LABORATORY PROTECTION RATE OF TORN BEDNETS TREATED WITH THREE DOSAGES OF PYRETHROIDE AGAINST *ANOPHELES CULICIFACIES*

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Abstract- For the prevention of human malaria, vector control is an important component of the global strategy. The objective of the present study was to observe the effect of impregnated torn bednets with three dosages of cyfluthrin 5% on the number of bites by *Anopheles culicifacies*. A glass made tunnel test was designed and hungry female mosquitoes were induce to pass through holes cut in the pyrethroid treated nets. A guinea pig used as bait to attract mosquitoes through circular holes in the netting. With untreated netting, 72-87% of laboratory-reared females passed through the holes overnight and 64-92% blood-fed successfully. When the netting was treated with cyfluthrin at doses of 25, 50 and 100 mg a.i./m^2, the entry index (the proportions that passed through the holes overnight) were 43.37%, 42.82% and 24.72%; mortality rates were 66.31%, 81.45% and 95.99%; and the feeding rate were 45%, 27% and 3%, respectively. Pyrethroid impregnated bednets using "tunnel tests" showing acceptable protection rate both in lower and higher dosages. In addition, the higher dosages of these three dosages pyrethroid provided good levels of protection against *An. culicifacies*.

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INTRODUCTION

For the prevention of human malaria, vector control is an important component of the global strategy (1). Vector control aims to prevent the transmission of malaria parasites by methods such as house spraying with residual insecticides (2) or personal protection by the use of pyrethroid-impregnated bednets (3).

Since Herodotus travel to Egypt, the bednets has assumed an important role in the control of malaria (4). Anti malaria bednets, treated with DDT, were first used during World War II by the Russian, German and US armies (5) but it was not until the 1980's that controlled trials demonstrate significant protection against the incidence of malaria (6).

Seasonal factors, patterns of use and question of cost are key factors likely to influence the effectiveness of bednets (7). Pyrethroid-treated bednets have been shown in recent trials to have an important impact on cases of malaria (8), incidence of infection and prevalence of anemia (9) and all-caused child deaths in several parts of the Africa (10). Treated nets are more acceptable and affordable than house spraying (9). The acceptance and usage of treated bednets is better than ordinary bednets (11). DDT spraying was more than six times

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more expensive per person per year than providing the bednets (2).

Bednets in perfect condition can prevent 90% of bites (2), but they are usually so damaged that mosquitoes are not stopped from taking a blood meal on the sleepers. A number of surveys have recently demonstrated the efficacy of torn bednets on reduction of man-vector contact. If a considerably torn bednet correctly be impregnated, it could be a useful method for limiting human-anopheline contacts (12). Damaged bednets with a large number of holes and impregnated with the pyrethroid etophenprox or organophosphate (OP) pirimiphos methyl did not provide protection against being bitten by pyrethroid resistance *An. gambiae* and *Culex* spp. (9).

The netting barrier under test was made of polyester with holes of standard size cut in it to simulate a torn bednet, as is often found in rural tropical houses. This has been used to test a series of different dosages of cyfluthrin, deltamethrin and permethrin, which have been developed by the pesticide industry for bednet impregnation.

MATERIALS AND METHODS

Mosquitoes

Mosquitoes were reared to the pupal stage on a diet of BEMAX®. Adults were kept at ≈75±5% RH and 28 ± 2°C in a photophase of 12:12 (L: D) h with unlimited access to 10 glucose.

Pyrethroid insecticides

Tests were performed with cyfluthrin 5% EW, obtained from Bayer®. A range of three concentration of each of these pyrethroid was used to examine the relationship between dose and response.

Impregnation

The netting was impregnated by immersion in a mixture of insecticide and water in any dose. The netting samples were dried in the laboratory. Testes were carried out 24 h after impregnation. The amount of insecticide required for treatment of the netting was calculated using the following formula:

[Recommended dosages × are of net/concentration of insecticide × 10] × 100

The volume of water absorbed by the samples of polyester was calculated by comparing the weight of five samples of each net when dry and when wet (dipped in water and excess water squeezed out).

Tunnel Test

Polyester 100 denier multifilament netting was tested to determine whether *An. culicifacies* females were able to pass through the holes having 1 cm diameter in sheets of treated or untreated netting, and whether the mosquitoes would take a blood meal there after with each of insecticides.

Testes were performed in a square glass "tunnel" (height 25 cm, width 25 cm, length 60 cm) with cage ends, as described by Elissa and Curtis (5) and Chandre (13), subdivided changeable piece netting with 9×1 cm holes (1% of total area) inserted on a cardboard frame across the tunnel. Netting was previously impregnated with insecticide to give a predetermined treatment rate (mg ai/m^2). After drying the impregnated piece of net used for testing 24 h post impregnation. In one end of the tunnel a guinea pig was placed as bait, held in a small metallic holder to prevent contact with the netting. In the other end of the tunnel, ≈ 100 unfed female mosquitoes (6-8 days old) were introduced at 17:00 hours and the apparatus was left overnight in a dark room maintained at 28° C and 80% RH. The next morning, at 07:00 hours, the numbers of mosquitoes in both compartments were counted and their mortality and blood-feeding rates were scored. Testes were replicated three times for each dose, EW 5% cyfluthrin 25, 50 and 100 mg/m^2, and control untreated netting.

Statistical analyses

Data was analyzed by observed percentage mortality were corrected by Abbott's formula for contemporary control mortality. For significant testing, corrected mortalities were Arcsine transformed and submitted to one-way ANOVA in SPSS.

A value of $P \leq 0.05$ was considered statically significant.

RESULTS

For three dosage of insecticide (cyfluthrin) tested in tunnel cage, mortality rates ranged from 3.9% to 4.34% with non-impregnated netting (Table 1). The proportions of mosquitoes that succeeded in passing through holes in the net (entry index) were from 77% to 99% and taking a blood meal (individual protection) from 64% to 92%.

Effects of impregnated torn nets on the numbers of mosquitoes entering from holes in three assessed of cyfluthrin are shown in Fig. 1. About 38% fewer *An. culicifacies* entered the tunnel containing cyfluthrin treated torn nets than untreated torn nets ($P < 0.01$). There were no significant differences between the numbers of *An. culicifacies* in treated torn nets with permethrin and cyfluthrin. In cyfluthrin (at 25 mg/m^2) about 25% of mosquitoes were succeeded in passing through holes ($P < 0.05$).

We observed about 100% mortality in high dosage of cyfluthrin (100 mg/m^2); therefore the test did not carried out with high dosage of this insecticide (1000 mg/m^2) (Table 1). For *An. culicifacies* with the torn nets, all the insecticides caused significantly greater mortality than the untreated torn nets ($P < 0.01$). There was significant difference between the numbers of *An. culicifacies* killed by cyfluthrin and control in low dosage. The highest percentage of mosquitoes was killed with cyfluthrin in 100 mg/m^2 (95%) concentration. In low dosages, fewer *An. culicifacies* was killed, but all of them killed more mosquitoes than untreated torn nets. The tunnel with untreated nets had a significantly higher percentage of blood feed females than any of treated nets ($P < 0.01$).

With cyfluthrin treated torn nets, there were significant differences in the proportions of blood feed with control in *An. culicifacies* females in low and medium dosages. Low dosage of cyfluthrin offered 20% individual protection, whereas it was 60% in control. The best individual protection against *An. culicifacies* was obtained with cyfluthrin at 100 mg/m^2 (nearly 100%) concentration (Fig 2).

In high dosage, cyfluthrin treated torn nets had a significantly lower percentage of blood feed females than control ($P < 0.01$).

DISCUSSION

Prevention of mosquito probing and blood feeding is a doubly important function of treated bednets, directly reducing transmission risk and allowing a good night sleep. With untreated bednets, mosquitoes can probe through the net or enter through torn holes to bite the sleeper.

Kolaczinski *et al.* reported that with increase of dosage of cyfluthrin, blood feeding is decrease (9). Yadava *et al.* stated that cyfluthrin impregnated bednets reduced blood feeding of mosquitoes (14). Present study is the first report from application of cyfluthrin against *An. culicifacies* in torn nets. In our study, in 100 mg/m^2 dosage of cyfluthrin observed the best efficacy in individual protection, Low dosages of this insecticide (25 mg/m^2) can reduce entry index, blood feeding and increase mortality rate of *An. culicifacies* in comparison with control (>80% reduced blood feeding). When cyfluthrin used, the trend of mortality rate was greater when the insecticide concentration was increased.

Table 1. Effect of netting impregnated with cyfluthrin 5% EW in tunnel cage on *An. culicifacies*

Insecticide Doses	Group	N	Mortality (%)	Passed through net (%)	Blood-fed	Survival rate	PI	PC
25 mg/m^2	control	285	4.21	77	204	78	72	71
	test	279	66.31	43	45	23	15.6	0.13
50 mg/m^2	control	282	3.9	85	182	89	64	64
	test	275	81.45	42	27	15	0.1	0.06
100 mg/m^2	control	276	4.32	93	255	96	92	91
	test	271	95.99	24	3	0.04	0.1	0

Abbreviations: N, number; PI, individual protection.

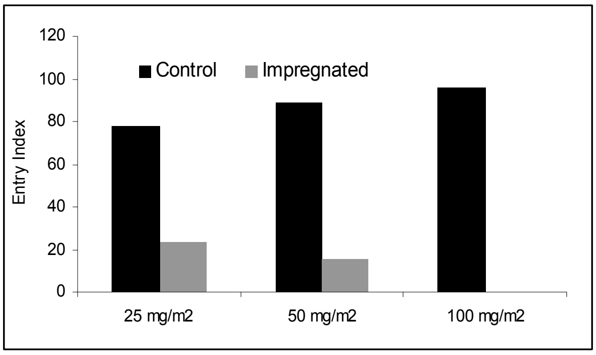

Fig. 1. Comparison of entry index of *An. Culicifacies* expose to torn nets impregnated with three dosages of cyflothrin 5% EW in tunnel test cage.

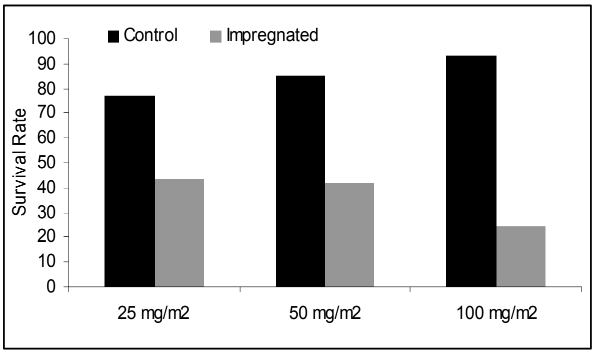

Fig. 2. Comparison of survival rate of *An. Culicifacies* expose to torn nets impregnated with three dosages of cyflothrin 5% EW in tunnel test cage.

Individual protection with this insecticide was 20-80%, depend on the dosages. Torn bednets impregnated with cyfluthrin (at 25 or 50 mg/m^2) reduced by nearly 50% the contact between humans and *An. gambiae* (15). In our study, torn impregnated nets reduced 50% contact between *An. culicifacies* and guinea pig at 50 mg/m^2. In *An. culicifacies*, in contrary with *An. gambiae* (12) mortality rate was increased when the insecticide was increased.

Tests of torn nets impregnated with cyfluthrin revealed the best individual protection (> 90%) in our study. It has been reported that torn bednets impregnated with 500 mg/m^2 of cyfluthrin reduced 60% entry of *An. Gambiae* (15), which reduced 80% of entry of *An. culicifacies* at same dosage in our study. Mortality rate of *An. culicifacies* exposed to cyfluthrin treated nets was dependent to insecticide concentration.

Different insecticides showed various degrees of irritancy and killing ability against mosquitoes when used for net impregnation (5). The results of this study indicate that different dosages of tested insecticides showed various degrees of irritancy and killing ability against *An. culicifacies*, when used for impregnation of torn nets. Pyrethroid-impregnated bednets in tunnel tests, showing that if insecticide and its dosage are selected properly, even torn bednets impregnated with pyrethroid provided good levels of protection against *An. culicifacies*.

Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

1. [No author listed]. Implementation of the Global Malaria Control Strategy. Report of a WHO Study Group on the Implementation of the Global Plan of Action for Malaria Control 1993–2000. Geneva, World Health Organization, 1993 (WHO Technical Report Series, No. 839).
2. Pampana EJ. A textbook of malaria eradication. Second ed. Oxford: Oxford University Press, 1969.
3. Lengeler C, Snow RW. From efficacy to effectiveness: insecticide-treated bednets in Africa. Bull World Health Organ. 1996; 74(3):325-332.
4. Nevill CG, Some ES, Mung'ala VO, Mutemi W, New L, Marsh K, Lengeler C, Snow RW. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. Trop Med Int Health. 1996 Apr; 1(2):139-146.
5. Elissa, N, Curtis CF. Evaluation of different formulations of deltamethrin in comparison with permethrin for impregnation of netting. Pesticide Science. 1995; 44: 363-367.
6. Luxemburger C, Perea WA, Delmas G, Pruja C, Pecoul B, Moren A. Permethrin-impregnated bed nets for the prevention of malaria in schoolchildren on the Thai-Burmese border. Trans R Soc Trop Med Hyg. 1994 Mar-Apr; 88(2):155-159.
7. Binka FN, Adongo P. Acceptability and use of insecticide impregnated bednets in northern Ghana. Trop Med Int Health. 1997 May; 2(5):499-507.
8. Jana-Kara BR, Jihullah WA, Shahi B, Dev V, Curtis CF, Sharma VP. Deltamethrin impregnated bednets against Anopheles minimus transmitted malaria in Assam, India. J Trop Med Hyg. 1995 Apr; 98(2):73-83.

9. Kolaczinski JH, Fanello C, Herve JP, Conway DJ, Carnevale P, Curtis CF. Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide impregnated bednets for mosquito control in an area of pyrethroid resistance. Bull Entomol Res. 2000 Apr; 90(2):125-132.
10. Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude GH, Armah GE, Kajihara B, Adiamah JH, Smith PG. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. Trop Med Int Health. 1996 Apr; 1(2):147-154.
11. Das PK, Das LK, Parida SK, Patra KP, Jambulingam P. Lambdacyhalothrin treated bed nets as an alternative method of malaria control in tribal villages of Koraput District, Orissa State, India. Southeast Asian J Trop Med Public Health. 1993 Sep; 24(3):513-521.
12. Carnevale P, Bitsindou P, Diomande L, Robert V. Insecticide impregnation can restore the efficiency of torn bed nets and reduce man-vector contact in malaria endemic areas. Trans R Soc Trop Med Hyg. 1992 Jul-Aug; 86(4):362-364.
13. Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P. Modifications of pyrethroid effects associated with kdr mutation in Anopheles gambiae. Med Vet Entomol. 2000 Mar; 14(1):81-88.
14. Yadava RL, Rao CK, Biswas H. Field trial of cyfluthrin as an effective and safe insecticide for control of malaria vectors in triple insecticide resistant areas.J Commun Dis. 1996 Dec; 28(4):287-298.
15. Zoulani A, Carnevale P, Penchenier L. [Influence of mosquito nets impregnated with deltamethrin on the aggressivity cycle of Anopheles gambiae in Djoumouna, Congo]. Ann Soc Belg Med Trop. 1994 Jun; 74(2):83-91. French.