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Assessing the toxicity of six insecticides on larvae of red palm weevil under laboratory condition

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ABSTRACT

Objective: The red palm weevil (RPW), is one of the most threatening pests of date palm trees worldwide, causing significant economic losses annually for date palm growers, both globally and in the Middle East, including Saudi Arabia. The primary objective of this research was to assess the insecticide market in Saudi Arabia, test various insecticides claimed to be effective against RPW, and evaluate their efficacy in laboratory settings. This evaluation aims to inform further trials under field conditions.
Methods: Six insecticides, including imidacloprid, thiamethoxam, fipronil, emamectin benzoate, deltamethrin, and fenitrothion, were tested to assess their toxicity against red palm weevil 8th instar larvae by diet incorporation under laboratory conditions. The insecticides were applied according to the manufacturer's recommendations with dosages of 1000 µl, 0.20 µl, 7.5 µl, 0.25 µl, 0.25 µl, and 0.5 µl for imidacloprid, thiamethoxam, fipronil, emamectin benzoate, deltamethrin, and fenitrothion, respectively.
Results: The results revealed that all tested insecticides exhibited 100 % mortality against 8th instar RPW larvae, with the exception of deltamethrin. However, the time required to achieve this mortality varied. Fenitrothion caused 100 % mortality after 72 h, while thiamethoxam, imidacloprid, and fipronil caused 100 % mortality after 96 h. Emamectin benzoate and deltamethrin resulted in 100 % and 93 % mortality, respectively, after 10 days. The variability in mortality rates may be attributed to differences in their active ingredients, which show varying levels of lethality. In conclusion, all tested insecticides showed effectiveness against RPW larvae and represent viable options for controlling this pest in date palm orchards in Saudi Arabia.

1. Introduction

The red palm weevil (RPW), scientifically known as *Rhynchophorus ferrugineus* (Olivier) (Olivier) (Coleoptera: Curculionidae), is indeed an invasive species that has caused significant damage to date palm plantations in many countries around the world (El-Mergawy and Al-Ajlan, 2011). Originally native to Southeast Asia, this invasive insect species has successfully spread to regions worldwide where palm trees are cultivated, including the Middle East and certain parts of Africa (Sadakathulla, 1991; Abozuhairah et al., 1996). The RPW is a serious threat to palm trees, as its larvae burrow into the trunk, feed on the internal tissues, and cause wilting, discoloration, and eventual death of the host tree (Abraham et al., 1998). The propagation of the date palm crop commonly involves the use of offshoots. However, there are chances that these offshoots might be infested with the neonates of the RPW. When infested offshoots are transferred and planted in new areas, they become a primary source of infestation and contribute to the spread of RPW individuals in the new locality.

Despite the use of possible alternative measures to manage insect pests affecting crops in the field and food grains under storage, chemical insecticides remain a crucial control measure under specific conditions. In general, numerous insecticides have proven effective against RPW, including malathion, fenitrothion, cypermethrin, beta-cyfluthrin, carbaryl, deltamethrin, imidacloprid, chlorpyrifos, and dimethoate (Hoddle et al., 2013; Chihaoui-Meridja et al., 2020; Rasool et al., 2021; Sabra et al., 2023). Certain insecticides, such as pirimiphos-methyl and oxydemeton-methyl, have been shown to be highly toxic to RPW adults and larvae (Ajlan et al., 2000). Similarly, imidacloprid has shown the

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best efficacy against different stages of RPW in both laboratory and field settings, with concentrations of 3.5 mL/L and 1000 mL/L, respectively (Kaakeh, 2006). In Spain, four active ingredients, such as chlorpyrifos, imidacloprid, phosmet, and thiamethoxam, have been recommended by the agriculture department for the management of RPW (Dembilio and Jaques, 2012).

In another study, fipronil (0.004 % active ingredient) applied via the dipping method resulted in 100 % mortality of different larval stages of RPW within 30 min after treatment (Al-Shawaf et al., 2013). Hamadah and Tanani (2013) observed that a concentration level of radiant at 200 ppm was very effective in eliminating the last instar larvae, pupae, and adults of the RPW. Furthermore, the toxicity assessment of various insecticides against RPW larvae and adults revealed that deltamethrin exhibited the most potent toxic effects among the investigated insecticides after 24 h of exposure (Shawir et al., 2014). Efforts have been made to evaluate the contact and fumigation toxicity of terpene (camphene) against different stages of the RPW under laboratory conditions, and LC_{95} values were calculated as 296.6, 1000.6, and 8113.9 μl/L for eggs, 4th instar larvae, and adults, respectively (Sharaby and Mona, 2016). Imidacloprid showed 84 % and 79 % mortality after 20 days for the 2nd and 4th instar larvae, respectively (Malik et al., 2016). However, overreliance and extensive use of insecticides can lead to resistance development, which ultimately increases the cost of chemical control. Therefore, insecticides should be used wisely, and only effective insecticides should be used for the control of RPW. The main objective of the present study was to evaluate the laboratory efficacy of different insecticides claimed to be effective against RPW.

2. Materials and Methods

2.1. Red palm weevil rearing

Red palm weevil adults were collected from infested date palm orchards in Riyadh, Saudi Arabia (24.4164°N, 46.5765°E). They were provided with a piece of cotton saturated with a 10 % sugar solution in a one kg plastic box (L: 17 cm; W: 11 cm; H: 7 cm). The RPW colony was maintained in a growth chamber at 25 ± 1 °C and 70 ± 5 % relative humidity, following the standard procedure already described by Aldawood et al. (2022).

2.2. Bioassay of insecticides

Six commercially formulated insecticides claimed to be the most effective by the sellers on the market were evaluated in laboratory trials against RPW larvae using the diet incorporation method. These insecticides included imidacloprid (Confidor 35 SC, Bayer, Germany), thiamethoxam (Actara 25 WG, Syngenta, Greensboro, NC, USA), fipronil (Fiprol 50 SC, Delta, Saudi Arabia), emamectin benzoate (Revive/Aretor 4 EC, (Syngenta, Greensboro, NC, USA), deltamethrin (Delthathrin 25 EC, Shanghai Bosman, China), and fenitrothion (Fentrol 50 EC). To assess the toxicity, the insecticides were applied to 8th-instar RPW larvae. The insecticides were applied according to the manufacturer's recommendations, with dosages of 1000 μl, 0.20 μl, 7.5 μl, 0.25 μl, 0.25 μl, and 0.5 μl for imidacloprid, thiamethoxam, fipronil, emamectin benzoate, deltamethrin, and fenitrothion, respectively. The insecticidal solution was prepared by diluting each insecticide concentration in 500 ml of distilled water. Subsequently, each concentration was thoroughly mixed with a 375-gram semi-artificial diet, while a control treatment consisting of distilled water (autoclaved) mixed with the diet was also prepared. The treated diet was transferred to plastic cups measuring approximately 3.5 cm in diameter and 4.5 cm in height. Five plastic cups were prepared for each replicate, and 25 g of treated diet was added to each plastic cup. An individual larva was placed in each plastic cup using forceps. A total of 105 larvae were used for the bioassay, and fifteen larvae were used for each treatment. Subsequently, all treatments were placed in an incubator (Steridium, Australia) set at 25 ± 1 °C with a relative humidity of 80 ± 5 % and a photoperiod of 6:18 h L:D. Mortality data were recorded at 24, 48, 72, 96, and 120 h post-treatment. Final data for the insecticides that did not kill the larvae until 120 h was taken after 10 days, when 100 % mortality occurred. The larvae that had not shown any movement after being touched with a fine brush were considered dead.

2.3. Data analysis

The data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of SAS (SAS, 2009), and means were separated using the least significant difference (LSD) at $P < 0.05$.

3. Results

The results revealed varying responses of the treated larvae to different insecticides. Specifically, after 24 h of exposure, thiamethoxam showed the highest mortality percentage compared to fenitrothion, imidacloprid, fipronil, deltamethrin, and emamectin benzoate. However, it is noteworthy that the effects of insecticides on RPW larvae observed in terms of mortality after 24 h of exposure were significantly different from the control (Table 1).

After 48 h of exposure, mortality indicated that fipronil was the most effective insecticide, followed by thiamethoxam, imidacloprid, and fenitrothion. The mortality rates of 8th instar larvae following exposure to fipronil (80 %), thiamethoxam (53 %), imidacloprid (53 %), and fenitrothion (47 %) were significantly higher compared to emamectin (27 %) and deltamethrin (20 %) (Table 2).

After 72 h of exposure, mortality indicated that fenitrothion was the most effective insecticide, followed by fipronil, thiamethoxam, and imidacloprid. The mortality rates of 8th instar larvae following exposure to fenitrothion (100 %), fipronil (93 %), thiamethoxam (80 %), and imidacloprid (73 %) were significantly higher compared to emamectin benzoate (47 %) and deltamethrin (47 %) (Table 3).

After 96 h of exposure, the mortality of 8th-instar RPW larvae showed significant differences compared to the control (Table 4). At this exposure duration, 100 % mortality rates were observed for thiamethoxam, imidacloprid, and fipronil, which were significantly higher compared to emamectin benzoate (73 %) and deltamethrin (53 %) (Table 4).

The efficacy of emamectin benzoate and deltamethrin was further tested against RPW larvae following a 120-hour exposure period, revealing a significant disparity in the percentage of mortality of both pesticides and the control (Table 5). However, the mortality rates were strikingly similar for both pesticides, exceeding 80 %. In the present study, emamectin and deltamethrin exhibited a slower response to 8th-instar RPW larvae compared to the other tested insecticides. In addition, the mortality rates of RPW larvae reached 100 % for emamectin and 93 % for deltamethrin after a 10-day exposure period (Table 6), indicating that longer exposure timing is required to achieve the maximum mortality for these pesticides. Based on our present findings, it can be concluded that all tested insecticides effectively killed the RPW larvae, although with differences in response time. While nearly all insecticides

Table 1
Mean percent mortality of red palm weevil larvae treated with different insecticides after 24 h exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Thiamethoxam	40.00 ± 13.09 a	15	2.54	6,104	0.0249
Fenitrothion	33.00 ± 12.59 ab	15			
Imidacloprid	27.00 ± 11.81 abc	15			
Fipronil	13.00 ± 9.08 abc	15			
Deltamethrin	7.00 ± 6.6 bc	15			
Emamectin benzoate	7.00 ± 6.6 bc	15			
Control	0.00 ± 0.0c	15			

Means followed by the same letter (s) are not significantly different (LSD test at P < 0.05).

Table 2
Mean percent mortality of red palm weevil larvae treated with different insecticides after 48 h of exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Fipronil	80.00 ± 10.69 a	15	5.41	6,104	<.0001
Thiamethoxam	53.00 ± 13.3 ab	15			
Imidacloprid	53.00 ± 13.3 ab	15			
Fenitrothion	47.00 ± 13.33 ab	15			
Emamectin benzoate	27.00 ± 11.81 bc	15			
Deltamethrin	20.00 ± 10.69 bc	15			
Control	0.00 ± 0.0c	15			

Means followed by the same letter (s) are not significantly different (LSD test at $P < 0.05$).

Table 3
Mean percent mortality of red palm weevil larvae treated with different insecticides after 72 h of exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Fenitrothion	100.00 ± 0.0 a	15	12.82	6,104	<.0001
Fipronil	93.00 ± 6.6 a	15			
Thiamethoxam	80.00 ± 10.69 a	15			
Imidacloprid	73.00 ± 11.81 ab	15			
Emamectin benzoate	47.00 ± 13.3b	15			
Deltamethrin	47.00 ± 13.3b	15			
Control	0.00 ± 0.0 e	15			

Means followed by the same letter (s) are not significantly different (LSD test at $P < 0.05$).

Table 4
Mean percent mortality of red palm weevil larvae treated with different insecticides after 96 h of exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Thiamethoxam	100.00 ± 0.0 a	15	29.79	5,89	<.0001
Imidacloprid	100.00 ± 0.0 a	15			
Fipronil	100.00 ± 0.0 a	15			
Emamectin benzoate	73.00 ± 11.8b	15			
Deltamethrin	53.00 ± 13.3b	15			
Control	0.00 ± 0.0c	15			

Means followed by the same letter (s) are not significantly different (LSD test at $P < 0.05$).

Table 5
Mean percent mortality of red palm weevil larvae treated with different insecticides after 120 h of exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Emamectin benzoate	80.00 ± 10.69 a	15	35.45	2,44	<.0001
Deltamethrin	87.00 ± 9.08 a	15			
Control	0.00 ± 0.0b	15			

Means followed by the same letter (s) are not significantly different (LSD test at $P < 0.05$).

Table 6
Mean percent mortality of red palm weevil larvae treated with different insecticides after 10 days of exposure under laboratory conditions.

Treatment	Mortality (%) ± SE	N	*F*	df	*P*
Emamectin benzoate	100.00 ± 0.0 a	15	211.00	2,44	<.0001
Deltamethrin	93.00 ± 6.6 a	15			
Control	0.00 ± 0.0b	15			

Means followed by the same letter (s) are not significantly different (LSD test at $P < 0.05$).

resulted in close to 100 % mortality, the duration required to achieve this varied. The varying timing of mortality could be attributed to the efficacy of the active ingredient in reaching the target site and its subsequent action. However, all of the insecticides tested demonstrated toxicity against the 8th instar RPW larvae.

The LT_{50} values for *R. ferrugineus* were 81.93 h for deltamethrin, 35.74 h for fenitrothion, 40.13 h for imidacloprid, 34.11 h for thiamethoxam, 36.32 h for fipronil, and 69.69 h for emamectin benzoate. The LT_{50} values of imidacloprid, thiamethoxam, and fipronil were significantly lower than that of deltamethrin (non-overlapping 95 % FL). There was no difference in LT_{50} values of deltamethrin and emamectin benzoate against *R. ferrugineus* (overlapping 95 % FL) (Table 7).

4. Discussion

Following a comprehensive survey of the pesticide market, six insecticides (Table 1) that were claimed to be effective against RPW were selected for evaluation in the present study. These insecticides were evaluated for their effectiveness against the 8th-instar RPW larvae using the diet incorporation technique under laboratory conditions. The concentrations of the insecticides were applied in accordance with the manufacturer's recommendations, and the mortality data were recorded at 24, 48, 72, 96, and 120 h post-treatment. The results were interesting; all insecticides tested against 8th-instar RPW larvae showed 100 % mortality. However, the time required to achieve this mortality varied. For example, fenitrothion induced 100 % mortality after 72 h, whereas thiamethoxam, imidacloprid, and fipronil achieved the same outcome after 96 h. Emamectin benzoate and deltamethrin reached 100 % and 93 % mortality, respectively, after 10 days. These variations may be attributed to differences in the active ingredients and the extent of their action, resulting in varying levels of lethality at different times.

In agreement with the results of the present study, similar observations were reported previously by Ajlan et al. (2000). For example, pirimiphos-methyl and oxydemeton-methyl exhibited the highest mortality against larval stages of the RPW after 24 h compared to chlorpyrifos. In another study, dimethoate, fipronil, deltamethrin, methidathion, methomyl, fenitrothion, salut, and chlorpyrifos with four different concentrations of each insecticide were tested against larval stages of the RPW in the laboratory, where fipronil was the most toxic insecticide (Al-shawaf et al., 2010). Similarly, Shawir et al. (2014) observed the highest mortality in 20-day-old RPW larvae after 24 h of exposure to deltamethrin, followed by emamectin benzoate and imidacloprid. According to Mohammed (2020), fipronil and dueracide proved to be the most effective insecticide against RPW larvae in Makkah Al Mukarramah Region, both through dipping and feeding treatments. Similarly, RPW larvae showed the highest susceptibility to sulfoxaflor and acetamiprid under laboratory conditions through direct spray application (Alhewairini, 2020). However, the extensive usage of insecticides can lead to resistance development against RPW. High levels of resistance were recorded in RPW field populations from Pakistan against phosphine, cypermethrin, deltamethrin, and profenofos compared to a susceptible strain (Wakil et al., 2018). Similarly, elevated levels of resistance in other insects from Saudi Arabia have been

Table 7
The LT_{50} values of different insecticide against 8th larval instar of *Rhynchophorus ferrugineus* under laboratory conditions.

Insecticide	LT_{50} (95 % FL) h	Slope ± SE	χ^2
Deltamethrin	81.93 (61.15–114.54)	2.47 ± 0.55	6.01
Fenitrothion*	35.74	3.83 ± 2.70	6.02
Imidacloprid	40.13 (27.91–50.88)	3.55 ± 0.86	2.77
Thiamethoxam	34.11 (19.68–44.99)	3.04 ± 0.83	3.36
Fipronil	36.32 (28.72–43.58)	5.92 ± 1.24	0.52
Emamectin benzoate	69.69 (55.10–87.53)	3.53 ± 0.78	0.42

* Fiducial limits (FL) were not estimated due to high calculated χ^2 than tabulated.

reported due to the unwise and extensive usage of various conventional and new chemistry insecticides (Hafez and Abbas, 2021; Hafez, 2022; Sabra et al., 2023; Abbas and Hafez, 2023). Therefore, insecticides should be used carefully and wisely against RPW in the Riyadh region of Saudi Arabia. Moreover, an integrated pest management strategy should be adopted to minimize the insecticide selection pressure for controlling RPW.

5. Conclusions

The results of all test insecticides, such as imidacloprid, thiamethoxam, fipronil, emamectin benzoate, deltamethrin, and fenitrothion, have shown remarkable effects and caused almost 100 % mortality against 8th instar RPW larvae when exposed to a treated diet under laboratory conditions. However, the time required to achieve 100 % mortality varied among the insecticides. For example, fenitrothion resulted in 100 % mortality after 72 h, while thiamethoxam, imidacloprid, and fipronil achieved the same level of mortality after 96 h Emamectin benzoate, on the other hand, reached 100 % mortality after 10 days, suggesting that toxicity increased with longer exposure times. These findings could aid in the selection of the most suitable insecticides under field conditions and their integration into RPW control programs as either protective or curative measures.

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CRediT authorship contribution statement

Khawaja G. Rasool: Conceptualization, Formal analysis, Methodology, Project administration, Software, Writing – review & editing, Writing – original draft. **Mureed Husain:** Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **Waleed S. Alwaneen:** Data curation, Formal analysis, Methodology, Software, Writing – review & editing. **Koko D. Sutanto:** Data curation, Investigation, Methodology, Software, Validation, Writing – review & editing. **Abdalsalam O. Omer:** Data curation, Investigation, Methodology, Software, Writing – review & editing. **Muhammad Tufail:** Investigation, Validation, Visualization, Writing – review & editing. **Abdulrahman S. Aldawood:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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