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An Electric Air Flow Olfactometer and the Olfactory Response of *Rhynchophorous ferrugineus* Weevil to Some Volatile

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Authors' contributions

Compounds

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

An electric air flow olfactometer was designed for testing the olfactory response of adult weevils (males and females). Thirty one natural plant volatile oils, eighteen terpenes and nine volatile chemical compounds were tested in the designed olfactometer for their stimulation on the adult weevil as attractants or repellents, data cleared some of the tested materials were attractants for both sex while others were repellents. They are arranged according to their intensity of reaction. The most attractive natural plant oil for both sexs and terpene were Juniper oil and terpene (-) Camphene, while Fenugreek oil was attracted to males and repellent for females. The repellent oils for both sex reached 15 of the tested oils. The attractive terpenes for females were (-) Camphene and Anethol. The attractive terpene for males was (-) Camphene. The other volatile chemicals (Alcohols, Aldyhydes and Ketones) were tested in 9 chemical compounds could be arranged in descending order, for females they were (Propionaldehyde>Furyl methyl Ketone), while Methylbenzaldehyde, Menthanal, Acetophenone, Benzaldehyde and Acetyl thiophene were repellents. All the nine chemical compounds were repellents for males. The attractive materials maybe used in bait traps in an IPM program, and repellent oils as a repellent by spraying on the wounded arias of palm trees.

Keywords: Red palm weevil; Rhynchophorus ferrugineus; olfactometer; volatile chemicals; olfactory response; attractants; repellents; plant oils; terpenes.

1. INTRODUCTION

The red palm weevil (RPW) *R. ferrugineus* (Olivier.) (*Coleoptera: Curculionidae*) is a major pest of various palms in the Middle East, South and South East Asia, North Africa and Southern Europe [1]. 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 and eventually kill the host [2]. The RPW has a wide geographical and host range. It was reported to infest 40 palm species worldwide [3]. Infestation with RPW starts with gravid females attracted to palm volatiles for laying eggs which hatch into damage inflicting larvae. Infestation date palms exhibit several symptoms depending on the stage of attack, e.g. oozing of brownish fluid mixed with palm tissue exerted by feeding larvae that has a typical fermented odor, tunneling of palm tissue by larval, presence of adults and pupae at the base of fronds, drying of infested offshoots, fallen pupae around an infested palm, drying of outer leaves and fruit bunches and topping of trunk in case of sever and extensive tissue damage [4].

Many plant essential oils and their constituents from terpenes show a broad spectrum of activity against pest insects ranging from insecticidal, repellents, attractants, oviposition, deterrents, growth regulators and anti vector activities. These oils have a long tradition of use in the protection of stored products. Recent investigations indicated that some chemical constituents of these oils interfere with the octopaminergic nervous system of insects [5,6,7]. As the target site is not shared with mammals, most essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests, and met the criteria of reduced pesticides risk. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential oil based pesticides owing to the complex mixture of constituents that characterize many of these oils. One of important components of IPM strategies for *R.ferrugineus* is mass pheromone traping system using aggregation pheromone, food bait with or without kairomons [8,9]. For controlling R. ferrugineus, synthetic pesticides are used in preventive sprays and curative [10,11,4,12]. However, inconsistent control of pest and high frequency of insecticide applications may contributes to environmental pollution. Recent studies revealed pesticide residues exceeding permissible limits in date fruits probably due to large scale and indiscriminate use of pesticide for the control of RPW, thus contaminating date fruit and also the environment with toxic substance [13,14]. Therefore it is desirable to reduce the number of synthetic pesticide sprays by alternate sprays of potent environmental friendly safe natural products. This will reduce the pesticide load in the resistance management. Bioactive natural products from plants are known to be both biorational and environmental friendly [15,16] Amongst the variety of natural products available, essential oils are being investigated to provide environmentally safe alternative to pesticides [17,18]. The aim of the current research is to estimate the olfactory response of the adult weevils of RPW towards some volatile compounds e.g. (natural plant essential oils, commercial terpens and volatile chemicals) that can be interfere in the IPM program of the RPW as attractants in bait traps and prevent new infestation. To test our hypothesis, an air flow `olfactometer was designed to analyze whether the weevils are able to discriminate the odor of the tested materials and their role as attractants or repellents.

2. MATERIALS AND METHODS

2.1 Insect Rearing

The RPW culture was maintained in laboratory from larvae, pupae and adults collected from infested palm trees in Al Khrj region of Saudi Arabia. The insect culture was established under laboratory condition at 25±2c° temperature and 30% RH, and L: D10:14h.Sugarcane stem split longitudinally in 10cm., pieces to provide a food source for (larvae and adult weevils) as well as oviposition substrate. The adults after emergence were sexed and kept in separate containers with sugarcane pieces. Laboratory bred adults were collected, sexed and maintained in separate 1 Liter jars with sugarcane pieces as food.

2.2 Volatile Compounds

2.2.1 Natural essential oils

Essential plant oils were obtained from (Bafahrat Company, Geddah, Saudi Arabia).

2.2.2 Terpenes

Eighteen commercial terpenes were obtained from Fragrance Co. (IFF) New York. USA., also from Sigma Chemical Company. Limited, UK.

2.2.3 Chemical volatile materials

Nine volatiles chemicals (Alcohols, Aldehydes and Ketones) were obtained from Aldrich chemical Company, Limited, UK.

2.3 Olfactometer

Electric air flow olfactometer has been designed according to specification do [19] with modifications. The device shown in (Figs. 1, 2a and 2b), it is a transparent Plastic box (Perspex material), olfactometer test cage (A) 30cm.long, 15 width and 15cm.height). The box or the screen cage (A) has a central hole (8) provided with a stopper to prevent air escape. The cage is divided into two equal compartment or air ways by incomplete plastic wall partition (1) (20cm. long and 15 cm height) leaving basal space for the weevil walking and choosing between the two test compartments, leaving 10cm longx15cm.width at the back side of the box for ease of movement the tested weevil and choice between the two air streams that passing through the two test chambers (6), one chamber contained the odor of the tested material and the other without odor as a control. At the front side of the box and at the end of each air way there were two circular opening (5) 5cm. diameter, fastened on each opening an inverted plastic funnel with end opining of 2. cm. diameter (2) to prevent the responded and cached weevil from returning back to the box after they have entered the test chambers. Two rectangular test chambers (10x10 x15cm.) (6) mounted above each funnel at the front side of the screen cage along with the flight way, at the terminal side of each test chamber there a is wide opening 10x10cm. (7) covered with a wire screen for air entrance from outside the device towards the screen cage then exhausted to outside the device by the aid of two small electric suction fans (4) fastened at the back side of the test box. The fans were fastened on two opening covered with metallic wire to prevent the insects from contact (3). The fans supplied with electric source through electric cord (9) and electric button. The test samples are placed on a filter paper inside the test chamber. When the device turned on the air current come from outside passing through the test chamber carrying the odor molecules of the volatile materials through the flight way to the screen cage in which the test weevil was housed in. The odor stimulate the insect which responded as attracted and captured into the chamber with odor or as repellent by escaping into the control chamber without odor, the remaining insect in the movement area or not entered any of the two chamber considered non-responded. The air flow goes outside through the suction fans.

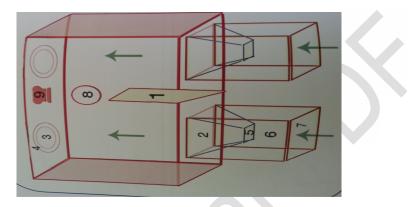


Fig. 1. Diagram of the electric air flow olfactometer

2.4 Olfactory Response of the Insects

Preliminary test was made to determination the end point of the weevil response to the volatile material., 10 μ l. of the tested material introduced on the filter paper inside the test chamber. Ten weevils were used separately in the screen cage. After the olfactometer was turned on the tested weevil showed movement with wing vibration, and flew for several centimeters, enter then leave each of the two air ways. After an average time of 1.30 hr. the insect stopped movement in the screen cage. This was the end point of the test.

Olfactory response of the volatile materials. A filter paper was impregnated with 10 µl. of the tested oil then introduced inside one of the test chamber of the olfactometer while the other chamber left blank as a control. The tested weevils were introduced into the screen cage through the central hole. A newly emerged weevil (males 3 and females 9) was starved 2 h before each test. The insect respond to the odor and discriminated between the two air ways by attraction or repulsion. For each volatile material 10 3 and 109 were tested individually. Mean numbers of the total insects that entered each of the air way or test chamber was recorded. The average intensity of reaction (IR) was calculated according [20] from the equation.

While IR= Intensity of reaction, S = Number of insects attracted to the scented chamber of the olfactometer. C= Number of insects moving to the non scented control chamber. From 1-10% the odor was very weakly attractive, 11-20% weekly attractive, 21-40% was attractive, and greater than 40% was highly attractive. If less than 1% the odor was considered repellent.

After each test of one odor the olfactometer was thoroughly washed with distilled water then left to dry to get rid of the odor. All experiments were conducted in constant temperature dark room $28\pm2c^{\circ}$.

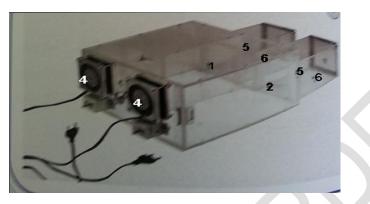


Fig. 2a. Photograph of the olfactometer (Lateral view)

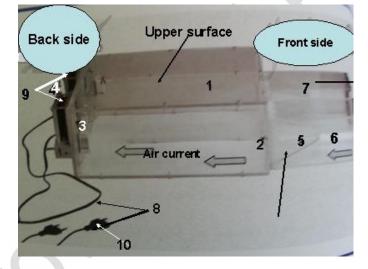


Fig. 2b. General photograph of the olfactometer.

(A) Olfactometer box or the screen cage, (1) incomplete wall barrier, (8) central hole,
(9) electric button, (4), suction fan., (2) Basal slot of the inverted funnel, (5) terminal slot of the inverted funnel, (6) test chamber, (7) slot for air entrance covered with wire gauze, (8) electric cord. Arrows that shown inside the olfactometer cleared the air current direction from outside to inside. (10) electric plug

3. RESULTS AND DISCUSSION

Data in Table 1 shows the intensity of reaction of 31 natural plant essential oils on the olfactory response of the adult weevils of *R. ferrugineus*. Great differences in the calculated values of the intensity of reaction for all the tested oils as well as differences between males and females responses. Oils of Parsley, Olbanum, Clove, Sweet basil, Black seed, Thyme and Henna were attractive only for females. Parsely and Sweet Basel oils gave the highest

positive reaction of (60%) highly attractive followed by Clove, Thyme and Henna with (40%). Finally Olbanum and Black seed oils showed the lowest positive reaction (20%). Oils were repellents for females were Helba with a negative reaction (-20%) although it was attractive to males with (40%) reaction. Other oils were repellent to males only with IR arranged as follows, Cumin= Parsely (-80%) > Olbanum = Thyme (-60%) > Clove = Henna (-40%) > Chamomile=Fennel=Rosemary (-20%). Table 1 shows that Cress, Castor, Coconut, and Juniper oils were attractive to both sexes. Of the 31 oils tested Radish, Almond, Garlic, Onion, Mint, Camphor, Nettle, Sesame, Orange, Lemon, Majoram, Saffron, Olea, and Mstard were repellent for both sexes, showing a response ranging from (-20 to-80%).

Scientific name of the	Common name	Family of the plant	IR %	
plant essential oil	of the plant		Females	Males
Ttrigonella foenum	Helba	Leguminosae	- 20	40
Urtica pilulifera	Nettle	Urticaceae	- 20	-40
Ocimum basilicum	Sweet basil	Labiatae	- 60	-20
Ricinus communis	Castor	Euphorbiaceae	60	40
Rosmarinus officinalis	Rosmary	Lbiatae	0	- 20
Cocos nucifera	Coconut	Arecaceae	20	40
Juniperas communsi	Common	Cupressaceae	80	80
Nigella sativa	Black seed	Ranunculaceae	- 20	- 80
Artemisia herba	Worm	Compositae	- 20	- 80
Sesamum indica	Sesame	Pedaliaceae	- 60	- 80
Citrus sinensis	Orange	Rutaceae	- 20	- 60
Citrus limon	Lemon	Rutaceae	- 80	- 60
Origanum vulgare	Margoram	Labiatae	- 40	- 60
Raphanus sativus	Radish	Curciferae	-100	- 60
Prunus communis	Almond	Rosaceae	-100	- 60
Cuminum cyminum	Cumin	Umbelliferae	0	-80
Matricaria aurea	Camomile	Compositae	0	- 20
Allium sativum	Garlic	Labiaceae	- 40	- 20
Eruca sativa	Cress	Curciferae	40	20
Allium cepa	Onion	Labiaceae	- 20	- 40
Petroselinum sativum	Parsley	Umbelliferae	60	- 80
Boswellia sp.	Olbanum	Butsiracae	20	- 60
Eugenia caryophylla	Clove	Caryophllacea	- 40	- 40
Mentha lavandulacea	Mint	Labiaceae	- 20	- 40
Eucalyptus camaldulensis	Camphor	Myrtaceae	- 80	- 80
Foeniculum valgare	Fennel	Umbelliferar	0	- 20
Thymus vulgaris	Thyme	Labiatae	40	- 60
Crocus sativa	Saffron	Laridaceae	- 20	-80
Lawsonia inermis	Henna	Lythraceae	40	-40
Aloe barbadensis	Aloe	Liliaceae	- 60	-60
Brassica rapa (1- 10%) very weakly attractive	Mystard	Cruciferae	- 60	-06 - 100%) Highly

Table 1. IR of plant essential oils on the olfactory response of *R. ferrugineus* weevil

(1- 10%) very weakly attractive., (11- 20%) weekly attractive ., (21- 41%) Attractive (41 - 100%) Highly attractive., (Less than 1%) Repellent

Data in Table 2 shows positive or negative response towards 18 commercial terpenes. Anethol and (-) Camphene were attractive for females their intensity of reaction were 20 and 40% respectively. The terpenes that were repellents for females arranged as follows

Cdenenine(-100%) > Ethyl Cinnamate= Carvone= Thymole= $\dot{\alpha}$ -Pinene(-80%) > Cinnamone= Eugenol= Myrcine (-60%) > Phyllandral= Iso Eugenol= $\dot{\alpha}$ – Ionone= Geraneol (-40%), Carveol was the lowest acting terpen as a repellent for the RPW. Four terpenes were attractive for males, (-) Camphene (60% highly attractive) > $\dot{\alpha}$ - Ionone= Fenchone= Anethol(40% attractive) while Geraneol = Cinnamon(20% weakly attractive). Cadenine and Carvone were repellents with negative IR of (-100%) followed by Myrcine= Thymole= Iso Eugenol= $\dot{\alpha}$ -Pinene (-80%) > Eugenol(-60%) > Ethyl Cinnamate= Phyllandral=(-)Carveol (-20%). The previous results showed that Anethol and (-) Camphene were attractive for both sexes, while (-)Carveol, Carvone, Phynllanddral, Myrcine, $\dot{\alpha}$ -Pinene, Cadenine, Ethyl Cinnamate, Eugenol, Iso Eugenol and Thymol were repellents for both sex.

Terpene	Chemical grou	р	IR %
-	_	Female	♀ Male ♂
(-)Crveol	Alcohols	-20	-20
Nerol		-20	0
3-phenyl-1-propanol		0	-60
Geraneol		20	-40
ά-lonone	Ketones	40	-40
Carvone		-100	-80
Fenchone		40	0
Phyllandral	Aldehyde	-20	-40
Myrcine	Hydrocarbons	-80	-60
ά-pinene		-80	-80
Cadenine		-100	-100
(-)Camphene		60	40
Cinnamone	Esters	20	-60
2-Ethyl cinnamate		-20	-80
Anethol	Fenoles	40	20
Eugenol		-60	-60
Iso Euganol		-80	-40
Thymole		-80	-80

Table 2. IR of terpenes on the olfactory response of *R. ferrugineus*

(1- 10%) very weakly attractive., (11- 20%) weekly attractive ., (21- 41%) Attractive., (41 - 100%) Highly attractive., (Less than 1%) Repellent

Table 3 shows IR of RPW to nine volatile chemical compounds classified as three chemical groups 1-Alcoholic group (Iso-Amyl alcohol, Methanol, Butan-2-ol. 2- Aldyhyde group (Methoxy benzaldehyde, Propionaldehyde and Benzaldehyde. 3- Ketone group (Acetyl thiophene, Furyl methyl Keton and Acetophenone. Data showed that Propionaldehyde and Furyl methyl keton were attractive with IRs of (40 and 20% respectively, whereas the repellent compounds for females were Benzaldehyde and Methoxy benzaldehyde (-80%), Methanol and Acetophane (-40%), Benzaldehyde and Acetyl thiophone (-20%). Iso amyl alcohol and Butan-2-ol the intensity of reaction were zero, this indicated that the response of these compounds were not attractive or repellent. All tested chemical compounds were repellents for males. The repellent compounds for both sex were Methoxy benzoate, Acetophenone, Acetyl thiophene, Menthanol and Benzaldehyde.

Our results recorded Lemon and orang oils were repellents for both sexes of *R. ferrugineus* agreed with that mentioned by [21]. Thy recorded that Lemon grass oil was toxic to *Sitophelus oryzae* weevil during the fumigant toxicity test with an LC_{50} value of 0.035g/l air,

and the major component of the oil were geraneol, eugenol and 1,8- cineol, the repellency to S. orvzae increased with increasing dose of the oil. In the present research Eugenol. Isoeugenol were repellents for R. ferrugineus weevil, while Geraniol was weakly attractive for females and repellent for males. The behavioral response of the female cigarette Lasioderma serricorne to plant essential oils cleared that Thyme, Peppermint were strongly repelled the beetle at 1µL . Present results recorded that Thyme was attractive to females and repellent for males. The variable effects of volatile oils due to the presence of their major constituents of terpens which interfering with various biosynthetic process of the insect. [19] found that from 55 volatile oils tested on adult moths of S. littoralis some of oils and terpenes were attractive and others were repellent for both sexes. For example, Cadenine was repellent for females, whereas Geraneol was attractive for both sexes. [22] mentioned that Geraneol and $\dot{\alpha}$ -lonone were repellents for the females of *H. armigera* moths, while $\dot{\alpha}$ -lonone was attractive for males. Our the present results are in contrast to [19] who recorded Cadenine and Nerol to be Attractive to males of S. littoralis moths. Our results showed that the two previously mentioned terpenes were repellents for the RPW. Also Iso -Eugenol and ά-Pinene were attractive for both sexes of S. littoralis moths whereas they were repellent to the RPW. Our results recorded that Geraneol was repellent for females and attractive for males of the indicated RPW, [19] found that Geraneole was attractive for both sexes of S.littoralis moths. [22] found $\dot{\alpha}$ -Pinene was attractive for females of H. armigera moths, while Geraneol and α -lonone were repellent. Nerol was attractive for both sexes, while in our results it was repellent for males of RPW and neutral for females. [5.23] recorded many plant essential oils that showed abroad spectrum of activity against pest insects and plant pathogenic fungi ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities. Recent investigations indicate that some chemical constituents of the essential oils interfere with the octopaminergic nervous system in insects. [12] mentioned that essential oils demonstrate fumigant and contact insecticidal activities to a wide range of pests. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine [7] by some oil and with GABA- gated chloride channels by others [24] Essential oils as Lemon grass (Cimbopogon winteriana), Eucalyptus (Eucalyptus globule), Rosmary (Rosmarium officinalis), Vetiver (Vetivera zizanoids), Clove (Eugenia caryophyllus) and Thyme (Thymus vulgaris) are known for their pest control properties, while peppermint (Mentha piperita) repels ants, flies, lice and moths; Pennyroyal (Mentha pulegium) wards off fleas, ants, lice, mosquitoes, ticks and moths. Spearmint (Mentha spicata) and Basil (Ocimum basilicum) are also effective in warding off flies. Similarly, essential oil bearing plants like Artemisia vulgaris, Melaleuca leucadendron, Pelargonium roseum, Lavandula angustifolia, Mentha piperita and Juniperus virginiana are also effective against various insects and fungal pathogens [25]. Citronella (Cymbopogon nardus) essential oil has been used for over fifty years both as an insect repellent and an animal repellent. Combining a few drops each of citronella lemon (Citrus limon), rose (Rosa damascene) Lavender and Basil essential oils with one liter of distilled water is effective to ward off indoor insect pests [26]. According to [27] orange oil extracted from citrus peel (containing 92% d- limonene) caused 96 and 68% mortality to Formosa subterranean termite, Coptotermes formosanus Shiraki with 5 days and there was significant reduction in feeding as compared to control at 5 ppm. concentration (v/v). Many essential oils and their monoterpenic constituent are known as mosquito repellent and have activity against Culex species [28,29] Cinnamaldehyde, Eugenol, Cinnamyl acetate and essential oils from different Cinnamomum species are effective mosquito larvicides [30]. Geraniol and Eugenol are effective attractants and are used as lures in traps for the Japanese beetle, Popillia japonica Newman, and Methyleugenol has been used to trap oriental fruit fly, Dacus dorsalis Hendel [31]. Based on what was shown by the results of this research, many of these essential oils, terpenes, and other volatile chemicals can be used as attractant bait in lire traps to monitor RPW population as well as to disrupt maiting. Furthermore Radish oil, Almond oil, Garlic oil, Carvone. Methyl benzaldehyde, Methanal, Acetophenone and Acetylthiophene can be used as repellent spray to prevent egg laying on the more susceptible areas such as wounds or damaged areas of the palm tree. The tested volatile materials were safe for the environment, biodegradable, and locally available so they can be included as an integral part of an IPM program against the RPW.

Chemical compound	Chemical group	Molecular structure	IR %	
			Male 👌	Female 2
Iso-Amyl	Alcohols	C5HI2OH	- 40	0
Methanol		СНЗОН	- 20	- 40
Butan-2-ol		C4H8OH	- 80	0
Methoxy bnzaldehyde	Aldehyds	C8H4O2	- 60	- 80
Propionaldehyde	-	C3H6O	- 80	40
Benzaldehyde		C7HO	- 20	- 20
Acetyl thiophene	Ketones	C6H3OS	- 80	- 20
Acetophenone		C8H30	- 20	- 40
Furyl methyl ketone		C6H3O2	- 60	20

Table 3. IR of chemical compounds on the olfactory response of *R. ferrugineus*

(1- 10%) very weakly attractive., (11-20%) weekly attractive., (21-41%) attractive., (41-100%) highly attractive., (less than 1%) repellent

4. CONCLUSION

An electric air flow olfactometer was designed for testing the olfactory response of adult weevils (males and females). Data cleared some of the tested materials were attractants both sex while others were repellents. The most attractive natural plant oil for both sexs and terpene were Juniper oil and terpene (-) Camphene, while Fenugreek oil was attracted to males and repellent for females. Oils of Parsley, Olbanum, Clove, Sweet basil, Black seed, Thyme and Henna were attractive only for femalesOils were repellents for females were Helba, although it was attractive to males. Cress, Castor, Coconut, and Juniper oils were attractive to both sexes. Radish, Almond, Garlic, Onion, Mint, Camphor, Nettle, Sesame, Orange, Lemon, Majoram, Saffron, Olea, and Mstard were repellent for both sexes, Anethol and (-) Camphene were attractive for females .The attractive terpene for males was (-) Camphene. Anethol and (-) Camphene were attractive for both sexes, while (-)Carveol, Carvone, Phynllanddral, Myrcine, & Pinene, Cadenine, Ethyl Cinnamate, Eugenol, Iso Eugenol and Thymol were repellents for both sex. The attractive volatile chemicals were Propionaldehyde>Furyl methyl Ketone, Methylbenzaldehyde, Menthanal, while Acetophenone, Benzaldehyde and Acetyl thiophene were repellents. The repellent compounds for both sex were Methoxy benzoate, Acetophenone, Acetyl thiophene, Menthanol and Benzaldehyde. volatile chemicals can be used as attractant bait in lure traps to monitor RPW population as well as to disrupt mating. Furthermore Radish oil, Almond oil, Garlic oil, Carvone. Methyl benzaldehyde, Methanal, Acetophenone and Acetylthiophene can be used as repellent spray to prevent egg laying on the more susceptible areas such as wounds or damaged areas of the palm tree. The tested volatile materials were safe alternatives of chemical insecticides, for the environment, biodegradable, and locally available so they can be included as an integral part of an IPM program against the RPW.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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