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Wash-resistance and field evaluation of alphacypermethrin treated long-lasting insecticidal net (Interceptor®) against malaria vectors *Anopheles culicifacies* and *Anopheles fluviatilis* in a tribal area of Orissa, India

Surya K. Sharma[a,*], Ashok K. Upadhyay[a], Mohammed A. Haque[a], Prajesh K. Tyagi[a], K. Raghavendra[b], Aditya P. Dash[c]

[a] *National Institute of Malaria Research (NIMR), Field Station, Sector-5, Rourkela 769 002, Orissa, India*
[b] *National Institute of Malaria Research (NIMR), Indian Council of Medical Research, Sector-8, Dwarka, New Delhi-110 077, India*
[c] *World Health Organization, Regional office for South-East Asia, Indraprastha Estate, Mahatama Gandhi Marg, New Delhi 110 002, India*

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ABSTRACT

A field trial was conducted on the efficacy of Interceptor nets—a long-lasting insecticidal net (LLN) factory treated with alphacypermethrin 0.667% (w/w) corresponding to 200 mg/m^2, against malaria vectors *Anopheles culicifacies* and *Anopheles fluviatilis* in one of the highly endemic areas of Orissa. The study area comprised 19 villages which were randomized into three clusters and designated as Interceptor net cluster, untreated net cluster, and no net cluster. Baseline studies showed that both the vector species *An. culicifacies* and *An. fluviatilis* were 100% susceptible to alphacypermethrin. Results of wash-resistance and bio-efficacy of Interceptor nets showed 100% mortality in *An. culicifacies* and *An. fluviatilis* even after 20 washings. Bioassays on the Interceptor nets while in use in the field conditions showed a knockdown effect on 70–90% mosquitoes during different months of intervention after 3 min of exposure and 100% mortality was recorded after 24 h of recovery period. The median knockdown time for these species ranged between 4.10–5.25 min and 4.00–5.00 min respectively during intervention period. In Interceptor net study area, there was a significant reduction of 88.9, 96.3 and 90.6% in the entry rate of *An. culicifacies, An. fluviatilis* and other anopheline species respectively with an over all reduction of 87.5% in total mosquitoes. The overall feeding success rate of mosquitoes in the trial villages was only 12.8% in comparison to 35.0 and 78.8% in villages with untreated nets and no nets respectively. A significant reduction was also recorded in parity rate and human blood index of vector species in Interceptor net area. The results of the study showed that Interceptor nets are effective against the malaria vectors and may be used as a suitable intervention strategy in high-risk areas.

1. Introduction

Over 40% of the World's population in some 100 countries are exposed to varying degrees of malaria risk and about 2.5 million people die of malaria annually. In India, about 1.5 million confirmed malaria cases and about 1000 deaths are reported annually. The highest number of malaria cases, *Plasmodium falciparum* cases and malaria related deaths are recorded from Orissa state located in the eastern part of India. The World Health Organization called for a renewed attack on malaria through community-based action oriented programme (World Health Organization, 1993). This necessitates development and application of control strategies that are socially acceptable and cost-effective. Towards this objective, insecticide-treated mosquito nets play an important role for the control of malaria and other vector-borne diseases. The insecticide-treated nets (ITN) have been extensively evaluated in many malaria endemic countries against different vector species and these studies have shown their usefulness in reducing morbidity and mortality from malaria (Lengeler, 2004). In India the efficacy trials of insecticide-treated nets carried out in Orissa and Assam have shown promising results for its large-scale introduction in the operational malaria control programme in India. The deltamethrin treated nets were found effective against malaria transmitted by *Anopheles minimus* (Jana-Kara et al., 1995). Other trials on nets treated with deltamethrin SC and tablet formulation or lambda-cyhalothrin against *Anopheles culicifacies* were successful in reducing high vector density (Sampath et al., 1998; Yadav et al., 2001; Sharma et al., 2005). ITNs are now a part of operational strategies adopted by the National Vector Borne Diseases Control Programme (NVBDCP), Government of India.

* Corresponding author. Tel.: +91 661 2647300; fax: +91 661 2641207.
E-mail addresses: suryaksharma@gmail.com, mrcrkl@dataone.in (S.K. Sharma).

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A number of insecticide formulations are available for the treatment of mosquito nets. However, these formulations are required to be accurately weighed or measured for getting the right concentration over the nets. These measurements at time, pose problems and accuracy is difficult to be sustained under field conditions and particularly for large-scale community level treatment programme. To overcome these problems, long-lasting insecticidal nets (LN) have been developed during recent years (Guillet et al., 2001). These nets are treated at the manufacturing level with insecticide either incorporated into or coated around fibres, and are resistant to multiple washes. The biological activity lasts as long as the net itself (3–4 years for polyester nets, 4–5 years for polyethylene nets). In India, studies on the bio-efficacy of LNs against local malaria vectors have shown good results (Sharma et al., 2006a, 2009a). Interceptor® nets manufactured by M/S. BASF Chemical Company Limited, Germany are 100% texturized multifilament polyester fabric in which the insecticide (alphacypermethrin or FENDONA®) is incorporated directly into the polymers at a dose of 200 mg/m^2. For the treatment of the nets, BASF has used FENDOZIN®—a proprietary polymer that forms a thin slow release coating containing the insecticide alphacypermethrin so that the active ingredient diffuses in a controlled manner to the surface of the netting. Interceptor nets have been evaluated by the WHO Pesticide Evaluation Scheme (WHOPES) and interim recommendations have been given for its use in malaria prevention and control. Interceptor nets have only recently been introduced in India, therefore, a field trial was conducted to assess the wash-resistance and bio-efficacy of Interceptor nets against malaria vectors in one of the highly endemic areas of Orissa. The results of a 1 year trial are presented in this paper. The study was approved by the Scientific Advisory Committee as well as Institutional Ethical Committee of the National Institute of Malaria Research, Delhi.

2. Materials and methods

2.1. Study area

The study was conducted in three clusters of villages under Gurundia and Birkera Primary Health Centres (PHCs) of Sundargarh district in northern part of Orissa state, India. The study area was located within a distance of about 20–35 km from Rourkela city and is accessible round the year. The typical breeding sites are streams, ponds, pools and rice fields. All the study villages are under the influence of two primary malaria vector species *An. culicifacies* and *Anopheles fluviatilis*, the former breeds in ponds, pools and rice fields, whereas the later breeds exclusively in slow running streams. The weather conditions can be divided into four seasons, summer (March–May), monsoon (June–August), autumn (September–October) and winter (November–February). The annual rainfall ranges between 160 and 200 cm. The maximum temperature during summer goes up to 46 °C and the minimum temperature during winter falls to 10 °C.

Malaria is a major public health problem in the area. The annual parasite index (API) or number of malaria cases per 1000 population of the Gurundia and Birkera PHC during 2003, 2004 and 2005 were 21.6, 22.6, 25.9 and 47.4, 37.7, 28.1 respectively. *P. falciparum* malaria is widely prevalent and accounts for 80–90% of total malaria cases (Sharma et al., 2006b). The malaria peak occurs during autumn and winter coinciding with the high prevalence of *An. fluviatilis*. The study population has low socio-economic status and mostly lives in thatched houses clustered in small hamlets either in the forest area or on deforested land. Their economy is basically dependent on forest products and subsistence farming. The nearest medical facilities are situated at a distance of 6–10 km.

2.2. Selection of study villages

On the basis of available epidemiological data with the Health Centres, some villages were short-listed and preliminary rapid fever surveys and entomological surveys were carried out to get some idea of the number of active malaria cases and mosquito productivity. On the basis of average incidence rate, sample size of the population for each arm of the study was calculated as ∼2000. Accordingly, 19 villages selected on the basis of preliminary data were randomized into three clusters and assigned to as either LN (Interceptor net) or controls with or without untreated nets. Cluster 1 (Interceptor net) comprised 6 villages (pop. 2314) and cluster 2 (untreated net) had 5 villages (pop. 2178) and remaining 8 villages with a population of 2204 constituted cluster 3 (no net). The treated and untreated net villages were located in Gurundia block, whereas no net villages were located in Gurundia (6 villages) and Birkera (2 villages) revenue blocks. The demographic information of the study villages was collected through house-to-house census surveys.

2.3. Specifications of Interceptor nets and distribution

Interceptor nets manufactured by M/S BASF India Limited, Mumbai were supplied by the company. The Interceptor nets were made of white polyester multifilament (minimum 32 filaments) of 75 denier with mesh size of 24 holes/cm^2 and were factory treated with alphacypermethrin at 0.667% wt:wt corresponding to 200 mg/m^2. All the nets were of double size (width, 160 cm; length, 180 cm; height, 150 cm). Plain nets with same specifications were used as untreated control. The requirement of the nets was ascertained through sleeping pattern survey in the trial villages. Before the start of the trial, community group meetings were organized in the study villages and inhabitants were educated on proper and regular use of nets and importance of the study. The distribution of nets as per sleeping pattern survey was started in the last week of October 2006 and completed during November 2006. The number of nets distributed to each household was recorded in the register and signatures of the recipient were obtained. 938 Interceptor nets and 911 untreated nets were distributed in cluster 1 and 2 respectively so as to achieve 100% net coverage for the population of these clusters. A village committee consisting of panchayat (governing body) members and other opinion leaders was constituted in consultation with the community to monitor proper use and maintenance of mosquito nets in each study village.

2.4. Data generation

After selection of study villages the data generation was started w.e.f. August 2006 and baseline data were collected up to October 2006. Intervention phase started from November 2006 and continued till July 2007. The following studies were conducted.

2.5. Insecticide susceptibility status of malaria vectors

Baseline insecticide susceptibility status of wild caught malaria vectors *An. culicifacies* and *An. fluviatilis* against alphacypermethrin (0.1%) was determined as per standard procedure (World Health Organization, 1998). The field collected full fed mosquitoes from unsprayed dwellings were exposed for 1 h to alphacypermethrin impregnated papers using WHO adult susceptibility kit. The mosquitoes were kept in the recovery tubes for 24 h and mortality was recorded. In test replicates where mortality in the control tubes was more than 5%, the mortality of the exposed mosquitoes was corrected using Abbott's formula (Abbott, 1925).

2.6. Wash-resistance and bio-efficacy of Interceptor nets

The wash-resistance of Interceptor nets was tested through washing the net with detergent up to 20 washes and subjected to cone bioassays after each wash. First four washings were done at weekly intervals and subsequent washings were performed at fortnightly intervals. Surf Excel procured from the market was used as detergent in all washings. Five grams (one tea spoon) of the detergent was dissolved in 5 l of water and Interceptor net was dipped in the detergent solution for 10 min. Subsequently, the net was washed and thoroughly rinsed with tap water. The cone bioassays on washed nets against malaria vector species were performed as per the standard procedure (World Health Organization, 1998). Wild caught fully fed female *An. culicifacies* and *An. fluviatilis* were used in the bioassays. Ten mosquitoes were released in each cone with the help of suction tube and exposed to Interceptor net for 3 min. The mosquitoes knocked down after the exposure period and mortality after 24 h were recorded. Mosquitoes were provided with cotton swab soaked in 10% glucose solution during recovery period. For each species four replicates were used. Unwashed Interceptor nets and washed untreated nets were taken as control. Percent-corrected mortality was calculated using Abbott's formula.

The persistence of insecticide on nets in regular use was determined every month during intervention phase by contact bioassays using the standard procedure described above. The untreated nets were taken as control. The percent-corrected mortality was calculated using Abbott's formula. In addition to cone bioassays, ring-net bioassays were performed using a netting apparatus consisting of two intersecting circles of wires of 15 cm diameter, welded together and wrapped with Interceptor net in such a way that a sleeve is left through which mosquitoes were introduced and removed with an aspirator. 11 mosquitoes were exposed and time taken for the knockdown of 1st, 6th and 11th mosquito was recorded. The time required for the knockdown of 6th mosquito was taken as median knockdown time.

2.7. Mosquito densities

Adult mosquito densities were measured in four fixed houses and four houses selected randomly each in trial and control village. The total number of mosquito in a house was measured every month in the morning hours between 0600 and 0900 h by the following methods:

2.7.1. Floor sheet collection

In the evening, white cloth sheets were spread over the entire floor of the houses before the occupants retired to bed. Next morning, dead and morbid mosquitoes lying on the floor sheets were picked up, identified to species and their abdominal condition was recorded.

2.7.2. Exit trap collection

One rectangular exit trap of size $12'' \times 15''$ having conical cone of plastic material with an orifice of 1 cm^2 was fixed in the mud walls of four houses fixed for entomological monitoring. Trap cloth cages were fixed in each exit trap before the sunset and next morning all the mosquitoes from the exit traps were collected. The live mosquitoes were transferred to a cage and brought to laboratory to check the mortality after 24 h under optimal conditions. All the mosquitoes collected from exit traps were identified to species and their abdominal conditions were recorded.

2.7.3. Hand catches

Indoor-resting mosquitoes were collected in the fixed and random houses for 15 min in each dwelling with the help of suction tubes using flashlights. The mosquitoes were brought to laboratory in cloth cages, identified and kept under observations for 24 h under optimal conditions. The mean monthly density of indoor-resting mosquitoes was calculated as person-hour density. The percentage of reduction in the density of mosquitoes was calculated as:

$$\%\,\text{Reduction} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

where DC is mosquito density in control villages and DT is mosquito density in trial villages.

2.8. Parity and human blood index

Vector mosquitoes collected through random sampling by hand catch from human dwellings and cattle sheds were dissected for ovaries as per WHO technique based on distended tracheolar skeins and were categorized as parous and nulliparous. Parity rate was calculated as percentage of mosquitoes with parous ovaries from the total mosquitoes dissected. For determining the human biting rate of vector species, direct observations through mosquito-landing catches on human volunteers were not carried out because of ethical considerations. However, human blood index (HBI), which is the proportion of mosquitoes fed on humans, was ascertained through blood meal analysis of vector species by gel diffusion technique (Collins et al., 1986).

2.9. Data recording and analysis

Data recording was done on pre-designed forms and analyzed using the Student's *t*-test to determine the significance of differences in the different parameters between Interceptor net, untreated nets, or no net.

3. Results

3.1. Susceptibility status of malaria vectors

Insecticide susceptibility tests on vector species showed that both the *An. culicifacies* and *An. fluviatilis* were susceptible to 0.1% alphacypermethrin.

3.2. Wash-resistance and bio-efficacy of Interceptor nets

Results of the bioassay tests on washed Interceptor nets showed 100% mortality with 3-min exposure in both *An. culicifacies* and *An. fluviatilis*, even after 20 washes. 100% mortality was also recorded in both the vector species on unwashed Interceptor nets, whereas mortality was ranging between 2.5 and 5.0% on serial washed untreated nets.

Cone bioassay tests performed every month during intervention phase on Interceptor nets in use by the inhabitants under field conditions showed a knockdown rate ranging between 70.0–85.0% and 77.5–90.0% in *An. culicifacies* and *An. fluviatilis* respectively after 3 min of exposure and there was 100% mortality in both the vector species during 24 h recovery period (Fig. 1). The mortality on untreated nets was negligible that varied from 0 to 5%. Results of ring-net bioassay tests on Interceptor nets conducted every month during intervention phase are presented in Table 1. The median knockdown time (6th mosquito) for *An. culicifacies* and *An. fluviatilis* during first month of intervention (November 2006) was 4.10 and 4.00 min respectively. However, there was gradual increase in the median knockdown time and after 9 months of use, the median knockdown time for these vector species was 5.25 and 5.00 respectively.

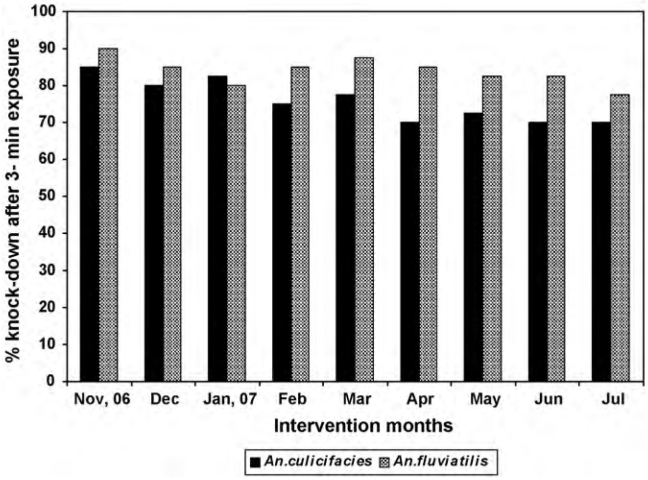

Fig. 1. Knockdown rate of malaria vectors in cone bioassays after 3 min exposure to field distributed Interceptor nets after monthly intervals. 100% mortality was recorded in both the vector species after 24-h recovery period.

3.3. Entry rate of mosquitoes

Entry rate of mosquitoes per room during the study period was calculated by pooling all the mosquitoes collected through hand catch, floor sheet and exit traps. The entry rate of different species of mosquitoes during pre-intervention and intervention phase is given in Table 2. The entry rate of *An. culicifacies*, *An. fluviatilis*, other anopheline species, C*ulex* species and total mosquitoes during pre-intervention period in the houses with Interceptor nets was 2.8, 2.8, 5.3, 2.0 and 12.8 respectively, whereas, it was 2.2, 2.3, 4.0, 2.3 and 10.8 in control houses having untreated nets and 2.1, 1.8, 3.7, 2.7 and 10.4 in houses without mosquito nets. The entry rate of different mosquito species in trial and two control villages was almost similar and no significant difference was observed ($P > 0.05$). During intervention phase in Interceptor net villages, the entry rate of *An. culicifacies*, *An. fluviatilis*, other anophelines, culicines and total mosquitoes had come down to 0.3, 0.1, 0.5, 0.6 and 1.6 (Table 2). There was a reduction of 88.9, 96.3 and 90.6% in the entry rate of *An. culicifacies, An. fluviatilis* and other anopheline species respectively with an over all reduction of 87.5% in the entry rate of total mosquitoes in comparison to pre-intervention phase. There was a significant reduction ($P < 0.01$) in entry rate of mosquitoes in Interceptor net houses in the absence of indoor residual spray.

Table 1
Results of ring-net bioassays on Interceptor nets to determine median knockdown time of the vector species in Sundargarh district.

Month, year	Vector species	Knockdown time of mosquitoes (min)		
		1st	6th	11th
November, 2006	*An. culicifacies*	2.15	4.10	9.40
	An. fluviatilis	2.10	4.00	8.35
December, 2006	*An. culicifacies*	2.50	4.15	9.45
	An. fluviatilis	2.05	4.00	8.30
January, 2007	*An. culicifacies*	2.20	4.35	9.45
	An. fluviatilis	2.10	4.25	8.40
February, 2007	*An. culicifacies*	2.25	4.50	9.55
	An. fluviatilis	2.15	4.30	8.45
March, 2007	*An. culicifacies*	2.30	5.00	9.50
	An. fluviatilis	2.20	4.35	8.50
April, 2007	*An. culicifacies*	2.55	4.55	9.45
	An. fluviatilis	2.25	4.45	8.40
May, 2007	*An. culicifacies*	3.00	5.05	10.00
	An. fluviatilis	2.30	4.50	8.55
June, 2007	*An. culicifacies*	3.05	5.15	10.00
	An. fluviatilis	2.35	4.55	9.00
July, 2007	*An. culicifacies*	3.10	5.25	10.05
	An. fluviatilis	2.35	5.00	9.00

11 mosquitoes of each vector species were exposed per ring net. The time taken to knockdown 6th mosquito was taken as median knockdown time.

Table 2
Total number of mosquitoes caught per room (entry rate) by resting catches, exit traps and on floor sheets, in houses with Interceptor nets, untreated nets and no net during the pre-intervention and intervention periods.

Species	No. of mosquitoes collected from houses with		
	Interceptor nets	Untreated nets	No nets
Pre-intervention (August–October 2006)			
An. culicifacies	66 (2.8)	52 (2.2)	51 (2.1)
An. fluviatilis	64 (2.8)	56 (2.3)	44 (1.8)
Other anophelines	127 (5.3)	96 (4.0)	89 (3.7)
Culex spp.	49 (2.0)	56 (2.3)	65 (2.7)
Total (all species)	306 (12.8)	260 (10.8)	249 (10.4)
Intervention phase (November 2006–July 2007)			
An. culicifacies	23 (0.3)	66 (0.9)	121 (1.7)
An. fluviatilis	10 (0.1)	19 (0.3)	34 (0.5)
Other anophelines	38 (0.5)	113 (1.6)	137 (1.9)
Culex species	46 (0.6)	214 (3.0)	287 (4.0)
Total (all species)	117 (1.6)	412 (5.7)	579 (8.0)

8 dwellings were searched for mosquito collection every month.
Numbers in parenthesis denote entry rate/room.

3.4. Impact on density of malaria vectors

The person-hour density (PHD) of *An. culicifacies* during pre-intervention period in trial villages, untreated net villages and no net area ranged between 8.5–14.0, 6.0–11.5 and 7.5–10.5 respectively, showing that all the three clusters of villages were similar in relation to mosquito productivity of *An. culicifacies* (Fig. 2). However, with the commencement of intervention during November 2006, there was sharp decline in the density of *An. culicifacies* (Fig. 2) in Interceptor net area (range: 0.0–1.5) as compared to areas with untreated nets (range: 1.0–6.0) and without net (range: 2.0–9.5). Similarly density of *An. fluviatilis* during pre-intervention period in Interceptor net, untreated net and no net area was ranging between 4.0–7.0, 3.5–5.5 and 2.5–8.0 respectively (Fig. 3) indicating that mosquito productivity of this species was more or less similar in

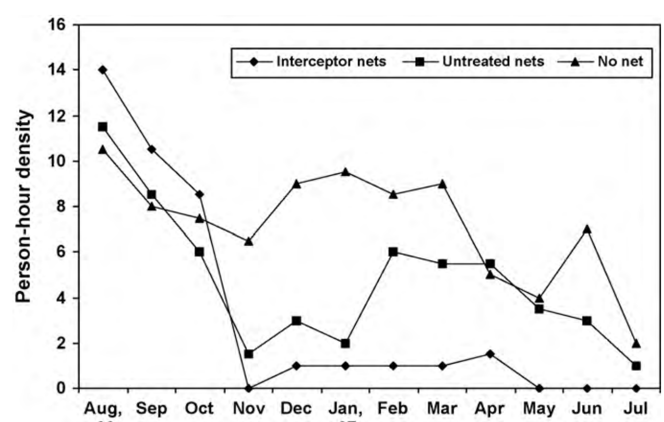

Fig. 2. Density of *An. culicifacies* in houses with Interceptor nets, untreated nets and no net during pre-intervention (August 2006–October 2006) and intervention phase (November 2006–July 2007).

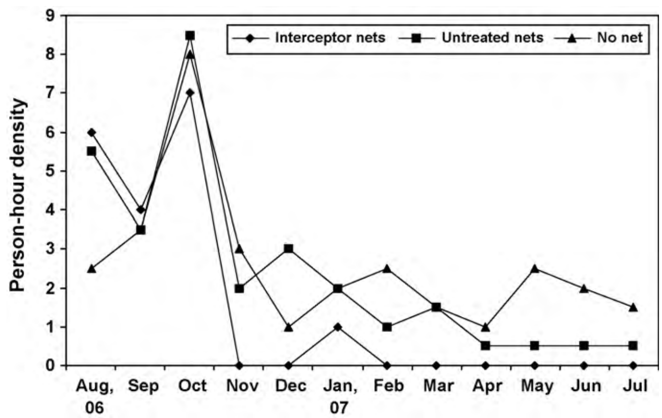

Fig. 3. Density of *An. fluviatilis* in houses with Interceptor nets, untreated nets and no net during pre-intervention (August 2006–October 2006) and intervention phase (November 2006–July 2007).

all these areas. With the introduction of Interceptor nets in the trial villages, the person-hour density of *An. fluviatilis* came down to zero and remained so during most part of the intervention phase (Fig. 3), whereas in control villages with untreated nets and no nets the density was ranging between 0.5–3.0 and 1.0–3.0 respectively during intervention period. There was a significant reduction of 82.3 and 90.9% in the density of *An. culicifacies* as compared to untreated net and no net area respectively. Similarly, a reduction of 91.3 and 94.1% was recorded in the density of *An. fluviatilis* in houses with Interceptor nets in comparison to houses with untreated nets and no net respectively.

3.5. Immediate and delayed cumulative mortality

Immediate mortality was recorded in mosquitoes collected through floor sheet collections and exit traps and delayed mortality was observed in all the mosquitoes collected through hand catch and exit traps from trial and control villages and the data are presented in Table 3. There was 30.8% immediate mortality in all the mosquitoes collected from houses with Interceptor nets, whereas, no immediate mortality was found in villages with untreated nets and without nets. Observations on delayed cumulative mortality in houses with Interceptor nets indicated 64.1% mortality after 24 h and 100% within 48 h of collection. The delayed mortality of mosquitoes in houses with untreated nets and no nets was only 2.4 and 0.2% respectively. The difference in delayed mortality of mosquitoes in houses with treated nets against untreated nets and no nets was statistically significant ($P < 0.001$).

3.6. Feeding success rate of malaria vectors

Blood fed mosquitoes collected through hand catch, floor sheet and exit traps showed that the feeding success rate of *An. culicifacies* and *An. fluviatilis* in houses with Interceptor nets was only 8.7 and 0.0% respectively in comparison to 24.2 and 15.8% in houses with untreated nets and 70.2 and 85.3% in houses without nets respectively. The overall feeding success rate of all the mosquitoes in the trial village was only 12.8% in comparison to 35.0 and 78.8% in villages with untreated nets and no nets respectively. The blood feeding inhibition of mosquitoes in the Interceptor net houses was statistically significant ($P < 0.001$) in comparison to untreated net or no net.

3.7. Parity rate and human blood index of vectors

The unfed mosquitoes collected from the houses using Interceptor net, untreated nets, and without nets were dissected to determine parity rate of *An. culicifacies* and *An. fluviatilis* during pre-intervention and intervention period and the data are presented in Table 4. The parity rate of *An. culicifacies* during pre-intervention period in all the three clusters was ranging between 37 and 41%. However, during intervention, the parity rate in Interceptor net area had come down to 9.4% and there was 77% decline in the parity rate. Similarly, the parity rate in *An. fluviatilis* during intervention phase was reduced from 41.2 to 11.1% and registered a decline of 73%.

The human blood index (HBI), which is the proportion of mosquitoes with human blood in their stomach was determined for both the vector species during pre-intervention and intervention period from all the three clusters and the data are presented in Table 4. *An. culicifacies* is primarily a zoophilic species with HBI of 0.02–0.03 in all the three clusters during pre-intervention period. On the other side *An. fluviatilis* is a highly anthropophilic species with an HBI of 1.0 in all the study area. The HBI of both the vector species was zero in Interceptor net area during intervention phase.

Table 3
Immediate and delayed mortality of mosquitoes in houses with Interceptor nets (LN), untreated nets (UN) and no nets (NN) during intervention phase (November 2006–July 2007).

Species	Houses with	Total no. of mosquitoes collected dead and alive[a]	Immediate mortality (dead when collected) (%)[b]	Delayed mortality after 24 h (%)[c]
An. culicifacies	LN	23	10 (43.5)	13 (56.5)
	UN	66	0	2 (3.0)
	NN	121	0	1 (0.8)
An. fluviatilis	LN	10	8 (80.0)	2 (20.0)
	UN	19	0	1 (5.3)
	NN	34	0	0
Other anophelines	LN	38	11 (28.9)	27 (71.0)
	UN	113	0	3 (2.6)
	NN	137	0	0
Culex species	LN	46	7 (15.7)	33 (71.7)
	UN	214	0	4 (1.9)
	NN	287	0	0
Pooled (total mosquitoes)	LN	117	36 (30.8)	75 (64.1)
	UN	412	0	10 (2.4)
	NN	579	0	1 (0.2)

[a] Mosquitoes collected by hand catch of resting mosquitoes, on floor sheet and in exit traps.
[b] Dead mosquitoes on floor sheet and in exit traps.
[c] Delayed mortality observed in mosquitoes collected by hand catch and exit traps.

Table 4
Parity rate and human blood index (HBI) of vector species in Interceptor net, untreated net and no net area during pre-intervention (August–October, 2006) and intervention phase (November 2006–July 2007).

Species	Period	Interceptor net		Untreated net		No net	
		No. dissected (% parous)	HBI	No. dissected (% parous)	HBI	No. dissected (% parous)	HBI
An. culicifacies	Pre-intervention	41 (41.5)	0.03	37 (37.8)	0.02	35 (37.1)	0.02
	Intervention	53 (9.4)	0.0	55 (21.8)	0.01	58 (37.9)	0.02
An. fluviatilis	Pre-intervention	17 (41.2)	1.0	12 (38.5)	1.0	8 (37.5)	1.0
	Intervention	9 (11.1)	0.0	14 (28.6)	0.8	18 (38.9)	1.0

HBI: proportion of mosquitoes showing human blood in stomach.

3.8. Excito-repellent action

The excito-repellent rate (percentage of mosquitoes collected in exit traps to total entry) of female mosquitoes in houses with Interceptor nets and untreated nets during different months of intervention phase ranged between 16.6–44.4% and 0–10.3% respectively (Fig. 4). The exit rate of *An. culicifacies* in houses with Interceptor nets and untreated nets was 39.1% (9/23) and 7.6% (5/66) respectively. The excito-repellent action on *An. fluviatilis* in trial and untreated net houses was 20.0% (2/10) and 0.0% (0/19) respectively. Other anopheline species and *Culex* species exhibited excito-repellent rate of 47.4% (18/38) and 13.0% (6/46) respectively in houses with Interceptor nets compared to 3.5% (4/113) and 5.1% (11/214) respectively in houses with untreated nets. The total excito-repellent rate of all the mosquitoes in houses with Interceptor nets and untreated nets was 29.9 and 4.8% respectively.

4. Discussion

Long-lasting insecticidal nets (LNs) have been developed to overcome the operational problems of re-treatment of conventional nets (ITN) in a community-based operational malaria control programme in malaria endemic countries. Several companies have developed long-lasting insecticide-treated nets (LNs) that retain lethal concentrations of insecticide for at least 3 years. Of the six brands of LNs currently recommended by the WHO Pesticide Evaluation Scheme (WHOPES), the interim recommendation has been given to Interceptor net for its use in malaria prevention and control. However, it becomes imperative to evaluate any brand against local malaria vector species before introducing it in the operational malaria control programme. In this endeavour, Interceptor net has qualified the field evaluation under Indian conditions. The results of the present field trial are based on comparative data collected from 3 different clusters of villages using Interceptor net, untreated net and no net over a period of 9 months of intervention in an area where the primary malaria vectors *An. culicifacies* and *An. fluviatilis* are fully susceptible to alphacypermethrin. The present study showed that the Interceptor nets maintained high bio-efficacy against *An. culicifacies* and *An. fluviatilis* even after 20 washings. Bioassays on Interceptor nets in use by the inhabitants under field conditions also showed a knockdown rate ranging between 70.0–85.0% and 77.5–90.0% in *An. culicifacies* and *An. fluviatilis* respectively after 3 min of exposure and there was 100% mortality in both the vector species during 24 h recovery period thereby indicating that the bioavailability of insecticide on Interceptor net remain effective for longer period even after multiple washings and also while in use in natural field conditions.

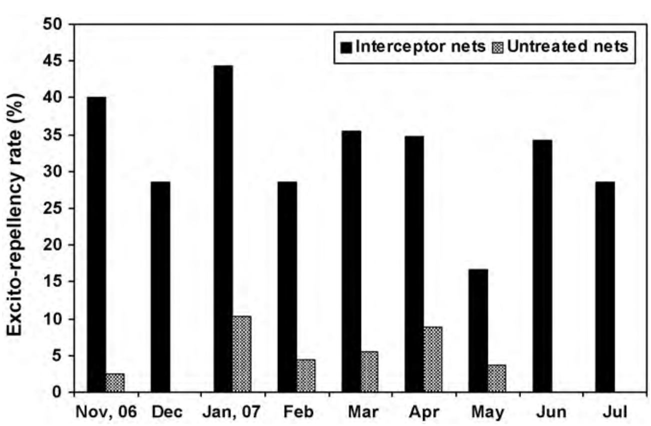

Fig. 4. Excito-repellent rate of female mosquitoes in houses with Interceptor nets and untreated nets.

The median knockdown time for both the vector species on Interceptor nets compare very well with the earlier study on permethrin treated Olyset nets in Sundargarh district of Orissa (Sharma et al., 2009a) and there was marginal increase in the median knockdown time for both the vector species over a period of 9 months of field use. For fast acting insecticides such as pyrethroids, the median knockdown time is directly correlated to the insecticide concentration over the surface (World Health Organization, 1998). There was a significant reduction of 88.9, 96.3 and 90.6% in the entry rate of *An. culicifacies*, *An. fluviatilis* and other anopheline species respectively with an over all reduction of 87.5% in houses using Interceptor nets. There was also a small reduction in the entry rate of mosquitoes in control houses with untreated nets. This was caused by the physical barrier due to untreated net and therefore, diversion of mosquitoes to cattle sheds for feeding. The overall density of *An. culicifacies* was significantly lower in houses with Interceptor nets in comparison to houses with untreated net or no net. With the introduction of Interceptor nets, the density of *An. fluviatilis* was almost decimated in the trial area because the vector species is highly susceptible to insecticides and mainly anthropophagic, so the survival of the species became difficult because of toxicity of alphacypermethrin and also blood feeding inhibition during intervention phase. The data on immediate mortality in all the mosquitoes entering the houses and delayed cumulative mortality suggested that Interceptor nets lead to a 'mass effect' on the village mosquito population. Similarly, introduction of Interceptor nets for personal protection in trial villages also resulted in significant reduction in feeding success rate and also in parity status of malaria vector species. The human blood index of vector species in the trial area had become zero during intervention phase, thereby indicating success of intervention in completely interrupting human biting by the mosquitoes. The Interceptor nets also exerted an excito-repellent effect on all the mosquitoes as documented with deltamethrin treated nets (Sharma et al., 2005) and alphacypermethrin (Maxwell et al., 2003; Soremekun et al., 2004). Thus, the results obtained from the field trial on all the entomological parameters such as entry rate of mosquitoes, indoor-resting density, immediate and delayed cumulative mortality, feeding success rate and parity rate of malaria vector species between trial and two control areas showed the effect of Interceptor nets as a suitable personal protection intervention tool.

Earlier studies in India on long-lasting insecticidal nets also showed high efficacy of these intervention tools against different mosquito species (Ansari et al., 2006; Sharma et al., 2006b, 2009a; Jeyalaksmi et al., 2006). There are also reports of high efficacy of long-lasting insecticidal nets in reducing man-vector contact and entry of mosquitoes in rooms (Itoh and Okuno, 1996; Nguyen et al., 1996; Maxwell et al., 2006). The long-lasting insecticidal nets performed better than conventionally treated nets in reducing man-vector contract as they significantly reduced entry and blood feeding rates (N'Guessan et al., 2001). However, there are also reports of poor efficacy of LNs in the laboratory and in the field against pyrethroid resistant *Anopheles gambiae* in Western Kenya (Lindblade et al., 2005; Gimnig et al., 2005). However, the results from the Indian trials confirm the utility of LNs as the best option for personal protection from disease vectors and nuisance mosquitoes.

The acceptability of Interceptor nets by the community was largely good as indicated by 70–90% compliance rate of net usage during different months of intervention. The perceived side effects such as skin rashes, itching, headache and lacrimation were minimal and the extent was almost similar to reported earlier in permethrin treated LNs (Sharma et al., 2009b). The present trial showed that the Interceptor nets are effective personal protection tool and may be used as an intervention strategy to control vector population in the high transmission difficult areas. The distribution of LNs in the operational malaria control programme will help to reduce the technicalities involved in the conventional treated nets and holds a good promise for use in large-scale community level interventions, which are envisaged under the malaria control strategies in high-risk malarious areas.

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Conflict of interest

The authors have no conflict of interest.

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