LABORATORY EVALUATION OF LAMBDA-CYHALOTHRIN A MICROENCAPSULATED FORMULATION ON MOSQUITO NETS FOR CONTROL OF VECTOR MOSQUITOS

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Abstract. Two formulations of lambda-cyhalothrin (EC-Emulsion concentrate and MC-Microencapsulated) were impregnated into bednets made of polyethylene and polyester. The nets were treated at a dosage of 15 mg/m². For bioassay of insecticidal efficacy, female *Anopheles maculatus* and *Aedes aegypti* were exposed to the nets for two minutes and mortality was scored 24 hours later. The nets were also tested after repeated washings with water and with soap and water. Microencapsulated (2.5CS) formulation was more effective than emulsion concentrate (2.5EC) formulation on both net materials - polyethylene and polyester. Repeated washing with water and soap reduces the efficacy of all bednet treatment combinations. Microencapsulated formulation on polyethylene gave best results; it could sustain up to five washes with water and two with soap and water.

INTRODUCTION

Impregnation of bednets with insecticides has now become a favorable method in malaria vector control because it is simple and effective in personal protection (Schreck and Self, 1985) even when they are slightly torn and have holes in them. In early trials, permethrin was the insecticide of choice; however studies have shown that lambda-cyhalothrin was found to be more effective than cypermethrin, deltamethrin, permethrin (Miller and Gibson, 1994), primiphos methyl (Miller *et al,* 1990) and bendiocarb (Weerasooriya *et al,* 1996) when bioassayed with vector mosquitos.

With more advanced technology, a new type of insecticide known as microencapsulated formulation (Baker *et al,* 1993: Kawada *et al,* 1995) has been developed. Lambda-cyhalothrin 2.5CS microcapsule were prepared by interfacial polymerization and condensation of oil water surface to produce polymerase wall, which encapsulated the droplets of insecticides. Mode of action is by diffusion (indirect contact) and rapture (direct contact) of capsule to produce active ingredient.

One major problem with current combination of netting materials, insecticides and methods of treating nets is that regular washing quickly diminish the insecticidal effect of the nets. Therefore it is important that a combination of netting material, insecticide and method of treatment be developed that will allow normal washing without loss of effectiveness. Thus the objective of this study was to compare the longevity of the insecticidal effect of microencapsulated and emulsion concentrate formulation of lambda-cyhalothrin against polyethylene monofilament and polyester multifilament nets after repeated washing with water or with soap and water by two mosquito speicies - *Anopheles maculatus* and *Aedes aegypti.*

MATERIALS AND METHODS

Two types of nets, polyethylene monofilament and nylon multifilament nets were used for the study. The polyethylene nets were locally manufactured by Mahsing Synthetic Inds Sdn Bhd and the mesh size is 1 mm x 1 mm, while the polyester nets were manufactured by Bangla Klamboe in Bangkok, Thailand and the mesh size 1 mm x 1 mm.

Impregnation of bednets with lambda cyhalothrin

The method described by Schereck and Self (1985) was used to treat the nets. The dosage of lambda-cyhalothrin used was 15 mg/m². 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 the nets laid flat on the ground in the shade to dry. Every half hour the nets were flipped to make it completely dry. After drying, all nets were hung in a safe, clean room ready for bioassay.

Test mosquitos

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

Bioassay of mosquitos on treated nettings

Assays were carried out using WHO insecticide resistance test kits. Treated net pieces of 15 cm x 15 cm were cut and 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. The mosquitos were exposed to the treated netting for 2 minutes after which they were transferred to holding tubes. An exposure time of two minutes was used as explained by Magesa *et al*, (1994). Number knocked down after 1 hour and mortality after 24 hours were recorded.

Washed nets

Portions of nets were cut from the nets and washed a total of six times at intervals with water. Other portions were cut from the same nets and washed with soap and water for six times as well. The pieces of nettings 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 detergent "Fab" and gently squeezed and scrubbed. After washing, the nets were laid flat in the shade and allowed to dry. For controls untreated nets were used.

Scanning electron microscopy (SEM)

In and attempt to visualize the structure of the pyrethroid deposit on the fibers of polyethylene and polyester, a study using scanning electron microscopy was undertaken. Samples approximately 100 mm^2 of both impregnated and control nets were fixed on EM holder and coated with approximately 20 nm of gold. The procedure of coating was found necessary to prevent surface changing during observation in Hitachi S430 scanning electron microscope. Secondary electron micrographs were recorded onto Ilford HP5 type 120 B/W film at an accelerating potential of 15-20 KV.

RESULTS

Bioassay test on impregnated nets over time

The results of bioassay test on *An. maculatus* and *Ae. aegypti* carried out on the unwashed nets for a period of 9 months showed no significant difference in the four combinations. All four combinations gave more than 90% mortality for a period of 9 months (except EC on polyester with *An. maculatus).* There was slightly higher average percentage knockdown detected on *Ae. aegypti* compared to *An. maculatus*.

Bioassay of impregnated nets washed with water

The percentage mortality and knockdown of *An. maculatus* exposed to 2 different formulations of lambda-cyhalothrin on 2 different materials of bednets are shown in Fig 1 and Table 1. The polyester nets treated with MC formulation and subjected to washes provided higher mortality compared to the polyester nets treated with EC formulation ($p < 0.001$). With MC formulation more than 90% mortality was obtained with both nets up to 4 washes. However, there was no significant difference in the MC formulation on either fabric. Although MC ployethylene gave higher knock down values than MC polyester this was not significantly different ($p > 0.05$).

Lambda-cyhalothrin EC formulation gave more than 90% mortality with polyethylene up to 2 washes, while with the polyester the mortality was 63% after the second wash. There was a significant difference between EC formulation on polyethylene and polyester ($p < 0.001$). The results showed that the MC formulation on polyester nets was more effective when compared to EC formulation on polyester ($p < 0.001$) (Table 2).

When tested against *Ae. aegypti*, the polyethylene nets treated with MC formulation gave more than 90% mortaility up to 4 washes while MC polyester had more than 90% mortality for only 3 washes (Fig 1). With EC formulation polyethylene net gave more than 90% for 2 washes while polyester nets gave more than 90% only for the first wash. There was a significant difference in the mortality of *Ae. aegypti* exposed to MC polyester and EC polyester after washing with water ($p < 0.01$). Although MC polyethylene gave higher mortality than EC polyethylene it was not significantly different. Knockdown values of more than 90% were achieved for up to 5 washes with both formulations and nets (Table 1).

Bioassay of impregnated nets washed with soap and water

The % mortality and knock down of *An.*

Table 1
Percentage knockdown of *An. maculatus* and *Ae. aegypti* after 2 minutes exposure to polyethylene(P) and polyester(N) nets impregnated with lambda-cyhalothrin microencapsulated (MC) and emulsion concentrate (EC) formulation subjected to six washings with water.

No. of	*An. maculatus*				*Ae. aegypti*			
washes	MC(P)	MC(N)	EC(P)	EC(N)	MC(P)	MC(N)	EC(P)	EC(N)
0	84.82 (9.82)	88.70 (2.19)	98.72 (1.81)	88.90 (1.46)	100 (0)	100 (0)	100 (0)	100 (0)
1	98.33 (2.36)	94.67 (7.54)	98.15 (2.62)	98.71 (1.81)	100 (0)	100 (0)	100 (0)	97.37 (1.92)
2	99.78 (1.58)	96.15 (0.57)	95.50 (3.37)	87.11 (2.06)	100 (0)	100 (0)	100 (0)	93.16 (5.57)
3	94.36 (1.44)	88.33 (10.60)	88.95 (4.38)	74.43 (16.27)	100 (0)	100 (0)	95.00 (7.07)	89.60 (1.58)
4	98.33 (2.36)	88.93 (9.90)	93.64 (1.93)	50.13 (5.50)	100 (0)	98.61 (1.96)	98.72 (1.81)	66.40 (21.59)
5	100 (0)	98.81 (1.68)	88.65 (8.22)	82.25 (12.41)	100 (0)	98.81 (1.68)	90.00 (4.50)	91.94 (3.75)
6	85.94 (17.95)	95.53 (1.90)	86.29 (1.33)	64.92 (14.07)	87.10 (4.87)	93.86 (2.70)	74.38 (6.34)	60.39 (33.54)

( ) denotes standard deviation

Table 2
Mean of percentage mortality of *Anopheles maculatus* and *Aedes aegypti* exposed to treated nets after washing with water for 6 times.

	MC polyester	MC polyethylene	EC polyester	EC polyethylene
An. maculatus	[a]88.88	89.99	[a]61.29[b]	79.92[b]
Ae. aegypti	[c]86.58	84.78	[c]66.85	72.58

Significance of difference: [a]p and [b]p < 0.001; [c]p < 0.01 by ANOVA

maculatus exposed to 2 different nets after washing with soap and water are shown in Fig 2 and Table 3. When tested against *An. maculatus*, the MC polyethylene nets washed with soap and water performed better than MC polyester net ($p < 0.005$) (Table 3). However washing with soap and water showed that a mortality of 90% was obtained only after the first wash (Fig 2). With *Ae. aegypi* MC formulation gave more than 90% mortality with both polyethylene and polyester nets for two washes (Fig 2). However there was no significant difference in any combination using *Ae. aegypti*. *Ae. aegypti* knockdown values were higher with MC polyester and EC polyester compared to polyethylene combination (Table 4).

These results showed that higher value of knock down recovered back after 24 hours among *Ae. aegypti* than *An. maculatus*, also washing with soap than with water and among emulsion concentrate formulation combination.

Scanning electron microscopy

Pictures obtained of polyester and polyethylene netting samples impregnated with MC and EC formulation of lambda-cyhalothrin are shown in Plate 1. The crystals of the MC formulation appear to have a crystaline structure and rather evenly cover the surface of the nets compared to the EC formulation. In the polyester nets the deposits can be seen concentrated in spaces between the multiple fibers.

Table 3
Percentage knock down of *An. maculatus* and *Ae. aegypti* after 2 minutes exposure to polyethylene (P) and polyester (N) nets impregneted with lambda-cyhalothrin microencapsulated (MC) and emsulsion concentrate (EC) formulation subjected to six washings with soap and water.

No. of	*An. maculatus*				*Ae. aegypti*			
washes	MC(P)	MC(N)	EC(P)	EC(N)	MC(P)	MC(N)	EC(P)	EC(N)
0	84.82	88.70	98.72	88.91	100.00	100.00	100.00	100.00
	(9.82)	(2.19)	(1.81)	(1.46)	(0.00)	(0.00)	(0.00)	(0.00)
1	98.61	88.49	94.12	88.43	100.00	100.00	100.00	92.88
	(1.96)	(10.30)	(8.32)	(4.88)	(0.00)	(0.00)	(0.00)	(2.40)
2	95.15	91.30	94.32	85.70	100.00	98.72	96.54	96.25
	(1.67)	(3.96)	(0.29)	(5.39)	(0.00)	(1.81)	(2.45)	(2.85)
3	72.76	72.07	73.92	78.68	87.16	83.93	72.36	96.15
	(23.00)	(8.20)	(13.70)	(6.70)	(9.73)	(9.11)	(9.48)	(5.44)
4	67.24	44.77	46.86	58.13	63.23	81.89	62.94	81.27
	(27.25)	(16.30)	(17.70)	(10.00)	(26.09)	(7.54)	(20.23)	(4.03)
5	80.82	55.92	66.20	73.94	84.12	81.21	81.26	91.78
	(11.20)	(17.96)	(11.61)	(8.27)	(6.67)	(6.36)	(11.89)	(4.46)
6	50.63	47.86	66.57	62.86	9.10	19.01	10.94	42.52
	(10.74)	(7.16)	(8.80)	(6.06)	(6.66)	(19.70)	(8.08)	(25.69)

( ) denotes standard deviation

Table 4
Mean of percentage mortality of *An. maculatus* and *Ae. aegypti* exposed to treated nets after washing with soap and water six times.

	MC polyester	MC polyethylene	EC polyester	EC polyethylene
An. maculatus	^{a}54.22	^{a}68.79^{b}	54.12	56.59^{b}
Ae. aegypti	59.02	51.57	58.19	54.95

Significance of difference: ^{a}p and $^{b}p < 0.005$ by ANOVA

DISCUSSION

This study showed that the residual activity of lambda-cyhalothrin microencapsulated formulation (MC) and emulsion concentrate (EC) at 15 mg/m^2 on two different types of nets tested were effective for a period of 9 months. Overall percentage mortality of above 90% was achieved by all treatment combinations. These results concur with that of other workers, where pyrethroid treated bed-nets have prolonged insecticidal effect of 6-12 months without washing (Miller *et al*, 1995; Jana-Kara *et al*, 1994). Curtis *et al* (1996) also showed that lambda-cyhalothrin microencapsulated formulation of 10 mg/m^2 on bednets performed well after 15 months of domestic use.

Washing with water alone reduced the effectiveness of the insecticide with both polyethylene and polyester nets as demonstrated by bioassays using *An. maculatus* and *Ae. aegypti*. However, it was shown that micorencapsulated formulation was more effective than emulsion concentrate. In the study, where permethrin was used to treat nets (0.5 g/m^2), the mortalities of *An. maculatus* and *Ae. aegypti* remained above 80% for up to 10 washes (Vythilingam *et al*, 1996). This could be due to longer exposure time of 10 minutes used in that study. In this study the exposure time was only 2 minutes. It is also note worthy that using short exposure times of a few seconds to a couple of minutes is more likely to represent the real period of contact between mosquitos and treated netting (WHO, 1989).

Since the microencapsulated formulation of insecticide gave more than 85% mortality for up to 5 washes suggest that capsules of the microencapsulated (Baker *et al*, 1993) formulation are not easily washed away with just washing with water. With a dosage of as little as 10 mg/m², bioassay involving 3 minutes exposure with *Anopheles gambiae* to nets in routine use, showed nearly 100% mortality over many months of use, despite washing the nets (Njunwa *et al*, 1991).

In this study, two washings of the treated nets with soap and water still resulted in a mortality of above 90% for MC formulation and above 80% for EC formulation. Perhaps in the MC formulation, the capsules were well bonded to the net fibers and are thus not easily removed by soap and water combinations. Similar results were obtained by Miller *et al* (1991) where he found lambda-cyhalothrin (EC) treated nets washed 3 times with cow-fat soap, reduced the bioassay mortality to 40%. These results show that MC formulation capsules provides greater protection of the active ingredient from degradation compared to crystalline form of EC formulation. This seems to explain the fact that MC formulation has an evaporatively generated airborne effect against mosquitos (Kawada *et al*, 1995; Baker *et al*, 1993).

With regards to the type of fabric and the formulation of insecticides used, it was shown that the MC formulation on polyethylene monofilament nets gave higher mortality with both *An. maculatus* and *Ae. aegypti* compared to MC formulation on polyester multifilament nets. Although the polyethylene net is monofilament it seems to have higher binding capacity towards MC formulation and thus able to

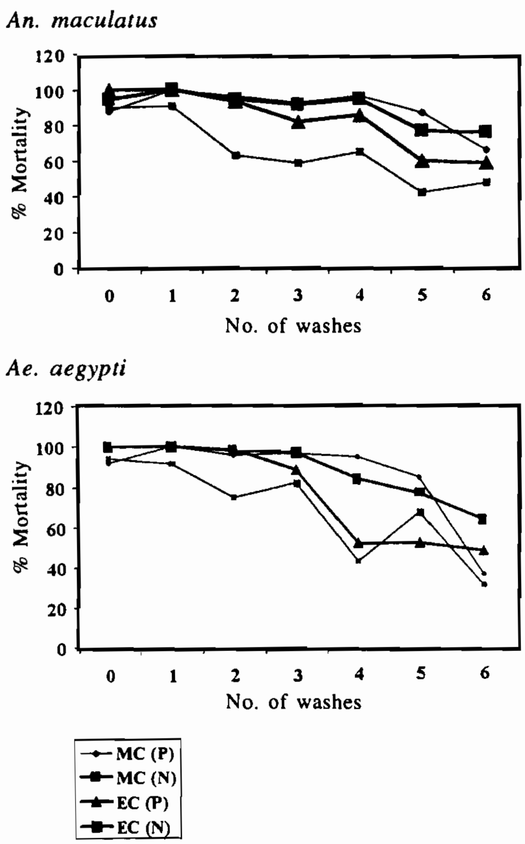


Fig 1–Percentage mortality of *An. maculatus* and *Ae. aegypti* after 2 minutes exposure to polyethylene (P) and Polyester (N) nets impregnated with lambda-cyhalothrin microencapsulated (MC) and emulsion concentrate (EC) subjected to six washes with water.

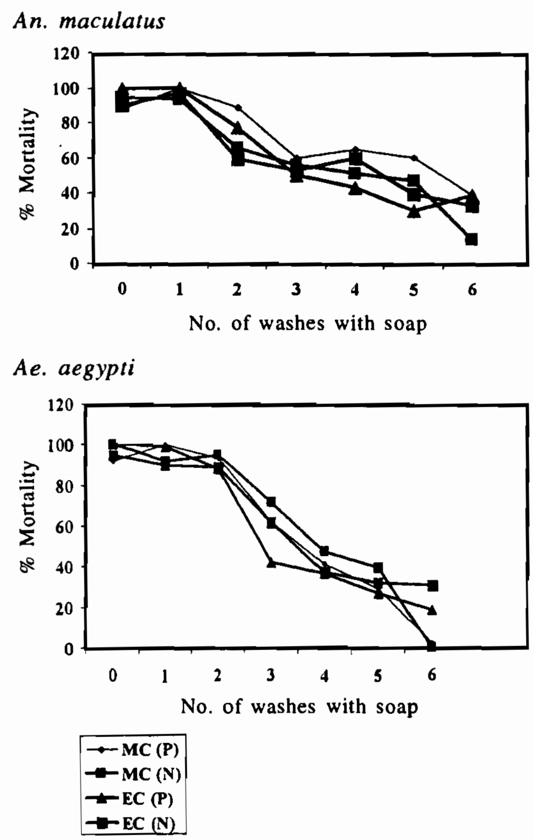
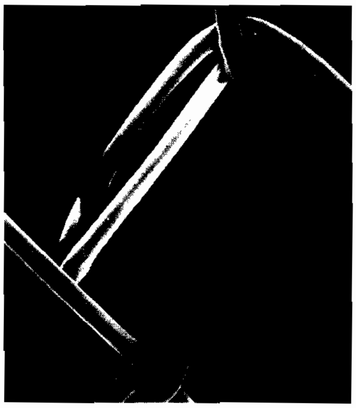
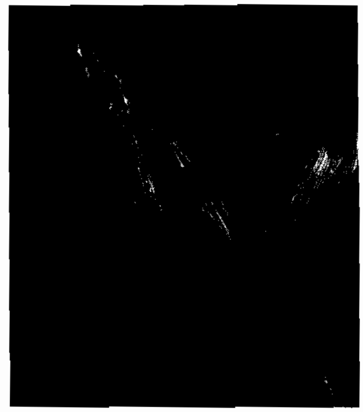


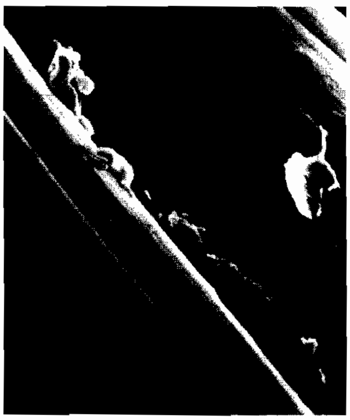
Fig 2–Percentage mortality of *An. maculatus* and *Ae. aegypti* after 2 minutes exposure to polyethylene (P) and Polyester (N) nets impregnated with lambda-cyhalothrin microencapsulated (MC) and emulsion concentrate (EC) subjected to six washes with soap and water.



A
Polyethylene 75x magnification (unimpregnated)

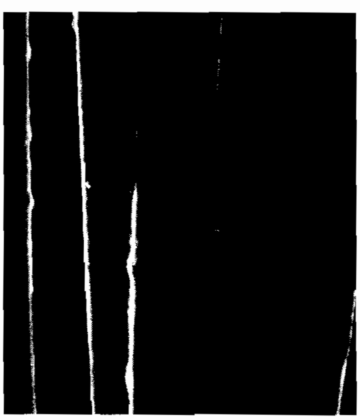


B
Polyester 75x magnification (unimpregnated)



C
Polyethylene EC formulation

D
Polyethylene MC formulation



E
Polyester EC formulation

F
Polyester MC formulation

Plate 1–SEM structure of impregnate and unimpregnated netting at 1,000x magnification.

withstand washes.

The results achieved is specific and unique on its own, since different insecticide formulations have different binding capacity to materials and fabrics used (Curtis *et al,* 1994). Lambda-cyhalothrin 2.5 CS (Miller *et al,* 1991) and 2.5 EC (Miller, 1990) has best effect on nylon while Curtis *et al* (1996) found that lambda-cyhalothrin 2.5 CS perform better on cotton and polyester.

The results of the present study showed that the polethylene fiber is better than the polyester fiber for impregnation with insecticide. This can be explained by observing the scanning electron micrographs. Most of the insecticide was deposited on the smooth surface of the polyethylene fiber but some insecticide was concentrated in the spaces between multifilaments of the polyester netting. The effectiveness of an insecticide will depend to a large extent on the amount of insecticide available on the net fiber surface for contact with mosquitos that rest on the net or probe with their head and proboscis between the fibers.

An 80% mortality level will portray the practical protection given to the communities in the field rather than 50%. Thus it is shown that lambda-cyhalothrin MC formulation impregnated to polyethylene nets at 80% cut off level performed best, where it could sustain up to 5 washes with water and 2 washes with soap and water. These results should only be taken as preliminary, as true effectiveness of pyrethroids on bednet materials should be tested in the field to evaluate the mortality, irritant and deterrent effect (Lindsay *et al,* 1991) on local vector mosquitos and degradation of insecticide due to normal washing performed by communities.

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