Starvation effect on bioactive components of the red palm weevil *Rhynchophorus ferrugineus* (Olivier); (Coleoptera : Curculionidae)

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**ABSTRACT**

*Rhynchophorus ferrugineus* (Olivier) (Coleoptera : Curculionidae) is nowadays considered the biggest threat to palm trees worldwide. Due to the current trend towards the use of environmentally-friendly control measures, increasing interest is being shown in trapping as a way of dealing with this pest. The present study assessed the influence of starvation on the red palm weevil bioactive components. The obtained results showed that *R. ferrugineus* larvae were more affected by starvation with the rate of 31.82 - 50.00 % and 34.91 & 48.65 % reduction, while the rate of reduction in adults were 5.60 & 22.40 % and 40.67 & 46.00 % in total proteins and carbohydrates after 48 and 120 h., respectively. It was, also noticed that larvae could not renew their activity again after feeding them again after 5 days of starvation. Data also showed that, the ability of *R. ferrugineus* adults to survive in face of adverse conditions is very strong. The physiological underpinnings of bio-chemical components and enzymes activity in face of starvation could be useful to assist in pest control. This study provides useful information for improving current management strategies against *R. ferrugineus*. Knowledge about the effect of starvation on the insect’s bioactive components may help for improving the applied control methods to decrease the damage caused by this insect.

**Key words:** Starvation, Red palm weevil, bioactive components

**Introduction**

Date palm is the most common and widely cultivated plant in the arid regions of the Middle East and North Africa where, in many areas, its fruits provided the staple carbohydrate food of people for nearly 5000 years (Purseglove, 1972 and Jones, 1995). The date palm crop in these countries now is under threat for the direct attack by the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera : Curculionidae) which is, fairly, considered one of the most invasive and destructive palm tree pests worldwide (Faleiro, 2006). Larvae feed inside the trunk and frequently destroy the apical growth area, causing death of the palm trees (Murphy and Briscoe, 1999). Infestations are usually detected after the palm tree has been seriously damaged (Blumberg, 2008), and only well-trained technicians can detect early symptomatology, so that numbers of plants are lost and the consequent financial impact is considerable. The pest has a broad range of hosts and is able to breed in a wide variety of climatic conditions (Murphy and Briscoe, 1999), thus increase its invasive ability and potential for damage. The presented investigation was conducted to find out the effect of starvation on different bioactive components and also the effect of starvation periods on the ability of the insect to move from one place to another under starvation conditions. Knowledge about the effect of starvation on the insect’s bioactive components may help for improving the applied control methods to decrease the damage caused by this pest.

**Materials and Methods**

Samples of male and female adults of the red palm weevil (*R. ferrugineus*) were collected from Qassasin, Ismailia Governorate. The collected adults provided as the initial samples for rearing. The rearing process was carried out in a rearing room with the means of 28±1°C and 75±5% R.H. and the photoperiod was approximately 12 : 12 L/D.

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The sample of adult weevils collected from the field were kept as groups of paired males and females in plastic cups (6x7 cm) supplied with sugarcane slices. Female weevils used the sugarcane slices as oviposition sites.

The larvae were fed on sugarcane small cuttings until being transferred to the pupal stage. Larvae made their cocoons from the fibers of sugarcane residues after feeding. Cocoons were placed in a plastic container, sprayed with a mist of water as needed. Cocoons were checked daily until adults emergence. Adults were handly picked and placed in plastic cups (6x7 cm). Red Palm weevil was successfully reared in the laboratory on stem cuttings as sugarcane is one of the natural food of the *R. ferrugineus*.

**Starvation treatments:**

Freshly formed last instar larvae and freshly emerged adults were kept starved in the mentioned containers. Starvation periods of 48, 72 and 120 hours were used to study their effects on biochemical contents of insect compared with control.

**Methods of biochemical assays:**

A group of larvae and adults were placed in small plastic cups (4 x4 cm), a single larva and adult in each cup. The samples of starved larvae and adults were taken at 0 time and after 48, 72 and 120 hours, and the different analyses were carried out compared with the control. The larvae and adults life period were also recorded after starvation. The adults were kept starved for 5 days, after which those were fed on sugarcane small cuttings for 24, 48, 72 and 120 hours, then samples of adults were taken for biochemical analysis.

**Chemicals used:**

Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Commasie brilliant blue G-250 was from sigma (Sigma chemicals co.). P- nitroanisole (purity 97%) was obtained from Ubichem Ltd.(Ham pshire), while nicotinamide ademine dinucleotide phosphate (reduced form, NADPH) was from BDH chemicals Ltd.(Poole, England). The rest of chemicals were of high quality and purchased from commercial local companies.

**Apparatus:**

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST – 2 Mechanic - Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at -20 °C till used for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

**Preparation of insects for analysis:**

The insects were prepared as described by Amin (1998). Those were homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 °C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, could be stored for at least one week without appreciable loss of activity when stored at 50 °C.

**Biochemical assayed:**

**1-Lipase:**

Lipase was determined colorimetrically by spectrum diagnostic kit (www.spectrum-diagnostics.com). A synthetic substrate (DGMRE) was splitted by lipase to yield the colored final
product methylresorufin. The increasing absorbance of the red methylresorufin was measured photometrically at 578 against air.

2-Determination of Trehalose activity

Digestive enzymes were determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976) using trehalose, sucrose, and soluble starch as substrates for determination of trehalose, invertase and α-amylase, respectively.

3-Determination of total lipids:

Total lipids were estimated by the method of Knight et al. (1972) using phosphor vanillin reagent.

4-Determination of total carbohydrates:

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois et al. (1956).

Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

5-Triglycerides determination:

Triglycerides were assayed using Stanbio kit (Stanbio Laboratory, Inc. 2930 East Houston street, San Antonio, Texas 78202). Triglycerides area was generally determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of glycerol released. Glycerol is then phosphorylated to glycerol-3-phosphate.

6-Total proteins:

Total proteins were determined by the method of Bradford (1976).

7-Transaminases determination:

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to the method of Reitman and Frankle (1957).

Statistical Analysis

All data were analysed by using ANOVA with three factors at 0.05 significance level for the whole results using SPSS (ver. 22). Data were treated as complete randomization design according to Steel et al. (1997). Multiple comparisons of significance were carried out by applying LSD values.

Result and Discussion

Effect of starvation on last larval instar and freshly emerged adults:

Data in Table (1) showed that adults were more resistant to starvation than larvae where the mean period of adults lived after starvation was longer than in larvae recording 7.7 and 6.8 days for adults and larvae, respectively. Starvation make movement and activity being less in adults and larvae, but in case of larvae the size became smaller with darkened colour (Figure 1). After refeeding of starved adults and larvae for five days the individual adults had recovered and became active. Larvae could not restore their activity after being refed again. Also, larvae were more affected by starvation than adults and this could be attributed to the reductions of protein and carbohydrates in
larval body were higher than adults (Table, 3). In this respect, many authors worked on energy metabolism in other animals; however, the synthesis and utilization of trehalose is unique to insect energy metabolism in that the blood sugar in insects is trehalose instead of glucose (Silva et al., 2004 and Tang et al., 2010). Trehalose and glycogen are the key energy sources in insects and are known to play an important role in physiological adaptation (Qin et al., 2012). Glycogen accumulates during growth and is hydrolyzed under conditions of starvation (Johnston and Carlson, 1992).

Table 1: Effect of starvation on the period of lifetime (days) of both larvae and adults

<table>
<thead>
<tr>
<th>Stage</th>
<th>Life time</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>7.7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Larva</td>
<td>6.8</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 1: Freshly emerged adults and freshly formed last instar larvae after different periods of starvation of R. ferrugineus

Table 2: Effect of starvation and refeeding on the weight of both larvae and adults:

<table>
<thead>
<tr>
<th>Starvation periods (hr.)</th>
<th>Larva after starvation</th>
<th>Adult after starvation</th>
<th>Adult after refeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (g)</td>
<td>Change (%)</td>
<td>Weight (g)</td>
</tr>
<tr>
<td>0</td>
<td>6.80 ±0.52a</td>
<td>-</td>
<td>1.47 ±0.26a</td>
</tr>
<tr>
<td>24</td>
<td>6.44 ±0.49a</td>
<td>-5.29</td>
<td>1.37 ±0.26a</td>
</tr>
<tr>
<td>48</td>
<td>6.00 ±0.47a</td>
<td>-11.76</td>
<td>1.31 ±0.27a</td>
</tr>
<tr>
<td>72</td>
<td>5.88 ±0.43a</td>
<td>-13.53</td>
<td>1.15 ±0.28a</td>
</tr>
<tr>
<td>120</td>
<td>5.01 ±0.52b</td>
<td>-26.32</td>
<td>1.00 ±0.28a</td>
</tr>
</tbody>
</table>

Data in Table, 2 indicated that the mean weight of mean weight of larva and adult decreased as a result of starvation, where the percentage of decrease in weight of larva recorded -5.29, -11.76, -13.53 and -26.32, but in adults, the decrease in weight recorded -6.80, -10.88, -21.77 and -31.97% after 24, 48, 72 and 120 h. of starvation, respectively. On the other hand, when adults were refed again after starvation, their weight re increased again and the rate of increase in weight recorded 2.06, 8.25, 21.65 and 26.80 after 24, 48, 72 and 120 h., respectively. The parameter of weight after refeeding was not determined for larvae.

Evaluation of biochemical components in both larvae and adults of red palm weevil after different periods of starvation and refeeding.

As a result from data in Table (3), starvation of the red palm weevil for 48, 72 and 120 h., decreased the total protein, total carbohydrates, total lipids and trehalose, while starvation caused increase in the rate of triglycerides and GPT compared with those recorded in the control in both larvae and adults. As for lipase content, it showed detectable increase in starved larvae, while in adults, it showed slight or no decrease.

In case of larvae, the rate of decrease in total protein, total carbohydrates, total lipids and trehalose ranged from (-31.82 to -50.00 ), (-34.91 to -48.65), (-10.11 to -34.83 ) and (-0.08 to 0.18) respectively.

In case of adults, the rate of decrease in total protein, total carbohydrates, total lipids and trehalose ranged from (-0.08 to 0.21), (-0.11 to 0.18), (-0.10 to 0.06) and (-0.08 to 0.02) respectively.
- 23.51% ) after starvation for 48 and 120 h., respectively. While, the rate of increase in triglycerides, GPT and lipase ranged from (28.36 to 46.27), (1.70 to 10.04) and (26.13 to 56.76%) after 48 and 120 h., respectively.

In case of adults, the rate of decrease in total proteins, total carbohydrates, total lipids and trehalose ranged from (-5.60 to -22.40), (- 40.67 to - 46.00), (- 0.41 to -21.16) and (-2.99 to - 32.63 %), and that of lipase (-1.08 to 0.00) after starvation for 48 and 120 hours, respectively. On contrary, the rate of increase in triglycerides and GPT ranged from (0.81 to 5.65) and (15.60 to 59.34 % ) after 48 and 120 h., respectively. Trehalose plays an important role in energy storage, metabolism, and protection from extreme environmental conditions in insects. It is the main blood sugar in insects, and it can be rapidly used as an energy source whenever needed. To elucidate the mechanisms of the starvation response, the effects of starvation on trehalose activity was followed. The results showed that levels of trehaloses decreased significantly in the first 48 h of starvation. This observation agreed with, Zuo-Kun Shi et al. (2017) who compared trehalose and glycogen contents in response to different starvation periods in the coccinellid beetle Harmonia axyridis. In general, the results showed that both trehalose and glycogen contents decreased as starvation time was prolonged. This results showed also that the highest level of trehalose content observed was 98.21±11.31 mg /g. Larvae and adults which are often under threat of starvation, have a greater capacity to resist starvation by undergoing a suite of physiological changes. In insects, starvation during the two feeding stages has been shown to reduce metabolic rates resulting in an increase in the energy stores of triglycerides (Wang et al., 2016). Also, results indicated that a decrease in the content of trehalose with associated prolongation of starvation period (Schilman and Roces, 2008). The lipids were found to be markedly influenced by starvation, this result is in agreement with Paulina and Alicja (1965), who studied the composition of lipids in the haemolymph of waxmoth larvae during feeding and starvation. Total lipids which represented about 2·4 % of the haemolymph were found to be markedly influenced by starvation. Eylem et al. (2010) found that the lipid, glycogen, and total sugar levels of the parasitoid, Bracon hebetor female were affected by sugar feeding. Bracon hebetor females emerged with high reserves of lipids. In their study, lipid reserves appeared irreplaceable through feeding; those decreased with time even in fed females. Their results, also indicated that a strong relationship existed between the parasitoid’s nutritional state and their utilization of metabolic reserves. Tests of whole females showed that total sugar levels of completely starved and sugar-starved individuals were significantly lower than those fed honey and sucrose. Wyckhuys et al. (2008) and Salee and Shakoori (1986) found that the effect on some enzyme activities after total starvation for 10 days led to a significant reduction in almost all enzyme activities of sixth instar larvae of Tribolium castaneum except amylase which showed an elevated activity.

As shown in Table (4), after starvation of the adults for 5 days, recharge was repeated. Enzymes rates were estimated after 24, 48, 72 and 120 h. A gradual increase in the rates of all enzymes occurred in all adults. There was gradual increase in the rate of total protein, total carbohydrate, total lipids, trehalose, triglycerides, GPT and lipase. In this scale’ Salee and Shakoori (1986) studied on five and 10 days of total starvation led to a drastic loss in the body weight of sixth instar Tribolium castaneum larvae. Refeeding did not cause an unusual increase in the body weight. The glucose and glycogen content decreased significantly after starvation for 5 and 10 days. Lipids, on the other hand, remained unchanged initially but showed decreased levels during an extended period of starvation. Under total deprivation, food energy was, therefore, obtained by mobilization of carbohydrates reserved during the initial stages, and later by mobilization of lipids, as evidenced by their significant decrease during starvation for 10 days.
Table 3: Effect of starvation of the last larval instar and adult of the red palm weevil, *R. ferrugineus*, on the amount of biochemical components

<table>
<thead>
<tr>
<th>Stage</th>
<th>Period (hour)</th>
<th>Total protein</th>
<th>Total carbohydrate</th>
<th>Total lipids</th>
<th>Triglycerides</th>
<th>GPT</th>
<th>Trehalose</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/g</td>
<td>% change</td>
<td>mg/g</td>
<td>% change</td>
<td>mg/g</td>
<td>% change</td>
<td>(μg glucose/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>min/g.b.wt.)</td>
</tr>
<tr>
<td>Starved larvae</td>
<td>0 control</td>
<td>15.40 ±0.77a</td>
<td>-</td>
<td>44.40 ±2.02a</td>
<td>-</td>
<td>69.00 ±3.21a</td>
<td>-</td>
<td>6.70 ±0.37f</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>10.50 ±0.11e</td>
<td>-31.82 ±1.55b</td>
<td>28.90 ±2.60b</td>
<td>-10.11±0.32e</td>
<td>8.60 ±28.36</td>
<td>719 ±23.3b</td>
<td>1.70 ±6.0a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10.35 ±0.44e</td>
<td>-32.79 ±1.86b</td>
<td>27.80 ±2.08b</td>
<td>-13.48 ±0.16e</td>
<td>9.03 ±34.78</td>
<td>738 ±3.2b</td>
<td>4.38 ±3.04b</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>7.70 ±0.36e</td>
<td>-50.00 ±2.15c</td>
<td>22.80 ±3.21c</td>
<td>-34.83 ±0.25d</td>
<td>9.80 ±46.27</td>
<td>778 ±17.6a</td>
<td>10.04 ±31.0c</td>
</tr>
<tr>
<td>Starved adults</td>
<td>0 control</td>
<td>12.50 ±0.5b</td>
<td>-</td>
<td>6.00 ±0.16d</td>
<td>-</td>
<td>24.10 ±1.08d</td>
<td>-</td>
<td>12.40 ±0.18b</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>11.80 ±0.36b</td>
<td>-5.60 ±0.20e</td>
<td>3.56 ±0.07e</td>
<td>-</td>
<td>24.20 ±1.70d</td>
<td>-</td>
<td>12.50 ±0.21abc</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>9.80 ±0.35cd</td>
<td>-21.60 ±0.05e</td>
<td>3.24 ±0.104d</td>
<td>-</td>
<td>22.70 ±0.909e</td>
<td>-</td>
<td>12.90 ±0.22ab</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>9.70 ±0.26cd</td>
<td>-22.40 ±0.09e</td>
<td>5.50 ±0.95c</td>
<td>-21.16 ±0.26a</td>
<td>13.10 ±5.65</td>
<td>674 ±10.0c</td>
<td>59.34 ±22.0h</td>
</tr>
</tbody>
</table>

*a, b & c: There is nonsignificant difference (P>0.05) between any two means having the same superscript small latter in the same column.*

Table 4: Effect of feeding *R. ferrugineus* adults after 5 days starvation on its chemical contents

<table>
<thead>
<tr>
<th>Period (hour) of feeding</th>
<th>Total protein</th>
<th>Total carbohydrate</th>
<th>Total lipids</th>
<th>Triglycerides</th>
<th>GPT</th>
<th>Trehalose</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td>% change</td>
<td>mg/g</td>
<td>% change</td>
<td>mg/g</td>
<td>% change</td>
<td>(μg glucose/ min/g.b.wt.)</td>
</tr>
<tr>
<td>0 control</td>
<td>9.70 ±0.26cd</td>
<td>-</td>
<td>3.50 ±0.09e</td>
<td>-</td>
<td>19.00 ±1.05e</td>
<td>0.00</td>
<td>6.30 ±0.26f</td>
</tr>
<tr>
<td></td>
<td>7.80 ±0.17c</td>
<td>-19.59 ±0.26d</td>
<td>4.60 ±31.43</td>
<td>31.43</td>
<td>19.00 ±1.05e</td>
<td>0.00</td>
<td>6.30 ±0.26f</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>8.90 ±0.38 d</td>
<td>-8.25 ±0.50d</td>
<td>60.00</td>
<td>20.00 ±5.26</td>
<td>5.26</td>
<td>8.60 ±15.1f</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>9.60 ±0.14 ed</td>
<td>-1.03 ±0.20d</td>
<td>45.71</td>
<td>20.00 ±11.5e</td>
<td>5.26</td>
<td>10.20 ±32.0d</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>9.60 ±0.25 ed</td>
<td>-1.03 ±0.55d</td>
<td>60.00</td>
<td>22.40 ±17.89</td>
<td>12.00</td>
<td>12.00 ±45.4c</td>
</tr>
</tbody>
</table>

*a, b & c: There is nonsignificant difference (P>0.05) between any two means having the same superscript small latter in the same column.*


