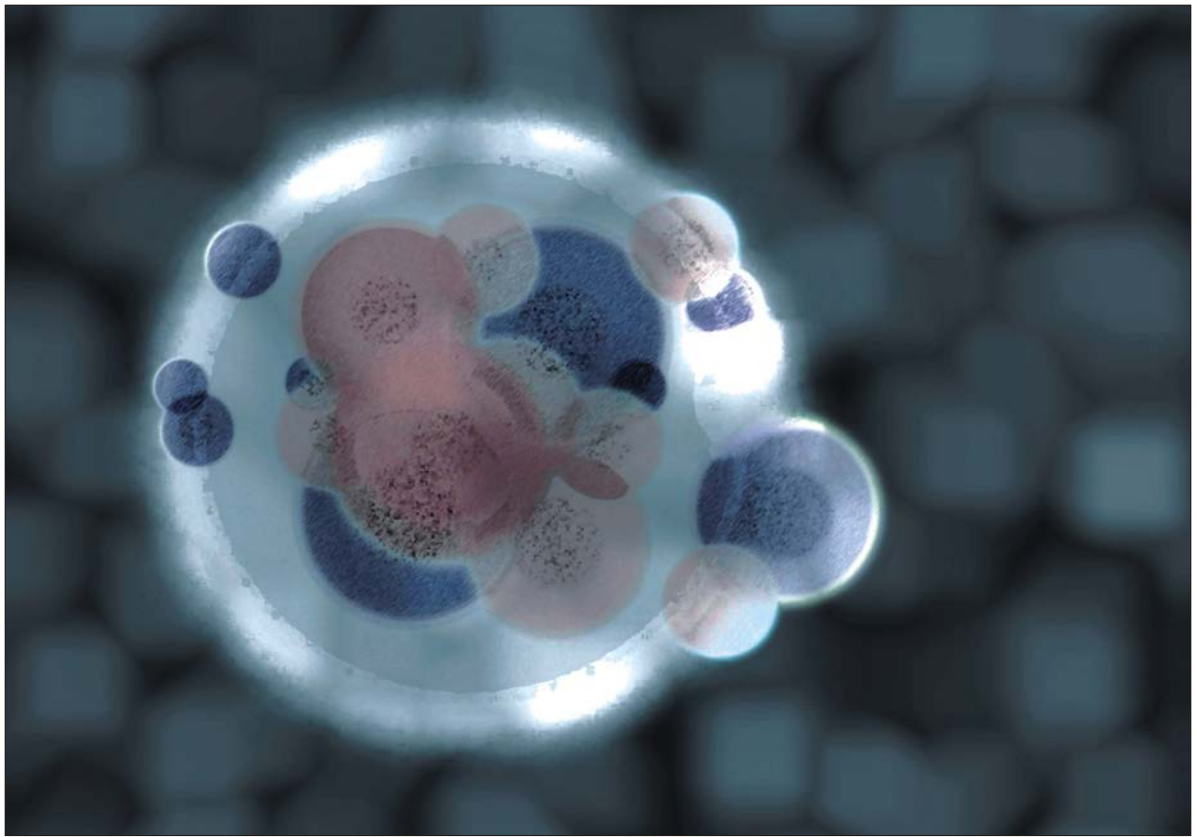




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Laboratory Efficacy of Protection Rate of Torn Nets Treated with Pyrethroids, Cyfluthrin, Deltamethrin and Permethrin Against *Anopheles stephensi* (Diptera: Culicidae)

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Abstract: The effect of torn bednets treated with three dosages of cyfluthrin 5% EW, deltamethrin 10% SC and permethrin 10% EC were evaluated under laboratory condition in. The objective of the present study was to observe the effect of impregnated torn bednets on the number of bites by *An. stephensi*. A glass tunnel test was designed to induce hungry female mosquitoes 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, 81-95% of laboratory-reared females passed through the holes overnight, 75-93% blood-fed successfully and 0.36-4% died. 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, 41.82 and 23.72%; mortality rates were 66.31, 81.45 and 95.99%; and the feeding rate were 16.13, 9.82 and 1.09%. Experiments with deltamethrin treated nets at dosages of 12.5, 25 and 50 mg a.i. m⁻², the entry index was 78.10, 61.54 and 27.34%, respectively, mortality rates were 53.26, 58.85 and 82.35%; blood feeding rate was 46.72, 31.15 and 13.49%. When the netting was impregnated with permethrin at dosages of 200 and 500 mg a.i. m⁻², the entry index was 33.58 and 14.95%; mortality rates were 93.80 and 100% and blood-feeding rate was 5.11 and 0.36%. In conclusion it should be stressed that efficacy of pyrethroid impregnated bednets using "Tunnel Tests" showing acceptable protection rate both in lower and higher dosages as well as cause dead in the blood-fed mosquitoes. In addition, the higher dosages of these three pyrethroids provided good levels of protection against *An. stephensi*.

Key words: *Anopheles stephensi*, torn impregnated nets, Tunnel test, pyrethroids, Iran

INTRODUCTION

In 1897, Ronald Ross discovered that malaria is transmitted through the bites of *Anopheles* mosquitoes. Malaria parasites (*Plasmodium*) are picked up when a mosquito bites an infected person; they mature inside the mosquito and are passed on to a new victim when the mosquito bites again. Soon after making this discovery, Ross realized that because these mosquitoes bite at night, bednets should be a useful protection against infection. In the 1980s, insecticides began to be applied to bednets, which greatly increased their effectiveness-even when torn. Yet, despite the enormous toll that the disease takes on millions of poor Africans, treated bednets are still used far too little (Curtis, 2005).

Pyrethroids are the only insecticides currently recommended by the World Health Organization for treatment of mosquito nets owing to their strong insecticidal activity at low concentrations and their low mammalian toxicity (Zaim *et al.*, 2000). Pyrethroid-treated

nets are effective in reducing malaria morbidity and mortality (Lengeler *et al.*, 1996) and may also provide community protection through mass impact on vector mosquito populations, when used at a high coverage rate (WHO, 1993; Takken, 2002).

Seasonal factors, patterns of use and question of cost are key factors likely to influence the effectiveness of bednets (Binka and Adondo, 1997). Pyrethroid-treated bednet have been shown in recent trials to have an important impact on cases of malaria (Jana-Kara, 1995), incidence of infection and prevalence of anemia (Curtis *et al.*, 1998) and all-caused child deaths in several parts of the Africa (Nevill *et al.*, 1996; Binka *et al.*, 1996). Treated nets are more acceptable and affordable than house spraying (Curtis *et al.*, 1998). The acceptance and usage of treated bednets is better ordinary nets (Das *et al.*, 1993). DDT spraying was more than six times more expensive per person per year than providing the bednets (Neng *et al.*, 1993). Recently, Hougard *et al.* (2003) compared the efficacy of seven pyrethroid

insecticides for impregnation of mosquito nets, six currently recommended by WHO and one candidate (bifenthrin), under laboratory condition.

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. Other studies on the efficacy, under laboratory conditions have been conducted in situations that did not permit direct comparison in terms of impact on mosquito mortality, irritancy and blood-feeding inhibition. The present study was undertaken to find that whether torn pyrethroids can prevent mosquito entering and biting even at the lower dosages? The aim of current study is establishing a novel method for performance of mosquito to different dosages of various pyrethroids.

MATERIALS AND METHODS

Mosquitoes: This study was conducted in School of Public Health and Institute of Health Research in Tehran University of Medical science in 2003. Laboratory strain of *An. stephensi* was used. Mosquitoes were reared to the pupal stage on a diet of BEMAX®. Adults were kept at, $\approx 75 \pm 5\%$ RH and $28 \pm 2^\circ\text{C}$ in a photophase of 12:12 (L: D) h with unlimited access to 10% glucose.

Pyrethroid insecticides: Most tests were performed with cyfluthrin 5% EW, obtained from Bayer®, deltamethrin 10% SC and Permethrin 10% EC obtained from Aventis®. A range of three concentration of each of these pyrethroides 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:

$$\frac{\text{Recommended dosage} \times \text{Area of net}}{\text{Concentration of insecticide} \times 10} \times 100$$

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).

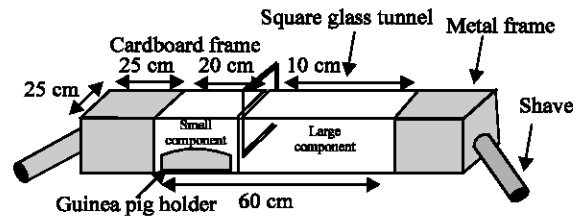


Fig. 1: Design of tunnel test cage

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 transformed with Arcsine and submitted to One-way-ANOVA in SPSS software.

Tunnel test: Polyester 100 denier multifilament netting was tested to determine whether *An. stephensi* 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 (1995) and Chandre *et al.* (2000), subdivided changeable piece netting with 9×1 cm holes (1% of total area) inserted on a cardboard frame across the tunnel (Fig. 1). Netting was previously impregnated with insecticide to give a predetermined treatment rate (mg a.i. 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 h and the apparatus was left overnight in a dark room maintained at 28°C and 80% RH. The next morning, at 07:00 h, 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, cyfluthrin 25, 50 and 100 mg m^{-2} , deltamethrin, 12.5, 25 and 50 mg m^{-2} and permethrin, 200, 500 and 1000 mg m^{-2} and control untreated netting.

RESULTS

For three dosages of three insecticides (Table 1) tested in tunnel cage (Table 2-4), mortality rates ranged from less than 5% with non-impregnated netting. The proportion of mosquitoes that successive in passing through holes in the net (entry index) was about 88.4% and taking a blood meal (Individual protection) around 84.14%.

Table 1: Specification of insecticide used for impregnation of nets

Insecticide	% a.i	Formulation	Manufacturer
Cyfluthrin	5	EW	Bayer
Deltamethrin	10	SC	Aventis
Permethrin	10	EC	Aventis

Table 2: Effect of netting impregnated with cyfluthrin 5% EW in tunnel cage on *An. stephensi*

Insecticide dosages (mg m ⁻²)	No. tested	Mortality (%)	Passed through net (%)	Blood-fed (%)
25 Control	285	4.21	81.4	78.25
25 Test	279	66.31	43.37	16.13
50 Control	285	3.9	82.98	75.18
50 Test	275	81.45	41.82	9.82
100 Control	278	4.32	87.05	91.73
100 Test	274	95.99	23.72	1.09

Table 3: Effect of netting impregnated with deltamethrin 10% SC in tunnel cage on *An. stephensi*

Insecticide dosages (mg m ⁻²)	No. tested	Mortality (%)	Passed through net (%)	Blood-fed (%)
12.5 Control	270	1.01	93.33	85.86
12.5 Test	274	53.26	78.10	46.72
25 Control	261	2.3	95.40	93.10
25 Test	260	58.85	61.54	31.15
50 Control	282	2.15	91.49	91.4
50 Test	289	82.35	27.34	13.49

Table 4: Effect of netting impregnated with permethrin 10% EC in tunnel cage on *An. stephensi*

Insecticide dosages (mg m ⁻²)	N	Mortality (%)	Passed through net (%)	Blood-fed (%)
200 Control	257	1.95	89.88	88.33
200 Test	274	93.80	33.58	5.11
500 Control	286	2.11	91.26	85.26
500 Test	281	100	14.95	0.36
1000 Control	*	*	*	*
1000 Test	*	*	*	**

We observed 100% mortality in medium dosage of permethrin (500 mg m⁻²), therefore we don't accomplished tests with high dosage of this insecticide (1000 mg m⁻²)

Effects of impregnated torn nets on the numbers of mosquitoes entering from holes were assessed in three dosages of cyfluthrin, deltamethrin and permethrin (Fig. 2). About 38% fewer *An. stephensi* entered the tunnel containing permethrin treated torn nets (at 200 mg m⁻²) than untreated torn nets ($p < 0.01$). There were no significant differences between the numbers of entering *An. stephensi* in treated torn nets with permethrin and cyfluthrin. In low dose of deltamethrin about 89% of mosquitoes were successes in passing through holes ($p < 0.05$). Significantly fewer mosquitoes were found in the presence of permethrin treated torn nets compared with other treated nets in medium dosage ($p < 0.01$). In high dosage, there were no significant differences between the numbers of mosquitoes in cyfluthrin and deltamethrin treated torn nets. We observed 100% mortality in medium dosage of permethrin; therefore the test did not carried out with high dosage of this insecticide.

All three insecticides had significantly greater mortality than the untreated torn nets ($p < 0.01$). There was no significant difference in mortality rate in cyfluthrin

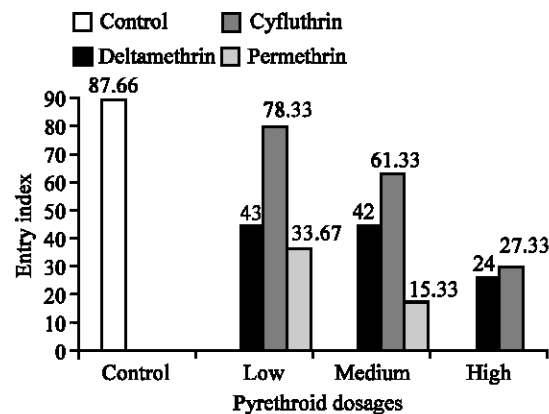


Fig. 2: Comparison of Entry Index of *An. stephensi* (IND-St) exposed to torn nets impregnated with three dosages of cyfluthrin, deltamethrin and permethrin in tunnel test cage

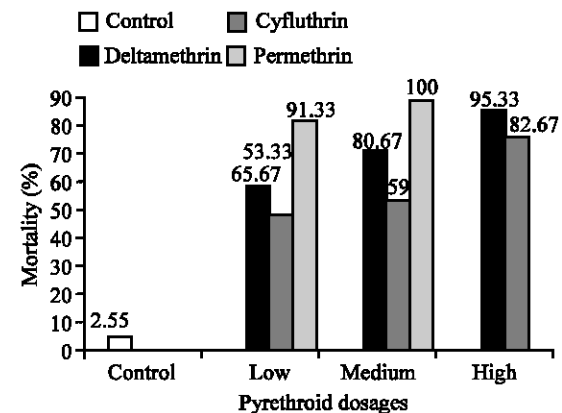


Fig. 3: Comparison of Mortality rate of *An. stephensi* (IND-St) exposed to torn nets impregnated with three dosages of cyfluthrin, deltamethrin and permethrin in tunnel test cage

and deltamethrin treated torn nets in low dosage. The highest percentage of mosquitoes was killed with permethrin in 500 mg m⁻² and cyfluthrin in 100 mg m⁻². Fewer were killed with deltamethrin at lower dose than other insecticides (Fig. 3). In low dosages, mortality rate was not significant in three insecticides, but was significant in comparison with control.

The tunnel with untreated nets had a significantly higher percentage of blood feed females than any of treated nets ($p < 0.01$). In cyfluthrin and permethrin treated torn nets, there were no significant differences in the proportions of blood fed *An. stephensi* females in low and medium dosages. The highest blood fed percentage was with deltamethrin dosages. In other

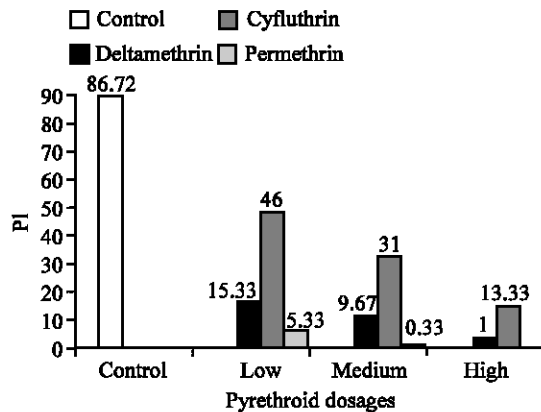


Fig. 4: Comparison of Individual Protection (PI) rate of *An. stephensi* (IND-St) exposed to nets impregnated with three dosages of cyfluthrin, deltamethrin and permethrin in tunnel test cage

hand, Permethrin treated torn nets had the best individual Protection (PI) in low dosage in comparison with deltamethrin and cyfluthrin. The best PI obtained with permethrin at 500 mg m⁻² dosage against *An. stephensi* (Fig. 4). In high dosage, cyfluthrin treated torn nets had a significantly lower percentage of blood feed females than deltamethrin ($p < 0.01$).

DISCUSSION

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

Curtis (1997) reported that with increase of dosage of cyfluthrin, blood feeding is decrease. Yadava *et al.* (1996) stated that cyfluthrin impregnated bed nets reduced blood feeding of mosquitoes. This study is the first report from application of cyfluthrin against *An. stephensi* in torn nets. In our study, in 100 mg m⁻² dosage of cyfluthrin observed the best efficacy in individual protection (PI), lower dosages of this insecticide (25 mg m⁻²) can reduce entry index, blood feeding and increase mortality rate of *An. stephensi* in comparison with control (>80% reduced blood feeding).

When deltamethrin were used, the trend of mortality rate was greater when the insecticide concentration was increased. Individual protection with this insecticide, was 47-80%, depend on the dosages. In *An. gambiae*, 12.5 or 25 mg m⁻² of deltamethrin had not influence on

biting cycle but reduced biting 50% up to (Zoulani *et al.*, 1994). Torn bed nets impregnated with deltamethrin (at 25 or 50 mg m⁻²) reduced by nearly 50% the contact between humans and *An. gambiae* (Carnevale *et al.*, 1992). In our study, torn deltamethrin impregnated nets reduced 50% contact between *An. stephensi* and guinea pig at 12.5 mg m⁻². In *An. stephensi*, in contrary with *An. gambiae* (Carnevale *et al.*, 1992) mortality rate was increased when the insecticide was increased.

Tests of torn nets impregnated with permethrin revealed the best PI (>90%) in our study. Miller *et al.* (1991) reported that torn bed nets impregnated with 500 mg m⁻² of permethrin reduced 60% entry of *An. gambiae*, which reduced 80% of entry of *An. stephensi* at same dosage. Corbel *et al.* (2004) in Tunnel tests showed that nets treated with permethrin at 250 and 500 mg m⁻² induced higher mortality and blood feeding reduction among susceptible and heterozygous (RS) females of *An. gambiae* as compared to the lower concentration (100 mg m⁻²). They concluded that nets treated with high permethrin concentrations provided better blood feeding prevention against pyrethroid-resistant *An. gambiae* than did lower concentrations. In our study mortality rate of susceptible strain of *An. stephensi* exposed to permethrin treated nets dependent on insecticide concentration. This means that mosquito nets were effective in high dosage; however the difference was not significant. It should be noted that our results are not comparable to that of finding of Corbel *et al.* (2004) due to testing on different species as well as resistant status of mosquito used.

Different insecticides showed various degrees of irritancy and killing ability against mosquitoes when used for net impregnation (Chandre *et al.*, 2000; Miller *et al.*, 1991; Curtis *et al.*, 1996). The results of this study indicate that different dosages of tested insecticides showed various degrees of irritancy and killing ability against *An. stephensi*, when used for impregnation of torn nets. Pyrethroid-impregnated bed nets in tunnel tests, showed that if insecticide and its dosage are selected properly, even torn bed nets impregnated with pyrethroids provided good levels of protection against *An. stephensi* but the biological factor that need to be considered is that mosquitoes might evolve resistance to the insecticide used to treat nets. The fact that so far all the insecticides used on nets are chemically similar because resistance to one might mean resistance to all. However, it is important to investigate the full effects of genes that can help insects resist insecticides in field conditions. It is postulated that if different sides of nets are impregnated with irrelevant

insecticides it may overcome the resistance mechanisms which may occur due to insecticide pressure. In this regards recently Darriet *et al.* (2005) studied the efficacy of mosquito nets treated with bifenthrin (pyrethroid) and chlorpyrifos-methyl (organophosphate), alone and in mixture against the pyrethroid-resistant strain of *Anopheles gambiae* using the tunnel test technique. They concluded that using mixture or rotation of different irrelevant insecticides on net can be a new strategy for combating against malaria vectors even in resistant strains. Present finding exhibited that even at the lower dosage of insecticides treated torn pyrethroids they are able to be effective against main malaria vector in Iran as well as the results should be attributed somewhere else where *An. stephensi* play a role in malaria transmission.

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REFERENCES

- Binka, F.N., A. Kubaje, M. Adjuik, L.A. Williams, C. Lengeler, G.H. Maude, G.E. Armah, B. Kajihara, J.H. Adiamah and P.G. Smith, 1996. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: A randomized controlled trial [see comments]. *Trop. Med. Intl. Health*, 1: 147-54.
- Binka, F.N. and P. Adongo, 1997. Acceptability and use of insecticide impregnated bednets in northern Ghana. *Trop. Med. Intl. Health*, 2: 499-507.
- Carnevale, P., P. Bitsindou, L. Diomande and V. Robert, 1992. 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.*, 86: 362-4.
- Chandre, F., F. Darriet, S. Duchon, L. Finot, S. Manguin, P. Carnevale and P. Guillet, 2000. Modification of pyrethroid effects associated with kdr mutation in *Anopheles gambiae*. *Med. Vet. Entomol.*, 14: 81-88.
- Corbel, V., F. Chandre, C. Brengues, M. Akogbeto, F. Lardeux, J.M. Hougard, P. Guillet, 2004. Dosage-dependent effects of permethrin-treated nets on the behavior of *Anopheles gambiae* and the selection of pyrethroid resistance. *Malar J.*, 8: 3-22.
- Curtis, C., 2005. Insecticide-treated bednets to prevent malaria. Science and Development Network.
- Curtis, C., 1997. Prevention of malaria with pyrethroid treated bednets. *Afr. Health*, 19: 17-8.
- Curtis, C.F., 1996. Insecticide-induced lupus erythematosus. *Intl. J. Dermatol.*, 35: 74-5.
- Curtis, C.F., J.E. Miller, M.H. Hodjati, J.H. Kolaczinski and J.H. Kasumba, 1998. Can anything be done to maintain the effectiveness of pyrethroid impregnated bednets against malaria vectors? *Philosophical Trans. R. Soc. London, Series B, Biol. Sci.*, 353: 1769-1775.
- Darriet, F., J.M. Hougard, V. Corbel, 2005. Effect of pyrethroid and organophosphate bi-treated nets on the behaviour of Kdr⁺ *Anopheles gambiae* mosquitoes. *Bull. Soc. Pathol. Exot.*, 98: 201-204.
- Das, P.K., L.K. Das, S.K. Parida, K.P. Patra and P. Jambulingam, 1993. Lambdaacyhalothrin 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*, 24: 513-521.
- Elissa, N. and C.F. Curtis, 1995. Evaluation of different formulations of deltamethrin in comparison with permethrin for impregnation of netting. *Pest. Sci.*, 44: 363-367.
- Jana-Kara, B.R., W.A. Jihullah, B. Shahi, V. Dev, C.F. Curtis and V.P. Sharma, 1995. Deltamethrin impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. *J. Trop. Med. Hyg.*, 98: 73-83.
- Hougard, J.M., S. Duchon, F. Darriet, M. Zaim, C. Rogier and P. Guillet, 2003. Comparative performance under laboratory conditions of seven pyrethroid insecticides used for impregnation of mosquito nets. *Bulletin of World Health Organization*, 81: 324-333.
- Lengeler, C. and R.W. Snow, 1996. From efficacy to effectiveness: insecticide-treated bednets in Africa. *Bull. World Health Organ.*, 74: 325-32.
- Miller J.E., S.W. Lindsay and J.R. Armstrong, 1991. Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in The Gambia. *Med. Vet. Entomol.*, 5: 465-76.
- Neng, W. *et al.*, 1993. Field evaluation of bednets impregnated with deltamethrin for malaria control. *Southeast Asian J. Trop. Med. Public Health*, 24: 664-671.

- Nevill, C.G., E.S. Some, V.O. Mung'ala, W. Mutemi, L. New, K. Marsh, C. Lengeler and R.W. Snow, 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast [see comments]. *Trop. Med. Intl. Hlth.*, 1: 139-146.
- Takken, W., 2002. Do insecticide-treated bednets have an effect on malaria? *Trop. Med. Intl. Hlth.*, 7: 1022-1030.
- WHO, 1993. Mise en oeuvre de la Strategie Mondiale de lutte Antipaludique. Technical Report Series No. 839. World Health Organization, Geneva .
- Yadava, R.L., C.K. Rao and H. Biswas, 1996. Field trial of cyfluthrin as an effective and safe insecticide for control of malaria vectors in triple insecticide resistant areas. *J. Commun. Dis.*, 28: 287-98.
- Zoulani, A., P. Carnevale and L. Penchenier, 1994. [Influence of mosquito nets impregnated with deltamethrin on the aggressively cycle of *Anopheles gambiae* in Djoumouna, Congo]. <Original> Influence des moustiquaires impregnees de deltamethrine sur le cycle d'agressivite d'*Anopheles gambiae* a Djoumouna, Congo. *Ann. Soc. Belg. Med. Trop.*, 74: 83-91.
- Zaim, M., A. Aitio and N. Nakashima, 2000. Safety of pyrethroid-treated mosquito nets. *Med. Vet. Entomol.*, 14: 1-5.