Laboratory Evaluation of Supernemic Insecticide (Neem Oil) and its Effect on Larvae of Big Headed myiasis Blow Fly Chrysomya Megacephala (Fibricius, 1794) (Diptera: Calliphoridae)

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Abstract

This study was carried out to evaluate the efficiency of Supernemic in the control of larvae of big headed myiasis fly Chrysomya megacephala by direct spraying and food treatment, the results of direct spraying of the second instar larvae with concentrations 0.3, 0.6 and 1ml.l⁻¹ led to cumulative mortality reached 25, 37 and 59% respectively, with a decrease in the emergence number of adults insects to 75, 36 and 41%, respectively, as well as 76% of the adults which produced from larvae were treated with 1 ml.l⁻¹ of supernemic died after 24 hours, some of malformation in the dead larvae was noted such as blackening the whole body of the larva or appearance of black spots on it. The treatment of larval food with same concentrations showed a cumulative mortality reached 27, 64 and 65%, respectively, and the adults emergence percentage were 67, 36 and 35 respectively, we conclude that the larvae of this fly were sensitive to the insecticide, as well as food treatment was more efficient in controlling of this fly compared to the direct spraying treatment.

Keywords: Neem oil, Myiasis, Chrysomya megacephala.

Introduction

Chrysomya megacephala (Blow fly) belong to Calliphoridae family, it is one of the important species medically, veterinary and economically, because of the its nature of life and feeding on the waste and the remains of the bodies of animals cadavers, it contributes to the transfer of many pathogens for humans and animals, especially bacteria. It was found that the transmission of this type of pathogens by adults is much greater than that of domestic fly [1].

In addition, this type of flies causes various facultative myiasis, including wound myiasis, vaginal myiasis, aural myiasis and umbilical myiasis [2, 5]. It is therefore necessary to control it by using the known methods such as chemical pesticides, but as a result of the repeated and indiscriminate use of these pesticides, the resistance of the insecticide has grown in insects, as well as the epidemiological explosion of a number of secondary pests, also the harmful effects of these pesticides in the natural and non-target areas and the environment and human health. [6], therefore, attention has been paid to the development of alternative control strategies, and the use of organic pesticides such as plant-based pesticides because of their high effectiveness and their degradation and its low toxicity against many non-target organisms and the lack of resistance it [7].

Various species of plants have been studied in order to obtain effective substances, characterized by pesticide properties against pests, the plant Azadirachta indica has received considerable attention in this field, the studies have shown that the all parts of neem tree (leaves, fruits, seeds, roots and stem bark) contain effective chemicals against many insect pests, most notably the azadirachtin, many commercial preparations have been produced in America, Germany and India for this purpose [8,9]. The objective of this research is to study the toxic effect of the insecticide of plant origin (Supernemic, neem oil) in the larvae of big headed myiasis fly Ch. megacephala by direct spraying and feed treatment.
Materials and Methods
Breeding the Colony of Big Headed Myiasis Fly Chrysomya Megacephala

Adult insects of species Ch. megacephala collected from a governorate of Baghdad in November 2015, by leaving a sheep lung for four days in an open container, then adults collected using aerial net and transferred to a laboratory in the Department of biology - college of Education for Pure Sciences - Ibn Al-Haitham. The insects were placed in a cubic metal cage (side length 30 cm) covered by tulle cloth, leaving the cloth from one side of the cage looks like shirt sleeve, the cage was provided with a mixture of milk powder and sugar (1:1) for insects feeding. For lay the eggs, amount of minced beef (fat free) (20 gm) was placed in a bowl with drops of diluted blood with distilled water [10].

The larvae were monitored by adding new minced meat and removing part of the old meat to avoid food dryness and rot, when the larvae extend to the end of the third larval instar, they were transferred to a disposable containers (500 ml) containing sawdust for the purpose of pupation, the pupae were moved to cubic metal cage (with side length 25cm) for the purpose of adults emergence [11]. The insect was breeded to four generations before the experiments and its identified at the Research Center and Museum of Natural History- University of Baghdad.

Effect of Supernemic Concentrations on Larvae of Big Headed Myiasis Flies Ch. Megacephala.
A- Direct Spraying Method

This experiment was carried out to estimate the effect of spraying the larvae with concentrations of the insecticide (0.3, 0.6, 1 mL-1), 240 of 2nd instar larvae from the breeding colony were distributed in 24 disposables (45cm base diameters and height 3.5 cm) with three containers replicates each concentration, the larvae sprayed with 2 ml of each concentrations individually with adding of Tween-80 (0.01%) using a hand spray, direct spray conducted from 10-15 cm distance, while the control treatment was sprayed with distilled water with Tween-80 (0.01%), the larvae were transferred to a small plastic disposable containers with 10 gm of minced beef (fat free), the containers covered with plastic caps were punctured with very small holes for ventilation and non-exit larvae, containers were placed in incubator with 27±2 °C and relative humidity 80±5 % and duration of lighting 12 hours, the experiment was monitored daily, the number of larvae and pupae were recorded to determine their mortality percentage, and the malformation were photographed by a digital camera type Sony. Also, the adults emergence percent and dead adults percent after 24 hours[12].

B- Food Treatment Method

This experiment was carried out to determine the effect of the treatment of the larval food with concentrations (0.3, 0.6, 1 mL-1) of Supernemic, 24 disposable containers each contain 10 gm of minced beef (fat free) (three replicates are allocated for each concentration), then addition 2ml of each of the concentrations to the food (minced meat) and mix well, while the control treatment, 2ml of distilled water with Tween-80 (0.01%) was added, 10 larvae were transferred to the containers, the containers covered with plastic caps were punctured with very small holes for ventilation and non-exit larvae, the experiment containers were placed in a temperature incubator with 27±2 °C and relative humidity 80±5 % and duration of lighting 12 hours, the experiment was monitored daily, the number of dead larvae and pupae were recorded to determine their mortality percentage, and the malformation were photographed by a digital camera type Sony. Also, the number of emergence adults to determine emergence percentages and perished adults after 24 hours [12].

Statistical Analysis

All experiments were designed according to the Completely Randomized Design (CRD), and the larval mortality percent were corrected according to the Schneider-Orellis equation [13]:

\[
\text{% mortality} = \frac{\text{No. of treatment mortality} - \text{No. of control mortality}}{100 - \text{No. of control mortality}} \times 100
\]

Then analyze the results using SPSS version 20, which includes the Duncan's New Multiple Range Test (L.S.R) to compare the means in all treatments and to calculate the
Results and discussion

The results in Table (1) showed that the direct spraying of the second instar larvae of blow fly with 0.3, 0.6, 1 mL⁻¹ gave mortality percent reached up to 2.60%, 6% and 26% respectively, recorded a significant difference. Some malformations were observed on the treated dead larvae, including their complete blackness, the appearance of black spots on their bodies (Fig. 1). The effect of insecticide concentrations continued and mortalities of pupae were 23.00, 31.00 and 33.50%, respectively. Table (1) shows also that the cumulative mortality percent of treated larvae was 25.00, 37.00 and 59.00%, respectively. There was a significant difference between the mortality percent.

Table 1: Effect of Three Concentrations of organic insecticide Supernemic on larvae of big headed myiasis fly using direct spraying method

<table>
<thead>
<tr>
<th>Concentration ml.L⁻¹</th>
<th>% of corrected larval mortality ± S.E.</th>
<th>% of corrected pupas mortality ± S.E.</th>
<th>% of cumulative mortality ± S.E.</th>
<th>% of adults emergence ± S.E.</th>
<th>% of adults mortality after 24 hours ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>0.26±2.600b</td>
<td>3.70±23.00a</td>
<td>3.20±25.00b</td>
<td>1.45±75.00a</td>
<td>29.00±55.00b</td>
</tr>
<tr>
<td>0.6</td>
<td>0.60±6.00b</td>
<td>9.70±31.00a</td>
<td>9.00±37.00b</td>
<td>7.00±36.00b</td>
<td>14.00±75.00b</td>
</tr>
<tr>
<td>1</td>
<td>7.00±26.00a</td>
<td>6.00±33.50a</td>
<td>9.00±59.00a</td>
<td>7.40±41.00b</td>
<td>2.40±76.00a</td>
</tr>
</tbody>
</table>

* The similar letters in each column mean that there are no significant differences according to Duncan's New Multiple Range Test (L.S.R) at a significant level (P <0.05). S.E. Standard error.

Table (1) shows that the adults emergence percentage were 75.00, 36.00 and 41.00%, respectively, but a large proportion of them were found died within 24 hours of their emergence, by 55.00, 73.00 and 76.00%, respectively. The mortality percentages malformation recorded is due to the toxic effect of the active compound contained in this insecticide, such as azadirachtin and other toxic compounds that are interacted with the biochemical and metabolic reactions of the cells and that the blackening of the treated larval bodies or the presence of black spots is due to the melanization phenomenon caused by these compounds. Figure (1) shows that larval survival rate after direct spray treatment decreased gradually over time, larval survival at concentration 1 mL⁻¹ was reduced to less than 60% after 10 days of treatment compared with control treatment that gave larval survival 90% at the same duration. This results in agreement with results of Bhardwaj and Ansari [14], they point out that spraying of *Earias vitilla* (cotton plant pest) with concentrations of insecticides Neemzal and Nimbecidine that extracted from the neem trees, led to decrease emergence of adults and control of this pest.

Ibraheem et al. [15] reported that spraying of olive black scale insect *Saisssetia oleae* with concentration 5ml.L⁻¹ of Nimbecidine led to decrease this insect by 96.06% after seven weeks of treatment.
Fig 1: Survival larval rates of big headed myiasis fly Ch. megacephala which treated with concentrations of supernemic using direct spraying method.

The results of this study are in a good agreement with results of Shoba et al. [16] who reported that the insecticide Nimbecidine is a toxic to pupae of Sphaerodema rusticum species and the LC$_{50}$ is 0.0028 ppm after 96 hours of treatment. Tine et al. [17] also reported that spraying cockroaches Blatta orientalis (under laboratory conditions) with concentrations of azadirachtin compound in the form of an oil solution ranging from 300-9000 ng.insect$^{-1}$ resulted mortalities by 9.39-96.96 %, respectively, after 10 days of treatment. LC$_{50}$ and LC$_{90}$ were 1349 and 6346 ng.insect$^{-1}$ respectively. Sutherland et al. [18] reported that spraying small rice stinkbugs Orealus poecilus (Dallas), with 12.5 mL$^{-1}$ of Neemactin and Agroneem (a commercial formulation of neem trees), led to mortalities by 40 and 35% respectively after three days of treatment. Results treatment of larval food of Ch. megacephala with concentrations (0.3, 0.6, 1 mL$^{-1}$) of supernemic showed mortality percentages dependent on concentrations by 13.00, 44.00 and 33.00 %, respectively, with a significant effect obtained from concentration 1 mL$^{-1}$ compared to other two concentrations, and mortality percentages of the pupae resulting from the treated larvae of 15.30, 20.00, 32.50 %, respectively (Table 2).

Table 2: Effect of Three Concentrations of Supernemic on larvae of big headed myiasis fly using food treatment method

<table>
<thead>
<tr>
<th>Concentration mL$^{-1}$</th>
<th>% of corrected larval mortality ± S.E.</th>
<th>% of corrected pupal mortality ± S.E.</th>
<th>% of cumulative mortality ± S.E.</th>
<th>% of adults emergence ± S.E.</th>
<th>% of adults mortality after 24 hours ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>8.90±13.00c</td>
<td>7.70±15.30b</td>
<td>1.80±27.70b</td>
<td>6.20±67.00a</td>
<td>6.30±54.00c</td>
</tr>
<tr>
<td>0.6</td>
<td>3.80±44.00a</td>
<td>4.70±20.00b</td>
<td>2.40±64.00a</td>
<td>2.70±36.00b</td>
<td>2.00±78.00b</td>
</tr>
<tr>
<td>1</td>
<td>7.70±33.00b</td>
<td>10.00±32.50a</td>
<td>3.00±65.00a</td>
<td>4.00±35.00b</td>
<td>6.70±93.00a</td>
</tr>
</tbody>
</table>

* The similar letters in each column mean that there are no significant differences according to Duncan’s New Multiple Range Test (L.S.R) at a significant level (P <0.05). S.E. Standard error.

Results in Table (2) also shows that cumulative mortality percentages increased by 27.70, 64.00 and 65.00 %, respectively and the percentages of adults emergence were 67.00, 36.00 and 35.00 %, respectively. It was also noted that the percentage of the mortality of the emerging adults were 54.00, 78.00, 93.00 %, respectively, after 24 hours of its emergence. These results can be due to the toxic effect of the active compounds in supernemic, the most important is azadirachtin compound, which affects the metabolism of the cells then stops the chemical reactions which lead to the larval mortality. Fig (2) showed that the survival rate of treated feed larvae with concentrations 0.3, 0.6 and 1 mL$^{-1}$ decreased over time, treating food larvae with concentrations 0.6 and 0.06 mL$^{-1}$ decreased survival rates to less than 60% and 50 % after 10 days compared to the control treatment, which had a 90% survival rate after the same period. These results in agreement with results of Abdel-Razek et al. [12] in an experiment they conducted on the potato plant infected with Phthoremia.
**operculella** at a concentration 5 and the 2.5 mL\(^{-1}\) of Nembeidine resulted in the mortality of insect larvae by 79.6 and 43.8%, respectively, after three doses of the insecticide, this led to increased potato yield by 11.53 and 9.8 kg.10 plants\(^{-1}\), respectively, compared with untreated plants which gave lowest yield 7.12 kg.10 plants\(^{-1}\).

Senthil-Nathan et al. [20] reported that the insecticide of Parecoil and Neema, which manufactured from neem trees, caused in mortality of Brown plant hopper *Nilaparvata lugens*, a serious pests of rice plant, as well as reducing its survival and consumption of food, also its noted that spraying rice plant at a concentration 2.5 mL\(^{-1}\) of Parker oil and Neema led to the pupal mortality by 75 and 65%, respectively. El-Hawary and Abdel El-Salam [21] also indicated that food treatment of the Aphis *Aphis craccivora* at a concentration 0.6 mL\(^{-1}\) from same insecticide reduces the duration of its presence on the plant to 3.7 days and recorded the mortality of treated pupae (on treated plant) with the same insecticide reached 92.2%, compared with the control treatment that gave the presence of insect on the plant 17.9 days. Siriwattanarungsee et al. [22] revealed that the effect of insecticide extracted from neem trees, which include 0.24% of azadirachitin on *Ch. megacephala* and *M. domestica* by mixing the insecticides with the larval food of these insects azadirachitin led to a high mortality percentage of larvae and pupae which feeding on the treated food, also the house flies are more sensitive to the insecticide than blow flies.

**References**


