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Original research

Formaldehyde's Insecticidal Potential on Immature Stages of Red Palm Weevil (*Rhynchophorus ferrugineus*)

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Abstract:

Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is the main date-injury palm insect in Egypt. This insect became the most destructive pest for date palms. All research seeks out safe methods for controlling this pest. This study aims to use eco-friendly and safer methods to manage R.P.W. However, the effect of Formaldehyde needs more research. The phrase "formalin" refers to the gas formic aldehyde, CH_2O, which is a 40% solution of Formaldehyde in water. The results showed that concentration (40%) had a significant impact on larvae in their second instar and newly hatched red palm weevil among all concentrations after exposure to a toxic zone for ten days. The results revealed that there was an increase in larvae mortality by increasing the duration of exposure to Formalin, and this may depend on the accumulation of Formalin on target organs. Data reported that Formalin is effective for the larvae. Therefore, Formalin could interrupt the life cycle of red palm weevil and might be suitable for integrated pest management of this pest as a protective or curative agent. In terms of LC_{50} and $LC_{90,}$ the relative toxicity of the tested concentrations at the LC_{50} and LC_{90} levels showed that the toxicity of Formalin on newly hatching was the most effective among other larvae of red palm weevil. Regarding LT_{50} and LT_{90} values, formalin application demonstrated great activity against *R. ferruginous*, and the needed time causing 50 and 95% mortality (LT_{50} and LT_{90}) decreased when the formalin concentration increased. Also, exposure of red palm weevil to ascending concentrations of Formalin showed a significant effect among all concentrations ($p>0.05$) of Formalin (40, 20, 10, 5 and 2.5 %) when used on larvae of the fourth instar. In comparison to control, the total lipids and total carbs dropped. As for total lipid and total carbohydrate content, it showed a detectable decrease in treated larvae, while other biochemical components (total protein) showed a slight or no decrease. In the field application of (Formalin) on palm trees for *R. ferrugineus* control, the results concluded that the infection began to stop seven days after treatment of formalin concentrations (40% and 20%). Also, the treated date palm trees manifested complete recovery after 30 days of the treatment.

Keywords: *Rhynchophorus ferrugineus*, Insecticidal activity, Formalin bioactive components

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1- Introduction

The date palm (*Phoenix dactylifera* L.), which is widely grown in Egypt and other Arab and Asian nations for its millions of tons of fruit goods as well as other well-known wonderful resources like fiber, fuel and furniture, is regarded as an economically significant crop in the Middle East and Arabian countries' most significant ornamental plant and fruit crop is the date palm (**FAO, 2019; Moursy and Saker, 1996**).

Date palms in Egypt and the world face various pests that affect date palms. The red palm weevil is one of the worst pests ever to affect palm plants in the Middle East and North Africa, *R. ferrugineus* (Olivier). Since its discovery in Egypt in 1993, the weevil has spread throughout the palm-growing region **(Murphy and Briscoe, 1999)**. The larva stage of the bug, which attacks palm tree trunks and bores tunnels into them until they reach the palm's head, is the detrimental stage that results in the death of the palm **(El-Mergawy and Al-Ajlan, 2011)**.

When the grubs are buried, and there are no obvious symptoms until it is too late, an infestation could go undetected for a while. The huge issue persists, and there is no obvious indication that it can be effectively controlled, leaving the probability of the date uncertain, risky palm trees in Egypt. When a pest is detected early, the palms can be saved, but early detection is difficult because the symptoms appear late, and this makes it difficult to control the palm weevil **(Faleiro *et al.*, 1998 and 1999)**. Insecticides are administered in a variety of preventive and remedial treatments meant to limit the infestation. There are numerous studies connected to reducing and controlling the damage caused by palm weevils at the moment **(Abuzuhairah *et al.*, 1996)**. Chemical treatments for the control of *R. ferrugineus* have recently been the focus of studies on integrated pest management. What you want to alter should go here. After that, click the button below **(Abraham *et al.*, 1998)**. The chemicals currently in use in the field were initially produced through laboratory research on potential compounds. In addition to other approaches, Formaldehyde is thought to be beneficial in controlling this pest, especially in reducing the usage of toxic chemical pesticides. As a result, this research allows for both laboratory and field evaluations of Formaldehyde's effectiveness against red palm weevil larvae.

The current study's objective is to assess Formaldehyde's biological activity on newly hatched, 2^{nd}, 4^{th}, 6^{th} and 11^{th} larvae employing the dip-food procedure in the laboratory for *R. ferrugineus*.

2. Materials and Methods

2.1. Insect rearing

After pruning the injury areas on infected palm trees in the Edfu district of the Aswan Governorate in 2021, several stages of *R. ferrugineus* were manually collected. Weevils were easier to remove from palm trees that had been badly infested using a portable wood saw to keep insects from escaping during transit, precautions were taken, and conditions were limited. For this, 500 cc glass jars with circular ventilation holes in the lids were employed. The adults who were collected served as the initial samples for raising. The process of raising *R. ferrugineus* (at 27±2ºC and 70±2 R.H. %).

Additionally, the photoperiod was roughly 12: 12 L/D and was carried out in the insectary of insect biology, Plant Protection Department, Faculty of Agriculture, Aswan University. The main culture of *R. ferrugineus* was reared on a semi-artificial diet as substantive by **(El-Zoghby and Abdel-Hameid, 2018).** Natural diets are designed for *R. ferrugineus* bulk raising due to the availability of sugarcane in Upper Egypt in cultivating this bug. Diets were also created to prevent the usage of pricey artificial diets for the culture of weevils. The field-collected adult weevils were maintained in pairs in plastic cups (6x7 cm) filled with sugarcane pieces, which served as the female weevils' oviposition sites. When needed, all plastic containers were kept at room temperature. For egg acquisition, larvae were put on diets and daily given 10 cm long strips of reed. The deposited eggs were collected and kept under similar circumstances in Petri dishes (9 cm in diameter) with wet filter paper until hatching in order to produce the larvae used in the research. After that, each larva was put into a tiny plastic container and fed sugarcane clippings until it reached the pupal stage. Larval stages were used in bioassay experiments.

2.2. Bioassay experiments by Formaldehyde on larvae of *R. ferrugineus*:

To test the efficacy of formaldehyde dilutions, laboratory tests were carried out utilizing the dipping food bioassay method **(Ajlan *et al.*, 2000).**

2.3. Larvicidal activity:

To examine the efficiency of the commercial formulation of Formaldehyde on red palm weevil, 5^{th} instar larvae (newly hatching, 2^{nd}, 4^{th}, 6^{th} and 11^{th}) were treated with five concentrations [40, 20, 10, 5 and 2.5%]. Treated larvae and the control were inspected after 2, 5, 7 and 10 days. Using reed pieces, 1^{st} instar larvae were evaluated as follows: The stems of the reeds were divided into 8 cm long slices and submerged for 1 minute in various Formaldehyde concentrations. The larvae were exposed to the treated sugarcane pieces. Ten starving larvae were fed treated pieces at a rate of two pieces per petri dish of treated pieces, which were arranged underneath filter paper. Triplicates from each concentration were done. As a control, identical sections of sugarcane stem were soaked in distilled water. After 24 hours from exposure, insect stages were evaluated, and the percentages of mortality that displayed symptoms of inactivity were tracked and recorded. It was noted the insects were dead.

LC_{50} values with their 95% fiducial limits, as well as regression equations of normal equivalent deviates (y) versus log concentration (x), was estimated according to **Finney (1971)**. Relative toxicity (L.T.) of the insecticide based on LC_{50} and LC_{90} levels was also estimated. LT_{50} and LT_{90} for Formalin were recorded.

2.4. Field Study

Treatments were completed upon infections in the Kom Ombo region in the Governorate of Aswan in 2020, where four replicates of an infested date palm tree each were randomly chosen. A red palm weevil detection tool and optical detection were used to identify the infected palm for infection-related purposes. Four palms per concentration formalin injections were used to treat infected trees, and the following treatment procedures have been used:

2.5. Tested pesticides in the field.

- For each treatment, four replicates (4 palm trees) were found. The cultivar (Zaghlul) was chosen at random from palm plantations that had red date palms (*R. ferrugineus*) invaded them in the Kom Ombo district, Aswan Governorate, in 2020.
- To test the effectiveness of the Formalin after 14, 21 and 30 days, three holes were drilled around the site of the infection area 10 cm away from the injury area using the injection device (Plate, 2), a large iron pin and plastic piping (Plate, 1).

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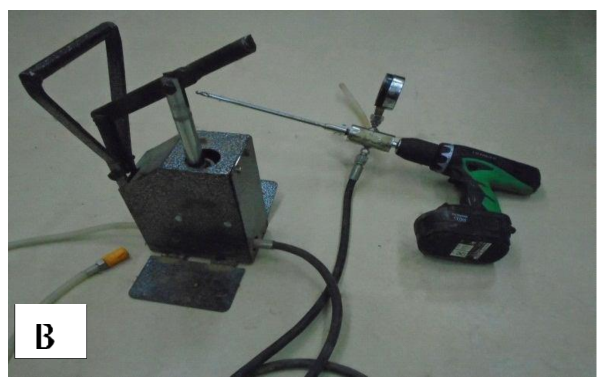
A **B** **C**



D **E** **F**

Plate 1: (A, B, and C), Early infection with red palm weevil (oozing of brownish Fluid). (D, E, and F), Filling the injection hole with a piece of fiber and mud after the injection.



Plate, 2. (A) Red palm weevil detection device. (B) Palm injection device.

- Each replication was performed three times using conidia of Formalin 40% commercial product; the concentration of formulation used was (40, 20, 10, 5 and 0%) in each injury. The same procedure was repeated a week after the first treatment.
- One liter of the used concentration was injected into each palm at a time (**Abdel-Salam *et al*., 2014**).
- Clay and fiber were used to fill the injection holes **(Abbas, 2013).**
- After 14, 21 and 30 days, the treated palm was evaluated to determine the extent of the harm according to **Abbas (2013); Kaakeh (2006)**, after the first application date, evaluated the Formalin used based on the disappearance of the obvious signs of infection by skimming and cleaning the afflicted areas, noting the dead *R. ferrugineus* individuals in all stages:
    1. The oozing substance dried up and ceased **(Abbas, 2013; Abdel-Salam *et al*., 2014**)

2. Dry tunnels.

Only 1 liter of water was used to treat the control group **(Abdel-Salam *et al.*, 2014)**. The Zaghloul cultivar, with ages ranging from 4 to 15, lengths of 1 to 4 meters and widths of 30 to 60 cm, underwent the treatments. Additionally, the injuries' height from the ground ranged from 20 to 140 cm.

2.6. Experimental design

It was a complete randomization design. The experimental units utilized were uniform in size and age and repeated, and coefficients were allocated at random among them.

2.7. Effect of Formalin on some biochemical parameters in red palm weevil.

The fourth instar larval was chosen to evaluate the biochemical activity of red palm weevil (total lipids, total proteins and total carbohydrates) under laboratory conditions. One larva per small (4x4 centimeter) plastic cup was used to house the larvae. The samples of treated larvae were taken and various analyses were performed in comparison to the control using the following concentrations: 40, 20, 10, 5 and 2.5%. Larval samples were collected for biochemical examination.

2.8. Methods of biochemical assays:

2.8.1. Chemicals used:

The Stanbio laboratory (Texas, U.S.A.) provided the bovine albumin standard that was purchased. Sigma (Sigma Chemicals Co.) produced Commasie Brilliant Blue G-250. *p*-nitroanisole, which has a purity of 97%, was provided by Ubichem Ltd. in Hampshire, whereas B.D.H. Chemicals Ltd. in Poole, England, provided the reduced form of NADPH. The other chemicals were of a high caliber and were bought from reputable local businesses.

2.8.2. Apparatus:

Red palm weevil larvae were homogenized for biochemical examination using a glass Teflon tissue homogenizer (S.T. - 2 Mechanic - Preczyina, Poland). Supernatants were maintained after homogenization until they were needed for biochemical experiments in a deep freezer at -20°C. Spectronic 1201, manufactured by Milton Roy Co. in the U.S.A., is a double-beam ultraviolet/visible spectrophotometer that was used to measure the absorbance of colored chemicals or metabolic products.

2.8.3. Preparation of insects for analysis:

The preparation of the insects followed the instructions described by **Amin (1998)**. The insects were homogenized in distilled water (50 mg/ml) and centrifuged using a chilled centrifuge at 8000 rpm for 15 min at 2°C. The enzyme extract supernatants were then kept at 50°C in a refrigerator.

2.9. Biochemical assayed:

2.9.1. Determination of total lipids:

Using the technique described by **Knight *et al.* (1972)**, the total lipids were calculated.

2.9.2. Total proteins:

The **Bradford (1976)** method was used to determine the total amount of proteins.

2.9.3. Determination of total carbohydrates:

By using the phenol-sulphuric acid reaction, the total carbohydrates in the sample's acid extract were calculated (**Dubois *et al.*, 1956**). In accordance with **Crompton and Birt (1967)**, total carbohydrates were isolated and prepared for analysis.

2.10. Statistical analysis.

Using SPSS, ver. 27 (I.B.M. Corp. Released 2013), two-way ANOVA was used for the statistical analysis. **Steel *et al.* (1997)** handled the data as a complete randomization design. The cutoff for significance was fixed at 0.05.

3. Results and Discussion

3.1. Larvicidal activity

The effect of Formalin on larvae of red palm weevil after 2, 5, 7 and 10 days of feeding on sugarcane treatment with different concentrations (40, 20, 10, 5 and 2.5%) formalin is presented in Table (1), the outcomes showed that every concentration was efficient in killing the larvae. After ten days, concentration (40%) had the greatest impact on fresh hatching and second larvae; the mortality to the larvae was (100.00±0.00 and 100.00±0.00), respectively, after ten days of exposure, while other concentrations showed low to moderate effect (Table, 1).

Table (1): Effect of laboratory treatments of the Formalin on larvae of the Red Palm Weevil, *R. ferrugineus* Olivier.

	Conc. (%)	Period (days)				Mean
		2	5	7	10	
Newly hatching	40	93.33±3.33^{ab}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	98.33±1.12^{a}
	20	93.33±3.33^{ab}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	98.33±1.12^{a}
	10	90.00±0.00^{b}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	97.5±1.31^{a}
	5	33.33±6.67^{c}	73.33±6.67^{b}	96.67±3.33^{b}	96.67±3.33^{a}	75.00±8.12^{b}
	2.5	21.67±1.67^{d}	41.67±1.67^{c}	61.67±1.67c	78.33±1.67^{b}	50.83±6.45^{c}
	Mean	66.33±8.65^{D}	83.00±6.28^{C}	91.67±4.07^{B}	95.00±2.34^{A}	
2nd	40	80.00±0.00^{ab}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	95.00±2.61^{a}
	20	70.00±5.77^{bc}	96.67±3.33^{b}	100.00±0.00^{a}	100.00±0.00^{a}	91.67±4.05^{b}
	10	40.00±0.00^{cd}	60.00±0.00^{c}	80.00±0.00^{b}	85.00±0.00^{b}	66.25±5.37^{c}
	5	23.33±3.33^{d}	36.67±3.33^{d}	50.00±0.00^{c}	70.00±0.00^{c}	45.00±5.29^{d}
	2.5	11.67±1.67^{e}	21.67±1.67^{e}	36.67±3.33^{d}	40.00±0.00^{d}	27.5±3.56^{e}
	Mean	45.00±7.12^{D}	63.00±8.42^{C}	73.33±6.95^{B}	79.00±6.00^{A}	
4th	40	80.00±0.00^{a}	93.33±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}	91.67±2.41^{a}
	20	66.67±6.67^{b}	83.33±8.82^{b}	90.00±7.64^{b}	90.00±7.64^{b}	82.50±4.37^{b}
	10	56.67±3.33^{c}	73.33±6.67^{c}	90.00±10.00^{b}	90.00±10.00^{b}	77.50±5.38^{c}
	5	20.00±0.00^{d}	40.00±0.00^{d}	48.33±1.67^{c}	48.33±1.67^{c}	39.17±3.53^{d}
	2.5	13.33±1.67^{e}	23.33±1.67^{e}	31.67±1.67^{d}	31.67±1.67^{d}	25.00±2.38^{e}
	Mean	47.33±7.12^{C}	62.67±7.38^{B}	71.33±7.36^{A}	71.33±7.36^{A}	
6th	40	73.33±3.33^{ab}	93.33±3.33a	93.33±3.33a	93.33±3.33^{a}	88.33±2.97^{a}
	20	53.33±8.82^{b}	86.67±3.33^{b}	86.67±3.33^{b}	86.67±3.33^{b}	78.33±4.90^{b}
	10	10.00±0.00^{d}	30.00±0.00^{c}	50.00±0.00	60.00±0.00^{c}	37.50±5.79^{c}
	5	20.00±0.00^{c}	20.00±0.00^{d}	40.00±0.00^{d}	51.67±1.67^{d}	32.92±4.10^{d}
	2.5	6.67±1.67^{e}	26.67±1.67^{e}	35.00±5.77^{e}	38.33±7.26^{e}	26.67±4.23e
	Mean	32.67±7.19^{D}	51.33±8.54^{C}	61.00±6.60^{B}	66.00±5.78^{A}	
11th	40	46.67±3.33^{a}	61.67±1.67^{a}	71.67±1.67^{a}	71.67±1.67^{a}	62.92±3.23^{a}
	20	30.00±0.00^{b}	43.33±3.33^{b}	63.33±3.33^{b}	63.33±3.33^{b}	50.00±4.44^{b}
	10	3.33±1.67^{c}	23.33±1.67^{c}	43.33±1.67^{c}	53.33±1.67^{c}	30.83±5.83^{c}
	5	3.33±1.67^{c}	6.67±3.33^{d}	16.67±3.33^{d}	21.67±3.33^{d}	12.08±2.57^{d}
	2.5	0.00±0.00^{d}	3.33±1.67^{e}	8.33±1.67^{e}	13.33±1.67^{e}	6.25±1.64^{e}
	Mean	16.67±4.99^{C}	27.67±5.99^{B}	40.67±6.72^{A}	44.67±6.24^{A}	

L.S.D. at 0.01	Conc.	Period	Group	C*P	C*G	P*G	C*P*G
	2.08	2.08	1.86	4.17	4.66	4.17	9.32

a, b & c: There is no significant difference (P>0.01) between any two means within the same column that have the same superscripted letters.

The relative toxicity of the quantities evaluated at the LC_{50} and LC_{90} levels revealed that Formalin's toxicity on newly hatching was the most effective among other red palm weevil larvae, at 2.35 and 8.61 mg/100 ml, respectively, followed by 2^{nd}, 4^{th} and 6^{th} (5.41, 5.78 and 8.73; 23.64, 29.20 and 55.96 mg/100 ml), respectively, (Table, 2).

Table (2): Lethal concentrations (L.C.'s, LC_{50} & LC_{90}) and toxicity index of different concentrations of Formalin against *R. ferruginous* at 27 ± 2 ºC and 70 ± 2 R.H%.

	LC_{50}	LC_{90}	Slope	P-value	R (Tab. 0.878)
Newly hatching	2.35	8.61	2.2750±0.2644	0.0076	0.9203
2^{nd}	5.41	23.64	2.0006±0.1750	0.3494	0.9876
4^{th}	5.78	29.20	1.8211±0.1591	0.0598	0.9745
6^{th}	8.73	55.96	1.5879±0.1520	0.0007	0.9432
11^{th}	22.44	139.50	1.6164±0.1631	0.6725	0.9938

Data of 11^{th} old larva instar was the less effective; LC_{50} and LC_{90}'s (22.44 and 139.50 mg/100 ml) were about ten times of the newly hatching larva. The data in Table (1) revealed that the larvae mortality had insignificant effects between all concentrations means ($P<0.01$) of Formalin (40, 20 and 10 %) when applied to the newly hatching larva. In contrast, there was a significant decrease between all concentrations ($p \geq 0.01$) of Formalin (40, 20 and 10) compared with 5 and 2.5 % concentrations.

When Formalin was exposed to larvae for a period of 2 to 10 days, the toxicity of the substance increased. The findings showed that lengthening the formalin exposure duration significantly increased the mortality of the larvae. Additionally, the percentage of larval mortality showed that the lack of Formalin had a considerable negative impact on all other larval instars. In the meantime, the results exhibited that the young larvae were more susceptible than the older larvae to Formalin. The *R. ferruginous* small ages (newly hatching, 2^{nd} and 4th instar larvae) were also reported to be more susceptible to concentrations than the later ages (6^{th} and 11^{th} instar larvae), i.e., mortality% decreased with increasing larval age from 2^{nd} instar until 11^{th} instar.

3.2. The estimated lethal times (L.T.s) for treatments with Formalin against *R. ferruginous* at 27±2ºC and 70±2 R.H%.

To study the efficiency of the commercial formulation of Formaldehyde to control, fifth instar larvae (newly hatching, 2^{nd}, 4^{th}, 6^{th} and 11^{th}) were treated with five concentrations (40, 20, 10, 5 and 2.5%). The LT_{50} and LT_{90} values were tested and estimated, as reported in (Table, 3). Formalin application exhibited a strong effect on *R. ferruginous*, and the LT_{50} and LT_{90} were reduced with increasing the concentration of the Formalin (Table, 3).

Table (3): Lethal times (L.T.'s, LT_{50} & LT_{90}) values estimated at different concentrations of Formalin for the tested instars of *R. ferruginous* at 27 ± 2 ºC and 70 ± 2 R.H%.

Stage	Conc. (%)	LT_{50}	LT_{90}	Slope	P-value	R (Tab. 0.95)
Newly	20	0.70	1.77	3.1604±11494	0.6927	0.9121
	10	0.86	1.99	3.4989±1.1535	0.5672	0.9121
	5	2.78	6.63	3.3940±0.3147	0.0259	0.9611
	2.5	5.09	19.54	2.1943±0.2713	0.1416	0.9747
2^{nd}	20	1.44	3.26	3.5999±0.5475	0.6842	0.9687
	10	2.92	14.07	1.8770±0.2602	0.1992	0.9731
	5	6.25	35.40	1.7010±0.2640	0.0905	0.9504
	2.5	14.47	114.62	1.4261±0.2906	0.4488	0.9737

Stage	Conc. (%)	LT_{50}	LT_{90}	Slope	P-value	R (Tab. 0.95)
4th	20	0.93	8.55	1.3276±0.2818	0.6893	0.9775
	10	1.67	9.58	1.6904±0.2723	0.1545	0.9496
	5	5.28	17.09	2.5116±0.2834	0	0.8838
	2.5	27.03	72.97	0.9663±0.2818	0.6953	0.9768
6th	20	1.57	9.39	1.6526±0.2735	0.0601	0.9121
	10	7.64	28.00	2.2723±0.2994	0.5528	0.9926
	5	11.82	115.22	1.2962±0.2720	0.0135	0.8403
	2.5	13.01	73.03	1.7108±0.3068	0.4222	0.9818
11th	40	2.38	43.84	1.0129±0.2478	0.6714	0.9755
	20	5.18	46.72	1.3417±0.2522	0.2028	0.9504
	10	8.83	25.45	2.7866±0.3605	0.5464	0.9955
	5	30.42	182.29	1.6462±0.4079	0.4278	09561
	2.5	26.68	86.54	2.5077±0.7100	0.8477	0.9955

When larvae of *R. ferruginous* were treated with Formalin at different concentrations, the LT_{50} at concentration 20% were 0.70, 1.44, 0.93, 1.57 and 5.18 days for larval instars (newly hatching, 2nd, 4th, 6th and 11th), respectively and, these values at concentration 2.5% recorded 5.09, 14.47, 27.03, 13.01 and 26.68 days for the same fourth larval instars of red palm weevil, respectively. LT_{90} values, on the other hand, were 1.77, 3.26, 8.55, 9.39 and 43.84 days for the larval instars (newly hatching, 2nd, 4th, 6th and 11th), respectively, after treating with Formalin at 20% concentration. Also, these values were 19.54, 114.62, 72.97, 73.03 and 86.54 days for the same fourth larval instars, respectively, after treating with Formalin 2.5% concentration. These results clearly indicated that the older instars of red palm weevil, *R. ferruginous*, were more tolerant to toxicity by Formalin at low concentrations (at 27±2ºC and 70±2 R.H.%) than the younger instars, which were more susceptible.

3.3. Formalin is applied in the field to palm plants to suppress *R. ferruginous*

The results of the current study presented in Table (4) could be concluded that seven days after the formalin concentrations were treated, the illness started to subside (40% and 20%). Also, the treated date palm trees manifested complete recovery after 30 days of the treatment. No damage to the palm trees occurred after three months of treatment with Formalin. The palms were examined and dead individuals were found.

Table (4): Field treatments of date palm trees with Formalin at the concentration at Al Kom Ombo **region in the Aswan Governorate in 2020 year:**

Date of inspection (day)	The period after application/days														
	40%			20%			10%			5%			Control		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
	Tree (5 Holes)			Tree (5 Holes)			Tree (5 Holes)			Tree (5 Holes)			Tree (5 Holes)		
7	D	D	D	D	D	D	D	X	X	X	X	X	X	X	X
14								X	X	X	X	X	X	X	X
21								X	X	X	X	X	X	X	X
30								X	X	X	X	X	X	X	X

N.b: X= still infected and D = recovery (Dry)

The toxicity of Formalin increased with increasing time of exposure. This toxicity could be related to the concentration injected by insects sufficient to inhibit biochemical targets. There may be further advantages if Formalin is employed in integrated pest management strategies to control pests. Soaking into the damaged areas of the palm may offer a practical approach to get rid of some palm pests. This form of treatment may also have additional advantages **(Howard and Stopek, 1999)**. A field experiment was conducted in 2008 and 2009 by **Sewify *et al.* (2014)** to evaluate the combined efficiency of entomopathogenic fungus *B. bassiana* or pesticide *B. bassiana* for controlling *R. ferrugineus* in the Aswan Governorate of Egypt. They discovered that the total mean decline in *R. ferrugineus* population brought on *B. bassiana*, a fungus or insecticide, was mass trapped at 61.40 and 40.16%, respectively.

3.4. Evaluation of biochemical elements in red palm weevil larvae following treatment with various formalin concentrations.

Results in Table (5) showed that exposure of larvae to increasing levels of Formalin had a substantial impact across all concentrations ($P \geq 0.05$) of Formalin (40, 20, 10, 5 and 2.5 %) when applied on 4th larval instar. Total lipids and carbohydrate levels dropped, when compared with those obtained in the control. As for total lipid and total carbohydrate content, it showed a detectable decrease in treated larvae, while other biochemical components **(**total protein) showed a slight or no decrease.

The data in Table (5) revealed that the total lipids had a significant decrease by increasing formalin concentration compared with the control, with the highest value for the control (706.08 mg/ml). In comparison, the lowest value was (493.99 mg/ml). On the contrary, total proteins had a significant increase by increasing formalin concentration used compared with control. In addition, total proteins had a significant decrease by formalin conc. Deficiency applied on larvae, the least value for control (195.20 mg/ml), while the highest value was (322.00 mg/ml) when applied by 5% formalin. Total carbohydrates had a significant decrease by increasing formalin concentration used, with the highest value for control (489.91mg/ml), while the least value was (305.28 mg/ml) when applied by 40 % formalin.

Table (5): Effect of Formalin of the larval instars (newly hatching, 2nd, 4th, 6th and 11th) of red palm weevil on the number of biochemical components (mean ± S.E.).

Treatment	Concentration (%)	Total lipids (mg/ml)	Total protein (mg protein/ml)	Total carbohydrate (mg glucose/ml)
1	40	493.99±15.01^{d}	289.41±7.37^{b}	305.28±8.77^{c}
2	20	532.18±16.72^{cd}	272.88±3.93^{b}	319.92±9.19^{c}
3	10	557.72±17.45^{c}	201.42±5.48^{c}	459.89±13.22^{ab}
4	5	647.51±25.19^{b}	322.00±13.94^{a}	335.44±9.64^{c}
5	2.5	705.97±21.89^{a}	215.54±7.85^{c}	441.72±12.69^{b}
6	0 (Control)	706.08±10.01^{a}	195.20±6.61^{c}	489.91±14.08^{a}
LSD at 0.05 for		56.59	25.14	35.33

As shown in Table (5), after treating with descending concentrations of Formalin, enzyme rates were estimated, with the exception of total protein. All enzyme rates gradually increased in all larvae. The rate of total lipids and total carbs increased over time. In this scale, **Ragheb *et al.* (2018)** discovered that the total protein in the fourth larval instar of the red palm weevil significantly decreased ($P \geq 0.05$) between days 5 and 7 after being treated by an entomopathogenic fungus. In contrast, the total lipid significantly increased between days 3 and 7 compared to the control.

The use of many of these pesticides has been constrained due to their adverse environmental effects **(Gush, 1997)**. Currently, biocides are being created, and integrated pest management (IBM), which includes surveillance, pheromone lures, cultural control and chemical treatments, is being employed to manage *R. ferrugineus*. (**Moura *et al.*, 1995**; **Abraham *et al*., 1998** and **Kaakeh, 2010**). For the control of the red palm weevil, integrated pest management (IBM) uses Formalin, a contact poison with broad spectrum activity, long-lasting efficacy, and a different mode of action from pesticides. It also has low toxicity to mammals (**Cox, 2001**; **Kaakeh, 2010**).

4. Conclusion

Overall, the findings demonstrated that after ten days of exposure to the poisoned medium, concentration (40%) had the greatest impact on fresh hatching and second larval instars of the red palm weevil than other concentrations. The toxicity of larvae increased with exposure time from 2 to 10 days and there was a noticeable increase in larval mortality with longer exposure times. The amount of Formalin ingested by insects may be sufficient to suppress the biochemical targets. As a result, Formalin is effective against the red palm weevil larvae, which means it could disrupt the pest's life cycle and be included in management programs as a preventative or remedial measure. The toxicity of Formalin on freshly hatched was the most effective among other red palm weevil larvae, according to the relative toxicity of the measured concentrations at the LC_{50} and LC_{90} levels.

Regarding the LT_{50} and LT_{90} values, formalin application demonstrated a robust activity against *R. ferruginous*, and the LT_{50} and LT_{90} values showed that the time required to produce 50 and 95% death (decreased with increasing formalin concentration). The findings showed that the red palm weevil was significantly affected by all formalin concentrations (40, 20, 10, 5 and 2.5%) when exposed to them when administered to larvae in their fourth instar ($P\geq0.05$). In comparison to those found in the control, the total lipids and total carbohydrates decreased. Total lipid and total carbohydrate content both decreased significantly in treated larvae, but total protein content only decreased little or not at all. After exposure to declining formalin concentrations, there was a highly noticeable rise in total lipids and total carbohydrates in the fourth larval instar that was statistically significant ($P\geq0.05$). The findings demonstrated that treatment of larvae with Formalin at LC_{50} caused a statistically significant ($P\geq0.05$) decrease in the total protein content of the fourth larval instar. The 4^{th} instar larvae treated with LC_{50} of Formalin, on the other hand, showed a substantial ($P\geq0.05$) decrease in total proteins, according to the data. The findings of a field application of (Formalin) on palm trees for *R. ferruginous* control showed that seven days after treatment with formalin concentrations (40% and 20%), the infection started to halt. Additionally, the treated date palm plants showed full recovery 30 days following the treatment. Last but not least, Formalin is a reliable integrated pest management tool for managing *R. ferrugineus* since it has an efficient ingredient against larvae.

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