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Determination of homozygous susceptible strain in *Culex quinquefasciatus* (Say), using single raft sib-selection method

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Abstract. The standard laboratory strain was found to be heterozygous for susceptibility. Hence, an attempt was made to obtain a homozygous susceptible strain in *Culex quinquefasciatus* (Say) using single raft sib-selection method. Lab-bred females of *Cx. quinquefasciatus* from insectariums, Unit of Medical Entomology were used in the experiment. After blood feeding *Cx. quinquefasciatus* mosquitoes laid eggs in raft form, ten rafts selected randomly for the test. Each egg raft was introduced into a plastic tray from number one to number ten. Twenty-five third stage larvae from each tray were exposed to 17.5µl from 500mg/l malathion in a paper cup label number 1 to number ten. In the bioassay, which had 100% mortality, the respective larva in that particular tray was bred to adult stage for the following generation. Less than 7days old female mosquitoes that emerged from F_0 were used in the test. The F_0 and the subsequent adult and larval stage generations were subjected to adult and larval bioassay. After selection for about 10 generations, a homozygous susceptible strain in *Cx. quinquefasciatus* was obtained.

INTRODUCTION

Culex quinquefasciatus mosquitoes are worldwide nuisance biting pests, vectors of urban filariasis, Japanese encephalitis and are one of the vector mosquito species most studied for insecticide resistance. Throughout most of its range, the female feeds intensely and actively only at night and causes nuisance (Richard & David, 1959; Selvi *et al.*, 2007). They breed and thrive abundantly in stagnant polluted water. In some countries, their breeding sites have been sprayed with organophosphorous insecticides and this has created the problem of resistance development (Nazni *et al.*, 1998). Study conducted by Leicester in 1908 stated that *Cx. quinquefasciatus* could be the vector of urban filariasis in Malaysia (Nazni *et al.*, 2005).

Insecticides play a central role in controlling major vectors of diseases and have been used for a very long time. Studies to detect incipient resistance in the field, and its mechanisms, are very important to design effective strategies to avoid resistance development (González *et al.*, 1999). Hidayati *et al.*, (2005), observed that the resistance development rate in *Cx.quinquiefasciatus* is more rapid to malathion and permethrin compared to that in *Ae. aegypti* and *Ae. albopictus*. Nazni *et al.*, (2000) also reported that larvae of *Cx.quinquiefasciatus* are more resistant compared to those of *Aedes* species towards malathion and temephos.

Insect resistance to insecticide is an increasing problem. Resistance genes in insect can be passed from one progeny to another over a period of several generations. Resistance develops more quickly under heavy doses of insecticides or very frequent applications of insecticide. The type of insect pest makes a difference on how fast resistance can develop. Contrary to social

insects, such as ants, where resistance is seldom a problem since usually only the queen is reproductive, each individual mosquito is reproductive and can allow resistance to develop more quickly. The time needed to become a reproductive adult also determines the development of resistance.

The objective of this study was to obtain a homozygous susceptible strain in *Cx. quinquefasciatus* (Say) using single raft sib-selection method. By using the method, the susceptible mosquitoes were isolated and reared for subsequent generations until pure susceptible strain was obtained.

MATERIAL AND METHODS

Adults of *Cx. quinquefasciatus* F_{623} were taken from laboratory strain and after blood feeding they were allowed to lay eggs. The larvae, which emerged, were named as F_0. The F_0 and the subsequent adult and larval stages in continuous generations were subjected to larval and adult bioassay. Malathion was used in both larval and adult bioassay.

The sib-selection procedure was conducted as described by Nazni *et al.* (1998). In the larval bioassay procedure, malathion with 93.3% active ingredients obtained from Cynamide was used and the insecticide was diluted in ethanol to obtain a concentration of 500mg/l. 17.5µl from this stock was used in the larval test. Larvae were tested for susceptibility status by the WHO standard bioassay methods (WHO, 1981b). After blood feeding, *Cx. quinquefasciatus* mosquitoes laid eggs in raft form, of which ten were selected randomly for the test. Each raft of eggs was introduced into plastic tray from number one to number ten. Twenty-five third instar larvae from each tray were used in the larval bioassay test. The insecticides were added and mixed into the 250ml water in a paper cup, which was labelled from number one to number ten and left for 10 minutes before adding the larvae. Larval mortality was recorded after 24 hours of exposure. Moribund larvae, if any, were counted as dead.

In adult bioassay, less than 7 day-old female mosquitoes were used. Three replicates, with 15 females per replicate were exposed to 5% malathion impregnated papers for 1 hour exposure period using standard diagnostic WHO Test Kits procedure (WHO, 1981a). Exposure test kits consisting female mosquitoes were covered with black cloth to ensure that they rest on the impregnated papers. Mortality in mosquitoes was recorded at every five minutes until the end of exposure period. Right after the defined time exposure, mosquitoes were transferred to clean tubes and were provided with cotton soaked in sugar. The test mosquitoes were held for a 24 hours recovery period and the mortality was recorded. Percentage mortalities were calculated for each exposure time and the mortality data were analyzed by probit analysis (Finney, 1971), using a computerised program of Raymond (1985). Resistance ratio (RR) was calculated by comparing LT_{50} of each generation with LT_{50} of F_{10} generation.

RESULT AND DISCUSSION

The larvae and adults have been selected for 10 generations with malathion. Data on the mortality of *Cx. quinquefasciatus* larvae in sib-selection method is shown in Table 1. It was observed that after 5 generations of sib-selection a pure homozygous strain was obtained as shown 100% mortality in the other five subsequent generations. LT_{50} for adult bioassay against malathion is presented in Table 2 and Figure 1. In LT_{50} value of adult *Cx. quinquefasciatus* to their respective generations of selections, the resistance level was decreasing at each generation and showed that the resistance in F_{10} decreased by 1.81 fold in adult when compared to F_0. This study has shown that larval stage more rapid decreases their resistance level compared to adult. After 5 generations, larval stages give 100% mortality but in adult stage, it still shows resistance level at a low frequency.

Table 1. Percentage of mortality value against laboratory selected *Cx. quinquefasciatus* larvae in different generations with sib-selection test

Sample	% of mortality larvae *Cx. quinquefasciatus* after 24 hours										
	F_0	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
1	96	100	100	100	100	100	100	100	100	100	100
2	100	98	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100	100	100
4	100	100	98	100	100	100	100	100	100	100	100
5	100	100	100	100	98	100	100	100	100	100	100
6	100	98	100	100	100	100	100	100	100	100	100
7	100	100	100	100	100	100	100	100	100	100	100
8	98	100	100	98	100	100	100	100	100	100	100
9	98	96	100	100	100	100	100	100	100	100	100
10	100	100	98	100	100	100	100	100	100	100	100

Table 2. LT_{50} value and linear regression against laboratory selected C*x. quinquefasciatus* adults

Generation	LT_{50} (minute)	Linear regression	*RR
0	34.2155 (32.8826 – 35.5588)	y = 5.47x – 58.07	1.81
1	33.5809 (32.0013 – 35.1575)	y = 5.05x – 53.25	1.76
2	34.9471 (33.2482 – 36.7172)	y = 5.41x – 57.43	1.85
3	30.3130 (28.4477 – 32.3220)	y = 4.97x – 35.40	1.60
4	25.9798 (24.0353 – 27.9817)	y = 2.42x – 22.58	1.37
5	25.3689 (23.6604 – 27.1548)	y = 3.44x – 34.25	1.34
6	26.2467 (24.4848 – 28.0193)	y = 3.22x – 31.78	1.39
7	25.8169 (24.2628 – 27.3701)	y = 3.60x – 36.08	1.36
8	22.3362 (21.1123 – 23.5396)	y = 4.34x – 44.30	1.18
9	21.6833 (20.3641 – 23.0119)	y = 3.80x – 38.09	1.15
10	18.9145 (17.5670 – 20.2564)	y = 3.23x – 31.48	–

*RR = Resistance ratio

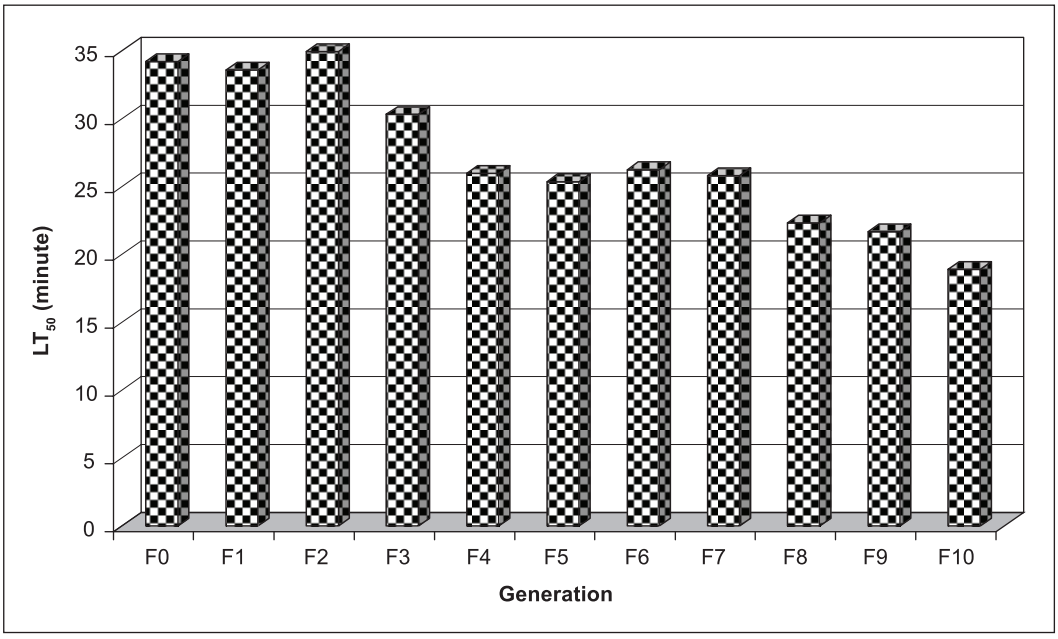

Figure 1. Comparison of LT_{50} value of *Culex quinquefasciatus* laboratory strains in different generations with sib-selection.

Resistance is genetically based and any decrease in the susceptibility of population to an insecticide, is due to changes in the allele frequencies in a population. In *Cx. quinquefasciatus*, under high insecticidal concentrations, the evolution of resistance was shown by gene amplification by three or more alleles. In insects, it was expressed by an autosomal recessive or incomplete recessive trait by one or few gene loci (Poopathi *et al.*, 1999). Insecticide resistance are more likely to be associated with biochemical basis of resistance and even small genetic changes can alter the target's shape, so the protein in the mosquito is no longer susceptible to the insecticide (Selvi *et al.*, 2007).

According to Darwinian theory, gene(s) responsible for insecticide resistance is(are) existing in a small segment of the population. The gene(s) will be activated on exposure to insecticidal pressure. The speed and degree of development of resistance depends on the frequency of resistance gene(s) in the population, the type of gene, which is responsible for resistance, the insecticide dosage applied, and the frequency of application (Nazni *et al.*, 1998). These studies showed that once the resistance population were not exposed to any insecticide, the level of resistance would decrease consistently. Since it has shown that the *Cx. quinquefasciatus* mosquitoes are able to decrease level of resistance to insecticide, it would be valuable if the insecticides are used on rotational basis to slow down the selection pressure of insecticides against mosquito species.

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