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Relative Efficacy of Different Synthetic Pyrethroids Impregnated Fabrics (ITNs) Against *Anopheles stephensi* in Iran

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Abstract: The efficacy of different synthetic pyrethroid-impregnated fabrics was evaluated against susceptible strain of *An. stephensi* under laboratory condition using a new method of WHO. LD_{50}, LD_{90} and diagnostic dose of pyrethroids were calculated for each type of insecticides and fabrics. Results revealed that LD_{50} of lambdacyhalothrin, deltamethrin, cyfluthrin and etofenprox were 1.84, 8.97, 10.15 and 40.6 mg m^{-2} on polyester; 3.19,11.73,10.94 and 103.01 mg m^{-2} on nylon and 9.95, 26.68, 46.75 and 9.53 mg m^{-2} on cotton. The polyester net need the least concentration of insecticide and it showed the maximum mortality and minimum values of LD_{50}, LD_{90} and diagnostic dose. So it is the best suitable net for impregnation. The cotton was the best for its persistence of insecticides. In all types of nets lambdacyhalothrin was significantly superior in comparison to deltamethrin, cyfluthrin and etofenprox. Some exception was seen in cotton nets impregnated with etofenprox. LD_{50}, LD_{90} and diagnostic dose were highly decreased in comparison with other pyrethroids.

Key words: Pyrethroid impregnated bednet, *Anopheles stephensi*, Deltamethrin, Cyfluthrin, Lambdacyhalothrin, Etofenprox

INTRODUCTION

The world strategy of malaria control is concentrated on vector control. In last decade Insecticide Treated Nets (ITNs) are the most suitable replace for residual insecticide treatments. The use of (ITNs) is an important part of Roll Back Malaria (RBM) programs which is recommended for malaria control in warm climate areas by WHO. Because of varieties in types of nets, insecticides and their formulations as well as species of mosquitoes, determination of diagnostic dose of insecticides is through necessary (Curtis *et al*., 1996; Curtis, 2005). Pyrethroids are today the only insecticides recommended for treatment of mosquito nets (Zaim *et al*., 2000). This is due to the rapid knock down effects and high insecticidal potency of pyrethroids at low dosages combined with relative safety for human contact and domestic handling. For treatment of nets WHO (2002) lists some insecticide products which have been passed the World Health Organization Pesticide Evaluation Scheme (WHOPES). The concentration which is recommended depends on texture of net, but recommended dosage is suggested to be effective against malaria vectors. Due to operational use of insecticide treated nets (ITNs) for malaria control is now advocated as a component of roll back malaria led by the WHO (1999, 2002 and 2005).

MATERIALS AND METHODS

An. stephensi, a principal vector of malaria in Iran, was used for laboratory evaluation. This species is highly susceptible to pyrethroid insecticides and provided by London School of Tropical Medicine and Hygiene. Emulsifiable Concentration formulation (EC) of deltamethrin (25%) (Russel Uclaff company), Suspension Concentration formulation (SC) of lambdacyhalothrin (2.5%) (Zeneca company), Emulsifiable oil-in Water formulation (EW) of cyfluthrin (5%) (Bayer company) and Emulsifiable Concentration formulation (EC) of etofenprox (10%) (Mitsui company) were used during the study.

The following local fabrics were used; Polyester, with 21 horizontal and 17 vertical threads per $inch^2$, 350 mesh. One meter square of this fabric absorbed 27 mL water. Nylon with 39 horizontal and 24 vertical threads per $inch^2$, 940 mesh. Each meter square of this fabric absorbed 43 mL water. Cotton with 35 horizontal and 26 vertical threads per $inch^2$, 910 mesh. One meter square of this fabric absorbed 75 mL water. All of fabrics were impregnated with the different synthetic pyrethroids with concentrations 0.312, 0.625, 1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320 mg m^{-2}. Etofenprox impregnated fabrics were impregnated with higher concentrations of 3.12, 6.25, 12.5, 25, 50, 100, 200 and 400 mg m^{-2}.

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Mosquito colonies were maintained in the insectarium at 28-30°C and 75-80% relative humidity and tested mosquitoes were maintained in the same condition and fed with 10% sugar solution for 24 h. Bioassay test was carried out with simple netting apparatus proposed by the WHO (1999). A spherical metal wire frame was covered by impregnated net. In each concentration five unfed female mosquitoes were released into each cone and exposed for 3 min to the insecticide impregnated net. Each concentration was repeated at least 20 times and mortality were recorded after 24 h allocated to a recovery period. Median lethal dose (LD_{50}) and 90% lethal dose (LD_{90}) values were calculated as described by Finney (1971). Diagnostic dose was regarded as the concentration yielding 100% mortality. This study was carried out in Tehran University of Medical Science, School of Public Health and Institute of Health Research, Tehran Iran, from September to December 2000.

IMPREGNATION TECHNIQUE

The absorption rate was determined by dipping one meter square of each fabric into water. The volume of insecticide required to give the target dosage as mg m^{-2} was added to the water absorbed by one meter square of soaked fabric and so one meter square of the same fabric was soaked in this solution in a non absorbent container tube and then were rubbed and squeezed to obtain uniform distribution of the insecticide over the entire piece of fabric. The target dose was exactly calculated. The impregnated fabric was then spread on a nylon surface and allowed to dry in the shade. The dried and impregnated fabrics were labeled and maintained into black sacks in the shade away from heat and sunshine. The bioassay test were conducted the nets (cages) within one week after impregnation of the fabrics.

RESULTS

In this study, it was found that different insecticides in different types of nets had different efficacies. There is direct relationship between concentration of insecticide and mortality rate. Polyester nets need less concentration that create maximum mortality, i.e., least LD_{50}, LD_{90} and diagnostic dose of insecticides, in the contrary the cotton acts differently (Table 1).

As shown in Fig. 1-3 the regression lines move from the left to the right side of diagrams from polyester to cotton nets. Increasing the LD_{50}, LD_{90} and diagnostic dose of four pyrethroids from polyester to cotton nets. In all three figures, regression line of lambdacyhalothrin is on the left side followed by cyfluthrin, deltamethrin and etofenprox. Except on cotton nets impregnated by etofenprox that suddenly move to the left side of diagram (Fig. 1-3).

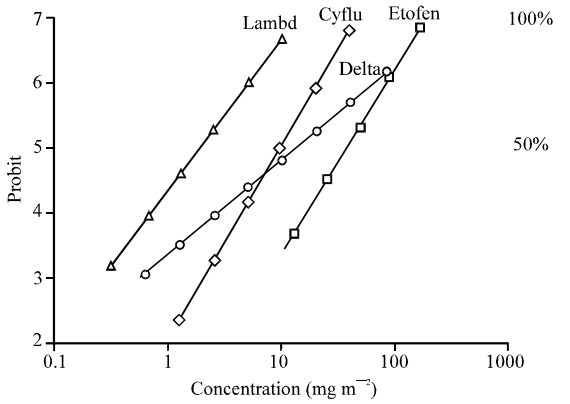

Fig. 1: Probit regression lines of *An. stephensi* (IND-St) exposed to different concentrations of pyrethroids on polyester net

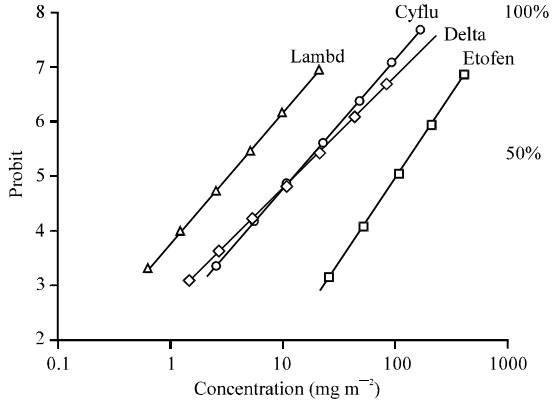

Fig. 2: Probit regression lines of *An. stephensi* (IND-St) exposed to different concentrations of pyrethroids on nylon net

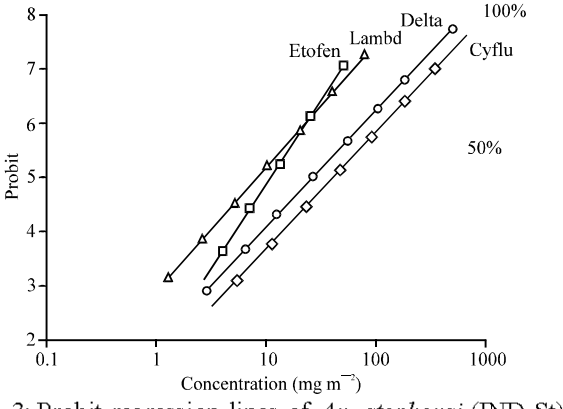

Fig. 3: Probit regression lines of *An. stephensi* (IND-St) exposed to different concentrations of pyrethroids on cotton net

Table 1: Relative efficacy of synthetic pyrethroids on different fabrics against *An. stephensi*

	Insecticide											
	Lambdacyhalothrin			Deltamethrin			Cyfluthrin			Etofenprox		
Fabric	LD_{50} 95% C.I	LD_{90} 95% C.I	DD	LD_{50} 95% C.I	LD_{90} 95% C.I	DD	LD_{50} 95% C.I	LD_{90} 95% C.I	DD	LD_{50} 95% C.I	LD_{90} 95% C.I	DD
Polyester	1.30	4.50	10	6.20	37.1	80	7.40	19.0	40	15.2	65.2	200
	1.84	7.03		8.97	56.36		10.15	28.12		40.6	128.0	
	2.60	15.2		13.3	138.0		14.3	58.9		92.0	198.0	
Nylon	2.00	6.5	20	7.40	35.3	80	7.6	23.6	80	63.5	164.8	400
	3.19	10.98		11.73	58.40		10.94	36.28		103	268.2	
	5.11	33.6		17.2	125.0		15.6	80.2		168	933.0	
Cotton	6.80	23.7	80	20.6	73.2	160	34.4	127.3	320	5.40	15.0	50
	9.95	33.5		26.7	105		46.8	193.7		9.53	25.24	
	11.7	55.5		34.9	181.6		64.2	377		17.0	131.4	

LD_{50}: 95% confidence interval (mg m^{-2}) = Lethal dose cause 50% mortality, LD_{90}: 95% confidence interval (mg m^{-2}) = Lethal dose cause 90% mortality, DD (mg m^{-2}) = Diagnostic dose cause 100% mortality

DISCUSSION

It was observed that polyester nets in comparison to cotton and nylon nets had minimum quantity of LD_{50}, LD_{90} and diagnostic dose. So in definite concentration mosquitoes exhibited maximum mortality. Therefore the polyester nets are the most suitable ones for impregnation and are recommended to be used for malaria control.

There was some exceptions in cotton nets impregnated with etofenprox. LD_{50}, LD_{90} and diagnostic dose of etofenprox was highly decreased and it was the most effective insecticide on cotton nets. It is assumed that probably the molecules of etofenprox are not able to penetrate in cotton fibers and remain on the surface of net or perhaps some materials of cotton nets play the roll of a synergist for etofenprox.

In the reports of the WHO (2005, 2002, 2001 and 1999) they recommended different concentrations for treatment of mosquito nets: 10, 25, 50 and 200 mg m^{-2} for lambdacyhalothrin 2.5% (C.S), deltamethrin 1% (S.C), 25% (WT), cyfluthrin 5% (EW) and etofenprox 10% (EW), respectively (WHO, 2005, 2002, 2001 and 1999).

Curtis (2005) recommended the diagnostic dose of 10, 25 and 200 mg m^{-2} for lambdacyhalothrin, deltamethrin and etofenprox.

Sharma *et al.* (2005) recommended one tablet of K-O TAB for impregnation of 10 m^2 net (about 25mg m^{-2}) for 100% mortality against main malaria vectors in India.

Hodjati *et al.* (2005) recommended 25 and 25 mg m^{-2} for lambdacyhalothrin and deltamethrin. In our study diagnostic doses were calculated as 10, 80, 40 and 200 mg m^{-2} for lambdacyhalothrin, deltamethrin, cyfluthrin and etofenprox, respectively. There is close correlation between these results and WHO recommendation and above-mentioned studies. Results from the test on deltamethrin was different. These differences are due to different geographical areas, different species of mosquitoes, different types of nets, insecticides, formulations and active ingredient of pesticides. So in each study, the specific diagnostic doses must have been determined.

In the other study concentration of 25 mg m^{-2} of deltamethrin yielded 100% mortality in *An. fluviatilis* whereas *An. culicifacies* showed a decline from 100 to 71% mortality. Therefore different species of *Anopheles* have different susceptibility to one specific concentration of insecticides. On the other hand differences in diagnostic dose in different studies is due to different species of *Anopheles* and their susceptibility status (Sharma *et al.*, 2005). In conclusion, in local circumstances any insecticide recommended for impregnation of bednets should be tested against local vector and on bednets which are being used by the community. In the reports of the WHOPES working group meeting in 2001 and 2005, WHO recommended polyester and polyethylene nets to nylon and cotton nets, perfectly. Since wash-off insecticide may be higher on other fabrics, it was recommended that the conventional net tested should be polyester nets. Long lasting insecticidal mosquito nets are recommended from polyester and polyethylene texture (WHO, 2005).

In this study the polyester nets for their low LD_{50}, LD_{90} and diagnostic dose of pyrethroids are recommended. The synthetic (polyester and polyethylene) nets have the smooth and soft surface and don't able to absorb enough insecticides. Because of high contact between these types of nets and mosquitoes, it makes high degree of mortality in vector population. The natural (cotton) nets due to existence of spaces in their texture, it absorbes a high quantity of insecticides and allow the insecticide to be away from mosquito access. So they decrease the mortality rate and increase LD_{50}, LD_{90} and diagnostic dose of insecticides.

Ansari *et al.* (1998) determined that deltamethrin, cyfluthrin and lambdacyhalothrin are the more effective insecticides, respectively against malaria vectors in India.

They also noted that the cotton nets are the most effective nets and diagnostic dose and in increasing residual effect of pyrethroids.

In our study the polyester nets are recommended for their low LD_{50}, LD_{90} and diagnostic dose of pyrethroids, as WHO recommendation of polyester nets.

In other study, bifenthrin, lambdacyhalothrin and DEET were respectively more effective on mortality rate and blood-feeding inhibition. There was direct relationship between concentrations of two pyrethroids and DEET and blood-feeding inhibition and mortality rate of *An.stephensi*. However, this relationship was reverse in passing mosquito through the holes (Vatandoost *et al*., 2005).

In this study there was direct relationship between different concentrations of pyrethroids and mortality rate of *An.stephensi* in pyrethroids impregnated bednets. Lamdacyhalothrin had the least values of LD_{50}, LD_{90} and diagnostic dose (10 mg m^{-2}) in comparison to other pyrethroids.

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