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ORIGINAL PAPER

Evaluation of permethrin-impregnated military uniforms for contact toxicity against mosquitoes and persistence in repeated washings

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Abstract The loss of permethrin from impregnated uniforms due to repeated washings was studied by chromatographic estimation of the residues. The mean ($\pm$ SE$_{mean}$) percentage losses of permethrin after one to five washings were 16.7 $\pm$ 2.3, 22.5 $\pm$ 3, 29.6 $\pm$ 2.9, 40.2 $\pm$ 2 and 52.2 $\pm$ 2.4, respectively. The reduction in contact toxicity against mosquitoes after each washing was studied by World Health Organization tube and cone bioassays against *Aedes albopictus* mosquitoes. The median knockdown time for 5-min exposure to the treated uniforms increased from 5.9 to 71.8 min after five washings. Mosquito mortality 24 h post-exposure in cone bioassays was <80 % after the fifth washing, indicating the loss of efficacy. The uniforms need to be retreated after five washings so as to ensure adequate protection against disease vectors. The washing water should be properly disposed off to prevent environmental contamination and toxicity to aquatic organisms. Methods for treatment of military uniforms, which ensure high resistance to washing, need to be adopted so as to avoid frequent re-impregnations with permethrin.

Keywords Bioassay · Median knockdown time · Repellent · Residue · Synthetic pyrethroid

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Introduction

Defence forces deployed in the tropical areas are at the risk of contracting vector-borne infections, and hence, personal protection measures are to be adopted in these areas so as to protect the service personnel. The personal protective measures such as the use of repellents, insecticide-treated bed nets, clothings and tents form the first line of defence against arthropod-borne diseases (Faulde et al. 2003). Impregnated clothes are the only effective way to protect people and troops against day-biting mosquitoes such as the vectors of dengue and chikungunya (Pennetier et al. 2010). The military uniforms are usually impregnated with permethrin, a synthetic pyrethroid insecticide, which along with skin repellents is found to be effective in preventing insect bites (Deparis et al. 2004; Faulde and Uedelhoven 2006). The use of repellents for clothing impregnation is recommended by the World Health Organization (Rozendaal 1997). During and after the Second World War, the military uniforms were treated with repellents for protection against scrub typhus and mosquito-borne diseases. Initially, the products like dimethyl phthalate and DEET (*N*, *N*-diethyl-m-toluamide) were used for clothing impregnation, which were later replaced by permethrin (Pennetier et al. 2010). Currently, permethrin is the repellent of choice for fabric impregnation due to its high repellent efficacy and low mammalian toxicity. It has high potency against a wide range of arthropods, rapid reactivity, excellent photostability and resistance to weathering (Faulde et al. 2003). The Australian Defence Forces have been using permethrin-treated uniforms since the early 1990s (Frances and Cooper 2007), whereas the French Army has developed long-lasting permethrin-treated battlefield uniforms (Deparis et al. 2001). Apart from mosquitoes, the impregnated uniforms were found to provide protection against ticks, sand flies and lice (Schreck et al. 1982; Sholdt et al. 1989; Asilian et al. 2003).

The north-eastern region of India, with its hot and humid climate, offers favourable environment for the proliferation of mosquitoes and other disease vectors. The use of impregnated uniforms would be helpful in protecting the Indian armed forces personnel operating in the region. The contact toxicity of permethrin-impregnated uniforms against mosquitoes under the weather conditions prevailing in the region has not been evaluated previously. Moreover, the loss of residual efficacy after subjecting the impregnated uniforms to washing–drying cycles is yet to be studied against mosquitoes in north-eastern India. Hence, the present study was carried out to estimate the loss of permethrin in washing water and the reduction in contact toxicity against mosquitoes after each washing. The environmental effects of permethrin treatment of military uniforms and the residues in washing water were also discussed. The studies were conducted during March—May 2010 at the Defence Research Laboratory, Tezpur, in north-eastern India.

Materials and methods

Impregnation of uniforms

Samples of fabric (dimensions 60 × 60 cm, 50 % cotton and 50 % polyester) were used for permethrin impregnation and the evaluation of mosquito knockdown efficacy. The fabric of the samples was identical to the fabric used for the Indian army uniform. An emulsifiable concentrate (25 %) formulation of permethrin was obtained from the Scientific Fertilisers Co. Ltd., Coimbatore, India. The rates of permethrin treatment were 0.5, 0.75, 1, 1.25 and 1.5 g/m^2 for which the solutions of required strength were made by diluting the formulation with sufficient quantity of water calculated as per the absorption coefficient and dimensions of the cloth. The uniform cloth pieces, one for each concentration, were rolled and placed in a polyethylene pouch to which the permethrin solutions were added. The pouches were sealed properly and were kept for 3 h after which they were taken out and dried under shade. Washing of the clothes was done according to standard World Health Organization protocol (WHO 2005).

Detection of residues

The detection of residues was carried out from the cloth pieces treated with permethrin at the rates of 0.5, 0.75, 1, 1.25 and 1.5 g/m^2. The residues were detected after each washing, and four replicates were analysed per concentration. The percentage reduction in permethrin residues after each washing in comparison with the unwashed cloth pieces was calculated for each of the five concentrations. The overall percentage loss of permethrin after each washing was estimated as the mean of the percentage losses from the five different concentrations; 2 cm^2 pieces of the treated clothes were cut and placed in microcentrifuge tubes before and after each washing. Permethrin was eluted by adding 1 ml acetonitrile to the tubes and centrifuging at 1,000 rpm for 10 min followed by incubation at room temperature for an hour. Chromatographic quantification of permethrin was carried out with HPLC system (Waters Corporation, Milford, USA) equipped with Spherisorb 5 µm ODS2 4.6 × 150 mm C-18 analytical column and acetonitrile–water (80:20) isocratic mobile phase. The flow rate was 1 ml/min, the detector was set at 225 nm, and the column temperature was 40 °C. The sample (20 µl) was injected after filtering with Whatman 0.45 micron nylon membrane filter. The standard curves were plotted using permethrin analytical standard (Sigma-Aldrich, USA). The detector used was Waters 2,996 Photodiode array detector (Waters Corporation, Milford, USA), and the data were processed using Empower Pro software.

Contact toxicity

World Health Organization tube and cone bioassays were carried out to evaluate the efficacy of the treated uniforms (WHO 2005, WHO 2006). Since 1.25 g/m^2 is the recommended rate for permethrin impregnation of uniforms (Frances and Cooper 2007), only the cloth pieces treated at this concentration were subjected to the bioassays after each washing. Adult female *Aedes albopictus* mosquitoes (2–5 days old) were obtained from the rearing facility at the Defence Research Laboratory, Tezpur, India. In the tube bioassay, the treated clothes were taped to cover the inner surfaces of the WHO tubes and the mosquitoes (20–25 numbers) were introduced and exposed for 5 and 10 min. The mosquitoes were transferred to the holding tubes at the end of the exposure periods. In the cone bioassay, five mosquitoes were exposed to treated cloth pieces for 3 min under standard WHO cones, after which they were transferred to paper cups. The observations on knockdown were made at regular intervals during the first hour, and the percentage mortality 24 h post-exposure was recorded. The percentage knockdowns at 15 and 60 min post-exposure (KD_{15} and KD_{60}) and the mortality 24 h post-exposure (MR_{24}) after each washing were compared. The WHO tube bioassays were replicated four times, whereas the cone bioassays were replicated ten times. The median knockdown times (KT_{50}) were estimated by probit analysis using IBM SPSS Statistics 19 (IBM, USA) software. The criteria used in cone bioassays for evaluating the efficacy after washing were >95 % knockdown 1 h or >80 % mortality 24 h post-exposure (WHO 2005).

Results and discussion

The mean (± SE_{mean}) percentage losses of permethrin after one to five washings were 16.7 ± 2.3, 22.5 ± 3, 29.6 ± 2.9, 40.2 ± 2 and 52.2 ± 2.4, respectively (Fig. 1). Earlier studies have shown that the loss of permethrin due to washings varies widely with the method of treatment of the uniforms (Faulde et al. 2003). In the polymer-coated uniforms (UTEXBEL), the initial permethrin content of 1,300 mg/m^2 was reduced to 280 mg/m^2 after 100 washings, which was equivalent to the residues after three and six washings of the uniforms treated using Peripel 10 and IARFT methods, respectively, indicating the higher residual efficacy of polymer coating over the dipping methods (Faulde et al. 2003). The permethrin residue estimated from the cloth piece subjected to bioassay was 1.28 g/m^2 initially, whereas 1.03, 0.94, 0.87, 0.75 and 0.56 g/m^2 after 1, 2, 3, 4 and 5 washings, respectively. The KD_{15} for 5-min exposure to the treated cloth piece was 77.6 ± 2.3 % initially, which got reduced to 15.9 ± 1.3, 9.5 ± 2.3 and 3.5 ± 1.2 % after 1, 2 and 3 washings, respectively, while there was no knockdown at 15 min post-exposure from the fourth washing onwards. The MR_{24} remained 100 % till the third washing and 96.6 ± 2.1 and 88 ± 2.9 % after the fourth and fifth washings. The KD_{15} for 10-min exposure, which was 100 at the start, decreased to 37.8 ± 2.8, 22.6 ± 4.8, 9.2 ± 1.7 and 0 % after the first, second, third and fourth washings, respectively. Ten-minute exposure to the treated clothes resulted in MR_{24} of 100 % even after the fourth washing, while it got reduced to 95.4 ± 1.8 after the fifth washing (Table 1). Earlier studies have shown that the mosquito mortality and knockdown on exposure to permethrin-treated clothes are primarily dependent on the number of washings and secondarily on the time of exposure (Fryauff et al. 1996).

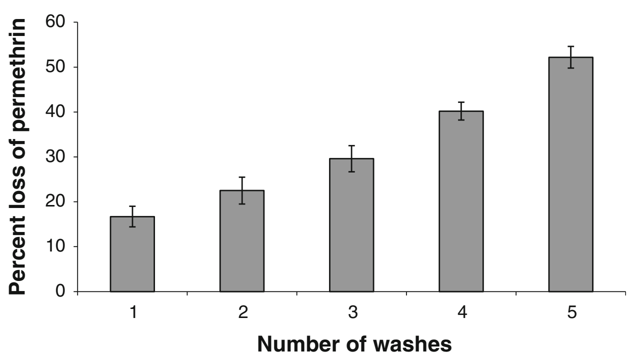

Fig. 1 Percentage loss of permethrin due to repeated washings of impregnated military uniforms (mean ± SE_{mean} of five concentrations with four replicates each per washing)

The median knockdown time (KT_{50}) for 5 and 10 min of exposure (95 % confidence interval) initially were 5.9 (3.76–7.40) and 4.61 (3.69–5.34) min, respectively. The KT_{50} (5 min) increased to 28.7 (25.7-31.5), 40.9 (37-44.7), 47.2 (42.7–53.2), 63.9 (55.7–89.9) and 71.8 (61.6–100.6) min after the first to the fifth washing, respectively. The corresponding KT_{50} (10 min) were 20.2 (17.3–22.7), 27.4 (24.6–31.1), 34 (31–37.2), 49.3 (46–53.9) and 59.7 (54.1–70.6) minutes (Table 2). The median knockdown time is considered to be a suitable method for the evaluation of insecticide-treated textiles as it measures the bioavailability of an insecticide on treated surface and the regeneration time (Skovmand et al. 2008).

Comparisons of the mosquito knockdown in cone bioassays indicated that there was substantial loss of mosquito knockdown efficacy due to washing. The KD_{15}, KD_{60} and MR_{24} on exposure to unwashed cloth were 50.6 ± 3.1,

Table 1 Effect of repeated washings of permethrin-impregnated military uniforms on the efficacy against mosquitoes

No. of washes	Permethrin residue (g/m^2)	5 min exposure		10 min exposure	
		KD^a_{15}	MR^b_{24}	KD^a_{15}	MR^b_{24}
0	1.28	77.6 ± 2.3	100	100	100
1	1.03	15.9 ± 1.3	100	37.8 ± 2.8	100
2	0.94	9.5 ± 2.3	100	22.6 ± 4.8	100
3	0.87	3.5 ± 1.2	100	9.2 ± 1.7	100
4	0.75	0	96.6 ± 2.1	0	100
5	0.56	0	88 ± 2.9	0	95.4 ± 1.8

All values are mean ± SE_{mean}

[a] Percentage knockdown 15 min post-exposure

[b] Percentage mortality 24 h post-exposure

Table 2 Effect of repeated washings of permethrin-impregnated military uniforms on the median knockdown time (KT_{50}) against mosquitoes

No. of washes	5 min		10 min	
	KT_{50}	95 %CI	KT_{50}	95 %CI
0	5.90	3.76–7.40	4.61	3.69–5.34
1	28.7	25.7–31.5	20.2	17.3–22.7
2	40.9	37–44.7	27.4	24.6–31.1
3	47.2	42.7–53.2	34	31–37.2
4	63.9	55.7–89.9	49.3	46–53.9
5	71.8	61.6–100.6	59.7	54.1–70.6

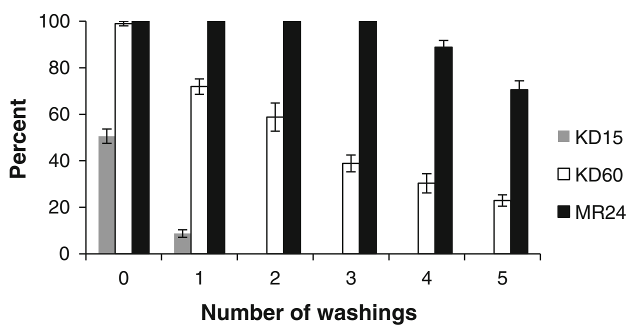

Fig. 2 Effect of repeated washings of permethrin-impregnated military uniforms on the efficacy against mosquitoes in cone bioassay (mean ± SE_{mean} of ten replicates per washing)

99 ± 1 and 100, respectively. Thus, there was 100 % mortality 24 h post-exposure even though complete knockdown was not achieved within 1 h post-exposure (Fig. 2). The MR_{24} for 10-min exposure of *Culex pipiens* mosquitoes to treated unwashed fabrics was 74 % in an earlier study (Fryauff et al. 1996), whereas 100 % in the present study with *Ae. albopictus*. The KD_{15} after the first washing was 8.7 ± 1.6 %, which got reduced to 0 % from the second washing onwards. The KD_{60} after the first to the fifth washings were 71.9 ± 3.3, 58.8 ± 6.1, 38.9 ± 3.6, 30.3 ± 4.1 and 22.9 ± 2.4 %, respectively. The MR_{24}, which was 100 % till the third washing, decreased to 88.9 ± 2.9 and 70.5 ± 3.9 % after the fourth and fifth washings, respectively (Fig. 2).

Shorter periods of exposure are considered to represent the actual contact toxicity effects during mosquito feeding (Frauff et al. 1996), and hence, the results of cone bioassays with 3-min exposure time were used for determining the loss of efficacy due to washings. The exposure to the cloth subjected to five washings resulted in less than 95 % knockdown 1 h and <80 % mortality 24 h post-exposure, which indicated that the efficacy against mosquitoes was lost after five washings (Fig. 2). Therefore, the treatment schedule for the uniforms should necessarily involve retreatment with permethrin after the fifth washing to protect the armed forces personnel from mosquitoes in the field. The permethrin residue in the treated fabric after the third washing was observed to produce little knockdown effect on *Cx. pipiens* mosquitoes but was still effective against sand flies (Fryauff et al. 1996). Permethrin-impregnated uniforms along with DEET have provided complete protection against mosquitoes in the field bioassays conducted in Pakistan (Sholdt et al. 1988). The field trials of permethrin-impregnated uniforms in Iran have shown that the percentage protection provided against mosquitoes, mainly *Cx. pipiens*, was 87 % (Khoobdel et al. 2005).

In the present study, WHO tube bioassays were useful to estimate the median knockdown times on exposure to impregnated uniforms, whereas cone bioassays with short exposure periods were used to devise the re-impregnation schedule. Washing of the permethrin-treated uniforms resulted in substantial loss of permethrin from the treated clothes, and more than half of the initial permethrin content was lost after five washings. This loss of permethrin through washing water would be significant in field deployments where a large amount of uniforms may need to be washed. Worldwide, the majority of permethrin use is for non-agricultural purposes, such as the use in residential areas and for clothing treatment. As in the case of other insecticides, the presence of permethrin has been reported in the water bodies receiving run-off, in the United States and the United Kingdom. Permethrin when received in an aquatic system is potentially harmful to the fauna and flora (Moore et al. 2009). Hence, the washing of the impregnated uniforms in river water should be discouraged, and the wash water needs to be properly disposed so as to prevent contamination of the environment. The washing water may be used as a mosquito larvicide by directing it to sewage where larval breeding is detected (Khoobdel et al. 2005). However, the use of permethrin-impregnated uniforms by a large number of armed forces personnel stationed in an area may create a herd effect which leads to reduction in mosquito population in that area (Kitchen et al. 2009). This in turn would reduce the need for chemical larviciding, fogging or spraying of insecticides in the camp area. Permethrin-impregnated uniforms, thus in the long run, would help to reduce the use of insecticides for mosquito control, thereby reducing environmental contamination.

Attempts should be made to develop permethrin formulations, which provide high resistance to washing so as to minimise the losses through wash water. Factory-impregnated uniforms, such as the ones used by the United States Marine Corps, have a long-lasting impregnation of permethrin where the losses due to washing are minimised (Kitchen et al. 2009). Such ready-to-wear uniforms are recommended due to their long-lasting efficacy, standardised method of application and minimal exposure threat to humans. Uniforms polymer-coated with permethrin after the dyeing process and before tailoring were found to substantially retain the active ingredients even after 100 washings (Faulde et al. 2003). Development of such products and technologies for permethrin impregnation of the military uniforms would help in avoiding the need for frequent retreatment, thereby reducing the operational costs for implementing the use of protective clothing.

Conclusion

Permethrin-impregnated uniforms are regarded as one of the most effective ways to protect the military personnel from arthropods and arthropod-borne diseases. The use of impregnated uniforms in the north-eastern India, which is endemic to malaria, would help to prevent the disease incidence and transmission among the soldiers. Laboratory studies indicated that the uniforms are effective against mosquitoes for up to five washings after which retreatment is recommended. It was shown that more than half of the initial permethrin residue was lost after five washings. Although the impregnated uniforms reduce the use of chemicals for mosquito control, there is a possibility of the washing water contaminating the aquatic environment. The use of methods such as factory treatment, which ensures high wash resistance of permethrin-impregnated uniforms, is important in the context of protecting the armed forces personnel from vector-borne diseases. In addition to the environmental benefits, these methods might also provide a more reliable protection from mosquitoes and other disease vectors.

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