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Insecticidal potential of cardamom and clove extracts on adult red palm weevil *Rhynchophorus ferrugineus*

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ABSTRACT

Toxicity of cardamom and clove seed powder and extracted compounds against the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae), was assessed in laboratory exposure experiments. The treatments comprised different amounts of seed powder of cardamom (0.8, 1, 3, and 5 mg) and clove (1, 3, 5, 7 mg), and extract concentrations (0.2, 0.4, 0.5, 0.6, 0.7, and 0.8) for both plants using ether petroleum or chloroform. Data showed that 5 mg of cardamom powdered seed resulted in 93% mortality after one day and 100% mortality after two days. Whereas after two days, lower amounts (0.8, 1, and 3 mg) resulted in 26%, 40%, 46%, respectively. A similar result was obtained for clove seed powder, where 7 mg caused 53% mortality after one day and 100% mortality after three days, other amounts (1, 3, and 5 mg) resulted in 33%, 73%, and 80%, mortality respectively, after three days. We found that all amounts of extract of both plants resulted in 100% mortality after three days. GC-MS analysis of the cardamom and clove extracts revealed the presence of a large number of terpenes of particular note was eugenol and two novel compounds Hydroxy-alpha-Terpenyl Acetate and Labda -8(17),13(E)-Diene- 15. The current work aims at the possibility of benefiting from natural plants pesticides as being safer as well as on the separation of volatile oils, which was known to be important in the control pests.

1. Introduction

The Red palm weevil (RPW)) *Rhynchophorus ferrugineus* (Olivier.) (Coleoptera: Dryophthoridae) is a major pest of various palms in the Middle East, South and South East Asia, North Africa and Southern Europe (Nirula, 1956). Females lay eggs in damaged or wounded plants. Upon hatching, the larvae burrow into the fresh tissue and migrate to the bud region and heart of the crown where they feed for two to four months eventually killing the host plant (Abraham, 1971). The RPW has a wide and host range and has been reported to infest 40 species palm worldwide (Vidyasagar and Subaharan, 2000). Infested date palms exhibit several symptoms depending on the stage of attack, such as producing of brown fluid with a fermented odor that is mixed with palm tissue exerted by feeding larvae, tunneling of palm tissue, presence of adults and pupae at the base of fronds, dried of infested offshoots, pupae around the palm base, dried outer leaves, and topping of the trunk in casees of sever and extensive tissue damage (Vidyasagar and Subaharan, 2000).

Management of agricultural pests of field and post-harvest crops over the past half century has largely depended on the use of synthetic pesticides (Ferry et al., 2004). Several reviews of control strategies for RPW including integrated pest management are available. The development of insect resistance to synthetic pesticide and the associated high operational cost and environmental pollution haves created a need alternative approaches to control insect pests. The use of essential oils is a potential alternative to synthetic pesticides in the control of numerous field and household insect pests (Sarwar et al., 2005, 2012, 2013). Essential oils are natural, volatile and complex compounds, and their characteristic odors are attributed to secondary metabolites in plants. Many plant essential oils and their constituents from terpenes show a broad spectrum of insecticidal, repellents, attractants, inducement and deterrent of oviposition, growth regulating and anti-vector activity against pest insects (Bakkali et al., 2008). Plant essential oils and monoterpenes act as botanical pesticides. One of the most important features of botanical pesticides is the range of effects against specific insects. Most compounds present in essential oils have been shown to be relatively non-toxic to mammals and fish

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via toxicological tests indicating reduced risk. Monoterpenes, the chemical constituents of essential oils found in plants, are biologically active compounds. Further, essential oils and their constituents demonstrate fumigant and topical toxicity as well as antifeedant and repellent effects (Shaya et al., 1997; Sharaby and EL-Dosary, 2014; Sharaby and EL-Nugiban, 2015) in insects. Spice essential oils contain a complex mixture of volatile monoterpenes, sesquiterpenes, and phenols, which play important defensive roles against insect herbivory (Isman, 2006). Some of these essential oil constituents were found to exhibit contact or fumigant toxicity, while others had only insect repellent, and antifeedant effects (Isman, 2000; Sedy and Koschier, 2003; Kanat and Alma, 2004; Nerio et al., 2010; Caballero Gallardo et al., 2011).

The cardamom (*Elettaria cardamomum* [L.] Maton) from the ginger family (Zingiberaceae) is considered as the queen of spices in India. Its seeds contain a clear to pale yellow essential oil with a pungent odor. The cardamom oil is mainly composed of two major constituents, 1, 8-cineole and α-terpinyl acetate, up to 50% each (Weiss, 2002). It was reported to be toxic to different life stages of the coleopteran and lepidopteran stored-product pests through contact and fumigant actions (Abbasipour et al., 2011). Also, Clove essential oil has been widely studied for its insecticidal and repellent activities against many species of pests (Chaieb et al., 2007, Kafle and Shih, 2013, Cortés-Rojas et al., 2014). However, there is no report on the bioactivity against *R. ferrugenius.* So this study aims to evaluate the acute toxicity of cardamom and clove extracts against adult red palm weevil in the laboratory.

Here, we evaluated the toxicity of extracted oils and powder of cardamom and clove against adult *R. ferrugineus*.

2. Materials and methods

2.1. Insects

Adult RPW were obtained from Ministry of Agriculture and Water at- Al Kharj. Transferred to the laboratory entomology Prince Sattam bin Abdel Aziz University, Al-Kharj, KSA. Rearing of adult red palm weevil was according to Al-Rajhy et al. (2005).

2.2. Plants

Cardamom (*Elettaria cardamomum*) and clove (*Eugenia caryophyllus*) seed were get from the market, washed in cold water, dried and extracted using petroleum ether or chloroform, and used for bioassays.

2.3. Extractions

Compounds from known weights of seed, washed in cold water, dried and then ground in a high-speed blender. The resulting powder was soaked in different organic solvents for 48 h. For extraction, 500 ml of petroleum-ether (B.P.4-60 °C C) was added to flask containing 250 g of powdered cardamom seeds (Su, 1985). After 48 h, the flask was shaken vigorously for almost with whatman filter paper (No.4) through anhydrous sodium sulphate. The solvent was then evaporated in a water- bath at 40 °C and the filtrate was thoroughly dried and put back into the flask. Extraction was repeated with chloroform. Extracts were then kept in screw-capped glass vials at 4 °C until needed (Islam, 1983, Afifi et al., 1988).

2.4. Chemical analysis

Extractions were analyzed using gas Chromatography-mass spectrometry (GC-MS), where molecules were separated due to differences in molecular weight and ionized. Chemical compounds were identified, based on the relative intensity of the ions using GC-MS ion trap configuration that then allowed mass spec/mass spec (MS/MS) analysis of samples, reduce the rate of false positive. GC-MS parameters included: Shemadzu, 5050 GC-MS 2 supplied samples model 20S-Aoc, and automatic injector 20i-Aoc characteristic fragmentation split/splitless by column separation Rtx-30 m, 0.25 mm ID, detector ETP, helium gas at 1.5 ml/min, temperature 50–310 °C for oven at high 10 m/min and 250 °C for injector, 280 °C for detector injected with 1 μl of each extract separately (Al-Dawsary, 2014).

2.5. Treatments

Three replicates of five adult RPW were exposed to a slice of sugarcane that had been mixed with 0.8, 1, 3 or 5 mg of powdered cardamom seed or 1, 3, 5, or 7 mg of powdered clove seed. Untreated sugarcane was a control. Mortality was assessed after 24, 48, and 72 h of exposure and LC50 and LC95 were calculated.

Three replicates of RPW were treated with the extracted compounds. 1 ml of extract was mixed with 10 ml of distilled water and three drops of Triton × 100 to create an essential oil emulsion that was applied to the insects. Using a small plastic hand sprayer. An aqueous solution of the same amount of water + emulsifier was applied as a control. Following treatment Insects were allowed to dry and then placed in a glass jar for mortality observation after 24, 48, and 72 h. All experiments were conducted in constant temperature at 28 ± 2 °C.

2.6. Statistical analysis

Used SPSS (24), and used Anova two ways for analysis data.

3. Results

Terpene Content namely eugenol, linalool, 1,8-cineole, chavicol, myrtanol, 1-octadecanol, acetyl eugenol, 3-allyl eugenol, acetyl eugenol, and many compounds were present in all extracts (see Table 1 and 2, Figs. 1 and 2).

3.1. Effect of seeds powder

Effect of cardamom seed powder on RPW mortality rate varied with concentration. After one day of treatment mortality rate at the highest concentration 5 mg was 93%, but zero at the other concentration; after two days mortality at the highest concentration had reached 100%. A three days mortality rates for the 0.8- 1- and 3- mg treatments were 40%, 26%, and 46% respectively (Table 3) the effect of cardamom seed powder was significantly different from that at 0.05%.

3.2. Effects of clove

Seeds powder on RPW mortality also varied with concentration mortality rate at the highest concentration (7 mg) after one day was, and 100% after three days. Mortality rates in the 1- 3- and 5 mg treatments were 33%, 80%, and 73% respectively (Table 3) the effect of cardamom seed powder was significantly different from that at 0.05%.

3.3. Effect of extracted compounds

Cardamom compounds extracted using petroleum ether mortality after one day of exposure; after three days, mortality rates had risen to 100% response to the 0.4-0.6, and 0.7 ml treatments

Table 1
Terpenoids found in cardamom extracts.

Chloroform		Petrolium ether	
Name compound	RT	Name compound	RT
.ALPHA.-PINENE	8.34	1,8-CINEOLE	11.40
SABINENE	9.56	TRANS-SABINENE HYDRATE	12.68
.BETA.-MYRCENE	10.10	LINALOOL	13.64
1,8-CINEOLE	11.44	.DELTA.-TERPINEOL	15.70
TRANS-SABINENE HYDRATE	12.68	3-CYCLOHEXEN-1-OL	15.96
LINALOOL	13.64	LINALYL PROPIONATE	
.DELTA.-TERPINEOL	15.72	4-TERPINENYL ACETATE	16.88
3-CYCLOHEXEN-1-OL	15.98	Z-CITRAL	17.54
LINALYL PROPIONATE		LINALYL ACETATE	17.88
CIS-SABINENE HYDRATE ACETATE	16.88	TRANS-GERANIOL	18.02
Z-CITRAL	17.56	Z-CITRAL	18.38
LINALYL ACETATE	17.86	.DELTA.-TERPINYL ACETATE	19.58
TRANS-GERANIOL	18.00	ALPHA-TERPINYL ACETATE	20.70
CITRAL	18.38	EUGENOL	20.82
.DELTA.-TERPINYL ACETATE	19.58	NERYL ACETATE	21.28
2,6-OCTADIENOIC ACID		TRANS-CARYOPHYLLENE	22.40
1-P-MENTHEN-8-YL ACETATE		1-METHYL-4-(1-ACETOXY-1-METHYL	22.74
EUGENOL	20.78	GERMACRENE-D	32.92
NERYL ACETATE	21.28	.BETA.-SELINENE	24.14
2-OCTENYL ACETATE	21.60	(-)-.ALPHA.-SELINENE	24.32
TRANS-CARYOPHYLLENE	22.40	3-ALLYL-6-METHOXYPHENOL	24.82
1-METHYL-4-(1-ACETOXY-1-METHYL	22.76	HYDROXY-.ALPHA.-TERPENYL ACETATE	25.04
.BETA.-SELINENE	24.14	D-NEROLIDOL	26.20
.ALPHA.-SELINENE	24.32	(E,E)-4,8,12-TRIMETHYL-1,3,7,1	
ACETIISOEUGENOL	24.80	(. + -.) 2-EXO-HYDROXYCINEOLE	
HYDROXY-.ALPHA.-TERPENYL ACETATE	25.04	CIS-FARNESOL	30.50
NEROLIDOL 1	26.20	TRANS, TRANS-FARNESAL	31.00
(E,E)-4,8,12-TRIMETHYL-		FARNESYL ACETATE 3	
1,3,7,1,1-TRIDECATETRAENE	26.48	GERANYL LINALOOL ISOMER B	37.26
(. + -.) 2-EXO-HYDROXYCINEOLE		NONADECANE	42.28
CIS-FARNESOL	30.52	1-OCTADECANOL	45.08
TRANS-CARYOPHYLLENE	31.00	DOCOSANE, 11-DECYL-	45.50
(+)-LABDA-8(17),13(E)-DIENE-15	32.56	HEXADECANOIC ACID	46.60
FARNESYL ACETATE 3		TRITETRACONTANE	48.46

Table 2
Terpenoids found in clove extracts:

Chloroform		Petrolium ether	
Name compound	RT	Name compound	RT
CHAVICOL	18.62	CYCLOHEXANE	4.26
.ALPHA.-COPAENE	20.48	CYCLOHEPTANE	4.44
3-ALLYL GUAIACOL	21.24	HEPTANE	5.38
TRANS-CARYOPHYLLENE	22.54	BENZENE	6.52
.ALPHA.-HUMULENE	23.36	NONANE	7.36
GERMACRENE-D	23.96	CHAVICOL	18.54
1-NAPHTHALENOL	24.38	ALPHA.-COPAENE	20.46
E,E-.ALPHA.-FARNESENE	24.48	EUGENOL	21.02
ACETYL EUGENOL	25.12	ALPHA.-COPAENE	21.26
CADINA-1,4-DIENE	25.36	BICYCLO[7.2.0]UNDEC-4-ENE	22.46
1-METHYLENE-2B-HYDROXYMETHYL-3		ALPHA.-HUMULENE	23.30
−4B-(3-METHYLBUT- 2-ENYL)CYCLOHEXANE	26.88	GAMMA.-CADINENE	23.76
HUMULENE OXIDE	27.68	GERMACRENE-D	23.92
2′,3′,4′ TRIMETHOXYACETOPHENON	29.76	CYCLOHEXANOL	24.32
	53.82	(E,E)-.ALPHA.-FARNESENE	24.46
		PHENOL, 2-METHOXY-4-(2-PROPENYL	
		CADINA-1,4-DIENE	25.30
		CARYOPHYLLENE OXIDE	26.80
		9-METHYL-10,12-HEXADECADIEN-1-	27.62
		CARYOPHYLLA-4(12),8(13)-DIEN-5	28.46
		(-)-CARYOPHYLLENE OXIDE	29.42
		2′,3′,4′ TRIMETHOXYACETOPHENONE	
		TRITETRACONTANE	53.80

(Table 4). In contrast, compounds extracted using chloroform achieved 100% mortality rates for the 0.7, and 0.8 ml treatments after one day of exposure. After three days of exposure, 100% mortality was observed in response to all treatments for both types of extract (Table 4) the effect of petroleum ether and chloroform was not significantly different from that 0.05%.

Clove compounds extracted using petroleum ether extract resulted in low levels of adult mortality, after one day, for the

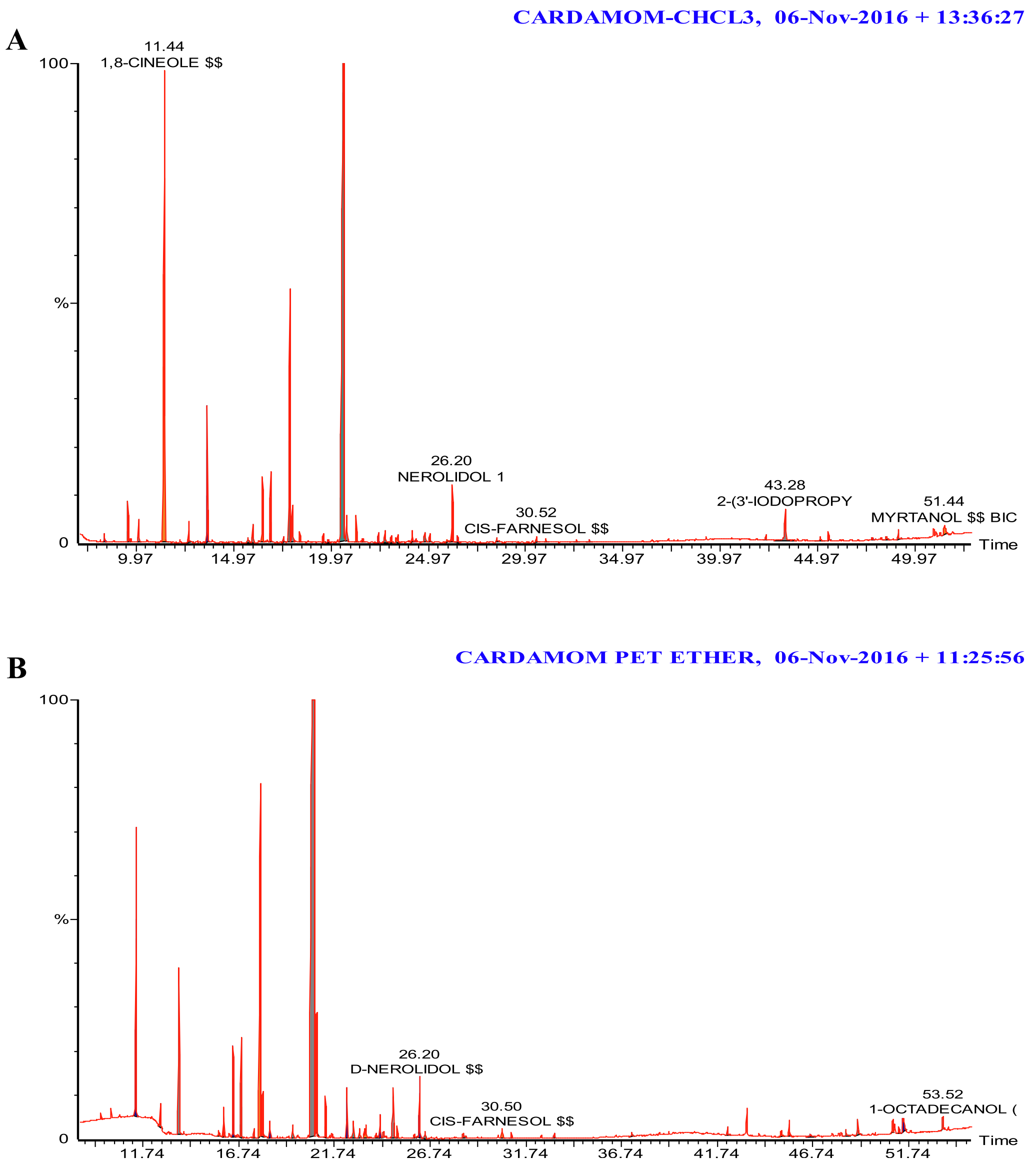

Fig. 1. Chromatograph analysis (GC-MS) of chardamom extracts. (A) Chloroform; (B) Petroleum ether.

0.1, 0.2, and 0.3 ml treatments; the mortality rate the lower levels of concentration after one day of exposure, ranging between 40 and 100% across the treatments (Table 5). After three days, mortality was observed in response to all treatments for both types extract all concentrations with the exception of the 0.2 and 0.5 ml chloroform extracts. The effect of petroleum ether extract was not significantly at 0.05%, while chloroform extract were significant different from that of 0.05%.

4. Discussion

Our study supports previous works on the use of aromatic oils and herbicides in pest control, that have demonstrated the efficacy of natural products insect pests. Consistent with previous studies, our results demonstrated mortality of up to 100% using extracts of cardamom and cloves against adult RPW. Further, we report the presence of a large number of terpenes, including eugenol in the extracts (Tables 1 and 2) that are known to exert insecticidal effects against a large number of insects. (Shaya et al., 1991; Phillips et al., 1995; Huang et al., 2002, Waliwitiya et al., 2005).

Similar to our results, Olivero-Verbel et al. (2010) stated that the main components found in the volatile oil from *E. cardamomum* were 1,8-cineol and α-terpineol acetate. Korikontimath et al. (1999), Lawrence (1979).

In our study cardamom seed extract using chloroform solvent was initially more effective than extracted using petroleum ether solvent at the highest concentrations (0.7 and 0.8 ml). All insects died after 24 h of treatment; however, after 3days of treatment mortality was 100% at all concentrations of the method of extraction.

The effects of clove seed extract were similar to those of cardamom seeds: 100% mortality was recorded after 24 h using highest concentrations (Tables 4 and 5) and mortality at the lower concentrations increased after 72 h, occasionally reaching 100%. Powdered seed of both plants was effective against adult RPW mortality for both treatments were 100% high concentrations after 72 h. However; cardamom was initially most effective 5 g of powdered cardamom seeds (93% mortality) after 24 h (Table 3).

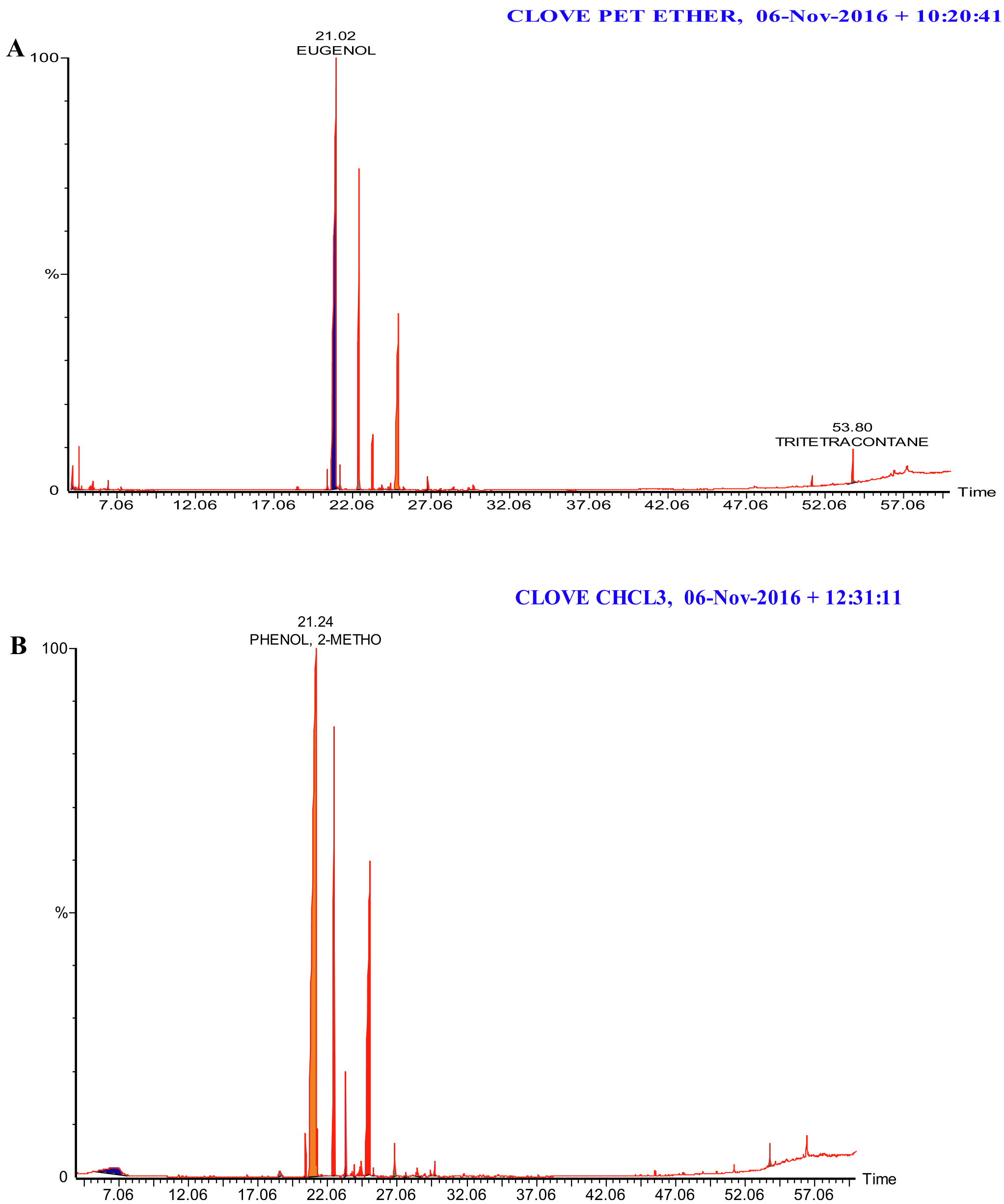

Fig. 2. Chromatograph analysis (GC-MS) of clove extracts. (A) Petroleum ether; (B) Chloroform.

Table 3
Toxicity of cardamom seeds and clove against adult red palm weevil.

Conc.	%Mortality after Exposure to cardamom seeds			LC50	LC95	F*	Conc.	%Mortality after Exposure to clove seeds			LC50	LC95	F*
	24 h	48 h	72 h					24 h	48 h	72 h			
0.8	0	20%	40%				1	13%	26%	33%			
1	0	7%	26%				3	7%	33%	80%			
3	26%	26%	46%	2.2	5.2	132.827	5	0	20%	73%			
5	93%	100%	100%				7	53%	73.3%	100%	1.78	6.42	8.283
control	0	0	0				control	0	0	0			

* Significantly different between treatments at 0.05%.

Chromatographic analysis of the plant extracts (Tables 1 and 2) showed the presence of terpenes that were likely to have caused the recorded mortalities in this experiment. The most important terpene detected was eugenol, which has been to be an effective insecticide. Huang et al. (2002) reported contact toxic effects of eugenol, iso eugenol, and methyl eugenol adult *Sitophilus zeamais.* Waliwitiya et al. (2005) noted toxic effects of a number of mono terpines and volatiles including: thymol, cetronellal, and eugenol on final larval instars of *Agriotes obscurus.* Here, we observed high mortality rates in adult RPW exposed to a number of terpenes: our

Table 4
Toxicity of cardamom extracts against adult red palm weevil.

Conc.	%Mortality after treatment with petroleum ether extract			LC50	LC95	F	%Mortality after treatment with chloroform extract			LC50	LC95	F
	24 h	48 h	72 h				24 h	48 h	72 h			
0.2	13%	13%	13%				13%	13%	86.6%			
0.4	26.6%	53.3%	100%	0.33	0.62	2.282	33.3%	73.3%	100%			
0.5	33.3%	40%	53.3%				40%	53.3%	100%	1.96	0.83	0.301
0.6	46.6%	73.3%	100%				60%	100%	–			
0.7	86.6%	100%	–				100%	–	–			
0.8	80%	100%	–				100%	–	–			
control	0	0	0				0	0	0			

Table 5
Toxicity of clove extracts against adult red palm weevil.

Conc.	%Mortality after treatment with petroleum ether extract			LC50	LC95	F	Conc.	%Mortality after treatment with chloroform extract			LC50	LC95	F*
	24 h	48 h	72 h					24 h	48 h	72 h			
0.1	6.6%	13%	33.3%				0.2	40%	66.6%	86.6%			
0.2	6.6%	6.6%	20%				0.4	73.3%	73.3%	100%			
0.3	6.6%	6.6%	26.6%	0.44	0.74	0.383	0.5	53.3%	66.6%	93.3%			
0.4	86.6%	100%	–				0.6	100%	–	–	1.7	0.45	16.810
0.6	100%	–	–				0.7	100%	–	–			
0.8	100%	–	–				0.8	80%	100%	–			
control	0	0	0				control	0	0	0			

* Significantly different between treatments at 0.05%.

results support those Sharaby and Al Dosary (2014) who showed that camphene is attractive to both sexes of RPW adults, eliciting 60 and 40% mortality on males and females respectively. Camphene has a range of effects on insects; the most important is the blocking of the air holes (spiracles) through which the insect breathes, causing asphyxiation. AL-Sharook et al. (1991) recorded effects of essential oils on insect respiratory and nervous systems and hormone regulation that lead to death. In some cases essential oils may also act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism. Previous research has demonstrated that essential oils have neurotoxic, cytotoxic and mutagenic effects in different organism, since they act at multiple levels the possibility of developing resistance is (Bakkali et al., 2008). Here, we identified novel terpenes in clove and cardamom extracts: hydroxy-alpha-Terpenyl acetate, and labda -8(17), 13 (E)-diene-15, (Table 1) that may exert synergistic toxic effects insects.

In conclusion, as a natural insecticide, the cardamom and clove extracted could be exploited in developing more effective strategies to prevent and control on insects. Furthermore, as clove and cardamom extracted is widely used as an herbal medicine and spice, it is generally recognized as safe to human health (Zheng et al., 1992; Naveena et al., 2006).

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