from 7.5 to100%. Also with increasing the concentration from 125 to 650 µl/l air the mortality of *L. serricorne* was increased from 37.5 to 95% and with increasing the concentration 2.3 times, the mortality of rice weevil was increased 1.66 times.

The fumigant toxicity of *Pistacia lentiscus* L. leaves essential oil against adult of *Tribolium castaneum* (Herbst) and *L. serricorne* has shown that cigarette beetle was more sensitive to this oil than flour beetle. The LC_{50} values against these pests were 8.44, 28.03 µl/l air respectively [2]. Therefore toxicity of *P. lentiscus* essential oil against *L. serricorne* was higher than *C. aurantium* essential oil, however in our research *L. serricorne* was more sensitive to *C. aurantium* essential oil than rice weevil. The LC_{50} value of *Cuminum cyminum* L. and *Piper nigrum* L. essential oils against rice weevil were 0.67 and 580 µl/l air respectively [3]. But, in our research the LC_{50} value of *C. aurantium* essential oil against rice weevil was 364.25 µl/l air which is lower than the *C. cyminum* essential oil and higher than the *P. nigrum* essential oil against this pest.

Persistency of essential oil

Result of essential oil persistency showed on table 2-4. The results have shown the suitable persistency of all concentration especially high concentration (600 µl/l air), against these pests. *O. surinamensis* with high concentration (600 µl/l air) of oil has 100% mortality 24 hours post-treatments also medium concentration (315 µl/l air) has high persistency (70% mortality). The lowest concentration (175 µl/l air) up to 4 hours post-treatment has acceptable mortality but, after this time mortality was significantly decreased. The mortality was significantly decreased with the medium concentration of essential oil 12 hours post-treatment however, the significantly reduce mortality with high concentration was occurred 24 hours post-treatments.

Table 2: Mortality percentage of *O. surinamensis* exposed to various concentrations of *C. aurantium* essential oil in persistency experiment

Time (h)	Concentration (µl/L air)		
	175	315	600
1	90±5.77 a*	96.66±3.33a	100±0a
2	90±0 a	93.33±3.33a	100±0a
4	86.66±3.33 a	93.33±3.33a	100±0a
12	60±0 b	90±5.77 a	100±a
24	10±5.77c	70±0b	100±a
48	0±0 d	0±0c	53.33±6.66b

* Different letter in each column showed significant difference (P≤0.05)

All concentrations of essential oil against *L. serricorne* had upper 80% mortality 4 hours post-treatments. High concentration (1425 µl/l air) had 100% mortality 12 hours post-treatments and with low concentration (600 µl/l air), the mortality was significantly decreased with a period of time but with high concentration, the mortality was not significantly decreased.

Table 3: Mortality percentage of *L. serricorne* exposed to various concentrations of *C. aurantium* essential oil in persistency experiment

Time (h)	Concentration (µl/L air)		
	600	950	1425
1	90±0 a*	100±0 a	100±0 a
2	90±0 a	96.66±3.33 a	100±0 a
4	86.66±6.66 a	90±0 b	100±0 a
12	60±5.77 b	80±5.77 c	100±0 a
24	6.66±3.33c	60±0d	96.66±3.33 a
48	0±0 d	46.66±3.33e	80±5.77 b

* Different letter in each column showed significant difference (*P*≤0.05)

With concentration of essential oil increasing, the persistency of toxic effect also increased. The rice weevil treated with high concentration (1280 µl/l air) of essential oil had 100% mortality 24 hours post-treatments. Medium concentration (800 µl/l air) also had 100% mortality 12 hours post-treatments but, the mortality was significantly decreased after this time. The lowest concentration (500 µl/l air) had upper 50% mortality 12 hours post-treatments on rice weevil but, the mortality were significantly decreased after one hour of treatments.

Table 4: Mortality percentage of *S. oryzae* exposed to various concentrations of *C. aurantium* essential oils in persistency experiment

Time (h)	Concentration (µl/L air)		
	500	800	1280
1	80±0 a*	100±0 a	100±0 a
2	63.33±8.81 b	100±0 a	100±0 a
4	60±11.54 b	100±0 a	100±0 a
12	53.33±6.66 b	100±0 a	100±0 a
24	30±5.77 c	70±5.77 b	100±0 a
48	0±0 d	10±5.77 c	30±5.77 b

* Different letter in each column showed significant difference (P≤0.05)

The result of this research has shown that the persistency of high concentration of essential oil was very high and had further 90% mortality after 24 hours post-treatment.

Repellency effect

The repellency effect of four concentration (0.1, 0.2, 0.41 and 0.83µl/cm^2) of essential oil, has been shown in table 5. The result has shown that in all concentration except (0.1 µL/cm^2) for *L. serricorne*, Increasing the treatment time in other species raised the repellency percentage. The highest repellency (80%) with high concentration (0.83 µL/cm^2) and the lowest repellency (20%) with low concentration (0.1 µL/cm2) were observed in *S. oryzae* and *L. serricorne* respectively one hour post-treatment. The highest and lowest repellency effect with high and low concentration of essential oil against *S. oryzae* and *L. serricorne* was the same as one hour at two hour post-treatments. The repellency effect of high concentration on *S. oryzae*, *O. surinamensis* and *L. serricorne* were increased to 100%, 100% and 73.33% respectively three hours post-treatments. The lowest concentration has considerable percentage of repellency. The repellency effect of this concentration was 60, 93.33 and 73.33% respectively three hours post-treatments.

Table 5: Repellent effect of different concentration of *C. aurantium* essential oil against three coleopteran stored product pests

Test insect	Concentration (μL/cm^2)	Repellency (%) Time (h) 1	2	3	Mean repellency (3 h after treatment)	Repellency class
O. surinamensis	0.1	53.33±17.63	66.66±17.63	93.33±6.66	96.66 [a*]	V
	0.2	73.33±17.63	86.66±6.66	93.33±6.66		
	0.41	73.33±17.63	86.66±13.33	100±0		
	0.83	73.33±13.33	86.66±13.33	100±0		
L. serricorne	0.1	20±20	20±20	73.33±6.66	73.33 [b]	IV
	0.2	60±11.54	66.66±13.33	73.33±17.63		
	0.41	60±11.54	66.66±13.33	80±11.54		
	0.83	60±0	73.33±17.63	86.66±6.66		
S. oryzae	0.1	26.66±13.33	33.33±6.66	60±11.54	78.33 [b]	IV
	0.2	26.66±13.33	40±11.54	66.66±13.33		
	0.41	53.33±6.66	40±0	66.66±13.33		
	0.83	80±0	93.33±6.66	100±0		

*Significantly different (*P*<0.05%, Duncan-test).

Average repellency of four concentration of essential oil against *O. surinamensis*, *L. serricorne* and *S. oryzae* were 96.66, 73.33 and 78.33 respectively. This index in *O. surinamensis* was significantly higher than two other species (P≤0.05). Based on repellency percentage, average repellency for *O. surinamensis*, *L. serricorne* and *S. oryzae* were grades V, IV and IV respectively of the sextet classification.

In similar research, the average repellency of four concentrations, 0.125, 0.25, 0.5, 0.75 and 1% of *Cinnamomum camphora* (L.) and *Simmondsia chinensis* (Link) essential oils *against O. surinamensis* were 37.12 and 41.09% respectively [1]. Average repellency of three concentration, 0.012, 0.06 and 0.003g/gm seeds of citrus peel *C. sinensis* essential oil against *Zabrotes subfasciatus* (Bohemann) were 25.75, 33.5 and 37.87% respectively [21]. In our research although the highest average repellency was on *O. surinamensis* but the highest concentration of essential oil had 100% repellency effect on *S. oryzae* and O. *surinamensis*. According to the different researches, the repellency effect and fumigant toxicity are not the same direction for example, the essential oil of *Evodia rutaecarpa* Hook had higher fumigant toxicity against *S. oryzae* than *Tribolium castaneum* but this oil had higher repellency effect against *T. castaneum* than *S. oryzae* [9]. In our research the fumigant toxicity of essential oil against *L. serricorne* was higher than *S. oryzae* however, the average repellency of this oil against *S. oryzae* was higher than *L. serricorne*.

Conclusion

The essential oil from the leaves of *C. aurantium* has remarkable fumigant toxicity, repellent activity and persistency effect on three above coleopteran pests. According to the abundant of the citrus leaves and also the uses dangerous of chemical pesticide we can use this oil for the effective control of these pests. Of course, it is necessary careful studies in store environment to be more confidence on this serious complainant.

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