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Evaluation of the impact of ZeroFly®, an insecticide incorporated plastic sheeting on malaria incidence in two temporary labour shelters in India

P.K. Mittal[1], U. Sreehari[1], R.K. Razdan[1] & A.P. Dash[2]

[1]*National Institute of Malaria Research (ICMR), New Delhi;* [2]*WHO-SEARO, New Delhi, India*

ABSTRACT

Background & objectives: Prevention of malaria is a major technical and operational problem in displaced and mobile populations such as refugee camps and temporary labour settlements. Insecticide incorporated plastic sheeting is a new technology to control mosquitoes in emergency shelters and also temporary habitations at different locations. In view of this, efficacy of ZeroFly®, an insecticide incorporated plastic sheeting (factory treated with deltamethrin 2.0 g/kg or 265 mg/m^2) was evaluated for its efficacy against malaria vectors and its impact on malaria incidence in temporary labour settlements in two urban areas in India.

Methods: This trial was conducted in two labour settlements in two urban areas, Delhi and Noida (U.P.), India with ~ 250 populations. In an area, two localities were selected for intervention with ZeroFly and untreated plastic sheets (control). Entomological and epidemiological data were collected using standard methods for one year.

Results: Baseline studies on the susceptibility of mosquitoes in Delhi and Noida areas revealed 100% susceptibility of the malaria vector species *Anopheles culicifacies* and *An. stephensi* to deltamethrin. Cone bioassay tests performed against *An. culicifacies* and *An. stephensi* to determine the efficacy of ZeroFly sheets showed 100% mortality against *An. culicifacies* and *An. stephensi* with 3 min exposure and after 24 h recovery period. Against *Culex quinquefasciatus* and housefly 100% mortality was obtained after 30 min of exposure period. Intervention with the ZeroFly plastic sheets resulted in almost complete reduction in the resting density of *An. culicifacies* and *An. stephensi,* the two major malaria vectors and also in the reduction of malaria cases in ZeroFly camps as compared to control camps. The ZeroFly plastic sheeting was found to be safe for human. Barring some complaints of skin irritation and itching, which were temporary in nature, no adverse health effects were reported by the users. The community acceptance was high.

Conclusion: Results of the present study revealed that ZeroFly® plastic sheeting is highly effective in reducing the indoor resting density of mosquitoes, man-vector contact and malaria incidences in labour populations living in temporary shelters.

Key words *Anopheles culicifacies*, feeding success; malaria; parity rate; resting density; ZeroFly® plastic sheeting

INTRODUCTION

Control of malaria vectors has relied mainly on indoor residual spraying of DDT, malathion and synthetic pyrethroids such as deltamethrin and cyfluthrin in India in rural areas by targeting mainly *Anopheles culicifacies* (Diptera: Culicidae), a major malaria vector which is attributed for the transmission of 60–70% of cases in rural and peri urban areas of India[1,2]. Vector borne diseases are one of the problems in displaced populations, refugee camps, labour settlements, temporary habitations, etc. In general, the shelters provided for such populations are temporary structures with canvas tents or plastic sheetings. Spraying on canvas tents with residual pyrethroid insecticide is an established method of vector control in refugee camps [3-7] . In recent times, plastic sheeting (polythene tarpaulins) has replaced canvas as the shelter material for displaced populations in complex emergencies. During the last decade efforts have been made to address the problem of protecting the population by spraying the tents with either pyrethroid or by impregnating the polyethylene tarpaulins with insecticide during manufacture[8]. Few studies with insecticide impregnated tarpaulins demonstrated entomological impact and these were found effective in mosquito control[8,9]. Recently, Sharma *et al*[10] evaluated plastic sheeting for control of tribal malaria in Orissa. However, such interventions need evaluation in different ecological conditions in India. In view of this, ZeroFly®, a deltamethrin incorporated plastic sheeting at a dosage of 265 mg/m^2, was evaluated to assess its efficacy against mosquitoes, particularly malaria vectors and its impact on malaria transmission in temporary labour settlements in Delhi and Noida (Uttar Pradesh), India.

MATERIAL & METHODS

ZeroFly® plastic sheeting measuring 4 × 5 m made up of HDPE flat yarns and laminated with blue LDPE film

and incorporated with deltamethrin @ 265 mg/m^2 ± 10% and untreated plastic sheeting with same material without insecticide were procured from M/s. Vestergaard Frandsen Pvt. Ltd, New Delhi, India.

The study was undertaken in some temporary labour settlements in Delhi and Noida, Uttar Pradesh, India. Preliminary rapid fever surveys and mosquito collections were carried out in some temporary labour settlements in Delhi and Noida areas for selection of study sites. Based on this information two labour camps (agricultural labourers) situated at the embankment of the River Yamuna in Delhi, having similar topography and same population size were selected. Similarly, two temporary labour camps (Construction site at Sector 125, Noida) with similar topography and population size were randomly selected for the trial. In one of the settlements, in both the study sites, temporary 'λ' shaped shelters were made from ZeroFly® sheeting. In the other labour settlements of both sites, tents were made with untreated plastic sheeting, which served as control. The sheets were either fixed as inner lining on all surfaces or temporary 'λ' shaped shelters (a ridge pole and two upright poles) were made. The sheets were open at the ends and pegged to the floor along the edges. Four fixed and four randomly selected structures, one each in four arms (north, south, west and east) were used for collecting mosquitoes and other insects. The demographic information of these labour camps in Delhi and Noida is given in Table 1. Free informed written consent was obtained from the inhabitants before inclusion in the study. Information, education and communication activities were undertaken to sensitize the communities regarding the usefulness of these sheets, safety, precautions, etc.

Insecticide susceptibility/resistance status of *An. culicifacies* and *An. stephensi* in both the study sites was ascertained using 24 h mortality after exposure to diagnostic concentration of insecticide test papers of DDT (4%), malathion (5%) and deltamethrin (0.05%). These insecticide test papers were procured from University Sans Malaysia (*www.usm.my*). Susceptibility tests were carried out using standard WHO procedure and kits[11]

Cone bioassays were performed on 3–5 day old female mosquitoes following standard WHO bioassay procedures[11] to assess the efficacy and persistence of efficacy of insecticide incorporated plastic sheeting on prevailing mosquito vectors. In addition to mosquito vectors, bioassays were also performed against houseflies. Cone bioassays were performed for 3 min exposure period against *An. stephensi* and *An. culicifacies*; and in case of *Culex* mosquitoes and houseflies, bioassays were performed for 3 min as well as 30 min exposure periods. Number of knocked-down mosquitoes or flies were recorded after 3/30 min exposure and 24 h mortality was scored. Mosquitoes were provided with cotton swab soaked in 10% glucose solution during the recovery period. All the tests were replicated four times along with control and corrected mortality was calculated by Abbott's formula[12]. Bioassays were conducted at monthly intervals to assess the persistence and efficacy up to a period of one year.

Mosquito collections were made fortnightly using suction tube and flashlight in early morning hours. Four structures were selected randomly (one each from north, south, east and west directions of the settlements) for sampling adult population of mosquitoes. Collections were done for 15 min in each selected structure and density per man-hour (MHD) was determined. Field-collected mosquitoes were brought to the laboratory for species identification and further processing. Sampled adult females collected from the experimental and control areas were dissected for gut and gland infections[13].

The unfed mosquitoes collected through different sampling techniques were dissected for ovaries as per WHO technique[13] based on distended tracheolar skeins and were categorized as parous and nulliparous. Vector incrimina-

Table 1. Demographic profile of the study sites and number of plastic shelters distributed in the study sites

Type of intervention	No. of dwelling units	Population			No. of plastic sheets distributed
		Male	Female	Total	
Study site # 1: Delhi					
ZeroFly	59	152	82	234	59
Untreated plastic sheet	71	167	72	239	71
Study site # 2: Noida					
ZeroFly	82	190	80	270	82
Untreated plastic sheet	67	173	87	260	67

tion was done by dissecting gut and salivary glands to detect oocyst/sporozoites.

During the study period point prevalence (mass blood survey) surveys were carried out in the settlements. The first survey was carried out prior to the intervention with ZeroFly® or untreated plastic sheeting in the month of July 2006 and subsequent surveys were carried out in the post-intervention period in the month of October–November 2006. Every fourth house was surveyed and blood smears of all family members were collected. Further, active surveillance was also carried out fortnightly in all the study sites. Blood smears of all fever cases were collected, stained with JSB stain and examined under microscope for the presence of malaria parasites. Presumptive treatment was given to all fever cases, while radical treatment was given to only positive cases as per the drug schedule of National Vector Borne Disease Control Programme (NVBDCP), Delhi, India.

Cross-sectional surveys were also conducted among ZeroFly plastic sheet users using a structured questionnaire for assessing their perception about the ZeroFly plastic sheeting, side-effects and collateral benefits.

Statistical analysis: Student's *t*-test was performed using statistical package for social sciences (SPSS version 9.0) to test the significance between ZeroFly and untreated plastic sheetings. Fischer's exact test was used to compare the parasitological data. Values were compared at 0.05 level of significance.

RESULTS

Insecticide susceptibility tests against *An. culicifacies* and *An. stephensi* collected from the study sites revealed that both the mosquito species are resistant to DDT (26–45% mortality) and fully susceptible to malathion and deltamethrin (100% mortality) in both the study sites. Results of cone bioassays on ZeroFly plastic sheet revealed 100% mortality in *An. culicifacies* and *An. stephensi* (Table 2) in August 2006. After one year of continuous use of ZeroFly sheeting (July 2007), the mortality in *An. culicifacies* and *An. stephensi* was 100% and 90%, respectively. In *Cx. quinquefasciatus,* only 55% mortality was obtained after 3 min exposure in cone bioassays (Table 2), while exposure for 30 min resulted in 100% mortality. However, the percent mortality declined slightly after one year of field use to 86.6% in 30 min exposure and 43.3% in 3 min exposure. Exposure of houseflies on ZeroFly revealed 100% mortality initially and after one year of continuous use the mortality reduced to 80% in 30 min exposure.

The per man hour density (MHD) of indoor resting vector species, *An. culicifacies* and *An. stephensi* in both the study sites are shown in Table 3. Results revealed a sharp reduction in the densities of *An. culicifacies* and *An. stephensi* in the ZeroFly area. There was a significant reduction in the density of *An. culicifacies* after intervention with ZeroFly, when compared to the untreated plastic sheeting ($p < 0.05$) in both the study sites.

Observations on the blood fed mosquitoes collected through hand catch showed no vector species in the ZeroFly (experimental) areas in both the study sites. The feeding success rate of *An. culicifacies* and *An. stephensi* in tents with untreated plastic sheets was 11.96 and 17.14% in the study site # 1 (Delhi) and 9.8 and 18.8% in the study site # 2 (Noida) respectively (Table 4). The observed variations in the feeding success rate between the ZeroFly and control areas indicate the effect of intervention measure.

Table 2. Efficacy and persistence of the ZeroFly® plastic sheeting against mosquitoes and houseflies assessed from cone bioassays

Insect/mosquito species	Exposure time in min	Month/Yr	% corrected mortality after 24 h
An. culicifacies	3	August 2006	100
		July 2007	100
An. stephensi	3	August 2006	100
		July 2007	90
Cx. quinquefasciatus	3	August 2006	55
		July 2007	43.3
	30	August 2006	100
		July 2007	86.6
Houseflies	3	August 2006	45
		July 2007	5
	30	August 2006	100
		July 2007	80

ZeroFly plastic sheetings were fixed in the structures of the study areas in August 2006.

Table 3. Mean ± S.D. of per man hour density of malaria vectors in two study sites with ZeroFly® and untreated plastic sheetings

Period	Type of intervention	No. of collections	Study site # 1: Delhi		Study site # 2: Noida	
			An. culicifacies	*An. stephensi*	*An. culicifacies*	*An. stephensi*
Pre-intervention July 2006	ZeroFly	2	4 ± 1.4	4 ± 0	2 ± 0	4 ± 0
	Untreated plastic sheet	2	1 ± 0	2 ± 1.3	0	3 ± 1.4
Post-intervention August 2006 to July 2007	ZeroFly	24	0	0.16 ± 0.2	0.16 ± 0.38	0.08 ± 0.2
	Untreated plastic sheet	24	10.91 ± 9.4	7.91 ± 9.08	6.25 ± 7.6	4.66 ± 8.7
P-value	ZeroFly *vs* Untreated plastic sheet		$t = 3.64$ $p = 0.0016$	$t = 2.99$ $p = 0.0068$	$t = 2.75$ $p = 0.012$	$t = 1.81$ $p = 0.085$

Table 4. Feeding success of malaria vectors collected from ZeroFly® and untreated plastic sheeting structures during August 2006–July 2007 from the study sites

Study sites	Type of intervention	Mosquito species	Total mosquitoes observed	No. of fed mosquitoes (%)	P-value ZeroFly *vs* Untreated plastic sheeting
Study site # 1: Delhi	ZeroFly	*An. culicifacies*	0	0 (0)	–
	Untreated sheet		117	14 (11.96)	
	ZeroFly	*An. stephensi*	1	0 (0)	$\chi^2 = 0.211$, $p = 0.646$
	Untreated sheet		105	18 (17.14)	
Study site # 2: Noida	ZeroFly	*An. culicifacies*	2	0 (0)	$\chi^2 = 0.217$, $p = 0.641$
	Untreated sheet		112	11 (9.8)	
	ZeroFly	*An. stephensi*	1	0 (0)	$\chi^2 = 0.231$, $p = 0.631$
	Untreated sheet		69	13 (18.8)	

No vector species was collected in the ZeroFly area, hence, these parameters were not determined. In the control labour camp in Delhi, 14 unfed females of *An. culicifacies* and 12 *An. stephensi* were dissected for parity status. Parity rate of the collected female *An. culicifacies* was 71.4 and that of *An. stephensi* was 90.9%. Similarly, in the control labour camp in Noida, 9 unfed females of *An. culicifacies* and 10 *An. stephensi* were dissected for parity status. Parity rate of the collected females of *An. culicifacies* was 88.8% and *An. stephensi* was 70%. However, none of the mosquito dissected for the detection of oocysts and sporozoites was found positive in both the study sites. Due to low sample size, no conclusion can be drawn.

Results of the mass blood surveys (irrespective of fever) carried out in these areas are presented in Table 5 and the results of active surveillance during the post-intervention period August 2006 to July 2007 are shown in Table 6. Comparison of malaria cases from the labour camps showed significant reduction in malaria cases in the ZeroFly used settlements when compared to untreated plastic sheet used settlements. These results substantiate that ZeroFly sheetings are quite effective in controlling malaria.

Cross-sectional surveys were conducted among ZeroFly plastic sheet users in labour population in Delhi and Noida areas in the month of September 2006 (n = 91) to assess their perception on protection from mosquitoes, the adverse effects and collateral benefits and the analysis of data revealed that 100% of the respondents knew about the purpose of fixing ZeroFly plastic sheeting for protection from mosquitoes and malaria. There were complaints of skin irritation and itching (43%) and eye irritation (27%). However, these effects were observed initially on the first exposure for 1–2 days and only transitory in nature. The community asserted the use of these sheets as they provided them relief not only from mosquitoes but also from other household pests such as cockroaches, ants and houseflies.

DISCUSSION

Control of malaria is a major technical and operational problem in displaced and mobile populations. Physical structures in displaced camps and temporary habitations may not support indoor residual spray or hanging insecticide treated nets[7]. The present study revealed that ZeroFly plastic sheets may be used as an effective intervention strategy to control *An. culicifacies* and *An.*

Table 5. Malaria incidence (mass survey) in ZeroFly® and control sheet camps

Period	Study site	Intervention	BSE	Total +ve cases	*Pf*	*Pv*	P-value ZeroFly *vs* Untreated plastic sheeting
July 2006 (Pre-intervention)	Study site # 1: Delhi	ZeroFly	50	1	0	1	$\chi^2 = 0.156$, $p = 0.901$
		Untreated	42	1	0	1	
	Study site # 2: Noida	ZeroFly	41	1	0	1	$\chi^2 = 0.662$, $p = 0.416$
		Untreated	32	2	0	2	
Oct –Nov 2006 (Post-intervention)	Study site # 1: Delhi	ZeroFly	55	0	0	0	$\chi^2 = 3.4$, $p = 0.065$
		Untreated	50	3	1	2	
	Study site # 2: Noida	ZeroFly	36	0	0	0	$\chi^2 = 0.978$, $p = 0.323$
		Untreated	75	2	2	0	
July 2007 (Post-intervention)	Study site # 1: Delhi	ZeroFly	40	0	0	0	$\chi^2 = 0.964$, $p = 0.326$
		Untreated	42	1	0	1	
	Study site # 2: Noida	ZeroFly	40	0	0	0	$\chi^2 = 0.956$, $p = 0.321$
		Untreated	40	1	0	1	

BSE—Blood slides examined; *Pf— Plasmodium falciparum; Pv—Plasmodium vivax.*

Table 6. Results of active surveillance for malaria incidence in labour camps with ZeroFly and untreated (control) plastic sheeting

Month/Year	Intervention	No. of slides examined	No. malaria positive	*Pf*	*Pv*	P-value ZeroFly *vs* Untreated plastic sheeting
Study site # 1: Delhi						
Pre-intervention July 2006	ZeroFly	9	3	0	3	$\chi^2 = 1.02$, $p = 0.312$
	Untreated	8	1	0	1	
Post-intervention	ZeroFly	86	1	0	1	$\chi^2 = 13.8$, $p = 0.0001$
(August 2006–July 2007)	Untreated	103	18	6	12	
Study site # 2: Noida						
Pre-intervention July 2006	ZeroFly	2	1	0	0	$\chi^2 = 4.44$, $p = 0.035$
	Untreated	8	0	0	0	
Post-intervention	ZeroFly	62	0	0	0	$\chi^2 = 10.4$, $p = 0.001$
(August 2006–July 2007)	Untreated	85	13	3	10	

stephensi, the major indoor resting malaria vectors in temporary labour settlements. In the present study, drastic reduction was observed in the indoor resting density of vector mosquitoes by using ZeroFly plastic sheetings as an alternative to untreated sheetings in the temporary shelters. The ZeroFly plastic sheets were found to be highly effective against malaria vector *An. culicifacies* and *An. stephensi* as an exposure period of 3 min in cone bioassays resulted in 100% mortality. The efficacy of ZeroFly sheeting persisted up to one year of use in the field. In addition, there was also a sharp reduction in the density of other mosquitoes and also other insect pests in the ZeroFly camps as compared to untreated plastic sheet (control) labour camps. A significant reduction in the feeding success rate of all the mosquitoes was also observed in ZeroFly camps as compared to control camps. These are in conformity with earlier workers who have reported high efficacy of insecticide-treated plastic sheetings, tarpaulins and tents[3-10, 14-16]. Results of the study may provide long-lasting solution to outstanding problems as an additional tool. These insecticide-treated plastic sheetings are of much use in displaced populations, temporary labour settlements, refugee camps, military personnel camping in forested and remote areas etc.

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Correspondence to: Dr P.K. Mittal, Scientist 'E', National Institute of Malaria Research (ICMR), Sector 8, Dwarka, New Delhi–110 077, India.
E-mail: pk_mittal52@yahoo.co.in

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