

Evaluation of Fiprol, Imidaprid and Dueracide Insecticides against Larval Stage of Red Palm Weevil *Rhynchophorus Ferrugineus* (Olivier) in Makkah Al-Mukarramah Region

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae) is one of the most threatening pests for date palm trees in Kingdom of Saudi Arabia. It is considered as an insect pest from category I in the Gulf region and the Middle East, according to the Food and Agriculture Organization (FAO). The difficulty of controlling the weevil chemically is due to its hidden life habits inside the trunk of palm. The excessive use of large quantities of insecticides made it possess resistance against many of them. Therefore, it was important to carry out this research to investigate the level of sensitivity of weevil larvae against some common insecticides as used in Makkah AL Mukarramah region. The effectiveness of three pesticides was tested (Fiprol: cyclodines ;Imidaprid: neonicotinoid and Dueracide: organophosphate) through feeding and dipping techniques. The results revealed that Fiprol was the most effective against *R. ferrugineus* larvae where LC_{50} was (0.896-71.2 ppm) by feeding and dipping methods respectively. Then, Dueracide and Imidaprid were tested with LC_{50} of (54.2 -115.9 ppm), respectively. The LC_{50} of Imidaprid by dipping method showed (112.5 ppm) while the Dueracide was found the least effective by dipping treatment where the LC_{50} was (1003.8 ppm). According to the values of resistance ratio(RR), Fiprol was more effective than Dueracide and Imidaprid by (60.53 - 129.37) times respectively, by feeding method and about (1.57 and 14.68) times respectively, compared to Imidaprid and Dueracide by dipping. Generally, treatment by feeding was (59.58) times more effective than by dipping.

Keywords: Control; Cyclodines; Makkah Al Mukarramah Region; Neonicotinoid ; Organophosphorus; *Rhynchophorus Ferrugineus*.

The date palm trees, *Phoenix dactylifera* have an economic importance at the global level as one of the food sources in many countries, especially in the Middle East and North Africa countries. Dates are one of the main crops in Kingdom of Saudi Arabia, where the Kingdom came at the second rank worldwide in the production of dates, the amount of production

is estimated at 1,122,820 million tons, scoring 14.96% of the global production of dates 1 and 2 and Kingdom of Saudi Arabia cultivates more than 400 palm varieties³.

Kingdom of Saudi Arabia is one of the most consuming countries for dates, as the Saudi citizen occupies the first rank globally in the consumption of dates, with an annual consumption rate of 34.8 kg / person^{4; 5; 6, 7}.

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The date palm trees recorded infestation by many insect and non-insect pests, but the red palm weevil *Rhynchophorus ferrugineus* (Olivier) was classified an insect pest from the first category I in the Gulf region and the Middle East, according to the Food and Agriculture Organization (FAO) 1;3; 8 and 9, where it infects 29 species of palm trees, the most important of them is date palm trees¹⁰.

One of the most important challenges which faces the controlling of red palm weevil return to its hidden life cycle inside the trunk of the palm tree;6 and 11 mentioned that it is among many different controlling methods applied against red palm weevil *R. ferrugineus* chemical control is considered as an essential, fast and reliable way to recover infested date palm trees with red palm weevil. Many organophosphate, carbamate and pyrethroid insecticides have been used in reducing this insect^{12;13;14;15;16;17}, which proved effective in controlling red palm weevil. However, the safety and security of use; environmental pollution and development of insect resistance to the used pesticides are among the most important factors that limit the effectiveness of using these insecticides in controlling red palm weevil^{10,12}.

In addition, excessive use of preventive insecticides and frequent spraying for unlimited periods lead to economic, environmental and health impacts. The presence of pesticide residues in dates at some areas of cultivation and the production of dates is associated with the excessive application of pesticides against red palm weevil¹⁸.

There is a scarcity of previous studies in the field of evaluating the effectiveness of some traditional pesticides currently applied in Makkah Al-Mukarramah region on red palm weevil control programs. The importance of carrying out this study lies in identifying the sensitivity; tolerance and resistance of red palm weevil to these insecticides and setting a database for them as reference when planning for its control programs and making the right decision toward the used insecticide.

MATERIAL AND METHODS

Insect Specimens

The adult insects were collected from different farms from the study areas (Taif– Makkah – Jeddah), using pesticide-free traps, according to

the method 19 or by manual collection directly from the infested date palm trees.

Rearing of the Red Palm Weevil

The red palm weevil was reared on offshoots date palm under laboratory conditions at temperature of $27 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$ to obtain a sufficient number of insects to carry out the experiments of this research²⁰.

The offshoots were chosen from three to four years, fronds and roots were removed, then the trunk was longitudinally split into two halves and a square cavity was made to put insects inside it. Male were placed for females in a ratio of (1: 2). The trunk was closed again using a strong thread, where it was well bonded to prevent the escape of the insects. Then, the trunk was covered from the outside with a metal mesh. The infested offshoots were placed in a special box designed for this purpose with three facades with a metal mesh for ventilation (upper – two sides) and the rest from aluminum to increase safety and ensure that the insects do not leak outside the laboratory.

After finishing from the offshoots, they were cut into small pieces and doused by kerosene in plastic bags in preparation for burning them to prevent contributing spread of the infestation. To get enough number of one age larvae, the red palm weevil was also reared on fresh sugarcane according to 20 and 21 to follow up the laying of eggs, where the insects were placed inside 300 ml plastic boxes with perforated covers for ventilation containing pieces of sugarcane.

The eggs inside the sugarcane were extracted by using soft brush. Then, it incubated in Petri dishes (12.5 cm diameter) where containing a wet filter papers to provide appropriate moisture and follow up the hatching within two to five days.

After hatching, the neonate was transferred to artificial diet according to 22 to follow up larval development. The larvae were individually placed in plastic boxes (200-500 ml) perforated for ventilation and it was kept in units designed for this purpose. The units consist of aluminum frame with several shelves and surrounded by three side by metal mesh to ensure proper ventilation and save samples from escape and external parasites.

Bioassay Experiments

Bioassay experiments of insecticides which are used to control red palm weevil in

Makkah Al-Mukarramah region were carried out at the central laboratory for bioassay of pesticides in the Department of Biological Sciences at King Abdulaziz University. A special laboratory was prepared to rear the red palm weevil to obtain a sufficient number of weevil larvae which were necessary to implement the experiments and fulfill the objectives of this search.

Tested Insecticides

The bioassay experiments were conducted for three commonly used insecticides to control the red palm weevil in Makkah region as follows:

Fiprol

It is a cyclodienes insecticide and the active ingredient Fipronil 5% (W/V); (RS)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile.

Imidaprid

It is a neonicotinoid insecticide and the active ingredient Imidacloprid 5% (W/V); N-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine.

Dueracide

It is an organophosphate insecticide and the active ingredient Methidathion 40% (W/V); 3-(dimethylphosphinothioylsulfanylmethyl)-5-methoxy-1,3,4-thiadiazol-2-one.

These insecticides were obtained from the branch of Ministry of Environment, Water and Agriculture, Makkah Al-Mukarramah Region.

Method of Treatment

The sensitivity level for the eight-week-old of red palm weevil larvae for the tested insecticides were evaluated by two techniques:

Feeding Technique

According to the method 23 with some modification. 60g of the diet was weighed and then added 20ml from the tested concentration of insecticide. There were five concentrations for each insecticide with five replicates for every concentration, while at the diet in a control was added to it 20 ml water and the results were recorded after 24 and 48 hours.

Dipping Technique

According to the 24, with some modifications the larvae were immersed in the tested concentration for 30 seconds using hill bags and transferred to diet. For each insecticide, five concentrations were tested with five replicates for

each concentration, while in a control the larvae were immersed in water for 30 seconds and then transferred to the diet. The results were recorded after 24 and 48 hours. The larvae were considered dead if it did not move or give a backlash to any movement or touch.

Statistical Analysis

A standardized statistical program (Ldp Line Program) was used to analyze the results of laboratory toxicity according to the method of Finney (1971) of drawing Laboratory toxicity curve for the tested insecticides and extracting statistical constants using a specialized statistical program²⁵.

RESULTS

Bioassay Experiments

The present study aimed to measure the sensitivity of the red palm weevil larvae *R. ferrugineus* to commonly used insecticides in the Makkah AL-Mukarramah region. The results were as follows:

Fiprol Insecticide

Feeding Technique

The results shown in Table (1) and Fig. (1) revealed that the effective concentrations of Fiprol after 48 hours by feeding treatment ranged between (0.5-2.5 ppm) and the corresponding mortality ratio was (33.3 – 86.6)% , while there wasn't a mortality recorded in the control and the LC₅₀ was (0.89 ppm) with 95% confidence level and the confidence intervals (1.02-0.760), whereas the LC90 was (3.39 ppm) at confidence intervals (2.74-4.65) at 95% confidence level. On the other hand, the slope of the toxicity line was (2.21±0.24).

Dipping Technique

The data indicated that the effective concentrations of Fiprol by dipping method was between (20-140 ppm) after 48 hours and corresponding mortality ratio ranged between (9.69-86.04)%. The LC₅₀ reached to (71.22 ppm) with 95% confidence level and confidence intervals was (64.03-78.99 ppm), while LC90 was (209.89 ppm) at confidence intervals (174.5–270.4) at 95% confidence level. The slope of the toxicity line was (2.73±0.25), also there wasn't a mortality recorded in the control (Table, 1 and Fig. 1).

Noteworthy, the treatment method had a clear effect on the efficacy of the Fiprol insecticide against the red palm weevil larvae as shown at

(Table 1 and Fig. 1). The treatment by feeding was the most effective compared to dipping method by about (79.49) times.

Imidaprid Insecticide

Feeding Technique

The effective concentrations of Imidaprid ranged between (50-140 ppm), the corresponding mortality ratio was (13.3–60.0)% ,without a mortality recorded at the control and the LC₅₀ after 48 hours by feeding method was (115.9 ppm) with 95% confidence level. The confidence intervals showed (105.7-115.9 ppm), while the LC90 was

(291.2 ppm) at confidence intervals (227.8- 436.1 ppm) at 95% confidence level. The slope of the toxicity line was(3.2±0.41)as shown in Table (1) and Fig. (1).

Dipping Technique

The results of statistical analysis in (Table, 1 and Fig. 1) showed that the effective concentrations of Imidaprid by dipping method ranged between (70-190 ppm) and corresponding mortality ratio was between (21.88- 86.04)% for lowest and highest concentration respectively. TheLC₅₀ was to (112.5 ppm) with 95% confidence

Table 1. Susceptibility levels of red palm weevil larvae to commonly used insecticides in Makkah region using feeding and dipping techniques

Insecticide	Treatment method	Con. (ppm)	Mortality (%) ^a	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2 ^b	slope
Fiprol	Feeding	0.5	33.3	0.89 (0.760-1.021)	3.39 (2.74-4.65)	4.42	2.21 ±0.24
		1	46.6				
		1.5	66.6				
		2	80				
		2.5	86.6				
	Dipping	20	9.69	71.22 (64.03-78.99)	209.89 (174.5- 270.4)	7.48	2.73 ±0.25
		50	30.521				
		80	47.917				
		110	68.75				
		140	86.04				
Imidaprid	Feeding	50	13.3	115.9 (105.7-115.9)	291.2 (227.8-436.1)	1.71	3.2 ±0.41
		70	20				
		90	40				
		110	46.6				
		140	60				
	Dipping	70	21.88	112.5 (104.03-120.75)	244.01 (212.8 -298.1)	4.354	3.81 ±0.40
		100	44.38				
		130	58.33				
		160	65.21				
		190	86.04				
Dueracide	Feeding	30	26.6	54.2 (48.8-59.5)	146.3 (123.7-186.7)	4.37	2.97 ±0.31
		50	40				
		70	60				
		90	73.3				
		110	86.6				
	Dipping	400	20	1003.8 (669.2-1403.3)	3131 (2806.6-9919.8)	9.46	2.59 ±0.26
		800	33.3				
		1200	53.3				
		1600	66.6				
		2000	86.6				

a: Five replicates, 20 larvae each.

b: Tabulated $\chi^2=7.8$, When tabulated χ^2 larger than calculated at 0.05 level of significance indicates the homogeneity of results

level and confidence intervals was (104.03-120.75), whereas the LC90 was (244.01 ppm) at confidence intervals showed(212.8–298.1) at 95% confidence level. The slope of the toxicity line was (3.81±0.40) also without a mortality recorded at the control.

Dueracide Insecticide Feeding Technique

The results shown in Table (1) and Fig. (1) revealed that the effective concentrations of Dueracide after 48 hours was between (30-110 ppm) and the corresponding mortality ratio was

(26.6-86.6)%and there wasn't a mortality recorded in the control. The LC₅₀ was (54.2) ppm after 48 hours of feeding treatment with 95% confidence level and confidence intervals (48.8-59.4), while LC90 was (146.3) ppm at confidence intervals (123.7-186.7) at 95% confidence level. On the other hand, slope of the toxicity line was (2.9±0.3124).

Dipping Technique

The data indicated that the effective concentrations of Dueracide after 48 hours was (400-2000 ppm) and the corresponding mortality ratio ranged between (20.0-86.6%)for highest

Table 2. Comparison between insecticides used against *Rhynchophorus ferrugineus* larvae on basis of LC₅₀ and resistance ratio values

No.	Line name	LC ₅₀	Lower limit	Upper limit	RR
1	Fiprol(feeding 48h)	0.896	0.761	1.022	1
2	Dueracide(feeding 48h)	54.2	48.821	59.455	60.527
3	Fiprol(dipping 48h)	71.2	64.033	78.998	79.494
4	Imidaprid(dipping 48h)	112.5	104.04	120.752	125.558
5	Imidaprid(feeding 48h)	115.9	105.709	131.284	129.369
6	Dueracide(dipping 48h)	1003.8	669.266	1403.343	1120.327

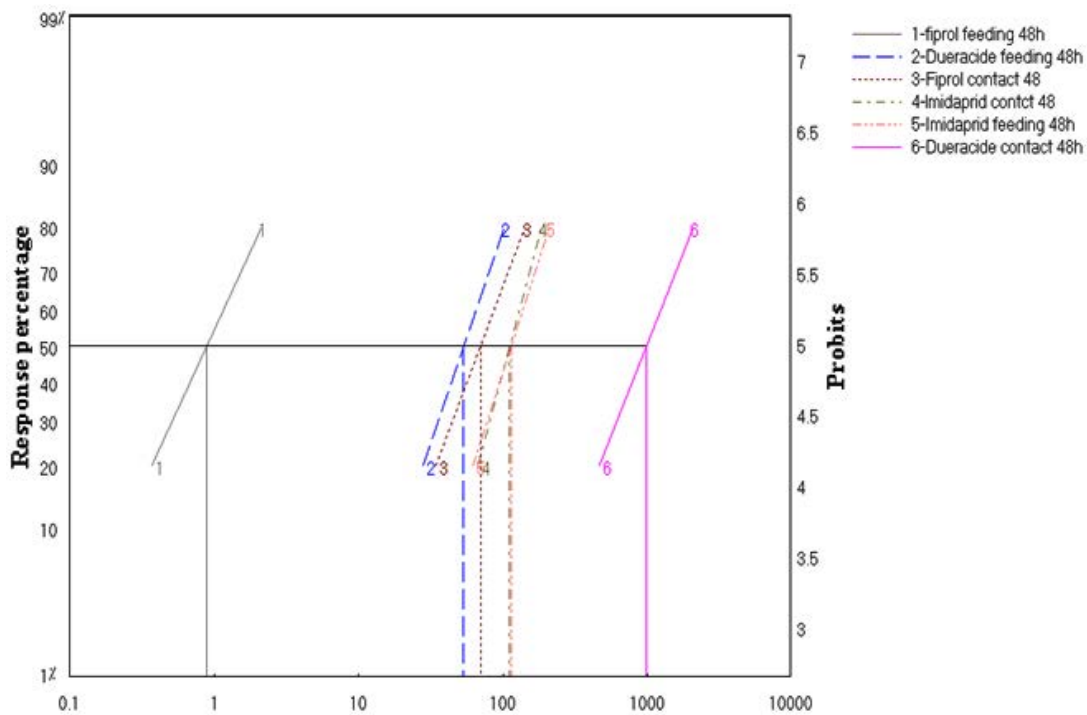


Fig. 1. Regression lines for some insecticides against *Rhynchophorus ferrugineus* larvae using feeding and dipping techniques

and lowest concentration respectively. The LC_{50} reached (1003.8) ppm at 95% confidence level and confidence intervals (669.2-1403.3) ppm. The LC_{90} was (3131.0) ppm at confidence intervals (2806.6–9919.8) and also no mortality was recorded in control. The slope of the toxicity line was (2.59±0.26) (Table, 1 and Fig. 1).

It can be concluded that the treatment method had a clear effect on the efficacy of the insecticide against red palm weevil larvae as shown clearly in (Table 1 and Fig. 1). The treatment by feeding was the most effective compared to dipping treatment by about 59.58 times.

According to the values of LC_{50} at table (2) and Fig. (1), the most effective insecticide by feeding treatment against *R. ferrugineus* larvae was Fiprol where the LC_{50} was (0.896) ppm, followed by Dueracide with LC_{50} (54.2) ppm, while the Imidaprid was the least effective compared to the previous insecticides with LC_{50} reached (115.9) ppm. According to the relative resistance index values (RR) the Fiprol insecticide was more effective than Dueracide and Imidaprid by about (60,527 and 129,369) times, respectively.

In addition, the data after 48 hours indicated that the Fiprol was the most effective insecticide by dipping technique, with LC_{50} was (71.2)ppm followed by Imidaprid , Dueracide with LC_{50} were (112.5 , 1003.8) ppm respectively .For(RR), Fiprol insecticide, it was the most effective compared to the Imidaprid and Dueracide by (1.57 and 14.68) times, respectively.

DISCUSSION

The use of pesticides in the past and now is considered the main way for reducing the agricultural pests, but the resistance of these pests against many pesticides is the main problem for its control, which was an important reason for the failure of control programs in the field and warehouses.

Therefore, it has become important to test sensitivity of insect to pesticides before using pesticides as a vital and important component of the success to control programs. The results of bioassay of pesticides are real indicators for understanding the sensitivity of the targeted pests and therefore proper planning of their control programs and making the right decision towards the pesticide

was done, which is the concern of many previous research and studies²⁶ .

The red palm weevil is one of the agricultural pests most exposed to insecticides at the present time. Therefore, the current study focused on evaluating its sensitivity to the insecticides which used in control programs in Makkah Al-Mukarramah region.

One of the objectives of this study was to determine the sensitivity, tolerance and resistance of red palm weevil to commonly insecticides used in its control. The results of the this study showed that there is a difference in the level of sensitivity of red palm weevil larvae to the tested compounds, which appeared clearly in the different concentrations of tested insecticides and the LC_{50} values. The reason for this may be due to the different active substances in these compounds, or it may be due to the different mode of action of it against the red palm weevil larvae, in addition to the history of the use of insecticides and the frequency of spraying operations. These results come in consistent with many previous studies whereas^{16; 27 and 28} found a difference in the sensitivity level of *R. ferrugineus* according to the tested insecticides.

On the other hand, the current study showed that there is a positive correlation between the concentrations used and the percentage of mortality of larvae treated with these concentrations. This may be due to the effect of sub-lethal concentrations on sensitive larvae only and some of them were tolerant to insecticide, while the high concentrations had more effect on both sensitive and tolerant individuals which increase the mortality with increasing the concentration. These results agree with those obtained by²⁹ where they found increased percentage of mortality from 60% when exposed to concentration of 600 ppm of Abamecticide to 100% at concentration 1000 ppm.

This study indicated that there was positive correlation between the exposure time and mortality percentage of larvae. This may be due to the increased exposure time which enabled the active substances of insecticide to bind to target -sites inside weevil body and thus make toxic effect, while the short time exposure of insecticide may be one of the reasons the insecticide fails to reach and bind to the targeted sites. These results come in line with many previous studies, which

demonstrated that increasing the effectiveness of the pesticide increases with exposure time due to the presence of a residual toxicity effect of insecticide, where 30 pointed out that exposing larvae and adults of *R. ferrugineus* to concentration 25 ppm of Fipronil until one week resulted in 100% mortality for both stages.

The tested insecticides had most effect when used with feeding technique mixed with diet. The effectiveness increased compared to its use through dipping technique. The reason for this may be that the effect of insecticide treatment through feeding on weevil was in two ways: the first one is effect as stomach toxins and the second one is the adhesion of treated food particles by the insecticide to the joints of the insect's body and thus the effect was by contact. In dipping technique, the effect is by contact only.

These results agree with several previous studies where 18; 31; 32;33 and 34 reported that date palm trunk injection with insecticide under field conditions -which simulates mixing insecticide with diet under laboratory conditions- was better than spraying treatment, which may be ineffective due to hiding the insects in bases fronds palm, and thus difficulty reach to these areas by insecticide .On the other hand, 18 attributed the efficacy by injection treatment compared to spraying treatment to the presence of many compounds in insecticide which has photolysis through the light of the sun. Therefore, inserting the active substance into the plant body or mixing with diet lead to protect it from weather factors and reduces the decomposition and breakdown of active ingredients.

The results of this study differed relatively with the findings by 11 that they said the absence of significant differences in percentage mortality could be attributed to different treatment conditions due to the different conditions between 11 study which was applied under field conditions and taken estimated results, while the current study was conducted under laboratory with a known number of treated larvae with correctness and ease in following up the live and dead larvae. Therefore the laboratory results were more accurate, which was indicated by researchers through the recommendations to implement advanced research through acting an artificial infection with specific numbers of larvae to obtain more accurate results.

Generally, according to the results of bioassay of tested insecticides and (RR) values index showed that Fiprol insecticide was the most effective in reducing the red palm weevil larvae compared to other tested insecticides where (RR) values were (7.817 – 157.186) times and. This matches with 30; 35 and 36 where they reported that the Fiprol was the most effective in controlling during all stages of *R. ferrugineus* with mortality percentage reaching 100% in treated larvae.

The current study also agreed with 14 in which he recorded the lowest LC₅₀ values to Fiprol among the tested insecticides and that was (0.896) ppm. The reason may be due to its different way of effect as neurotoxins which effect on polarization of neurons. This result is consistent with 37 where they recorded different levels of relative resistance to insecticides tested against red palm weevil.

The results also showed a positive relationship between increasing the efficacy of the pesticide and increasing the exposure time, which agrees with what 27 mentioned.

CONCLUSIONS

This study concludes that the Fiprol insecticide was the most effective against red palm weevil larvae and treatment by feeding was better than dipping . Therefore, it is recommended to inject the trunk of date palm tree by the Fiprol insecticide alternately with the Dueracide which have different mode of action to avoid the resistance of red palm weevil to these groups of pesticides to control the weevil.

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