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Full Length Research Paper

Repellency and fumigant toxicity of clove and sesame oils against American cockroach (*Periplaneta americana* (L.)

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Repellency and fumigant toxicity of clove (*Syzygium aromaticum*) and sesame (*Sesamum indicum*) oils were investigated against American cockroach (*Periplaneta americana* (L.) in the laboratory of the Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah Province, Saudi Arabia at 25 ± 3°C and 75 ± 5% relative humidity. Both clove and sesame oils showed variable percentages of repellency according to concentrations, exposure periods and stages. Clove oil exhibited strong repellent activity than sesame oil. First nymph was more sensitive than the fourth one followed by adults. After 48 h of exposure, complete repellency (100%) was recorded against first nymph at concentration of 2% for clove oil and 6% for sesame oil. Same result was obtained against fourth nymph at concentration of 10% of sesame oil after 48 h. While clove oil completely repelled all fourth nymphs after 24 h at concentration of 8%. For adult stage, the greatest repellency percentages were recorded by clove oil (90.00 ± 5.77%) and sesame oil (83.33 ± 3.33%) after 48 h at a concentration of 10%. Regarding the fumigant toxicity, clove oil provided highly significant effects against nymphs and adults of *P. americana* after 24 and 48 h, respectively. Complete mortality (100%) was recorded at a concentration of 7.5 µl/L of air for first nymph, 10 µl for fourth one and 17.5 µl for adults after 48 h of fumigation. First nymph was the most sensitive to clove oil by LC_{50} value 1.06 µl followed by 3.12 µl for fourth nymph and 8.20 µl for adults. The fumigant tests showed that sesame oil did not exhibit toxicity against *P. americana* at concentrations range of 5 to 20 µl/L of air. In conclusion, both clove and sesame oils can be used as repellent botanical insecticides, but only clove oil can be used as fumigant agent against *P. americana* because sesame oil gave no effect at tested concentrations.

Key words: Sesame oil, clove oil, American cockroach, repellency, fumigant toxicity.

INTRODUCTION

The American cockroach is one of the most important cockroach species (3500) existing in the world; they are world travelers, living with humans in almost everywhere (Ogg et al., 2006). American cockroach are more common in restaurant, grocery stores and bakeries as well as other sites where food is prepared and they can develop to enormous numbers (Hahn and Ascerno, 2005). They may be become pests in homes after being introduced in cartons, grocery bags and containers (Whitworth and Ahmed, 2007).

Although, many pathogens have been isolated from American cockroach, *Periplaneta americana* (L.) (Cotton et al., 2000) and it acts as a reservoir for infection pathogens (Tatfeng et al., 2005). There are no documented cases directly linking cockroaches as the definitive of any human and animal diseases (Kopanic et al., 1994). On the other hand, being a carrier of germs they could transfer them. Cockroaches transfer germs mechanically

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by crawling over bacteria–laden substances and later walking over dishes and eating utensils (Kirbay and Dill, 2004). Cockroaches represent a real hazard for human health and must be controlled in order to maintain acceptable hygiene standards (Chaichanawongsaroj et al., 2004).

Plant oils and monoterpenoid components are selective chemicals and bioactive as insect-control agent (Weaver and Subramanyam, 2000; Lee et al., 2001; Papachristos and Stamopoulos, 2002; Kim et al., 2003; Jogar et al., 2006; Park et al., 2006; Bisseleua et al., 2008). Plant oils have fumigants, antifeedant and repellent effects as well as inhibiting the reproduction in cockroaches (Ahmad et al., 1995; Regnault–Roger, 1997; Apple et al., 2001). Based on the fact that essential oils and monoterpenoid components degrade quickly and some are very pest specific and relatively safe to human and wildlife, they may be potential alternatives to presently use synthetic chemicals against *P. americana*.

Ngoh et al. (1998) recorded that a number of diverse terpenoids in essential oils, such as citronella, citral, gernaniol and eugenol have repellent activity against the American cockroach *P. americana*. Appel et al. (2001) evaluated the repellency and toxicity of mint oil to American cockroach; mint oil-based formulations could provide good integrated pest management tool for cockroach control. Hyperactivity was followed by hyperextension of the legs and abdomen, and fast knockdown followed by death was also demonstrated by Enan (2001) under the effect of three essential oil constituents (eugenol, alpha-terpineol and cinnamic alcohol) against American cockroach. Thavara et al. (2007) investigated the repellent activity of seven commercial essential oils extracted from plant species; they found that the essential oil derived from *Citrus hystrix* showed the best repellency (100%) against *P. americana*.

The present work aimed to evaluate the repellent and fumigant effects of clove (*Syzygium aromaticum*) and sesame (*Sesamum indicum)* oils on American cockroach *P. americana*.

MATERIALS AND METHODS

Tested insect

Field strain of American cockroach *P. americana* (L.) was collected from dark and damp places (sewer) from Jeddah Province (Saudi Arabia) using food jars surrounded by dark cloth as a trap (Wang and Bennett, 2006). The trap was placed into sewers where cockroaches were collected every two days and placed in glass containers. The collected adults and nymphs were separated in glass containers (30 × 60 × 30 cm). The containers were glued 2 cm from the top with petroleum jelly to prevent the escape of cockroaches, and supplied with water, dry dog pellets and cardboard harborage as shelter. The cultures were kept in laboratory of the Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah Province, Saudi Arabia at 25 ± 3°C and 75 ± 5% relative humidity. After two weeks, adult cockroaches (males and females) were used for the experiments. Collected nymphs were maintained at the laboratory until they emerged to the adult stage, then, the deposited egg cases were removed to other rearing containers until hatching. Small nymphs (1st instar) and medium nymphs (4th instar) were used in the experiments.

Tested plant oils

Clove bud (*S. aromaticum*) and sesame (*S. indicum*) oils were extracted and used to evaluate the repellent activity and fumigant toxicity against field strain of American cockroach. Clove was extracted using steam distillation according to Wenqiang et al. (2007). Solvent extraction method was used to extract sesame oil in petroleum benzene (Rajaei et al., 2008).

Repellent tests

Repellent activity of clove and sesame oils was tested against small nymph (1st instar), medium nymph (4th instar) and adult of American cockroach in the laboratory. The experiments were kept at 25 ± 3°C and 75 ± 5% relative humidity. The cockroaches that were located in the treated and control area were carefully observed and counted after 24 and 48 h of exposure. Accumulative mean repellency percentages were calculated from the repellency obtained from three replications. For concentration repellency studies, the clove bud and sesame oils were chosen based on their significantly repellency effect on nymph (1st and 4th instars) and adult stages in the initial repellency experiments.

First nymph

Circular white filter paper (Whatman No. 40, 9 cm diameter), divided into two halves (treated and control area) were used (Liu et al., 2011). One of the halves was treated with 0.5 ml of acetone; the other half was treated with 0.5 ml acetone solutions of oil as much as possible to cover the entire treated area. Both oil and acetone – treated inserts were dried for 5 min under laboratory fume hood before being placed into plastic Petri dish with perforated cover (10 cm). Ten first nymphs (two to five days old) were released into the middle of each Petri dish. Food and water were placed at each half of the Petri dish and subsequently covered. Three replicates were used for each concentration and control in a completely randomized design. Repellency percentage against nymph (1st instar) was calculated using the following equation:

$$\text{Repellency (\%)} = 100\text{-}[T \times 100]/N$$

Where T stands for the number of nymph (1st instar) located in the treated area and N stands for the total number of nymph (1st instar). The tested concentrations were 0.5, 1.0, 1.5, 2.0 and 4.0% for clove oil and 2, 4 and 6% for sesame oil.

Fourth nymph

The same method used for 1st instar was conducted for 4th nymph. The concentrations used for clove oil were 2, 4, 6 and 8% and 2, 4, 6, 8 and 10% for sesame oil.

Adult

A glass square-box (50 × 50 × 15 cm, with the top open) was employed in the repellent tests. All four walls of the box were

smeared with vaseline to prevent escaping of cockroaches. A piece of filter paper (Whatman No.1, 50 × 50 cm) was marked by a pen to divide it into two equal parts (treated and control areas) and then placed at the bottom of the box. The tested oils were dissolved in acetone and 1.25 ml was applied on the treated area to cover the whole of treated part of the filter paper, whereas the control area was treated by acetone. Both oil and acetone – treated inserts were dried for 5 min under laboratory fume hood before being placed into the box. Food and water were placed at both sides of the box. Twenty (20) adults after two weeks of collection were released into the box at the central point. Three replicates were used for each concentration and control in a completely randomized design. The treatments were maintained in the laboratory conditions. Active concentrations used for both clove and sesame oils were the same (2, 4, 6, 8 and 10%).

Fumigant toxicity tests

Clove and sesame oils were also tested for fumigant toxicity against small nymph (1st instar), medium nymph (4th instar) and adult of American cockroach in the laboratory.

First nymph

Fumigant activities of clove and sesame oils were assessed by sealing 10 first nymphs (two to five days olds) in 1 L glass jars with a 1 cm diameter cotton ball (20 g) treated with 50 µl oil dissolved in acetone whereas the control cotton ball was treated by acetone (Ling et al., 2009). Oil was injected into the center of each cotton ball to allow volatilization and to prevent direct contact with the residue. Both oil and acetone – treated inserts were dried for 5 min under laboratory fume hood before being placed into the jars. The tested concentrations for clove oil were 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 and 5, 10, 15, and 20 µl/L of air for sesame oil. Food and water were placed in the treated jars and control. Three replicates were used for each concentration and control in a completely randomized design. The treatments were maintained in the same conditions as previously mentioned in the repellent tests against nymph (1st instar). Mortality was recorded after 24 and 48 h of exposure and accumulative mean mortality percentages calculated, respectively.

Fourth nymph

Same methods as previously mentioned for the treatment of nymph (1st instar) were used for nymph (4th instar 5 to 15 days old). The tested concentrations for clove oil were 2.5, 5.0, 7.5 and 10.0 and 5, 10, 15, and 20 µl/L of air for sesame oil.

Adult stage

Same methods used for nymph (1st instar) were applied for the adult stage. The tested concentrations for clove oil were 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 and 5, 10, 15, 20 µl/L of air for sesame oil. Probit analysis (POLO-PC statistics software) (LeOra, 1987) was used to determine LC_{50}, LC_{90} and the slope. The toxicity lines were drawn on log probit graph paper (Finney, 1952; Matsumura, 1975).

Statistical analysis

All data were statistically analyzed using SPSS statistical analysis one way analysis of variance (ANOVA, version 11) (Bluman, 2007). Means were compared using least significant difference (L.S.D).

RESULTS

Repellency and fumigant toxicity of clove (*S. aromaticum*) and sesame (*S. indicum*) oils were evaluated against the different stages of American cockroach, *P. americana*. Data presented in Table 1 shows that both clove and sesame oils caused highly significant repellency against first nymph of *P. americana* at different concentrations and exposure periods. After 24 h of exposure, clove oil recorded complete repellency (100 ± 0.00%) to the first nymph of *P. americana* at a concentration of 4% while sesame oil provided a high degree of repellency (80.00 ± 5.77%) at the highest concentration, 6%. After 48 h of exposure, the repellent activity of both clove and sesame oils was same (100 ± 0.00%) at different effective concentrations of 2 and 6%, respectively.

Repellent activity of clove and sesame oils against forth nymph is summarized in Table 2. Repellency was highly significantly increased as it was influenced by the concentrations. Clove oil had the greatest effect; it gave moderate level of repellency (86.67 ± 3.33%) at concentrations of 6% after 24 h and after 48 h increased to 96.67 ± 3.33%, while complete repellency for all forth nymphs was recorded at the highest concentrations of 8% after 24 h. Sesame oil exhibited a high repellency (86.67 ± 3.33%) after 24 h and complete repellency (100 ± 0.00%) after 48 h at the highest concentration, 10%. Using concentrations range of 2 to 10% for adult stage, the repellency after 24 and 48 h of both clove and sesame oils showed highly significant increase as the concentrations increased (Table 3). The lowest repellency was recorded at concentration of 2% for both oils. After 24 h, the highest repellency was 70.00 ± 5.77 for clove oil and 66.67 ± 3.33% for sesame oil at the highest concentration 10% and after 48 h increased to 90.00 ± 5.77 and 83.33 ± 3.33%, respectively.

Regarding the fumigant toxicity, responses of highly significant effects varied according to the concentrations of clove oil (Table 4). Data reported no control mortality for any tested stages during study after 24 h. The lowest mortality percentages (16.67 ± 3.33, 6.67 ± 3.33 and 3.33 ± 3.33%) were recorded at the lowest concentrations of 0.5, 2.5 and 5.0 µl/L of air for first nymph, fourth one and adults of *P. americana*, respectively. Clove oil recorded complete mortality (100 ± 0.00%) at the highest concentrations 10.0 µl/L of air for first and fourth nymphs and 20.0 µl/L of air for adults. Sesame oil gave no fumigant toxicity at tested concentrations (5, 10, 15, and 20 µl/L of air).

After 48 h of fumigation by clove oil, complete mortality (100.00 ± 0.00%) was recorded at effective concentration 7.5 µl/L of air against first nymph, 10.0 µl/L of air against fourth one and 17.5 µl/L of air against adult stage as indicated in Table 5. Highly significant responses varied according to concentrations. The first nymph was more

Table 1. Repellent activity of clove and sesame oils on nymph (1st instar) of *P. americana.*

Oil	Concentration (%)	Interval (h)							
		Repellent % ± SE		F		$L.S.D._{0.05}$		$L.S.D_{0.01}$	
		24h	48h	24 h	48 h	24 h	48 h	24 h	48 h
Clove	0.0	0.00±0.00[a]	0.00±0.00	101.90**	183.20**	11.86	8.57	16.63	13.44
	0.5	10.00±5.77[ab]	23.33±3.33						
	1.0	16.67±3.33[b]	33.33±3.33						
	1.5	36.67±3.33	50.00±5.77						
	2.0	70.00±5.77	100.00±0.00						
	4.0	100.00±0.00	-						
Sesame	0.0	0.00±0.00	0.00±0.00	57.37**	156.00**	14.38	10.87	20.92	15.82
	2.0	33.33±3.33	60.00±5.77[a]						
	4.0	50.00±5.77	66.67±3.33[a]						
	6.0	80.00±5.77	100.00±0.00						

The means followed by the same letter (in the same column) are not significantly different at the 0.05 level of probability. **Differences between means are highly significant at 0.01 level of probability.

Table 2. Repellent activity of clove and sesame oils on nymph (4th instar) of *P. americana.*

Oil	Concentration (%)	Interval (h)							
		Repellent % ± SE		F		$L.S.D._{0.05}$		$L.S.D_{0.01}$	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Clove	0	0.00±0.00	0.00±0.00	88.32**	52.82**	14.99	13.33	21.80	19.37
	2	16.67±3.33	36.67±3.33						
	4	53.33±3.33	76.67±3.33						
	6	86.67±3.33[a]	96.67±3.33						
	8	100.00±0.00[a]	-						
Sesame	0	0.00±0.00	0.00±0.00	46.17**	102.40**	14.53	10.14	20.37	15.90
	2	20.00±5.77[a]	40.00±5.77						
	4	30.00±5.77[a]	53.33±3.33						
	6	53.33±3.33[b]	70.00±0.00						
	8	66.67±6.67[b]	90.00±5.77[a]						
	10	86.67±3.33	100.00±0.00[a]						

The means followed by the same letter (in the same column) are not significantly different at the 0.05 level of probability. **Differences between means are highly significant at 0.01 level of probability.

Table 3. Repellent activity of clove and sesame oils on adult stage of *P. americana.*

Oil	Concentration (%)	Interval (h)							
		Repellent % ± SE		F		$L.S.D._{0.05}$		$L.S.D_{0.01}$	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Clove	0	0.00±0.00[a]	0.00±0.00[a]	39.54**	40.44**	12.12	14.84	19.01	23.28
	2	3.33±3.33[a]	6.67±3.33[a]						
	4	16.67±3.33	30.00±5.77						
	6	33.33±3.33	46.67±8.82[b]						
	8	46.67±6.67	56.67±3.33[b]						
	10	70.00±5.77	90.00±5.77						
Sesame	0	0.00±0.00[a]	0.00±0.00[a]	49.03**	44.96**	10.14	13.91	15.90	19.50
	2	6.67±3.33[ab]	10.00±5.77[ab]						
	4	13.33±3.33[b]	23.33±3.33[b]						
	6	30.00±5.77	40.00±5.77[c]						
	8	43.33±3.33	50.00±5.77[c]						
	10	66.67±3.33	83.33±3.33						

The means followed by the same letter (in the same column) are not significantly different at the 0.05 level of probability. **Differences between means are highly significant at 0.01 level of probability.

Table 4. Fumigant toxicity of clove oil against *P. americana* after 24 h.

Stage		Concentration (µL/L of air)	Mortality % ± SE	F	$L.S.D._{005}$	$L.S.D_{0.01}$
Nymph	1st	0.0	0.00±0.00	247.18 **	7.09	9.94
		0.5	16.67±3.33[a]			
		1.0	23.33±3.33[a]			
		2.5	50.00±0.00			
		5.0	63.33±6.67			
		7.5	80.00±0.00			
		10.0	100.00±0.00			
	4th	0.0	0.00±0.00[a]	127.70**	12.40	18.03
		2.5	6.67±3.33[a]			
		5.0	53.33±6.67			
		7.5	73.33±3.33			
		10.0	100.00±0.00			
Adult		0.0	0.00±0.00[a]	182.26**	8.65	11.92
		5.0	3.33±3.33[a]			
		7.5	23.33±3.33			
		10.0	46.67±3.33[b]			
		12.5	53.33±3.33[b]			
		15.0	73.33±3.33			
		17.5	96.67±3.33[c]			
		20.0	100.00±0.00[c]			

The means followed by the same letter (in the same column) are not significantly different at the 0.05 level of probability. **Differences between means are highly significant at 0.01 level of probability.

sensitive to clove oil by LC_{50} and LC_{90} values (1.06 and 4.22 µl) than for fourth nymph (3.12 and 6.04 µl) followed by adult stage (8.20 and 15.10 µl), respectively (Figure 1). Homogeneity of response (slope of the log-dose probit relationship) was least for first nymph (2.14 ± 0.35) and greatest for fourth nymph (4.48 ± 0.42) followed by adult

Table 5. Fumigant toxicity of clove oil against *P. americana* after 48 h.

Stage		Concentration (µl/L of air)	Mortality % ± SE	F	L.S.D.$_{0.05}$	L.S.D.$_{0.01}$	LC_{50}	LC_{90}	Slope ± SE	Intercept	95% confidence	
											Lower	upper
Nymph	1st	0.0	0.00±0.00	123.56**	11.02	15.67	1.06	4.22	2.14 ± 0.35	4.95	4.45	5.44
		0.5	33.33±3.33[a]									
		1.0	40.00±0.00[a]									
		2.5	73.33±3.33									
		5.0	93.33±3.33[b]									
		7.5	100.00±0.00[b]									
		10.0	-									
	4th	0.0	0.00±0.00	110.20**	3.33	19.37	3.12	6.04	4.48 ± 0.42	2.78	2.25	3.32
		2.5	36.67±3.33									
		5.0	76.67±3.33									
		7.5	96.67±3.33[a]									
		10.0	100.00±0.00[a]									
Adult		0.0	0.00±0.00	84.75**	8.01	11.04	8.20	15.10	4.83 ± 0.78	0.58	-1.59	2.75
		5.0	20.00±5.77									
		7.5	43.33±3.33									
		10.0	63.33±6.67[a]									
		12.5	70.00±5.77[a]									
		15.0	93.33±3.33[b]									
		17.5	100.00±0.00[b]									
		20.0	-									

The means followed by the same letter (in the same column) are not significantly different at the 0.05 level of probability. **Differences between means are highly significant at 0.01 level of probability.

stage (4.83 ± 0.78).

DISCUSSION

The laboratory repellency results indicated highly significant differences in susceptibility to clove and sesame oils among the nymph (1st and 4th instars) and adult stages of American cockroach after 24 and 48 h. First nymph was the most sensitive, followed by the fourth one and adults as the least. Clove oil was more effective than sesame oil. Also, repellency percentages increased as exposure period prolonged. After 48 h, clove and sesame oils provided complete repellency (100%) against first nymph at concentrations of 2 and 6%, respectively. Same percentage was obtained against fourth nymph at the highest concentration 10% of sesame oil after 48 h and at 8% of clove oil after 24 h, whereas after 48 h of exposure at concentration of 6%, clove oil exhibited high degree of repellency (96.67 ± 3.33%). Concerning, the adults of American cockroach, clove and sesame oil showed high repellencies (90.00 ± 5.77 and 83.33 ± 3.33), respectively at a concentration of 10% after 48 h.

Studies regarding the repellency of clove and

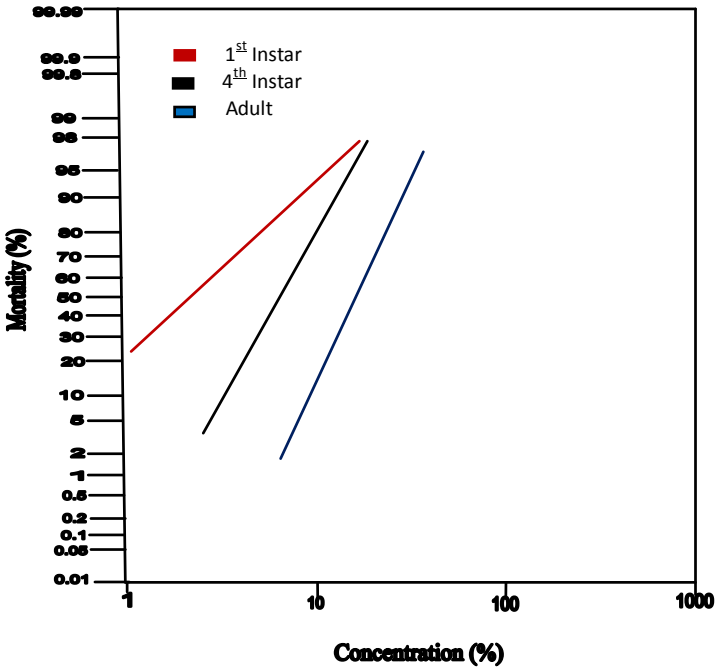

Figure 1. Fumigant toxicity lines of clove oil on nymph (1st, 4th instar) and adult of *P. americana* after 48 h.

sesame oils against American cockroach are limited. Appel et al. (2001) mentioned that mint oil provided complete repellency to adults of American and German cockroaches. Thavara et al. (2007) recorded that essential oils (100%) derived from kaffir lime leaves was the most effective repellency tested showing complete repellency against *P. americana* and *Blattella germanica* and essential oil (100%) derived from ginger rhizomes showed moderate level of repellency (85%) against *P. americana*. Previous studies showed that some essential oils and components from peels of orange (*Citrus* spp.), eucalyptus, grapefruit, lemon, lime, and orange oils had repellent activity against American and German cockroaches (Imjai et al., 2008; Yoon et al., 2009). Recently, some essential oil components can serve as repellents to American cockroach (Ling et al., 2009) and to German cockroach (Phillips and Appel, 2010; Phillips et al., 2010; Alzogaray et al., 2011; Liu et al., 2011).

Regarding the fumigant toxicity, the present results shows that the effective concentration of 20.0 µl/L of air of clove oil to get 100% mortality for adult *P. americana* was double the effective concentration 10.0 µl/L of air for both first and fourth nymphs after 24 h. The results indicate that the sensitivity of first nymph was higher than the fourth one to clove oil, followed by the adult stage. In this respect, Appel et al. (2001) found that fumigation with 50 µl of mint oil revealed 100% mortality of *B. germanica* adult after 24 h. Fumigant toxicity of essential oil derived from *Allium sativum* (L.) (*Liliales: Liliaceae*) was recorded against adult of *B. germanica* (Tunaz et al., 2009) and same results was obtained by essential oil derived from genus *Eucalyptus* against first nymph of the same species (Alzogaray et al., 2011). Concerning the slope of the log-dose probit relationship of the current fumigant test, there was no significant homogeneity to clove oil between adults (4.83 ± 0.78) and fourth nymph (4.48 ± 0.42). While the slope for first nymph was low 2.14 ± 0.35 and revealed no significant heterogeneity to clove oil.

In conclusion, both clove and sesame oils can be used as repellent botanical insecticides, but only clove oil can be used as a fumigant agent against *P. americana* because sesame oil gave no effect at tested concentrations (5, 10, 15, and 20 µl/L of air).

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