Influence of Temperature Change on the Growth and Susceptibility of the Common House Mosquito, *Culex pipiens* in Egypt to Some Insecticides

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Abstract: By transmitting major human diseases, mosquito species represent a serious threat worldwide in terms of public health. Most vector control programmes aiming to control life-threatening mosquitoes rely on the use of chemical insecticides. For the reason that only a few insecticides are used for public health, maintaining the efficacy of control programmes mostly relies on resistance management strategies. Development of such strategies requires understanding the factors influencing resistance together with characterizing the mechanisms involved. In this context, the present study aims to update current knowledge about the effect of temperature on the mosquito *Culex pipiens* population response to chemical insecticides. The results demonstrated that alteration of the temperature significantly affects *Cx. pipiens* populations. High temperature (25, 30°C) resulted in high survival rate (90, 95% respectively); while at temperature 20°C the survival rate was 80%. Egg hatching percentage was 95% after 24 h, at temperature 30°C and 50% after 24h, 50% after 48h at 25°C; however at 20°C egg hatching percentage was 100% after 48 h. In case of *Cx. pipiens* larvae that were reared under various temperatures pupated on day 5, 9 and 12 at 30°C, 25°C and 20°C, respectively. At high temperature 30°C, females emerged before males. On the other hand resistance of all *Cx. pipiens* populations to the selected chemical insecticides decreased with raising temperature. The obtained results also showed that there was significant change in acetylcholinesterase and glutathione-S-transferase level in both larvae and adult due to temperature changing. These results indicate that temperature is an important parameter that must be considered during the application of chemical assays or control of *Cx. pipiens* populations.

Keywords: *Culex pipiens*, Insecticide Resistance, Temperature, Biochemical Assay

1. Introduction

Many of the most dangerous diseases such as malaria, filariasis, or dengue fever, are transmitted to humans by mosquitoes. Most vector control programmes largely based on the application of chemical insecticides. Today, insecticides resistance has been reported in all main mosquito vector species and geographical regions with high parasite-related mortality and morbidity [1, 2].

For Culicidae, as in other arthropods, temperature is one of the most important abiotic factors affecting the development and survival of the immature stages [3]. Mosquito growth and development rates are temperature dependent, with development times typically shortening [4], and body size either increasing [5] or decreasing [6] as temperature increases.

Insecticides are the most common strategy used for mosquito management [7, 8]. However, insecticide toxicity does not only depend on the active ingredient. The efficacy of chemical insecticides against its target is also a function of
the formulation, the biology of the insect, and the environment in which these interact [9]. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms [10].

Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species, while carbamates are known to exhibit a slightly negative correlation between ambient temperature and mortality for many insect species [11]. Environmental temperature has been shown to influence the outcome of insecticide exposure; temperature differences expected to occur naturally under field conditions can lead to notable variations in chemical efficacy [12].

This study aims to investigate the effect of temperature changing of mosquito larval habitats on subsequent development and relation of such factor with insecticides resistance.

2. Materials and Methods

2.1. Test Insects

Three Cx. pipiens populations were used in this study: resistant, susceptible and laboratory ones. All populations were reared in the Research Institute of Medical Entomology (Ministry of Health & Populations, Giza, Egypt) under standard conditions (26-28°C, 12 h:12 h light/dark period, 70-80% relative humidity) [13]. The susceptible and resistant populations were brought from The American Naval Medical Research 3 (NAMRU3) insectary and laboratory population was collected from field and reared in the laboratory for seven generation at the same conditions. The susceptible population was not exposed to any control agents since 1986 [14].

Mosquito eggs were transferred to different containers with different temperatures (20, 25 and 30°C), till emergence of the 4th larval instar. Part of the 3rd larval instars from each group was used for larval bioassays, while the remaining larvae were reared to the adult stage and used for adult bioassays. The larvae were fed on a fish food. The adults were fed on 10% sucrose solution [15]. The mosquitoes were reared and maintained in habitat free from insecticides and repellents. The tests were carried in incubators set at 20, 25 and 30°C.

2.2. Biological Studies

2.2.1. Hatching Period

The newly laid eggs were placed in three water dishes for each population (resistant, susceptible and laboratory). For embryonation, a single raft of eggs (approximate 80-100 eggs) was placed in egg-hatching water dish contained dechlorinated water. Nine egg-hatching containers (three replicates for each population) were labeled with the kind of population, the temperature at which the test was set, and the date of the starting of the test and given an identifying number. Observations were reported at intervals of 24 hours and the numbers of larvae hatching from the eggs were recorded. A record of the number of larvae hatching was taken until maximum hatching was obtained. The hatching period was calculated according to the following equation [16].

\[
\text{The hatching period} = \frac{\text{The numbers of larvae} \times \text{the number of days taken to hatch}}{\text{The maximum number of larvae} \text{hatched for that replicate}}
\]

2.2.2. The Larval Duration

Once hatching had taken place, the larvae were observed every 24h and the number of pupae formed in each egg-hatching dish was recorded.

The larval period was calculated according to the following equation [16].

\[
\text{The larval duration} = \frac{\text{The number of pupae at each observation} \times \text{the number of days since the larvae were hatched}}{\text{The total number of pupae for that replicate}}
\]

2.2.3. The Pupal Duration

The pupae were collected with a little water using a large pipette and moved into plastic cup and put in special mosquito cage. Observations were reported every 24 hours and the number of adults emerging recorded until all adult emerged. The duration of the pupal period was calculated according to the following equation [16].

\[
\text{The duration of the pupal period} = \frac{\text{The total number of adults emerged} \times \text{the number of days}}{\text{The total period in days in which all the adults emerged}}
\]

2.2.4. The Survival Rate and Emergence of Male and Female

Surviving of all stages was monitored and recorded daily. The emerged adult males and females were counted and observed to determine which of them emerged first.

2.3. Toxicalogical Studies

2.3.1. Larvicides

Three recommended organophosphorus insecticides by the World Health Organization’s Pesticide Evaluation Scheme (WHOPESS) for control mosquito larvae [17]: temephos, chloropyrifos and pirimiphos methyl were used in the study.

To perform bioassays, single-concentration diagnostic tests were conducted as proposed by [18] to monitor insecticide resistance. The diagnostic concentrations were: 0.02 ppm for temephos, and 0.01 ppm for chloropyrifos, while the diagnostic concentration for pirimiphos methyl was calculated by measuring LC50 of susceptible population and then duplicate (0.1 ppm).
Larval bioassay was performed according to the WHO standard method [19]. For bioassay, four replicates for each selected insecticide were conducted against newly 3rd larval instar. Test beakers of 500 ml capacity, each containing 249 ml tap water were prepared. In each, 1 ml of each concentration was infiltrated under the water surface with a pipette. After 30 min. of preparing the insecticide solution a group of 25 larvae were placed in each beaker. The test was run at the same three temperature degrees (20, 25 and 30°C) at which the larvae were previously reared. Larvae were left for 24 h and mortality was then recorded. Moribund larvae were considered as dead ones. Another group of larvae was used as control. The control tests were set up by adding 1 ml of ethanol (the solvent of used chemical insecticides) into water and mortality never exceeded 4%.

2.3.2. Adulticides

Three pyrethroid insecticides (Lambda- cyhalothrin, Deltamethrin and Permethrin) commonly recommended for mosquito adult control were tested against the three studied Cx. pipiens populations. The proposed diagnostic concentration by [18] for each insecticide was used to monitor insecticide resistance (0.05% Lambda- cyhalothrin, 0.05% Deltamethrinand0.75% Permethrin). Twenty sucrose-fed 4 day adult female mosquitoes per replicate were used for this bioassay. The principle of this test is to expose of females of each different larval breeding habitat (temperature: 20, 25, 30°C) for one hour in a specially designed plastic tube lined with a filter paper impregnated with diagnostic concentration of the insecticide. After the exposure the mosquitoes were transferred to another tube lined with clean filter paper and mosquito adult mortality is observed after 24 hours. For the control, impregnated-papers containing 1 ml ethanol were used.

2.4. Biochemical Assay

To determine the effect of temperature on enzymes of the mosquitoes, adults and larvae of the three tested C. pipiens populations that reared at different temperature degrees (20, 25, 30°C) were submitted to biochemical assays for general acetylcholinesterase ACHe, glutathione S-transferase GST and total protein.

2.4.1. Preparation of Insects for Analysis

The survived larvae and adults of C. pipiens were collected after insecticide application for each different temperature then weighted and mechanically homogenized in distilled water by a chilled glass teflon homogenizer (ST- Mechanic- Preezyina, Poland). Homogenates were centrifuged by refrigerated centrifuge (6 MR, USA) at 8000 r.p.m. for 15 min at 5°C. The deposits were discarded and the supernatant was kept in a deep freezer till use.

2.4.2. Determination of Total Protein Content

Total protein quantification of mosquito homogenates was performed using Bradford reagent with bovine serum albumin as the standard protein [20] to normalize enzyme activity levels by protein content.

2.4.3. Determination of Acetylcholinesterase (ACHe) Activity

Acetylcholinesterase (ACHe) activity was measured by using acetylcholine bromide (AChBr) as substrate, the produced color was measured calorimetrically using double beam ultraviolet/visible spectrophotometer (Sectronic 1201, Milton Roy Co., USA) used to measure absorbance of colored substances at 515 nm [21].

2.4.4. Determination of GST (Glutathione S-Transferases) Activity

Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. Enzyme activity was measured by a reaction mixture of 10 µL of the insect homogenate plus 200 µL of reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) solution (10 mM reduced glutathione dissolved in 0.1 M phosphate buffer pH 6.5 and 3 mM CDNB originally dissolved in 1 mL methanol) [22]. The mixture was incubated at 30°C for 5 min then the enzyme activity was measured at 340 nm with spectrophotometer. The enzyme activity was reported as µM/min/mg protein using the extinction co-efficient corrected for the path length of the solution in the microtitre plate well.

2.5. Statistical Analysis

Mortality data of toxicological tests and values of biochemical assay were calculated by using Microsoft Excel (Microsoft Corporation, 2007). Data of the biological parameters and biochemical results were statistically analyzed using t- tests and ANOVA using SPSS (20) [23] for Windows to find out if there were significant differences in all tested result.

3. Results

3.1. Biology

The obtained data demonstrated that changing temperature had the same effect on the survival rate, growth duration of the three tested mosquito populations (resistant, susceptible and laboratory):

Table 1 showed that temperature has an effect on egg hatching, larval and pupal periods as well as survival rate of mosquitoes. There were significant differences in the embryonation periods under each of the three experimental temperatures at (p <0.05). At 20°C, the eggs of Cx. pipiens took an average of 2.4 days for embryonation while at25°C; the eggs need an average of 1.7 and at 30°C, the eggs hatched after 1.13 days.

There was inverse relationship between the temperature and larval duration as well as pupal duration, that as the temperature rose, the duration of these immature stages reduced. With rising temperature, the survival rate of the mosquitoes increased, that at 30°C the survival rate was 95%. Also it was observed that, the female emerged before male at temperature 30°C.
3.2. Insecticide Bioassays

3.2.1. Larvae

Table 2 demonstrated the mortality percentage of the three mosquito populations due to the exposure to the selected larvicides at different temperature.

The result showed that all tested populations were more sensitive to temephos at all temperature degrees with mortality percent 100% followed by pirimiphos methyl and the lowest effective insecticide was chloropyrifos. On the other hand, all populations were more susceptible at low temperature than at high temperature, that the minimum mortality percent occurred at 30°C followed by 25°C and maximum mortality percent occurred at 20°C. As expected, susceptible population exhibited the lowest resistance to larvicides then laboratory population followed by resistant population which has the highest degree of resistant especially at temperature 30°C.

3.2.2. Adults

From the tabulated data in table 3 it could be observed that all populations were more susceptible at low temperature than at high ones. The minimum mortality percent occurred at 30°C followed by 25°C and highest mortality occurred at 20°C. Lambda-cyhalothrin was the most effective insecticide followed by deltamethrin, while permethrin was the least effective insecticide. Also, the susceptible population recorded the highest mortality percentage, followed by the laboratory one then the resistant population.

3.3. Biochemical Assay

3.3.1. Larval Biochemical Assay

The biochemical assay results were represented in figures (1 and 2). The resistant population had highest level of the enzymes especially at temperature 30 °C, followed by laboratory population, while susceptible population recorded the lowest enzyme activity levels. In all populations, by increasing the temperature degrees the levels of AChE and GST increased.

Figure 1 showed that the lowest levels of AChE observed at 20°C (1.9, 2.3 and 4.11µg AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. On other hand temperature 30°C resulted appearance of the highest level of AChE (3.6, 4.1 and 7 µg AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. According to fig. 2, the enzyme GST level of susceptible population was (1004, 1214 and 1659 n mole sub conjugated/min/mg protein) at temperature 20, 25 and 30°C, respectively, while GST level of laboratory population was recorded (1421, 1655 and 1796 n mole sub conjugated/min/mg protein) at temperature 20, 25 and 30°C, respectively. The resistant ones had GST levels (1609, 1974 and 2094 n mole sub conjugated/min/mg protein) at temperature 20, 25 and 30°C, respectively.

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Hatchability (Mean day ± S.D)</th>
<th>larval duration (Mean day ± S.D)</th>
<th>pupal duration (Mean day ± S.D)</th>
<th>survival rate%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.4 ± 0.15*</td>
<td>12.5 ± 0.25*</td>
<td>3.6 ± 0.15*</td>
<td>80</td>
</tr>
<tr>
<td>25</td>
<td>1.7 ± 0.15*</td>
<td>9 ± 0.2*</td>
<td>2.13 ± 0.08*</td>
<td>90</td>
</tr>
<tr>
<td>30</td>
<td>1.13 ± 0.08*</td>
<td>5.1 ± 0.2*</td>
<td>2 ± 0.12*</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 1. The effects of different temperature degrees on hatchability, larval and pupal duration and survival rate of Culex pipiens.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Mortality percentage</th>
<th>laboratory population</th>
<th>susceptible population</th>
<th>resistant population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature °C</td>
<td></td>
<td>Temperature °C</td>
<td>Temperature °C</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>20</td>
<td>89% 79% 64%</td>
<td>100% 93% 79%</td>
<td>66% 60% 46%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100% 84% 71%</td>
<td>100% 100% 84%</td>
<td>70% 65% 50%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100% 100% 100%</td>
<td>100% 100% 100%</td>
<td>100% 100% 100%</td>
</tr>
<tr>
<td>Pirimiphos methyl</td>
<td>20</td>
<td>90% 80% 74%</td>
<td>90% 90% 88%</td>
<td>80% 75% 70%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100% 85% 77%</td>
<td>100% 100% 85%</td>
<td>100% 100% 100%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100% 100% 100%</td>
<td>100% 100% 100%</td>
<td>100% 100% 100%</td>
</tr>
<tr>
<td>Temephos</td>
<td>20</td>
<td>80% 60% 60%</td>
<td>90% 78% 70%</td>
<td>70% 43% 25%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>85% 82% 65%</td>
<td>93% 90% 75%</td>
<td>75% 50% 40%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>87.5% 85% 68%</td>
<td>95% 93% 78%</td>
<td>78% 55% 45%</td>
</tr>
</tbody>
</table>

Table 2. Susceptibility status of Culex pipiens larvae exposed to different insecticides at different temperatures.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Mortality percentage</th>
<th>laboratory population</th>
<th>susceptible populations</th>
<th>resistant population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature °C</td>
<td></td>
<td>Temperature °C</td>
<td>Temperature °C</td>
</tr>
<tr>
<td>Permethrin</td>
<td>20</td>
<td>80% 60% 60%</td>
<td>90% 78% 70%</td>
<td>70% 43% 25%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>85% 82% 65%</td>
<td>93% 90% 75%</td>
<td>75% 50% 40%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>87.5% 85% 68%</td>
<td>95% 93% 78%</td>
<td>78% 55% 45%</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>20</td>
<td>80% 60% 60%</td>
<td>90% 78% 70%</td>
<td>70% 43% 25%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>85% 82% 65%</td>
<td>93% 90% 75%</td>
<td>75% 50% 40%</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>87.5% 85% 68%</td>
<td>95% 93% 78%</td>
<td>78% 55% 45%</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>20</td>
<td>80% 60% 60%</td>
<td>90% 78% 70%</td>
<td>70% 43% 25%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>85% 82% 65%</td>
<td>93% 90% 75%</td>
<td>75% 50% 40%</td>
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<td>87.5% 85% 68%</td>
<td>95% 93% 78%</td>
<td>78% 55% 45%</td>
</tr>
</tbody>
</table>
3.3.2. Adult Biochemical Assay

The biochemical assay results of the adult mosquitoes were plotted in figures 3, 4. The resistant population recorded high levels of the enzymes elevation followed by laboratory population, while susceptible population had the lowest enzyme activity. Temperature 30°C elevated the enzymes activity in all tested populations if compared with the two other degrees. Figure 3 showed that the least levels of AChE observed in temp. 20°C (5.5, 5.2 and 6 (µg AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. Temperature 25°C showed moderate level AChE (4.9, 4.8 and 5.9 µg AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. On other hand, temperature 30°C showed the highest level of AChE (6.8, 7.4 and 7.9 µg AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. GST activity for susceptible population was (1254, 1177 and 1745 n mole sub conjugated/min/mg protein) at temperature 20, 25 and 30°C, respectively, while GST activity for laboratory population was (1284, 1493 and 1937 n mole sub conjugated/min/mg protein) for temperature 20, 25 and 30°C, respectively. While the resistant strain exhibited elevation in activity of GST (1425, 1377 and 2000 n mole sub conjugated/min/mg protein) for temperature 20, 25 and 30°C, respectively.
4. Discussion

Climate change is expected to lead to global and regional changes in environmental temperature and other climatic variables [24], which are likely to have an impact on different disease vectors distribution [25, 26]. It is thought that global warming may make currently in hospitable regions amenable to vector expansion along altitudinal gradients [27]. In order to generate useful predictions of any disease transmission and the impact of intervention programmes, the full impact of environmental conditions on the life history parameters and population dynamics of disease vectors needs to be taken into account when forecasting transmission [28].

The results demonstrate that alteration of the temperature affects egg hatching, duration of the larval and pupal stages of the mosquito, emergence of adult in all studied populations of mosquito, Cx. pipiens. At high temperature Cx. pipiens life cycle became shorter (mosquito development occurred faster) while at low temperature mosquito took long time for complete development. The present study proved that temperature had great effects on biology as well as susceptibility of Cx pipiens mosquitoes.

These findings agree with [29, 30] who found that temperature has effects on the emergence, survival and the subsequent behavior of the adult Cx. pipiens mosquitoes and
**5. Conclusion**

The current findings suggested that high larval rearing temperatures cause changes in the activity levels of AChE and GST enzymes which lead to make larvae being more resistant to insecticides. This means that any modifications in the levels of resistance of the mosquitoes to any applied chemical control may be due to the impact of the temperature, so the environmental conditions at which the insecticides are used must be took in consideration.

**References**


