EFFECT OF *VARROA DESTRUCTOR* ON DIFFERENT HAEMOCYTE COUNT, TOTAL HAEMOLYPH PROTEIN ON LARVAE, PUPAE AND ADULTS OF *APIS MELLIFERA* DRONES

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**ABSTRACT**

The effect of infestation with *Varroa destructor* Oud. (Acarina: Varroidae) on the haemolymph of the different stages in honey bee drons were studied. It decreased the total haemocyte count (THC) by 47.4%, 13.04% and 65% for infested larvae, pupae and adults, respectively. Differential haemocyte count (DHC), haemocyte surface area, corpora allata surface area (CA) and total haemolymph protein (THP) were decreased also due to infestation with varroa mite.

Key words: *Varroa destructor* - haemolymph - *Apis mellifera* Drones.

**INTRODUCTION**

The varroa mite, *Varroa destructor* Oud. (Acarina: Varroidae) is one of the most serious obligatory ectoparasite of honey bee all over the world.

Varroa immature and adults feed on honey bee haemolymph causing morphological abnormalities and transmitting virus. Also, it transmit bacterial and fungal infections to the recipient host (El-Zen *et al.*, 2001; Allen and Ball, 1996, Glinski and Jarosz, 1984; 1988 and 1992). Varroa mite infestation was associated with less brood and fewer bees. The infested colonies were failing and produced smaller adult and brood populations (Downy and Winston, 2001).

It is possible that the parasite *V. destructor* female enter the cell just before it is capped and impairs the internal defense. It lays eggs on the pupae and puncture the bee to feed on its haemolymph Weinberg and Madel 1985 & 1988. The mite and its offspring usually don’t kill the bee, they emerge with it to undergo a phoretic period on adult bee before they enter another cell to reproduce. *V. destructor* is a highly destructive pest responsible for reduction honey and reduced the brood production.

In Egypt, *V. destructor* at its maximum rate during late summer and early autumn is considered a serious pest of honey bee colonies (Gadelhak, 1999).

The aim of this study is to estimate some physiological parameters of different stages of honey bee under the infestation of the parasitic mite *V. destructor*.

**MATERIALS AND METHODS**

This experiment was performed on the last larval instar,newly capped pupae (white eye) and adults of drone honey bee *Apis mellifera* (Hymenoptera: Apidae) during spring which infested with varroa mite. Groups, each of ten individuals of healthy and infested larvae, pupae and adults were chosen from three different frames to estimate the total haemocyte count (THC) by using Haemocytometer. The specimen was severing in the dorsal vessel and a drop of blood was received on a clean glass slide. The haemolymph was quickly smeared to a thin film, air dried and stained with Wright’s blood stain, (Conn, 1948). They were examined under light microscope by using emersion oil lens (100X) and classified according to Essawy (1999). The different haemocyte types (DHC) were count for each individual. Haemocyte surface area was measured as described by Arnold and Hinks (1976) by using micrometer slide. To estimate total haemolymph protein (THP) the individual was bled by pricking the dorsal part and haemolymph was collected in an Eppendorf tube containing few crystals of Phenyl thioureae and stored in the freezer until THP analyzed according to Lowry *et al.* (1951). Ten individuals, of the different stages of *Apis mellifera* were taken to evaluate the effect of *V. destructor* on corpora allata (CA) surface area. Head capsules were kept in alcoholic Boun’s solution for fixation. Heads were dissected under Zeiss steriomicroscope (OPM-6). The CA gland were pulled out with fine forceps and mounted in glass slide in a drop of glycerin jell. Using the top-view Camera lucida, CA was drawn then the surface area was calculated by Plannimeter.
The data were statistically analyzed to check the differences between healthy and infested individuals. Data in table were represented as means±SE.

RESULTS AND DISCUSSION

Varroa mites are external honeybee parasites that attack both the adults and the brood (Figure, 1) with a distinct preference for drone brood. They suck the blood from both the adults and the developing brood, weakening and shortening the life span of the ones they fed. Emerging brood may be deformed with missing legs or wings.

![A: Larvae](image1)  ![B: white eye pupae](image2)

Figure (1): Showing the infested stages of honey bee

A) Larvae     B) white eye pupae

The obtained data presented in Table (1) indicated that all stages were affected by infestation with varroa mite. The reduction percentage in total haemocyte count were 47.4%, 13.04% and 65% for infested larvae, pupae and adult drones, respectively. The THC were 20000 h/mm³, 10000 h/mm³ and 14000 h/mm³, in infested larvae, pupae and adult, respectively, while they were 38000 h/mm³, 11500 h/mm³ and 40000 h/mm³ for healthy larvae, pupae and adult, respectively. These results confirmed those of Ginski and Klimont (1987) and Gadelhak (1999) who stated that the total haemocyte count were affected by varroa infestation.

The obtained data in Table (1) showed the effect of infestation of the drone with *V. destructor* on different haemocyte counts (DHC). Four types of haemocytes were identified. These haemocyte types namely prohaemocyte (pr), granulocyte (Gr), plasmatocyte (pl), and oenocytoides (Oe). Muller (1925) described four types of haemocyte in *Apis mellifera* while Gadelhak (1999) recorded five haemocyte types in honey bee drone during varroa infestation.

A reduction in the number of all types of DHC was detected in the last larval instar, (Table, 1). The prohaemocytes under infestation decreased from 19.8±0.1 to 14.3±0.3 and the surface area of these cells also decreased (34.3 to 19.8 μm²). The same trend was observed in the granulocyte where it decreased from 36.2±4 to 31.2±1.7. Also, the surface areas of these cells were decreased from 54.1 μm² to 39.6 μm² according to infestation. The reduction in number of plasmatocytes ranged from 55.7±2 to 46±1.7 and the surface area decreased from 56.8 to 43 μm². The number of oenocytoides decreased from 3.5±0.6 to 2.3±0.6 and the surface area were decreased from 84.5 μm² to 61.4 μm².

The DHC of the pupal stage were characterized by a reduction in the granulocyte due to infestation. The numbers of granulocyte were decreased from 40±1.4 to 30±1.4 while the surface area decreased from 54.7 μm² to 38.9 μm². The same trend was observed with plasmatocyte where it decreased from 65±3 to 47±0.3 and from 52.8 to 40.9 μm² for DHC and surface area, respectively. A slight decrease was observed in the number of oenocytoides in the infested pupae where it was 4±0.5 in the healthy pupae and. 2.5±0.2 in the infested pupae. Surface area of healthy pupae was 87.1 μm² and decreased to 72.6 μm², in the infested pupae (Tables 1 & 2). Gadelhak (1999) recorded an increase in both plasmatocyte and oenocytoides in infested pupae.

The same trend of decrement was also observed in the infested adults where the prohaemocyte was decreased from 40±1.2 to 26±1.4 and the surface area was decreased from 52.8 to 38.3 μm². The granulocyte also decreased from 40±1.2 to 26±1.4 and the surface area was decreased from 52.8 to 38.3 μm². The plasmatocyte also decreased from 33±1.1 to 28±1.2 and the surface area decreased from 51.2 to 39.6 μm².

This finding agree with Ginski and Klimont (1987) who noted a decrease in the total haemocyte count but they did not showed any change in the metabolic activity of haemocytes in the haemolymph of honey bees infested with varroa mite. Also, Shapiro, 1979 suggested that haemocyte counts may change quantitively and qualitatively during growth and development and also due to different internal and external factors.

The corpora allata (CA) volume was used as an indicator of the glandular secretory activity of Juvenile hormone (Wirtz, 1973). As shown in Figure (2) the corpora allata surface area decreased to be 0.334±0.01 mm² in the infested larvae comparing with healthy larvae 0.499±0.01 mm².
Table (1): Effect of *Varroa destructor* on total haemocyte count (THC). On the different stages of honey bee *Apis mellifera* drones

<table>
<thead>
<tr>
<th>Stage</th>
<th>Av. No. of Mites/Individual</th>
<th>Reduction %</th>
<th>Total haemocyte counts (THC) (mm³)</th>
<th>Different haemocyte count (DHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. larvae</td>
<td></td>
<td></td>
<td>38000</td>
<td>Pr: 19.8±0.1, Gr: 36.2±4.0, Pl: 55.7±2.0, Oe: 3.5±0.6</td>
</tr>
<tr>
<td>I. larvae</td>
<td>2</td>
<td>47.7</td>
<td>20000</td>
<td>Pr: 14.3±0.3, Gr: 31.2±1.7, Pl: 46.0±1.7, Oe: 2.3±0.6</td>
</tr>
<tr>
<td>H. pupae</td>
<td></td>
<td></td>
<td>11500</td>
<td>Pr: 11.0±0.1, Gr: 40.0±1.4, Pl: 65.0±3.0, Oe: 3.0±0.5</td>
</tr>
<tr>
<td>I. pupae</td>
<td>3</td>
<td>13.04</td>
<td>10000</td>
<td>Pr: 10.0±0.4, Gr: 30.0±1.4, Pl: 47.0±0.3, Oe: 2.5±0.2</td>
</tr>
<tr>
<td>H. adult</td>
<td></td>
<td></td>
<td>40000</td>
<td>Pr: 15.6±0.1, Gr: 40.0±1.2, Pl: 33.0±1.1, Oe: 3.0±0.1</td>
</tr>
<tr>
<td>I. adult</td>
<td>2</td>
<td>65</td>
<td>14000</td>
<td>Pr: 9.0±0.4, Gr: 26.0±1.4, Pl: 28.0±1.2, Oe: 1.2±0.2</td>
</tr>
</tbody>
</table>

H = Healthy  I = Infested  Pr = Prohaemocyte  Gr = Granulocyte  Pl = Plasmatocyte  Oe = Oenocytoids

Table (2): Effect of *Varroa destructor* of haemocyte surface area µm² of different stages of Honey bee *Apis mellifera*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Haemocyte surface area µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. larvae</td>
<td>Pr: 34.32 ± 0.3, Gr: 54.1 ± 0.6, Pl: 56.8± 0.7, Oe: 84.48 ± 1.1</td>
</tr>
<tr>
<td>I. larvae</td>
<td>Pr: 19.8 ± 0.1, Gr: 39.6 ± 0.4, Pl: 43.6± 0.4, Oe: 61.4 ± 0.7</td>
</tr>
<tr>
<td>H. pupae</td>
<td>Pr: 35.6 ± 0.2, Gr: 54.7 ± 0.5, Pl: 52.8± 0.6, Oe: 87.1 ± 0.9</td>
</tr>
<tr>
<td>I. pupae</td>
<td>Pr: 20.46 ± 0.2, Gr: 38.9 ± 0.3, Pl: 40.9± 0.6, Oe: 72.6 ± 0.8</td>
</tr>
<tr>
<td>H. adult</td>
<td>Pr: 35.64 ± 0.6, Gr: 52.8 ± 0.6, Pl: 51.2 ± 0.4, Oe: 92.4 ± 1.3</td>
</tr>
<tr>
<td>I. adult</td>
<td>Pr: 21.4 ± 0.2, Gr: 38.3 ± 0.4, Pl: 39.6 ± 0.3, Oe: 81.18 ± 0.8</td>
</tr>
</tbody>
</table>

H = Healthy  I = Infested

Figure (2): The change in CA surface area of larval and pupal stages of drone honey bee.

The same trend was observed in the pupal stage, the CA surface area were 0.368±0.005 mm² and 0.211±0.01mm² in the healthy and infested pupae, respectively.

The haemolymph protein (THP) was also recorded in the healthy and infested larvae and pupae of the drone honey bee. The data in Figure (3) showed that the infestation with varroa mite decreased the THP in larvae by about 146% and pupae by about 99%. These results agree with Bozena and Andrezej (2003) who recorded a sharp decrease in the THP in the haemolymph of the immature stages under varroa infestation. Also, Kolev and Habanov (1989) studied the change in the haemolymph protein of all stages of drons parasitized by varroa mite.

Pathak (1983) suggested that parasite produce some unknown factors which affects the neuro endocrine system of insects in producing more hormone and influencing the haemocytes. Verrett and Mills (1976) mentioned that both nervous and endocrine system directly affected the concentration of most blood constituents and normal patterns of change in the blood cycle and reflected the release and interactions of the neuro endocrine system.

In conclusion, the infestation with *V. destructor* has affected the haemolymph protein, corpora allata activity, the total haemocyte counts, the differential haemocyte counted and its surface areas in both infested larvae, pupae and adult drone.
REFERENCES


