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Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus*, principal vector of bancroftian filariasis from filaria endemic areas of northern India

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ABSTRACT

Objective: To understand the insecticide resistance status of *Culex quinquefasciatus* Say (Diptera: Culicidae) *(Cx. Quinquefasciatus)* to deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin, DDT and malathion in filarial endemic areas of Uttar Pradesh, India. **Methods:** Insecticide susceptibility assays were performed on wild-caught adult female *Cx. quinquefasciatus* mosquitoes to deltamethrin (0.05%), cyfluthrin (0.15%), permethrin (0.75%), lambdacyhalothrin (0.05%), malathion (5.0%) and DDT (4.0%), the discriminating doses recommended by the World Health Organisation (WHO). **Results:** The data showed that *Cx. quinquefasciatus* is highly resistant to DDT and malathion; the mortality was 28.33% and 27.5%, respectively and incipient resistance to synthetic pyrethroids (deltamethrin, cyfluthrin, permethrin, and lambdacyhalothrin), where mortality ranged from 95.83% in permethrin to 98.33% in cyfluthrin and lambdacyhalothrin. Knockdown times (KDT_{50}) in response to synthetic pyrethroids varied significantly between different insecticides ($P<0.01$) from 31.480 min for permethrin to 21.650 for cyfluthrin. **Conclusions:** The results presents here provide the status report of the insecticide resistance/susceptibility of *Cx. quinquefasciatus* in major filaria endemic areas of northern India.

1. Introduction

Lymphatic or bancroftian filariasis is the predominant infection in the Southeastern Asia[1]. As per WHO estimates, India contributes about 67% of the 700 million endemic populations in the Southeastern Asian region. Of the 60 million persons either harbouring microfilariae (mf) or suffering from clinical manifestations of the disease in the Southeastern Asian region, about 82% are found in India. About 465 million people including 28 million mf carriers and 21 million clinical cases are spread in 13 States and 5 Union Territories in India[2]. Lymphatic filariasis (LF) is a major impediment to socioeconomic development (estimated loss $ 1 billion per annum and is responsible for immense psychosocial suffering among the affected [3,4]. Uttar Pradesh (UP) is one of the major filaria endemic states in northern India, and held the third position in endemicity (14.6%) in India after Bihar (Over 17%) and Kerala (15.7%) (*cf* 4). As per the report of Regional Filaria Training and Research Centre (RFTRC), Varanasi (UP), over 7 600 filariasis patients were examined and treated at the RFTRC in 2006 while the number of patients grew up to 8 000 in 2007 and 9 000 in 2008[5] *Culex quinquefasciatus (Cx. quinquefasciatus)* is the main vector of the parasitic worm *Wuchereria bancrofti*, the agent of lymphatic filariasis throughout the continental Asia. *Cx. quinquefasciatus* is a continuous biting nuisance, mostly for those living close to larval habitats.

Vector control is the key component in disease management. Vector control is also important for diseases like filariasis that are controlled primarily by preventive mass drug administration (MDA). The current strategy to

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eliminate lymphatic filariasis is unlikely to achieve complete elimination of infection if MDA is not supplemented by transmission-control interventions in some areas. The strategy of the national vector control programme is based on the use of insecticides for indoor residual spraying (IRS) and insecticide-treated nets (ITNs) with synthetic pyrethroids. Due to intense selection pressure, mosquitoes are developing resistance against major insecticides used in public health programmes.

Recent information on the susceptibility/resistance status of mosquito species against different insecticides in Uttar Pradesh is scarce. There is a concern that this updated information is needed to ensure that the pattern of insecticide used in this disease endemic area is optimized to avoid increasing resistance that could threaten the sustainability of the vector control strategy. Thus, continued monitoring of insecticide susceptibility and/or resistance status and establishment of a baseline data bank for the areas are of prime importance.

The current study presents the report of the insecticide susceptibility and/or resistance status of adult *Cx. quinquefasciatus* to deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin, DDT and malathion, in filarial endemic areas of Uttar Pradesh, India. This study also assessed the susceptibility of temephos, the major larvicide against *Cx. quinquefasciatus* in the areas. The results are of importance to the development of future insecticide resistance management strategies and will inform the selection of insecticides for mosquito control in these areas.

2. Materials and methods

2.1. Study sites and mosquito collection

The study was carried out in two major filaria endemic districts (Chandauli and Varanasi) of Uttar Pradesh, northern India. Majority of population of these districts is at risk of filariasis. These areas are under regular application of DDT, malathion, deltamethrin, permethrin, and temephos under the vector control programme (malaria, filariasis, Kala-azar, *etc*.) of government of India. As a result, wild *Cx. quinquefasciatus* mosquito is regularly exposed to these insecticides. Due to unavailability of Geographical Positioning System (GPS) during the study, we are unable to provide the exact GPS coordinate of the study sites.

Adult mosquitoes for testing the resistance/susceptibility status of each population were collected by standard entomological techniques and bioassays were performed on wild-caught adult female mosquitoes following the WHO adult test kit method[6]. For testing of larval susceptibility against temephos, blood fed mosquitoes were brought to the central laboratory at National Centre for Disease Control, Delhi and eggs obtained from these females were placed for hatching. Late third instar or early fourth instar larvae of the F1 generation were subjected to the bioassay with diagnostic concentration of temephos (0.02 mg/L).

2.2. Insecticide susceptibility bioassays: Adult

Insecticide susceptibility assays were performed on wild-caught adult female mosquitoes. The age and number of blood feed were unknown and variable respectively for wild caught females, which may slightly influence the bioassay results. Mortality and knockdown resulting from tarsal contact with insecticide-treated filter papers were measured using WHO test kits[6]. The tests were carried out using deltamethrin (0.05%), cyfluthrin (0.15%), permethrin (0.75%), lambdacyhalothrin (0.05%), malathion (5.00%) and DDT (4.00%), the diagnostic doses recommended by WHO. For each of insecticides tested, mosquitoes were divided into batches of 20 per test and exposed to insecticide-treated papers for four hours in the case of DDT (4%) and one hour for other insecticides. The effects of papers treated with only carrier oils were assayed in parallel as a control. At the end of the exposure period, mosquitoes were transferred into tubes with untreated white filter papers (known as holding tubes) and allowed a 24-hour recovery period.

For mosquitoes exposed to DDT and malathion mortality rates were recorded only after the recovery period, and for mosquitoes exposed to synthetic pyrethroids (*eg*., deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin) the numbers knockdown were recorded every 10 minutes for up to one hour during exposure. All mosquitoes were provided with 10% glucose water during the 24-hour recovery period.

2.3. Insecticide susceptibility bioassays: Larvae

Single concentration larval diagnostic tests were conducted[7] in order to separate the susceptible and the resistant individuals. The third and early fourth instar larvae of the F1 generation of the strain were exposed to the diagnostic concentration of temephos (0.02 mg/L). Twenty-five larvae were exposed in 250 mL of water containing the diagnostic concentrations. All the tests were repeated at least three times. Mortality was recoded after 24 hours of the post exposure recovery period. All the experiment was carried out under the laboratory condition at (28 ± 2) ℃ and 70%-80% relative humidity.

2.4. Statistical analysis

Mean mortality of replicates was determined for each insecticide. The WHO criterion for evaluating resistance or susceptibility was used[8]; mortality of less than 80% indicate resistance, while those greater than 98% indicate susceptibility. Mortality between 80%-98% suggest the possibility of resistance that needs to be verified. Analysis of variance (ANOVA) was used to compare knockdown rates after 10 min intervals up to the 1-hour exposure period

between different insecticides tested. The times to 50% knockdown (KDT_{50}) were estimated by regression analysis between percent knockdown and exposure time, using the log-probit method[9]. Descriptive statistical analysis was used to calculate mean, standard deviation, confidence intervals, and chi-square for the samples collected from different study sites and exposed to the diagnostic dose of different insecticides.

3. Results

Results of susceptibility status of *Cx. quinquefasciatus* to diagnostic dose of deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin, malathion and DDT were shown in Table 1. The data shown that *Cx. quinquefasciatus* was highly resistant to DDT and malathion; the mortality was 28.33% and 27.5% respectively and incipient resistance to synthetic pyrethroids (deltamethrin, cyfluthrin, permethrin, and lambdacyhalothrin), where mortality ranged from 95.83% in permethrin to 98.33% in cyfluthrin and lambdacyhalothrin. Adult bioassays were performed in batches of 20 mosquitoes per test, with replicates, and the mean mortality values of replicates being presented in Table 1.

Table 1 also showed the 50% knockdown times, *ie*. KDT_{50}, of *Cx. quinquefasciatus* after continuous exposure to deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin for up to one hour and the percent mortality after 24-hour post-exposure holding period. The species was found to be slightly tolerant of these insecticides, and the ranges of KDT_{50} values were 31.480 min for permethrin to 21.650 for cyfluthrin. Knockdown rates were significantly different between different insecticides (ANOVA, $F = 7.132$, $df = 18$, $P < 0.01$). Knockdown effect of mosquitoes against different synthetic pyrethroids at 10 min intervals up to the 1-hour exposure period was plotted in Figure 1. From Figure 1, it was apparent that permethrin was less effective comparing to other pyrethroids followed by lambdacyhalothrin, deltamethrin, and cyfluthrin.

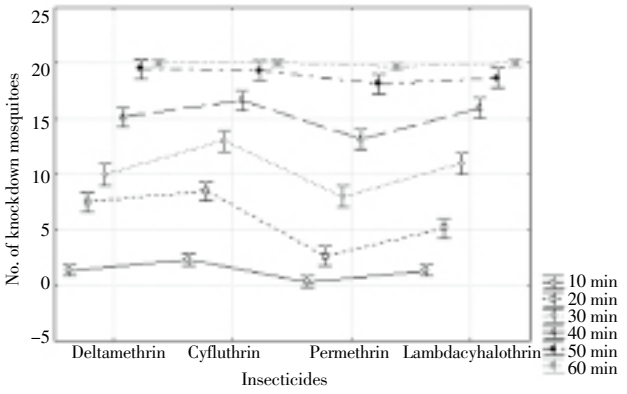

Figure 1. Knockdown rates (10 min time intervals) of *Cx. quinquefasciatus* against different insecticides.
Figure shows the mean knockdown times calculated by the ANOVA (Wilks lambda = 0.011, $F = 7.132$, $df = 18$, $P< 0.01$). Vertical bars denote confidence intervals. Therefore, knockdown rates significantly varies between different insecticides.

Cx. quinquefasciatus larvae were found to be resistant to temephos, the major chemical larvicide used in public health programme in India (Table 1). Only 30% mortality was observed in mosquitoes collected from the study sites (Table 1).

Table 1
Susceptibility and / or resistance status of *Cx. quinquefasciatus* to diagnostic dose of different insecticides.

Insecticides (dose/concentration)	% Mortality (Sample size)	#Mean mortality	KDT_{50}	95% Confidence interval	χ^2 (*df*)
Deltamethrin (0.05%)	96.66 (120)	19.33±0.82	24.855±2.517	17.806-31.210	8.213 (4)
Cyfluthrin (0.15%)	98.33 (120)	19.66±0.82	21.650±1.472	17.488-25.500	3.255 (4)
Permethrin (0.75%)	95.83 (120)	19.16±0.75	31.480±2.022	25.760-36.787	13.926 (4)
Lambdacyhalothrin (0.05%)	98.33 (120)	19.66±0.82	25.701±1.825	20.540-30.456	9.467 (4)
DDT (4.0%)	28.33 (120)	5.67±1.63	ND	ND	NA
Malathion (5.0%)	27.50 (120)	5.50±1.05	ND	ND	NA
Temephos (larvicide) (0.02 mg/L)	30.00 (300)	15.00±2.90	ND	ND	NA

Table shows the mean mortality values (replicate mean) of mortality rates, 50% KDT_{50} in minutes and *Chi*-square (χ^2) value for synthetic pyrethroids (deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin). # Represents the mean mortality of replicates. ND = Not determined; NA = Not applicable. SD = Standard deviation, *df* = degree of freedom.

4. Discussion

The resistance status of *Cx. quinquefasciatus* mosquitoes to multiple insecticides (deltamethrin, cyfluthrin, permethrin, lambdacyhalothrin, malathion and DDT) was investigated in filaria endemic areas of northern India. Based on the WHO criteria for characterizing insecticide resistance/ susceptibility, where susceptibility is defined by mortality rates greater than 98% after 24-hour post-exposure, evidence for high resistance to DDT and malathion was found at the study areas. The mortality after 24-hour post exposures obtained in knockdown bioassays for synthetic pyrethroids suggests incipient resistance to these insecticides; however, further data verification is needed for any strategic conclusion.

In this part of the world, the public health department in

the recent past introduced synthetic pyrethroid-treated bed nets. Knockdown rates at 10 min intervals were significantly different ($P< 0.01$) between different insecticides. There are similar reports for *Anopheles fluviatilis* by James from Orissa[10] and *Anopheles culicifacies* Giles from Tamil Nadu, India[11-14], where delayed knockdown effects were observed, although there were 100% mortality 24 hours post- exposure against 0.05% deltamethrin.

In this study, a high level of DDT and malathion resistance was observed in *Cx. quinquefasciatus*, which may be correlated with the use of DDT and malathion for vector control in these areas for many years. The use of DDT is discontinued in most parts of India due to development of resistance in vector populations. However, it is still being used for control of Kala-azar vector and some parts of northeastern India for malaria vectors. Nevertheless, persistence of DDT in the environment may have resulted in the continued selection for resistance. Sarkar *et al*[4,15] also reported high DDT resistance in *Cx. quinquefasciatus* at army cantonment areas of northeastern India after many years of DDT withdrawal.

Proper management of insecticide resistance of mosquitoes in the wild populations can only achieve effective vector control. There are several methods to delay the onset of resistance that is based on the strategic use of available insecticides, such as the avoidance of using insecticides that simultaneously select resistance to other chemically related insecticides, and the use of a number of insecticides in rotation[16]. Thus, there is a need, not only for continuous monitoring of the status of insecticide resistance and its possible mechanisms in different settings, but also for the assessment of the impact of any observed resistance on the effectiveness of vector control programmes[15]. We believe that adoption of alternative method of vector control like use of biological control, and bioenvironmental methods may help to tackle the problem of insecticide resistance. The data provide baseline information that is essential for monitoring the development of insecticide resistance in northern India.

Conflict of interest statement

We declare that we have no conflict of interest.

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