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RESPONSE OF *ANOPHELES FARAUTI* TO PERMETHRIN-TREATED NET AND CLOTH FABRICS IN THE LABORATORY[1]

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ABSTRACT. The median effective dose of permethrin for knockdown and mortality of *Anopheles farauti s. s.* exposed to 2 net and 2 cloth fabrics was estimated. There were no significant differences in the knockdown or mortality of *An. farauti* or *Aedes aegypti* following 15–180-sec exposure to permethrin-treated bed nets and polyester/cotton fabric. Despite the presence of permethrin (0.007–0.068 mg/cm^2) in bed net material, 18.2–50% of *An. farauti* adults successfully obtained a blood meal through the nets, and although all of them were knocked down after 60 min, up to 77.8% recovered within 24 h and 57.1–100% of the survivors were bloodfed. The results indicate that this important vector of malaria can obtain a blood meal through treated bed net material and emphasizes the importance of avoiding contact with the net while sleeping.

Recent studies have indicated the potential of treating mosquito bed nets with permethrin for protection against malaria (Curtis 1989, Rozendaal 1989). This method has been introduced to several countries in the Southwest Pacific region, where the main vectors of malaria are members of the *Anopheles punctulatus* complex (Samarawickrema et al. 1992, Genton et al. 1994). Recent reports also show the use of permethrin-impregnated clothing, in combination with topical repellents, protects individuals from mosquitoes (Gupta et al. 1987). In this note we report laboratory experiments on the response of *Anopheles farauti* Laveran *senso stricto* (*s. s.*) following exposure to bed net and cloth fabric treated with permethrin.

Four fabric types were used: 1) Thai net, a nylon bed net material produced by Siamdutch, Bangkok, Thailand; 2) Australian net, a nylon bed net material produced for the Australian Army; 3) 100% white cotton fabric, Australian Wool Testing Authority standard; and 4) 50% polyester/50% cotton fabric.

Fabrics were treated with permethrin from Peregin®, an emulsifiable concentrate with 500 g/liter permethrin (cis : trans ratio 25:75) produced by Wellcome (Sydney, Australia), prepared as a water emulsion. Individual fabric pieces (15 × 12 cm) were weighed, then submerged in a permethrin emulsion for 2 min to ensure saturation, removed, and excess emulsion allowed to run off for 30 sec. The fabric piece was then weighed, and allowed to air dry for up to 20 h. The fabric was then wrapped in aluminum foil and stored at room temperature until use.

Test mosquitoes were 2–6-day-old nulliparous female *An. farauti s. s.* from a colony established with specimens collected in Rabaul, Papua New Guinea, in 1972. In some experiments 2–6-day-old nulliparous *Aedes aegypti* (Linn.), from a colony originally established from the Queensland Institute of Medical Research, in 1981, were also used.

In the first experiment *An. farauti* were exposed to the test fabrics following treatment with a range (0.002–0.021 mg/cm^2 for nets, 0.016–0.061 mg/cm^2 for 100% cotton, and 0.009–0.077 mg/cm^2 for polyester/cotton) of permethrin concentrations to determine the median effective dose (ED_{50}) for knockdown after 60 min and mortality after 24 h. World Health Organization (WHO) susceptibility test kits, which allowed mosquitoes to be exposed to test fabrics for short defined periods of time (Schreck et al. 1978, Hossain et al. 1989), were used to expose 4 batches, each of 10 mosquitoes, for 3 min. Pieces of net fabric (15 × 12 cm) were attached to blotting paper using double-sided tape (Scotch™, 3M Corp., 12.7-mm wide). This was then rolled into a tube shape and fixed into the text kit treatment cylinder using metal clips. For experiments with cloth fabric, samples were not attached to paper, but fixed directly into the treatment cylinder using metal clips.

Following exposure to the test fabrics, mosquitoes were transferred to the holding cylinder of the WHO kit. The cylinders were placed into a polystyrene container and covered with moist cotton wool. The knockdown of mosquitoes was scored 60 min after exposure, and mortality was scored after 24 h. A mosquito was scored as knocked down if it was lying on its back or side,

[1] The views of the authors do not purport to reflect the position of the Australian Defence Force or Department of Defence (Australia). Mention of a commercial product does not constitute an endorsement of the product by the Department of Defence (Australia). The volunteer gave informed consent to participate in the study.

Table 1. Estimated median effective dose (ED_{50}) of permethrin for knockdown (60 min) and mortality (24 h) of *Anopheles farauti* adults after 3-min exposure to 4 treated materials.

Fabric type	ED_{50} (mg/cm²)	
	60-min knockdown	24-h mortality
Thai net (nylon)	0.0015 a[1] (0.0012–0.0019)	0.0053 a (0.0045–0.0061)
Australian net (nylon)	0.0059 b (0.0050–0.0069)	0.0111 b (0.0091–0.0136)
100% cotton	0.0129 c (0.0088–0.0188)	0.0302 c (0.0245–0.0373)
Polyester/cotton	0.1099 d (0.0656–0.1842)	0.1047 d (0.0560–0.1960)

[1] ED_{50} in the same column followed by a different letter is significantly different using the method of overlap of 95% confidence limits (shown in parentheses below).

and was unable to maintain flight following a gentle tap on the cylinder. The ED_{50}s and 95% confidence limits were determined for knockdown and mortality using probit analysis (Finney 1971). The results showed lower ED_{50} values for knockdown and mortality of *An. farauti* exposed on nylon nets than either cotton or polyester fabrics (Table 1). The ED_{50} for mortality after 24 h was also significantly higher than the concentration needed for knockdown after 60 min for 2 net fabrics and 100% cotton. In contrast, the ED_{50} for knockdown and mortality of mosquitoes exposed to polyester/cotton fabric were not significantly different. Hossain et al. (1989) reported the LD_{50} of permethrin on cotton nets was 3 times greater than on nylon nets. This was partly attributed to the smoother surface of nylon fibers resulting in more permethrin adhering to the surface and being available to mosquitoes, whereas on rougher cotton fabric permethrin was also deposited in the crevices of fibers and less accessible to mosquitoes (Hossain et al. 1989).

In a second experiment the effect of exposure time on the knockdown and mortality of mosquitoes exposed to treated nets were conducted. *Anopheles farauti* were exposed to Australian net material treated with 0.024, 0.034, 0.047, and 0.067 mg/cm² permethrin for 15, 30, 60, and 180 sec. Two batches of 10 mosquitoes were exposed to each concentration and exposure time using the WHO susceptibility test kit. The mean knockdown of *An. farauti* 60 min after exposure to all concentrations and times was 90–100%, and mortality after 24 h was 60–100%. There were no significant differences in knockdown ($P = 0.44$, df = 3) or mortality ($P = 0.12$, df = 3) due to exposure time. Additional tests were conducted to study the knockdown of mosquitoes exposed for 15, 30, 60, and 180 sec to polyester/cotton fabric treated with 0.043, 0.064, 0.088, and 0.130 mg/cm² of permethrin. The knockdown 60 min after exposure of *An. farauti* was 33.3–100% and of *Ae. aegypti* was 75–100%. There were no significant differences in knockdown ($P = 0.12$, df = 3) or mortality ($P = 0.05$, df = 3) due to time of exposure to the fabric at the concentrations of permethrin tested. Hossain et al. (1989) showed that permethrin was disproportionately effective at shorter exposure times due to the rate of penetration of permethrin into the mosquito. The time of exposure of mosquitoes to treated fabrics is also important, as foraging mosquitoes spend less than 3 min on the surface of permethrin-treated materials (Hossain and Curtis 1989), and exposing test mosquitoes to treated fabrics for relatively short exposure times more closely resembles the field situation. In experiments with *Anopheles gambiae* Giles the time spent on treated nets was dependent on the concentration of permethrin in the fabric (Hossain and Curtis 1989). In the present experiment, mosquitoes were exposed to net and polyester/cotton fabric treated with doses recommended for the impregnation of nets and clothing (Schreck et al. 1978, Schreck and Self 1985), and the results show that *An. farauti* are rapidly affected following short exposure to treated material.

In a third experiment the ability of 5–6-day-old *An. farauti* and *Ae. aegypti* to obtain a blood meal through permethrin-treated net material was tested using a method adapted from that of Hossain and Curtis (1989). Batches of 10 adult mosquitoes were placed into wax paper cups (diam = 7 cm) covered with untreated Australian net material. The mosquitoes were then transferred to cups covered with Australian net material treated with 0.025, 0.034, 0.043, and 0.068 mg/cm² permethrin and Thai net material

Table 2. Biting response, knockdown, and mortality of *Anopheles farauti* and *Aedes aegypti* following 10 min of exposure to Australian and Thai bed net material treated with permethrin.

Permethrin dose (mg/cm²)	*Anopheles farauti*				*Aedes aegypti*			
	Fed (%)	Knock-down (60 min) (%)	Mortal-ity (24 h) (%)	Sur-vivors blood-fed (%)	Fed (%)	Knock-down (60 min) (%)	Mortal-ity (24 h) (%)	Sur-vivors blood-fed (%)
Australian net								
0	55.2	0	0	55.2	78.9	0	10.5	82
0.025	44.4	100	22.2	57.1	28.6	100	100	—
0.034	18.2	100	81.2	100	20.0	100	100	—
0.043	30.0	100	75.0	60.0				
0.068	31.6	100	80.0	100				
Thai net								
0	67.9	0	0	67.9	100	0	0	100
0.007	20.0	100	70.0	66.9	6.3	100	100	—
0.012	50.0	100	50.0	80.0	0	100	100	—
0.018	45.0	100	75.0	80.0				
0.020	21.0	100	94.7	100				

treated with 0.007, 0.012, 0.018, and 0.020 mg/cm² permethrin. The cups were then adpressed to the stomach of a volunteer (Caucasian male) and the mosquitoes allowed to feed for 10 min. Mosquitoes were then aspirated from the test cups and returned to holding cups and provided with a moist cotton wool pad. The number of bloodfed adults was recorded; then, for all mosquitoes, the knockdown scored at 60 min and mortality scored after 24 h. Two batches of mosquitoes were exposed to each concentration of permethrin and net type. Despite the presence of permethrin on mosquito net material, 18.2–50% of *An. farauti* were successful in obtaining a blood meal (Table 2). Also, 57.1–100% of those *An. farauti* that fed successfully also recovered from the effects of permethrin. In contrast, <30% of *Ae. aegypti* adults obtained a blood meal through the same nets, and none of these survived (Table 2). Hossain and Curtis (1989) reported a similar response with *An. gambiae* and *Ae. aegypti,* and they found that adult *An. gambiae* touched the net with only 2 or 4 of their legs. They found that a dose rate of 2.5 g/m² (0.25 mg/cm²) permethrin prevented feeding of *An. gambiae* through nylon nets. In the current test the highest dose rate used was 0.068 mg/cm² (0.68 g/m²), which allowed 31.6% of *An. farauti* to obtain a blood meal, but higher doses of permethrin were not tested.

Charlwood (1986) reported a differential response of *Anopheles* and *Culex* mosquitoes to untreated bed nets in studies conducted in Papua New Guinea. Although fewer *Anopheles koliensis* Owen and *An. farauti* were collected in a room containing people sleeping under a bed net, the proportion of bloodfed adults in rooms with and without nets were not significantly different, but significantly fewer *Culex* were collected in a room with a net. He suggested that insecticide-treated nets would be more effective against culicines than anophelines. The laboratory results reported here show that *An. farauti* can successfully obtain a blood meal through permethrin-treated net material, and indicate that for optimal protection people sleeping under bed nets should avoid contact with the net surface.

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