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Original Article

Insecticide Susceptibility Status of *Culex tritaeniorhynchus* Giles, Vector of Japanese Encephalitis in Delhi

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SUMMARY: Laboratory studies were carried out to ascertain the current susceptibility status of adult and larval stages of the *Culex tritaeniorhynchus* mosquito, vector of Japanese encephalitis, to various insecticides used under public health programs in India. The present study revealed that exposure of adult mosquitoes to diagnostic concentrations of DDT - 4.0%, malathion - 5.0%, fenitrothion - 1.0%, and propoxur - 0.1% could induce only 50.0, 10.0, 15.0, and 5.0% mortality, respectively, indicating that the species was resistant to all of these insecticides. The LT_{50} and LT_{95} values calculated using diagnostic concentrations of DDT, malathion, fenitrothion, and propoxur were found to be 56.4 and 136, 138 and 272, 185 and 258, and 187 and 249 min, respectively. However, when adult mosquitoes were exposed to the diagnostic concentration of synthetic pyrethroids, viz., deltamethrin -0.025%, permethrin - 0.25%, and lambdacyhalothrin - 0.1%, 100.0% mortality was observed, indicating that the species was highly susceptible to these adulticides. Larval susceptibility tests carried out using diagnostic dosages of DDT- 0.008, temephos- 0.02, fenthion- 0.008, fenitrothion- 0.125, and malathion- 0.005 mg/l failed to induce any mortality, indicating that larvae were resistant to these larvicides. The LC_{50} and LC_{90} values calculated for commonly used larvicides, viz., temephos and fenthion, were 0.1511 and 1.9098, and 0.6151 and 2.395 mg/l, respectively. Increase in tolerance level were estimated at 95.5- and 299.4-fold when these LC_{90} values were compared with diagnostic dosages of temephos and fenthion, respectively.

INTRODUCTION

Culex tritaeniorhynchus Giles is one of the vector mosquito species largely involved in the transmission of Japanese encephalitis in Southeast Asia, notably in India. It has been reported to be a widely distributed species in the Indian Subcontinent. This vector mosquito species has not only been exposed to the larvicides/adulticides used under public health programs but to the insecticides used for the control of agricultural pests, particularly in the case of the paddy crop, as the mosquitoes have outdoor resting and feeding habits. Therefore, it is essential, before contemplating use of any insecticide either as an indoor residual spray, application through ULV (ultra low value) spray or thermal fogging, or implementation as a larvicide, that the susceptibility status of adult and larval stages of the vector mosquito species be ascertained in order to ensure the effectiveness of vector control measures.

The present report deals with the results of laboratory bioassay tests carried out during 1999 to ascertain the current susceptibility status of larvae and adults of *Cx. tritaeniorhynchus* mosquito to various insecticides commonly used under public health programs in India for purposes of controlling vector mosquito species.

MATERIALS AND METHODS

The biological material was collected at the National Zoological Park, Delhi from various channels and moats around animal enclosures, where stagnant water collects with free water floating vegetation favorable for the breeding of *Cx. tritaeniorhynchus* mosquitoes. Mosquitoes at immature stages were collected from different breeding sites and transported to the laboratory, where samples were separated by genus. Late third instar larvae of *Cx. tritaeniorhynchus* were used for larval susceptibility tests. Fourth instar larvae were kept until emergence, for purposes of adult susceptibility tests.

Adult Bioassay: The *Cx. tritaeniorhynchus* adults emerged from the field-collected material in a mosquito cage (12 by 12 by 12 in.) and were allowed to feed on 10% glucose solution soaked into a cotton pad. Thereafter, three day-old female adult mosquitoes were exposed to a diagnostic concentration (concentration that has a high probability of killing all susceptible populations [1]) of DDT- 4.0%, malathion -5.0%, fenitrothion -1.0%, propoxur - 0.1%, deltamethrin - 0.025%, permethrin - 0.25%, and lambdacyhalothrin - 0.1%, respectively. Control experiments were run in parallel. For each run of insecticide, four replicates were run, each run containing 20 female glucose-fed mosquitoes. After the recommended exposure period (1-3 h) to insecticide-impregnated papers (12 by 15 cm), mosquitoes were transferred to recovery chambers provided with cotton pads soaked in 10% glucose solution as food. Mortality counts were made after a 24-h recovery period (2). The experiments were conducted at an ambient temperature of 27 ± 1°C and relative humidity of 75 to 80% under laboratory conditions (3,4).

To determine the lethal time (LT) inducing 50 and 95% (LT_{50} and LT_{95}) mortality, respectively, adult mosquitoes were exposed at intervals of 15 min (ranging from 15 min to 4 h 45

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min) to the diagnostic concentration of those insecticides to which they had shown resistance; viz., DDT, malathion, propoxur, and fenitrothion.

Larval Bioassay: Field-collected third instar larvae of *Cx. tritaeniorhynchus* were identified, separated, washed in tap water to remove debris, and kept for observation for 2 h to weed out the dead and moribund larvae. Healthy larvae were then exposed to the diagnostic dosages of DDT - 0.008, temephos - 0.02, fenthion - 0.008, fenitrothion - 0.125, and malathion - 0.005 ppm, respectively. Tests were carried out by placing 25 larvae in a 500-ml capacity beaker containing 250 ml of water and the required dosage of larvicide. For dosage of each larvicide, four replicates were run and control experiments were run in parallel. Mortality counts were made after a 24-h exposure period. To determine LC_{50} and LC_{90} (lethal concentration) values, the technical form of temephos (50% emulsion concentrate) (EC) and that of fenthion (82.6% EC), obtained from Cyanamid Ltd., Mumbai, India and Bayer Ltd., Mumbai, India, respectively, were used.

Bioassay tests showing more than 20% control mortality were discarded and repeated, although, in the control case mortality ranged between 5 to 20%, the corrected mortality was calculated using Abbot's formula (2).

RESULTS

Adult Bioassay: The results of the susceptibility tests carried out using the adult *Cx. tritaeniorhynchus* mosquito are given in Table 1. In adult mosquitoes exposed to diagnostic concentrations of DDT, malathion, fenitrothion, and propoxur, 50%, 10%, 15%, and 5% mortality rates, respectively, were induced, indicating that the species has developed resistance to all four of these insecticides. However, in the mosquitoes exposed to diagnostic concentrations of deltamethrin, permethrin, and lambdacyhalothrin, 100% mortality was induced, which showed that the species is highly susceptible to synthetic pyrethroids.

In order to determine the LT_{50} and LT_{95} values, the adult mosquitoes were exposed to diagnostic concentrations of DDT, malathion, fenitrothion, and propoxur, respectively, for a range of exposure periods. The data obtained were subjected to probit analysis to calculate the regression equation, LT_{50}, LT_{95} values, and heterogeneity (5). The results are given in Table 2. The LT_{50} and LT_{95} values calculated using diagnostic concentrations of DDT, malathion, fenitrothion, and propoxur were found to be 56.4 and 136, 138 and 272, 185 and 258, and 187 and 249 min, respectively. The heterogeneity (P=0.05) was found to be significant, implying that the adult popula-

Table 1. Results of susceptibility tests exposing *Culex tritaeniorhynchus* adult mosquitoes to various insecticides at diagnostic concentrations

Insecticide	Concentration (%)	Exposure time (h)	No. of adults exposed	No. dead (after 24 h)	Mortality (%)	Susceptibility/ Resistant status*
DDT	4.0	1.0	160	80	50.0	R
Malathion	5.0	1.0	160	16	10.0	R
Fenitrothion	1.0	2.0	160	24	15.0	R
Propoxur	0.1	2.0	160	8	5.0	R
Deltamethrin	0.025	1.0	160	160	100.0	S
Permethrin	0.25	3.0	160	160	100.0	S
Lambdacyhalothrin	0.1	1.0	160	160	100.0	S

*S: susceptible (> 98% mortality), VR: verification required (80-98% mortality), R: resistant (< 80% mortality) (2).

Table 2. Dosage mortality response of adult *Culex tritaeniorhynchus* mosquitoes, used to determine LT_{50} and LT_{95} values and heterogeneity

Insecticide	Regression Equation	LT_{50} values (min)	LT_{95} values (min)	Heterogeneity χ^2 (df*)
DDT-4.0%	–7.4997±1.8596x	56.4	136	20.312(6)
Malathion-5.0%	1.8883±2.4165x	138	272	29.1912(5)
Fenitrothion-1.0%	25.9389±4.965x	185	258	17.0607(4)
Propoxur-0.1%	3.8087±3.8682x	187	249	12.2598(4)

*df: degrees of freedom.

Table 3. Results of susceptibility tests carried out using *Culex tritaeniorhynchus* larvae against different larvicides at diagnostic dosages

Larvicides	Diagnostic dosage (mg/l)	No. of larvae exposed	No. dead (after 24 h)	Mortality (%)	Susceptibility/ Resistant status*
DDT	0.008	200	Nil	0.0	R
Temephos	0.02	200	Nil	0.0	R
Fenthion	0.008	200	Nil	0.0	R
Fenitrothion	0.125	200	Nil	0.0	R
Malathion	0.005	200	Nil	0.0	R

*S: susceptible (> 98% mortality), VR: verification required (80-98% mortality), R: resistant (<80% mortality) (2).

Table 4. Dosage-mortality response of *Culex tritaeniorhynchus* larvae to various dosages of temephos and fenthion, used to determine the LC_{50} and LC_{90} values, and heterogeneity

Insecticide	Regression Equation	LC_{50} values (mg/l)	LC_{90} values (mg/l)	Heterogeneity χ^2 (df*)
Temephos	0.9547±0.5052x	0.1511	1.9098	25.5986(5)
Fenthion	0.4582±0.9427x	0.6151	2.395	26.9387(4)

*df:degrees of freedom.

tion tested was heterogeneous.

Larval Bioassay: The results of the larval susceptibility tests carried out using respective diagnostic dosages of DDT, temephos, fenthion, fenitrothion, and malathion revealed that these larvicides failed to induce any larval mortality after a 24-h exposure period, indicating that larvae were resistant to these larvicides (Table 3).

The results of larvae exposed to various dilutions of the technical form of larvicides revealed that the LC_{50} and LC_{90} values estimated for temephos and fenthion were 0.1511 and 1.9098 and 0.6151 and 2.395 mg/l, respectively (Table 4). The heterogeneity (P=0.05) was found to be significant, implying that the larval population tested was heterogeneous.

DISCUSSION

During 1958, the first report on the development of DDT resistance in *Cx. tritaeniorhynchus* mosquito appeared from Okinawa Island, Japan (6). In India, DDT and dieldrin resistance were reported from different parts of the country by several researchers (7,8). DDT resistance in the *Cx. tritaeniorhynchus* mosquito has also been reported from additional countries, including Bangladesh, Benin, China, Nigeria, Korea, Sri Lanka, and Thailand (4). During the present study, this species was also found to be resistant to DDT, and the high LT_{95} values estimated for DDT- 4.0% (136 min) is indicative of development of a high level of DDT resistance in adult *Cx. tritaeniorhynchus*. It appears likely that the use of DDT creates a DDT-containing dust in and around animal enclosures erected to protect the captive animals from other crawling insects at National Zoological Park, and this dust has contributed significantly in precipitating the DDT resistance evident in increased LT_{50} and LT_{95} values.

Malathion resistance in the *Cx. tritaeniorhynchus* mosquito was reported from Japan during 1987 (9). In India, malathion resistance in larval and adult stages of *Cx. tritaeniorhynchus* mosquito has been reported from Arthala, Uttar Pradesh, and Kota, Rajasthan (10,11). Pennington (12) reported a 48.4-fold increase of malathion resistance in the *Cx. tritaeniorhynchus* mosquito larvae within 5 years after the introduction of malathion. Malathion resistance in the *Cx. tritaeniorhynchus* mosquito has been reported from several countries, including China, Japan, Korea, and Sri Lanka (4). The LT_{95} values for malathion, fenthion, and propoxur reported from Kota (Rajasthan) were 123 min, 132 min, and 168 min, respectively, at diagnostic concentrations (11). In the present study, the LT_{95} values recorded for malathion, fenthion, and propoxur were 272 min, 258 min, and 249 min, respectively, which are much higher than the levels reported earlier in *Cx. tritaeniorhynchus*, indicating increasing tolerance to these insecticides.

Temephos resistance in the larvae has been reported from Japan, Korea, China, and Taiwan, and resistance to fenthion has been reported from Japan and Korea (13). Susceptibility tests carried out earlier in Delhi against the larvae of the *Cx. tritaeniorhynchus* mosquito recorded LC_{50} and LC_{90} values for temephos and fenthion of 0.03831 and 0.2214, and 0.02939 and 0.1614, respectively (14). In the present study, LC_{50} and LC_{90} values recorded for temephos and fenthion were found to be 0.1511 and 1.9098, and 0.6151 and 2.395, respectively; much higher than those previously reported. Comparatively, LC_{90} values found in the present study indicate a 95.5- and 299.4-fold increase against the diagnostic dosages of temephos and fenthion, respectively.

The development of DDT and malathion resistance in adults, and temephos and fenthion resistance in larvae, of *Cx. tritaeniorhynchus* mosquitoes warrants strict monitoring of the susceptibility status of this species to various insecticides in Japanese encephalitis prone areas before contemplating the use of these substances for ULV spray/thermal fogging, as a residual spray, or as a larvicide.

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