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Full Length Research Paper

Response of *Culex quinquefasciatus* to deltamethrin in Lahore district

Hafiz Muhammad Tahir[1]*, Abida Butt[1] and Shafaat Yar Khan[2]

[1]Department of Zoology, University of the Punjab, Lahore, Pakistan.

[2]Department of Biological Sciences, University of Sarghoda, Pakistan.

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In the present study, both male and females of *Culex quinquefasciatus* were sampled from five different localities of Lahore District of Punjab, Pakistan. (that is, Jaman, Mohlanwal, Noorpur Bhatta A1, Yohanaabad–2 and GCU Lahore). Bioassays against 5% deltamethrin were performed to evaluate the level of resistance in *Culex quinquefasciatus* against deltamethrin. For the adult bioassays standard protocol of WHO was adopted. Results of the bioassays showed that individuals of all populations were susceptible to 5% of deltamethrin. Male specimens from all populations showed significantly higher mortality as compared to females. Biochemical tests were performed to measure the esterase activity, both in males and females of *C. quinquefasciatus*. Significant variation was observed in the level of esterases in *C. quinquefasciatus* populations, collected from five different localities. Alpha naphthyl acetate was hydrolyzed faster than beta naphthyl acetate by mosquito homogenates of Yohanabad -2, GC University Lahore and Noorpur Bhatta A1 while mosquito homogenates of Mohlanwal and Jaman population hydrolyzed the beta naphthyl acetate faster than alpha naphthyl acetate. Only one band was observed in all populations for esterases A. For esterases B band pattern was similar in Yohanabad -2 and GC University. In these two populations there were three bands (B1, B2 & B3). In the population of Noorpur Bhatta A1, Mohlanwal and Jaman band B2 was absent. There was no resistance detected in *C. quinquefasciatus* from Jaman, Mohlanwal populations after 1 h exposure to 5% deltamethrin while population of GC University, Yohanabad and Noorpur Bhatta A1 showed a high level of tolerance to deltamethrin.

Keywords: Esterases, *Culex quinquefasciatus*, resistance, pyrethroids.

INTRODUCTION

Mosquitoes are known to be vectors of numerous diseases that collectively represent a major source of human morbidity and mortality (Khan, 1996). In Pakistan three main genera of mosquitoes are present (that is, *Culex, Anopheles* and *Aedes*). High population explosion of *Culex quinquefasciatus* in Lahore district has become a severe biting nuisance primarily in summer months. *C. quinquefasciatus* also serves as a vector of bancroftian filariasis (Wolf and Khan, 1971). Insecticide resistance has become a serious concern in all-insect vectors of emerging diseases (Hemingway and Ranson, 2000).

Resistance to Organochlorine, Organophosphate and carbamate insecticides predominantly involves either metabolic detoxification of insecticides before it reaches its target site or change in sensitivity of target site so that it is no longer susceptible to insecticide inhibition (Hemingway, 2000). The most common metabolic resistance mechanisms involve esterases and S- transferases or monooxygenases (Scott et al., 1998). Esterases are the most significant enzymes for insecticide detoxification in insects (Devonshire, 1991; Hemingway, 2000; Hsu et al., 2004) and their enhanced activity is thought to be a major mechanism of insecticide resistance (Oppenoorth, 1984). Organophosphate, carbamate and pyrethroids contain carboxylester and phosphotriester bonds that are subject to attack by esterase enzymes. Esterase-base re-

*Corresponding author. E-mail: hafiztahirpk1@yahoo.com

sistance mechanism in *C. quinquefasciatus* has been detected in more than 80% *Culex* mosquitoes worldwide (Hemingway and Karunaratne, 1998; Raymond et al., 1991). Polymorphism is also a notable characteristic of insect esterases (Brattsten, 1992; Dauterman, 1985). Resistance to pyrethroids has been reported in *Culex* and *Anopheles* from South America, Sudan, Sri Lanka, Nigeria, Burkina Faso, Egypt, Guatemala, USA, Turkey and Syria (Malcolm, 1988; WHO, 1992).

Most biochemical approaches developed thus for aim at the detection or measurement of enzymatic reaction in homogenates of a single insect and use electrophoretic analysis, filter paper test or microtiter plates essays. They are concerned with the detection of organophosphate and carbamate resistance due to reduced sensitivity of acetylcholinesterase (Raymond et al., 1985; Hemingway et al., 1987; Brogdon et al., 1988) or to increase detoxifi-cation by highly active esterases in mosquitoes (Georghiou and Pasteur, 1978; Rees et al., 1985).

In Pakistan (Punjab) an organophosphate, malathion was used to control mosquitoes before 1991 in public health program. However after 1991 malathion was replaced by a synthetic pyrethroid deltamethrin as resis-tance against malathion was reported in some areas of Pakistan (Malcolm and Boddington, 1989). Till 2002 resistance against deltamethrin was not reported in *C. quinquefasciatus* in Lahore distinct. The present study aims to investigate the following concerns:

1. Is there any population of *C. quinquefasciatus* in Lahore district, which is resistant against recommended of deltamethrin (pyrethroid) during 2003.
2. Is there any difference in the levels of esterases in different populations of *C. quinquefasciatus.*
3. Is there any difference in the amount of esterases in male and female specimens of a population?
4. How many types of esterases in different populations of *C. quinquefasciatus* in Lahore district.
5. Is there any correlation between levels of esterases and previous exposure to insecticides?

MATERIALS AND METHODS

Sampling

During this study, *C. quinquefasciatus* were collected from the following localities of Lahore District of Punjab, Pakistan: Jaman, Mohlanwal, Noorpur Bhatta A1, Yohanaabad–2 and GCU Lahore from April to September, 2003. The samples were collected from living rooms, stores, animal sheds and wash rooms of all localities using aspirator (WHO, 1992). Total sample size was 2000 (400 individual from each locality).

The identification was made by using Fauna of British India and specific M- shape band pattern on abdominal segments. Batches of mosquitoes collected were divided into two groups. One group of female mosquitoes was subjected to bioassay tests and the second was conserved in a freezer at –20°C for further biochemical estimation of esterases and polyacrylamide gel electrophoresis (PAGE).

Adult bioassays

Blood-fed females (n = 25) and males (n = 25) of *C. quiquefasciatus* were used for the bioassays (WHO, 1981). Mosquitoes collected from each locality were exposed for 1 h to 5% of deltamethrin impregnated papers using WHO standard exposure tubes (WHO, 1992) and then transferred to holding tubes (tubes without impregnated papers) to record mortality after 24 h.

Three replicate tests were performed for same concentration (5%) Mortality of mosquitoes in control and treated papers was recorded after 24 h post exposure.

Biochemical estimation of esterases

Esterase activity was measured in homogenates of single adult mosquito (n = 25). Each adult was homogenized in 50 ul of 40 mM phosphate buffer, pH 7.0, containing 0.01% (w/v) of Triton x-100 (Georghiou and Pasteur, 1978). The homogenate was centrifuged at 13000 rpm for 5 min. Supernatant was used in assay with alpha naphthyl acetate (substrate A) and beta naphthyl acetate (substrate B). The reaction mixture contained 30 ul homogenate, 60 ul substrate solution (0.1 M), 100 ul SDS (5%) and 1440 ul phosphate buffer. After the incubation period of 30 min at 37°C, optical density was recorded at the wave length of 620 nm for alpha naphthyl acetate (substrate A) and at 545 nm for beta naphthyl acetate (Substrate B). Amount of esterases was calculated by converting the optical density values into ng/ml of esterases using the standard curves which were prepared using different concentration of alpha and beta naphthol (Figures 1a and b).

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide Gel Electrophoresis was performed to separate different types of alpha and beta esterases in different populations of *C. quinquefasciatus*. Two concentrations of gel were used in PAGE that is, separating gel and staking gel. Clean gel plates were clamped firmly after placing the spacers on the two sides of the gel plates. The plates were surrounded by gas kit on the three sides to prevent any leakage. Separating gel (8%) was prepared by dissolving 9.7 ml distilled water, 5.3 ml acrylamide and bisacrylamide (30%), 5 ml tris buffer (P^H 8.8), 0.2 ml SDS (10%), 0.2 ml ammonium per sulphate (10%) and 0.012 ml TEMED. This solution was poured in space between two glass plates. Staking gel was prepared by mixing 3.4 ml distilled water, 830 ul acrylamide and bisacrylamide (30%), 630 ul tris buffer (pH 6.8), 50 ul SDS (10%), 50 ul ammonium per sulphate (10%) and 5ul TEMED.

Staking gel was poured over separating gel and a comb was inserted into staking gel to make the wells. Comb was removed after polymerization of gel. From each locality 15 mosquitoes were taken randomly and crushed in 300 ul phosphate buffer. The homogenate was centrifuged at 13000 rpm for five min. From supernatant 100 ul of the sample was mixed with 100 ul alpha/beta naphthyl acetate. After the incubation of 35 min at 37 °C, 150 ul sample buffer was also added to the sample and heat shock was given to the sample for five minutes. Gel plates were adjusted in vertical position to the gel apparatus and electrode buffer was added in both upper and lower tanks of the gel apparatus. The gel was run at 150 volts with continuous cooling at 4°C until the dye reached the lower plates. Power supply was disconnected and gel was taken out of the plates and stained with fast blue RR stain for

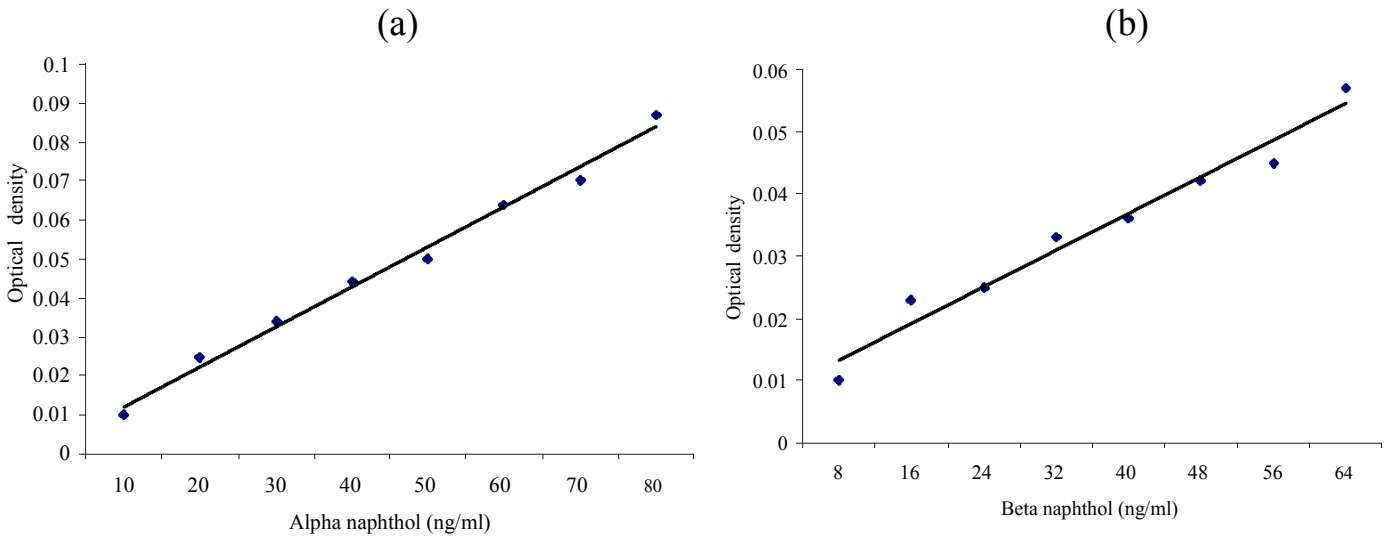

Figure 1. Standard curve for alpha nephthol (a) and beta nephthol (b).

ten minutes. Destaining was done to remove the extra stain. When bands of esterases became clear, gel pattern was studied. The procedure was repeated until the esterases band and background intensity were of sufficient quality.

Statistical analysis

Fisher's exact test was used to compare the mortality rate at different time intervals. Paired t-test was used to compare the mortality in male and female mosquitoes.

Levels of esterases in different populations of *C. quinquefasciatus* were compared using ANOVA. In order to analyze biochemical data, paired t- test was used to compare the level of esterases in male and female mosquitoes using Minitab Software (version 13.3)

RESULTS

Results of bioassay tests indicated that populations of *C. quinquefasciatus* were susceptible to WHO recommended dose of deltamethrin 5% giving 100% mortality, recorded 24 h after exposure. Mortality response of *C. quinquefasciatus* was higher in first 12 h post exposure as compared to earlier hours in all populations (Figure 2). Male specimens from all populations showed significantly higher mortality during the first 12 h than females ($t = 5.31$; $p < 0.001$).

One-way ANOVA performed to compare the levels of esterases in five different populations of *C. quinquefasciatus* showed statistically significant variation ($F_{4, 96} = 4.38$; $p = 0.015$). Highest level of esterase activity was recorded in GC University population while lowest in the population of Jaman. It was also observed that three populations (that is, Yohanabad-2, GC Uuniversity Lahore and Noorpur Bhatta A1) had high esterase activity against alpha naphthyl acetate (Substrate A) while two populations (that is, Mohlanwal and Jaman) had high esterases activity against beta naphthyl acetate (Substrate B) . Moreover, all five populations displayed a large variation in the levels of both estrases A and B. Females were found to have significantly higher level of esterase A ($t = 5.22$; $p < 0.001$) and esterase B ($t = 4.99.22$; $p < 0.001$) activity than males.

Only one band (A1) was observed for esterase A in all five populations (Figure 3a). Band A1 was highly intensified in GC University population while less intensified in Jaman population. For esterase B band pattern was similar in Yohanabad-2 and GC University populations (Figure 3b). In these two populations three bands (B1, B2 & B3) were recorded (present at the same position). In the population of Noorpur Bhatta A1, Mohlanwal and Jaman two bands were observed. Third band (B2), which was present in Yohanabad-2 and GC University populations, was absent in these three populations.

DISCUSSION

Several studies have reported the development of resistance against pyrethroids in *C. quinquefasciatus* in different parts of the world such as South America, Sudan, Sri Lanka, Nigeria, Burkina Faso, Egypt, Guatemala, USA, Turkey and Syria (Malcolm, 1988; WHO 1992a). However results of the bioassays of present study showed that individuals of all populations were suscep-tible to the recommended dose (5%) of deltamethrin. Significant variation was recorded in esterase levels in

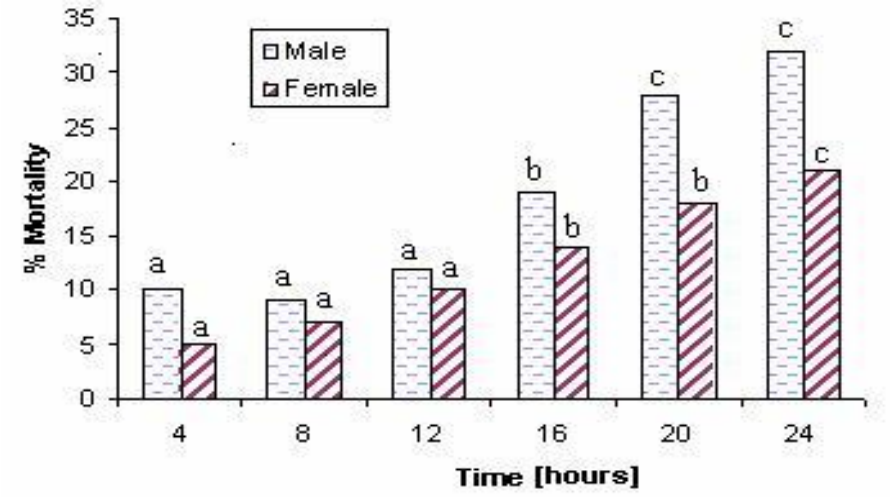

Figure 2. Percentage mortality of *Culex quinquefasciatus* after one-hour exposure to Deltamethrin (combined for five populations).
Note: Bars in the figure with different superscripts are showing significant difference in percentage mortality.

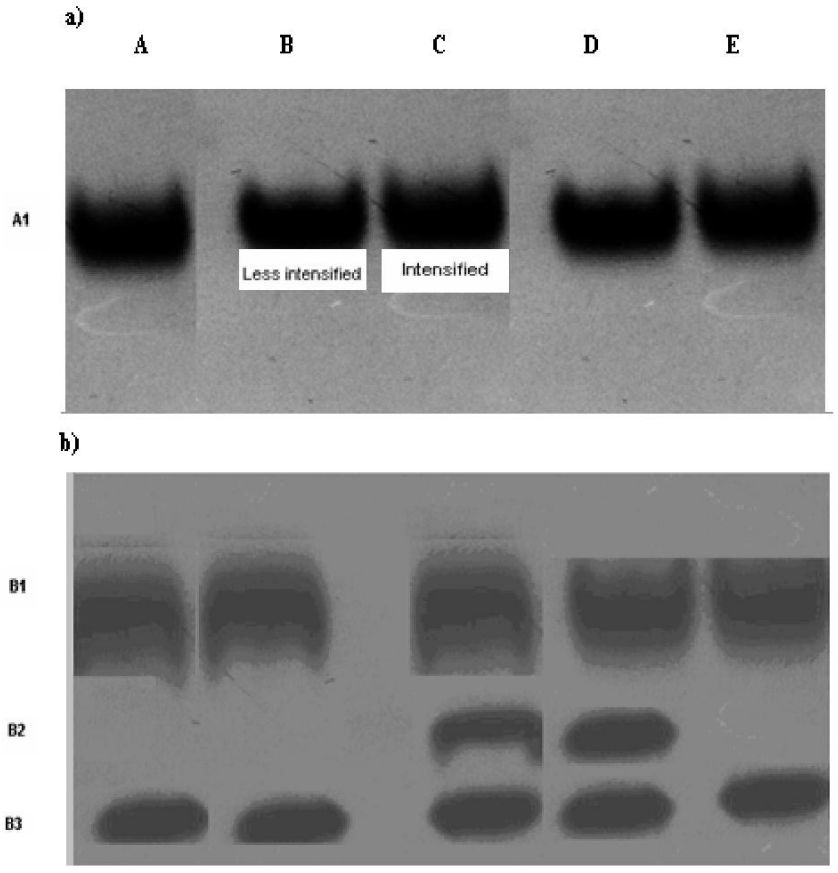

Figure 3. Polyacrylamide gel of *Culex quinquefasciatus* showing esterase A (a) and esterases B.
Note: A= Mohlanwal, B = Jaman, C = GCU Lahore, D = Yohanaabad–2 and E =. Noorpur Bhatta

five different populations of *C. quinquefasciatus*. This result is in accordance with the findings of Pasteur et al. (1981), who observed similar variation in quantity of esterases in field-collected mosquitoes. Alpha naphthyl acetate was hydrolyzed faster than beta naphthyl acetate by mosquito homogenates of Yohanabad-2, GC University Lahore and Noorpur Bhatta A1, indicating the predominance of esterases A in these populations. However mosquito homogenates of Mohlanwal and Jaman populations hydrolyzed the beta naphthyl acetate faster than alpha naphthyl acetate which is an indication for the dominance of esterases B. Increased levels of esterases B is an indication of organophosphates insecticide resistance among the *Culex* mosquitoes as described by Georghiou and Pasteur (1978).

GC University was never sprayed with pyrehroid and showed high levels of esterases A. This might be due to the fact that GC University is located in the central Lahore, which is highly polluted area where an intense traffic rush, emits a large number of chemical particles. Moreover, large numbers of chemicals are used in research work in the University. Such as insecticides (all types) to check their effects on fish, spiders and mice and other common laboratory chemicals. High levels of esterases A1 might be due to exposure of these chemicals. Esterases dependent cross-resistance between OPs and pyrethroids has been detected in several insect species (Hsu et al., 2004) . In OP resistant *M. domestica* and *Culex* mosquitoes, the esterases responsible for cross-resistance are also thought to be involved in pyrethroid hydrolysis (Soderlund and Bloomquist, 1990).

High levels of esterase B in Jaman and Mohlanwal may be due to higher exposure of organophosphate insecticides, which are widely used in these areas in agriculture practice. Esterase A is produced mainly against pyrethroids in *C. quinquefasciatus* and esterase B against organophosphates (Mouches et al., 1987). High levels of esterases A and esterases B in female *C. quinquefasciatus* may be due to these esterases being sex linked, with females receiving high doses and males receiving fewer doses because life span of male is very short. This result differ from the results of Krafsur and Ernst (1986) which showed that only esterases B levels are high in female but not A. Some studies have shown that both esterases A and B are high in females (Georghiou and Pasteur, 1978; Rees et al., 1985).

Highly intensified band in GC University population and less intensified band in Jaman population suggested that former population has high level of esterases A and later population low levels of esterases A. Bisset et al. (1990) reported two types of esterases A in *C. quinquefasciatus* from Cuba. For esterases B three bands (B1, B2 & B3) in Yohanabad-2 and GC University, while two bands in Noorpur Bhatta A1, Mohlanwal and Jaman were recorded. Third band (B2), which was present in Yohanabad-2 and GC University populations, was absent in these three populations (that is, Noorpur Bhatta A1, Mohlanwal and Jaman were recorded). Bisset et al. (1991) also reported three types of esterases B in *C. quinquefasciatus* from Cuba. Result the of present study are also in contrast with the result of Bull et al. (1988) who found greater esterases B in pyrethroid-resistant populations. This could be interpreted to mean that this esterase is involved in pyrethroid resistance but according to present study esterase A is involved in pyrehroid resistance because those populations which have greater exposure to pyrehroid showed high values of optical densities with alpha naphthyl acetate.

It is concluded that although resistance against recommended dose of deltamethrin has not developed in *C. quinquefasciatus* in Lahore district but tolerance level has become high in GC University, Yohanabad and Noorpur Bhatta population, which may lead to resistance in future. Males have low levels of esterases than females. There is only one type of esterase A in all populations while 2-3 types of esterases B. Esterases A are produced against pyrethroids and esterases B against organophosphates.

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