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ASSESSMENT OF A NEW TYPE OF PERMETHRIN IMPREGNATED MOSQUITO NET

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Abstrak: Sejenis kelambu baru yang dipanggil kelambu Olyset telah diuji dan dibandingkan dengan polietilen monofilamen dan nylon multifilamen yang telah dirawat dengan permetrin terhadap nyamuk *Anopheles maculatus* dan *Aedes aegypti*. Kelambu Olyset dibuat daripada serat polimer yang telah dirawat dengan racun serangga sebelum ditenun menjadi bahan kelambu. Kesan tiga jenis kelambu ini diuji selepas dicuci dengan air sahaja atau dengan air dan sabun. Mortaliti nyamuk *An. maculatus* ialah 95% untuk kelambu Olyset, 83% untuk nylon dan 26% untuk polietilen, selepas cucian sebanyak 15 kali dengan air. Walau bagaimanpun mortaliti nyamuk *Ae. aegypti* ialah 100% untuk kelabu Olyset, 91.7% untuk nylon dan 81.7% untuk polietilen, selepas cucian sebanyak 15 kali dengan air. Selepas kelambu dicuci sebanyak 4 kali dengan air dan sabun, mortaliti nyamuk *An. maculatus* ialah 86.7% untuk kelambu Olyset, 80.3% untuk nylon dan 3.3% untuk polietilen. Dengan nyamuk *Ae. aegypti* mortaliti ialah 90.3% untuk Olyset, 50% untuk nylon dan 5% untuk polietilen. Dalam kajian penembusan, semua nyamuk tidak berupaya menghisap darah dan mati dalam masa 12 jam walaupun mereka berupaya menembusi kelambu Olyset.

Abstract: A new type of mosquito net called Olyset net was tested and compared with polyethylene monofilament and nylon multifilament impregnated with permethrin. The Olyset net has the insecticide directly incorporated into the polymer fibers used to weave the netting material. The longevity of the insecticidal effect of Olyset net against two other commonly used nets after repeated washing with water or with soap and water was tested. The penetrability of mosquitoes due to large mesh used in the Olyset net was also measured. Two species of mosquitoes *Anopheles maculatus* and *Aedes aegypti* were used for the test. The percentage mortality of *An. maculatus* exposed to Olyset, nylon multifilament and polyethylene nets after 15 washes with water was 95%, 83% and 26% respectively, while for Ae. aegypti with mortality as 100%, 91.7% and 81.7% repectively. After the nets had been washed four times with soap and water, the percentage mortality of *An. maculatus* exposed to Olyset, nylon and polyethylene nets was 86.7%, 80.3% and 3.3% respectively, while for *Ae. aegypti* the mortality was 90.3%, 50% and 5% respectively. In the penetrability study, although the mosquitoes were able to penetrate the large mesh none were blood fed and all died within 12 hours.

INTRODUCTION

Bednets have long been used to protect people from mosquitoes and other biting insects. The traditional bednet looses its effectiveness when it becomes torn because insects can enter through the holes. One way of overcoming this disadvantage is to treat bednets with an insecticide, preferably a pyrethroid. Permethrin is a pyrethroid recommended for impregnation of bednets by the WHO Expert Committee on Vector Biology and Control (World Health Organisation 1985).

Among the available control measures, vector control using mosquito nets has recently received some attention because it is a simple and an effective means of personal protection (Schreck and Self 1985). The concept of treating bednets with an insecticide is based on

the fact that mosquitoes alighting on the net, on entering are killed, thereby reducing man/vector contact and overall vector density.

Impregnation of nets offered several advantages including extending the useful life of the net because treated nets are effective even when torn; providing a lethal resting site for mosquitoes even during the daytime when the net is not used and killing of mosquitoes attempting to bite sleeping persons after the mosquitoes have fed, thereby reducing the numbers of infective and non-infective mosquitoes.

One major problem with the current combination of netting materials, insecticides and methods of treating nets is that regular washing quickly diminish the insecticidal effect of the nets. Malaria control programs often ask people using the nets not to wash them between treatments. The frequency of washing nets varies from place to place and from culture to culture. However, it is unreasonable to expect families to sleep inside nets that have not been washed for a long time. It is therefore important that a combination of netting material, insecticide and method of treatment be developed that will allow normal washing without loss of effectiveness.

One possible solution is a new type of mosquito net called Olyset net that has been developed and marketed by Sumitomo Chemical Company. It has the insecticide directly incorporated into the polymer fibers used to weave the netting material. The manufacturers claim that the insecticidal effect will last for up to two years with several washing. They also claim that the large mesh size allows free flow of air into the net but still prevents mosquitoes from getting inside. If these claims are true the Olyset nets represent a significant improvement over currently available nets.

The objectives of this study were to compare the longevity of the insecticidal effect of Olyset net against two other commonly used nets after repeated washing with water or with soap and water and to measure the penetrability of large mesh used in Olyset net by two mosquito species - *Anopheles maculatus* and *Aedes aegypti*.

MATERIALS AND METHOD

Three types of nets, Olyset net, polyethylene monofilament and nylon multifilament nets were used for the study. The Olyset net was manufactured by Sumitomo and had the insecticide directly incorporated in the netting material. The mesh size is 3mm x 3mm and the active ingredient is permethrin (2% w/w). The polyethylene nets were locally manufactured by Mahsing Synthetic Inds Sdn Bhd and the mesh size is 1mm x 1mm, while the nylon nets were manufactured by Bangla Klamboe in Bangkok, Thailand and the mesh size is 1mm x 1mm.

Impregnation of bednets with permethrin

The method described by Self and Schreck (1985) was used to treat the nets. The dosage of permethrin used was 0.5 gm per square meter of the net. The nets were dipped in the insecticide solution and it was made sure that every portion of the net was completely soaked. The excess insecticide was allowed to drain off and were laid flat on the ground in the shade to dry. Every half an hour the nets were flipped to make it completely dry. After drying, all nets together with Olyset net were hung in a safe, clean room ready for bioassay.

Test mosquito

Two species of mosquitoes were used for the bioassay namely: *Anopheles maculatus* (Post Betau strain) and *Aedes aegypti* (Selangor strain). All were from colonies maintained in the insectary of the Institute for Medical Research, Kuala Lumpur, Malaysia. Female mosquitoes fed with sugar solution were used for the tests.

Bioassay of mosquitoes on treated nettings

Assays were carried out using WHO insecticide resistance test kits. Treated net pieces of 15cm x 15cm were cut and

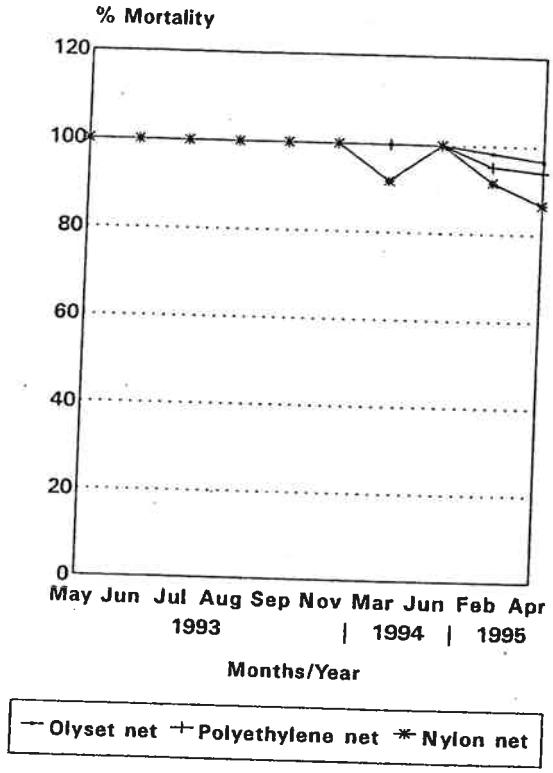

Figure 1: Percentage mortalities of *An. maculatus* to the three different unwashed nets.

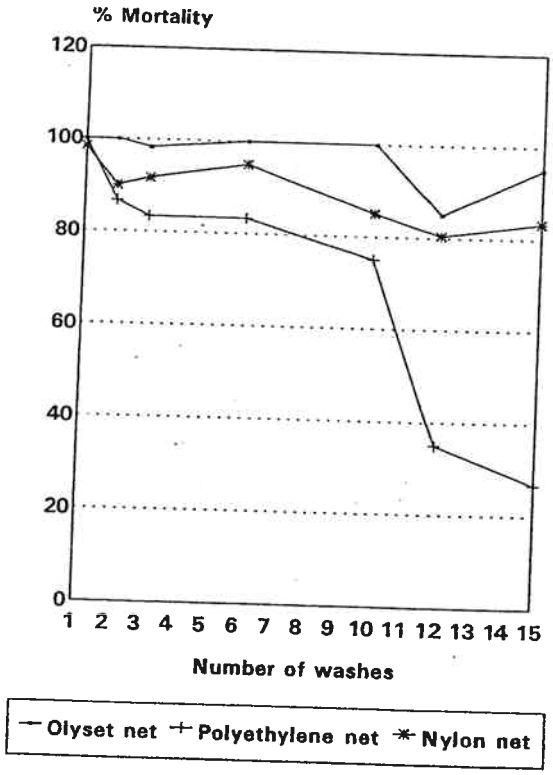

Figure 2: Percentage mortalities of *An. maculatus* exposed to three different nets after washing with water.

attached to smaller pieces of filter paper. The smaller size filter paper enables edges of the nets to be folded and fixed to the other side of the paper, thus eliminating the possibility of minimizing the exposure area which could have been brought about by the presence of sticking tape. The filter paper was also used to hold the nets firmly and to avoid absorption of permethrin into the walls of the test kits.

All bioassay tests were carried out in the laboratory maintained at 25°C and 65% RH. Three replicates were carried out for each test. About 20 sugar fed mosquitoes were collected in clean cups the day before the bioassay. They were then exposed for 10 minutes in the exposure tube. After exposure they were gently blown out into the holding tube. The number knockdown after 10 minutes was recorded and the mortality reading was noted 24 hours later.

Washed nets

Portions of the nets were cut from all three types of net. The portions were washed a total of fifteen times at intervals with water. Other portions were cut from the same nets and washed with soap and water four times.

The pieces of netting that were washed with water alone were soaked individually in a pail of water for about 3 minutes and then rinsed 3 times with gentle squeezing after each rinse. The nets were thoroughly dried in the shade.

For the pieces washed with soap and water, the same method was applied, but in this case the net was washed with a bar of soap and gently squeezed and scrubbed. After washing, the nets were laid flat in the shade and allowed to dry.

Penetrability Test

A small cage constructed with a wooden frame covered with Olyset net measuring 30cm x 17cm x 18cm was placed inside a larger cage covered with fine nylon mesh (1mm x 1mm) which measured 36cm x 28cm x 29.5cm. A guinea pig was placed inside the inner cage as bait. One hundred mosquitoes were then released into the larger cage and left for about 2 hours. At the end of that period mosquitoes from the outer large cage and smaller inner cage were collected separately and transferred into cups. A record was made of the number of mosquitoes that were fed and unfed in each group and after a period of 12 hours the number of dead mosquitoes were recorded.

For control, an untreated set of identical cages were made with the inner cage covered with fish net (3mm x 3mm), the same mesh size as the Olyset net. The same procedure as the above was followed.

RESULTS

The results of the bioassay test on *An. maculatus* carried out on the unwashed nets for a period of 2 years are shown in Figure 1. One hundred percent mortality was observed with *An. maculatus* exposed to unwashed Olyset net and polyethylene net for a period of one year. At the end of the second year, the mortalities of *An. maculatus* exposed to Olyset net, polyethylene net and nylon net were 96.7%, 95.3% ad 86.7% respectively. With *Ae. aegypti*, the mortality was 100% for all nets for the two year period.

The Olyset net gave 100% mortality of *An. maculatus* up to the 10th washing with water only (Figure 2). The mortality dropped to 85% at the 15th wash. The mortalities of *An. maculatus* exposed to nylon and polyethylene nets were 98.3% and 100%, respectively after the first wash. At the 15th wash the percentage mortality of *An. maculatus* dropped to 83.3% with nylon net and 26.7% with polyethylene net.

When tested against *Ae. aegypti*, the Olyset nets subjected to washes with water provided 100% mortality up to the 15th wash (Figure 3). With polyethylene net, 81.7% mortality was observed while with nylon net, the mortality was 91.7%.

When *An. maculatus* and *Ae. aegypti* were exposed to three different types of nets which had been washed four times with soap and water, total mortality of *An.*

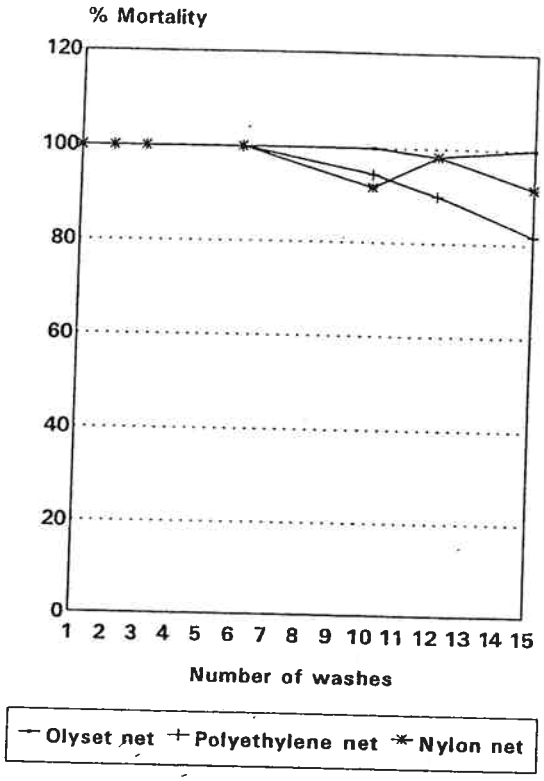

Figure 3: Percentage mortalities of *Ae. aegypti* exposed to three different nets after washing with water.

Table 1: Percentage mortalities of *An. maculatus* and *Ae. aegypti* exposed to the three different nets after washing with soap and water.

Net/Species	1st wash	2nd wash	4th wash
Olyset net			
An. maculatus	100	95.8	86.7
Ae. aegypti	100	93.8	90.3
Nylon net			
An. maculatus	96.7	84.1	80.3
Ae. aegypti	93.3	68.3	50.
Polyethylene			
An. maculatus	58.3	18.3	3.3
Ae. aegypti	62.5	41.7	5.0

maculatus was observed when tested against Olyset net after 1st wash with soap and water (Table 1). With subsequent 2nd and 3rd washes the mortalities were reduced. Mortality of *An. maculatus* exposed to nylon net after the first wash with soap and water was 96.7%, while with polyethylene net, the mortality was 58.3%.

Ae. aegypti when exposed to Olyset net washed with soap and water also gave high mortality. With nylon net, the mortality after the first wash was 93.3% and with polyethylene net was 62.5%.

On the penetrability test, 95% of *Ae. aegypti* and 82.5% of *An. maculatus* were recovered from the outer large cage (Table 2). Mortality in the outer large cage was higher for *Ae. aegypti* compared to *An. maculatus*. None were fed in the inner cage. *Ae. aegypti* and *An. maculatus* were found in the inner cage showing that they were able to penetrate the cage mesh. None however were fed and all died within 12 hours.

In the control experiment, 58% of *Ae. aegypti* and 89.5% of *An. maculatus* were recovered in the outer cage. Of which some were fed indicating that they had passed through the fish net and back out again.

Mortality of *Ae. aegypti* recovered from the outer cage was 25% and with *An. maculatus* was 8.9%. Both *Ae. aegypti* and *An. maculatus* were able to penetrate the mesh and out again. Only 45.2% of *Ae. aegypti* and 19% of *An. maculatus* were fed. Mortality of *Ae. aegypti* was low, but with *An. maculatus* mortality was high.

Analysis of variance showed that there was a significant difference in mortality of *An. maculatus* between Olyset net and polyethylene net ($P<0.005$) and also between the Olyset net and nylon net ($P<0.005$) through 15 washes with water. There was no significant difference observed in mortality between nylon and polyethylene ($P>0.05$).

As for *Ae. aegypti* there was a significant difference in mortality between Olyset net and polyethylene net ($P<0.005$) and there was no significant difference between Olyset net and nylon net ($P>0.05$).

These result suggest that there was a significant decline in effectiveness of the insecticide on the nylon and polyethylene nets but this change was only detectable using *An. maculatus* mosquitoes.

DISCUSSION

The present study showed that residual activity of permethrin on the three different types of nets tested were effective for a period of 2 years. Some workers have found that the residual activity declined after two to four months (Lines *et al.* 1987 and Darriet *et al.* 1984). Hossain *et al.* (1989) also observed a higher mortality value on permethrin impregnated cotton netting than impregnated nylon netting. Lines *et al.* (1987) also found that permethrin-impregnated synthetic netting performed better than permethrin impregnated cotton netting against field caught malaria vectors in Tanzania. However in another study, Hervy and Sales (1980) failed to find any difference in the efficacy between treated cotton and nylon netting. In this study, no difference in the different types of netting were found for both *An. maculatus* and *Ae. aegypti*. Difference in efficacy could also be due to different exposure periods. The exposure times in various studies have differed from 3 minutes to 30 minutes. In this study, an exposure time of 10 minutes was used, as it seemed more practical for release and transfer of specimens from the test kits.

The unwashed nets produced 100% mortality in the bioassay through the first year of testing. In the second year, mortality remained above 95% for Olyset net and polyethylene net while it dropped to below 90% for nylon net.

Washing with water alone reduced the effectiveness of the insecticide with both the nylon and polyethylene nets as demonstrated by the bioassay using *An.*

Table 2: Percentage mortalities of *Anopheles maculatus* and *Aedes aegypti* exposed to Olyset net and Fish net for penetrability study

	Number of mosquito released	Outer cage			Inner cage		
		Number collected	Number fed	Mortalities after 12 hrs	Number collected	Number fed	Mortalities after 12 hrs
Olyset net							
Ae. aegypti	200	109(95.%)	0	183(91.58%)*	10(5%)	0	10(100%)*
An. maculatus	200	165(82.5%)	0	85(42.5%)*	35(17.5%)	0	35(100%)*
Fish net							
Ae. aegypti	200	116(58%)	11(9.5%)	29(25%)*	84(42%)	38(45.28%)	3(3.5%)*
An. maculatus	200	179(89.55%)	11(8.9%)	16(8.9%)*	21(10.5%)	4(19%)	14(66.6%)*

* Values in brackets with asterisk represent percent mortality.

maculatus. A major drop was observed after the second washing. This was probably due to the washing out of insecticide crystals that were only partially bound to the net fibers. After the second washing, the mortality stayed fairly consistent right through the 10th washing.

Mortality of *An. maculatus* exposed to the Olyset net remained at close to 100% through all 10 washings with water. This may represent equivalent of 10 months of normal usage and washing. Even though the mortality of *An. maculatus* dropped after two washes with nylon and polyethylene nets, the levels remained high suggesting that washing with water alone should be recommended.

Washing the polyethylene net just once with soap and water resulted in marked drop in effectivenss with both *An. maculatus* and *Ae. aegypti*. With the Olyset net, the mortality still remained above 90% after 4th washing with soap and water, while for nylon net it was below 90%. This suggests that in polyethylene nets the insecticide is not well bonded to the net fibers and thus is easily removed by soap and water combination.

In this study, we were able to test the Olyset net for a period of 2 years during which it was washed 15 times and it appears that by incorporating the insecticide directly into the polymer used to make the fiber the "washability" of Olyset net is better than that of other types of nets now commonly used. Further testing in the field is needed to determine if the similar results can be obtained with actual usage. It is possible that several factors could atttribute to the effectiveness of the net such as chemical degradation over time, handling of the nets or deposition of soot on the nets.

The penetrability tests done as part of this study have clearly shown that the large size mesh used in Olyset net will protect people sleeping inside. Some mosquitoes were able to get through but none fed and all were killed. Obviously, people sleeping in untreated nets with mesh size of 3mm x 3mm would not be protected.

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REFERENCES

Darriet F, Robert V, Tho Vien N and Carvenale P. 1984. Evaluation of the efficacy of permethrin-impregnated intact and perforated mosquito nets against vectors of malaria. WHO mimiographed document WHO/VBC 84-899.

Hervy J P and Sales S. 1980. Evaluation de le remanence de deux pyrethrinoides se Synthese - OMS 1821 et OMS - 1998 apres impregnation de differents tissues entrant dans la confection de monstiquaires. 14pp. Centre muraz, Bobo Dion lasco, Burkire Faso (document no 40/ENT 80).

Hossain M I, Curtis C F and Heckin J P. 1989. Assays of permethrin-impregnated fabrics and bioassays with mosquito (Diptera, Culicidae). Bull Ent Res 79: 299-308.

Lines J D, Myamba J and Curtis C F. 1987. Experimental hut trials of permethrin impregnated nets and eve curtains against malaria vectors in Tanzania. Med Vet Ento 1: 37-51.

Schreck C E and Self L S. 1985. Treating mosquito nets for better protection from bites and mosquito-borne diseases. 6pp. Geneva, (WHO/VBC/ 85.914).

World Health Organization. 1985. Safe use of pesticides. WHO Technical Report Series No. 720, Geneva.