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PERSISTENCE AND WASH-RESISTANCE OF INSECTICIDAL EFFICACY OF NETTINGS TREATED WITH DELTAMETHRIN TABLET FORMULATION (K-O TAB®) AGAINST MALARIA VECTORS

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ABSTRACT. Persistence, wash-resistance, and shelf life of mosquito nets treated with a water-dispersible tablet formulation of synthetic pyrethroid insecticide deltamethrin (K-O TAB®) at 25 mg/m^2 was evaluated against malaria vectors in India. During June 2001, treated and untreated polyester, nylon, and cotton nets were separately distributed in 3 villages and cone bioassays were performed on *Anopheles culicifacies* and *An. stephensi* 1 day after treatment and thereafter every month for 12 months. The mosquitoes were exposed for 3 min on the nettings (treated and unwashed, or treated and washed once or twice in 3 months, and untreated) and knock-down (1 h) and 24 h postexposure mortality were recorded. Unwashed polyester nets, and those washed once 1 month after treatment, gave 100% mortality in *An. culicifacies* for 6 months. A 2nd wash at 3 months after treatment marginally reduced the insecticidal action. *Anopheles stephensi* was fully susceptible up to 4 months when exposed to unwashed nets but washing considerably reduced insecticidal action (65–78% after 2 washes). Treated nylon and cotton nets were effective for 4 months on both vectors. Treated nets kept on shelf retained 100% efficacy for 10 months. Overall, the treated nets gave a considerably long persistence of insecticidal action even after a single wash. Treated polyester nets were found most effective. Compared with our earlier experiences of using liquid formulations, the tablet formulation is likely to have a better community acceptance in treating nets.

KEY WORDS Mosquito nets, deltamethrin tablet, malaria vectors, bioassays, India

INTRODUCTION

Insecticide treated mosquito nets (ITNs) have proved an important complimentary tool in reducing malaria morbidity and mortality in malarious countries. In India, several field trials have shown effectiveness of ITNs and their operational-scale introduction in the National Anti-malaria Programme is currently underway. In all the previous Indian trials, mosquito nets were treated either with an emulsifiable concentrate (Sampath et al. 1998a, 1998b; Yadav et al. 1998), a flowable (Jana-Kara et al. 1995, Yadav et al. 2001), or an emulsion in water (Sharma and Yadav 1995) formulation of the pyrethroid.

The operational problems with ITN-oriented intervention inter alia are mainly community compliance and sustainability. Availability of the insecticide in small packaging for retail use and simplification of the treatment procedure is likely to help in scaling up ITN intervention. The presently marketed liquid formulations can pose hazards due to spillage in transportation, and require tools for accurately measuring the dosage required for net treatment. Accuracy of measurement is difficult to sustain under the field conditions, particularly at the hands of the individual users and in community-wide ITN interventions. K-O TAB® is a freeze-dried suspension concentrate of deltamethrin (25% weight per weight) made into a water-dispersible tablet formulation that offers to reduce the operational technicalities and environmental risk. K-O TAB was evaluated for its bioefficacy against the local malaria vectors, *Anopheles culicifacies* Giles, 1901, and *An. stephensi* Liston, 1901, by using 3 different types of nettings in Gujarat State, India, during 2001–02.

MATERIALS AND METHODS

Study villages: The study was done in the laboratory of the Malaria Research Centre at Nadiad and in 3 villages, namely Vaghjipura (population 100), Ambaipura (population 215), and Haripura (population 167), in Kheda District, Gujarat State, India. The villages lie in close proximity to a large irrigation tank. Most residents traditionally use mosquito nets for protection from persistent mosquito nuisance. Some households use large-size nets and curtains to protect their livestock. A population census was conducted in early June 2001 and houses were assigned codes. Availability of nets in each household was recorded. The people were informed about the purpose of the study and written consent of each householder to participate in the trial was obtained.

Treatment and distribution of nets: Single-size, rectangular mosquito nets without hems, measuring 10 m^2 (1.9 m long, 1 m wide, 1.4 m high) were procured from a local market in Delhi. Nylon and polyester nets were 100-denier strength with a mesh size of 156 holes/in.^{2} Cotton nets having square mesh were of high quality conforming to the Indian Standard 1143:1973. Water-absorbing capacity of each type of net was determined beforehand. Deltamethrin tablets were used in treating the nets. The

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Indian packaging of 1 g of K-O TAB contains 250 mg active ingredient of deltamethrin and is suitable for treatment of a 10-m^2 net to achieve the dosage of 25 mg/m^2. The tablet was dissolved in the required quantity of tap water in a plastic tub and it completely disintegrated in less than 2 min. Net impregnators wore hand gloves and dipped the net, gently soaking and kneading in the suspension. Nets were dried flat on plastic sheeting for some time and then hung in the shade for complete drying. The starched cotton nets were washed with ample cold water and dried before treatment. Each net was given an identification number with indelible ink.

In addition to the 86 nets already in use in 103 households, 31 nets each of polyester (20 treated and 11 untreated), nylon (20 treated and 11 untreated), and cotton (18 treated and 13 untreated) were distributed free of cost in Ambaipura, Vaghjipura, and Haripura villages, respectively, in late June 2001. Householders were advised not to expose the nets to the direct sunlight or wash them.

Two each of the treated and untreated nets of each type of fabric were sealed in polyethylene bags and kept in the dark at room temperatures (15–28°C) for evaluating the shelf life of the insecticidal efficacy.

Cone bioassays: Mosquitoes from cyclic colonies of *An. culicifacies* sibling species C and *An. stephensi* (Nadiad strain), maintained at the Nadiad Field Station of the Malaria Research Centre since 2000 at 28 ± 2°C and 70 ± 5% relative humidity, were used in the cone bioassays. Both of the colonized species were 100% susceptible to 0.05% deltamethrin test papers by the standard WHO susceptibility test. Freshly emerged females were maintained on 10% glucose solution and were held in the insectary for 48–72 h before using them in the bioassays.

The bioassays were performed in the laboratory on nets 1 day after treating them (in late June 2001) and once monthly thereafter until June 2002 inside the rooms in the study villages. For field bioassays, mosquitoes from the colonies were transferred into muslin cloth cages (30 × 30 × 30 cm). Cages were held in wooden boxes and transported to the study villages. Bioassays were performed during the morning hours between 0900 and 1100 h. Bioassay cones were fastened at different positions with rubber bands on the outer side of the net. Female mosquitoes in 4 replicates of 15 each were introduced through the hole into the cones by using a suction tube. Holes were plugged with cotton wool. Each time, 15 mosquitoes in 4 replicates (total of 60 females) were exposed on the treated nets. In houses provided with untreated nets, 2 replicates of mosquitoes (30 total) were simultaneously exposed to the untreated nettings, which served as control.

After a 3-min exposure, the mosquitoes were gently transferred into plastic holding cups covered with a netting piece fastened with rubber band. The holding cups were labeled, a cotton wool pad soaked in 10% glucose solution was placed on the netting piece covering the cups, and these were then placed in insulated polystyrene boxes. The number of the mosquitoes that were knocked down during the 1st hour of holding was recorded. Room temperature and humidity were recorded by using a digital thermohygrometer.

Insulated polystyrene boxes containing plastic cups holding the exposed mosquitoes were transported back to the insectary and mortality in mosquitoes was scored 24 h after exposure. The bioassays also were done in the laboratory to evaluate the shelf life of insecticidal action. Test mortality was corrected by applying Abbott's formula (Abbott 1925) if the mortality in control was between 5 and 20%.

To evaluate the effect of washing on the residual efficacy of the insecticide, 8 randomly selected treated nets of each fabric were washed in the villages 1 month after their initial treatment and use. The nets were soaked for about 5 min in tap water with washing powder (Nirma brand manufactured by Nirma Limited, Ahmedabad, India) containing caustic soda and rinsed twice in ample of clean water before hanging them in the shade to dry. Four untreated nets were similarly washed to serve as control. After a further use for 2 months (i.e., at 3 months after the initial treatment), 4 treated nets and 2 untreated nets that had earlier been washed once (from the 8 treated and 4 untreated nets washed at the 1-month interval) were washed a 2nd time. Treated and untreated nets thus washed once or twice were used in the subsequent bioassays along with unwashed treated and untreated nets.

Percent mortality on different netting combinations was compared by using 1-way analysis of variance, and differences among mortalities were detected by using Tukey's multiple comparison test.

RESULTS

Mortality rates in *An. culicifacies* exposed to polyester nets that were in use were 100% up to 6 months (Table 1). A single wash 1 month after treatment caused no decline in the insecticidal action but a 2nd wash at the end of 3 months reduced the mortality to 91.7%, which still remained >75% until the end of the 6-month period. Mortality rates were >80% during the 7- to 12-month interval irrespective of 1 or 2 washes.

Mortality in *An. stephensi* exposed to unwashed or washed polyester nets was 100% up to 4 and 2 months, respectively. Mortality caused by unwashed nets declined to 66.7% at the end of 6 months and varied from 65 to 78% on washed nets during 4–6 months after treatment. Mortality rates further declined to 65–75% at the end of the 12th month.

The treated nylon nets gave >93% mortality up to 4 months in both the species, irrespective of 1

Table 1. Mortality (%) in malaria vectors exposed on polyester netting treated with deltamethrin at 25 mg/m^2.

Interval of bioassay	*Anopheles culicifacies*			*Anopheles stephensi*			ANOVA[3]
	Unwashed	Washed once[1]	Washed twice[2]	Unwashed	Washed once[1]	Washed twice[2]	
Day 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 2	100.0	100.0	100.0	100.0	100.0	100.0	
Month 3	100.0^{a}	100.0^{a}	91.7^{a}	100.0^{a}	78.3^{b}	63.3^{c}	***
Month 4	100.0^{a}	100.0^{a}	80.0^{b}	100.0^{a}	78.3^{b}	70.0^{c}	***
Month 5	100.0^{a}	100.0^{a}	75.0^{b}	83.3^{ab}	70.0^{b}	66.7^{b}	***
Month 6	100.0^{a}	100.0^{a}	100.0^{a}	66.7^{b}	66.7^{b}	65.0^{b}	***
Month 7	93.3^{a}	86.7^{a}	80.0^{a}	81.7^{a}	93.4^{a}	61.7^{b}	***
Month 8	100.0	98.3	98.3	100.0	96.7	95.0	ns
Month 9	83.3^{ab}	96.7^{a}	98.3^{a}	65.0^{b}	90.0^{a}	86.7^{a}	**
Month 10	83.3^{ab}	93.3^{a}	91.7^{a}	66.7^{b}	86.7^{ab}	83.6^{ab}	*
Month 11	100.0^{a}	100.0^{a}	100.0^{a}	86.7^{ab}	81.7^{b}	80.0^{b}	**
Month 12	96.7^{a}	88.3^{a}	86.7^{ac}	65.0^{b}	75.0^{bc}	66.7^{b}	***

[1] At 1 month after treatment.
[2] At 1 and 3 months after treatment.
[3] ANOVA, one-way analysis of variance. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant ($P > 0.05$); figures followed by the same letter in superscripts within a row were not significantly different from each other ($P > 0.05$) when using Tukey's multiple comparison test.

or 2 washes (Table 2). Mortality rates in both vectors in the bioassays at the end of the 6th month ranged between 65 and 80%. Thereafter, an increase in the insecticidal action was observed because the mortality rates in both the species increased up to 95%. In the 12th month, bioassay mortality rates were higher in *An. culicifacies* (86.7–100%) than in *An. stephensi* (70–75%).

The treated cotton nets were 100% effective against *An. culicifacies* up to 4 months and >80% until 6 months (Table 3). Bioassays at the end of 6 months showed a decline in the mortality rates to 76.7% on nets washed once and to 70% on those washed twice. Variations in mortality rates ranged between 70 and 100% during the 7- to 12-month interval. The mortality in *An. stephensi* was 100% up to 2 months and 90% at 4 months, which declined to 66.7% at 6 months on unwashed nets. Washing further decreased the insecticidal action to 30–41%. Toward the end of the study, an increase in bioefficacy was observed (81.7–85%).

All 3 types of treated nets kept on the shelf in the laboratory for shelf-life evaluation gave 100% mortality up to 9 months in *An. stephensi* and up to 10 months in *An. culicifacies.*

The project staff that treated the nets reported that the use of 1 tablet for 1 net was an easy method and that it was easy to handle the tablets and prepare the liquid for net treatment.

DISCUSSION

The study showed that the unwashed nets retained high insecticidal power for over 6 months against *An. culicifacies* and up to 4 months against *An. stephensi.* Therefore, *An. culicifacies* apparently was relatively more susceptible to deltamethrin than *An. stephensi.* The insecticidal efficacy persisted longer on the polyester nets than on the nylon or cotton nets. Mortality rates on all 3 types of nets, whether unwashed or washed (once or twice), varied considerably during the latter half of the 1-year trial. An increase in insecticidal bioefficacy was observed in the bioassays performed in months 7 and 8 in all 3 nettings and wash conditions. Many villagers reported nonuse of nets during the winter months (mid-November to mid-February), mainly because of a reduced mosquito nuisance and possibly some protection provided by blankets. During that period, most nets were kept safely stored, which possibly resulted in the leaching effect of the insecticide and an increase in the bioefficacy in subsequent months when net use resumed.

It has been reported that deltamethrin-treated nylon nets retain insecticidal power against *An. culicifacies* well over 6 months (Yadav et al. 2001). The comparatively shorter effective period of bioefficacy on cotton nets is suggestive of a reduced availability of insecticide on the surface of the fabric due to absorption.

A single wash of nets 1 month after treatment caused only a marginal loss of insecticidal efficacy. A 2nd washing after 3 months further reduced insecticidal power. Washing is known to reduce the effectiveness of insecticide-treated materials (Ordonez Gonzalez et al. 2002). In conventional bioassays, cotton, nylon, and polyester nets treated with deltamethrin (suspension concentrate and wettable powder) or lambdacyhalothrin (emulsifiable concentrate) showed a reduction in the insecticidal activity of all netting–formulation combinations after washing (Jana-Kara et al. 1994). In a study in India, it was found that lambdacyhalothrin-treated nylon nets washed after 3 months of regular use gave 98.3% mortality, and those washed after 6 months gave about 42% mortality in wild-caught, bloodfed *An. culicifacies* (Sampath et al. 1998a).

Table 2. Mortality (%) in malaria vectors exposed on nylon netting treated with deltamethrin at 25 mg/m^2.

Interval of bioassay	*Anopheles culicifacies*			*Anopheles stephensi*			ANOVA[3]
	Unwashed	Washed once[1]	Washed twice[2]	Unwashed	Washed once[1]	Washed twice[2]	
Day 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 2	100.0	100.0	100.0	100.0	100.0	100.0	
Month 3	100.0	100.0	100.0	100.0	100.0	100.0	
Month 4	100.0	100.0	100.0	100.0	100.0	93.3	ns
Month 5	83.3^{ab}	73.3^{b}	76.7^{bc}	91.7^{ac}	93.3^{a}	86.7^{ab}	**
Month 6	80.0	70.0	73.3	80.0	80.0	65.0	ns
Month 7	93.3	91.7	95.0	93.3	95.0	95.0	ns
Month 9[4]	68.3^{a}	86.7^{a}	73.3^{a}	85.0^{a}	86.7^{a}	91.7^{a}	*
Month 10	95.0^{a}	95.0^{a}	95.0^{a}	90.0^{a}	71.7^{a}	76.7^{a}	*
Month 11	100.0	95.0	90.0	90.0	91.7	90.0	ns
Month 12	100.0^{a}	91.7^{ac}	86.7^{ab}	75.0^{bc}	70.0^{b}	73.3^{b}	***

[1] At 1 month after treatment.
[2] At 1 and 3 months after treatment.
[3] ANOVA, one-way analysis of variance. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant ($P > 0.05$); figures followed by the same letter in superscripts within a row were not significantly different from each other ($P > 0.05$) when using Tukey's multiple comparison test.
[4] Bioassays were not done in month 8.

There also was some evidence that the tablet formulation persisted better on unwashed nets after 5 months (despite the relatively low initial values). A single wash decreased the concentration of all 3 treatments about equally, by 50–60%.

The tablet formulation has certain advantages over the liquid formulations. It is easy to pack, handle, and carry the tablets. They are safer in transport and pose little risk of an accidental spill compared with the liquid formulations. K-O TAB contains a bitter agent to dissuade children from accidental consumption. In the present study, the net impregnators also perceived some of these advantages. In a community trial to assess the convenience and acceptability of sachets of lambdacyhalothrin and tablets of deltamethrin in Tanzania, it was observed that although in a few cases the tablet was broken before the packet was opened, none of the tablets was lost, and the entire contents could be added to water, in contrast with the sachets, which were found to be difficult to empty completely (Jones and Miller 1997).

In conclusion, unwashed, K-O TAB–treated polyester nets had more than 6-month persistence of insecticidal efficacy against both malaria vectors, and nylon and cotton nets were also effective for about 5–6 months. Although the bioassay mortalities in both the vectors fluctuated considerably thereafter, in areas with unstable malaria of about 6-months duration, predominantly by *An. culicifacies* in rural India, a single treatment of nets would provide effective protection against malaria. In ar-

Table 3. Mortality (%) in malaria vectors exposed on cotton netting treated with deltamethrin at 25 mg/m^2.

Interval of bioassay	*Anopheles culicifacies*			*Anopheles stephensi*			ANOVA[3]
	Unwashed	Washed once[1]	Washed twice[2]	Unwashed	Washed once[1]	Washed twice[2]	
Day 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 1	100.0	100.0	100.0	100.0	100.0	100.0	
Month 2	100.0	100.0	100.0	100.0	100.0	100.0	
Month 3	100.0	100.0	100.0	91.7	95.0	96.7	ns
Month 4	100.0^{a}	83.3^{b}	85.0^{ab}	90.0^{ab}	85.0^{ab}	93.3^{ab}	*
Month 5	83.9^{a}	80.3^{a}	78.6^{ab}	75.0^{ab}	40.0^{c}	55.0^{bc}	***
Month 6	80.0^{a}	76.7^{a}	70.0^{ab}	66.7^{ab}	35.0^{c}	41.7^{bc}	***
Month 7	81.7	91.7	88.3	75.0	81.7	80.0	ns
Month 8	96.7^{a}	91.7^{ab}	88.3^{ab}	81.7^{b}	88.3^{ab}	83.3^{b}	*
Month 9	73.3	76.7	71.7	56.7	70.0	70.0	ns
Month 10	76.7	75.0	70.0	71.7	76.7	58.3	ns
Month 11	100.0	93.3	90.0	93.3	91.7	86.7	ns
Month 12	93.3	91.7	88.3	85.0	88.3	81.7	ns

[1] At 1 month after treatment.
[2] At 1 and 3 months after treatment.
[3] ANOVA, one-way analysis of variance. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant ($P > 0.05$); figures followed by the same letter in superscripts within a row were not significantly different from each other ($P > 0.05$) when using Tukey's multiple comparison test.

eas with persistent transmission of malaria, treatments every 6 months might be required to allow for a single wash of nets by some people. K-O TAB has successfully passed through evaluation by the WHO Pesticide Evaluation Scheme elsewhere (Darriet et al. 1998, Javara et al. 2001). The present study in India also has shown the efficacy of K-O TAB against 2 important malaria vectors. In Southeast Asia, the ITN technology is at the verge of scaling up. Availability of deltamethrin tablets is expected to facilitate self-treatment of nets for personal protection. In community-wide trials, the net retreatment rates are often found to be low. Introduction of tablets may increase acceptability of the ITNs in such interventions.

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