

Oviposition deterrent effect of four essential oils against the date palm weevil, *Rhynchophorus ferrugineus* Olivier

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ABSTRACT

The Egyptian essential oils derived from the fresh green parts of clove (*Syzygium aromaticum*), eucalyptus (*Cinnamomum eucalyptusa*), lemongrass (*Cymbopogon citratus*), and sweet basil (*Osmium basilicum*) were evaluated under greenhouse conditions for their oviposition deterrent activity against *Rhynchophorus ferrugineus*. The highest oviposition deterrent activity was shown by *S. aromaticum* followed by *C. eucalyptusa* and *cv. citratus* oils (at 15% concentration) with values of 98.17%, 97.9% and 94.06 effective repellency, respectively. Cloves and eucalyptus essential oils at concentration of 10 %, significantly reduced egg laying and gave good practical oviposition deterrent effect (80.74 and 66.77% repellency). Moreover, a mixture of *S. aromaticum* and *C. eucalyptusa* oils (at 15% concentration) exhibited the higher oviposition deterrent activity (100%) than the other tested oils or their mixtures. These results clearly revealed that both essential oils (*S. aromaticum* and *C. eucalyptusa*) can be included as an integral parts of an IPM program against the date palm weevil.

Key words: *Ocimum basilicum*, Genovese, chemical fertilization, chicken manure, yeast extract, growth, chemical composition and oil productivity and constituents.

Introduction

Palm trees are an important resource for many societies in the Middle East and North Africa region. The number of palm trees today is about 120 million trees (FAO, 2013), 70% of them in the Arab countries (El-Juhany 2010). Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier, is a serious pest of numerous palm species in many countries (Hussain *et al.*, 2013) as well as in Egypt (Saleh, 1992). Heavy infestations of red palm weevils are mainly responsible for the destruction of palms that worth millions of dollars annually.

Synthetic insecticides, have been tried to manage the populations of *R. ferrugineus* (Al-Shawaf *et al.*, 2010; Shar *et al.*, 2012 and Aljabr *et al.*, 2014). Although several insecticides from these groups are found to be potent, however, environmental pollution and development of insecticide resistance limit their efficacy against red palm weevils (Kamel *et al.*, 2007; El-Saeid & Al-Dosari, 2010 and Al-Ayedh *et al.*, 2016). According to Al-Ayedh *et al.* (2016) synthetic pesticides do not provide an effective control against *R. ferrugineus*.

In the last few years, the Ministry of Agriculture aims to minimize the use of insecticides in integrated pest management programs. Therefore, it is essential to reduce the use of synthetic pesticide sprays by alternate sprays of potent environmental friendly safe natural products. Essential oils proved potential sources of alternative compounds. These oils are known to be environment-friendly (Isman, 2006; Dayan *et al.*, 2009 and Malik *et al.*, 2016).

So, the aim of the current research is to evaluate the oviposition deterrent effect of some natural plant essential oils against *R. ferrugineus* to prevent new infestation.

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Material and Methods

1. Evaluate the oviposition deterrent effect of essential oils against *R. ferrugineus*.

1.1. Insect Rearing

To start a culture of the red palm Weevil, *Rhynchophorus ferrugineus* Olivier, Larvae and pupae of red palm Weevil were collected from infested palm trees in Sharkia Governorates. The collected insects were incubated in an transparent plastic boxes (120 × 60 × 30cm) with easily removable perforated covers (Ahmed *et al.*, 2015) under laboratory condition. Sugarcane stem split longitudinally in 10 cm. pieces to provide a food source for (larvae and adults) as well as oviposition substrate. The newly emerged adults were sexually differentiated and kept in separate containers with sugarcane pieces as food for bioassay.

1.2. Essential oil extraction:-

The fresh green parts of Lemongrass (*Cymbopogon citratus*), Cloves (*Syzygium aromaticum*), Eucalyptus (*Cinnamomum eucalyptusa*) and sweet basil (*Osmium basilicum*) which shadow dried were collected from and 50 gm. from each was used for oil extraction by steam-distillation using a Clevenger-type apparatus according to the method of Giray *et al.*, (2008). Through the distillation time of 3 hours, each 50 gm. of the dried material yielded nearly 2 ml oil. So, the distillation was repeated to obtain the required oil quantity for research purposes.

Essential oils from dried plants were obtained by hydro distillation for 3h. Each essential oil was prepared as 5%, 10% and 15%, where dissolved in drops of ethyl alcohol and then filled with distilled water on the basis of volume/volume by mixing known volume from the oil with 100 ml. one drop of Triton x100 was added as emulsifier and stored at 4°C before testing.

1.3. Oviposition deterrent bioassay.

The present experiment was carried out in greenhouse (6 m width x 3 m length x 3 m height) containing date palm shoots (Zagloul var.) homogenous in size and age planting in pots.

Each concentration of the tested oils or their mixture was heavily sprayed on three shoots till run off by using a hand pump pressure sprayer as well as another 3 shoots was sprayed with distilled water as a check.

To evaluate the efficacy of tested oils against *R. perniciosus*, twenty five pairs (males and females) of newly emerged adults aged 20 days old, were introduced into the greenhouse for oviposition. The position of the pots were switched every day to avoid the position effects. After four weeks of treatment, the treated shoots were investigated and the eggs laid on each palm shoot were collected separately and counted using a stereomicroscope.

The percentage of effective repellency for each essential oil was calculated using the following formulae (Phasomkusolsil and Soonwera, 2012).

$$R\% = (NC - NT / NC) \times 100$$

(Where R % = Repellency percent, NC = the total number of eggs in the control and NT = the total number of eggs in each treatment).

1.4. Screening and identification of essential oil components

GAS chromatography - Mass spectrometry analysis: The obtained essential oils analyzed using GC-MS apparatus. Separation was performed on Trace GC Ultra Chromatography (Thermo Scientific, USA), equipped with ISQ-Mass (Thermo Scientific, USA) and 60 m x 0.25 mm x 0.25 µm film thickness TG-5MS capillary column (Thermo Scientific, USA). The column separation programmed from 50°C withhold time 3 minutes and temperatures increase at rate 4°C/minute to 140°C withhold time 5 minutes, then at rate 6°C /minute to 260°C with 5 minutes. Isothermal hold. The injector temperature was 180°C, Ion source temperature 200°C and the transition line temperature was 250°C.

The carrier gas was helium with constant flow rate 1.0 ml minutes⁻¹. The mass spectrometer had a scan range from m/z 40 to m/z 450. Ionization energy was set at 70 eV.

The identification of compound based on the comparison the MS computer library (NIST library version 2005), compared with those of authentic compounds and published data 20, and the relative percentage of the oil constituents was calculated from GC peak areas. A linear retention was calculated for each compound using the retention times of a homologous series of C6 – C26 n-alkanes (Adams, 1995).

Antimicrobial Activity

Petriplates 9cm, PDA media and Micro-organisms isolated from Plant Pathology department, Agriculture research center, Egypt.

Micro-organisms isolated from Plant Pathology Disease Research from (ARC) were tested for antagonism with essential oil of Lemongrass , Cloves , Eucalyptus and sweet basil on P DA plates for fungi with added into PDA media at the rates of (1, 3, 6 and 10 mg ml⁻¹) before plating. Plates were inoculated with 0.5 cm diameter discs of *Fusarium moniliforme* grown on PDA media for 12 days. Control of the experiment was non-amended exudate PDA plates. Growth diameter was recorded when fungus growth filled up a plate. The testing of the bacterial cultures for the inhibitory essential oil of lemongrass for different concentration the rates of (1, 3, 6 and 10 mg/ l) effect of for each treatment was replicated. Isolates exhibited convenient and antagonisms in accordance to (Haenseler and Allen, 1934).

Statistical analyses:

The data obtained were subjected to regular statistical analysis (one way ANOVA) and mean comparison were carried out using L.S.D. at 5%.

Results and Discussion

1. GC-MS analyses

1.1. GC-MS analyses of Egyptian clove extract:

Eight compounds were identified in clove extract by GC-MS, (Table 1), (Fig. 1). The major components present was Eugenol (71.56%) followed by Eugenyl acetate (8.99%).

Table 1: The main components of clove, *Syzygium aromaticum* essential oil.

No.	Compound	Area %	Identification method
1	5-Hexene-2-one 0.67 Guaiol	0.90	MS ^b & KI ^a
2	Thymol	0.87	MS & KI & ST
3	Eugenol	71.56	MS & KI & ST
4	Eugenyl acetate	8.99	MS & KI
5	Caryophyllene oxide	1.67	MS & KI & ST
6	Nootkatin	1.05	MS & KI
7	solongifolanone (trans)	0.86	MS & KI
8	Benzene-1-butylheptyl	0.55	MS & KI

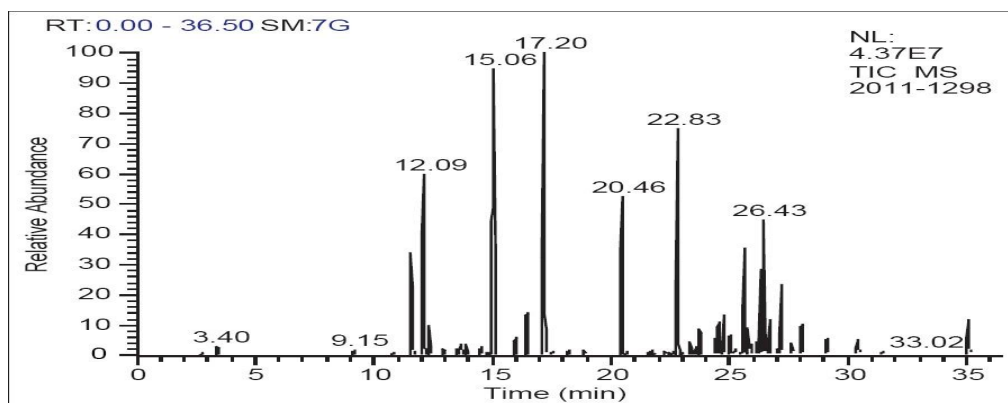


Fig. 1: Chromatogram of clove, *Syzygium aromaticum* essential oil.

1.2. GC-MS analyses of Egyptian sweet basil extract:

GS-MS chromatogram of extracts study showed several peaks in *Ocimum basilicum* L extract. The fragmentation patterns of the peaks were compared with that of the library of compounds. Seven compounds were identified by GC-MS. Five compounds were identified in sweet basil extract by GC-MS, (Table 2), (Fig. 2). As shown in Table (2) the major components present was Methyl chavicol (estragole) ($27.82 \pm 3.1\%$) followed by Linalool ($25.35 \pm 2.3\%$).

Table 2: The main components of sweet basil, *Ocimum basilicum* L oil.

No.	Compound	Area %	Identification method
1	Eucalyptol (1,8-Cineole)	4.92±0.8	MS & KI & ST
2	Linalool	25.35±2.3	MS & KI & ST
3	Terpinen-4-ol	2.06±0.3	MS & KI
4	Methyl chavicol (estragole)	27.82±3.1	MS & KI & ST
5	Eugenol	8.81±1.6	MS & KI & ST

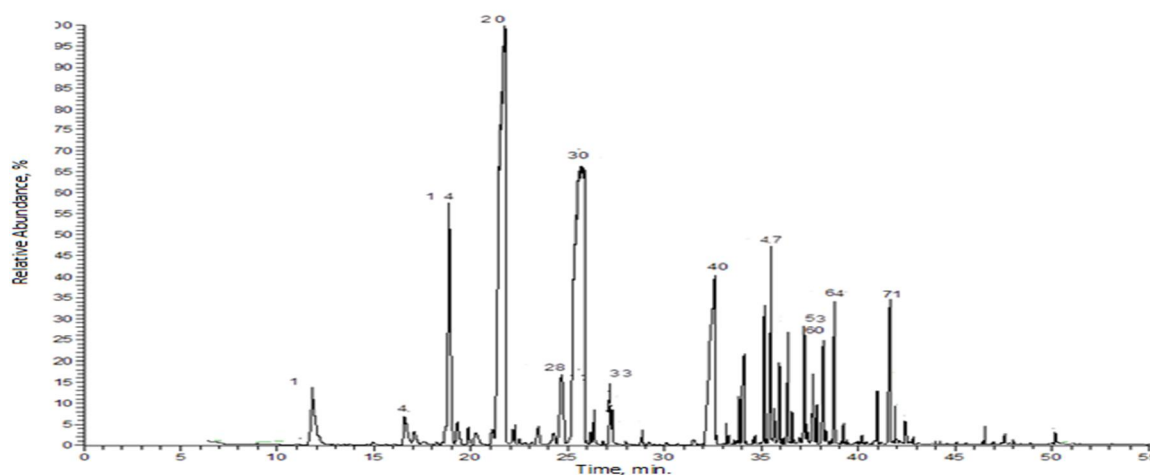


Fig. 2: chromatogram of methanol extraction of *Ocimum basilicum* L oil.

1.3. GC-MS analyses of Egyptian lemongrass extract:

As show in (Table 3) and (Fig. 3), ten compounds were identified in lemongrass extract. The major components present was Geranial ($20.9 \pm 2.8\%$) followed by Neral ($16.2 \pm 1.6\%$) and Geraniol ($8.3 \pm 1.2\%$).

Table 3: The main components of lemongrass, *Cymbopogon citratus* essential oil.

No.	Compound	Area %	Identification method
1	6-methyl-5-heptene-2-one	3.0±0.53*	MS ^b & K ^{1a}
2	Linalool	5.6±0.95	MS & KI& ST
3	Neral	16.2±1.6	MS & KI & ST
4	Carvon	2.5±0.88	MS & KI
5	Geraniol	8.3±1.2	MS & KI& ST
6	Methyl Citronellate	1.8±0.58	MS & KI
7	Geranial	20.9±2.8	MS & KI
8	Methyl Nerolate	2.0±0.47	MS & KI
9	Methyl Geranate	2.4±0.69	MS & KI
10	Geranyl acetate	4.1±0.66	MS & KI

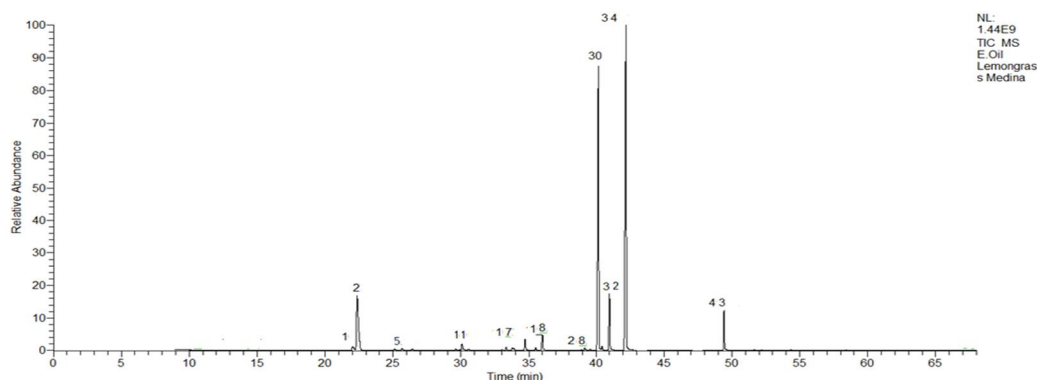


Fig. 3: Chromatogram of methanol extraction of Lemongrass, *Cymbopogon citratus* essential oil.

1.4. GC-MS analyses of Eucalyptus extract:

GS-MS chromatogram of the eucalyptus extracts showed ten peaks in *C. eucalyptusa* extract. The fragmentation patterns of the peaks were compared with that of the library of compounds. Ten compounds were identified in eucalyptus extract (Table 4) and (Fig. 4). As shown in table (4) the major components present was Citronello (33.52 %) followed by Pulegol (25.20 %) and Citronellyl acetate (14.70 %).

Table 4: The main components of Eucalyptus, *Cinnamomum eucalyptusa* essential oil.

S/N	Compound	% Area	Identification method
1	Hydroxy citronellol	1.52	MS ^b & KI ^a
2	Sopulegol	1.26	MS & KI& ST
3	Citronellyl acetate	14.70	MS & KI & ST
4	Longifolene	2.45	MS & KI
5	Pulegol	25.20	MS & KI& ST
6	Thujone	2.26	MS & KI
7	Cis-3-Pinanone	3.71	MS & KI
8	Isomenthone	1.52	MS & KI
9	Neomenthol	0.29	MS & KI
10	Citronellol	33.52	MS & KI

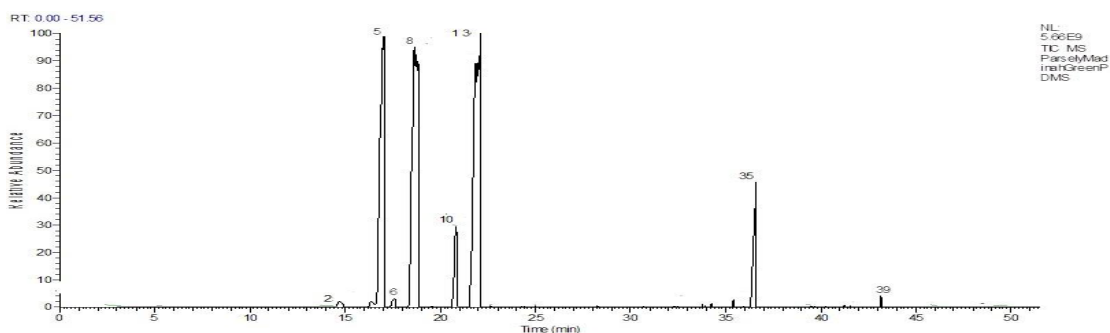


Fig. 4: Chromatogram for *Cinnamomum eucalyptusa* essential oil.

2. Oviposition deterrent effect of essential oils:

The results obtained in table (5) showed the fraction of eggs (mean number of eggs/ shoot) laid by 25 females of *R. ferrugineus* on treated and untreated date palm shoots with different concentrations (5, 10 and 15%) of essential oils extracted from clove, eucalyptus, lemon grass and sweet Basil.

The obtained results as shown in table 5, cleared that the date palm weevil females preferred to lay eggs in untreated shoots than treated once. All tested essential oils at a concentration of 15% significantly reduced egg laying, and exhibited high oviposition deterrent activity against *R. ferrugineus* females. However, the average number of eggs was 0.7 ± 0.6 , 2.0 ± 1.7 , 3.7 ± 3.2 and 15.0 ± 4.0 / shoot for clove, eucalyptus, lemon grass and sweet basil oils, respectively (represented by 98.88, 96.79, 94.06 and 75.92% repellency, respectively). Cloves and eucalyptus essential oils at concentration of 10 %, significantly reduced egg laying (12.0 ± 4.0 and 20.7 ± 5.0 eggs/ shoot) and gave good practical oviposition deterrent effect (80.74 and 66.77% repellency). So, the obtained results indicated that clove and eucalyptus essential oils proved to be good oviposition deterrent against *R. ferrugineus* females.

As shown in tables (5 and 6) the mixed oils of (clove and eucalyptus) caused more decreased in the egg numbers of *R. ferrugineus* than each essential oil alone. However, the ovipositional deter value was 73.72, 85.75 and 100% with the mixed oils (cloves and eucalyptus), while, it was 61.89, 80.74 and 98.88% with the cloves oil alone at 5, 10 and 15% concentrations, respectively. Also, the mixed oils of (eucalyptus and lemon grass) exhibited high oviposition deterrent effect against *R. ferrugineus* females, represented by 58.09, 74.14 and 95.85% in comparison with eucalyptus alone (42.22, 66.77 and 96.79 %) at 5, 10 and 15% concentrations, respectively. In contrary, the mixed oils of (clove and lemon grass or sweet basil), showed no more decreased in egg numbers than cloves oil alone.

With respect to mixed of all tested oils (Table, 6), it exhibited the highest ovipositional deter values (81.05, 95.44 and 100%) at concentration of 5, 10 and 15%, respectively.

Table 5: Oviposition deterrent effect of four essential oils against *Rhynchophorus ferrugineus* females on date palm shoots (zaghlolle cv), under greenhouse conditions (L.S.D. = 16.78, p = 0.05).

Treatment	Con. And	Av. No. of eggs	Repellency %
Clove <i>Syzygium aromaticum</i>	5	25.3 ± 5.5 ab	61.89
	10	12.0 ± 4.0 a	80.74
	15	0.7 ± 0.6 a	98.88
Eucalyptus <i>Cinnamomum eucalyptusa</i>	5	36.0 ± 6.6 b	42.22
	10	20.7 ± 5.0 ab	66.77
	15	2.0 ± 1.7 a	96.79
Lemon grass <i>Cymbopogon citratus</i>	5	52.3 ± 9.0 c	16.05
	10	32.3 ± 11.1 b	48.15
	15	3.7 ± 3.2 a	94.06
Sweet Basil <i>Oscimum basilcum</i>	5	55.7 ± 12.9 c	10.59
	10	34.7 ± 7.8 b	44.30
	15	15.0 ± 4.0 a	75.92
Distilled water (Control)	0	62.3 ± 27.5 c	-----

Table 6: Oviposition deterrent effect of essential oil mixture against *Rhynchophorus ferrugineus* females on date palm shoots (zaghlole cv), under greenhouse conditions (L.S.D. = 13.93, p = 0.05).

Treatment	Con.	Av. No. of eggs	Repellency %
Clove + Eucalyptus	5	19.0 ± 3.0 ab	73.72
	10	10.3 ± 4.5 a	85.75
	15	0 a	100
Clove + Lemon grass	5	24.7 ± 9.1 ab	65.84
	10	16.0 ± 3.6 ab	77.87
	15	3.3 ± 3.1 a	95.44
Clove + Sweet Basil	5	45.6 ± 7.1 c	36.93
	10	13.0 ± 10.1 a	82.02
	15	3.0 ± 2.0 a	95.85
Eucalyptus + lemon grass	5	30.3 ± 8.5 b	58.09
	10	18.7 ± 5.5 ab	74.14
	15	3.0 ± 2.0 a	95.85
Eucalyptus + Sweet Basil	5	41.7 ± 10.5 bc	42.32
	10	26.7 ± 5.9 ab	63.07
	15	9.0 ± 3.6 a	87.55
All oils	5	13.7 ± 5.9 a	81.05
	10	3.3 ± 1.5 a	95.44
	15	0 a	100
Distilled water (Control)	0	72.3 ± 27.5 d	-----

Effect of used essential oil of inhibitory to date whlite disease on mycelial growth of *Fusarium moniliforme*.

Essential oil Added into PDA medium v/v.	Mean Diam. Of mycelial growth in cm.				
	0.0 mg/l	0.1 mg/l	3.0 mg/l	6.0 mg/l	10.0 mg/l
Lemongrass	9.0	9.0	9.0	3.75	3.65
Cloves	9.0	4.75	4.55	2.67	2.3
Eucalyptus	9.0	8.57	6.97	5.62	4.75
sweet basil	9.0	9.0	9.0	9.0	5.67
water	9.0	8.7	9.0	9.0	9.0
L.S.D. at 5%	0.0	0.68	0.57	0.36	0.21
at 1%	0.0	0.92	0.78	0.49	0.29

L.S.D for treatments x rate at 5% = 1.60 at 1% = 1.69

Essential oil Cloves, Lemongrass and Eucalyptus significantly reduced mycelial growth of *Fusarium moniliforme*. (table.). While essential oil of sweet basil and water were not effective. However Essential oil and rates interaction exhibited significant effects at specific rates according to oils. For instance: the lowest myelial growth with oil Cloves was detected at 10.0 ml/l and Lemongrass .

Discussion

Oviposition deterrent effect of essential oils:

All tested essential oils had significant repellence to *R. ferrugineus* females and deterred oviposition compared to untreated controls. The essential oil extracted from *Syzygium aromaticum* followed by *Cinnamomum eucalyptusa* and *Cymbopogon citratus* exhibited the high oviposition deterrent activity against *R. ferrugineus* females. The repellent effects of various plant essential oils have been reported on the weevils, *Sitophilus granarius* (L.) and *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae), (Conti *et al.*, 2010, 2011; Mossi *et al.*, 2011).

Also, *S. aromaticum* oil showed high percentage of effective repellency against oviposition of mosquito species (Trongtokit *et al.*, 2005 and Phasomkusolsil & Soonwera, 2012) and *Musca domestica* (Soonwera, 2015). Tarkhani *et al.*, (2017) demonstrated that clove oil induces anaesthesia and blunts muscle contraction power. Also, essential oils from *C. odorata* and *Lippia alba* showed repellent properties against *Tribolium castaneum* (Coleoptera) (Gallardo *et al.*, 2011).

Soonwera (2015) added that *C. odorata* oil exhibited the excellent oviposition deterrent with 100% effective repellency against oviposition of house fly females. According to Burdock and Carabin (2008) the constituents of essential oil extracted from *C. odorata* were phenols, eugenol, methyleugenol, isoeugenol, limonene, geraniol and cinnamaldehyde. These constituents have properties to act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests (Koul *et al.*, 2008). According to Shapiro (2012) eugenol is a natural chemical found in oil of *S. aromaticum* and has been shown to be environmentally safe and nontoxic to humans (Trongtokit *et al.*, 2004). Moreover, *S. aromaticum* oil has been studied for its antibacterial, antimicrobial and antifungal properties against cutaneous infectious manifestations and has been shown to be environmentally safe (Trongtokit *et al.*, 2004).

The activities of *S. aromaticum*, *C. eucalyptusa* and *Cy. citratus* may be attributed to their major constituent. In the current study, the main compound of cloves, eucalyptus and lemongrass was eugenol, citronello and geraniol, respectively. According to Kordali *et al.*, (2005) 1,8-cineole, the major constituent of oils from eucalyptus; eugenol from clove oil; and carvacrol and linalool from many plant species. The essential oils of marjoram (*Origanum majorana* L.), and mint (*Mentha arvensis* L.) significantly deterred the feeding activity of *Thrips tabaci* Lindeman as a result of linalool and eugenol (Koschier and Sedy, 2001). Also, citronella (*Cymbopogon nardus*) essential oil has been used as an insect repellent. Citronella oil activity has been mainly attributed to its major monoterpene constituent citronellal (Zaridah *et al.*, 2003).

The present study cleared that Lemon grass oil (*Cymbopogon citratus*) was repellents for *R. ferrugineus*. These results are similar with those obtained by Adhikari *et al.* (2002) and Sharaby and Al-Dosary (2014). Parangama *et al.*, (2004) reported that the major component of the Lemon grass oil were geraneol, eugenol and 1, 8- cineol, the repellency to the weevil, *S. oryzae* increased with increasing dose of the oil.

Price and Berry, (2006) demonstrated that eugenol depressed spontaneous and stimulus-evoked impulses in the abdominal nerve cord of cockroaches, with an almost complete block of spikes. Geraniol had similar depressive effects but increased spontaneous firing at lower doses. Spontaneous firing was progressively reduced by increasing concentrations of eugenol, whereas geraniol and citral produced biphasic effects.

Essential oil of sweet basil exhibited low efficiency as oviposition deter effect on *R. ferrugineus* females. These results in agreement with those obtained by Sharaby and Al-Dosary, (2014) who mentioned that oil of sweet basil, was attractive for *R. ferrugineus* females, also, they are Sharaby and Al-Dosary, (2014) suggested that the attractive materials may be used in bait traps in an IPM program, and repellent oils as a repellent by spraying on the wounded ariars of palm trees.

So, it could be concluded that *S. aromaticum*, *C. eucalyptusa* and *Cy. citratus* oil in this study has high potential for development of new product or green product to *R. ferrugineus* management.

Further studies still needed on the application of essential oil as oviposition deterrent agents in combination with efficient trapping system, while *R. ferrugineus* females search for a suitable place to lay eggs for the management of this pest.

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