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BIOEFFICACY OF OLYSET® NETS AGAINST MOSQUITOES IN INDIA

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ABSTRACT. Olyset® nets are highly effective in killing anopheline mosquitoes within 3 min of exposure, as evidenced in cone bioassay tests. These nets also showed their efficacy on other vector mosquitoes (*Aedes aegypti* and *Cx. quinquefasciatus*) at higher exposure periods. The efficacy remained at ~90% even after 20 washings at an interval of 24 h against *Anopheles culicifacies* and *Cx. quinquefasciatus.* Use of the nets inside the house also resulted in drastic reduction of daytime resting density of mosquitoes because of high repellency, excitorepellency, and killing action of the nets. Pilot studies are indicated to evaluate the impact of use of Olyset nets on vector-borne diseases, particularly malaria, and its cost-effectiveness in comparison to conventional indoor residual spraying.

KEY WORDS Olyset® net, man-hour density, killing action, repellency action

INTRODUCTION

Insecticide-treated nets are widely used as a personal protection measure in vector control for their effectiveness in controlling vector-borne diseases, particularly malaria. Field trials with various insecticides such as deltamethrin, cyfluthrin, permethrin, lamdacyhalothrin, and others have demonstrated cost-effective control of malaria. However, low treatment rates, washing, and erratic dose of the insecticide in conventionally treated nets resulted in dilution of efficacy of the nets. During recent years, long-lasting insecticidal nets have been developed to overcome such problems encountered with conventional impregnation methods. These nets are treated at the factory with insecticide either incorporated into or coated around fibers, are resistant to multiple washes, and have biological activity that lasts as long as the net itself (up to 3 years for polyester nets and 5 years for polyethylene nets). Long-lasting insecticidal nets offer a practical solution in terms of wash resistance, safety, and ease of use (ready-to-use pretreated nets), pending that they fulfill specifications. At present there are 2 types of long-lasting insecticidal nets available in the market—Olyset® net, a polyethylene net with 2% permethrin incorporated within fiber, and PermaNet®, a polyester net with deltamethrin at 55 mg/m^2 (WHO 2001). Quality-control checks carried out by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) with the 2 long-lasting insecticidal nets (Olyset net and PermaNet) have shown excellent compliance to specifications on both insecticide treatment and netting specifications. The efficacy of Olyset nets was tested in several countries, including Cambodia, Vietnam, Tanzania, Solomon Islands, Malaysia, Senegal, and Côte d'Ivoire (Nguyen et al. 1996, Itoh and Okuno 1996, Ikeshoji and Bakotee 1997, Tami et al. 2004, Doannio et al. 1999, Guillet et al. 2001, N'Guessan et al. 2001, Henry et al. 1999, WHO 2001). Results of these studies revealed that Olyset nets have demonstrated cost-effective control of malaria vectors, reduction of malaria morbidity, or both. In view of this, it was considered desirable to investigate the bioefficacy of Olyset nets against major malaria vectors and other species of mosquitoes in India.

MATERIALS AND METHODS

Materials: Olyset nets manufactured by Sumitomo Chemicals, Mumbai, India, were used in the present study. Plain nets with 100 denier polyester netting were used as untreated controls. Field-collected fully fed mosquitoes from different sites collected with the help of a suction tube and flashlight during early morning hours (0600–0700 h) were used in all bioassay tests.

Study site: Beel Akbarpur village located in the Primary Health Centre Dadri, District Ghaziabad, Uttar Pradesh, India, was selected for the field trial. The population of the village is about 1,800 living in about 178 houses made of brick and cement. Density of *Anopheles culicifacies* Giles is very high throughout the year. Twelve human dwellings were selected for field studies. Olyset nets were distributed in 4 structures; whereas plain nets were distributed to each member of the family in 4 other structures. The remaining 4 human dwellings were used as controls, where no net was distributed.

Cone bioassay tests: Bioassay tests were performed as per WHO standard procedures by exposing field-collected bloodfed female mosquitoes. Initially, discriminating exposure times for different mosquitoes were determined by exposing mosquitoes for different time periods and once discriminatory exposure time was established it was invariably used for further experiments. Ten female mosquitoes (*Anopheles culicifacies, An. subpictus, An. stephensi* Liston, *Aedes aegypti* Linn, and *Culex quinquefasciatus* Say) were released in each cone with the help of a suction tube and exposed to the Olyset net. For bioassays, cones were affixed vertically on the netting to avoid the mosquitoes resting on the surface of the cone. Because of the wide mesh size of the Olyset net, 2 folds were used. After a 3- to 30-min exposure, depending on mosquito species, the mosquitoes were kept in holding

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Table 1. Bioassay test after washing with detergent.

	Anopheles culicifacies				*Culex quinquefasciatus*				
No. washings	No. mosquitos exposed to Olyset net	No. dead after 24 h	No. mosquitoes exposed to plain net	No. dead after 24 h	Corrected % mortality	No. mosquitoes exposed to Olyset net)	No. mosquitoes dead	No. mosquitoes exposed to plain net	No. dead after 24 h	% corrected mortality
1	40	40	40	1	100	40	40	40	0	100
5	40	40	40	1	100	40	40	40	2	100
10	40	40	40	2	100	40	40	40	1	100
15	40	40	40	2	100	40	38	40	2	94.7
20	40	40	40	2	100	40	36	40	2	89.4

kits, which were kept vertically, and a cotton wool swab soaked in 10% glucose solution was plugged in the opening. Mortality was recorded after 24 h as a postexposure sequel. Four replicates were used for each species. Plain nets were used as the control. All data were pooled together and percent corrected mortality was calculated by using Abbott's formula (Abbott 1925):

$$\%\ \text{corrected mortality} = \frac{\text{observed } \%\ \text{mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100.$$

Impact of washing on Olyset nets: Impact of washing on the efficacy of Olyset nets was tested after washing the net at an interval of 24 h. Surf Exel, a detergent powder manufactured by M/S. Hindustan Lever Ltd., Mumbai, 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 thoroughly rubbed for 5 min. Then the net was thoroughly rinsed three times in plain water to remove all the detergent. The washed nets were dried in sunlight for 5–6 h. The temperature range was 35–40°C during drying. Bioassay tests were performed as per the procedure described earlier on *An. culicifacies* and *Cx. quinquefasciatus.* Plain net was used as a control. Percent corrected mortality was calculated by using Abbott's formula.

Entomological evaluation: Altogether, 12 fixed catching stations were chosen randomly with 4 stations each for Olyset net, plain net, and without net. Catches of morning resting fauna were made every week in a month with an aspirator and flashlight. The mean monthly density of indoor-resting mosquitoes was calculated as per man-hour. Percent reduction in the density of mosquitoes was calculated by using the following formula:

$$\%\ \text{reduction} = [(\text{mosquito density in control} - \text{density in experimental}) \div (\text{density in control})] \times 100.$$

Action of Olyset nets: The action of Olyset nets was studied in field conditions at Beel Akbarpur village as per the procedure described earlier by Ansari and Razdan (2000). Weekly night collections were made in 4 structures using Olyset nets and 4 structures using untreated nets. In each structure, 1 volunteer used the net from 1830 to 0600 h. Two insect collectors were deployed in each room to collect mosquitoes with the help of aspirators and flashlights. Mosquitoes that entered were collected from walls, roof, and hanging objects hourly throughout the night by 1 insect collector and the other insect collector collected the mosquitoes that landed on the net. The tasks of insect collectors were rotated to avoid bias. Mosquitoes were iden-

Table 2. Man-hour density of mosquitoes in experimental and control structures (hand catch).[1]

Month	Olyset® net			Plain net			Without net (control)		
	An. culicifacies	*Cx. quinquefasciatus*	Total mosquitoes	*An. culicifacies*	*Cx. quinquefasciatus*	Total mosquitoes	*An. culicifacies*	*Cx. quinquefasciatus*	Total mosquitoes
Pre-experimental period									
Aug. 2003	36 ± 11	68 ± 11	287 ± 74	32 ± 16	62 ± 16	325 ± 98	28 ± 4	78 ± 20	252 ± 35
Sept.	52 ± 6	79 ± 33	428 ± 189	48 ± 8	65 ± 12	358 ± 45	48 ± 14	65 ± 5	386 ± 46
Oct.	64 ± 13	75 ± 14	365 ± 82	70 ± 28	82 ± 47	491 ± 98	54 ± 10	68 ± 20	377 ± 96
Nov.	63 ± 3.5	139 ± 6.5	163 ± 11.5	86 ± 5	162 ± 9	329 ± 17.5	92 ± 4	186 ± 8	385 ± 18.5
Average	5.75 ± 11.2	90.2 ± 28.4	310 ± 70.6	59 ± 20.6	92.7 ± 40.7	375 ± 67.7	55.5 ± 23.1	99.2 ± 50.3	350 ± 56.6
Post-experimental period									
Dec.	33 ± 1.5	75 ± 0.5	144 ± 3	55 ± 2.5	109 ± 2.5	214 ± 2	68 ± 2	128 ± 4	256 ± 0
Jan. 2004	0	15 ± 1	17.5 ± 0.5	3 ± 0.3	19 ± 1.5	26 ± 0.5	3.5 ± 0.5	26 ± 2	39.5 ± 0.5
Feb.	0	20 ± 1	22 ± 1.5	3.5 ± 0.7	41 ± 5.5	53 ± 6	6.5 ± 0.5	56 ± 5.7	76 ± 8
March	2 ± 0	60 ± 4	67 ± 4.5	16 ± 0	136 ± 10	173 ± 12.5	19 ± 0.5	148 ± 2	195 ± 3.5
April	4 ± 1	137 ± 27	147 ± 12.5	23 ± 2.5	277 ± 6.5	330 ± 9.3	30 ± 2	312 ± 9.6	378 ± 11.2
Average	1.8 ± 1	61.4 ± 48.8	79.5 ± 56.6	20.1 ± 19	116.4 ± 90.9	159 ± 110.8	25.4 ± 23.2	134 ± 99.6	188 ± 128.7
% reduction over control	92.9	47.2	50	20.8	13.1	15.4	—	—	—

[1] *An.*, *Anopheles*; *Cx.*, *Culex*.

tified by using a hand lens. Repellent action of nets on females entering in rooms and the excitorepellent action were calculated by the formula as earlier described by Ansari and Razdan (2000). Indoor-collected mosquitoes from experimental and control structures were kept separately in a muslin cloth cage ($10 \times 10 \times 10$ cm) for 24 h at a temperature of 28 ± 2°C and 70–80% relative humidity to determine the delayed mortality. Mortality was recorded and corrected by using Abbott's formula (Abbott 1925).

Repellent action (RA), excitorepellent action (ERA), and killing action (KA) were calculated by using the following formulae:

$$RA = [100 - (T/C)] \times 100,$$

$$ERA = [100 - (L/T)] \times 100, \quad \text{and}$$

$$KA = \frac{\text{observed mortality} - \text{control mortality}}{100 - \text{control mortality}} \times 100,$$

where, T is the total number of mosquitoes entering structures using Olyset nets, C is the total number of mosquitoes entering control structures, and L is the total number of mosquitoes that landed on the net.

RESULTS

Cone bioassay tests

Results of cone bioassay tests for determining discriminatory exposure time and bioefficacy revealed that Olyset nets produced 100% mortality in *An. culicifacies, An. subpictus,* and *An. stephensi* within 3 min of exposure and after 24 h of holding. In *Ae. aegypti,* 100% mortality was observed at 5 min of exposure, whereas in *Cx. quinquefasciatus,* 100% mortality was observed at 30 min of exposure and 24 h of holding. From the results, discriminatory exposure time was established as 3 min for *An. culicifacies, An. stephensi,* and *An. subpictus*; 5 min for *Ae. aegypti*; and 30 min for *Cx. quinquefasciatus.*

Results of susceptibility tests with 0.75% permethrin-treated papers in WHO susceptibility tubes revealed 100% mortality in field-collected *An. culicifacies, An. stephensi,* and *An. subpictus* at 3 min of exposure, whereas only 36% mortality was observed for *Cx. quinquefasciatus.*

Effect of washing on the efficacy of Olyset nets

Results of cone bioassay tests on washed Olyset nets at an interval of 24 h up to 20 washes are presented in Table 1. The results showed that washing did not dilute the efficacy of Olyset nets, as evidenced by the fact that 100% mortality was observed in *An. culicifacies* even after 20 washes, whereas in *Cx. quinquefasciatus,* 100% corrected percent mortality was observed only up to 12 washes. The results clear-

Table 3. Killing action of Olyset® net against different species under field conditions.[1]

	% corrected mortality							
	Anopheles culicifacies		Total anophelines		*Culex quinquefasciatus*		Total mosquitoes	
Date	E	L	E	L	E	L	E	L
Nov. 2003	50.1	—	48	—	38	—	46.6	—
Dec. 2003	42.9	—	39.9	100	39.0	100	47.7	100
Jan. 2004	100	—	45.9	—	26.3	—	46.2	—
Feb. 2004	34.7	—	47.0	—	29.9	—	49.5	—
March 2003	37.7	—	36.7	—	33.4	—	42.5	—
April 2004	39.7	—	31.7	—	37.0	100	36.5	100

[1] E, Mosquitoes entered room; L, mosquitoes landed on net. Percent (%) corrected mortality was calculated over untreated net by using Abbott's formula (see text). Each value is average of 16 replicates in 1 month.

ly show that Olyset nets are highly effective in killing anopheline mosquitoes even after repeated washings, which did not dilute the effect.

Entomological evaluation

Indoor-resting density of mosquitoes in structures with Olyset nets, plain nets, and without nets is depicted in Table 2. Results revealed that average man-hour density of *An. culicifacies* during the preexperimental period in structures selected for Olyset net use was 53.75 ± 11.2, as compared to 59 ± 20.6 and 55.5 ± 23.1 in structures with plain nets and without nets. However, the density of *An. culicifacies* was reduced drastically in structures with Olyset nets. The percent reductions in density of *An. culicifacies* in the structures with Olyset nets when compared with structures where nets were not used during pre- and postexperiment periods were 94% and 95.2% based on density in structures with plain nets and without nets, respectively. In case the of *Cx. quinquefasciatus* and total mosquitoes, the percent reductions in density observed based on structures with plain nets and without nets were 47.2% and 54.1% and 50% and 57.7%, respectively. No reduction in the density of mosquitoes was observed in structures with plain nets based on densities in structures without nets. The results clearly show that Olyset nets not only prevent mosquito bites but also keep mosquitoes away inside the house where nets were used by inhabitants. To confirm this hypothesis, another experiment was conducted to evaluate action of nets.

Action of Olyset nets

Action of Olyset nets was evaluated by studying the repellent action, excitorepellent action, and killing action under field conditions. Killing action was studied against mosquitoes that entered in the room as well as landed on the net. Results obtained for a period of 6 months are presented in Table 3. Landing rate on Olyset nets was drastically reduced. However, 100% corrected mortality was observed in those mosquitoes that landed on the Olyset nets. Mortality also was observed in indoor-resting–collected mosquitoes within 24 h. These mosquitoes were exposed to the net for few seconds and settled to rest on walls. The average corrected percent mortality was 50.8% in *An. culicifacies,* 41.5% in total anophelines, 33.6% in *Cx. quinquefasciatus,* and 44.8% in total mosquitoes. This clearly emphasizes that Olyset nets not only kill the landed mosquitoes but also kill the mosquitoes that enter the room having Olyset nets and that are exposed to the net.

Repellent action and excitorepellent action of Olyset nets are presented in Table 4. The results revealed that Olyset nets produced strong repellent action. Repellent action of Olyset nets was more pronounced in *An. culicifacies* as compared to total anophelines and *Cx. quinquefasciatus.* The repellent action of the Olyset nets was 55.2% in *An.*

Table 4. Repellent and excitorepellency action of Olyset® nets against different species of mosquitoes under field conditions.[1]

	Anopheles culicifacies		Total anophelines		*Culex quinquefasciatus*		Total mosquitoes	
Date	RA	ERA	RA	ERA	RA	ERA	RA	ERA
Nov. 2003	50.5 ± 9.2	100 ± 0	38.3 ± 4.3	100 ± 0	38.5 ± 7.5	100 ± 0	37.5 ± 6.8	100 ± 0
Dec. 2003	51.6 ± 9.4	100 ± 0	31.7 ± 6.8	99.1 ± 0	39.6 ± 9.4	99.9 ± 0	38.8 ± 8.2	99.8 ± 0
Jan. 2004	100	100 ± 0	34.6 ± 5.2	100 ± 0	37.9 ± 9.1	100 ± 0	37.5 ± 8.4	100 ± 0
Feb. 2004	60 ± 9.1	100 ± 0	33 ± 1.8	100 ± 0	37.5 ± 6.1	100 ± 0	37 ± 5.5	100 ± 0
March 2004	54.2 ± 7.1	100 ± 0	44.0 ± 2.0	100 ± 0	39.6 ± 2.0	100 ± 0	40 ± 1.8	100 ± 0
April 2004	52.6 ± 10.4	100 ± 0	37.8 ± 4.6	100 ± 0	39.8 ± 5.4	100 ± 0	39.7 ± 5.0	100 ± 0

[1] Each value is mean ± SD of 16 replicates in a month; RA, repellent action; ERA, excitorepellent action.

culicifacies compared to 38.6% in *Cx. quinquefasciatus.* Results also revealed that excitorepellency action was almost 100% against all the mosquito species over a period of 6 months.

DISCUSSION

The study revealed high efficacy of Olyset nets against *An. culicifacies,* the major malaria vector in the study area. In bioassay tests, the 24-h mortality in almost all anophelines exposed for 3 min was 100%. Nguyen et al. (1996) reported that *Ae. aegypti* were knocked down after 9–16 min of exposure to the Olyset nets and the adulticidal activity remained 100% up to 8 months. In trials in Tanzania, it was reported that 3 min of exposure induced 70–80% mortality in *Anopheles gambiae* after 6 months as well as after 12 months. Time for 80% knockdown was about 5–6 min even after 12 months of use.

Washing the nets with detergent and reuse after 24 h also produced similar results as use of unwashed nets. These results are in conformity with other studies (N'Guessan et al. 2001, WHO 2001). The high efficacy of Olyset nets even after repeated washing may be due to the reactivation of the insecticide. A major advantage of the Olyset net is that its biological efficacy is resumed by diffusion of the insecticide from inside the yarn to the surface, which is accelerated by heat. This might be the reason for high efficacy of these nets even after repeated washings, which also was observed in the present study, where the washed nets were dried under direct sunlight for 5–6 h (35–40°C).

In entomological studies, it was observed that the density of mosquitoes in structures with Olyset nets was drastically reduced when compared to structures with plain nets and without nets. This may be due to the repellent action, killing action, and excitorepellent action of the net. In a trial of Olyset nets by N'Guessan et al. (2001) in M'bé village in Côte d'Ivoire, 60% and 90% reduction in density of *An. gambiae* and *Anopheles funestus,* respectively, was reported. In a study carried out in Cambodia, >70% decline in indoor-resting density of vectors and ~60% decline in parous rate of mosquitoes was reported where Olyset nets were used (WHO 2001). Our results also are in conformity with these results. It may also be pointed out that no toxic, irritation, and allergic effects were observed in volunteers sleeping inside the net and in technicians doing bioassay tests on the Olyset nets.

In view of these observations, it may be concluded that Olyset nets have shown a high degree of efficacy against malaria vectors and can be safely used to prevent mosquito bites. However, large-scale trials are indicated to evaluate the impact of use of Olyset nets on transmission of disease, particularly malaria, and their cost-effectiveness in comparison to conventional indoor residual spraying.

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