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Physiological Studies on the House Fly *Musca Domestica Vicina* Muscidae, Diptera

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Abstract: The total and dry body weight of larvae treated with the bacterium *Bacillus thuringiensis israelensis* at LC_{30} (1.32%) was significantly decreased at different time intervals under investigation (6, 12, 24, and 48 hr) post-treatment. The body water content was decreased significantly at 6 hr post-treatment, while it was highly significant at the three other treatments. Also hemolymph volume was significantly decreased. A significant increase in hemolymph density was observed only at 12 hr post treatment. The mean of the total hemocyte count in the treated larvae at all inspected time was highly significantly increased, also there was a marked variation in the hemocytes percentage of untreated and treated larvae of *M. domestica* at time intervals.

Introduction

Filth fly *Musca domestica vicina* is one of the most serious and abundant medical insect pest in many countries in the world. Microbial pest control agents are considered as one of the new methods for the control of such insect pest, which have low impact on the peoples associated with the pest in environment. Thus the present investigation is preformed to encourage the using of *Bacillus thuringiensis israeliensis* as alternative method to chemical insecticide. This work is planned to investigate the following ; the effect of LC_{30} of the tested bacterium on some physiological parameters such as body water content, total and dry body weight as well as hemolymph voulume, hemolymph

density and also to determine its effect on the total and differential hemocyte counts of the 2nd instar larvae.

Materials and Methods

1. Test Insects

a. Sources of Colony

Adults susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from a well established colony originated from Biology Department Faculty of Science for girls, King Abdul Aziz University.

b. Rearing Technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27±2°C and 60±5% R.H). Each egg mass was placed in a clean petri dish (10cm diameter), previously washed with 10% formalin solution to avoid any contamination according to a constant technique described by Lelwallen [1]. Full grown larvae were allowed to pupate in clean glass petri dishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar 2% milk solution as a source of food.

2. *Source of the Bacterial Pathogen.*

The bacterium *Bacillus thuringiernsis israelensis* was chosen as a pathogen for this study because of its wide use in biological control. The power was obtained from Valent Biosciences, U.S.A.

3. *Experimental Larvae*

All the following investigated tests were accomplished on newly moulted 2nd instar larvae of *M.domestica* treated with sublethal concentration (LC_{30}) of the bio-insecticide B.t.i the tested larvae were obtained from the stock colony maintained in the laboratory of biology department- faculty of science for girls, King Abdul Aziz University. Just after moulting and starved for about 12hrs then allowed to feed on treated larval media with LC_{30} of the bacterial pathogen. All the experiment were carried out under lab conditions (25 ±2°C and 60 ±5% R.H).

4. *Phsiological Studies*

4.1 Determination of Total Body Weight and Body Water Contents of the 2nd Instar Larvae

Body water content was determined by the method by Lim and Lee[2]. Measurements were adopted on ten larvae per each time interval (6, 12, 24, and

48h) after treatment. the body water content was calculated as the difference between fresh(total) body weight and body weight after drying for one day at 80°C in an oven.

4.2 Estimation of Some Physical Parameters of Larval Hemolymph

a. *Hemolymph Density*

The density of hemolymph was determined for normal and treated larvae after the different time intervals [3], it was expressed as mg/ µ l. Ten measurements were used for each time interval.

b. *Hemolymph Volumes*

The blood density was determined as described above. The blood weight was determined as the difference between filter paper weighted before and after 10 larvae squeezed on this filter paper. The following equation was adopted to evaluate the blood volume:

$$\text{Blood volume} = \frac{\text{Blood weight}}{\text{Blood denisty}}$$

The blood volume is expressed as µl/Larva.

4.3 *Cellular Immune Response*

4.3.1 *Differential Hemolymph Counts (DHCs)*

Differential hemolymph counts were determined [4-5]. The smears were examined under oil immersion (× 1000) and 100 cells from random fields were differentiated on each slide to determine the percentage of each type. Cell – shape, diameter, nuclear, cytoplasmic ratio and cytoplasmic inclusions were used for classification of the hemocytes using the classification scheme [6], types calculated by the formula:

$$\% = \frac{\textit{Number of each hemocyte type}}{\textit{Total number of hemocytes examined}} \times 100$$

The measurements were replicated ten times for each time interval.

4.3.2 Total Hemolymph Counts (THC_S)

To determine the total (THC_S) counts, according a technique described by Jones[7] and Shapiro[8]. To avoid error duplicate counts were made with each sample to check counting error [9], the sample was discarded according to Wheeler [10]. The count was repeated ten times for each time interval.

Calculations

Dilution of blood = 1:20 ml

Side of each small square = ¼ mm

Depth of each small square = $\frac{1}{10}$ mm

$$\text{volume of each small square} = \frac{1}{4} \times \frac{1}{4} \times \frac{1}{10} = \frac{1}{160} \text{ mm}^3$$

Number of WBCs in 64 small square = x

$$\text{Number of WBCs\mm3} = \frac{x}{64} \times \frac{1}{\text{Vol. of each small square}} \times \frac{1}{\text{Dillution}}$$

$$= \frac{x}{64} \times 160 \times 20 \text{WBCs\mm3}$$

5. *Statistical Analysis*

1. Data were expressed as mean ± standard error; the statistical significance of differences between individual means was determined by student "t" test for paired observations. The level of significance of each experiment was stated to be non significant ($P< 0.05$) and highly significant ($P< 0.05$).

2. The percentage reduction was calculated [11].

3. The corrected mortality percentage was statistically computed[12]. From which the corresponding concentration Probit lines (Ld –p line) of the 2nd *Instar Larvae* were estimated in addition to determine 30% and 50% mortalities and slope value of the tested material.

Results

1. *Effect of B.t.i. at LC_{30} on the Total Body, Dry Body Weights and on the Body*

Water the Content of the 2nd Larval Instar.

The total body weight of the treated larvae was significantly decreased at different time intervals under investigation (6, 12, 24, and 48 h) post-treatment. The same trends were also observed in the case of measuring the dry body weight, (Table 1). It is clear from the present results that the body water content was decreased significantly ($P< 0.05$) at 6 h post treatment, whereas highly significant decrease was recorded at the other three time intervals post-treatment. The decrease in the total body weight (fresh weight) after larval treatment with microbial agent appeared to be mainly due to the decrease in the dry body weight and secondary due to the decrease in the body water content.

Table 1: Body water content of 2nd *instar larvae* of *M. domestica* determined at different time intervals post-treatment with LC_{30} (1.32%) of B.t.i. .

Hours post-treatment	Body water content (mg)	
	Control	Treated
	mean± S.E.	mean± S.E.
6	8.11 ± 0.241	7.07 ± 0.217 *
12	11.88 ± 0.555	8.32 ± 0.374**
24	20.68 ± 0.853	5.59 ± 0.401**
48	33.02 ± 1.431	9.17 ± 0.651**

n 10 larvae per test.
* Significant ($P< 0.05$) .
** Highly significant ($P< 0.01$).

2. *Effect of Microbial Agent (B.t.i.) at LC_{30} on the Hemolymph Volume and Density of the Treated Larvae:*

Results in Table 2 indicate that the hemolymph volume was significantly decreased at 12, 24 and 48 h post-treatment. These values were 2.23 ± 0.41, 1.56 ± 1.94 and 4.68 ± 0.245 µL as compared to 2.71± 0.111, 7.38 ± 0.331 and 13.44 ± 0.197 µL, respectively.

A significant increase in the hemolymph density was observed only at 12 h post treatment. On the other hand, there are no significant differences in the values of hemolymph density in the untreated larvae as well as in treated larvae at 6, 24,

3. *Effect of B.t.i. at Concentration of* 48h post-treatments, (Table 3).*LC_{30} on the Total Hemocyte Counts (THC_s) and Differential Hemocytes (DHC_s) of 2nd Larval Instar of M. domestica.*

A. *Total Hemocyte (THC_s)*

The blood cells, or hemocytes of insects form a part of defense mechanism against insecticides, bacteria and other foreign bodies, they are mesodermal in origin and analogous to the leucocytes of the vertebrates. In the present study, the blood cells of the 2nd instar larvae of *M. domestica* were classified into five types:- prohemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids . The mean THC_s at different time of treated and untreated 2nd instar larvae intervals was obtained as shown in Table 4; it is clear from the present results that the mean of THC_s (cells/mm³) in the treated larvae at all inspected times (6, 12, 24, and 48 h) was highly significantly increased; for example, the mean of THC_5 during the period was 33690 ± 395, 38000 ± 495, 24010 ± 403 and 20210 ± 409 (cells/mm³) respectively. The corresponding figures of the untreated larvae during these periods were 25685 ± 245, 26335 ±211, 16040 ± 284 and 18855 ± 230 cells/ mm³ respectively.

B. *Differential Hemocytes (DHC_s)*

Results in Table 5 indicated that the differentiated hemocytes were greatly affected as a result of post–treatment with B.t.i. When the 2nd instar larvae treated with tested material at $LC_{30,}$ the prohemocytes became smaller than untreated larvae. The injury causes other effects on the cytoplasm, break down of the cell wall; clumped with each other and extruding of their cytoplasm were observed.

1. *Plasmatocytes*

Some morphological changes were observed in those of treated larvae. There are numerous shapes such as podocytes, amaobocytes and spindle shape. Some forms of plasmatocytes contain no distinguishing inclusion bodies in their cytoplasm which separated from the nucleus and contained large vacuoles.

Table 2: Hemolymph volume (µL/larva) of 2nd instar of *M.domestica* determined at different time intervals post-treatment with LC_{30} (1.32%) of B.t.i.

Hours post-treatment	Hemolymph volume (µL /larva)	
	Control	Treated
	mean± S.E.	mean± S.E.
6	2.14 ± 0.159	1,79 ± 0.145
12	2.71 ± 0.111	2.32 ± 0.141*
24	7.38 ± 0.331	1.56 ± 0.194 **
48	13.44 ± 0.197	4.68 ± 0.245 **

n 3 replicates per test.
* Significant (P< 0.05) .
** Highly significant (P< 0.01).

Table 3: Hemolymph density (mg /µL) of 2nd instar of *M.domestica* determined at different time intervals post-treatment with LC_{30} (1.32%) of B.t.i..

Hours post - treatment	Hemolymph density (mg/µL)	
	Control	Treated
	mean± S.E.	mean± S.E.
6	0.86 ± 0.01	0.89 ± 0.008
12	0.87 ± 0.007	0.89 ± 0.014*
24	0.88 ± 0.007	0.88 ± 0.090
48	0.88 ± 0.005	0.88 ± 0.007

n 3 replicates per test.
* Significant (P< 0.05) .

Table 4: Total hemocyte counts (THC_s) (cells/ mm ³) of 2nd instar of *M. domestica* determined at different time Intervals post -treatment with LC_{30} (1.32%) of B.t.i..

Hours post - treatment	Hemocyte counts (THC_s) (cells/ mm ³)	
	Control	Treated
	mean± S.E.	mean± S.E.
6	25685 ± 245	33690 ± 395 **
12	26335 ± 211	38000 ± 495 **
24	16040 ± 284	24010 ± 403 **
48	18855 ± 230	20210 ± 409 **

n 10 replicates per test.
* Significant (P< 0.05).

Table 5: Differential hemocyte counts (DHCs) of 2nd instar of *M. domestica* determined at different time intervals Post- treatment with LC_{30}(1.32%) of B.t.i..

Hours post treatment	Percentage of hemocyte type Mean ± SE									
	Prohemocytes		Plasmatocytes		Granulocytes		Spherulocytes		Oenocytoids	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
6	10.50 ±0.05	7.70 ±0.21**	41.40±0 .33	48.70±0.4 3**	24.70±0. 35	18.30±0.2 0**	19.30±0 .23	23.40±0.3 2**	4.10 ±0.13	1.90±0.1 1**
12	4.90 ±0.14	2.20±0.0 9**	42.40±0 .16	49.80±0.4 5**	29.70±0. 35	18.50±0.4 2**	20.20±0 .31	27.20±0.6 2**	2.80±0. 09	2.30±0. 12*
24	1.60±0.08	3.64±0.1 5**	55.20±0 .12	56.46±0.2 9**	24.50±0. 29	20.60±0.2 7**	15.80±0 .32	16.50±0.4 3	2.90 ±0.18	2.80±0. 15
48	1.01±0.03	1.43±0.0 9**	52.89±0 .31	58.70±0.6 5**	28.60±0. 29	19.10±0.4 9**	15.40±0 .24	18.70±0.5 7**	2.10±0. 07	2.07±0. 20

* Significant (P< 0.05).
** Highly significant (P< 0.01) .

2. *Spherulocytes*

The effect of tested material on these cells was small, they often have an irregular shape, the membrane is broken, the nucleus moved to become laterally located.

3. *Granulocytes*

The injuries as a side effect of treatment were degeneration in cell wall with vacuolized cytoplasm, some cells were divided into two cells, and others are aggregated together. Extrusion of nucleus from the cytoplasm is also observed in infected cells.

4. *Oenocytoids*

Our observations clearly indicated that these cells become irregular in shape with extruded cytoplasm and divided nucleus. Few cells appeared small with two nuclei and vacuoles in their cytoplasm.

Discussion

1- *Effect of B. t.i. at Concentration of LC_{30} on the Total Body Weight, Dry Body Weight and on the Body Water Content of 2nd M. domestica:*

The total body weight, dry body weight and body water content of the treated larvae were highly significant decreased at different time intervals as compared to control (untreated larvae), and this decrease appeared mainly due to the decrease in the dry body mass, as reflected from the increase in water content percent, wet body weight and also due to the decrease in the body water content.This enables us to say feeding of *M.domestica* larvae on diet treated with LC_{30} B.t.i. decreased the larval dry body weight. these results are in conformity with those of [13-14], who found that in the Indian meal moth *Plodia interpunctella* larvae, the B.T. induced gradual decrease in the fresh, dry body weights and body water content at three time intervals (6, 12, 24, and 48h). [15] . Came to the same conclusion on the greater wax moth, *Galleria mellonella* larvae after injection with *Bacillus circus.*

2- *Effect of B.t.i. at Concentration of LC_{30} on the Hemolymph Volume and Density of 2nd Instar Larvae of M. domestica domestica :*

The hemolymph volume was significantly decreased at 12,24, and 48h post-treatment. The estimated significant decrease of the blood volume in the larvae may be attributed to water loss from blood and tissues as a result of bacterial infection. The present results are in accordance with that demonstrated [7,14,16] . The hemolymph density of untreated larvae showed non-significant difference in this parameter was observed at 12h post treatment, and this increase may be due to the increase in the total hemocyte counts and the increase of blood volume as well as the increase of bacterial metabolites. These results are in conformity with[14] on *Plodia interpunctella* larvae with staphylococcus [15] , on *Galleria mellonella* larvae injected with *B. circus.*[17,18 ,19,20,21] .

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دراسات فسيولوجية على الذبابة المنزلية

Musca Domestica Vicina Muscidae, Diptera

نجلاء يوسف أبو زنادة

قسم الأحياء – كلية العلوم للبنات

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المستخلص: عند معامله اليرقات بالجرعةLC_{30} (1.32%) من بكتريا *Bacillus thuringiernsis israelensis* انخفضت جميع الأوزان الكلية والجافة في جميع الفترات الزمنية موضع الدراسة (٦،١٢،٢٤،٤٨ ساعة) بعد المعاملة، وكما ظهر انخفاض معنوي في المحتوى المائي بعد ٦ ساعات من المعاملة، ولوحظ وجود انخفاض عالي المعنوية في المعاملات الثلاث الأخرى، وسجل حجم الدم انخفاضاً معنوياً. وظهرت زيادة معنوية في كثافة الدم فقط عند ١٢ ساعة من المعاملة. في حين رصدت زيادة معنوية في العدد الكلي لكرات الدم في اليرقات المعاملة في جميع فترات المعاملة, وأيضاً لوحظ وجود فروق واضحة في نسب خلايا الدم المختلفة في اليرقات المعاملة وغير المعاملة للذبابة المنزلية في مختلف الفترات.