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Research Article
Efficacy of Some Botanical Volatile Oils on Protection Dry Date Palm from *Oryzaephilus surinamensis* L. Infestation

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Abstract
Background and Objective: The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), is deemed one of the prevalent stored product pests which cause about 43% of the total physical and nutritional loss of grains. The control of this beetle is very difficult due to their ability to hide in many places in storage facilities and built resistance to pesticides. The experiments were targeted to evaluate the reaction of the saw-toothed beetle toward some of commercial and natural volatile oils as alternative safe materials and its role in reducing damage of dry date during storage. **Materials and Methods:** For this purpose, the olfactometer apparatus was designed, olfactory reaction, biological bioassay, sterility effect and persistence index of tested oils toward *Oryzaephilus surinamensis* were studied under laboratory conditions. **Results:** The most repellent oils toward the male and female beetle were fennel and clove oils fumes. The biological assay test was cleared that fumes of clove oils caused the highest reduction in eggs hatchability and mortality percentage of 1st instars' larvae and adults' stage. Continuous exposure larvae to fumes of fennel oils caused malformation in the formed pupae that lead to lost ability to change to normal adult. The cross mating of treatment female and male or each one separately by fumes of tested oils caused sterility ranged between 84-100%. **Conclusion:** The efficacy of clove oil fumes toward saw-toothed beetle was persisting for 5 days while fennel oil was persisting for 2 day. That mean fumes of clove oils was recorded persistence index more than fennel oil.

Key word: *Oryzaephilus surinamensis*, olfactometer, botanical volatile oil, mortality, sterility, damage index, persistence

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera:silvanidae), is secondary pests that attacks damaged grain and dry date[1,2]. It is consider one of the most widespread and wasteful stored product pests which mess and spoil sentient merchandise such as food products and animal feed. Furthermore, storage insect pests are reported to be the most detrimental of even all factors (biotic and abiotic factors) that cause about 43% of the total physical and nutritional loss of grains[3].

The most famous way was used in protecting the stored products, fumigation process by gaseous materials. Recently, many searches were confirmed the efficacy of botanical or commercial oils fumes toward different stored product pests[4-6]. The saw-toothed grain beetle is a very small insect which has the ability to hide in many places in storage facilities, making it difficult to be controlled by insecticides and it has built up resistance to several insecticides[7-9]. In the view of the abovementioned perspective, the present study was interested to test some natural plant oils against different stages of the saw-toothed grain beetle.

MATERIALS AND METHODS

Rearing technique: The saw-toothed beetles were reared under laboratory conditions as Moawad and Al-Ghamdi[2]. New hatched adults were collected and placed in Petri dish (20 cm) filled with a thin film of wheat flour which act as substrate for eggs deposition. After 24 h, the newly deposited eggs were collected. To obtain newly emerged adults, pupae were isolated in separated Petri dish. In general, the main culture were placed in glass container 1 L, supplied with food source (dry dates) and covered with double layer of muslin and kept inside incubator (27±1°C and 65±5% Rh).

Designing olfactory apparatus: Test the olfactory reaction of the saw toothed grain beetle adult stages toward some medicinal and aromatic plant oils.

The olfactory apparatus was designed to accommodate the size and behavior of tested insects following idea of Holtmann[10] and De Kogel *et al.*[11]. The apparatus was made from transparent plastic sheet and divided to five chambers as described in Fig. 1.

The preliminary experiments were carried out to evaluate its ability through sexual interaction. The intensity of reaction was calculated according to Gunn and Cosway[12] equation:

$$\text{Intensity of reaction} = \frac{S\text{-}C}{S+C} \times 100$$

Where:

S = No. of insects enter test chamber

C = No. of insect in control chamber

At these apparatus was tested eleven botanical oils and six commercial oils (Table 1, 2).

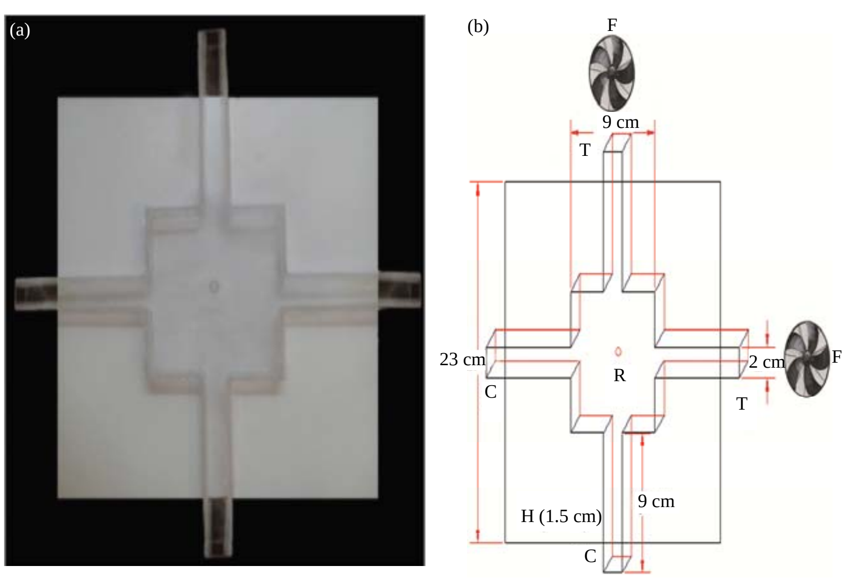
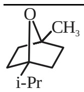
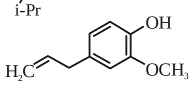
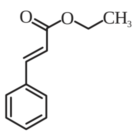
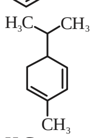
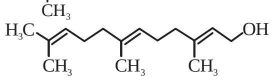
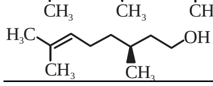


Fig. 1 : Olfactometer apparatus, (a) General view and (b) Apparatus
T: Treated site, C: Control site, R: Release hole, F: Fan (Source of air)

Table 1: Evaluation of natural and medicinal plant oils and extracted parts

English name	Scientific name	Family	Extracted part
Onion	*Allium cepa* L.	Alliaceae	bulb
Garlic	*Allium sativum* L.	Alliaceae	lobes
Cardamom	*Elettaria cardamomum*	Zingiberaceae	seeds
Thyme	*Thymus vulgaris*	Labiatae	leaves
Fennel	*Foeniculum valgare*	Umbelliferae	seeds
Strawberry	*Fragaria ananassa*	Rosaceae	fruits
Clove	*Syzygium aromaticum*	Caryophyllaceae	seeds
Caraway	*Carum carvi*	Apiaceae	seeds
Sage	*Salvia officinalis*	Labiatae	leaves and flowers
Wheat germ	*Triticum aestivum* L.	Graminaceae	seeds
Black seed	*Nigella sativa*	Ranunculaceae	seeds

Table 2: Tested commercial Terpenes oils and its chemical structures

Structure formula	Molecular structure	Terpene
	$C_{10}H_{18}O$	Cineole
	4-($H_2C = CHCH_2)C_6H_3$-2-(OCH_3)OH	Eugenol
	$C_6H_5CH = CHCOOC_2H_5$	Ethyl cinnamate
	$C_{10}H_{16}$	Phellandrene
	$(CH_3)2C = CHCH_2CH_2C(CH_3) = CHCH_2CH_2C(CH_3) = CHCH_2OH$	Farnesol
	$C_{10}H_{18}O$	Citronella

Biological activity of tested oils fumes: The experiments were carried out on the most repellent oils toward different stages of saw-toothed beetles. The different volumes of tested oils (0.02, 0.12, 0.22, 0.32 µL L^{-1}) was put on the filter paper which fixed on the tip of plastic container and then released the target tested stages (eggs, 1st instar larvae or newly emerged adults):

- In case of treatment eggs stage was calculated percentage of eggs hatchability
- While in state of remediation of 1st instar larvae or newly emerged adults were exposed to fumes of oils at interval times (6, 12 and 24 h) after that was removed oils from tested container. The percentage of mortality, pupation, adults' emergence and malformation were recorded
- The last test was done to record sterility effects of fumes of tested oils. Virgin male and female were exposed to vapour of tested oils (0.22 µL L^{-1}) for 4 h and after that isolated two pairs of males and females in separated Petri dish as follows:

- Treated female × treated male
- Treated female × untreated male
- Untreated female × treated male
- Untreated female × untreated male

After one week the experiments were investigated to calculate total number of deposited eggs/7 days, percentage of eggs hatching and percentage of sterility.

Percentage of sterility was calculated according to Tappozada *et al*.[13] as follow:

$$\text{Sterility (\%)} = 100 - \frac{\text{ma} \times \text{na}}{\text{mt} \times \text{nt}} \times 100$$

Where:

ma = Average number of deposited eggs in treatment
na = The percentage of hatching in treatment
mt = Average number of deposited eggs in control
nt = The percentage of eggs hatching in control

Residual effect and persistence index for tested oils fumes: Test efficacy of the most impact oils on adult stages after exposure at interval period during a week, i.e., put tested oils (0.22 µL L^{-1}) separately on filter paper and kept it inside plastic cup (500 mL) and then exposed four pairs of newly emerged adults of saw toothed-beetle for 24 or 48 or 72 h; after that collected exposed adults to liberate into other cups which contained 50 g of disinfested date. The plastic cups with treated adults were kept inside incubator for 54 days to calculate damage index, number of new generation and residual effect and persistence index. The experiments were replicated five times.

Statistical analysis: Obtained data was subject to statistical analysis using the computer program one way ANOVA and t-test.

RESULTS

Behavioural response: Preliminary tests were carried out to be decide the efficiency of the designed air-way olfactometer for the response of mouth to sex attraction and hence to volatile plant oils.

The results in Table 3 showed that intensities of reaction of virgin male toward virgin female (isolated inside tested chamber) increased by time till reaches to 91.3 after 24 h after that no more reactions. On other hand, intensity of reaction of virgin female toward isolated virgin male was recorded less attraction reach to 45.5% after 24 h.

The olfactory reactions of virgin male or female toward natural or commercial oils are cleared in Table 4. The obtained results were cleared that the most of tested oils had repellent effect toward both sexes, except in few cases. The fennel and clove oils (as natural oils) were recorded the highest repellent reaction (-100, -97.1, -83.33 and -80.95%) toward both female and male, respectively.

On other side the commercial oils as ethylcinnamate and phellandrene were recorded the high repellency effect -57.4 and 66.7% toward female while Citronella, Farnesol and phellandrene were elicited moderate repellent effect (43.5, 35.8 and 27.7%) toward male, respectively.

Biological effects of some volatile oils on *O. surinamensis* stages

On egg stage: The data indicated that fumes of clove and fennel oils caused reduction in eggs hatchability at different levels (Table 5). The most impressive one was fumes of clove oil which caused reduction in egg hatchability reached to 100% at 0.32 µL L^{-1}/24 h exposure.

Table 3: Intensity of reaction of virgin saw-toothed grain beetle in air way olfactometer

	Intensities of reaction (%)	
Time (h)	Male toward female	Female toward male
6	25.0	44.0
12	83.3	50.0
24	91.3	45.5

Table 4: Intensities of reaction of some plant essential oils on the olfactory response of *O. surinamensi*

	Intensity of reaction (%)	
Tested oil	Male	Female
Natural botanical oils		
Onion	0.0	71.40
Garlic	56.52	55.56
Cardamom	-42.86	-21.74
Thyme	10.0	-65.22
Fennel	-91.3	-100.00
Strawberry	0.0	-33.33
Clove	-80.95	-83.33
Caraway	-65.5	-16.67
Sage	41.18	33.33
Wheat germ	-20.0	40.00
Black seed	0.0	0.00
Commercial terpene oils		
Cineole	41.2	36.80
citronella	-43.5	-39.50
Ethyl cinnamate	-81.8	-57.40
Eugenol	5.3	11.10
Farnesol	-35.8	5.30
Phellandrene	-27.7	-66.70

-: Repellent response, +: Attractant response

Table 5: Effect of different oils concentrations of *F. valgare* and *S. aromaticum* fumes on *O. surinamensis* eggs hatchability

Tested oils	Concentrations (µL L^{-1} air)	Hatchability (%)
F. valgare	0.02	66.7
	012	58.3
	0.22	58.3
	0.32	50.0
S. aromaticum	0.02	66.7
	012	25.0
	0.22	5.6
	0.32	0.0
Control	0.00	100.0

On larval stage: First larval instars of saw-toothed grain beetle were greatly affected by fumes of *S. aromaticum* more than *F. valgare* when exposed to fumes for 24 h as described at Table 6. The fumes of clove oil caused mortality (%) reached to 86.7% at 0.32 µL L^{-1}/12 for 24 h exposure.

The remaining larvae which are able to live and continue till reach adult stages were followed as shown at Table 7. Fumes of clove oil at 0.32 µL L^{-1} had ability to prevent larval development and pupation compare to control. While fumes of fennel oils was recorded 33.3% adult emergence and 22.2% malformation into formed pupae or pupal-adult intermediate at 0.22 and 0.32 µL L^{-1} (Fig. 2).

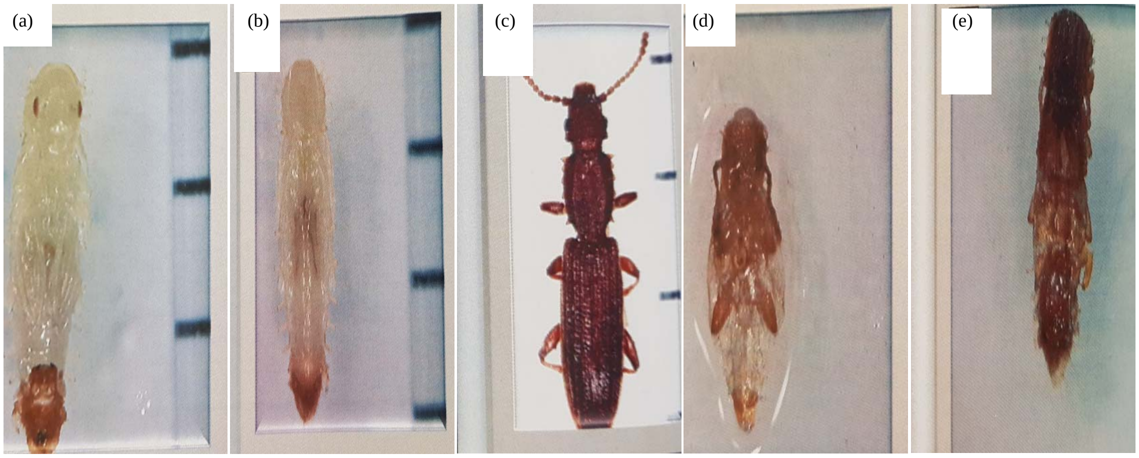


Fig. 2: Effect of continuous exposure of larval stage to fumes of Fennel oils at 0.22 and 0.32 µL L^{-1}, (a-c) Morphological normal pupae (from dorsal and ventral views) and (d, e) Morphological abnormalities in pupae (pupal-adult intermediated)

Table 6: Effect of *F. valgare* and *S. aromaticum fumes* at different concentrations on mortality (%) of 1st larvae stage of *O. surinamensis*

Tested oils	Concentrations (µL L^{-1} air)	Mortality (%) Exposure time (h) 6	12	24
F. valgare	0.02	0	0	0
	0.12	0	0	16.7
	0.22	0	0	53.3
	0.32	20	20	60
S. aromaticum	0.02	0	0	20
	0.12	16.7	66.7	66.7
	0.22	60	70	80
	0.32	60	86.7	86.7
Control	0.00	0	0	0

Table 7: Effect of treatment of 1st larval instar of *O. surinamensis* by fumes of volatile oils on pupation, adult emergence and malformation (%)

Tested oils	Concentrations (µL L^{-1} air)	Pupation (%)	Adult emergence (%)	Malformation (%)
F. valgare	0.02	100.0	100.0	0.0
	0.12	75.0	75.0	0.0
	0.22	66.7	33.3	22.2
	0.32	33.3	33.3	22.2
S. aromaticum	0.02	100.0	66.7	16.7
	0.12	50.0	50.0	16.7
	0.22	33.3	33.3	0.0
	0.32	0.0	0.0	0.0
Control	-	100.0	100.0	0.0

On adult stage: The data indicated that mortality (%) of male and female were increased with increase the period of exposure and concentrations of tested oils fumes (Table 8). The highest percentages of mortality for both sexes were noticed in case of treatment by fumes of clove oil reach to 100% at 0.22 and 0.32 µL L^{-1} air for 24 h.

On other hand, Table 9 cleared that exposure male and female individually or both together to 0.12 µL L^{-1} air fumes oil for 4 h caused significant ($p \leq 01$) depression in reproduction ability i.e., decrease number of deposited eggs, hatchability and increase sterility percentage. Fumes of *S. aromaticum* caused sterility effect reaches to 100% in case of exposure male and female.

Table 8: Effect of tested oils fumes at different concentrations on mortality (%) of adult stage of *O. surinamensis*

Tested oils	Concentrations (µL L^{-1} air)	Mortality (%) Female 6 h	12 h	24 h	Male 6 h	12 h	24 h
F. valgare	0.02	0	0	1.6	0	0	1.6
	0.12	0	0	12.5	0	0	31.5
	0.22	0	0	50	0	0	56.3
	0.32	20	20	56.3	20	20	68.8
S. aromaticum	0.02	0	0	37.5	0	0	62.8
	0.12	0	18	62.5	25	43.8	100
	0.22	0	25	100	25	50	100
	0.32	37.5	68	100	62.5	93.8	100
Control	0	0	0	0	0	0	0

Table 9: Sterility effect of tested oils fumes (at 0.12 µL L^{-1} air) on adult stage of *O. surinamensis*

Tested oil (0.12 µL L^{-1} air)	Crosses	Average No. of deposited eggs±SE	Eggs hatchability (%)	Sterility (%)
F. valgare	TF×UM	2.67±1.12^{b}	64.1	84.3
	TF×TM	2.33±0.76^{b}	56.5	88.2
	UF×TM	4.50±0.67^{b}	55.6	77.3
S. aromaticum	TF×UM	2.50±0.43^{b}	40	90.9
	TF×TM	3.00±0.37^{b}	16.7	95.4
	UF×TM	3.00±0.45^{b}	0.0	100
Control	UF×UM	11.33±0.8^{a}	97.3	0.0
Statistical analysis	$LSD_{0.05}$ = 2.16			
	$LSD_{0.01}$ = 3.7			

Residual effect and persistence index for fumes of tested oils: The date indicated that the fumes of clove oil had ability to effect along three days than fennel oil (Table 10). Fumes of

Table 10: Residual and persistence indices of tested oils fumes at (0.22 µL L^{-1} air) against adults of *O. surinamensis*

	Storage period															
	1 day		2 day		3 day		4 day		5 day		6 day		7 day			
Tested oils	F1	D.I	F1	D.I	F1	D.I	F1	D.I	F1	D.I	F1	D.I	F1	D.I	F/I	F/I× day
F. valgare	1.7±1.7	0.7±0.7 (97.7) (I)	25.0±1.7	16.0±1.2 (46.7) (F)	27.3±1.4	21.0±0.6 (30)	37.7±6	25.7±1.5 (14.3)	43.3±0.8	27.3±0.7 (9)	48.3±0.7	28±0.6 (6.7)	53.0±1.2	29.0±0.6 (3.3)	0.48	0.96
S. aromaticum	0.0±0.0	0.0±0.0 (100) (I)	0.0±0.0	0.0±0.0 (100)	9.0±0.6	9.0±0.6 (70)	13.3±1.3	16.7±0.7 (44.3) (F)	47.7±4	28.0±0.0 (6.7)	52.0±0.2	28±1.2 (6.7)	54.0±0.6	28.8±0.7 (4)	0.44	1.8
Control	59.0±3.5	29.3±0.7 (2.3)	59.0±3.7	29.3±0.7 (2.3)	59.0±3.5	29.3±0.7 (2.3)	59.0±3.5	289.3±0.7 (2.3)	29.3±0.7	59.0±3.5 (2.3)	29.3±0.7	59±3.5 (2.3)	29.3±0.7	59.0±3.5 (2.3)		

I: Initial, F: Final, D.I: Average of damage index, FI: Average number of progeny, F/I: Residual efficiency, F/I×day: Persistence index, The value between brackets are represent the protection (%) or reduction (%) in D.I

clove oil caused reduction in percentage of new progeny and damage index along 3 days, reaching to 9 individuals/54 days and 70% reduction in damage index. So, the residual and persistence indices of clove oil were recorded 0.443 and 1.772 more that other oil.

DISCUSSION

From the obtained results it is clear that there are different between reactions of the most of tested oils toward the attraction both sexes while only the fennel and clove oils was given the same repellent effect toward both sexes. These observations agree with Moawad *et al.*[14] who recoded the repellence effect of clove oil toward *Anacanthotermis ochraceus*.

In addition to the fumes of clove and fennel oils had ability to reduce infestation by *O. surinamensis* during storage through affects on biology of different insect stages at different degree. Microscopically examination to un-hatched eggs was noticed that the colour of eggs changed from crystal white to dark yellow and loss their ability to hatch that might be indicated to its hormonal effect as recorded by Moawad *et al.*[15,6] who test fumes of clove oil on eggs of both *Tuta absoluta* and *Galleria mellonella* L.

The current results data confirmed with Fouad[16] and Moawad[5] who recorded increase in mortality percentage to *Callosobruchus maculates* when exposed to fumes of clove oil. Ebadollahi *et al.*[17] recorded that fennel oils fumes caused mortality to *Sitophilus oryzae* and *S. granarius* and that increase with increase concentration and duration of exposure.

The efficacy of tested oils may be attributed to their chemical constituents. There are author confirmed that as Zeng *et al.*[18] who analysis the chemical constituents of clove oil and recorded 18 components (the most common and important one were 2-methoxy-4-(2-propenyl)-phenol and Trans-caryophyllene) which tested separately with clove oil on *T. castaneum* and *S. oryzae*. They noticed that clove oil and 2-methoxy-4-(2-propenyl)-phenol caused the highest mortality than Trans-caryophyllene compound while 2-methoxy-4-(2-propenyl)-phenol was recorded the highest repellent than clove oil and other compound. While Abo-El-Saad *et al.*[19] attributed the effect of clove oil toward *T. castaneum* to terpenoid elements content which were recorded as Eugenol, caryophyllene and α-caryophyllene. On other side, Kim and Ahn[20] separated different components from fennel oil as estragole, anthole and fenchone which appeared efficacy as fumes toward cowpea beetle and rice weevil.

The volatile oils toxicity effects might be attributed to present olice, linolice and palmice acid and other alkaloid, beside to their odour which closed tracheal system especially when applied as fumes[21]. Moawad[4] confirmed that fumes of some volatile oils make disturbance in the rate of O_2 uptake that led to abnormality and death to the potato tuber moth *Phthorimaea operculella* Zeller that was exposed.

CONCLUSION

The obtained results confirmed that the fumes of clove and fennel oils can decrease the damage of *O. surinamensis* to date during storage. The tested oils had sterility effect toward both male and female. Beside, their efficacy might be persisting for five day (clove oil) or two day only (fennel oils).

SIGNIFICANCE STATEMENT

This study gives valuable information around the importance of botanical volatile oils and their role in protection stored product. The botanical oils can be used as alternative materials to decrease use insecticide, especially on staple food.

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