

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].

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

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].

The larval duration =

The number of pupae at each observation X the number of days since the larvae were hatched / The total number of pupa

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

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. Toxicological Studies

2.3.1. Larvicides

Three recommended organophosphorus insecticides by the

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 24h 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].

World Health Organization's Pesticide Evaluation Scheme (WHOPES) for control mosquito larvae [17]; temephos, chlorpyrifos 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 chlorpyrifos, while the diagnostic concentration for pirimiphos methyl was calculated by measuring LC₉₉ 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% Deltamethrin and 0.75% 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 mosquitos, 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 telfon homogenizer (ST- Mechanic-Preczyina, 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 at 25°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.

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

| Temp. °C | Hatchability (Mean day \pm S.D) | larval duration (Mean day \pm S.D) | pupal duration (Mean day \pm S.D) | survival rate% |
|----------|-----------------------------------|--------------------------------------|-------------------------------------|----------------|
| 20 | 2.4 \pm 0.15 ^a | 12.5 \pm 0.25 ^a | 3.6 \pm 0.15 ^a | 80 |
| 25 | 1.7 \pm 0.15 ^a | 9 \pm 0.2 ^a | 2.13 \pm 0.08 ^a | 90 |
| 30 | 1.13 \pm 0.08 ^a | 5.1 \pm 0.2 ^a | 2 \pm 0.12 ^a | 95 |

a: means that there is significant difference between three groups(p <0.05).

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 larval insecticides then laboratory population followed by resistant population which has the highest degree of resistant especially at temperature 30°C.

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

| Insecticides | Mortality percentage | | | | | | | | |
|-------------------|-----------------------|------|------|------------------------|------|------|----------------------|------|------|
| | laboratory population | | | susceptible population | | | resistant population | | |
| | Temperature °C | | | Temperature °C | | | Temperature °C | | |
| | 20 | 25 | 30 | 20 | 25 | 30 | 20 | 25 | 30 |
| Chloropyrifos | 89% | 79% | 64% | 100% | 93% | 79% | 66% | 60% | 46% |
| Pirimiphos methyl | 100% | 84% | 71% | 100% | 100% | 84% | 70% | 65% | 50% |
| Temephos | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

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.

Table 3. Susceptibility status of *Culex pipiens* adults exposed to different insecticides at different temperatures.

| Insecticides | Mortality | | | | | | | | |
|---------------------|-----------------------|-----|-----|-------------------------|-----|-----|----------------------|-----|-----|
| | laboratory population | | | susceptible populations | | | resistant population | | |
| | Temperature °C | | | Temperature °C | | | Temperature °C | | |
| | 20 | 25 | 30 | 20 | 25 | 30 | 20 | 25 | 30 |
| Permethrin | 80% | 60% | 60% | 90% | 78% | 70% | 70% | 43% | 25% |
| Deltamethrin | 85% | 82% | 65% | 93% | 90% | 75% | 75% | 50% | 40% |
| Lambda- cyhalothrin | 87.5% | 85% | 68% | 95% | 93% | 78% | 78% | 55% | 45% |

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 4.11 μ g AChBr/min/mg protein) for susceptible, laboratory and resistant population, respectively. Temperature 25°C showed moderate level of AChE (1.97, 2.7 and 4.23 μ g 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 μ g 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.

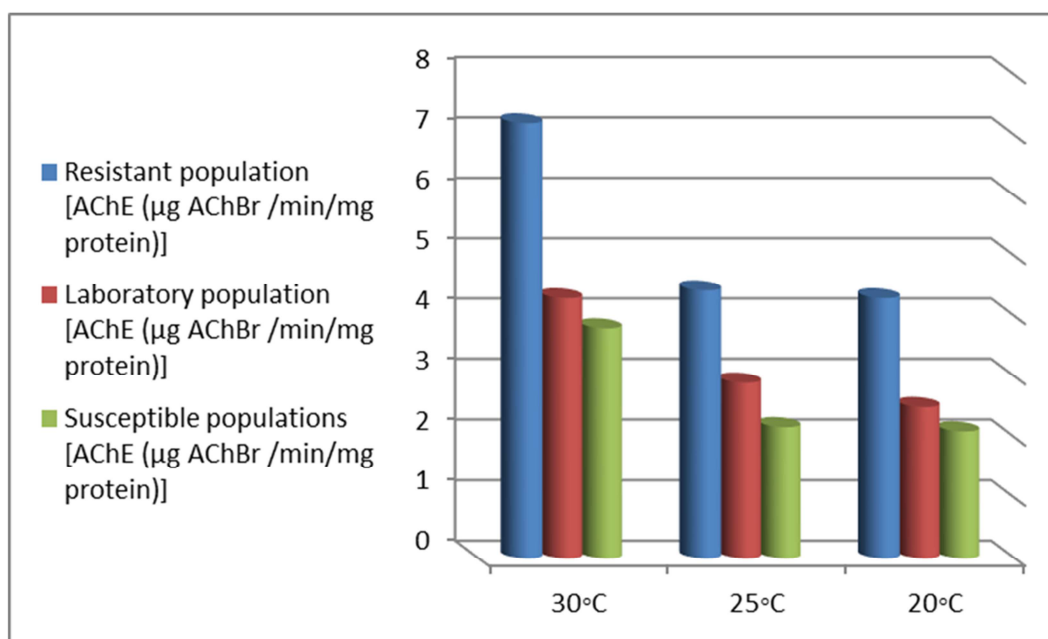


Figure 1. Mean activity levels of AChE in different *Culex pipiens* larval populations reared at three different temperature degrees.

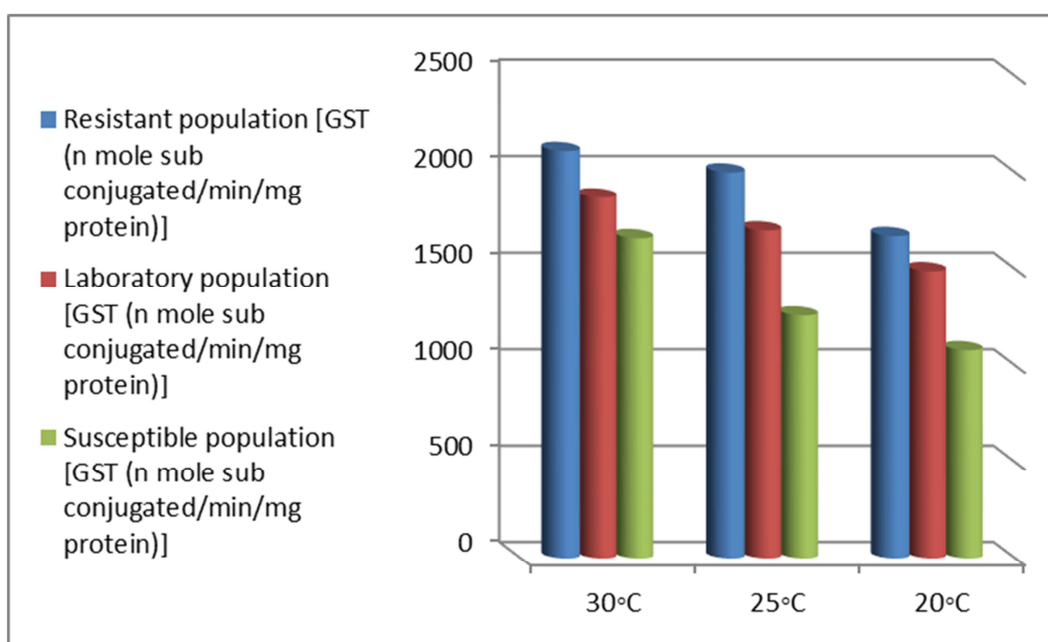


Figure 2. Mean activity levels of GST in different *Culex pipiens* larval populations reared at three different temperature degrees.

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, 5.2 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.

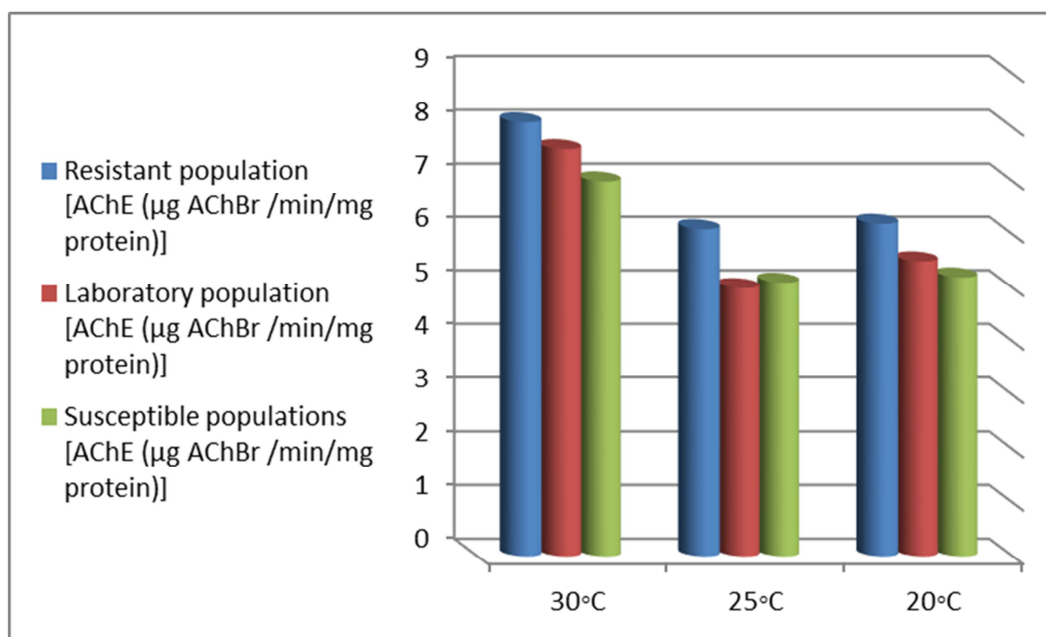


Figure 3. Mean activity levels of AChE in different *Culex pipiens* adult populations reared at three different temperature degrees.

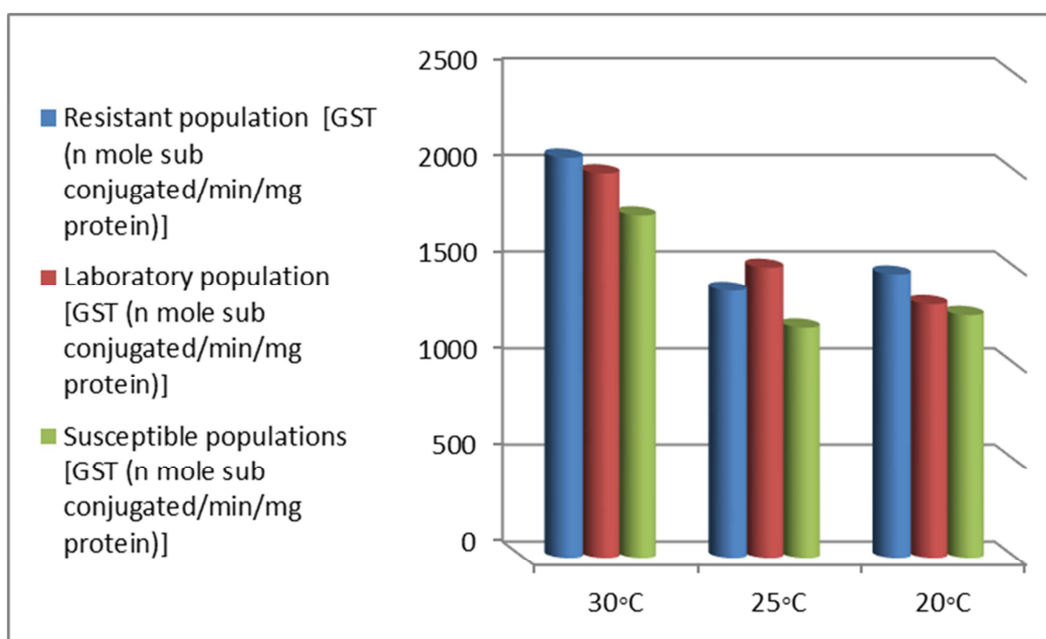


Figure 4. Mean activity levels of GST in different *Culex pipiens* adult populations reared at three different temperature degrees.

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 inhospitable 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

Ochlerotatus albifasciatus mosquitoes. Also [16, 31] observed that in higher temperatures the mosquitoes complete their life cycle faster but also died earlier.

Generally, the survival rate in this study was high at temperature between 25- 30°C and this finding agreed with [32, 33] who found that the rate of larval survive decreased at temperatures below 18°C and above 32°C in *Anopheles gambiae* and *Cx tarsalis* respectively. Also [31] established that when the larvae of *Aedes aegypti* were exposed to a range of varying temperatures, mortality was observed to increase with increase temperatures above certain levels with the temperature being completely lethal at 43°C. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms [10].

The present data showed that larval breeding conditions considerably influence the susceptibility of *Culex* mosquitoes to different insecticides. The susceptibility of *Cx. pipiens* increased as the temperature decreased. This findings agreed with [11, 34, 35, 36, 37] who found that the temperature increases often associated with decrease in toxicity of different insecticides against different mosquito species. Also, the results agreed with [38] who estimated that pre-exposing larvae to high temperatures resulted in increased tolerance to the insecticide.

On other hand the present results are disagreement with [39, 40], who observed that organophosphorus insecticides toxicity increases with increasing temperatures.

To gain satisfactory explanation for changing the response of mosquitoes to the used insecticides with temperature changing, a biochemical assay was conducted for certain two enzymes which are known to be implicated in insect resistance. The enzymes are acetylcholine esterase AChE and glutathione- s- transferase GST. AChE is an enzyme that found in the center nervous system and its functions is removing acetylcholine from its post synaptic receptors. The result of this action is the hydrolysis of acetylcholine into acetate and prolonged neuron excitation [41]. Inhibition of AChE by bio- insecticides causes a desensitization of acetylcholine receptors and leads to eventual death of the organism. The three gene families, GSTs, cytochrome P450 monooxygenases (cytochrome P450s) and hydrolases are mainly involved in the detoxification of insecticides, whereas, three proteins, including acetylcholinesterase, sodium channels and GABA receptor, have been mainly implicated in decreased target site sensitivity in insect nervous system [42].

At high temperature *Cx. pipiens* showed more resistance to the used insecticides and elevated activity of AChE as well as GST. The activation of GST and AChE may explain increase of resistance in the mosquito population.

Temperature affects not only insecticide ingredients but also enzymes and proteins, which play an important role in the survival of many organisms [43]. The biochemical studies agreed with [44] who found that decreased temperature may also increase the susceptibility of neurons of crayfish to pyrethroid insecticides making them more sensitive to excitation.

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.

5. Conclusion

The obtained results 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. It could be concluded that, water temperature plays a fundamental role not only on development and biodiversity of mosquito larvae but also on the susceptibility to some insecticides by affecting the level of detoxified enzymes which interfere with insecticide resistance and their assays. Mosquito larval mortality was observed to increase with increase temperatures.

References

- [1] Roberts, D. R. and Andre, R. G. (1994): Insecticide resistance issues in vector-borne disease control. American Journal of Tropical Medicine and Hygiene, 50:21–34.
- [2] Ranson, H.;Guessan, R. N.; Lines, J.; Moiroux, N.; Nkuni, Z. and Corbel, V. (2011): Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends in parasitology. 27:91–98.
- [3] Clements A. N. (1992): The Biology of mosquitoes. Vol. I. Development, Nutrition and Reproduction. London: Chapman & Hall.
- [4] Delatte H.; Gimonneau G.; Triboire A. and Fontenille D. (2009): Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. Journal of Medical Entomology, 46: 33–41.
- [5] Lounibos, L. P.; Suárez, S. and Menéndez, Z. (2002): Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? Journal of Vector Ecology, 27: 86–95.
- [6] Barton, P. S. and Aberton, J. G. (2005): Larval development and autogeny in *Ochlerotatuscamptorhynchus*(Thomson) (Diptera: Culicidae) from Southern Victoria. Proceedings of the Linnean Society of New South Wales, 126: 261–267.
- [7] Alves, S. B.; Alves, L. F. A.; Lopes, R. B.; Pereira, R. M. and Vieira, S. A. (2002): Potential of some *Metarhiziumanisopliae* isolates for control of *Culex quinquefasciatus* (Diptera, Culicidae). J. Appl. Entomol. Zeitschrift Fur Angewandte Entomology, 126: 504–509.
- [8] Swain, V.; Seth, R. K.; Raghavendra, K. and Mohanty, S. S. (2009): Impact of temperature on susceptible and resistant strains of *Culex quinquefasciatus* to synthetic pyrethroids. Acta Trop, 112: 303–307.

- [9] Miller, T. and Adams, M.(1982): Mode of action of pyrethroids. In: Coats JR, editor. Insecticide Mode of Action. Cambridge: Academic Press. p. 3–27.
- [10] Andersson, T.; and Forlin. L. (1992): Regulation of the cytochrome-P450 enzyme -system in fish. *Aquatic Toxicol.*, 24: 1–19.
- [11] Devries, D. H. and Georgiou, G. P. (1979): Influence of temperature on the toxicity of insecticides to susceptible and resistant house-flies (Diptera, Muscidae). *Journal of Economic Entomology*, 72: 48–50.
- [12] Boina, D. R.; Onagbola, E. O.; Salyani, M.; Stelinski, L. L. (2009): Influence of posttreatment temperature on the toxicity of insecticides against *Diaphorinacitri* (Hemiptera: Psyllidae). *J. Econ. Entomol.*, 102:685–91.
- [13] Chapman, H. C. and Barr, A. R. (1969): Techniques for successful colonization of many mosquito species. *Mosquito News*, 29(4).
- [14] Zayed, A. B.; Szumlas, D. E.; Hanafi A. H.; Fryauff, D. J.; Mostafa, A. A.; Allam, K. M. and Brogdon, W. G. (2006): Use of bioassay and microplate assay to detect and measure resistance in field populations of *Culex pipiens* from filariasis endemic areas of Egypt. *Journal of the American Mosquito Control Association*, 22(3):473-482.
- [15] Gerberg, E. J.; Bernard, D. R. and Ward, R. A. (1994): Manual for mosquito rearing and experimental techniques; American Mosquito Control Association Bulletin, 5: 61-62.
- [16] Martha, W.; Kiarie-Makara; Philip, M.; Ngumbi and Dong-Kyu Lee. (2015): Effects of Temperature on the Growth and Development of *Culex pipiens* Complex Mosquitoes (Diptera: Culicidae). *Journal of Pharmacy and Biological Sciences*, 10(6):01-10.
- [17] World Health Organization (2006): Pesticides and their applications, for the control of vectors and pests of public health importance. WHO/ CDS/ NTD/ WHOPES/ GCDPP/1.
- [18] World Health Organization (1981): Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/ VBC/ 81.807.
- [19] World Health Organization (1975): Manual on Practical Entomology in Malaria. Part II. WHO, Geneva.
- [20] Bradford, M. M. (1976.): A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analalytical Biochemistry*. 72: 248-254.
- [21] Simpson, D. R.; Bulland, D. L. and Linquist, D. A. (1964): A semimicrotechnique for estimation of cholinesterase activity in boll weevils. *Entomological Society of American*, 57: 367-371.
- [22] Habig, W. H.; Pabst, M. J. and Jakoby, W. B. (1974): Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry*, 249(22): 7130-7139.
- [23] Barrett, K. C.; Morgan, G. A.; Leech, N. L. and Gloeckner, G. W. (2012): IBM SPSS for introductory statistics: Use and interpretation. Routledge.
- [24] IPCC, (2013): Working Group 1 Contribution to the IPCC Fifth Assessment Report. In Climate change 2013: the Physical Science Basis.
- [25] Garske, T.; Ferguson, N.; Ghani, A. (2013): Estimating air temperature and its influence on malaria transmission across Africa. *PLoS One*, 8(2), e56487.
- [26] Lyons, C.; Coetzee, M. and Chown, S. (2013): Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*. *Parasit Vectors*, 6:104–112.
- [27] Siraj, A. S.; Santos-Vega, M.; Bouma, M. J.; Yadeta, D.; Carrascal, D. R. and Pascual, M. (2014): Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. *Science*, 343(6175):1154–1158.
- [28] Christiansen-Jucht, C.; Parham, P. E.; Saddler, A.; Koella, J. C. and Basáñez, M. G. (2014): Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* ss. *Parasites & vectors*, 7(1), 489.
- [29] Oda, T.; Uchida, K. and Mori, A. (1999): Effects of high temperature on the emergence and survival of adult *Culex pipiens* molestus and *Culex quinquefasciatus* in Japan. *J. Am. Mosq. Contr. Assoc.*, 15: 153 - 156.
- [30] Soledad, F. M.; Cristina, M. M.; Fischer, S; Pablo, W. O. and Schweigmann, N. (2000): Effects of flooding and temperature on *Aedes albifasciatus* development time and larval density in two rain pools at Bueno Aires University City. *Memórias do Instituto Oswaldo Cruz*, 95 (6): 787 –793.
- [31] Mourya, T. D.; Yadav, P. and Mishra, A. C. (2004): Effects of temperature stress on immature stages and susceptibility of *Aedes aegypti* mosquitoes to Chikungunya virus. *Journal of Tropical Medicine and Hygiene*, 7 (4): 346 – 350.
- [32] Bailey, S. F. and Geike, P. A. (1968): A study of the effect of water temperatures on rice field mosquito development. *Proceedings of the California Mosquito Vector Control Association*, 36: 53–61.
- [33] Reisen, W. K.; Milby, M. M. and Bock, M. E. (1984): The effects of immature stress on selected events in the life history of *Culex tarsalis*. *Mosquito News*, 44: 385–395.
- [34] Toth, S. J. and Sparks, T. C. (1988): Influence of treatment technique on the temperature toxicity relationships of cis-permethrin and trans-permethrin in the cabbage-looper (Lepidoptera, Noctuidae). *J. Econ. Entomol.*, 81: 115–118.
- [35] Alzogaray, R. A.; Picollo, M. I. and Zerba, E. N. (1998): Independent and joint action of cis- and trans-permethrin in *Triatoma infestans* (Hemiptera: Reduviidae). *Arch. Insect Biochem. Physiol.*, 37: 225–230.
- [36] Weston, D. P.; You, J.; Harwood, A. D. and Lydy, M. J. (2009): Whole sediment toxicity identification evaluation tools for pyrethroid insecticides: III. temperature manipulation. *Environ. Toxicol. Chem.*, 28: 173–180.
- [37] Ma, Y. H.; Gao, Z. I.; Dang, Z. H.; Li, Y. F. and Pan, W. L. (2012): Effect of temperature on the toxicity of several insecticides to *Apolygus lucorum* (Heteroptera: Miridae). *J. Pestic. Sci.*, 37: 135–139.
- [38] Patil, N. S.; Lole, K. S. and Deobagkar, D. N. (1996): Adaptive larval thermotolerance and induced cross - tolerance to propoxur insecticide in mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *Medical and veterinary entomology*, 10(3): 277-282.

- [39] Horn, D. J. (1998): Temperature synergism in integrated pest management. Temperature sensitivity in insects and application in integrated pest management, 311: 125–39.
- [40] Polson, K. A.; Brogdon, W. G.; Rawlins, S. C. and Chadee, D. D. (2012): Impact of environmental temperatures on resistance to organophosphate insecticides in *Aedes aegypti* from Trinidad. Revista Panamericana de Salud Pública, 32 (1): 1-8.
- [41] El- Kady, G. A. E.; Kamel, N. H.; Mosleh, Y. Y. and Bahght, I. M. (2008): Comparative toxicity of two bio-insecticides (Spinotoram and Vertemic) compared with methomyl against *Culex pipiens* and *Anopheles multicolor*. World Journal of Agricultural Sciences, 4(2): 198-205.
- [42] Feyereisen, R. (1995): Molecular biology of insecticide resistance. Toxicology Letter, 82: 83- 90.
- [43] Imasheva, A. G.; Loeschcke, V.; Zhivotovsky, L. A. and Lazebny, O. E. (1998): Stress temperatures and quantitative variation in *Drosophila melanogaster*. Heredity, 81(3): 246.
- [44] Salgado, V. L.; Herman, M. D. and Narahashi, T. (1989): Interactions of the pyrethroid fenvalerate with nerve membrane sodium-channels - temperature- dependence and mechanism of depolarization. Neurotoxicology, 10: 1–14.