Evaluation of bifenthrin treated mosquito nets against anopheline & culicine mosquitoes

Article *in* The Indian Journal of Medical Research · February 2005

Source: PubMed

9 authors, including:

Chandra prakash Batra
National Institute of Malaria Research
34 PUBLICATIONS **398** CITATIONS
SEE PROFILE

Raghavendra Kamaraju
Indian Council of Medical Research
160 PUBLICATIONS **2,349** CITATIONS
SEE PROFILE

Tripti Adak
University of Burdwan
119 PUBLICATIONS **1,901** CITATIONS
SEE PROFILE

Om P Singh
National Institute of Malaria Research
209 PUBLICATIONS **1,402** CITATIONS
SEE PROFILE

Indian J Med Res 121, January 2005, pp 55-62

Evaluation of bifenthrin treated mosquito nets against anopheline & culicine mosquitoes

C.P. Batra, K. Raghavendra, T. Adak, O.P. Singh, S.P. Singh, P.K. Mittal, M.S. Malhotra
R.S. Sharma* & S.K. Subbarao

*Malaria Research Centre & *National Anti Malaria Programme, Delhi, India*

Received July 21, 2003

***Background & objectives*: The main rural malaria vector *Anopheles culicifacies* has developed resistance to dichloro diphenyl trichloroethane (DDT), hexachloro cyclo hexane (HCH) and malathion in the state of Haryana in northern India. An alternative synthetic pyrethroid insecticide bifenthrin was therefore evaluated on mosquito nets against anopheline and culicine mosquitoes, in two villages Jagdishpur and Garh Mirakpur of Community Health Center (CHC) Badhkhalsa in district Sonipat, Haryana state.**

***Methods*: Two formulations of bifenthrin, suspension concentrate (SC) and micro-emulsion (ME) were compared with micro-capsule suspension (CS) of lambdacyhalothrin. The impact of three doses of bifenthrin (10, 25 and 50 mg/m^2) impregnated on mosquito nets was compared with lambdacyhalothrin (25 mg/m^2) and untreated control. Quality assessment of treatment on treated nets was carried out by residue analysis and the persistence of the insecticide on nets was determined by contact bioassays. Efficacy of treated nets on mosquito density was assessed by calculating mosquito entry rate, immediate mortality, delayed mortality and excito-repellency to the insecticides.**

***Results*: In susceptibility tests *An. culicifacies* was susceptible to bifenthrin (0.1% test papers) and to lambdacyhalothrin (0.05% test papers). Bioassays on treated nets against *A. culicifacies* recorded 100 per cent mortality up to tenth fortnight for all the doses of impregnation with bifenthrin (SC and ME) and lambdacyhalothrin (CS). Ring-net bioassays against *An. culicifacies* showed median knock-down time between 3.1 to 11.4 min. Behavioural indices were also studied for anopheline and culicine mosquitoes. The reduction in entry rates of anopheline and culicine mosquitoes into the rooms with treated nets compared to control indicated good efficacy with all the formulations and doses of the insecticides.**

***Interpretaion & conclusion*: Indoor (immediate) mortality of mosquitoes with bifenthrin ME formulation was relatively lower compared to SC fomulation of bifenthrin and based on delayed mortility and continued susceptibility in bioassays, bifenthrin ME at the rate of 10mg/m^2 dose was found suitable for the impregnation of mosquito nets for phase III trial.**

Key words .*Anopheles culicifacies* - bifenthrin - bioassays - efficacy - insecticide treated mosquito

In India, the current annual incidence of malaria is about 2-3 million cases as per National Anti Malaria Programme (NAMP) of Government of India[1]. Indoor residual spraying with insecticides dichloro diphenyl trichloroethane (DDT), hexachloro cyclo hexane (HCH) has been the main vector control strategy in rural areas since 1958 under the National Malaria Eradication Programme (NMEP)[2]. Malathion spray was introduced in 1969 to control double resistant *Anopheles culicifacies*[3]. HCH has been phased out of public health in 1997. Continuous usage of these insecticides has resulted in the development of resistance in *A. culicifacies* and the vector has become triple resistant *i.e.*, to DDT, HCH and malathion in many areas[4]. Indoor residual sprays with prethroids have shown encouraging results[5-7] and these insecticides are being used in public health sprays in some parts of the country.

Insecticide treated mosquito nets (ITMN) with pyrethroid were reported to be effective for control of malaria in several countries[8]. In India, trials with ITMN were carried out for the first time in mid 1980s at several locations. Deltamethrin treated nets were found effective against *A. minimus*[9], deltamethrin and lambdacyhalothrin against *A. culicifacies*[10] and cyfluthrin against *A. fluviatilis*[11]. Following these and other successful field trials, insecticide impregnated mosquito nets have been introduced under the National Anti Malaria Programme (NAMP; now known as National Vector Borne Diseases Control Programme) in the 1990s.

The state of Haryana is endemic for malaria and both *Plasmodium vivax* and *P. falciparum* malaria parasites are prevalent[12]. The major vector of malaria in this area was *A. culicifacies,* and was incriminated for sporozoites[13]. This species was found to be primarily endophagic and endophillic and comprises of two sibling species, A and B, with the predominance of the former[14]. Studies on insecticide susceptibility of *A. culicifacies s.l.* in Haryana showed that it was susceptible to deltamethrin, fenitrothion and propoxur, but was resistant to DDT, dieldrin and malathion[15]. A field trail was therefore carried out and a new pyrethroid insecticide, bifenthrin was evaluated on mosquito nets in villages of Community Health Center (CHC) Badhkhalsa, district Sonepat, Haryana. The main objectives of this trial were to assess the relative efficacy of mosquito nets impregnated with two different formulations of bifenthrin, suspension concentrate (SC) and micro-emulsion (ME) to vis-a-vis micro-capsule suspension (CS) formulation of lambdacyhalothrin and plain net (control).

Material & Methods

Baseline susceptibility for bifenthrin and lambdacyhalothrin was determined on laboratory reared 3 day old glucose fed-F1 female progeny of field collected *A. culicifacies*. Mosquitoes were exposed for 1 h with bifenthrin 0.1 per cent as per supply received from WHOPES and WHO recommended diagnostic dose of 0.05 per cent lambdacyhalothrin insecticide impregnated papers obtained from WHOPES using WHO test kit and method[8,16]. Tests were conducted in a room where the temperature was maintained at 27 ± 2^{o}C and 70-80 per cent relative humidity.

Two villages of CHC Badkhalsa, district Sonepat, Haryana, Garh Mirakpur (200 houses and population 1500) and Jagdishpur (80 houses and population 600) were selected for the field evaluation. These villages are situated 40 km north of Delhi on the banks of river Jamuna. The walls in these villages are plastered with cement or mud and most of the houses have thatched roofs. The climate in the area was divided into three seasons summer (April-June), monsoon (July-October) and winter (November-March). During extreme conditions in winter the temperatures fall to as low as 2-3^{o}C and in summer the temperature rises to as high as 46^{o}C. In view of high mosquitogenic conditions these villages were selected.

Efficacy studies of the individual formulation were carried out against the main malaria vector, *A. culicifacies*, other anopheline and culicine mosquitoes.

During the study period (August 1999 to March 2000) no other vector control measure was employed in the two study villages. The study protocol was approved by the ethics committee of the institution.

A total of 40 houses were selected in the two villages and distributed equally among 5 research arms. Each research arm consists of eight houses with different formulations and doses *viz*., bifenthrin SC (BSC) and ME formulation (BME) at the rate of 10, 25 and 50 mg/m^2; lambdacyhalothrin CS (L) at the rate of 25 mg/m^2 and a control house with plain net. Consent was taken from the families of the selected houses for inclusion into the trial. All the selected houses were numbered and coded. Thus the evaluation of the insecticide bifenthrin [2-methylbiphenyl-3-y1methyl (Z)-(IRS)-cis-3 (2-chloro-3, 3,3-trifluroprop-l-enyl)-2,2-dimethyl cyclopropane carboxylate] was designed to carry out in five replicate houses per dose against formulation of a given control. In each study house two rectangular exit traps of size 12"x15" having plastic cone with an orifice of 1 cm^2 were fixed preferably facing east.

Nets of polyester polyfilament fibre with 100-denier strength, 10 m^2 area, 156-mesh size (12x13 holes/$inch^2$) white in colour were used. A total of 148 nets, which included both, treated and untreated nets were distributed based on requirement.

Mosquito nets were treated individually following the dipping method[17]. Before distribution of nets, inhabitants were educated about the proper use of nets. All the nets were marked with a water-soluble ink to detect washing. Fortnightly, the supervisory staff randomly checked 10 households at night to ensure compliance of mosquito net use. To assess quality of treatment 10 cm x 10 cm pieces from the treated nets were cut after 2 days of impregnation. Eight pieces from minimum two treated nets were sampled randomly of each dose of the two insecticides. Samples were packed individually and coded. A total of 112 samples (2x56 pieces) were sent for residue analysis to WHO Pesticide Evaluation Scheme (WHOPES) reference laboratory at Department of Phytopharmacie, Gembloux, Belgium. Residues were estimated using capillary gas chromatography with 63 Ni electron capture detection using external standard calibration.

Persistence was determined by contact bioassays using cone and ring-net bioassays in the field conditions following the method of WHO[8] on day one and thereafter at fortnightly intervals. Exposures were made on distributed nets in use by the inhabitants. Laboratory reared, 3 day old glucose fed female *A. culicifacies* species A, were used for the assays. The mosquitoes after exposure were placed in plastic bowls and were provided cotton soaked in 10 per cent glucose solution in water while holding in field laboratory. Mortality at the end of 24 h holding was noted.

Assessment of impact was made every fortnight in the morning (0530 to 0800) by floor sheet collection, exit trap collection, hand catch and pyrethrum space spray collection of mosquitoes, in sequence as per the WHO procedures[16].

Entry rate was determined by pooling all the collected mosquitoes in each of the structure by different methods and expressed as total entry. Immediate mortality was determined as the per cent proportion of mosquitoes found dead on floor sheets and in exit traps in a house out of the total entered during the night. Delayed mortality was determined by keeping the live mosquitoes collected from exit traps and hand collections for 24 h and per cent mortality was calculated from total mosquitoes collected live by hand catch and in exit traps. To assess the extent of excito-repellency effect, exit to entry ratio was determined. The results were compared using Chi square test and one way ANOVA to assess the efficacy of the given formulation of insecticide.

Results

Baseline data on insecticide susceptibility of field collected *A.culicifacies* (F1 progeny) showed 100 per cent mortality with 0.1 per cent bifenthrin (n=173) and 0.05 per cent lambdacyhalothrin (n=85) impregnated papers. Mortality with 4 per cent DDT (n=40) impregnated test papers was 17.5 per cent. Thus, the baseline susceptibility tests showed that the *A.culicifacies* population was fully susceptible to bifenthrin and lambdacyhalothrin but was 82.5 per cent resistant to DDT.

Residue analysis of the impregnated strips indicated that bifenthrin suspension concentrate (BSC)

formulations targeted at 10, 25 and 50 mg/m^2 were 7.56 ± 1.15, 15.61 ± 0.58 and 26.58 ± 1.06 mg/m^2 respectively. The percentage of targeted dose ranged between 51.04 and 87.1. In contact bioassays, the results of BSC formulation against *A.culicifacies* on impregnated nets showed 100 per cent mortality up to tenth fortnight. In eleventh fortnight BSC (10 mg/m^2) caused 100 per cent mortality and BSC 25 and 50 mg/m^2, resulted in 92.2 and 88.4 per cent mortality respectively. Estimated median knock down time against BSC formulation in the ring net bioassays against *A.culicifacies* was between 3.1 and 11.4 min (Fig.1).

Results of residue analysis of BME formulations when targeted at 10, 25 and 50 mg/m^2 were 6.02 ± 0.54, 10.74 ± 0.49 and 18.79 ± 1.45 mg/m^2 respecetively. The percentage of targeted does was ranging between 34.68 and 65.6. For LCS, result of residue analysis at a target dose of 25 mg/m^2 was 9.42 ± 1.05 mg/m^2 with a range of 33.48-41.88. The results of BME in contact bioassays and ring net showed similar trend as in BSC formulation.

During field experiment in 13 fortnights the total anopheline and culicine mosquitoes entered in experimental houses were 2818. *A.culicifacies* proportion was 1.03 per cent and the prevalence of other mosquito species was; 0.11 per cent *A.stephensi*, 17.46 per cent *A.subpictus* and 81.41 per cent *Culex quinquefaciatus*. Total entry rate (average number of all mosquitoes/st ructure) of mosquitoes by all the mosquito collection methods was mean mosquito density 13.11 in untreated control. In treated houses with BSC formulation it ranged between 3.8 and 5.94. Monthwise entry rate was maximum in September and minimum in February (Table). Excito-repellency rate (proportion of mosquitoes collected in exit trap to total entered) for total mosquitoes with BSC formulation ranged between 13.47 and 20.0 compared to control rate of 2.58 (Table).

The observed immediate mortalities (proportion of mosquitoes dead to total entered in a night) for total mosquitoes in house with BSC formulation ranged between 10 to 21.5 compared to 0.59 in control (Fig.2). Immediate mortality was highest in first month up to 50.98 at does of BSC 10 mg/m^2 and declined to zero after 3 months period (Table). The total delayed mortality of mosquitoes ranged between 45.15 to 56.69 and month wise delayed mortality was above 80 per cent up to two months at all the doses of BSC formulation (Fig.2,Table).

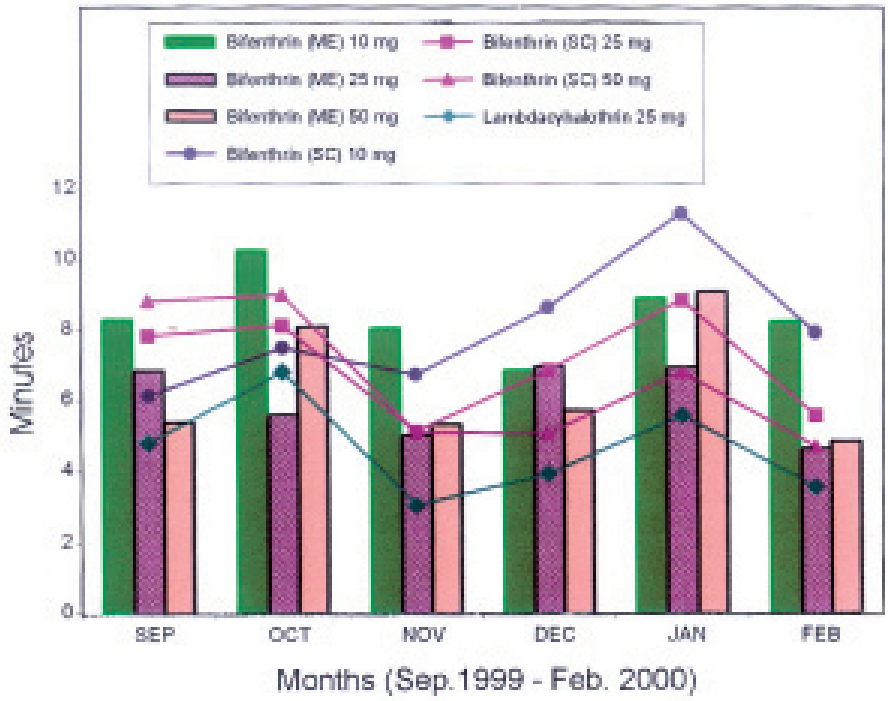

Fig.1. Average median knock-down times for *A. culicifacies* in ring-net bioassays.

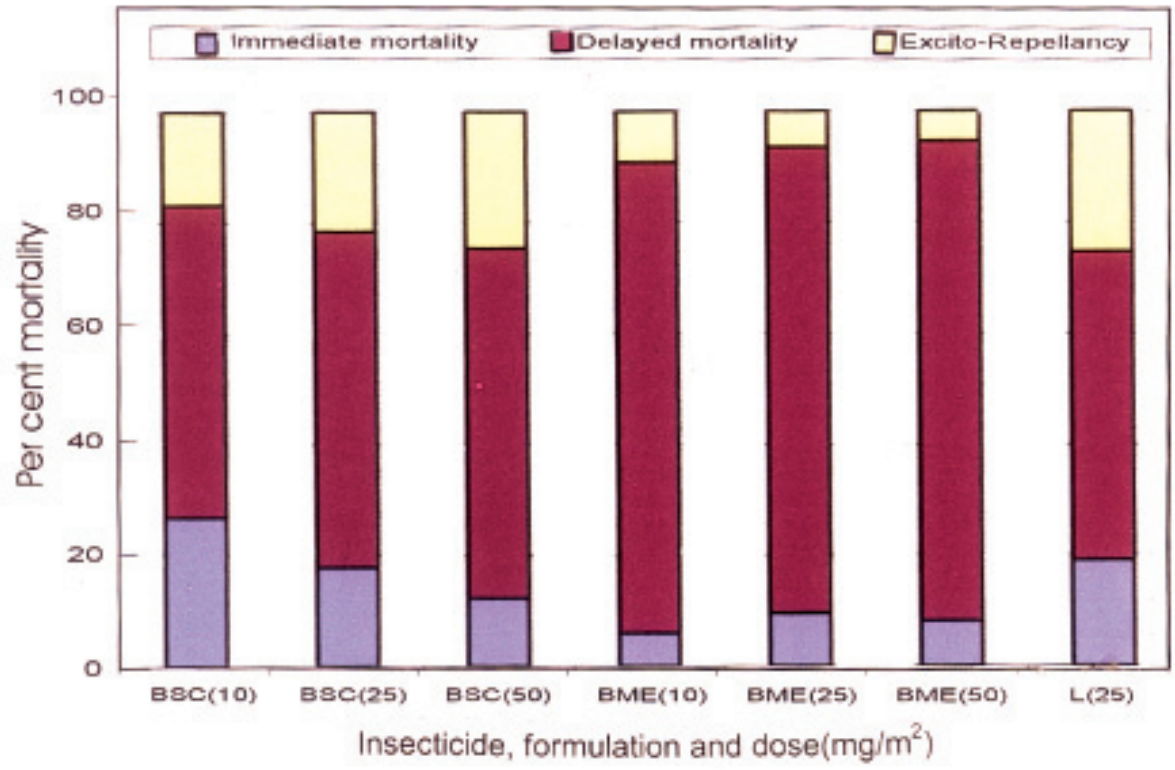

Fig.2. Cumulative effect of different doses and formulation of bifenthrin and lambdacyhalothrin on different entomological indices.

The observed entry rate of total mosquitoes with BME formulation ranged from 3.71 to 4.32, mean mosquito density in 13 fortnights compared to 13.11 in control and 4.0 in LCS formulation. Monthwise entry rate with all the three doses of BME formulations from 2.8 to 5.6. Excito repellency, immediate mortality and delayed mortality with BME formulations of total mosquitoes were ranging from mean density of 2.85 to 4.56, 2.90 to 4.98 and 41.95 to 45.23 respectively (Table).

Discussion

Persistence results of the impregnated nets determined by cone bioassay and ring net bioassay for 11 fortnights indicated that under the given experimental conditions in field, BSC and BME formulations were effective and bioavailability of insecticides on nets for complete efficacy was up to 5 months. In general, it was observed that the retention with both BSC and BME formulations was relatively more than LCS formulation which showed the least retention. Effective dose was always lower than the applied target dose and reasons for non uniform retention could be dose of impregnation itself, insecticide formulation, treatment method and net material *viz*., fiber composition, thickness weight, structure, number of fibres per cm^2 *etc*[18].

Results of entry rate of mosquitoes in all the formulations indicated a minimum of 50 per cent reduction in the entry rates in rooms with impregnated nets. The observed total entry of mosquitoes into the houses with treated nets was significantly different compared to houses with untreated nets ($P<0.001$). Further analysis by multiple comparison of the density of mosquitoes among the houses with treated nets were similar ($P<0.05$). Thus the results indicated no difference in the effect of different formulations of different insecticides on the entry of mosquitoes

Table. Monthly mean mosquito density in experimental houses

	Control	BSC (10 mg/m^2)	BSC (25 mg/m^2)	BSC (50 mg/m^2)	BME (10 mg/m^2)	BME (25 mg/m^2)	BME (50 mg/m^2)	LCS (25mg/m^2)
Entry rate:								
September	20.70	10.20	6.30	5.80	3.80	3.40	4.00	6.20
October	8.70	4.60	3.80	3.40	4.70	3.10	4.20	2.40
November	13.80	6.20	4.50	5.20	3.00	2.90	5.10	4.70
December	18.70	6.20	4.40	5.70	4.80	5.60	5.00	4.60
January	11.00	6.30	1.90	3.20	3.00	4.50	4.00	3.00
February	7.20	2.80	2.10	3.20	2.80	4.00	3.30	3.30
March	10.20	4.60	3.40	5.00	4.00	5.20	5.00	3.60
POOLED	13.11	5.94	3.80	4.46	3.71	4.02	4.32	4.00
Indoor (immediate) mortality:								
September	1.45	50.98	33.33	29.31	13.16	23.53	15.00	27.42
October	0.00	23.91	18.42	5.88	2.13	9.68	4.76	16.67
November	0.72	22.58	17.78	7.69	3.33	6.90	7.84	27.66
December	0.53	4.84	9.09	10.53	0.00	0.00	0.00	10.87
January	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
February	0.00	10.71	4.76	0.00	0.00	0.00	0.00	0.00
March	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
POOLED	0.59	21.50	16.60	10.00	2.90	4.98	4.27	15.00
Delayed mortality:								
September	4.31	100.00	100.00	100.00	100.00	100.00	100.00	100.00
October	9.52	80.00	87.50	82.76	83.33	94.74	100.00	92.86
November	9.89	33.33	38.46	45.95	20.69	38.10	33.33	35.71
December	5.69	30.00	36.67	31.58	32.61	25.00	27.91	24.24
January	8.82	43.48	63.16	25.81	17.39	40.00	38.24	27.27
February	4.76	27.27	31.58	24.00	42.86	28.00	37.50	32.14
March	3.03	31.58	23.08	68.42	25.00	38.10	18.18	20.00
POOLED	6.72	45.15	56.69	51.23	41.95	44.51	45.23	43.83
Excito-repellency:								
September	6.28	8.82	9.52	24.14	10.53	5.88	0.00	32.26
October	1.15	6.52	34.21	20.59	10.64	3.23	0.00	16.67
November	0.72	37.10	35.56	25.00	6.67	13.79	3.92	38.30
December	2.67	16.13	29.55	31.58	0.00	1.79	8.00	13.04
January	1.82	4.76	0.00	9.38	0.00	0.00	2.50	3.33
February	0.00	14.29	4.76	3.13	0.00	0.00	0.00	6.06
March	0.00	0.00	5.88	8.00	0.00	3.85	4.00	5.56
POOLED	2.58	13.47	20.24	20.00	4.56	3.45	2.85	20.00

BSC, bifenthrin suspension concentrate formulation; BME, bifenthrin micro-emulsion formulation, LCS, lambdacyhalothrin microcapsule suspension formulation

and at the same time a definite variation in densities among the houses with and without treated nets.

In contrast to the observed trend in total entry of mosquitoes in the experimental houses, the observed excito-repellency rates between the houses with untreated nets with BME formulation did not differ significantly (P>0.05). There was no difference in the excito-repellency rates in the houses with different doses of BME. Mosquito nets treated with BSC and LCS has exhibited increased excito-repellency and did not differ significantly. The excito-repellency rates between different doses of BSC and LCS also did not differ significantly. The observed results on excito-repellency with different formulations of BSC and LCS and between BME and control indicated similar rates of excito-repellency.

Observed rates of immediate mortalities indicated that houses with BSC 50 mg/m^2 and LCS 25 mg/m^2 treated nets did not show significant differences. All the three doses of BME formulation also did not show differences within the doses of treatment. However, BSC at doses 10 and 25 mg/m^2 have shown significantly higher immediate mortality as compared to control and other formulations (P<0.05).

The observed delayed mortalities in the mosquitoes collected from houses with bifenthrin and lambdacyhalothrin treated nets were significantly different from the houses with untreated nets (P<0.05). These results also indicated desired efficacy of the insecticides irrespective of formulations and compounds.

The cumulative per cent of immediate and delayed mortalities with different doses of BME was >90 per cent compared to < 80 per cent with other formulations and insecticides and thus indicated relatively increased efficacy of BME that was coupled with relatively lower excito-repellency rates. Of the two formulations of bifenthrin, SC and ME, ME was relatively more efficacious. Intrinsically bifenthrin has exhibited similar efficacy in both the formulations and doses with respect to median knock-down times, persistence, total entry and delayed mortalities. However BME differed significantly with respect to excito-repellency exhibiting lower rates and was found to be at par with the untreated nets. It caused immediate and delayed mortality as in SC formulation and is therefore advantageous for use as impregnant on mosquito nets especially in view of the lower excito-repellency but with potential killing effect.

Different doses of BME showed comparable results in all evaluation indices with the lowest of the doses BME (10 mg/m2) found as effective as other doses of this formulation and could be used for impregnation of mosquito nets for large scale phase III trial.

Acknowledgment

We thank WHO Pesticide Evaluation Scheme (WHOPES) for financial support and Prof. M.Zaim,Scientist,WHOPES, CPE/PVC Zeneva; Director, National Anti-Malaria Programme and to the health officials of the district Sonepat, Haryana for their support. We are grateful to Drs V.P. Sharma, M.K.K. Pillai and N.N. Singh for their valuable suggestions during data analysis.

References

1. Sharma VP, Malaria and poverty in India. *Cur Sci* 2003; *84* (4) : 514-5.

2. NMEP. Operational Manual for Malaria Action Programme (MAP) Govt. of India, National Malaria Eradication Programme; 1995 p.1-180.

3. Rajagopal, R. Malathion resistance in *Anopheles culicifacies* in Gujarat. *Indian J Med Res* 1977; *66* :27-8.

4. Pillai, MKK. Vector resistance to insecticides *Proc Natl Acad Sci India* 1996; *66* (B): 77-97.

5. Ansari MA, Sharma VP, Batra CP, Razdan PK, Mittal PK. Village scale trial of the impact of deltamethrin (K-othrine) spraying in areas with DDT and HCH resistant *Anopheles culicifacies. Indian J Malariol* 1986; *23* : 127-31.

6. Singh K, Rahman SJ, Joshi GC. Village scale trial of deltamethrin against mosquitoes. *J Commun Dis* 1989; *21*: 339- 53.

7. Yadava RL, Rao CK, Biswas H. Field trial of cyfluthrin as an effective and safe insecticide for control of malaria vectors in triple insecticide resistant areas. *J Commun Dis* 1996; *28* : 287-98.

8. World Health Organization. Report of the WHO Informal Consultation: Test Procedures for Insecticides Resistance Monitoring in Malaria Vectors, Bio-efficacy and Persistence of Insecticides on Treated Surfaces. 1998; WHO/CDS/CPC/MAL/98.12.

9. Jana-Kara BR, Jihullah WA, Shahi B, Dev, V, Curtis CF, Sharma VP. Deltamethrin impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. *J Trop Med Hyg* 1995; *98* : 73-83.

10. Sampath TR, Yadav RS, Sharma VP, Adak T. Evaluation of lambdacyhalothrin impregnated bednets in a malaria endemic area of India. Part 2. Impact on malaria vectors. *J Am Mosq Control Assoc* 1998; *14* : 437-43.

11. Sharma VP, Yadav RS. Cyfluthrin impregnated mosquito nets to control malaria in the mining settlement of Orissa, India. *Pub Hlth* 1995; *12* : 9-17.

12. Choudhary DS. Studies on resurgence of malaria in parts of northern India. In: Sharma VP, editor. *Proceedings of the Indo-UK Workshop on malaria*. Delhi: Malaria Research Centre; 1984 p. 61-74.

13. Subbarao SK, Adak T, Sharma VP. *Anopheles culicifacies*: sibling species distribution and vector incrimination studies. *J Commun Dis* 1980; *12* : 102-4.

14. Subbarao SK, Nanda N, Raghavendra K. Malariogenic stratification of India using *Anopheles culicifacies* sibling species prevalence. *ICMR Bull* 1999; *29* : 75-80.

15. Raghavendra K, Subbarao SK, Vasantha K, Pillai MK, Sharma VP. Differential selection of malathion resistance in *Anopheles culicifacies* A and B (Diptera: Culicidae) in Haryana state India. *J Med Entomol* 1992; *29* : 183-7.

16. World Health Organization,Geneva. Manual on Practical Entomology in Malaria. 1975; Part II : 1-191.

17. Sampath TRR, Yadav RS, Sharma VP, Adak T. Evaluation of lambdacyhalothrin - impregnated bednets in a malaria endemic area of India. Part I. Implementation and acceptability of the trial. *J Am Mosq Control Assoc* 1998; *14* : 431-6.

18. Rozendaal JA, Curtis CF. Recent research on impregnated mosquito nets. *J Am Mosq Control Assoc* 1989; *5* : 500-7.

Reprint requests: Dr C.P. Batra, Assistant Director, Malaria Research Centre, 2-Nanak Enclave
Radio Colony, Delhi 110009, India
e-mail: batracp@icmr.org.in