

Bio-efficacy and operational feasibility of alphacypermethrin (Fendona) impregnated mosquito nets to control rural malaria in northern India

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Bio-efficacy and operational feasibility of alphacypermethrin treated nets was evaluated in certain villages of District Ghaziabad (U.P.). Results revealed that poly-filament nylon nets treated with alphacypermethrin suspension concentrate (g/l) formulation @ 25 mg/m² has shown repellent action (26.5 ± 8.1), excito repellent action (93.7 ± 8.1) and killing action (100%) against *An. culicifacies* landed on treated nets. Significant reduction in indoor resting density of *An. culicifacies* in human dwellings was also observed in treated nets village ($p < 0.05$). Instant killing action of treated nets did provide complete protection to inhabitants sleeping inside the net from *An. culicifacies* bites. The persistent use of nets by the inhabitants has also resulted significant reduction in malaria cases ($p < 0.05$). It was interesting to note that not even a single case of *falciparum* malaria was observed after distribution of treated nets in spite of the fact that at no point of time cent per cent compliance of net usage was observed during the study period. Bio-assay tests revealed that treated nets can produce up to 70% mortality in *An. culicifacies* for about 22 weeks and as such only one treatment with insecticide is required in a year in seasonal transmission area to protect from malaria. The study also revealed that treated nets can be stored at room temperature for about 10 months without losing their efficacy suggesting thereby that malaria outbreaks can be tackled by the nets if adequate treated nets are stored in core problem districts. It was also revealed that *An. culicifacies* is a late night biter and as such treated nets can be used successfully against this species.

Key words Alphacypermethrin – *An. culicifacies* – *Cx. quinquefasciatus* – insecticide-treated nets – malaria control – social acceptability

Malaria is a serious public health problem in southeast Asian countries including India. After the implementation of Modified Plan of Operation (MPO) in 1976 in India, malaria incidence came down to 2–3 million from 7.5 million recorded cases¹. However, malaria continued to be endemic in certain pockets particularly in tribal districts. The emergence and wide-spread drug-resistance in malaria parasites in India, necessitated to use cost-effective and reliable alternate method for the vec-

tor control. Insecticide-treated mosquito nets have emerged as a potential low cost vector control tools, which reduce the man-vector contact thereby preventing transmission and reducing morbidity and mortality due to malaria. Most effective measure of vector control is the killing of adult mosquitoes with a residual insecticide applied to bed nets or sprayed on house walls and ceilings². During 1997 the National Anti Malaria Programme (NAMP) intensified the efforts to control malaria under the Enhanced Malaria Control Programme (EMCP) launched in 100 *falciparum* pre-

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dominant and tribal districts of India³. The EMCP project advocated the use of insecticide-treated mosquito nets (ITMNs) in rural areas involving the communities to reduce the reliance on chemically hazardous indoor residual spraying (IRS) carried out in blanket manner. Earlier studies carried out in India relates to evaluation of pyrethroid treated nets (deltamethrin or cyfluthrin or lambdacyhalothrin) in different ecological and malaria paradigms^{4–9}. Present evaluation relates to alphacypermethrin (Fendona)-treated nets against *An. culicifacies* and its impact on malaria incidence carried out from 1999 to 2001 is reported in this paper.

Material and Methods

Study sites

Field trials were carried out during 1999–2001 in Dasana and Dhaulana Primary Health Centres (PHCs) in District Ghaziabad (Uttar Pradesh) located in command area of the upper Ganga canal ~ 45 km southeast of Delhi in all three villages— Jadhonpur, Siddhipur (Dhaulana PHC), and Mubarakpur (Dasana PHC). The villages were selected for the present study due to high prevalence of malaria and man-hour densities of *An. culicifacies*. Insecticide-treated nets (ITNs) were used in Jadhonpur (population 802) while plain nets were used in Siddhipur village (population 975) and Mubarakpur village (Population 510) was taken as control where nets were not used. The man to cattle ratio in these villages was ranging between 1:1.8 and 1:1.20. All the three villages were comparable in respect of occupation, sociocultural status, mosquito-genic conditions and endemicity of the disease. *An. culicifacies* is the principal vector of malaria and breeds in irrigation channels, pools, ponds and wells.

Treatment procedure

Nets were treated as per the procedure earlier described elsewhere⁹. The average quantity of water sufficient to soak the whole net was calculated by using several replicates and found to be 380 ml. For the

treatment of a net 2.5 ml of 10% alphacypermethrin was mixed in 377.5 ml of water in plastic tub and nylon net was immersed in the solution. Hand gloves were used to avoid dermal exposure. When the net absorbed the whole solution uniformly, including cotton hem, it was taken out from the tub, spread on a plastic sheet and allowed to dry under shade. After complete drying, mosquito nets were packed in polythene sheets displaying name of insecticide, dosage, date of impregnation, etc. and stored in the laboratory under controlled conditions. Required number of treated nets (666) were distributed to each house in Jadhonpur village during 3–5 August 1999. During the same period plain nets (732) were also distributed in Siddhipur village. Children aged above 10 years were given a separate net, while one net was shared by two aged below 10 years. A separate record was maintained for distribution and redistribution of nets to inhabitants of both the villages.

Entomological evaluation

Resting density of mosquitoes was monitored fortnightly in experimental and control villages with the help of suction tube and flashlight for 15 min in 8 huts and 8 cattlesheds. Collected specimens were identified in the laboratory and the man-hour density (MHD) was calculated. Samples of adult females were collected from the experimental and control areas and dissected for gut and gland infections.

Epidemiological evaluation

Epidemiological evaluation was carried out by door-to-door fortnightly surveillance and blood smears of all the fever cases were prepared on glass micro slides and examined microscopically within 2–3 days. Presumptive treatment was given to all fever cases while radical treatment was given to only positive cases as per NAMP drug schedule in all the study villages. The blood examination rate (BER), slide positivity rate (SPR), slide falciparum rate (SfR), cases/000 and *Pf*/000 population were calculated using standard procedures.

Laboratory bio-assays

Bio-assay tests were performed on 3–5 days old blood fed female mosquitoes following WHO standard bio-assay procedures. The treated nets were fixed on cardboard with the help of drawing pins. Plastic cones were fixed with adhesive tape on the treated net. Fifteen female mosquitoes were released in each cone with the help of suction tube and exposed for 15 min. Later they were kept in holding tubes, which were kept vertically and a cotton wool swab soaked with 10% glucose solution was plugged in the opening. In the next 24 h, a vigil was kept and the mortality of the mosquitoes, if any, was recorded as a post-exposure sequel. All the tests were replicated 10 times and carried out at $25 \pm 2^\circ\text{C}$ and $70 \pm 10\%$ relative humidity. Tests were repeated when the control mortality was 20% or more. Corrected mortality was calculated by Abbott's formula¹⁰.

Laboratory bio-assay experiments were performed at monthly intervals to assess the effect of storage of

nets after treatment with the insecticide. The experiments were carried out continuously for about 24 months. Field bio-assay tests were performed fortnightly at the onset of first and second treatments. Action of treated nets was studied in the field. The data of all collections were pooled together and repellent action, excito-repellency action and killing action were calculated using the procedure and formulae described elsewhere⁹. In addition to this, biting rhythm of *An. culicifacies* and *Cx. quinquefasciatus* was also recorded on human volunteers for about two years to evaluate the suitability of using treated nets against these species of mosquitoes.

Results

Distribution pattern and social acceptability

Distribution, usage pattern and social acceptability of nets are depicted in Table 1. It was revealed that intensive health education is required for inhabitants to

Table 1. Social acceptance and usage pattern in different seasons in the selected villages

Month/Year	Jadhonpur (Treated)			Siddhipur (Untreated)		
	Total using net	No. lost	Total wear & tear	Total using net	No. lost	Total wear & tear
Aug 1999	649 (97.4) (666)*	1 (0.15)	0 (0)	695 (94.9) (732)*	0 (0)	0 (0)
Sep	605 (90.8)	3 (0.45)	3 (0.45)	652 (89.0)	4 (0.54)	4 (0.54)
Oct	600 (90.0)	5 (0.75)	5 (0.75)	615 (84.0)	7 (0.95)	8 (1.0)
Nov	600 (90.0)	8 (0.83)	10 (1.5)	601 (82.1)	11 (1.5)	13 (1.7)
Dec	486 (72.9)	12 (1.8)	15 (2.2)	476 (65.0)	14 (1.9)	16 (2.1)
Jan 2000	350 (52.5)	15 (2.2)	20 (3.0)	293 (40.0)	17 (2.3)	22 (3.0)
Feb	380 (57.0)	18 (2.7)	27 (4.0)	396 (54.0)	21 (2.8)	28 (3.8)
Mar	450 (67.5)	22 (3.3)	33 (4.9)	469 (64.0)	25 (3.4)	36 (4.9)
Apr	531 (79.7)	24 (3.6)	37 (5.5)	557 (76.0)	28 (3.8)	41 (5.6)
May	570 (85.5)	27 (4.0)	41 (6.1)	586 (80.0)	32 (4.3)	46 (6.2)
Jun	265 (39.7)	29 (4.3)	46 (6.9)	234 (31.9)	35 (4.7)	53 (7.2)
Jul	608 (91.2)	32 (4.8)	51 (7.6)	652 (89.0)	39 (5.3)	58 (7.9)
Aug	649 (97.4) (786)*	34 (5.1)	55 (8.2)	689 (94.1) (892)*	44 (6.0)	64 (8.7)
Sep	653 (98.0)	37 (5.5)	60 (9.0)	681 (93.0)	48 (6.5)	71 (9.6)
Oct	653 (98.0)	41 (6.1)	67 (10.0)	695 (81.2)	54 (7.3)	83 (11.3)
Nov	535 (80.3)	44 (6.6)	73 (10.9)	557 (76.0)	59 (8.0)	91 (12.4)
Dec	466 (69.9)	45 (6.7)	77 (11.5)	454 (62.0)	62 (8.4)	98 (13.3)

Nets were treated with alphacypermethrin @ 25 mg/m² on 2 August 1999 and 24 July 2000; *No. of nets distributed to the inhabitants (One net per person above 10 years age was distributed in each village); Figures in parentheses indicate percentage.

use and store the net at a safe place. This statement may be substantiated by the fact that nets were lost either due to theft or torn-out by the inhabitants due to rough use and improper storage. About 120 nets (treated) and 160 (untreated) were supplemented in Jadhonpur and Siddhipur villages respectively. Though the majority of the inhabitants realised the usefulness of the net but there was a small proportion, particularly drunken people, who defied the use under toxication. The use of net was also directly proportional to seasons. During the transmission period—August to October 1999 the percentage of users varied from 90 to 97.4 and the protection from mosquito bite and malaria has reinforced the faith in using treated nets during the corresponding period of the year 2000. Per cent users were ranging from 97.4 to 98 during August to October 2000. The use of nets was gradually reduced during winter season probably due to non-transmission of malaria and low density of mosquitoes. Similar trend was

observed in the plain net area though the overall percentage of users in comparison to the treated net area was slightly low—71.2% as against 76.6%. Similarly, the percentage loss varied from 8.5 to 10.1% in treated and untreated nets supplied villages. The natural wear and tear was 14.2% in treated net and 16.2% in untreated net supplied villages.

Impact of alphacypermethrin treated nets on target and non-target species of mosquitoes

Cumulative pre- and post-treatment: Man hour density (MHD) of *An. culicifacies* and other mosquitoes in different villages in both human dwellings and cattlesheds are shown in Table 2. It appears from the table that the density of mosquitoes was more or less the same in all the three villages up to July 1999, but use of treated nets in Jadhonpur village significantly reduced the density of mosquitoes particularly *An. cu-*

Table 2. Man hour density of resting mosquitoes in selected villages of Distt. Ghaziabad (U.P.)

Month and year	Total anophelines			<i>An. culicifacies</i>			<i>Cx. quinquefasciatus</i>		
	Treated	Untreated	Without net	Treated	Untreated	Without net	Treated	Untreated	Without net
Pre-treatment densities									
Jan–Mar 1999	21.6±1.2 (29.2±1.3)	17.6±0.8 (32.6±2)	21.3±1 (31.6±1.2)	15.3±0.3 (24.5±1)	10.3±1.1 (25±1.8)	11±0.6 (19.3±1)	24±1.1 (56±2.3)	22±1 (48±2)	22.3±1 (53.3±2)
Apr–Jul 1999	35±1 (66.7±1.8)	39.5±1 (56.2±1.4)	41.7±2.8 (55±2.5)	25±1.5 (48±1.5)	18.5±0.8 (41.7±0.1)	24.2±0.9 (46.7±1.8)	78±0.25 (152.5±2.5)	62.5±1.8 (124.2±3.4)	64.5±2 (129.7±3.7)
Post-treatment densities									
I impregnation Aug–Dec 1999	12±0 (187.6±2.2)	37.2±0.2 (110.8±3.2)	72±0.6 (95±0.7)	4.6±0.3 (87.6±0.2)	24±0.8 (48±1)	37±1.3 (50.2±2.3)	5.8±0.1 (113.8±0.1)	19.4±0.1 (85±0.1)	55±0.7 (77±1.3)
Jan–Jul 2000	6.2±0.6 (42.1±2.3)	5.8±0.7 (32±6)	20.8±0.5 (35.1±0.5)	3.2±0.1 (30.1±1.3)	2.7±1.2 (20.5±2)	11±0.75 (22.2±6)	17±0.5 (83.5±1.05)	17.1±2.5 (56.8±6.5)	45±0.25 (65.7±7.5)
II impregnation Aug–Dec 20001	2.7±1 (207±1.5)	22±1 (155±1.25)	104±3 (136±4)	5.2±0.2 (70.8±0)	12.6±1.75 (48.4±9.5)	37±8.2 (51±5.7)	12±0.4 (146±2.8)	24.4±1 (118±8.5)	59±0.25 (89±0.25)

Figures in parentheses indicate the MHD of mosquitoes in cattlesheds. The values are mean + S.D. of individual collections. The values are significant at $p < 0.05$ (insignificant for *Cx. quinquefasciatus* at $p > 0.05$ between untreated & without net and for total *Anopheles* between treated and untreated).

licifacies in comparison to untreated and without net villages ($p < 0.05$). It may be possible that the population deviated from the human dwelling or killing action of nets has resulted reduction in daytime resting density of vector species. The average density of anophelines from April to July 1999 was 35, which reduced to 12.7 as against 39.5 and 22 in untreated and 41.7 and 104 in without net villages respectively. It is also interesting to note that density of daytime resting anophelines in cattlesheds of treated net village was 207 as against 155 and 136 in untreated and without net villages respectively suggesting thereby that some proportion of mosquitoes deviated due to repellency action from human dwellings where treated nets were used and preferred to rest in cattlesheds (Table 2). Similar observations were also made against *An. culicifacies* and *Cx. quinquefasciatus*.

The action of alphacypermethrin treated net is shown in Table 3. The results revealed that treated net acts in three ways. Firstly it has moderate repellent action (RA). Secondly it has demonstrated very strong excito repellency action (ERA). In addition, it has also produced killing action (KA) of those females who have adequate exposure to treated nets. The repellent action was 19.5 ± 1.7 as against 97.6 ± 1.5 excito repellency action for total mosquitoes. The killing action was about 39.6 and 95.4% for females collected from inside treated net house and females collected from the surface of treated nets respectively.

Epidemiological impact of alphacypermethrin treated mosquito nets

Malaria prevalence in treated, untreated and without net villages is shown in Table 4 along with standard epidemiological indicators. Results revealed that alphacypermethrin treated nets have drastically reduced malaria related morbidity and prevented the mortality. The slide positivity rate (SPR) was 29.1, 18.7 and 23.3 in treated, untreated and without net villages respectively before July 1999. The use of treated nets in Jadhonpur village resulted drastic reduction in SPR in successive months and the trend continued after the

second impregnation in the month of May 2000. This was also reflected when slide falciparum rate (SfR), cases/000 and *Pf*/000 were compared. The use of plain nets in Siddhipur village has also resulted marginal reduction in all indicators in comparison to Mubarakpur village where nets were not distributed. Cases/000 and *Pf*/000 were 19.4 and 9.2 respectively in Siddhipur village after second impregnation as against 60.7 and 33.3 respectively in Mubarakpur village. Cases/000 and *Pf*/000 in treated net village (Jadhonpur) were 2.4 and 0 respectively in corresponding period suggesting thereby that alphacypermethrin treated nets were quite successful in preventing malaria related morbidity and mortality. Solitary cases of malaria in treated net village may be either due to relapse or visits to adjoining endemic villages. It may also be possible that some of them did not use the net properly or regularly.

Bio-assay test results are shown in Fig. 1. It was revealed that alphacypermethrin treated nets produced 100% mortality of *An. culicifacies* for about 10 weeks in the field followed by gradual decline in successive weeks. Nevertheless persistence at 70% level was observed up to 22 weeks suggesting thereby that treated nets require two treatments at an interval of six months in a year in areas of perennial transmission. In case of *Cx. quinquefasciatus* the persistence was marginally reduced and 70% level of mortality was observed only up to eight weeks.

Impact of storage on alphacypermethrin treated mosquito nets

Fig. 2 shows the per cent corrected mortality of adult mosquitoes obtained at periodic exposure to discriminating dose of alphacypermethrin. It is very clear from the figure that if the nets are properly treated, packed and stored at room temperature, will continue to produce more than 70% mortality up to 10 months both against *An. culicifacies* and *Cx. quinquefasciatus*. Thereafter, the efficacy is gradually diluted in successive weeks suggesting thereby that nets could be treated well in advance, stored and distributed at the

Table 3. Action of alphacypermethrin treated nets on mosquitoes in Jadhonpur village of Distt. Ghaziabad (U.P.)

Species	RA		ERA		KA	
	SPR	SfR	SPR	SfR	E (%)	L (%)
<i>An. culicifacies</i>	26.5 ± 8.1	(47)	93.7 ± 8.1	(3)	31.9 ± 4.1	100 ± 0
<i>Culex</i> spp.	23.1 ± 3.5	(47)	97.3 ± 1.7	(11)	50.4 ± 3.4	90.9 ± 1.2
Total mosquitoes	19.5 ± 1.7	(895)	97.6 ± 1.5	(22)	39.6 ± 2.4	95.4 ± 1.1

Average of 24 replicates for 1999 & 2000; Figures in parentheses indicate total no. of mosquitoes collected in eight night collections.

Table 4. Incidence of malaria in selected villages of Ghaziabad distt. (U.P.)

Month & Year	Treated				Untreated				Without net			
	SPR	SfR	Cases/000	Pf/000	SPR	SfR	Cases/000	Pf/000	SPR	SfR	Cases/000	Pf/000
Jan-Mar 1999	20 ± 0	10 ± 0	4.9 ± 0	2.4 ± 0	13.3 ± 3.4	6.6 ± 0	6.1 ± 0.55	3 ± 0	14.2 ± 3.6	7.1 ± 1.2	11.7 ± 0	3.9 ± 0
Apr-Jul 1999	29.1 ± 0.75	0	8.7 ± 0.6	0	18.7 ± 3.3	0	6.1 ± 1	0	23.3 ± 3.3	0	13.7 ± 0.98	0
	Introduction of nets (first treatment)											
I impregnation	Before introduction of net											
Aug-Dec 1999	0	0	0	0	13.3 ± 0.4	3.3 ± 1.8	8.2 ± 0	2 ± 0	38.8 ± 1	19.4 ± 6.2	27.4 ± 1.9	13.7 ± 0.98
Jan-Jul 2000	3.9 ± 0	0	2.4	0	9.8 ± 1.3	0	7.1 ± 0.55	0	15.5 ± 1.7	3.4 ± 0	17.6 ± 0.98	3.9 ± 0
	Post-introduction of nets (second treatment)											
II impregnation	Before introduction of net											
Aug-Dec 2000	1.7 ± 0	0	2.4	0	16.5 ± 0.75	7.8 ± 2.5	19.4 ± 0.5	9.2 ± 1.5	24 ± 2.5	13.1 ± 3.1	60.7 ± 4.9	33.3 ± 4.9

Values are significant at $p < 0.05$; Treatment date 2 August 1999 and 24 July 2000.

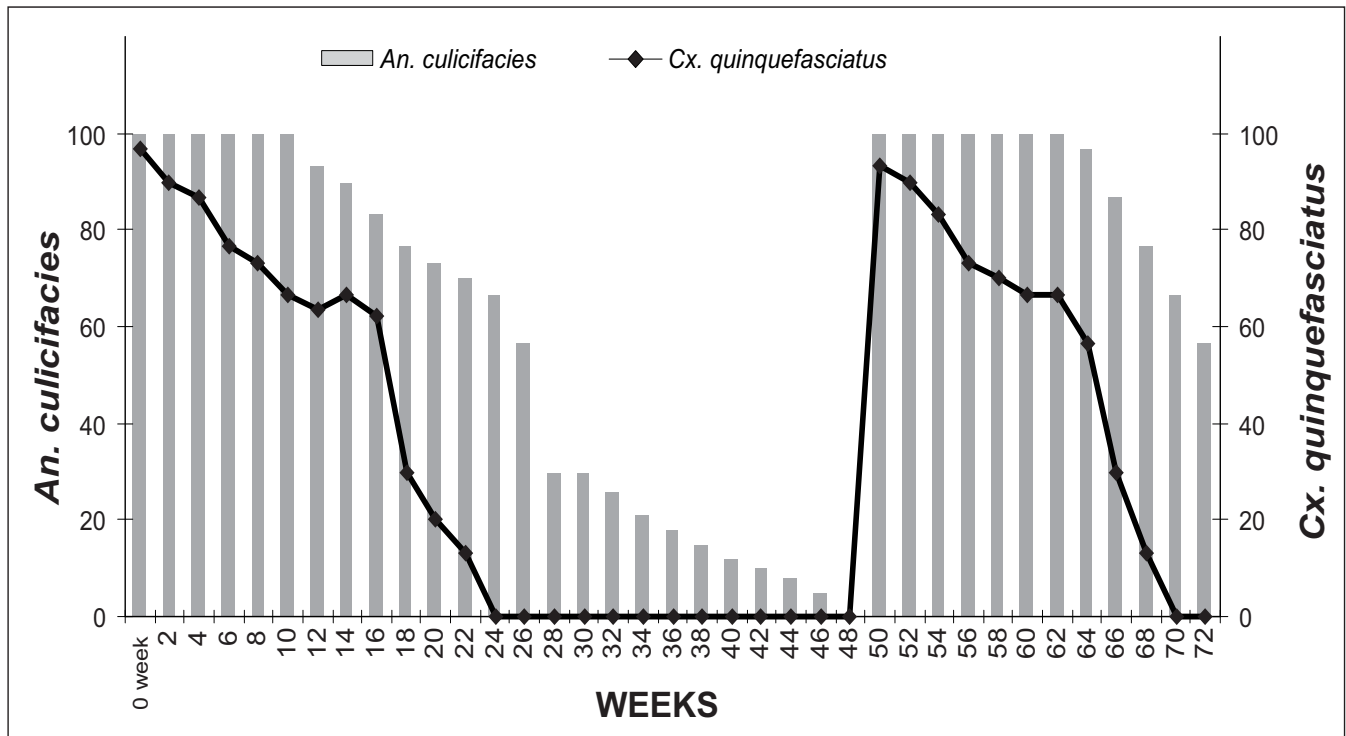


Fig. 1: Bio-assay test on alphacypermethrin treated net @ 25 mg/m² in village Jadhonpur Distt. Ghaziabad (U.P.)

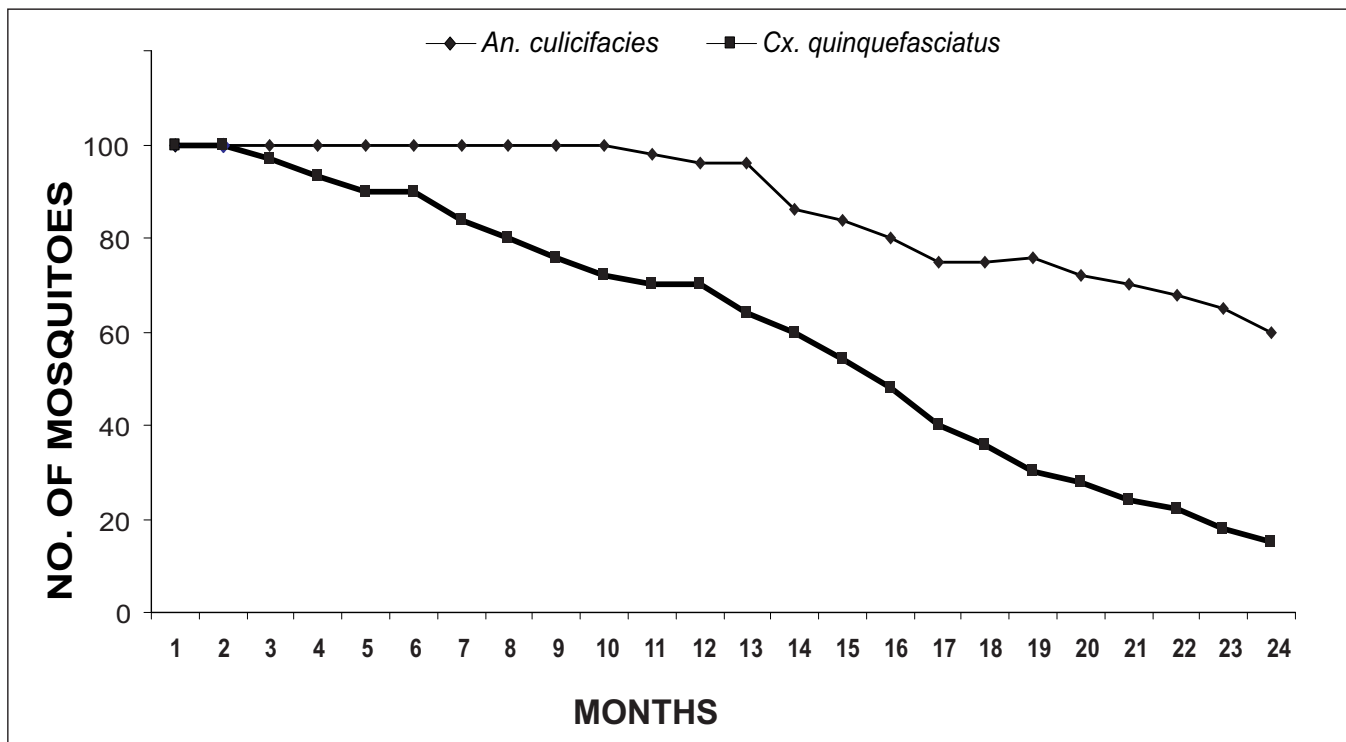


Fig. 2: Impact of periodic storage of alphacypermethrin treated nets of mosquitoes under laboratory conditions (1999–2000)

time of outbreak or in potential high risk areas to prevent epidemics.

Biting rhythm of vector species

The success of insecticide treated nets depends upon the biting rhythm of prevalent vector species and social behaviour of the community. A study was, therefore, carried out to observe biting behaviour of *An. culicifacies*. Results of six-night collections in each month are depicted in Fig. 3. It is clear from the figure that the *An. culicifacies* starts feeding on human bait after 2100 hrs and the feeding activity continued till dawn 0600 hrs. The peak biting activity was recorded between 0400 and 0600 hrs in the early morning. The biting behaviour fluctuates in each year and seasons depending upon climatic factors. The feeding does not take place in extreme winter (December to February), moderate in autumn and spring months (March and November) and highest in monsoon and post-monsoon seasons (July to October). In

view of this, insecticide-treated nets can be successfully used against this species. Contrary to this, *Cx. quinquefasciatus* starts feeding on the onset of dusk and the biting continues till dawn, however, the peak biting activity was observed in between 2100 and 0200 hrs like *An. culicifacies* biting activity vary in each month, year and seasons.

Discussion

The results of the present study revealed that inhabitants socially accepted alphacypermethrin-treated nets and compliance rate was more than satisfactory particularly during the transmission period. Treated nets were quite effective in reducing the indoor resting density of *An. culicifacies* in the study area. The reduction in vector density of *An. minimus* and *An. culicifacies* was also observed by earlier workers using lambdacyhalothrin treated nets in Assam⁴ and Orissa states⁷ and against *An. fluviatilis* by cyfluthrin treated nets¹¹ and against *An. farauti*¹² and *An. gambiae*¹³

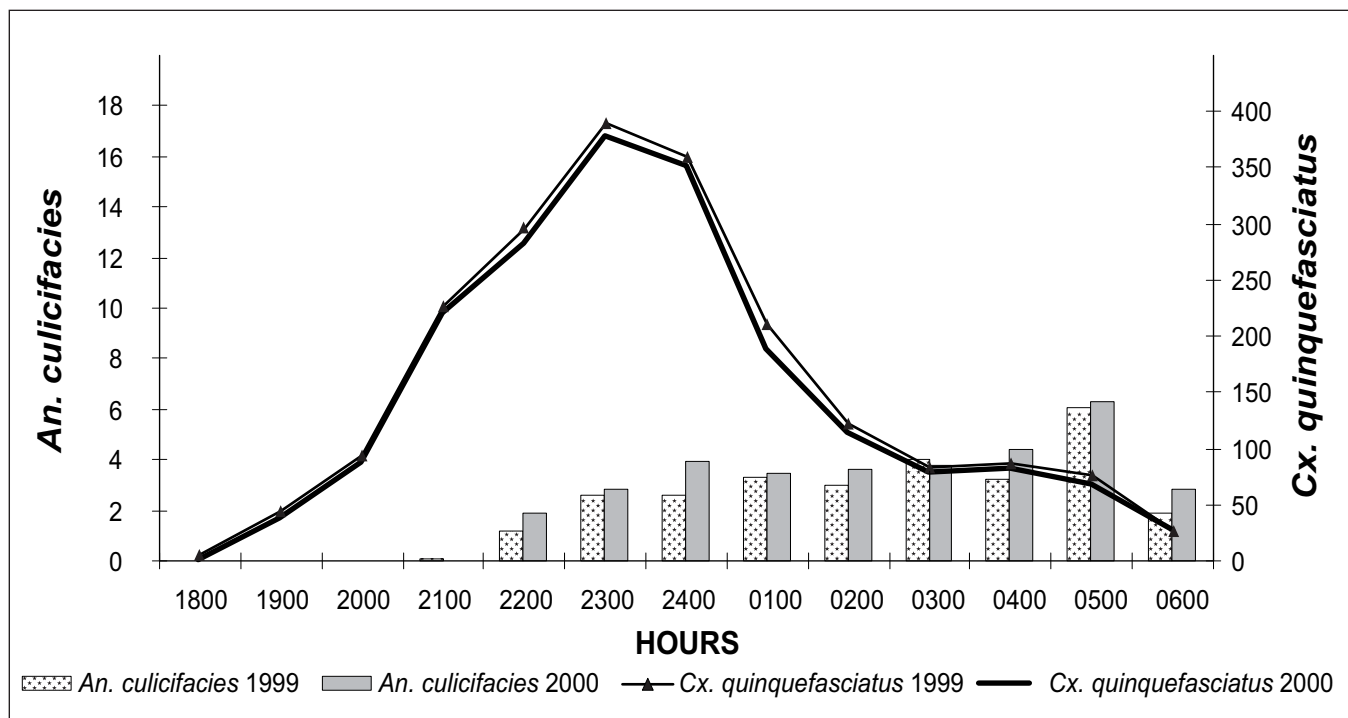


Fig. 3: Biting rhythm of *An. culicifacies* and *Cx. quinquefasciatus* under field conditions in selected villages of Distt. Ghaziabad (U.P.)

by introduction of permethrin treated nets. A significant reduction in indoor densities of *An. culicifacies* and other mosquitoes in the village where treated nets were used can be attributed to the killing action of treated nets¹⁴ or both killing and repellent action as reported earlier⁹. In the present study deviation in resting behaviour of mosquitoes was also observed as the density greatly reduced in human dwellings in treated net village and substantially increased in cattle sheds after the introduction of treated nets which can be due to excito repellent action of the insecticide. It may be appropriate to point out that no such diversion was observed in a village where plain nets were distributed. The results also revealed that *An. culicifacies* starts its feeding after 2100 hrs which coincides with sleeping time of inhabitants and peak biting activity of this species was observed in between 0400 and 0600 hrs, therefore, the treated nets can be effectively used in this epidemic prone areas. Similar observations were also made by earlier workers who reported that 70–80% feeding of *An. culicifacies* takes place in between 2100 and 0500 hrs in south¹⁵, west¹⁶ and central¹⁷ India. The study also revealed that alphacypermethrin persists on net for about six months and is quite effective against mosquitoes particularly against *An. culicifacies*, therefore, single treatment on the onset of transmission season (July to November) is required for effective control of malaria in northern India. However, in areas where more than two vectors are involved in the malaria transmission, malaria is transmitted throughout the year due to favourable climatic conditions treatment can be done at an interval of six months. The study also showed that alphacypermethrin treated nets can be stored at normal temperature for about one year without diluting its efficacy. In view of this, mass treatment of nets can be done well in advance of transmission season and can be distributed in high risk epidemic prone areas to tackle malaria outbreaks.

The introduction of alphacypermethrin treated nets also produced desirable impact on malaria incidence. The substantial reduction in cases/000 and Pf/000 clearly indicate that treated nets were highly effective

in preventing mosquito bites. Similar efficacy of nets has earlier been demonstrated in India and elsewhere by use of synthetic pyrethroid treated nets^{18–23}. In plain net used village marginal reduction of malaria incidence was observed due to wide-spread use of nets, which provided physical barrier to user but no such reduction was observed in a village where nets were not distributed. The use of insecticide-treated nets is more appropriate compared to indoor residual spraying in terms of safety to inhabitants as mass use of treated nets on sustained basis at recommended dosage does not pose any hazard either to those treating or using nets²⁴.

In view of this it is safe to conclude that use of alphacypermethrin treated nets was found to be operationally feasible, socially acceptable and produced desired impact both on vector density and malaria incidence in study villages. Based on the present findings and global recommendations the use of synthetic pyrethroid nets could be promoted in multi-resistant areas of *An. culicifacies* involving communities with appropriate partners for effective malaria control.

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