DIFFERENTIAL TOLERANCE OF TWO MORPHOLOGICAL VARIANTS OF *CULEX TRITAENIORHYNCHUS* (DIPTERA: CULICIDAE), A JAPANESE ENCEPHALITIS VECTOR, TO PYRETHROID INSECTICIDES IN MYSORE, INDIA

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Abstract: Five synthetic pyrethroids, deltamethrin, cypermethrin, permethrin, alpha-cypermethrin, and lambdacyhalothrin, were tested on the larvae of 2 morphological variants of *Cx. tritaeniorhynchus* collected from Mysore City (ground pools) and outside Mysore (paddy fields), in Karnataka State, India. The morphological characters, *ie*, length and width of siphon, siphonal index, comb scale numbers, pecten teeth numbers, length of anal gills and anal gill index of larvae of two *Cx. tritaeniorhynchus* populations were found to be significantly different ($p<0.05$). To elicit further detail of these two variants, pyrethroid bioassays were undertaken. In general, the toxicity ranking of these pyrethroids tested on the *Cx. tritaeniorhynchus* variants from Mysore City was deltamethrin > lambdacyhalothrin > cypermethrin > permethrin > alpha-cypermethrin. However, for the rural variety it was deltamethrin > lambdacyhalothrin > alpha-cypermethrin > permethrin > cypermethrin. Of the 5 pyrethroids tested, deltamethrin was the most effective on both variants. Alpha-cypermethrin and lambdacyhalothrin were respectively 2.17 and 2.09 times more effective on the city variety ($p<0.05$), based on LC_{50} values. The results suggest that, in addition to morphological differences, the 2 tested varieties of *Cx. tritaeniorhynchus* also differ in susceptibility status.

INTRODUCTION

Japanese encephalitis (JE) was first recognized in India in 1955 (Work and Shah, 1956). Several outbreaks of JE have occurred and hundreds of children have died of this disease since then (Dhanda *et al,* 1989). Outbreaks of JE of varying intensity have occurred in Kolar and Mandya districts of Karnataka State since 1977 (Mishra, 1984). From these areas, the JE virus has been isolated from > 10 species of mosquitoes; of these, 5 species, *ie*, *Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. gelides* and *Cx. fuscocephala* are considered important vectors for the transmission of JE. Another report shows that JE virus has been isolated from 16 species of mosquitoes in India, the *Culex vishnui* subgroup being the major vectors, consisting of *Cx. tritaeniorhynchus*, Giles; *Cx. vishnui*, Theobald; and *Cx. pseudovishnui*, Colless (Samuel *et al,* 2000). *Cx. tritaeniorhynchus* is considered to be the most important vector of JE virus in most parts of south-central Asia (Gingrich *et al,* 2001).

Fakoorziba and Vijayan (2003) revealed two morphological variants of *Cx. tritaeniorhynchus* based on length and width of siphon, siphonal index, comb scale numbers, pecten teeth numbers, length of anal gills and anal gill index from the 2 breeding sources in Mysore. The variety from Mysore City (ground pools), type A, has a comparatively shorter siphon index ratio and truncated tips, shorter anal-gill index ratio, fewer comb scales and longer basal hair tufts on the siphon. The variety from outside Mysore (paddy field) (type B) has comparatively longer siphon index ratio and slender tips, longer anal-gill index ratio, more comb scales, and shorter basal hair tufts on the siphon (Fakoorziba and Vijayan, 2003). On the basis of this finding, the current investigation was undertaken to assess the susceptibility status of these 2 morphological variants.

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MATERIALS AND METHODS

Study area and larval collection

For the purpose of comparison, 2 variants of *Cx. tritaeniorhynchus* from Mysore (12° 18', N; 76° 18' E), in Karnataka State, India, were employed. Larvae were collected from ground pools in Mysore and paddy fields outside Mysore, and brought to the Vector Biology Research Laboratory of the Department of Studies in Zoology. They were maintained at 28 ± 2°C and 70 ± 5% relative humidity in enamel trays (32   32   4 cm) employing dechlorinated water. The immatures were provided with finely ground dog biscuit and yeast at a ratio of 3:1. Early 4[th] instar larvae were used for measuring morphological characters and for bioassay.

Synthetic pyrethroid insecticides

Technical grade formulations of 5 synthetic pyrethroid insecticides were used for the larval bioassay study: deltamethrin (98% from M/s AgrEvo India, Mumbai), cypermethrin (93.7% M/s Bayer India, Mumbai), permethrin (94.7% from M/s Rousell Ucalf, UK), alpha-cypermethrin (94.2% from M/s Zenica India, Mumbai), and lambdacyhalothrin (90.8% from M/s Syngenta, India).

Larval bioassay

Susceptibility tests were conducted per the standard WHO method (WHO, 1981). Technical grade pyrethroids were used to prepare different concentrations of insecticide using absolute alcohol as solvent. Different concentrations (deltamethrin 0.0001-0.0120 mg/l, cypermethrin 0.0005-0.0320 mg/l, permethrin 0.0002-0.0112 mg/l, alpha-cypermethrin 0.0004-0.0080 mg/l, and lambdacyhalothrin 0.0004-0.0280 mg/l) were used to achieve mortality rates up to 90% after 24 hour exposure. For each concentration, at least 3 sets of replicates comprising 25 early 4[th]-instar larvae were maintained. Control experiments were conducted by adding 1 ml of absolute alcohol to 249 ml of dechlorinated water. The LC_{50} and LC_{90} values of the 5 pyrethroids for the 2 variants were calculated by probit regression analysis (Finney, 1971). Percent mortality was corrected using Abbot's formula (Abbott, 1925) when control mortality was < 20%.

RESULTS

From the finding on the morphotaxonomic studies on certain characters in the larvae of 2 populations of *Cx. tritaeniorhynchus* from different areas of Mysore, this species could be categorized into types A and B (Fakoorziba and Viyayan, 2003). The susceptibility status of the *Cx. tritaeniorhynchus* type-A larvae from Mysore City, and the type B from outside Mysore against the 5 synthetic pyrethroids are shown in Table 1, with a comparison of LC_{50} values for the 2 types. The LC_{50} values of 3 pyrethroids, deltamethrin, alpha-cypermethrin, and lambdacyhalothrin, show significant differences between types of *Cx. tritaeniorhynchus* ($p < 0.05$). *Cx. tritaeniorhynchus* type A from Mysore was more tolerant by 2.17-, 2.09-, and 1.24-fold against alpha-cypermethrin, lambdacyhalothrin and deltamethrin, respectively, than type B from outside Mysore.

The LC_{50} values indicated that, of the 5 synthetic pyrethroids tested, deltamethrin was the most effective for both types. Cypermethrin was least effective on type B, and alpha-cypermethrin on type A. In general,

Table 1

Susceptibility status of type A and type B of *Culex tritaeniorhynchus* types A and B against synthetic pyrethroids.

Insecticide	Type A		Type B		Fold difference (LC_{50})
	LC_{50} (mg/l)[a]	LC_{90}(mg/l)[a]	LC_{50}(mg/l)	LC_{90}(mg/l)	
	LCL UCL	LCL UCL	LCL UCL	LCL UCL	
Deltamethrin	0.00097 (0.00063-0.00141)	0.0216 (0.0112-0.06310)	0.00078 (0.00059-0.00099)	0.00603 (0.00419-0.01038)	1.24[b]
α-cypermethrin	0.00310 (0.00269-0.00357)	0.01525 (0.0114-0.02310)	0.00143 (0.00029-0.00325)	0.00636 (0.00293-0.99266)	2.17[b]
Cypermethrin	0.00262 (0.0009-0.00559)	0.06267 (0.0202-1.58690)	0.00271 (0.00218-0.00319)	0.00895 (0.0074-0.01163)	0.97
Permethrin	0.00265 (0.00146-0.00467)	0.00861 (0.0048-0.05997)	0.00228 (0.00193-0.00263)	0.0124 (0.0095-0.01817)	1.16
Lambdacyhalothrin	0.00226 (0.00169-0.00292)	0.03199 (0.02122-0.05628)	0.00108 (0.00087-0.00133)	0.00626 (0.0046-0.00943)	2.09[b]

LCL: Lower concentration limit; UCL: Upper concentration limit; [a]Calculated by probit analysis regression; [b]Indicates a significant difference between LC_{50} value of the two populations based on the non-overlapping or overlapping 95% CLs of LC_{50} values (Yang *et al*, 2002).

the toxicity ranking of these pyrethroids, tested on *Cx. tritaeniorhynchus* type A from Mysore City was deltamethrin > lambdacyhalothrin > cypermethrin > permethrin > alpha-cypermethrin; for type B it was deltamethrin > lambdacyhalothrin > alpha-cypermethrin > permethrin > cypermethrin. *Cx. tritaeniorhynchus* type B was more susceptible to most of the 5 pyrethroids tested than *Cx. tritaeniorhynchus* type A (Table 1).

DISCUSSION

Synthetic pyrethroid insecticides nowadays play a major role in vector control, as they are reported to be highly lethal to insects. By exhibiting low mammalian toxicity, under normal conditions they may not be hazardous to humans. They are photo-stable, with a potentially valuable residual activity (Mulla *et al*, 1980; Rajvanshi *et al*, 1982).

An earlier study has shown that *Cx. tritaeniorhynchus* from Mandya, near Mysore, was 2.2 and 1.8 times more tolerant to cypermethrin and deltamethrin, respectively, than the Mysore population (Vijayan and Revanna, 1994). However, they could not report the presence of two variants. In the present study, type A from Mysore City was 1.24, 2.17, and 2.09 times more tolerant to deltamethrin, alpha-cypermethrin and lambdacyhalothrin, respectively, than type B from outside Mysore. This shows that *Cx. tritaeniorhynchus* type A is a more tolerant variant than type B against pyrethroid insecticides. These two variants may diverge further, as they are restricted to different ecological habitats. Further studies on their vectorial capacity will be interesting.

According to Sirivanakarn (1975) and Colless (1975), the *Cx. tritaeniorhynchus* complex comprises *Cx. tritaeniorhynchus* and its infraspecific forms *summorosus* and *siamensis*. However, Bram (1967) and Reuben (1968) have considered both of them as a synonym for *Cx. tritaeniorhynchus*. In addition, it has been shown that *Cx. tritaeniorhynchus* is a plastic species, with clinal variation in certain anatomical features (Sucharit *et al*, 1989).

According to the morphotaxonomic studies on the larvae of *Cx. tritaeniorhynchus* from the Mysore area, and with the present observation on the susceptibility status of the 2 types, the opinions of Sirivanakarn (1975), Colless (1975), and Sucharit *at el* (1989) will hold good. However, additional studies, such as enzyme assay, isozyme profile, and mating experiments, will furnish more information about these two types of *Cx. tritaeniorhynchus*.

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