TOXICITY OF PYRETHROIDS TO *AEDES AEGYPTI* LARVAE IN RELATION TO TEMPERATURE

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ABSTRACT. The influence of temperature on the toxicity of the pyrethroids cypermethrin, permethrin, fenvalerate, *d*-phenothrin, flucythrinate and bioallethrin to 3rd instar *Aedes aegypti* larvae was determined. Based on LC_{50} levels, the toxicities of all pyrethroids were in the range of 1.33- to 3.63-fold greater at 20°C than at 30°C. Our laboratory results suggest that for larval control of *Ae. aegypti*, field performance of these pyrethroids may be reduced at warmer temperatures.

INTRODUCTION

The toxicity of natural pyrethrins to insects is greater with decreasing temperatures. One of the earliest reports was that of Hartzell and Wilcoxon (1932), with a number of others following. Synthetic pyrethroid insecticides have also been reported to be more active at cooler temperatures on the nervous system (Narahashi 1971), and also affecting mortality, as reported for bioresmethrin on house flies (Yoke and Sudderuddin 1975). Since then a great number of studies have supporting evidence for a negative temperature effect, but, as reported by Sparks et al. (1982), some exceptions have been noted with certain insect species or with certain pyrethroids.

The present study provides new information on the influence of posttreatment temperatures on pyrethroid toxicity to *Aedes aegypti* (Linn.) larvae. The posttreatment temperatures of 20° and 30°C were employed using 6 pyrethroids tested on 3rd instar larvae.

MATERIALS AND METHODS

Aedes aegypti eggs obtained from the Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, were incubated in distilled water in ceramic pans at 25 ± 1°C and 50 ± 10% R.H. Larvae that hatched within 24 hr were transferred to rearing pans and fed yeast powder until the 3rd instar.

Pyrethroids tested were analytical reference standards obtained from the U. S. Environmental Protection Agency. They were cypermethrin (94.2% purity), permethrin (approximately 60% *trans*, 40% *cis* isomers), fenvalerate (100%), *d*-phenothrin (mixed *cis*, *trans* isomers), flucythrinate (96% purity) and bioallethrin (*d-trans* allethrin), (90% purity). Stock solutions of insecticides were made in absolute ethanol and serially diluted. Several concentrations of each insecticide were made by adding 1 ml of insecticide solution to 50 ml of distilled water. Third instar *Ae. aegypti* larvae were separated from the rearing pans and placed as groups of 10 in 250 ml glass beakers containing 50 ml of distilled water. Insecticide solutions were added to the beakers containing larvae to bring the final volume to 100 ml. Untreated larvae received 1 ml of ethanol in 100 ml of distilled water. Five to 8 concentrations of each insecticide were employed, and replicated 3 to 6 times. Concentrations of bioallethrin used at both temperatures ranged from 15 to 75 ppb while those of other pyrethroids ranged from 0.05 to 3.75 ppb.

Beakers with larvae were incubated at 20 ± 1°C or 30 ± 1°C, both with 65 ± 10% RH. Larvae were examined for mortality at 24 hr. Immobile larvae were considered dead. Pooled concentration-mortality responses of replicates were corrected for control mortality (< 7%) and analyzed by probit methods (Finney 1971) to determine the LC_{50} values. The 95% confidence limits were used to determine significant differences between LC_{50}s at the two temperatures. Temperature coefficients were calculated for each insecticide as ratio of LC_{50} at 30° and 20°C.

Probit regression slopes at 20° and 30°C were tested for parallelism to determine if the difference in response between temperatures was valid at all concentrations. A one-tailed *t*-test was used to compare the difference between the slopes (Steel and Torrie 1980) at 20° and 30°C and alpha was fixed at 0.05.

RESULTS

Table 1 shows the number of larvae used, resulting LC_{50}s with 95% confidence limits, slopes and standard errors (SE), χ^2 values for heterogeneity of probit regression lines, and temperature coefficients. χ^2 values for all insecticide-temperature combinations indicated adequate fit of data to probit model ($P > 0.05$). The LC_{50}s of bioallethrin were not significantly different ($P > 0.05$) at 20° and 30°C, whereas the toxicities of remaining pyrethroids were significantly greater ($P < 0.05$) at 20° than at 30°C. Temperature coefficients for permethrin, *d*-phenothrin, cypermethrin, fenvalerate, bioal-

Table 1. Toxicity of six pyrethroids to 3rd instar *Aedes aegypti* larvae at 20° and 30°C.

Insecticide	Temperature (°C)	No. larvae tested	LC_{50} (ppb)	95% confidence limits		Slope (SE)[a]	χ^2 (df)[b]	Temperature[c] coefficient
				lower	upper			
Cypermethrin	20	420	0.16	0.13	0.18	1.93 (0.05)	0.76 (6)	2.13
	30	359	0.34	0.29	0.39	2.49 (0.14)	2.02 (5)	
Permethrin	20	360	0.27	0.22	0.31	2.01 (0.17)	2.68 (4)	3.63
	30	359	0.98	0.90	1.06	4.34 (0.09)	0.21 (4)	
Fenvalerate	20	330	0.46	0.38	0.54	1.99 (0.16)	2.37 (4)	1.91
	30	300	0.88	0.71	1.11	1.72 (0.16)	4.50 (4)	
d-Phenothrin	20	212	0.56	0.47	0.64	3.61 (0.10)	0.22 (3)	2.71
	30	180	1.52	1.29	1.81	2.87 (0.44)	4.27 (3)	
Flucythrinate	20	392	1.00	0.88	1.14	2.58 (0.09)	0.83 (5)	1.33
	30	389	1.33	1.16	1.57	2.33 (0.24)	5.25 (5)	
Bioallethrin	20	182	23.99[d]	21.10	26.79	4.10 (0.41)	1.47 (4)	1.67
	30	148	40.15	25.53	52.59	1.89 (0.50)	1.88 (3)	

[a] SE = slope standard error.
[b] All χ^2 values indicate adequate fit of data to probit model ($P > 0.05$).
[c] Temperature coefficient = LC_{50} at 30°/LC_{50} at 20°C.
[d] LC_{50}s not significant ($P > 0.05$).

lethrin and flucythrinate were 3.63, 2.71, 2.13, 1.91, 1.67 and 1.33, respectively (Table 1).

Concentration-mortality lines were parallel ($P > 0.05$) for fenvalerate, *d*-phenothrin, and flucythrinate, whereas lines for cypermethrin, permethrin, and bioallethrin were not parallel ($P < 0.05$). Therefore, the 1.91-, 2.71-, and 1.33-fold greater toxicity at 20° than at 30°C of fenvalerate, *d*-phenothrin, and flucythrinate, respectively (Table 1), was valid at all concentrations tested.

Toxicity comparisons, using LC_{50} values, showed cypermethrin to be most toxic with a value of 0.16 ppb at 20°C (Table 1). Less toxic, in descending order, was permethrin, fenvalerate, *d*-phenothrin, flucythrinate and bioallethrin. The latter compound was considerably less toxic, the LC_{50} value being 23.99 ppb at 20°C.

DISCUSSION

Our results showing greater toxic effects of pyrethroids at cooler temperatures agree with several other studies on adult dipterans. These include permethrin effects on adult *Hylemyia (= Delia) antiqua* (Meigen) showing a 3.6-fold greater effect at 15° than at 32°C (Harris and Kinoshita 1977). Toxicities of allethrin, flucythrinate, permethrin, and cypermethrin to an NAIDM strain of *Musca domestica* Linn. (Scott and Georghiou 1984) and permethrin and cypermethrin to *Glossina austeni* Newstead (Hadaway 1978), were negatively correlated with temperature. No apparent differences due to temperature were evident in studies with *Dacus dorsalis* Hendel when comparisons were made at the LD_{95} level (Tan 1982). Exceptions in which pyrethroid toxicity was greater at a warmer posttreatment temperature occurred with some Coleoptera, Lepidoptera and Orthoptera. Examples include a flea beetle, *Phyllotreta cruciferae* (Goeze) (Burgess and Hinks 1986), and 2 Lepidoptera, *Spodoptera frugiperda* (J. E. Smith) and *Heliothis virescens* (F.), even though *Trichoplusia ni* (Hübner) responded with greater sensitivity at a cooler temperature (Sparks et al. 1982, 1983). Cypermethrin and deltamethrin gave a positive correlation of toxicity with temperature and allethrin a negative correlation when tested against *Blattella germanica* (Linn.) (Scott and Matsumura 1983).

One set of comparative LC_{50} values for *Ae. aegypti* are those of Herald et al. (1980). They reported values of 9.19, 0.41 and 0.10 ppb for fenvalerate, Ectiban and Atroban, respectively. The latter two are formulations of permethrin. We are not in a position to explain the reason for the higher value which is about 10-fold less toxic than our value for fenvalerate.

Pyrethroids act on both the central and peripheral nervous systems of insects (Miller and Adams 1977), and the processes leading to death following pyrethroid poisoning are complex (Miller and Adams 1982). Therefore, the reasons for the greater toxic effects of pyrethroids at cooler temperatures are difficult to explain. Neurophysiological studies have indicated that the insect nervous system is more sensitive to poisoning by pyrethroids as the temperature is decreased (Narahashi 1971; Gammon 1978,

1979, 1980; Miller and Adams 1982). Cuticular pick-up and penetration are probably not involved, as early studies with DDT (Zubairi and Cutkomp 1964) and pyrethrum (Blum and Kearns 1956) showed a greater pick-up and penetration at higher temperatures. Insecticide metabolism in insects is greater at higher temperatures (Edwards 1946; physiological texts) and, therefore, is not a likely explanation for the increased toxicity of pyrethroids at cooler temperatures.

Aedes aegypti larvae can effectively develop between temperatures of 16° and 34°C (Christophers 1960). Extrapolation of laboratory data to field conditions is not without risk, but our results suggest that the field performance of the tested pyrethroids for *Ae. aegypti* larval control may be reduced at warmer water temperatures.

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