Wash resistance and repellent properties of Africa University mosquito blankets against mosquitoes

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Abstract

The effect of permethrin-treated Africa University (AU) mosquito blankets on susceptible female *Anopheles gambiae* sensu lato mosquitoes was studied under laboratory conditions at Africa University Campus in Mutare, Zimbabwe. Wash resistance (ability to retain an effective dose that kills ≥80% of mosquitoes after a number of washes) and repellence (ability to prevent ≥80% of mosquito bites) properties were studied. The AU blankets were wash resistant when 100% mortality was recorded up to 20 washes, declining to 90% after 25 washes. Untreated AU blankets did not cause any mortality on mosquitoes. However, mosquito repellence was 96%, 94%, 97.9%, 87%, 85% and 80.7% for treated AU blankets washed 0, 5, 10, 15, 20 and 25 times, respectively. Mosquito repellence was consistently above 80% from 0-25 washes. In conclusion, AU blankets washed 25 times were effective in repelling and killing *An. gambiae* sl mosquitoes under laboratory conditions.

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Introduction

Malaria is one of the killer diseases affecting Zimbabwe, mostly in the low veldt due to the conducive environment prevailing in these areas. The Ministry of Health and Child Welfare (MoHCW) has a mandate to control malaria through the National Malaria Control Program (NMCP) that has defined structures from head office, provincial, down to district levels. The NMCP co-ordinates all malaria control activities in the country using the integrated vector management approach that includes indoor residual spraying, insecticide treated materials (ITMs), larviciding and use of personal protection methods (Lukwa, 1994; Taylor & Mutambu, 1986).

Personal protection is one of the malaria control methods advocated for at community level. ITMs have been used for some time although the use of treated blankets is relatively uncommon. Long-lasting permethrin-treated nets used as blankets inhibited 35-60% of mosquitoes from entering the huts and eventually 88-98% of them died (Hougard *et al.*, 2007). The use of treated-blankets and sheets reduced malaria infection in the intervention group by 70% (Kimani *et al.*, 2006). In another study, insecticide-treated bedding protected people against malaria and leishmaniasis vectors, since the mean mortality rates were 16-30.3% (Graham *et al.,* 2002).

The results on acceptability clearly showed that providing treated bedding resulted in protection from mosquito bites (Graham *et al.,* 2002). Chaddars (head dress) treated with permethrin were evaluated in several villages in Afghanistan with a 7.2% incidence rate of leishmaniasis for the control group and 2.5% for people using permethrin-treated chaddars (Reyburn *et al.*, 2000). Up to 8% of the respondents using treated chaddars for the control of leishmaniasis were convinced that chaddars prevent bites from sand flies, as compared to 28% who did not feel any change in sand fly biting (control group) (Reyburn *et al.*, 2000). Permethrin-treated chaddars killed 36-70% of the mosquitoes exposed to the treatment as compared to 20-39% of the mosquitoes that were killed in the controls (Rowland *et al.*, 1999). The World Health Organization (WHO) (WHO, 2006) set the criteria for 24-h mortality at 80% or over.

Although the use of long-lasting insecticide-treated mosquito nets (LLINs) is an important part of malaria control in Zimbabwe, treated blankets have never been used for this purpose. Studies were carried out at Africa University, Mutare, on the possibility of using Africa University (AU) mosquito blanket for controling mosquitoes.

Materials and methods

Study area

Mosquitoes were collected from selected breeding sites from

Chakohwa village (19°51' S, 32°55' E) where malaria transmission is moderate. The inhabitants are mostly subsistence farmers who specialize in growing maize. Some irrigation is practised for vegetables. Laboratory tests were carried out using facilities at Africa University (18°52' S, 32°42' E) located near Mutare City, Zimbabwe.

Mosquito collection and rearing

The *Anopheles gambiae* sensu lato mosquito larvae were collected from the fringes of rivers (Figure 1) using soup ladles in breeding sites in Chakohwa village. The larvae were placed in rearing bowls (Figure 2) and later covered using mosquito netting. All the larvae were fed on fish food until they pupated. The pupa were picked up using a 2 mL plastic pipette, placed in a small dish before being placed in 5 L plastic mosquito cages to hatch. The upper part of the mosquito cage is open and has netting, and a sleeve is provided for introducing and retrieving mosquitoes. The adults were fed on 10% sugar solution that had been daubed in cotton wool and placed on top of the cage. The adult *An. gambiae* sl mosquitoes from this study area have not been identified to species level.

A sample of the resultant adults was used to determine levels of insecticide susceptibility and the rest were used for bioassays. The adult mosquitoes were susceptible to the 4 classes of insecticides, namely pyrethroids (lambda-cyhalothrin), organophosphates (malathion), organochlorines (dichloro-diphenyl-trichloroethane) and cabarmates (bendiocarb).

Repellent blanket

The AU mosquito blanket (also known as the Chicopee Insect Shield) measures 187.96×121.92 cm and weighs 567 g. It is manufactured by BuzzOff™ (S.C. Johnson & Son, Inc., St Racine, WI, USA) and is treated with 0.52% permethrin at the factory. The blanket can be used as a wrapper or top sheet (*i.e.* placed on top of blankets). The control blanket was made by the same manufacturer but it did not contain insecticide.

World Health Organization's washing procedure

The blanket was cut into 25×25 cm pieces, with 6 pieces for each wash (0, 5, 10, 15, 20 and 25). Each piece was individually introduced into a 1 L beaker containing 500 mL of distilled water (with 1 mL/L soap) added. The beakers were introduced into a water bath that contained water at a temperature of 30°C. The water bath was set at 155 movements per min for 10 min. After 10 min, the beakers were removed from the water bath and the pieces of blankets removed. Each piece of blanket was rinsed twice for 10 min in clean distilled water in the same shaking conditions and dried under cover. All pieces of blanket were kept uncontaminated before use. Bioassays (described under wash resistance properties) were only performed on dry blanket samples (WHO, 2005).

Wash resistance properties

Wash resistance properties were evaluated on either permethrin-treated or untreated samples of AU blankets that were washed using the standard WHO washing procedure (WHO, 2005). Each WHO cone measured 8.5 cm in diameter at the base and was 5.5 cm high. Each piece of was fastened to the WHO cone using a rubber band (6 replicates for each wash) (Figure 3). A total of 5 non-blood fed (2-5 days old) *An. gambiae* s.l adult female mosquitoes were aspirated using a sucking tube (carried out through the small hole in the cone) and exposed to each blanket sample for 30 min. Mosquitoes from each WHO cone were retrieved and placed in holding paper cups after being provided with 10% sugar solution in the form of a cotton swab used as a wick. Mortality was recorded 24 h after exposure. This procedure was repeated for all the 6 washes (0, 5, 10, 15, 20 and 25).

Repellence test

Mosquito cages were made from 5 L buckets consisting of a sleeve for introducing mosquitoes and covered with mosquito netting on top and used for each test. Five cages per wash were prepared simultaneously for each of the 6 washes. Fifty starved laboratory-reared female *An. gambiae* s.l were placed in each mosquito cage and left to acclimatize for 1 h in studies that were replicated 5 times. Each piece of blanket was wrapped around each hand (Figure 4) and placed in the mosquito cage (Figure 5). One hand was placed in the cage containing mosquitoes for 2 min and the number of mosquitoes probing to bite recorded as described by Maharaj *et al.* (2010). This was carried out for untreated and treated pieces of blanket. Repellence tests were performed following the test method previously described by Curtis *et al.* (1990). Percentage mosquito repellence was calculated following the method previously described by Mehr *et al.* (1985) as follows:

$$(\bar{B}_c - \bar{B}_t / \bar{B}_c) * 100$$

where

B_c is the mean number of bites on control; and

B_t is the mean number of bites on treated pieces of blanket.

Figure 1. Fringes of mosquito breeding site.

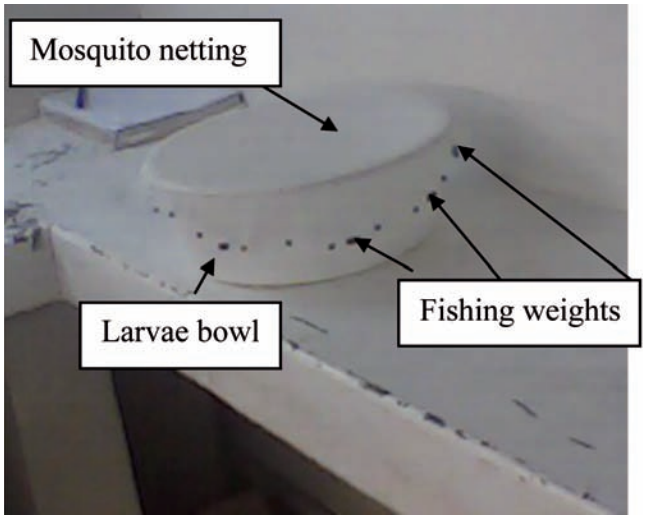


Figure 2. Mosquito rearing bowl with mosquito netting.

The cut-off point for repellence was 80% or over as described by Maharaj *et al*. (2010).

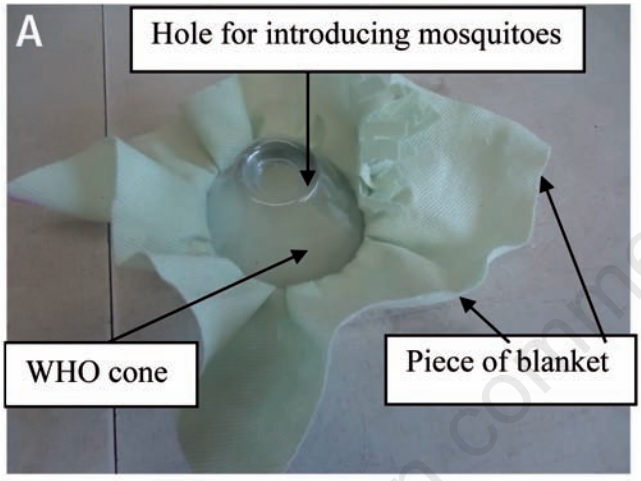
Data analysis

An analysis of variance (ANOVA) test of variance using the 95% confidence limit was carried out to test statistical difference.

Results

AU treated mosquito blankets were wash resistant for up to 25 washes since the mortality was above 80%, as stipulated by the WHO (2005) (Table 1). One hundred percent mortality was realized up to 20 washes before it decreased at 25 washes when treated blankets were used. No mortality occurred with the untreated blankets.

In mosquito repellent studies, more mosquitoes were biting the persons using untreated samples of the blanket than the treated, and the results were significantly different for all washes ($P<0.0001$) (Table 2). Mosquito repellent properties of treated AU blankets were observed for up to 25 washes since repellence was above 80% as reported by Maharaj *et al.* (2010).



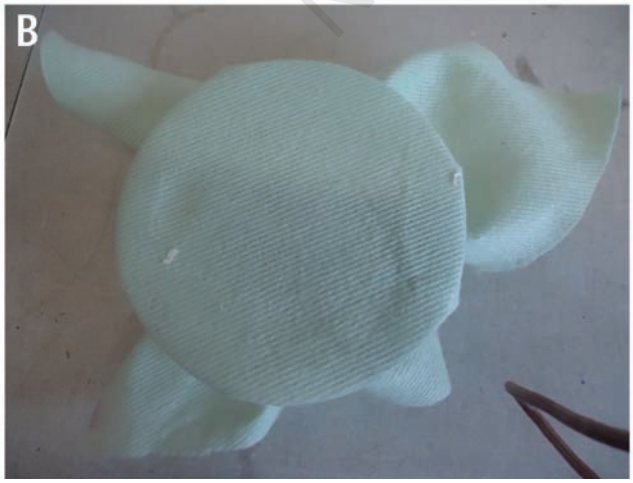


Figure 3. A) Bottom of World Health Organization's (WHO) cone; B) top of WHO cone covered with piece of blanket.

Discussion and conclusions

Studies on wash resistance properties showed that the treated AU blanket killed mosquitoes for up to 25 washes, in agreement with Rowland *et al.* (1999). Thus, enough lethal insecticide was retained on treated pieces of blanket for up to 25 washes. When blankets are not

Table 1. Wash resistance properties of Africa University treated mosquito blankets three days after washing.

No. of washes	Mortality (%)
0	30/30 (100)
5	30/30 (100)
10	28/28 (100)
15	31/31 (100)
20	27/27 (100)
25	27/30 (90)

Figure 4. Hand wrapped in piece of blanket during repellence test.

Figure 5. Hand introduced into mosquito cage for repellence test.

Table 2. Number of mosquitoes probing to bite people using treated and untreated blanket samples and their repellence effect.

No. of washes	Treated AU blankets No. of mosquitoes probing to bite Total (range)	Repellence [control-treated/control]*100 %	Untreated AU blankets No. of mosquitoes probing to bite Total (range)
0	5 (0-2)	96.0	125 (18-37)
5	7 (1-2)	94.0	118 (17-30)
10	3 (0-1)	97.9	145 (26-33)
15	16 (3-4)	87.0	123 (23-26)
20	18 (2-5)	85.0	120 (23-26)
25	26 (4-7)	80.7	135 (24-30)

AU, Africa University.

properly treated during manufacture, insecticide can be lost quickly during washing, thus losing the protective efficiency. When this happens, mosquitoes will no longer be killed and people living in malarious areas will be exposed to infective mosquito bites. The more wash resistant the treated blankets are, the longer they last in protecting vulnerable populations. The quality of the fiber used in blanket manufacture has a bearing on how long the treated blankets will last.

Observations of Graham *et al.* (2002) on mortality (16-30%) of mosquitoes were not as good as ours (90-100%), implying that AU treated blankets worked better than treated bedding. However, our results on mortality were slightly better than (LLINs) treated with permethrin but used as top sheets (88-98%) (Hougard *et al*., 2007).

On the other hand, treated AU blankets have proved to be good repellents for up to 25 washes and our results were better than those of Hougard *et al.* (2007). The treated AU blankets minimize the number of mosquitoes that land and, therefore, reduce malaria transmission. Results from this study show that treated blankets might offer protection from mosquito bites even if they have been washed 25 times. These results point to the use of a new concept (repellent blankets) in malaria control.

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