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Susceptibility of *Culex quinquefasciatus* (Diptera: Culicidae) to malathion in Sargodha district, Pakistan

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ABSTRACT

The present study was designed to evaluate the susceptibility of *Culex quinquefasciatuns* against malathion in Sargodha district of Pakistan. The activities of detoxifying enzymes *i.e.*, *β*-esterases, GSTs and monooxygenases were also estimated. Our results revealed 100 percent mortality in insecticide treated groups after 24 hours of exposure. There was no difference in the activities of insecticide detoxifying enzymes between control and treated groups. We concluded from our work that malathion is still effective against *C. quinquefasciatuns* in the area for control of mosquito population.

Keywords: *C. quinquefasciatuns*; Susceptibility; Esterases; Glutathione S-Transfereases; Malathion

1. INTRODUCTION

Mosquitoes are vector agents that cause diseases by transmitting the virus and parasite from one person to another. Mosquito-borne diseases are responsible for significant global morbidity and mortality, and are disproportionately affecting children and adolescents [1]. *Culex* mosquitoes, especially *C. quinquefasciatus*, are the chief vectors of *Wuchereria bancrofti* that cause a disease known as bancroftian filariasis. This disease is common in many regions of the world including the Middle East and Eastern Mediterranean countries [2]. *C. quinquefasciatus* may also cause protozoan, viral, parasitic and helminthic diseases.

Insecticides are considered as the most important components in the global mosquito control efforts [3], but due to their injudicious use, *Culex* mosquitoes have developed resistance against them [4]. More than 100 mosquito species are known to have developed resistance to one or more insecticides. Resistance to pyrethroids and organophosphates has been found in *C. quinquefasciatus* [5,6].

The insecticide detoxifying enzymes have been described to confer insecticide resistance in mosquitoes which are esterases, cytochrome P-450-dependent monooxygenases and glutathione *S*-transferases [7]. Esterases are mostly involved in detoxification of organophosphates and carbamates [8]. Glutathione S-transferases are important family of multifunctional enzymes [9]. The monooxygenases P450 are phase-I metabolic enzymes [10] and are considered as the only enzymes to oxidize insecticides in insects.

For the successful implementation of mosquito control in a specific area, it is necessary to assess their resistance status in that area. The aim of present study was to conduct the susceptibility tests of *C. quinquefasciatus* against malathion, so the resistance status of *C. quinquefasciatus* in the study area may be determined. Activity of insecticide detoxifying enzymes was also determined in insecticide treated and control group.

2. MATERIALS AND METHODS

Samples were collected from University of Sargodha, Punjab, Pakistan by using aspirator. Only blood fed female mosquitoes were used for study. Bioassays were performed to determine susceptibility of *C. quinquefasciatus* against malathion. Three concentrations of malathion insecticide [*i.e.*, recommended field dose (1.5 µl/500 ml), 3/4th of recommended field dose (1.12 µl/500 ml) and 1/2 of recommended field dose (0.75 µl/500 ml) were used]. For residual bioassay specimens were divided into control and treated groups (n = 30 in

each group). Treated group was exposed to insecticide impregnated filter paper, for one hour and then transferred to clean holding jar. Control group was exposed to water impregnated filter paper. Mortality was observed at discrete intervals for 24 hours. The bioassay tests were repeated thrice.

In order to evaluate the role of esterases, glutathione-S-ransferases and Monooxygenases in insecticide resistance, mosquitoes were exposed to sub-lethal dose of malathion for one hour and then shifted to clean jars. After 24 hours of exposure, survivors were frozen at −20˚C for 15 - 20 minutes in order to immobilize them, and then their wings, legs and abdomen were removed. Rest of the body was homogenized in 400 µl of chilled phosphate buffer (0.1 M, pH 7.0) containing 0.01% (w/v) of triton X-100. The crude homogenate was centrifuged at 13,000 rpm for 5 minutes. The supernatant was collected and used further as enzyme source for biochemical estimation of non-specific esterase (*β*-esterases), glutathione-S-transferases (GST) and monooxygenases.

To measure the activity of non-specific esterases method described by Baker *et al*. [11] was followed. B-naphthyl acetate (Substrate B) was used as substrate. The activity of Glutathione-S-transferases towards 1-chloro-2, 4-dinitrobenzene (CDNB) was estimated according to methods elaborated by Habig *et al*. [12]. The activity of monooxygenases was determined by the method described by Vulule *et al*. [13]. To compare the activity of enzymes between control and insecticide treated group two sample t-tests was applied using Minitab 13.2.

3. RESULTS

C. quinquefasciatus population was found susceptible to the all tested concentartions of malathion. After 24 hours of exposure 100 percent mortality of mosquitoes (all mosquitoes in a group died treated with each dose) was observed at each concentration. However, no mortality was recorded in control group (**Table 1**).

When the activity of Esterases between control and treated groups were compared, non-significance difference was observed (df = 11; T-value = −0.74; P-value = 0.474, **Figure 1**). Although the activities of Glutathione-s-transferases and Monooxygenases in the treated group were higher but statistically difference was non-significant (df = 18; T-value = −0.40; P-value = 0.697 for Glutathione-s-transferases and df = 11; T-value = −1.711; P-value = 0.148 for Monooxygenases) as depicted in

Table 1. Mortality in *C. quinquefasciatus* against different concentrations of malathion.

Concentraions (µl/500 ml) of malathion	Number of musquitoes died at different time intervals Total moqquitoes in each group were 30.				
	4 hrs	8 hrs	12 hrs	16 hrs	24 hrs
0.00 (Control)	0	0	0	0	0
0.75	20	23	24	26	30
1.12	25	25	25	25	30
1.5	25	28	28	28	30

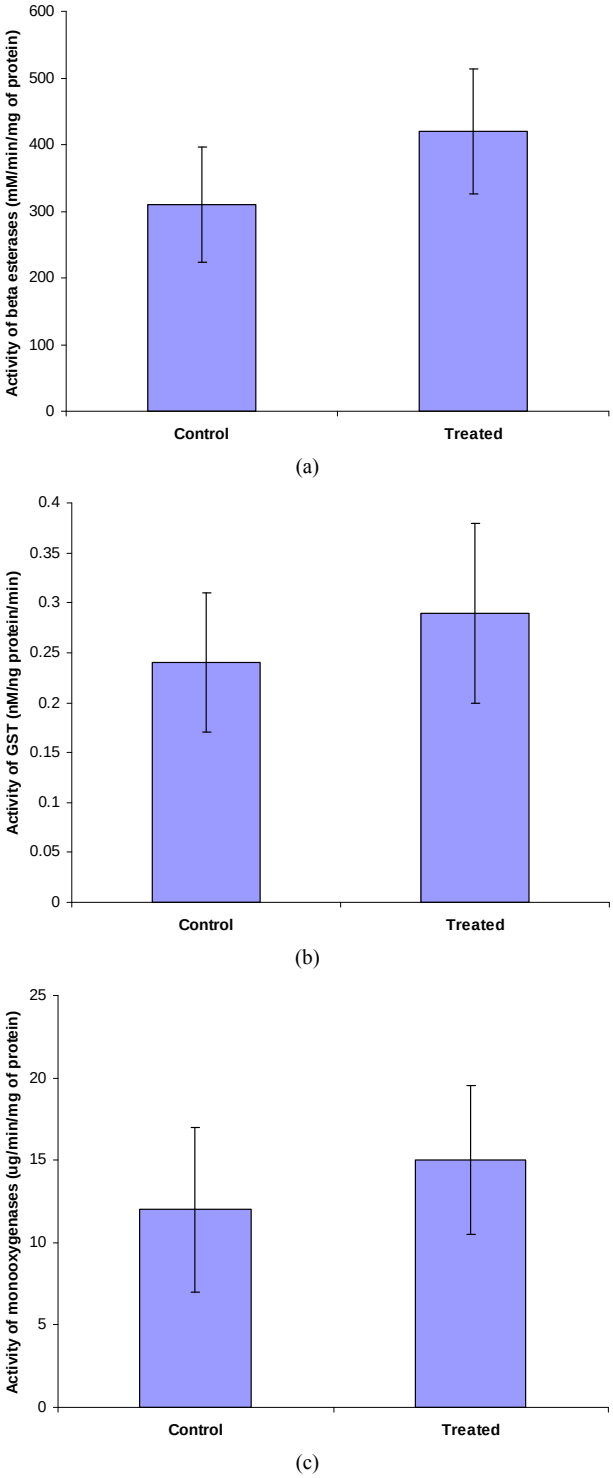

Figure 1. Activities of esterases (a) Glutathione-s-transferases (b) and Monooxygenases (c) in control and treated group.

Figure 1.

4. DISCUSSION

Mosquitoes are the major public health pests and are vectors for many diseases, such as malaria and West Nile Virus [14]. Various methods are being used by researchers to control the mosquitoes. Insecticides are frequently used to control the mosquitoes, but over time these can build up a resistance to insecticides [8].

Results of present study showed malathion to be the potent insecticide to produce a high level of mortality in *C. quinquefasciatus*. Duran and Stevenson [15] also reported malathion susceptibility in *C. quinquefasciatus*. However, these results are contrary to Hamdan *et al*. [16] who reported development of resistance in larvae of *C. quinquefasciatus* against malathion in Malaysia. Kumar *et al*. [17] also reported the malathion resistance in *C. quinquefasciatus* from India. Difference among our findings and those, who found resistance in *C. quinquefasciatus* against malathion, might be due to insecticide usage profile.

Resistance development is a slow process which takes several years and successive generations to set up. It also depends upon the dosage and frequency of insecticide applied [18]. According to the information collected, University of Sargodha is not sprayed with insecticides regularly. However, the use of insecticides in Laboratory for research purpose is common but this usage is not sufficient to develop resistance in insects. Along with insecticide usage profile, there are many other co-factors for development of insecticide resistance, such as temperature, humidity [19] and rainfall [20].

Biochemical methods were used to detect the possible resistance mechanism in insects. Our results from biochemical estimation of enzymes showed that the activities of non-specific esterases, GSTs and monooxygenases among treated and control groups are not different statistically. Our result of biochemical estimation is correlated with bioassay. Malathion caused high mortality in mosquitoes in the study area and insect's enzymatic detoxification pathways have also not been activated. So, we concluded from the present study that malathion is still effective in area for control of mosquito population.

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