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EFFICACY OF PERMANET® 2.0 AGAINST *ANOPHELES CULICIFACIES* AND *ANOPHELES STEPHENSI*, MALARIA VECTORS IN INDIA

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ABSTRACT. Bioefficacy of PermaNet® was evaluated in both the laboratory and field against *Anopheles culicifacies* and *An. stephensi*, major malaria vectors in India. Contact bioassays were carried out after repeated washings and ring net bioassays to determine the median knockdown time of mosquitoes. Three villages were selected for the field trial: in the 1st village PermaNets were distributed, in the 2nd village untreated nets were distributed, and the 3rd village was a control. Entomological data were collected using standard procedures. The PermaNet contact bioassays showed high mortality (>80%) even after 20 washes against both the vector species. The median knockdown time of *An. culicifacies* and *An. stephensi* was 392 and 480 sec when exposed to fresh PermaNets and 472 and 986 sec when exposed to PermaNets that had been washed 20 times, respectively. PermaNets showed high efficacy in reducing the person–vector contact as evidenced by reduced person-hour density in the PermaNet village. Long-term field trials are indicated to test the impact of use of PermaNets in controlling malaria.

KEY WORDS *Anopheles culicifacies*, *An. stephensi*, contact bioassays, PermaNet, person-hour density

Long-lasting insecticide-treated nets (LLINs) are emerging as promising personal protection measures against mosquitoes (Guillet et al. 2001). LLINs offer a practical solution to the need for frequent retreatment of insecticide-treated nets. PermaNet® polyester net with deltamethrin at 55 mg/m^2, developed by Vestergaard Frandsen (Kolding, Denmark), has been tested for its bioefficacy against mosquitoes and wash resistance in different countries (Muller et al. 2002, Ordonez-Gonzalez et al. 2002, Kroeger et al. 2004, WHOPES 2004, Gimnig et al. 2005, Graham et al. 2005, Lindblade et al. 2005). The results of these studies revealed that the insecticide efficacy of conventionally treated nets was gradually reduced after washing, whereas it was not exhausted even after 20 washes for PermaNets. Using standard laboratory washing methods, PermaNet retained high efficacy even after 20 washes, giving more than 97% mortality of anophelines in contact bioassay with 3-min exposures (Graham et al. 2005). Thus, a study was undertaken to evaluate the bioefficacy of PermaNet against 2 major malaria vectors in India—*Anopheles culicifacies* Giles and *An. stephensi* Liston in both the laboratory and field.

PermaNet 2.0, 100 denier fiber thickness, 156 mesh hole size with deltamethrin at 55 mg active ingredient/m^2 was manufactured by M/s Vestergaard Frandsen Pvt. Ltd. (New Delhi, India). Untreated polyester nets with 100 denier thickness also were used. Wild bloodfed mosquitoes collected from field were used in bioassays. Mosquitoes resting indoors were collected from houses by using a suction tube and flashlight from 0600 to 0700 h.

Cone bioassay tests were performed as per World Health Organization (WHO) standard procedures (WHO 1998) by exposing field-collected fully fed female mosquitoes. Four nets in total were used for each bioassay, and batches of 10 mosquitoes were exposed against each net. Data were pooled and percent-corrected mortality was calculated using Abbott's formula (Abbott 1925). Ring net bioassays were conducted fortnightly in the field following the procedure given in WHO (1998) to determine the median time to knockdown (MTKD). Two metal rings with 45-cm circumference and 15-cm diameter were welded to form a sphere. The net was wrapped around the ring, and batches of 11 mosquitoes were exposed continuously to the netting. The observed time to knockdown of the median (6th) mosquito was recorded. Four replicates of nets were used for each experiment.

Impact of washing on the efficacy of PermaNet was tested after each wash up to 20 washes. The gap between each wash was 5–6 days. Surf Exel, a detergent powder manufactured by M/s Hindustan Lever India Pvt. Ltd. (Bangalore, India), was used for washing. Ten grams of detergent was dissolved in 10 liters of water for 5 min. The pH of detergent water was ~9.5. The net was then soaked in the water for 30 min and rubbed for 5 min thoroughly. Then, the net was rinsed 3 times (3 min for each rinse) in tap water thoroughly to remove all the detergent. The washed nets were dried under sunlight for 5–6 h during daytime, which is a general practice in India. The temperature range was 33–40°C

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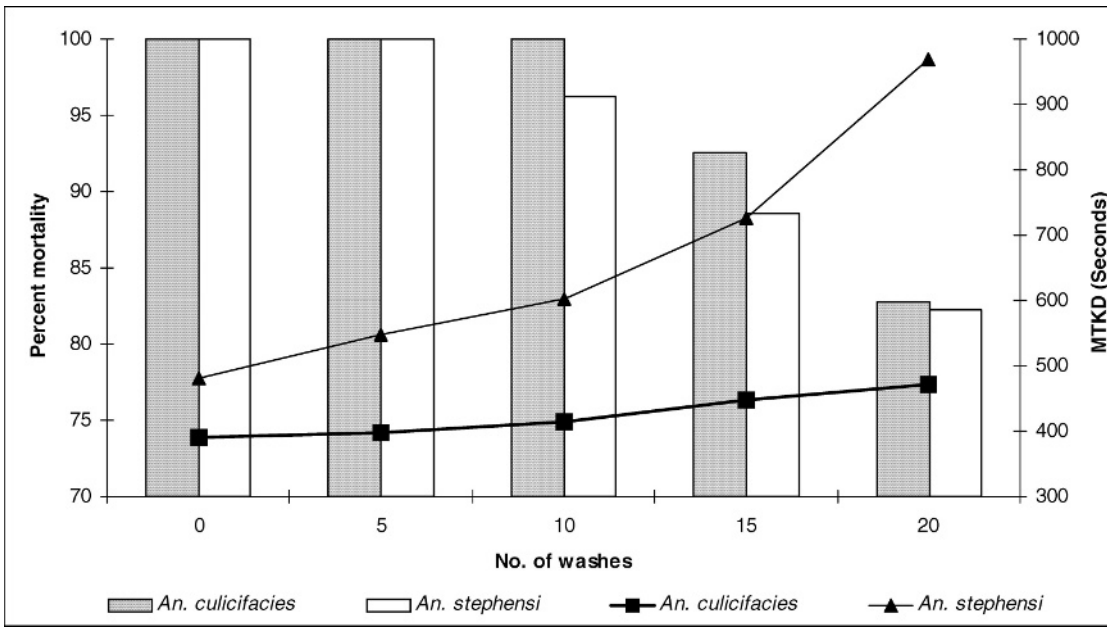

Fig. 1. Percent-corrected mortality (bars) in 3-min cone bioassays and median time for knockdown (MTKD; lines) in seconds in *Anopheles culicifacies* and *An. stephensi* exposed to washed and unwashed PermaNet® 2.0.

during drying. Bioassay tests were performed as per the procedure described above. Untreated net was used as a control. Percent-corrected mortality was calculated using Abbott's formula (Abbott 1925).

Nawada, Durgawali, and Harampur villages located in the belt of Jumuna River in Loni Primary Health Centre, District Ghaziabad (Uttar Pradesh), India, were selected for the field trial. The population of these villages is about 699, 685, and 398, respectively. *Anopheles stephensi* and *An. culicifacies* are the major malaria vector species in these villages. PermaNets were distributed in Nawada village, and untreated nets were distributed in Durgawali village. Harampur village was left as a control village where nets were not used. Nets were distributed using the formula of 1 net per person >10 years of age and 1 net per 2 people <10 years of age.

Indoor resting mosquitoes were collected fortnightly via the hand catch method by using a suction tube and flashlight in selected structures in the early morning. Villages were divided into quadrants, and 1 house from each quadrant was selected randomly for each fortnight collection. Approximately 15 min was spent in each structure for collecting the mosquitoes, and the same person collected the mosquitoes in all the structures selected for a particular collection. The mean monthly density of indoor resting mosquitoes was calculated as per person-hour.

Analysis of variance (ANOVA) was performed using SPSS 9.0 for Windows (SPSS Inc., Chicago, IL) for testing the significance among PermaNet, untreated net, and control villages and *t*-test was performed to test the significance between *An. stephensi* and *An. culicifacies*. P values <0.05 were considered statistically significant.

Percent-corrected mortalities of *An. culicifacies* and *An. stephensi* in 3-min contact bioassays performed on unwashed and washed PermaNet 2.0 are depicted in Fig. 1. The results showed high efficacy of PermaNet against both the species as evidenced by the finding that even after 20 washes, mortality in both the species remained >80%. Furthermore, there was no significant difference between the mortalities of the 2 species ($P > 0.05$). In ring net bioassays, the baseline median knockdown time against *An. culicifacies* is 393 sec, which increased progressively. However, after progressive washings, the MTKD also was increased, which is statistically significant. The MTKD of *An. culicifacies* was less than that of *An. stephensi*, and the difference was statistically significant ($P < 0.05$) (Fig. 1).

Person-hour densities of different mosquito species collected by hand catch method in Ghaziabad district are presented in Table 1. The results revealed that the person-hour densities of *An. culicifacies* and *An. stephensi*, the major malaria vectors in PermaNet, untreated net, and control villages during the predistribution period was 27 and 53, 22 and 32, and, 20 and 22 mosquitoes, respectively. After the distribution of nets, the densities reduced gradually in the PermaNet village. Although there was also a re-

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Table 1. Person-hour densities of *Anopheles* mosquitoes in PermaNet®, untreated net, and no-net villages.[1]

Month	PermaNet			Untreated net			No net		
	An. culicifacies	*An. stephensi*	Total mosquitoes[2]	*An. culicifacies*	*An. stephensi*	Total mosquitoes[2]	*An. culicifacies*	*An. stephensi*	Total mosquitoes[2]
Predistribution[3]									
April 2005	12 ± 1.5[2]	70 ± 1	492 ± 64	10 ± 1	44 ± 12	342 ± 70	9 ± 1	28 ± 2	314 ± 10
May	21 ± 1.5	60 ± 2	274 ± 62	13 ± 0.5	50 ± 15	193 ± 55	10 ± 2	24 ± 1.5	186 ± 24
June	27 ± 3	53 ± 1.5	199 ± 32	22 ± 1	32 ± 1.5	135 ± 45	20 ± 4.5	22 ± 0.5	123 ± 32
Postdistribution[4]									
July	20 ± 1	10 ± 1	251 ± 34	24 ± 0.5	15 ± 0.5	393 ± 10.5	32 ± 3.5	18 ± 1.5	337 ± 38
Aug.	14 ± 0.5	5 ± 1.5	198 ± 22	32 ± 0.5	12 ± 0.75	538 ± 75	40 ± 2.5	21 ± 0.5	584 ± 55
Sep.	8 ± 0.5	4 ± 0	161 ± 35	25 ± 2.5	19 ± 2.5	402 ± 42	34 ± 2.5	22 ± 0.5	504 ± 49
Oct.	4 ± 0.5	3 ± 1	101 ± 20	17 ± 0.5	16 ± 0.5	350 ± 45	19 ± 3	16 ± 0.5	372 ± 10
Nov.	2 ± 0.5	3 ± 1	84 ± 16	13 ± 1	14 ± 0.25	296 ± 32	22 ± 3.5	16 ± 0.5	322 ± 22

[1] Each value is a mean of 2 collections in a month.
[2] Total mosquitoes include *Anopheles culcifacies, An. stephensi, An. annularis, An. subpictus*, and *Culex quinquefasciatus*.
[3] ANOVA results: *An. culicifacies* $F_{2,15} = 1.974$, $P > 0.05$; *An. stephensi* $F_{2,15} = 28.3$, $P < 0.001$; total mosquitoes $F_{2,15} = 1.721$, $P > 0.05$.
[4] ANOVA results: *An. culicifacies* $F_{2,27} = 16.73$, $P < 0.0001$; *An. stephensi* $F_{2,27} = 46.91$, $P < 0.0001$; All mosquitoes $F_{2,27} = 23.33$, $P < 0.0001$.

duction in the village using untreated nets, the impact was less pronounced than that observed in the PermaNet village.

Safety of PermaNet use was studied by interviewing the users of the nets through structured questionnaires. Results revealed that eye and skin irritation were the main complaints reported by the inhabitants when they used the nets at night. Furthermore, the inhabitants mentioned that these symptoms were not perceived after washing the affected areas with clean water. This irritation may be due to high dose of deltamethrin on the PermaNet. Headache, vomiting, and nausea were not reported.

The results of the present study showed high efficacy of PermaNet 2.0 even after 20 washes in the laboratory and after several months under field conditions. In cone bioassays, >80% mortality was recorded in *An. culicifacies* and *An. stephensi*, even after 20 washes. The results of the present evaluation conform with those of previous studies (Kroeger et al. 2004, WHO 2004, Graham et al. 2005, Gimnig et al. 2005, Lindblade et al. 2005). Kroeger et al. (2004) reported >80% mortality in *Anopheles* mosquitoes exposed to PermaNet 2.0 that had been washed 23 times. Graham et al. (2005) reported >97% mortality in *Anopheles* mosquitoes exposed to PermaNet 2.0 that had been washed 21 times; Gimnig et al. (2005) reported high mortality rates even after 20 washes. In the present study, >80% mortality also was reported in both the vector species.

Median time to knockdown bioassays have the capacity to reveal small differences in performance when the amount of bioavailable insecticide remains on the net is sufficient to produce uniformly high mortality in 3 min. The difference in the median time to knockdown after 20 washes can be attributed to the susceptibility status of these 2 mosquito species. The MTKD of *An. stephensi* was 480 sec against unwashed PermaNet, and it reached 968 sec after 20 washes. These results conform with those of Kroeger et al. (2004) and Graham et al. (2005), who reported increases in MTKD after washing. The cause for increase in MTKD in both the species might be due to the loss of insecticide after repeated washing. However, the amount of insecticide left on the nets might have been sufficient to produce >80% mortality in these mosquitoes as evidenced in 3-min cone bioassay tests. This finding demonstrates the improved wash resistance of PermaNets. Because of the high loading dose of deltamethrin (55 mg/m^2) as reported by the manufacturer, even after repeated washings the insecticide lost could not affect the efficacy of nets in producing desired mortality against mosquitoes. Use of PermaNets also resulted in reduction of density of mosquitoes compared with untreated and no-net villages. Long-term field studies are needed to confirm the long-lasting efficacy of Perma Net 2.0 in reducing person–vector contact and their impact on curtailing the malaria transmission.

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