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Disruption impact of citronella and menthol insecticides on adults behavior and hemocytes morphology in the red palm weevil *Rhynchophorus ferrugineus* "Oliver" (Coleoptera: Curculionidae)

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Abstract
This study was conducted to evaluate some terpenes effect on the behavior and immune function of hemocytes in adults of the red palm weevil *Rhynchophorus ferrugineus*. Six individual different terpenes these are: (±)-menthol, B-citronellol, (+)-3-carene, (R)- (+)- limonene, citronella oil and orange terpenes. The results revealed significant differences between the terpenes used on the olfactory response on this insect, in that half of the compounds were very attractive while the other half were repellant to them. This behavior study results with olfactometer citronella oil exhibited an 80% attraction response rate for both sexes, while menthol exhibited a 60% attraction response rate for females and 100% for males. By contrast, menthol had a more significant effect on adults than citronella, lethal concentration at 50 scale (LC_{50}) values of 1.03, 0.89, and 0.9 mg, and LC_{95} values of 5.09, 2.01, and 1.59 mg, after 24, 48 and 72 h, respectively. For citronella oil, the LC_{50} values were 2.09, 1.76, and 1.70 mg after 24, 48, and 72 h, and the LC_{95} values were 5.5, 3.7, and 1.5 mg after 24, 48 and 72h, were noted. In the present study, the effects of citronella and methanol insecticides were observed on six types of hemocytes namely prohemocytes, granulocytes, plasmatocytes, oenocytes, coagulocytes and spherulocytes. Both citronella oil and menthol had a histopathological effect on the hemocytes of the adult red palm weevil, specifically, on the cell membrane, cytoplasm, and nucleus. The findings also revealed that the vacuoles in some hemocytes, specifically, the prohemocytes, plasmatocytes, and granulocytes were more sensitive than those in other hemocytes, which remained unaffected by the treatment.

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The effects of citronella and menthol on RPW immunity were demonstrated in this study, and this information may be applied to their usage in integrated pest control at sub-lethal dosages.

Keywords
Citronella, menthol, red palm weevil, olfactometer, histopathology

Introduction

In the Middle East, the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Oliv.) is a serious pest of the date palm Phoenix dactylifera.[1] In the mid-1980s, the insect was initially discovered in Saudi Arabia.[1–3] It has expanded significantly since then, infecting all of the date palm farming regions[4–5] by the propagation of diseased offshoots and palm trees. Because of the lack of early and obvious exterior indications of RPW infection in date trees, the latter develops predominantly in young 5- to 15-year-old trees.[6,7]

Essential oils extracted from plants are utilized as parasite and insect repellents. Numerous studies have demonstrated the contact and fumigant toxicity of plant essential oils and their components to many species of stored product insects at various life stages. Essential oils from plants such as Eucalyptus genus against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *Mentha piperita* L. against *Tribolim castaneum*, *Lasioderma sericoides* (Fabricius) (Coleoptera: Anobiidae) *Chenopodium ambrosioides* L. against *Sitophilus zeamais*, *Lasioderma sericoides.* Terpenes are secondary metabolites that are found all across the plant world. Plants contain a combination of terpenes, which may be readily separated as oils by hydro-distillation, and are often known as essential oils, such as clove, cardamom, thyme, tea, and citrus fruit.[8] Each plant species' oil makeup is unique, with one to three terpenes often constituting the primary components, but many more appearing as minor components.[9]

Terpenes are highly volatile compounds that are extensively employed as perfumes, flavoring agents, and in medical and alternative treatments (such as aromatherapy). They are vital in plant defense against a range of creatures in nature, including insects.[10,11] Terpenes are ideal prospects for the production of eco-friendly insecticides due to their documented toxicity against several insect pests such as mosquitoes, houseflies, beetles, and butterflies.[12–15] Pests have sensory receptors that allow them to discriminate between different host plants and their fundamental chemical components, such as carbohydrates, amino acids, and salts,[16–18] as well as secondary botanical compounds that influence whether the substance is appealing[19,20] or repulsive.[21] Chemoreceptors are a group of sensory receptors that include olfaction, taste, and touch and are studied and identified in pests.[18,22–23]

The development of new eco-friendly and effective methods and materials aimed at managing this pest is urgent and necessary to reduce dependence on hazardous chemical pesticides and maintain the natural balance and beneficial organisms as well as the preservation of human health.[13]

In weevils, their egg-laying behavior is limited to the chemical sensory response of olfaction and contact.[24] Fenemore[25,26] has argued that the weevil chooses its host plants for feeding and laying eggs based on the natural features of the plant, such as its surface texture, in addition to those chemical agents the weevil can identify by its olfactory sensory receptors. Moreover, the plant oils and their terpenes components can be effective at controlling the spread of these weevils.[27–31] Using an olfactometer, the researchers evaluated the influence of terpenes on the behavior of the adult red palm weevil as an attractant or repellent. The study concentrated on which terpenes appeal to adults, and found that poisonous citronella and menthol are more appealing to adults. Furthermore, it looked into the effects of LC_{50} and LC_{95} on hemocyte counts and histology in red palm weevils under laboratory circumstances.

Material and methods

Insects rearing

According to Al-Rajhy et al.[32] rearing the larvae of the red palm weevil *R. ferruginous* on the palm offset can be summarized follows: firstly, the offsets of the fibrilla, roots and fronds were cleaned, then the palm was cut open longitudinally into two halves with length and width incision that was similar to that used for the offset root. A number of the fertilized pairs of insects (male and female adults) were then placed into the incision appropriate to the size of the incision. Thereafter, the offset incision was closed and using steel wires it was then bound together tightly to ensure that the insects could not escape. The offset was then placed inside a reticular cage with dimensions of approximately ($70 \times 70 \times 150$ cm). Finally, the cage was placed inside a soil laboratory at a temperature of 27 ± 2°C, and relative humidity 60–70% to allow the adult pairs to mate and lay eggs. After 2–3 days, the offset is opened to allow the placed pairs to depart, then the root is closed and the cage is returned to its original state. The root is opened after fifty days to get the adult insects and this way aimed to get a continuous colony of the insects. While the second way is in which pieces of the palm offset are used to rear the newly hatched larvae (about 100 larvae) as an estimation of its life standards. As each piece is put inside a small plastic can then transferring the newly hatched larvae to these pieces as the pieces are changed from time to time to prevent fermentation and the growth of the fungi, then following the larvae growth till it researches to the adult phase.

Terpenes

Several terpenes were used to test the olfactory response of the weevil: Citronella oil (CAS-8000-29-1, 85/35%; Sigma Aldrich, USA), Menthol (CAS number, 89-78-1, 99%- Sigma Aldrich), Orange terpene (CAS 68647-72-3, natural, FG Sigma Aldrich), Citronella (CAS 106-22-9, 95%- Sigma Aldrich) and limonene (CAS, 5989-27-5, 94% Merck Millipore, UK) were procured. On the basis of terpene toxicity, we have selected menthol and citronella oil for further study.

Olfactometer 2.0

The olfactometer described by Sharaby and Al Dosary[33] was redesigned by Aramco's bader program in the Kingdom Saudi Arabia (Figure 1). Natural plant volatile oils,

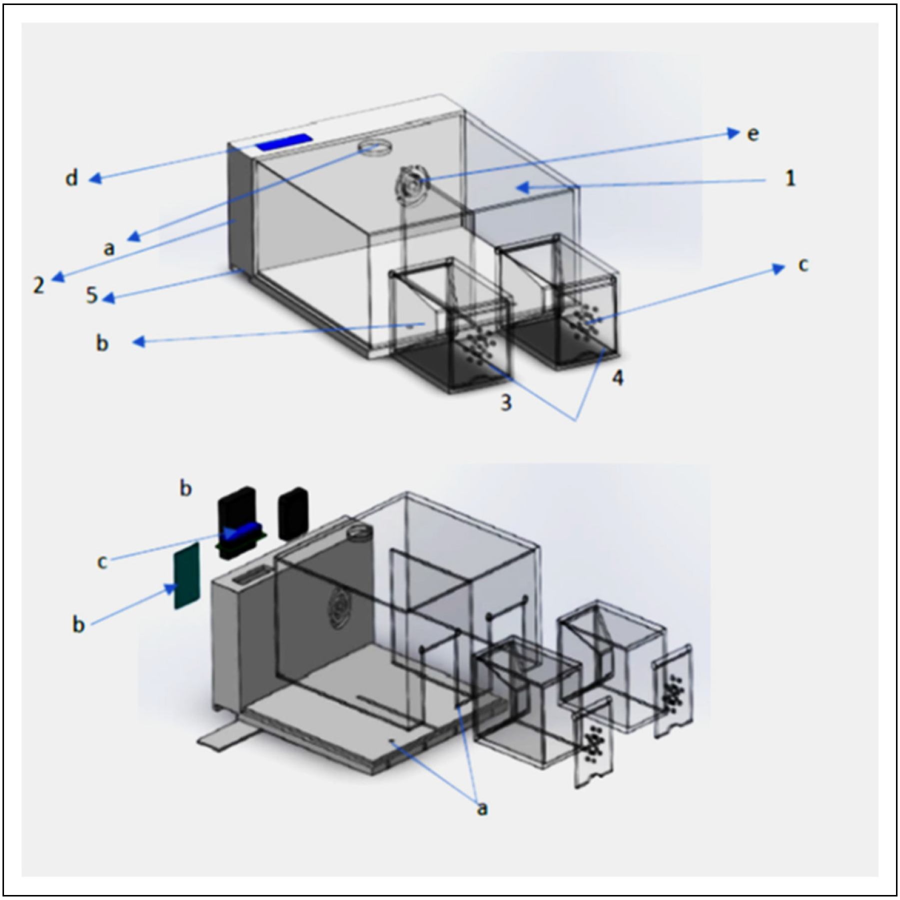

Figure 1. A, olfactometer 2.0 front view, 1: Box; 2: base; 3: control chamber; 4: test chamber; 5: sliding door; a: central hole; b: funnel; c: holes; d: LCD monitor, an arduino, e: Fan. b, exploded view, a: Sensors, b: Arduino, c: LCD screen.

terpenes and volatile chemical compounds were tested in the designed olfactometer for their stimulation on the adult weevil as attractants or repellents. This electric airflow olfactometer was specifically modified and updated as a second version, with the goal of optimizing the interaction and flow of the experiment. The device consists of five main modular components:(1) clear test box; (2) base; which serves as housing for the internal electrical components;(3)- control chamber; (4)- test chamber;(5)- sliding door to allow access to inside in the event of repairs (Figure 1). The device lid (1), is made of clear acrylic plastic that is 4- mm thick, and measures 24- cm long, 24-cm wide, and 12- cm high. This box has a central hole (a.) whose diameter is 4 cm; it is provided on the backside of the box, to enable easier movement of the tested weevil and its choice between the two airstreams that pass through the two test chambers. The test chamber (3) is provided with filter paper containing the odor of the tested material (i.e. treatment) and the other chamber (4) lacks any odor to serve as the control. At the base of each chamber lies an inverted plastic funnel with an end opening of 2 cm (b) to prevent the responding and collected weevil from returning to the box after each has entered the test chambers. At the other end of the two rectangular test chambers (3,4) each measuring 9 × 10 × 15cm, there is 9-cm × 10-cm opening that is closed off by a hinged acrylic door featuring holes (c) to allow for airflow and scent distribution.

The base of the device (2) serves as a housing for the internal components to prevent their contact with the experimental insects. The housing area is 6 cm long, 30 cm wide, and 15-cm high, while the base areás dimensions are 29 cm × 29 cm with a thickness of 1 cm. The base has grooves that allow the lid and divider wall to fit securely on top without any shifting. The internal parts consist of an LCD monitor (d), a dc fan, an ARDUINO motherboard, and a battery pack that provides power. The base is fitted with a vent (e) that allows for airflow from the fan housed inside and generates the suction needed to evenly distribute the scent of the materials being tested throughout the test space area.

Olfactory response experiment with the RPW

When the device is turned on, the fan carries the air current from the test chamber, carrying the odor molecules of the test material throughout the test area including the lid in which the weevil is placed. The insect will then respond by either being A, attracted to the scent, which will lead it to the odorous test chamber, or B, repelled by the scent, prompting it to escape into the odorless control chamber. An insect that remains in the movement area or odor not entered any of the two chambers is considered non-responsive.

In this, olfactory response, a filter paper was soaked with 10µl of essential oil inserted inside the chamber while another chamber left blank was marked as control and the volatile oil treatment was used for male and female insects individually. Normally, for each volatile material 10 males and 10 females weevils are tested individually. The treated weevils were entered into the screen cage by the central hole. An afresh appeared males and females weevil were famished 2 h before each test The insect interacts with odor and is favored by either attraction or repulsion mechanism between the two airways following the method of Sharaby and Al Dosary.[33] According to the method described by Gunn and Cosway[34] a microcontroller programmer was used to calculate average intensity % IR (final value) using the following formula:

$$IR = 100x\frac{s - c}{s + c}$$

wherein IR = Intensity of reaction, S = is the number of insects attracted to the scented chamber of the olfactometer, and C is the number of insects that moved into the non-scented control chamber (i.e. were repelled).

To automate the calculation of the experiments' results, this updated device (a) additional motion sensors that record which side the insect was attracted to, and it now includes the ARDUINO (b), which has been programmed to input the results from the sensors and plug them into the above equation and finally, (c) an LCD screen to conveniently display the experimental results, follows:

Less than 1%: the odor is considered repellent; or 1–10%: the odor is very weakly attractive; or 11–20%: weakly attractive; or 21–40%: attractive; or more than 40%: highly attractive. All experiments were conducted in constant in a dark room at a constant temperature ($28 \pm 2°C$). After testing a single odor, the olfactometer 2.0 was thoroughly washed with distilled water then left to dry. This was made easier by the modularity of the device as it allows each component to be individually removed and properly washed to eliminate different odors. Data were analyzed using SAS[35] specifically its GLM (general linear model) procedure. The fitted model included the effects of treatment, sex and interaction between treatment and sex:

$$Y = m + T + Sex + T * Sex + e$$

where: Y was the observed value, T was the effect of treatment (six levels; carene, citronella, citronellol, limonene, menthol, orange); sex (two levels; male, female); T * was the sex (= the interaction between treatment and sex); and E was the residual error term (unexplained variation).

Toxicity effect of citronella and menthol terpenes on adult

After testing the insectś response behavior to terpene odor, we selected the terpenes that were more attractive to the adults and tested them on hemolymph, and selected menthol and citronella, because they are more attractive to insects of both sexes (citronella oil has an 80% attraction response for both sexes, menthol 60% response for females and 100% for males). The effect of the terpenes as contact poison was explored in accordance with the method described by Salama et al.[36]

Contact toxicity

The adult insects (males and females) were sprayed with strong concentrations of citronella and menthol, were prepared by dilution with distilled water (1ml of terpenes was mixed with 10 ml of distilled water) with various concentrations of 1, 2, 3, 5, and 7 mg respectively. These concentrations were based on our previous study Next, 0.1% Triton X-100 was added as an emulsifier for treating the adults, then the insects were transferred to natural food palm offset, in order to observe the changes and calculate the mortality rate at different time points (24, 48, 72h), so as to identify the lethal concentration for 50% (LC_{50}) and lethal concentration for 95% (LC_{95}). The control jars were treated with only water + 0.1% Triton X-100. For each treatment, three replicates were used, and in each replication, 10 insects were used per concentration.

Effect of the LC_{50} and LC_{95} of the citronella and menthol on the insects' hemocytes

A hemolymph study was performed to explore the effect of citronella and menthol on the hemocytes the adult insects. This study was carried out on those insects that survived treatment with a lethal concentration of LC_{50} and LC_{95} by contact.

Preparation samples to hematology

One leg of either a male or female was amputated and drops of hemolymph were extracted and fixed onto a glass slide with McDowells solution.[37] Each hemolymph drop was gently spread into a thin film using a glass rod. The hemolymph drops were air-dried, fixed in 70% ethyl alcohol for 3 min, stained with Gimsa stain for 5 min, and rinsed in distilled water. The slides were then mounted in natural D.P.X. medium and examined under a microscope. The cell types were identified under a light microscope with a digital camera (x1000 magnification).

Statistical analyses

The RPW mortality was calculated for normal equivalent deviates, Chi square, and LC_{50} and LC_{95} and their fiducial limits were calculated using SPSS statistical software, version 16.0. A 5% least significant difference (LSD) test was used to compare the mean mortality percentages where a P value of ≤ 0.05 was considered statistically significant.[38]

Results

Effect of terpenes on the behavioral olfactory response of adults

The results presented in Table 1 demonstrate there were significant differences in the degree by which volatile oils and terpenes influence the adult olfactory response of the RPW, where the degree by which insects were attracted towards the tested materials

Table 1. Behavioral response of adults red palm weevil *R. ferrugineus* in olfactometer 2.0 to different terpenes.

Terpenes	% intensity of reaction males	Results	% intensity of reaction females	Results
Carene	60%	Repellent	20%	Repellent
Citronella oil	80%	Highly attractive	80%	Highly attractive
Citronellol	60%	Highly attractive	40%	Attractive
Limonene	20%	Repellent	20%	Repellent
Menthol	100%	Highly attractive	60%	Highly attractive
Orange	40%	Repellent	40%	Repellent

varied either by their attraction to the treatment material itself or towards the control as an expelling response to it. Among the tested treatment materials, menthol terpenes elicited the highest attraction rate, 100% for males but lower (60%) for females, followed by citronella oil at 80% for both sexes, while citronella terpene elicited an attraction response rate of 60% for males and 40% for females. Given the percentages, these could be considered as the degree of RPW affinity towards for materials.

The findings for the tests on adult insects' behavior are shown in Table 1. The statistical analysis revealed significant differences in the olfactory response of the insects (irrespective of sex) to the terpene treatments, as shown in Table 2. By contrast, in terms of the behavior of the adult insects, their olfactory response was one of being repelled by the other terpenes used in this study. Carene elicited the strongest repelling response by a terpene, recorded at −60% for males and −20% for females, followed by orange oil at −40% for both sexes, and the least repellent terpene limonene at 20% for both sexes. These interactions had a significant effect on the response of the adult insects, but sex was not a significant determinant of this. (Table 2). This was evident in the affinity and similarity of the response ratios, whether for attraction or expulsion, found between the sexes.

Effect of toxicity of citronella on adults RPW

The percent mortality of different concentrations of citronella against adults RPW 24, 48, and 72 h after treatment have shown in Tables 2 and 3 and Figure 1. After 24 h the percent mortality was 100% at the high concentration 7mg, while it reached 6.7% at the lower concentration of 1mg. Additionally, the other concentrations had a toxic effect on the adults; 73.33%, 56.6% and 86.6% at 2, 3 and 5mg respectively. After 48h the remaining insects died at 5mg, and mortality increased at 1mg and 2mg 13.33% and 86.66% respectively. Table 4 shows the LC_{50} and LC_{95} values of citronella against adults RPW, with LC_{50} values at 2.09, 1.76 and 1.70 mg after 24, 48 and 72 h respectively and LC_{95} values at 5.52, 3.78, and 1.59 mg respectively.

Table 2. Effect of treatment on olfactory response adults red palm weevil *R. ferrugineus*.

Treatment	Mean ± SD
Carene	0.50 ± 0.52^{bc}
Citronella oil	0.90 ± 0.32^{a}
Citronellol	0.75 ± 0.45^{ab}
Limonene	0.30 ± 0.47^{c}
Menthol	0.90 ± 0.21^{a}
Orange	0.30 ± 0.48^{c}

Means with the same letter are not significantly different ($P < 0.01$). The mean values refer to effect of treatment on olfactory response adults red palm weevil.

Table 3. Effect of interaction treatment on olfactory response gender adults red palm weevil.

Treatment	Sex	Mean ± SD
Carene	Male	0.2 ± 0.422
	Female	0.4 ± 0.516
Citronella oil	Male	0.9 ± 0.316
	Female	0.9 ± 0.316
Citronellol	Male	0.8 ± 0.422
	Female	0.7 ± 0.483
Limonene	Male	0.6 ± 0.516
	Female	0.4 ± 0.516
Menthol	Male	1.0 ± 0.000
	Female	0.8 ± 0.422
Orange	Male	0.3 ± 0.483
	Female	0.3 ± 0.483

Table 4. Mortality of red palm weevil after treatment with citronella and menthol.

	%Mortality after treatment with citronella			%Mortality after treatment with menthol		
Conc.	24h	48 h	72	24 h	48 h	72 h
1	6.7	13.33	13.33	40	60	60
2	73.33	86.6	100	86.6	100	-
3	56.6	56.6	56.6	90	93.33	100
5	86.6	100	-	96.6	96.6	100
7	100	-	-	90	100	-
control	0	0	0	0	0	0

Effect of toxicity of menthol on adults RPW

Table 3 and Figure 2 show that terpene menthol had a more toxic effect than citronella on the mortality adult RPW, where the mortality was 40%, 86.6%, 96.6%, and 90% at 1, 2, 3, 5, and 7 mg concentrations respectively. Moreover, all insects died at 2mg and 7mg after 48h and mortality increased at the other concentrations 60%, 93.33%, and 96.6 at 1, 3, and 5 mg respectively. After 72h, the remaining insects died at 3 mg and 5 mg concentrations, resulting in 100% mortality. The LC_{50} and LC_{95} values LC_{50} values (at concentrations of 1.03, 0.89, and 0.91 mg) and LC_{95} values (at concentrations 5.09, 2.01, and 1.59 mg.) are shown in Table 5. Figure 3.

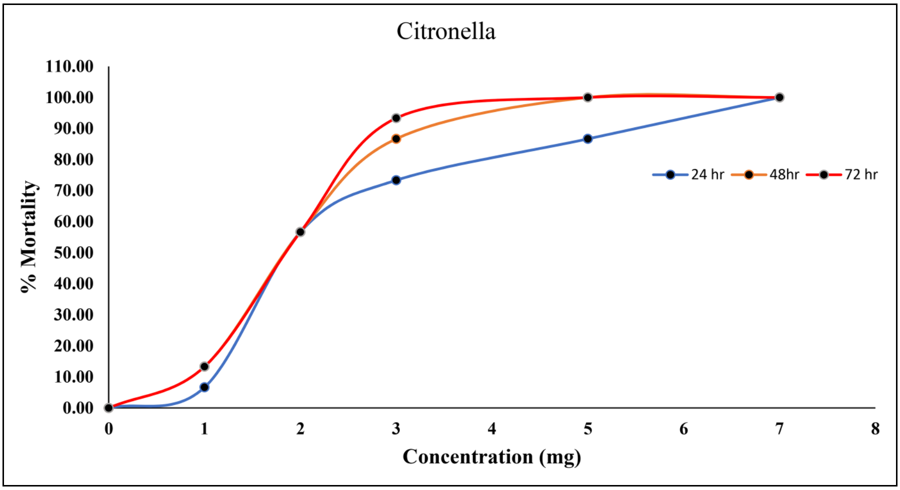

Figure 2. Percent mortality of RPW after exposure to citronella for various time periods and concentrations (n = 30).

Table 5. Estimated LC_{50} and LC_{95} values of citronella against RPW at different time intervals after treatment.

Time	(LC_{50} (mg) (95% fiducial limits))	(LC_{95} (mg) (95% fiducial limits))	DF	Chi X^2	slope	P value
24	2.09 (1.74-2.42)	5.52 (4.43-7.86)	3	4.07	1.24 ± 0.25	0.25
48	1.76 (1.50-2.02)	3.78 (3.12-5.14)	3	0.79	1.22 ± 0.26	0.52
72	1.70 (1.469-1.94)	1.59 (2.87-4.63)	3	1.497	1.25 ± 0.27	0.68

Microscopic hemocytes study

In this study, we have analyzed the six types of RPW hemocytes (Figure 4). These are:

> prohemocytes (round/ oval cells with a high nucleus with small hemocytes), granulocytes (cells with a heterochromatic nucleus containing a few cytoplasmic granules), oenocytes (cells with a central nucleus with homogeneous cytoplasm), plasmatocytes (cells that exhibit a characteristic spindle shape) coagulocytes (cells with a central nucleus with homogenous cytoplasm) and spherulocytes (cells with contain numerous large granules (Figure 4).

Effect of citronella treatment at LC_{50} and LC_{95} on hemocytes morphology

Generally, the treatment with terpene citronella in this study affected the cell membrane, cytoplasm and nucleus of the hemocytes. The most sensitive cells were found to be the

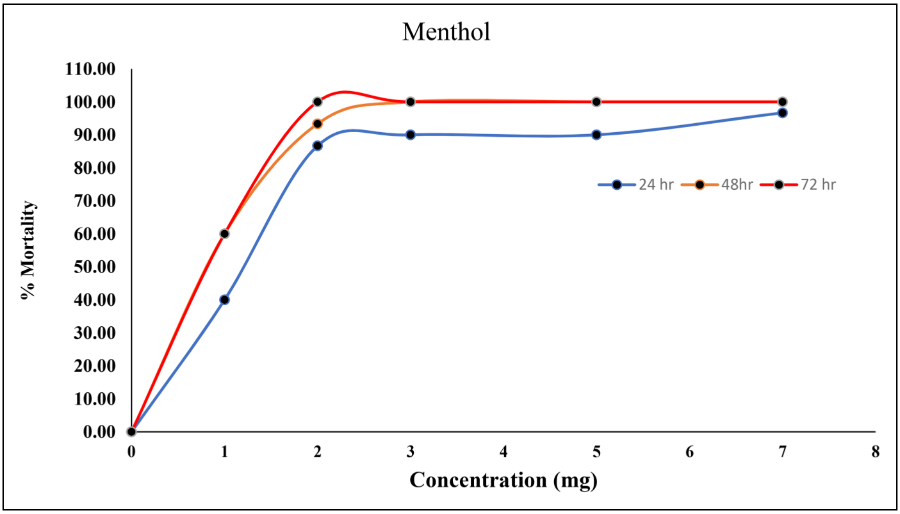

Figure 3. Percent mortality of RPW after exposure to menthol for various time periods and concentrations (n = 30).

prohemocyte, followed by the granulocyte, and the plasmatocyte, but no effect was observed on the other hemocytes. After 24 h citronella treatment at LC_{50} we observed some vacuoles in the hemocyte specific prohemocytes, granulocytes, and plasmatocytes, and also some lysed cytoplasm, and nucleus and stick cells (Figure 5 A F). However, after 48 h treatment with the same terpene, we observed some stick cells and lysed prohemocytes (Figure 6A), some changes in the shape of plasmatocytes, and vacuoles, lysed cytoplasm (Figure 6B), and completely lysed this cell after 48h (Figure 6C). We also observed some changes in the shape of granulocytes, and complete lysis (Figure 6 D). Although the histopathological effect of citronella treatment at LC_{50} on hemocytes also increased with treatment at LC_{95} from this terpene, we observed the same change after 24 and 48 h in prohemocyte shape and an increased number of vacuoles (Figure 7A), along with an advanced stage, lysed cytoplasm and nucleus (Figure 7 B, C). Vacuoles were observed in the granulocytes along with initial lysis of the cytoplasm, and complete lysis of the plasmatocytes and cell membrane (Figure 7 D, E).

Effect of menthol treatment at LC_{50} and LC_{95} on hemocytes morphology

Figure 8 shows the effect of menthol treatment at LC_{50} of menthol on the hemocytes morphology of RPW after 24 h, changes in the shapes of prohemocytes, granulocytes, and plasmatocytes were observed, but there was no effect on the other hemocytes. However, after 48 h menthol treatment at LC_{50}, a complete distortion of plasmatocytes was observed (Figure 9 A, B, C). Additionally, 24 h menthol treatment at LC_{95} caused sticks cells plasmatocytes, and vacuoles were observed in granulocytes and

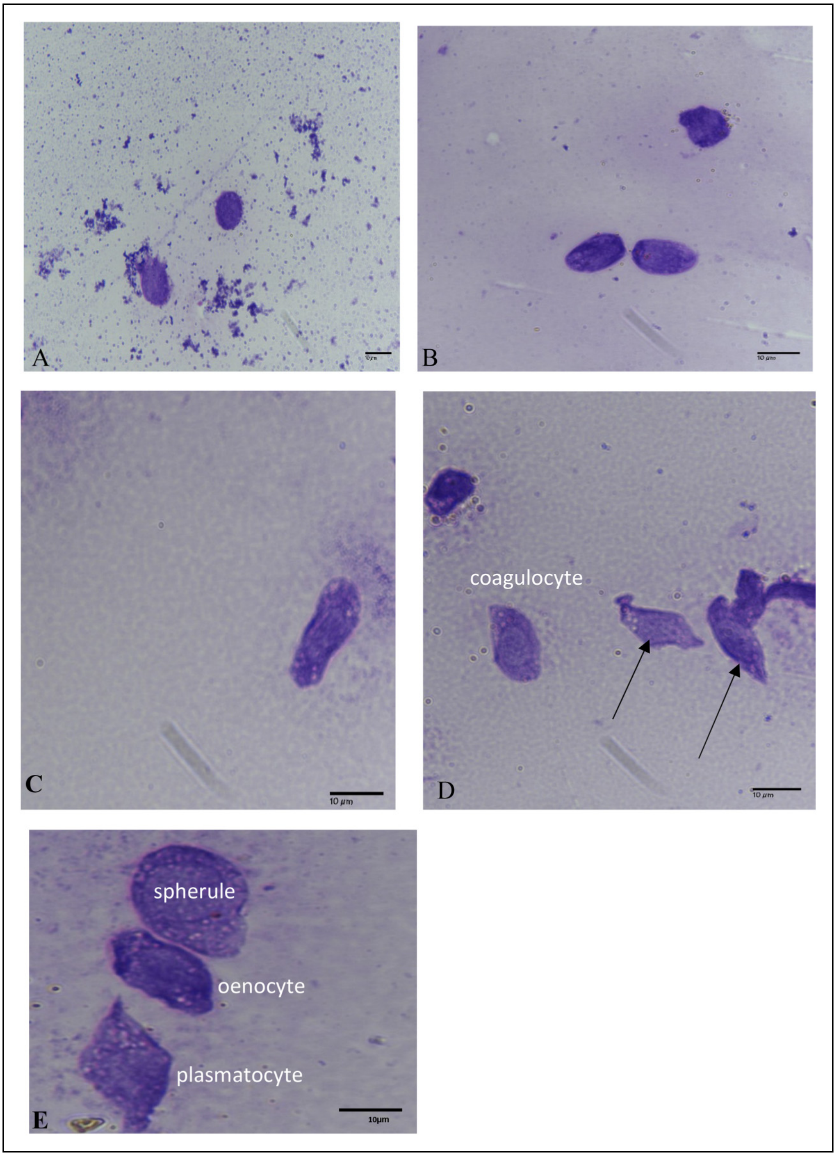

Figure 4. Light microscopy photo, showing types of hemocytes identified from the hemolymph of the untreated (control) red palm weevil *R. ferrugineus*. A: Prohemocyte identified by the high nucleus/ cytoplasm. B: Granulocyte with an acentric nucleus. C: Oenocyte have a central nucleus with homogenous cytoplasm. D and E: Coagulocyte show a heterochromic nucleus with few cytoplasmic granules. Plasmatocyte showing the shape spindle (arrow). F: Spherule cell with numerous large granules.

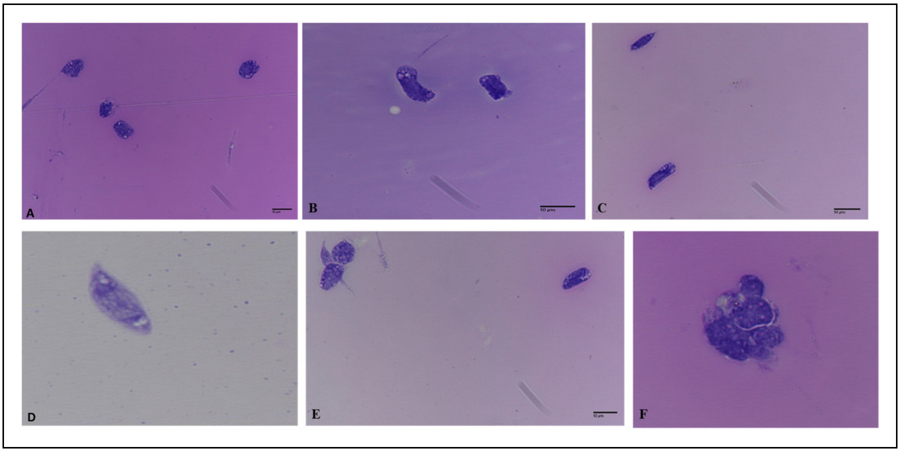

Figure 5. Changes hemocytes *R.ferrugineus* after 24h treatment with LC_{50} of citronella. A: Change shape of prohemocyte and lysed nucleus, found vacuoles. B: Oenocyte, showing vacuoles, lysed nucleus. C- D: Plasmatocyte shrank shape and found vacuoles, lysed cytoplasm and nucleus. E: Lysed cytoplasm granulocyte, and found vacuoles. F: Stick cells and lysed cell, nucleus, and found vacuoles.

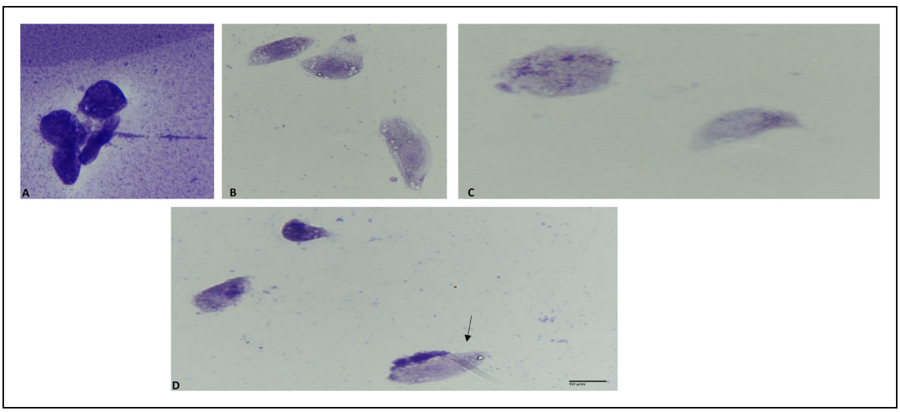

Figure 6. Morphology changes hemocytes *R. ferrugineus* after 48h treatment with LC_{50} citronella. A: Sticks and lysed of prohemocyte, vacuoles. B: Change shape of plasmatocyte and vacuoles, and lysed cytoplasm. C: Completely lysed cytoplasm of plasmatocyte. D: Vacuoles and lysed cytoplasm and cell of granulocyte, (arrow) of plasmatocyte completely lysed.

prohemocytes. As an additional effect of this terpene after 48 h treatment with the same LC_{95}, we observed plasmatocytes, granulocytes, and prohemocytes. Compared with untreated hemocytes, we observed an increased number of vacuoles along with lysis of the nucleus and cell membrane (Figure 10, Figure 11).

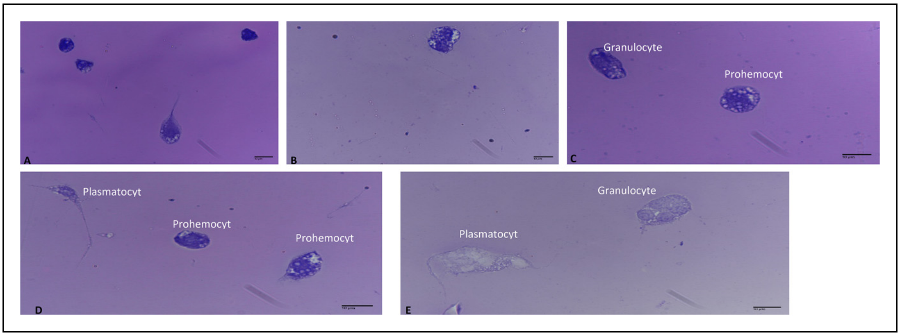

Figure 7. Morphology changes hemocytes *R. ferrugineus* after treatment with LC_{95} of citronella. A: Prohemocyte show change shape and vacuoles. B: Lysed cytoplasm and nucleus of prohemocyte. C: Granulocyte, vacuoles and start lysed cytoplasm. D: Plasmatocyte, change shape, vacuoles. E: Completely lysed of plasmatocyte and cell.

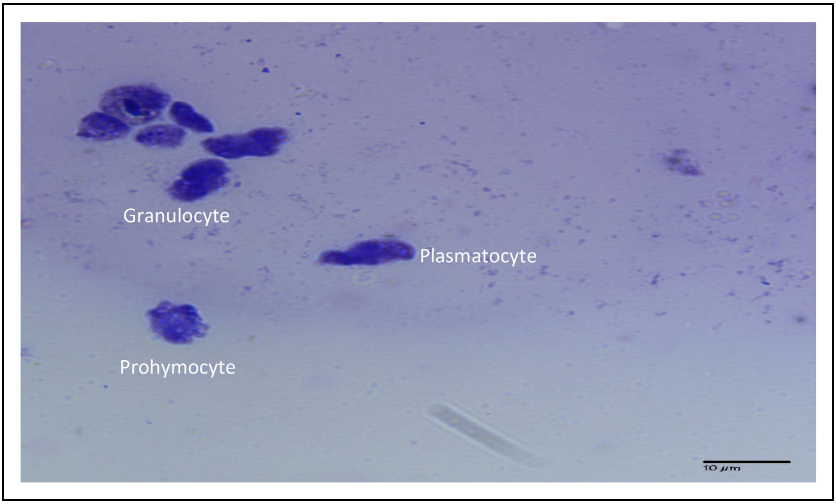

Figure 8. Morphology changes hemocytes *R. ferrugineus* after 24h treatment with LC_{50} menthol.

Discussion

In previous research by Sharaby and Al-Dosary,[33] essential oils and terpenes were tested for their impact on the behavior of the adult red palm weevil in previous research by Sharaby and Al-Dosary, and the findings were measured as either an attraction or an expulsion by the insects. We observed that the terpene menthol had the greatest attraction rate of 100% for RPW males and 60% for RPW females, followed by citronella oil at 80% for both sexes, and lastly, citronellol at 60% for RPW males and 40% for RPW females. These findings are consistent with those of Sharaby and Al Dosary,[33] whose investigation

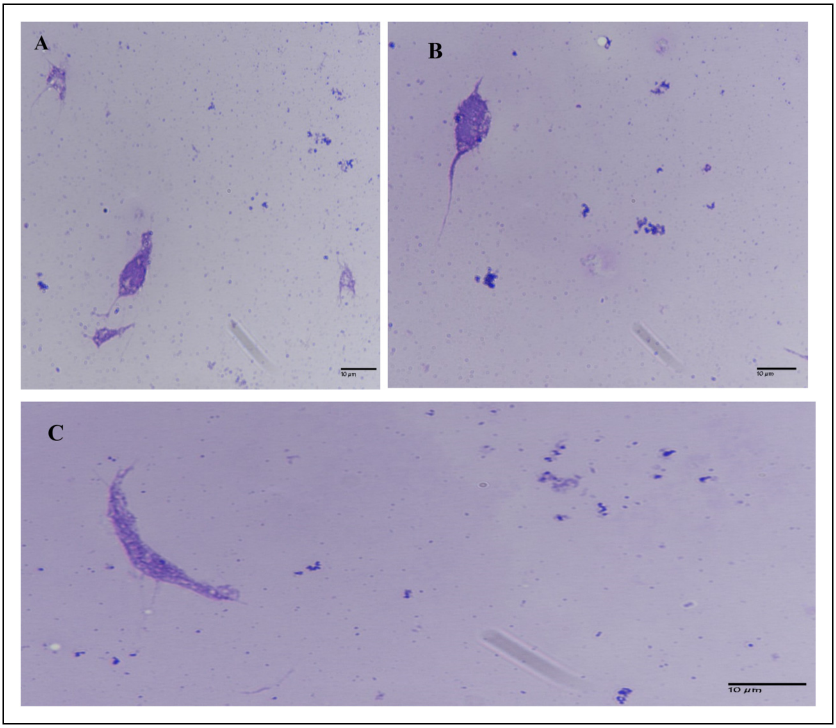

Figure 9. Morphological changes of hemocytes *R. ferrugineus* after 48h treatment with LC_{50} menthol. Change shape of plasmatocytes, A: Lysed cytoplasm and nucleus, vacuoles. B: Lysed hemocyte. C: Completely lysed plasmatocyte.

examined the rate of attraction or repelling reaction of RPW adults to a vast range of objects. Both sexes of RPW were attracted to anethol and (-) camphene in that study, but terpene and -pinene, phyllandral, and eugenol[33] were repelled by both sexes of RPW. The current study's findings are consistent with prior findings, in that both sexes showed equal degrees of attraction or repelling reactions to the therapy, with no significant variations in their responses to the treatment (the main sex had no significant effect on the rate of attraction or repellant response). When it came to the repellent response of adult RPW to the tested substances, males showed a diminishing strength of the repellent response of 60% for carene, 40% for orange, and 20% for limonene, whereas females showed a slightly different ranking of the repellent response of 40% for orange, and 20% for both carene and limonene. These findings matched those of Sharaby,[22] who found that cadenine oils were repellent to female cotton leaf worms, while geraneol was attractive to both sexes, among 55 volatile oils tested on adults of the cotton leafworm *Spodoptera littoralis* using an Olfactometer regarding the attraction vs. repellent response of terpenes. Additionally, by looking at the results of the toxicity of citronella and menthol, as shown in Table 3 in addition to their attractive effect on the adults, they also exert a lethal poisonous effect upon contact methanol exerted a stronger

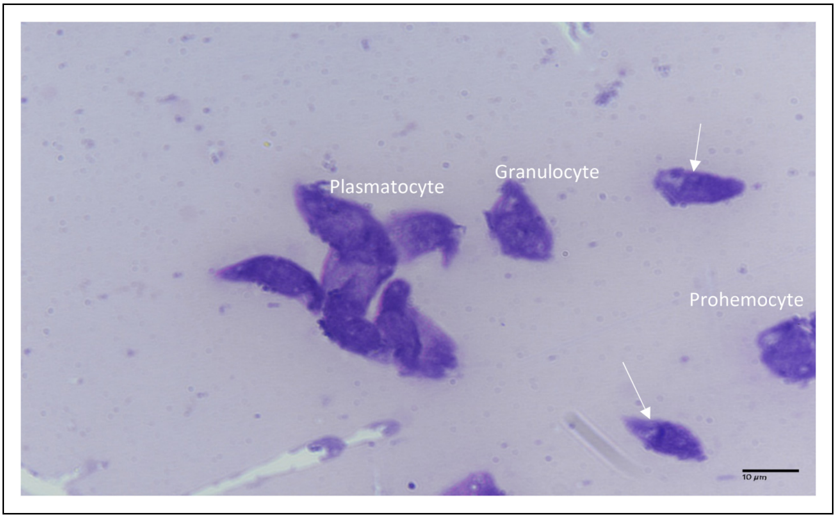

Figure 10. Morphological changes of hemocytes *R. ferrugineus* after 24h treatment with LC_{95} of menthol. Stick plasmatocyte (arrow), granulocyte and prohemocyte vacuoles.

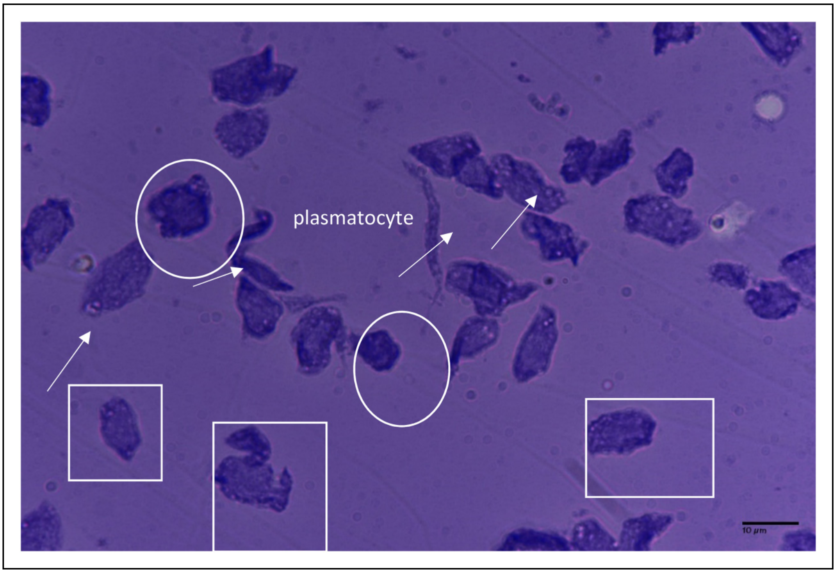

Figure 11. Morphological changes of hemocytes *R. ferrugineus* after 24 and 48h treatment with LC_{95} of menthol. Showing sticks cells, and change shape and size cells, lysed nucleus vacuoles. Arrows: Plasmatocytes; slender, lysed nucleus, cytoplasm, vacuoles. Circles: Prohemocytes; vacuoles, change shape, lysed nucleus, Squares: Granulocytes; change shapes, vacuoles, lysed nucleus.

effect on adults than citronella where LC_{50} values of 1.03, 0.89, and 0.9 mg, and LC_{95} values of 5.09, 2.01, and 1.59 mg were found after 24, 48 and 72 h respectively. For citronella, an LC_{50} of 2.09, 1.76 and 1.70 mg, and an LC_{95} of 5.5, 3.7, and 1.5 mg was found at 24, 48 and 72h respectively. These results agree with those of Sharaby and El-Dosary[33] who observed that juniper oil exhibited insecticidal activity against red palm weevil, whereas (-) camphene exhibited a more poison effect on adults than juniper oil.[33] Furthermore, Huang et al. found the terpenes eugenol, iso-Eugenol, and methyl eugenol acted as contact poisons against the adults of *Sitophilus zeamais.*[39] The obtained results also agreed with those of Hashemi and Rostafer[40] who observed that juniper oil fruit exerted insecticidal activity against red palm weevil. In some cases, the oils may act as poisons, interacting with the fatty acids of the insects and interfering with normal metabolism. Because these oil and terpene were characterized by their high toxicity to insects, as was evident from the LC_{50} and LC_{95} values associated with both citronella and menthol, this study chose to explore the effect of such agents on RPW hemocytes. Six types of hemocytes were observed prohemocytes, granulocytes, plasmatocytes, oenocytes, coagulocytes, and spherulocytes (Figure 4–11). The same six types were identified by Gadelhak[41] and Manachini et al.[42] while six types (prohemocytes, granulocytes, plasmatocytes, oenocytes, coagulocytes, and spherulocytes) were identified by Alkhalifa and Siddiqui.[43] Precise clear morphological changes were observed in the shapes of hemocytes after treatment with LC_{50} and LC_{95} at 24 h, and 48 h. These changes were represented by a number of histopathological symptoms in the cell membrane, cytoplasm, nucleus, and found vacuoles. The hemocytes found to be more sensitive were prohemocytes, granulocytes, and plasmatocytes, and no changes to the other hemocytes were observed. These results agreed with Hamadah and Tanani[44] who studied the effect of pyriproxyfen, Neem Azal and spinetoram on the hemocytes of adults RPW, specifically, the histopathological effect of these compounds on the cell membrane, cytoplasm, nucleus and found vacuoles. These effects were similar to the effects observed with some of the insecticides,[43–47] insects growth regulators,[46–50] and phytochemicals.[44,51] The sensitivity of these cells might be due to phagocytic cells' attraction to any foreign material, which would have a negative impact, or to the activity of actin, which is found in the cells' lamellar extensions.[48,52] Table 6.

Table 6. Estimated LC_{50} and LC_{95} values of menthol against RPW at different time intervals after treatment.

Time	(LC_{50} (mg) (95% fiducial limits))	(LC_{95} (mg) (95% fiducial limits))	DF	Chi X^2	slope	P value
24	1.03 (0.59-1.39)	5.09 (3.68-9.55)	3	4.85	0.36 ± 0.024	0.86
48	0.891 (0.57-1.00)	2.01 (1.61-3.36)	3	0.379	0.23 ± 0.022	0.95
72	0.91 (0.60-1.06)	1.59 (1.304-3.959)	3	0.307	0.25 ± 0.028	0.95

Conclusion

The present study showed the effects of citronella and menthol on RPW immunity, and this knowledge can be applied to their use in integrated pest management at sublethal concentrations. However, the extent to which immunity is affected will depend on the insect life cycle because hemocytes play a key role in protecting against foreign invaders, wound repairing and molting.

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