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Anopheles subpictus Complex: Distribution of Sibling Species in Sri Lanka

T.A. ABHAYAWARDANA, S.R.E. WIJESURIYA and R.K.C. DILRUKSHI

To determine the sibling species composition of *An. subpictus* complex in Sri Lanka polytene chromosomes of ovarian nurse cells were examined. Samples of *An. subpictus* s.l. were collected from 65 of the 73 Health Areas surveyed. Of 3095 ovaries collected 869 polytene chromosomes preparation were identified as sibling species A and 77 B. Species B was present only in coastal localities of the Island whereas species A was found both in coastal and inland areas with predominance in inland areas. This is the first report on the occurrence of two sibling species of *An. subpictus* in Sri Lanka. Some biological characteristics of coastal and inland populations such as susceptibility to different insecticides, human blood index and human biting rates are discussed.

Keywords: *Anopheles subpictus*, Human biting rates, Human blood indices, Insecticide-susceptibility, Sri Lanka

INTRODUCTION

Anopheles subpictus Grassi is the secondary vector of malaria in Sri Lanka[1]. Recent studies in system C of the Mahaweli Irrigation Project in north eastern province have revealed the role of this species as a major vector[2].

Two sibling species of *An. subpictus*, species A and B, were identified from India on the basis of an inversion of the x-chromosome and egg morphology[3]. Since, close proximity of Sri Lanka and India, the occurrence of sibling species in the Island was suspected. The present study was under-

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taken to examine the existence and distribution of these sibling species.

MATERIALS AND METHODS

Female mosquitoes of *An. subpictus* were collected from different climatic and eco-epidemiological areas during August 1990 to February 1992. A total of 16 Health Areas were selected for routine collections at monthly/bi-monthly intervals. Additional collections were also made from 57 other Health Areas. Mosquitoes used in the present study were from cattle-baited Cadjan hut collections, pyrethrum-spray collections (PSC), indoor hand collections, cattle-baited net trap collections, exit-window trap collections, human-bait night collections and larval collections.

Larvae collected were reared in plastic basins (about 35 cm diam) in a field laboratory until pupation, and pupae were placed in a cage for the emergence of adults. The emerged female adult mosquitoes and the unfed females collected by other techniques were fed on cattle or human blood.

Blood-fed mosquitoes were kept in paper cups until the ovaries developed up to Christopher stage III[4]. Mosquitoes having ovaries at this stage were killed by tapping in an aspirator. Ovaries of these mosquitoes and those already knocked down during PSC were removed and fixed in glacial acetic acid and methanol (1:3 v/v).

The fixed ovaries were transferred to the Central Laboratory in Colombo for further analysis. Polytene chromosomes were stained using the method described by Green and Hunt[5]. Polytene chromosomes were examined and species were identified following the line drawings and photomicrographs of two sibling species[3], A and B. The diagnostic inversion genotypes of sibling species were species A – $X+^a$ and species B – X^a.

Insecticide susceptibility/resistance test

The standard adult testing procedure was followed[4]. Samples of blood-fed females of *An. subpictus* populations gathered by cattle-baited net trap and hut collections from inland and coastal areas were used. 1% fenitrothion and 5% malathion impregnated papers were used within three weeks of opening the box and each paper was used for not more than 15 tests. Morphological characters of sibling species observed by Reuben and Suguna[6] were used in the identification of adult mosquitoes.

Blood meal identification: Samples of female blood-fed *An. subpictus* mosquitoes from PSC and indoor-hand collections were used to prepare blood smears on filter papers. Blood meals were assayed for the host source by "gel diffusion technique". In this analysis adults were identified using either morphological or cytotaxonomical characters[3,6].

Human-bait all night collections: A single biting/landing catch on human bait was conducted on August 15, 1991 at Vannathivu, a coastal area in the Puttalam Health Area. The indoor collection involved 2-4 baits, while in the outdoor collection 3-5 baits were engaged.

RESULTS

An. subpictus were collected from 65 to 73 Health Areas surveyed. The cytospecies was identified from 34 Health Areas (Table 1). A total of 3095 ovary preparations were examined during the study period. Health Areas Batticaloa, Chilaw, Hambantota, Puttalam, Tissamaharamaya and Trincomalee that recorded the presence of *An. subpictus*, are in the coastal region of the Island.

Both the sibling species A and B were identified (Fig. 1). The majority, 91.9%, were of species A and the rest 8.1% of species B, the latter species being found only in the coastal areas. All the specimens identified from Echchantivu and Kannankuda in Batticaloa Health Area were of species B. Both sibling species were collected from some coastal localities: Godana in Health Area Tissamaharamaya and Daluwa, Kalpitiya, Kurignapitiya, Palvavi, Sethupola and Vannathivu in Health Area Puttalam.

The majority 63.8% of species A identified were from the collections of indoor resting mosquitoes, 15.5% from cattle-baited hut collections, 12.8% from cattle-baited net traps and remaining from other collections. Most of the species B (46.8%) identified were from cattle-baited net traps, 32.5% from larval collections, 15.6% from in-

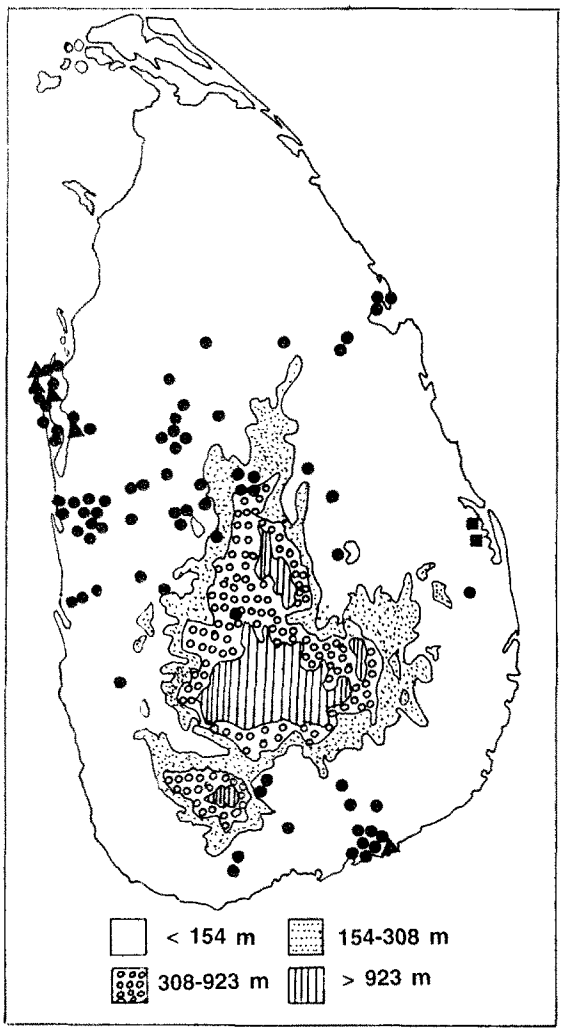

Fig. 1: Map of Sri Lanka showing the distribution of sibling species of *An. subpictus*: Species A (●), Species B (■), and Species A and B sympatric (▲)

Table 1. Bimonthly identification of *Anopheles subpictus* species A in different Health Areas of Sri Lanka

Health Area	Aug 90	Sep/ Oct 90	Nov/ Dec 90	Jan/ Feb 91	Mar/ Apr 91	May/ Jun 91	Jul/ Aug 91	Sep/ Oct 91	Nov/ Dec 91	Jan/ Feb 92	Total
Ampara	0(0)	–	–	–	–	–	–	4(0)	–	–	4(0)
Anuradhapura	–	–	–	7(0)	–	–	–	–	–	–	7(0)
Atakalanpanna	–	81(0)	18(0)	–	–	–	–	–	–	2(0)	101(0)
Batticaloa	–	–	–	–	–	–	–	–	0(12)	–	0(12)
Bingiriya	–	–	–	16(0)	–	–	–	–	–	7(0)	23(0)
Chilaw	0(0)	–	–	20(0)	4(0)	106(0)	3(0)	5(0)	–	2(0)	140(0)
Dambulla	8(0)	1(0)	14(0)	39(0)	10(0)	–	–	–	5(0)	–	77(0)
Embilipitiya	–	–	–	–	–	–	–	–	1(0)	–	1(0)
Galgomuwa	–	–	4(0)	20(0)	–	–	6(0)	–	4(0)	–	34(0)
Gokarella	–	1(0)	1(0)	–	–	–	–	–	–	5(0)	7(0)
Hambantota	1(0)	–	–	–	–	–	–	–	–	–	1(0)
Hingurakgoda	2(0)	–	–	–	–	–	–	–	–	–	2(0)
Kahatagasdigiliya	–	–	1(0)	4(0)	–	–	–	–	–	–	5(0)
Kandy	–	–	–	4(0)	–	–	–	–	–	–	4(0)
Katana	–	0(0)	1(0)	11(0)	–	–	3(0)	–	–	–	15(0)
Kekirawa	–	–	–	–	–	–	–	2(0)	–	–	2(0)
Kurunegala	0(0)	0(0)	30(0)	69(0)	–	20(0)	–	–	–	–	119(0)

contd...

Table 1. (*contd.*)

Health area	Aug 90	Sep/ Oct 90	Nov/ Dec 90	Jan/ Feb 91	Mar/ Apr 91	May/ Jun 91	Jul/ Aug 91	Sep/ Oct 91	Nov/ Dec 91	Jan/ Feb 92	Total
Mahiyanganaya	0(0)	–	1(0)	–	–	–	–	–	–	–	1(0)
Maho	–	–	–	–	–	–	–	–	–	1(0)	1(0)
Monaragala	–	3(0)	–	13(0)	–	–	4(0)	–	–	–	20(0)
Narammala	–	–	–	–	–	–	–	–	–	1(0)	1(0)
Padukka	–	–	–	–	1(0)	–	–	–	–	–	1(0)
Panduwasnuwara	–	–	–	–	–	–	–	11(0)	–	–	11(0)
Pannala	–	–	–	5(0)	–	–	–	–	–	–	5(0)
Polgahawala	–	–	–	–	–	1(0)	–	–	–	–	1(0)
Polonnaruwa	0(0)	1(0)	11(0)	20(0)	–	–	–	–	1(0)	–	33(0)
Puttalam	–	2(0)	3(0)	8(0)	3(3)	24(44)	1(15)	4(2)	–	–	45(64)
Sandunpura	–	–	–	30(0)	–	–	–	–	–	–	30(0)
Thambuttegama	–	6(0)	–	9(0)	–	–	–	–	–	–	15(0)
Tissamaharama	–	1(0)	–	28(0)	–	–	41(1)	–	–	2(0)	72(1)
Trincomalee	–	–	–	–	–	23(0)	12(0)	–	–	–	35(0)
Walasmulla	–	–	1(0)	–	–	–	–	–	–	–	1(0)
Wariyapola	–	0(0)	–	35(0)	7(0)	–	–	–	–	6(0)	48(0)
Wellawaya	–	1(0)	–	–	–	–	–	–	–	6(0)	7(0)
Total	11(0)	97(0)	85(0)	338(0)	25(3)	174(44)	70(16)	26(2)	11(12)	32(0)	869(77)

Figures in parentheses indicate number of species B identified.

door resting populations, 3.9% from cattle-baited huts, and only 1.2% were from human-bait night collections.

Indoor biting density of species B (the only anopheline species collected in this single night at Vannathivu-coastal area) during 1800-2200 hrs ranged from 0.25-1.5 per man hour and the highest density was observed during 2100-2200 hrs. No indoor biting was observed from 2200-0600 hrs.

Outdoor biting density of species B ranged from 0.2-5.0 per man hour during 1800-2100 hrs collections with highest biting during 1800-1900 hrs. From 2100 hrs onwards biting occurred only for 3 h with biting rates of 0.33 per man hour during 2200-2300 hrs, 0.33 per man hour during 0300-0400 hrs and 0.33 per man hour during 0500-0600 hrs (Fig. 2)

Susceptibility/resistance test: In inland areas, *An. subpictus* exposed to 5% malathion and 1% fenitrothion for an hour showed 68.7% mortality to malathion (237 exposed) and 54.3% to fenitrothion (46 exposed).

A total of 393 blood-fed females from Batticaloa Health Area and 45 from Puttalam Health Area (coastal area) were fully susceptible to malathion (100% mortality). Mosquitoes exposed to 1% fenitrothion from the same populations (64 and 90 in numbers respectively) were also fully susceptible to fenitrothion.

Blood meal identification: A total of 35 blood-smears were identified from specimens collected from coastal areas. Out of these 3 were from Tissamaharamaya, 29 from Puttalam and the rest from Batticaloa. Of these 7

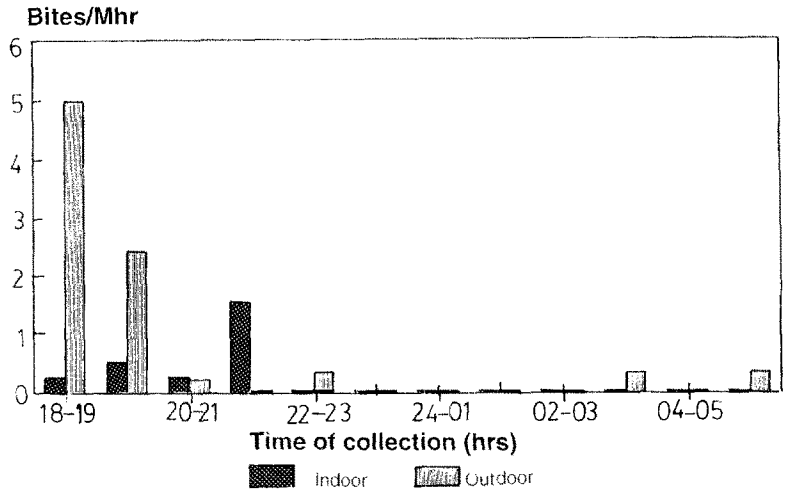

Fig. 2: Hourly biting rates of *An. subpictus* B in a coastal locality: Vannathivu of Puttalam Health Area

(20%) were identified as human blood, 19 (54.3%) as cow blood and the rest 9 (25.7%) did not respond to cow or human antisera.

In the sample of 1187 blood smears of *An. subpictus* from inland areas examined for source of blood, 0.25% were of human origin, 87.2% were cow blood and the rest, 12.55% did not respond to human or cow antisera.

DISCUSSION

During the study mosquito collections were made throughout the Island except Jaffna Peninsula, Mannar, Mulathivu and Vauniya. Recently, Suguna *et al.*[7] reported four sibling species in the *An. subpictus* complex. The present study is based on the earlier report by Suguna[3] that the two sibling species A and B can be identified based on inversion 'a' on the x-chromosome.

The distribution of species B of *An. subpictus* was restricted to coastal localities of the Island. The species was collected from the eastern coast (Batticaloa), north western coast (Puttalam) and the southern coast (Kirinda). All the specimens identified from Batticaloa were of species B. In other two localities both the sibling species were found. Just after the rainy period when the salt content in water sources was diluted, the density of species A increased in coastal areas. With the increase in salt concentration of water sources there was a gradual increase in the density of species B, while species A disappeared from the locality, indicating the preference of species B to salinity and in tolerance in species A. Species B was always in breeding sites associated with floating algae. Even in the same water body, the species was not found in the absence of water plants. This may be because the larvivorous fish (which were freely available in such brackish waters, e.g. *Oreochromis mossambicus*) could not reach larvae when the floating water plants were abundant.

Coastal populations were fully susceptible to 5% malathion and 1% fenitrothion, while inland population was moderately resistant. Since all three coastal areas were under malathion spray coverage for a long period, the failure of species B to develop resistance may be due to its exophilic behaviour. However, it may be noted that coastal population of *An. subpictus* is resistant to permethrin, while inland population (species A) is highly susceptible to this insecticide (unpublished entomological data — AMC).

As coastal populations were highly susceptible and all the coastal areas were under malathion spraying, the indoor resting mosquitoes were few. Most of the specimen collected were blood-fed, suggesting either low survivorship indoors or exophily after feeding. However, human blood index between inland and coastal populations were remarkably different. There

was no significant difference in the man : cattle ratio in coastal and inland areas investigated.

Species B was found biting both indoors and outdoors. The peak biting period was in early part of the night. Though there was a single night biting collection, it appeared that species has a high human biting rate. It is important to study the role of species B of *An. subpictus* in malaria transmission. It was noticed that not even a single *An. culicifacies* mosquito was collected during the study period in coastal areas of Puttalam, but malaria transmission was there at its peak according to the records of patients attending for blood film testing at the central dispensary, Kalpitiya.

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Evaluation of Methoprene (Altosid) and Diflubenzuron (Dimilin) for Control of Mosquito Breeding in Tezpur (Assam)

I. BARUAH and S.C. DAS

Insect growth regulators (IGRs) namely, Isopropyl (E-E)-(RS)-11-methoxy-3,7,11-trimethyldodeca-2, 4-dinoate (Methoprene) and 1-(4-cyclophenyl)-3-(2,6-diflerobenzoyl) urea (Diflubenzuron) were evaluated against mosquito larvae in laboratory as well as in different breeding habitats in Tezpur, Assam. LC_{90} values of diflubenzuron against *Culex quinquefasciatus* and *Aedes albopictus* were 0.0022 and 0.0027 ppm respectively, while it was 0.0027 and 0.0022 ppm respectively in case of methoprene. However, LC_{50} values of both the IGRs were almost same in case of *Ae. albopictus* and *Cx. quinquefasciatus* (varies between 0.0009 and 0.0011 ppm). In case of methoprene, maximum mortality was observed in pupal stage though the exposure was given in all the cases to the III instar larvae. Field trials were conducted in cemented drains, small ponds and ditches. At 0.2 ppm (0.020 kg/ha) both diflubenzuron and methoprene were found to eliminate 92-96 per cent *Culex* and *Anopheles* larvae. Methoprene and diflubenzuron were found equally effective for control of mosquito breeding in different breeding habitats and provide better efficacy than conventional larvicides and biocides.

Keywords: Emergence inhibition, Field trial, Insect growth regulator, Larvicides

INTRODUCTION

Resistance of mosquitoes to conventional insecticide is increasing day-by-day. Requirement of higher and higher dosage and indiscriminate use of insecticides causes irreversible environmental pollution and also affects the

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non-target organisms. Search for alternative methods have been tried by several workers which resulted in selection of IGRs as fourth generation insecticides due to their specificity and selective action[1]. Chemical compounds mimic/analogue to IGRs are now available and found quite effective for mosquito control[2-7]. The biological and environmental dynamics of IGRs have been reviewed and most were found safer than conventional insecticides[8]. Though several IGRs have been effectively evaluated against mosquito vectors, only two namely, methoprene (Altosid) and diflubenzuron (Dimilin) were found effective under field conditions[2,3,9,10].

The laboratory and field evaluation of altosid and dimilin against different species of mosquitoes in various breeding habitats are presented in this paper.

MATERIALS AND METHODS

Chitin inhibitor diflubenzuron [Dimilin, 1-(4-cyclophenyl)-3-(2,6-diflurobenzoyl)] urea 25% wettable powder marketed by M/s. Philip Duphar BV Amsterdam, Holland and juvenoid hormone analogue methoprene [Altosid, Isopropyl (E-E)-(RS)-11-methoxy-3,3,11-trimethyldodeca-2, 4-dinoate)] EC, provided by M/s. Zoecon Corporation, California were evaluated in the present study.

Laboratory evaluation: Laboratory reared larvae of *Cx. quinquefasciatus* and *Ae. albopictus* were used as test insect. Stock solution of 1 ppm concentration was prepared in distilled water and later concentrations were made as per the requirement of the studies.

Tests were conducted in 500 ml glass beaker containing 250 ml filtered water. 25 late III or early IV instar larvae were introduced in each beaker with desired concentration for continuous exposure. Four sets of test were conducted for each concentration with eight replicates and concurrent controls. Larval food (mixture of powdered liver and brewer's yeast) was provided both to exposed and control beakers. Beakers were covered with fine cloth netting to secure emerging adults. Observations were taken at every 24 h to record dead/moribund larvae and emerged adults. Morphologically abnormal adults unable to leave the water surface were recorded as dead. Tests were conducted under controlled conditions of temperature 27±2°C and relative humidity 75±5% RH.

The probit values of the recorded mortality were plotted against log concentration and LC_{50} and LC_{90} values of IGRs were determined.

Field evaluation: Field evaluation of IGRs were carried out in different types of breeding habitats namely, cemented drains, ditches and small ponds against immature stages of culicine and anopheline mosquitoes. Quantity of IGRs required for each treatment was determined by the size of the water body. Required quantity of IGR was

mixed well in a known volume of water and sprayed uniformly on the surface of the water with a knapsac sprayer. One adjacent plot was left untreated as control.

A sample was collected before the treatment to record the species composition of the breeding site. Density of larval population was monitored daily to calculate the per cent emergence inhibition (%EI).

RESULTS AND DISCUSSION

Results of the laboratory evaluation of dimilin and altosid against *Cx. quinquefasciatus* and *Ae. albopictus* are presented in Table 1. Results revealed that LC_{90} values of dimilin against *Cx. quinquefasciatus* and *Ae. albopictus* were 0.0022 and 0.0027 ppm respectively; while LC_{90} values of altosid against these two species were 0.0027 and 0.0022 ppm respectively. LC_{50} values of dimilin and altosid against *Ae. albopictus* was almost same (0.001 ppm), whereas LC_{50} values of dimilin and altosid against *Cx. quinquefasciatus* were 0.0011 and 0.0018 ppm respectively. LC_{90} values of altosid is higher than dimilin against *Cx. quinquefasciatus*, while LC_{90} values of dimilin is higher than altosid against *Ae. albopictus*. This indicates that *Ae. albopictus* was more susceptible to altosid and *Cx. quinquefasciatus* was slightly more susceptible to dimilin. Similar observations were reported by Rathburn *et al.*[11] against *Cx. nigripalpus* and *Ae. taeniorhynchus*. Dame *et al.*[12] obtained 90% EI in case of *Cx. quinquefasciatus* at 0.05 and 0.015 ppm of altosid and dimilin respectively. Sharma *et al.*[4] reported 0.001 ppm as LC_{95} value of dimilin against *Cx. pipiens fatigans*. At 2 ppm total inhibition of adult emergence was observed in larvae with altosid against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*[13]. In the present study low dosages were used for total inhibition of

Table 1. Results of the laboratory dosage mortality test of dimilin and altosid against *Cx. quinquefasciatus* and *Ae. albopictus* larvae

IGRs	Species*	Lethal concentration in ppm	
		LC_{50}	LC_{90}
Dimilin (Diflubenzuron)	*Cx. quinquefasciatus*	0.0011	0.0022
	Ae. albopictus	0.0009	0.0027
Altosid (Methoprene)	*Cx. quinquefasciatus*	0.0018	0.0027
	Ae. albopictus	0.0010	0.0022

* Continuous exposure till the entire lot of test immatures was exhausted; Control mortality nil.

Table 2. Field trials of IGRs against mosquito larvae in different breeding habitats

IGRs	Species	Habitat	Dosage kg/ha	% reduction (days)		
				1	2	3
Dimilin (Diflubenzuron)	*Cx. quinquefasciatus*	Cemented drain-2	0.01	46-50 (48)	-	-
	Cx. quinquefasciatus	Cemented drain-6	0.02	90-92 (91)	94	94-96 (94.8)
	An. vagus *An. crawfordi*	Ditch-2	0.02	80-86 (83)	90	93-95 (94)
	An. annularis *Cx. vishnui* group	Small pond-2	0.02	90-92 (91)	95	95-96 (95.5)
	Cx. quinquefasciatus	Cemented drain-2	0.04	92	93-94 (93.5)	96
	An. vagus *Cx. vishnui* group	Small pond-2	0.04	90	92-93 (92.5)	95
Altosid (Methoprene)	*Cx. quinquefasciatus*	Cemented drain-2	0.02	54	72	91-94 (92.5)
	Cx. gelidus *An. vagus*	Ditch-3	0.02	45-58 (51.7)	80-85 (82.3)	93-95 (94.3)
	An. vagus *An. crawfordi*	Ditch-2	0.02	50-55 (52.5)	82	94-96 (95)
	Cx. quinquefasciatus	Cemented drain-2	0.04	81-86 (83.5)	90	95-98 (96.7)
	Cx. vishnui group	Ditch-4	0.04	82-85 (83.5)	88	92-94 (92.8)
	An vagus *An. crawfordi*	Ditch-2	0.04	86	92-93 (92.5)	96-97 (96.5)

Figures in parentheses are mean values of per cent reduction.

adult emergence. There seems to be a wide range of variations in the LC_{50} and LC_{90} values of both altosid and dimilin with those of other investigators. Many of the variations were due to different strains of mosquitoes, larval instars, rearing temperature, type of water, food etc[11].

Present findings are in confirmation with Mulla *et al.*[14] who observed that dimilin has good larvicidal activity,

while bulk of mortality occurred in the pupal stage following larval exposure to altosid.

Field trial: The relative effectiveness of dimilin and altosid in different types of habitats are summerized in Table 2. At 0.01 kg/ha (0.1 ppm) of dimilin, reduction of 46-50% *Cx. quinquefasciatus* larvae was observed in cemented drains after 24 h of treatment. Higher dosages, 0.02 to 0.04 kg/ha is required for achieving 80-92% and 93-96% mortality after 1 and 3 days of treatment respectively against *Culex* and *Anopheles* breeding. In case of altosid, 45-58% larval reduction at 0.02 kg/ha and 81-86% at 0.04 kg/ha was observed after one day of treatment that increased to 92-98% after 3 days of treatment in both the cases. This indicates that larval mortality after 24 h was higher in case of dimilin than altosid, while total reduction after 3 days was almost same in both the IGRs.

Sharma *et al.*[4] reported 14 and 80% larval reduction with dimilin at 0.1 and 0.25 ppm respectively, while more than 90% control of *Cx. quinquefasciatus* breeding in drain was achieved with 0.5 ppm within 4 days of treatment. In the present trial similar results were obtained with comparatively lower dosages. This is in confirmation with the observation of Amalraj *et al.*[7] who has observed that unlike *kutcha* drains or cesspits, relatively low dosage was required in cemented drains to obtain 100% inhibition of emergence for a longer period. Mulla and Darwazeh[2] found complete emergence inhibition of *Psorophora confinis* with dimilin and altosid at the dosages of 0.011 and 0.028 kg/ha respectively. Dame *et al.*[12] carried out field evaluation of four formulations of IGRs and found effective at the rate of 0.011-0.056 kg/ha. Among these compounds, methoprene and dimilin were found most effective at 0.028 and 0.022 kg/ha respectively against *Cx. nigripalpus* and *Cx. salinarius*. This conforms the results obtained in the present trials conducted against field population of *Culex* and *Anopheles* larvae.

It is concluded from the present study that both the IGRs (Dimilin and Altosid) are equally effective for control of larval population of culicine and anopheline mosquitoes in different breeding habitats. At the dosages of 0.02-0.04 kg/ha residual effect of the spraying lasted for 3-4 days. In view of their effectiveness and low dosage requirement both the IGRs can be introduced as an agent of integrated vector control programme in north eastern region.

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In-vitro Cultivation of Exoerythrocytic Stages of *Plasmodium cynomolgi* in Hepatocytes of *Macaca radiata*

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Hepatocytes from bonet monkey (*Macaca radiata*) obtained by perfusion of a liver biopsy were infected *in-vitro* with *Plasmodium cynomolgi bastianellii* sporozoites raised in *Anopheles stephensi*. The development of exoerythrocytic (EE) stages was seen under phase contrast microscope and by Giemsa staining. Multinucleated EE-stages were seen in the cultured hepatocytes on day 7-8 post-sporozoite inoculation.

Keywords: EE-stage, Hepatocyte, *Macaca radiata*, *Plasmodium cynomolgi*

INTRODUCTION

Malaria infection is started by the bite of an infected female *Anopheles* mosquito that injects sporozoites into the blood stream. The sporozoites invade the liver cells and develop into EE forms, which generate several thousand merozoites by schizogony. When mature, these merozoite are released from ruptured parenchymatous cells and enter red blood cells to begin erythrocytic schizogony. Much is known about the sporozoite, the asexual blood forms and the sexual forms of plasmodia. However, the EE-stages which constitute an essential link between the sporozoite and the erythrocytic stages are poorly understood. Investigations on the EE-stages have been greatly hampered because these stages occur in a deep organ, the liver, and hence are not easily accessible.

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Studies on EE-stages has been facilitated by the development of techniques permitting their culture *in-vitro*. EE-stages of some avian[1], rodent[2] and simian[3,4] plasmodia have been successfully cultured. The cultivation of EE-stages of all the four human plasmodia species has also been successfully achieved[5-8].

In-vitro studies on EE-stages of human malaria parasite are restricted by the difficulty of obtaining sporozoites and human hepatocytes. *P. cynomolgi*, a simian malaria parasite closely resembles human malaria parasite, *P. vivax* in morphology, tertian periodicity, pattern of life cycle[9] and shares certain antigenic similarities[10]. It infects a wide variety of simian hosts. Recently, we have reported complete development of EE-stages of this parasite in primary cultures of hepatocytes of rhesus monkey (*Macaca mulatta*)[11]. The present study reports the successful cultivation of this parasite in primary cultures of hepatocytes of bonet monkey (*M. radiata*).

MATERIALS AND METHODS

Parasite: The *bastianellii* strain of *P. cynomolgi* used in the present study was obtained from Dr. W.E. Collins, Centre for Disease Control, Atlanta, Georgia, USA. The parasite is routinely maintained at this Institute by cyclical transmission through *An. stephensi* mosquitoes.

Infection of *An. stephensi* mosquitoes: Two days old *An. stephensi* mosquitoes were starved for six hours and then fed on rhesus monkey infected with *P. cynomolgi* and showing gametocytes in the blood smears. The mosquitoes were fed during the secondary peak of infection, when there was enhancement of mosquito infectivity[12]. The infected mosquitoes were maintained at 26^0C in 70-80% humidity. Seven days following infected blood meal, a batch of ten mosquitoes were dissected for determining the oocyst count. Batches of mosquito on an average having 50-100 oocysts per gut were selected for the study. On day 14 post-infected blood meal, salivary glands were dissected for the presence of sporozoites.

Collection of liver biopsy: A small piece of liver (approx. 2 x 2 cm) by operating under sterile conditions was taken out and transferred into pre-warmed (37^0C) SMEM (Supplemented minimum essential medium containing 2 g/l of bovine albumin, 10 mg/l of bovine insulin, 0.1 mM non-essential amino acids, 292 mg/l of L-glutamine, 150 IU/ml penicillin and 150 µg/ml streptomycin). The tissue was immediately transferred to the laboratory.

Isolation of hepatocytes: Liver tissue was transferred to a 100 mm petridish and washed gently with five changes of MEM. The tissue was then perfused with approx. 100 ml of calcium free Hepes buffer (NaCl, 8 g/l; KCl, 200 mg/l; Hepes, 2.38 g/l), pH 7.4. This was followed by perfusion with Hepes buffer supplemented with 5 mM Ca^{2+} and collagenase (30 mg type I + 30 mg type IV for

100 ml Hepes buffer). The perfusion was carried out inside the laminar flow with the help of a peristaltic pump and a flow rate of 5-7 ml/min was maintained. The temperature of the perfusing solution was adjusted to 38-39°C. After perfusion, the dissociated hepatocytes were gently dispersed in Hepes buffer and cell suspension was passed through a nylon mesh to remove clumps of cells. The hepatocytes were washed twice in Hepes buffer by centrifugation at 600 rpm for 3 min. The cells were resuspended in MEM and the viability were assessed by staining with 0.1% trypan blue solution.

Culture of hepatocytes: Primary cultures of hepatocytes were obtained as described earlier[11]. Briefly hepatocytes were suspended in SMEM containing 10% fetal bovine serum. These were deposited on the bottom of 35 mm plastic tissue culture dish (Nunclon). The hepatocyte cultures were maintained at 37°C in 5% CO_2 and 95% air.

Isolation of sporozoites: Fourteen to sixteen days after the infected blood meal, mosquitoes earlier tested positive for the presence of oocysts served as donors of sporozoites. These mosquitoes were washed in 70% ethyl alcohol for 30 s. Salivary glands from individual mosquitoes were dissected under dissecting microscope and transferred into SMEM containing 20% normal monkey serum. Pooled salivary glands were gently disrupted in a glass homogenizer and counted in a haemocytometer.

Inoculation of sporozoites into hepatocyte cultures: Forty-eight hours after hepatocyte monolayer formation, most of the culture medium over the monolayers was removed. Fifty microlitre of culture medium containing about 5 x 10^4 freshly harvested sporozoites was added to the cultures. The cultures were then transferred to CO_2 incubator. After three hours, the culture medium overlying hepatocyte monolayers was replaced with fresh medium. After another 2 h, the medium was replaced with SMEM containing 0.5 μg/ml 5-fluorocytosine (antifungal agent). The culture medium was changed daily taking care to expose the cultures to the outside atmosphere for minimum duration and keeping temperature close to 37°C.

Vizualization of EE forms: The infected hepatocyte monolayers were observed daily under the phase contrast microscope. In addition, cultures were terminated every alternate day and the monolayers in the petridishes were rinsed thrice with warm medium and air dried in cool atmosphere. The cells were then fixed with chilled methanol for 10 min and stained with 10% Giemsa for 30 min. The stained cells were finally washed with PBS dried and observed under the microscope.

RESULTS AND DISCUSSION

Studies on the EE forms of human malaria are often hindered because of restricted supply of sporozoites and poor accessibility to human liver. On the other hand simian hosts are easily available and can be employed for similar studies. *P. cynomolgi* has been

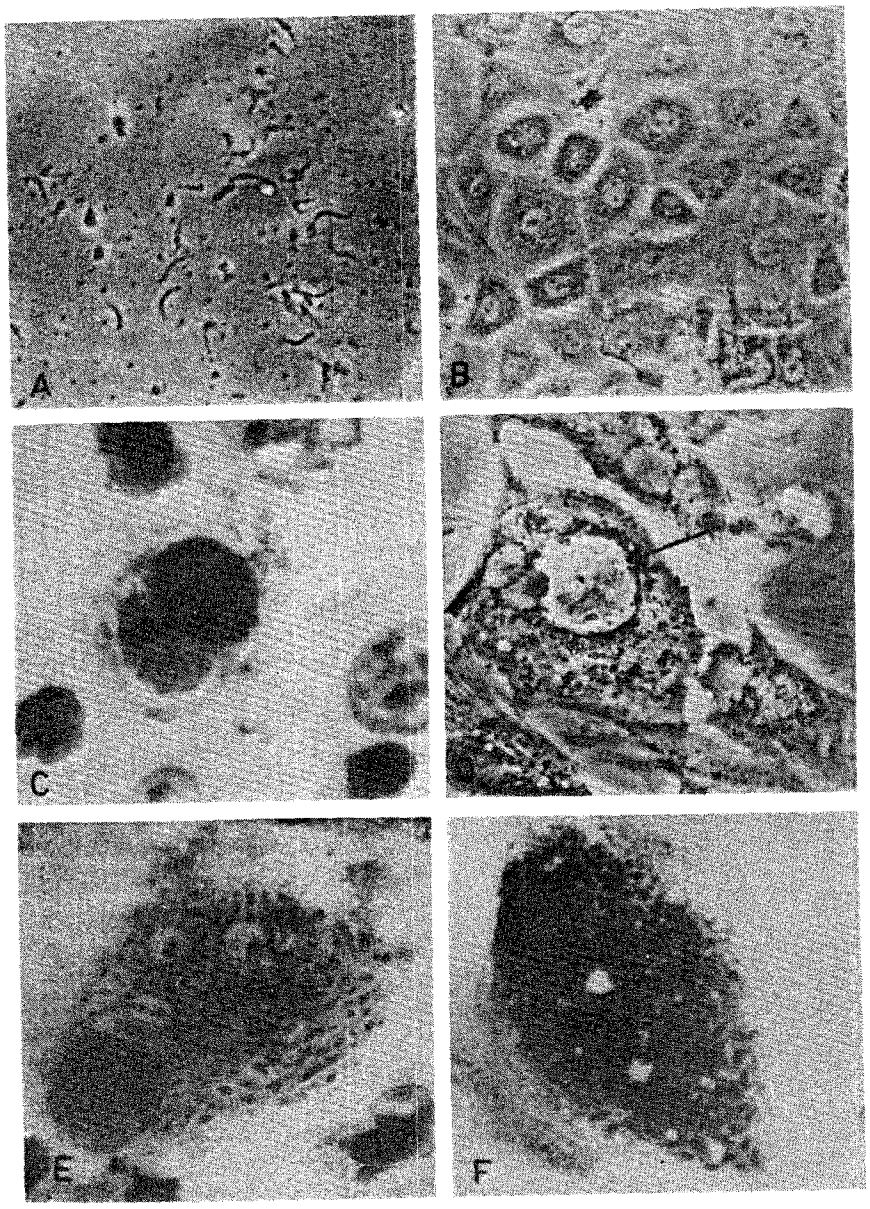

Fig. 1: Development of exoerythrocytic forms of *Plasmodium cynomolgi*. A — Sporozoites isolated from *An. stephensi*, B — Hepatocyte monolayer cultures under phase contrast, C — Giemsa stained 3 day old EE-stage, D — Phase contrast picture of 4 day old EE form, E — Giemsa stained picture of 7 day old EE schizont, and F — Giemsa stained picture of 8 day old EE form (Magnifications before printing — A and B: 50x; C-F: 200x)

widely used because of its close homology with human malaria parasite, *P. vivax*. It can infect some commonly occurring simian hosts.

In the present study complete development of EE-stages of *P. cynomolgi* was obtained in primary cultures of hepatocytes of *M. radiata*. Perfusion of a piece of liver resulted in isolation of hepatocytes with more than 90% cell viability, Sedimentation, adherence and resulting growth of the cells formed monolayers. Cultured hepatocytes remained viable for four weeks. No multiplication of the cultured hepatocytes was noted. The infectivity rate of the sporozoites was quite low and approximately 0.7% of the inoculated sporozoites developed into exoerythrocytic schizonts. It was possible to identify 3 days or older EE forms in hepatocyte cultures under phase contrast microscope. This direct *in-vitro* observation of EE-stage development of Plasmodium species offers opportunities to observe visually the activity of specific drugs or biochemical agents at various stages of exoerythrocytic development[13]. In Giemsa stained preparations the 7-8 days old schizonts contained several hundred nuclei. In some schizonts, intracytoplasmic vacuoles were seen. The presence of intracytoplasmic vacuoles has been described in most simian parasites[9]. At Day 8 fully mature forms were noted (Fig. 1).

The usefulness of *in-vitro* cultivation of hepatic stages has been amply demonstrated in research on tissue schizontocidal drugs[14] effect of cytokines[15,16], antibodies[17,18] and vaccines[19,20]. The culture system has also allowed detailed understanding of ultrastructure of EE-stages[21].

The study has established that EE-stages of *P. cynomolgi* can also be cultivated in primary cultures of *M. radiata* hepatocytes. Such a model may be valuable in studies dealing with chemotherapy and immunology of EE-stages of the parasite.

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Cytotaxonomical Examination for Sibling Species in the Taxon *Anopheles culicifacies* Giles in Sri Lanka

T.A. ABHAYAWARDANA, R.K.C. DILRUKSHI and S.R.E. WIJESURIYA

Ovarian polytene chromosomes of *Anopheles culicifacies* collected from different climatic and eco-epidemiological localities were examined to study the composition and distribution of sibling species in Sri Lanka. During the study period 4328 ovaries were prepared for examination, of which 1937 were suitable for the reading of polytene chromosomes. Among these 458 specimens were identified as belonging to species B based on diagnostic inversion on x-chromosome and 1479 definitively were identified as sibling species B on the basis of x-chromosome and chromosome arm-2 inversion. Other sibling species were not encountered among the specimens identified from 31 Health Areas during the study period. The occurrence of sibling species B of *An. culicifacies* in Sri Lanka is confirmed but no evidence of other sibling species (A, C or D) was found.

Keywords: *An. culicifacies*, Cytotaxonomy, Sibling species

INTRODUCTION

An. culicifacies. Giles was incriminated as a vector of malaria in Sri Lanka during the early part of this century[1,2]. Four sibling species based on paracentric inversions on x-chromosome and chromosome arm-2 have been identified in this taxon till date in Asia. These are species A, B[3], C[4] and D[5,6]. The standard arrangement of chromosomes for species A is $x+^{a}+^{b}$ $2+g^{1}+h^{1}+i^{1}/i^{1}$, species B xab $2g^{1}+h^{1}$, species C xab/$2+g^{1}h^{1}$ and for species D $x+^{a}+^{b}$ $2i^{1}+h^{1}$.

Sibling species B of *An. culicifacies* is regarded as the malaria vector in Sri

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Lanka. However, this cytotaxonomical identification is based on a few specimens of *An. culicifacies* from southern Sri Lanka identified as species B by Green and Miles[3]. This determination was based exclusively on the x-chromosome prior to the identification of species C that has similar x-chromosome banding pattern as of species B but differs in the pattern of chromosome-2 due to two inversions g^1 and h^1. Thus, the cytotaxonomic status of Sri Lankan *An. culicifacies* cannot presently be considered definitively established. Also, given the close proximity of the Island to the Indian mainland where four sibling species exist[5,7]. It is important that the cytotaxonomic status of *An. culicifacies* be examined throughout its range in Sri Lanka. This is especially so because species B is a poor vector in India, the major vectors being species A, C, and D[7,8]. The present paper reports the results of a study conducted to examine the existence and distribution of sibling species of *An. culicifacies* throughout Sri Lanka.

MATERIALS AND METHODS

Specimens of *An. culicifacies* were collected from different climatic and eco-epidemiological situations from July 1990 to February 1992. Sites in 16 Health Areas were selected for monthly/bimonthly routine collections. In addition, occasional collections were also made from localities in 56 other Health Areas. Different sampling techniques namely, pyrethrum-spray collections (PSC), cattle-baited hut collection, cattle-baited net trap collection, indoor hand collection, human-baited night collection, window (exit) trap collection, outdoor collection and light trap collection were used for collecting mosquitoes[9]. This was done to ensure that sibling species displaying different behavioural patterns may not be missed in the collections.

Unfed females of *An. culicifacies* collected by these methods were allowed to feed until repletion on cattle or human blood in field laboratories. These, and the already engorged individuals collected from the field were kept in paper cups under field conditions for ovarian development to proceed. Females with ovaries developed up to Christopher stage III were killed by tapping in an aspirator. The ovaries were dissected out and fixed in 1:3 glacial acetic acid: methanol.

The ovaries were fixed and transported to the Central Laboratory in Colombo for further analysis. Staining and preparation of the ovarian polytene chromosomes was done according to Green and Hunt's method[10]. Slides were examined under a compound microscope at 400x magnification. If readable chromosomes were present, the species was identified by comparing with chromosomal maps of sibling species[7]. Photomicrographs were obtained using ASA 32 black and white film.

RESULTS

An. culicifacies s.l. was collected from 53 out of 72 surveyed Health Areas. Readable chromosomes were obtained in specimens from 31 Health Areas (Table 1).

From a total of 1937 identified preparations during the study, the majority (52.5%) were from specimens collected from cattle-baited cadjan huts, while 28.5% were from indoor resting catches. Cattle-baited net trap collections produced 2.3% specimens. Human-biting catches contributed 0.2%, while other methods contributed <1%.

From a total of 4828 preparations made 1937 (44.8%) had readable chromosomes. In 1479 (76.4%) preparations both chromosome arm-2, and x-chromosome could be examined while from 458 only x-chromosomes could be studied. All preparations with readable chromosome arm-2 were similar to the pattern of sibling species B, in the case of those with readable x-chromosomes only, the banding pattern was that of xab, which includes sibling species B and C. Thus definitive cytospecies identification was not possible on the basis of x-chromosome characters alone.

On the basis of the banding pattern on chromosome arm-2, sibling species B was identified from the dry (1460 ovary preparations), intermediated (435), and wet (42) zones.

The occurrence of *An. culicifacies* at different elevational ranges is shown in Fig. 1. *An. culicifacies* was not col-

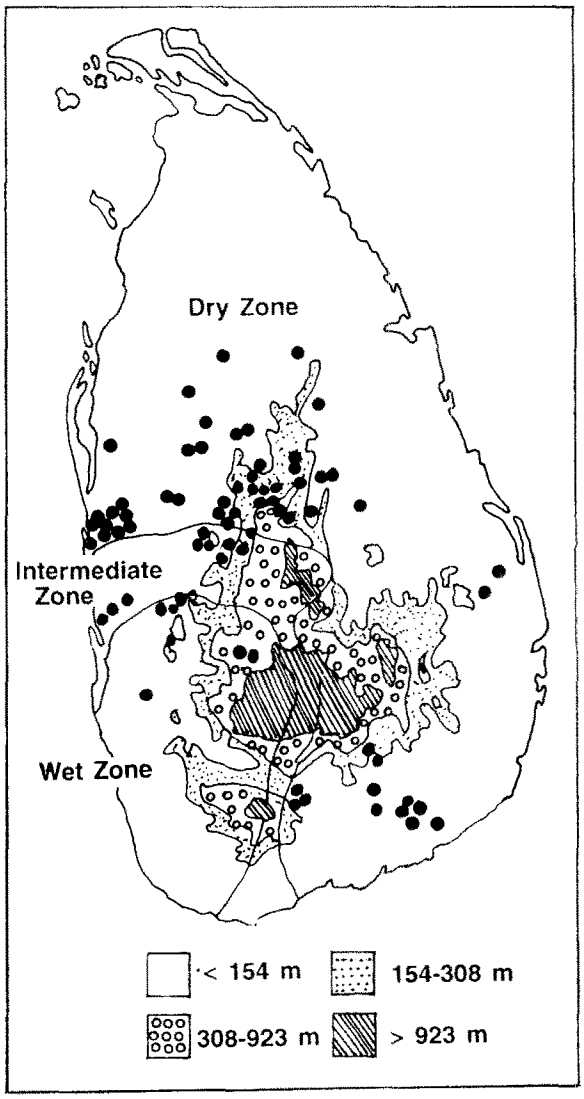

Fig. 1: Map of Sri Lanka showing the distribution of *Anopheles culicifacies* B at different climatic zones (Demarcated according to Vishvalingum, 1961) and altitudes. Solid dots indicate localities where *An. culicifacies* were found

Table 1. Bimonthly identification of *Anopheles culicifacies* species B in different Health Areas

Health Area	Jul/ Aug 90	Sep/ Oct 90	Nov/ Dec 90	Jan/ Feb 91	Mar/ Apr 91	May/ Jun 91	Jul/ Aug 91	Sep/ Oct 91	Nov/ Dec 91	Jan/ Feb 92	Total
Ampara	1(0)	–	–	–	–	–	–	1(3)	–	–	2(3)
Anuradhapura	–	–	1(0)	–	–	–	–	–	–	–	1(0)
Atakalanpanna	–	36(10)	74(9)	–	–	–	3(0)	–	–	0(1)	113(21)
Bingiriya	–	–	15(2)	14(1)	–	–	–	–	0(3)	0(1)	29(7)
Chilaw	43(16)	83(46)	15(2)	39(1)	55(19)	3(0)	2(0)	36(4)	2(1)	–	278(90)
Dambulla	51(27)	55(15)	38(13)	3(0)	17(0)	–	–	2(0)	5(4)	–	171(59)
Galewela	–	–	2(1)	20(4)	–	–	–	–	–	–	22(5)
Galgamuwa	–	5(6)	5(0)	7(0)	–	–	–	–	–	–	17(6)
Gokarella	9(9)	16(3)	28(7)	8(1)	19(7)	–	42(3)	14(1)	9(7)	0(1)	145(39)
Hingurakgoda	–	–	–	–	2(0)	6(1)	–	–	–	–	8(1)
Kahatagasdigiliya	–	6(1)	–	–	–	–	–	–	–	–	6(1)
Katana	3(1)	1(03)	13(0)	11(0)	–	–	2(0)	–	–	–	30(4)
Kekirawa	–	–	–	2(0)	–	–	–	1(0)	–	–	3(0)
Kirindiwela	–	2(0)	–	–	–	–	–	–	–	–	2(0)
Kurunegala	–	41(22)	100(25)	99(10)	37(4)	21(2)	3(0)	–	–	–	301(63)
Mawathagama	–	6(7)	1(0)	–	2(0)	–	0(1)	–	–	–	9(8)
Mirigama	–	2(2)	–	–	–	2(0)	1(0)	–	–	1(1)	6(3)

contd...

Table 1. (*contd.*)

Health Area	Jul/ Aug 90	Sep/ Oct 90	Nov/ Dec 90	Jan/ Feb 91	Mar/ Apr 91	May/ Jun 91	Jul/ Aug 91	Sep/ Oct 91	Nov/ Dec 91	Jan/ Feb 92	Total
Monaragala	16(24)	4(2)	–	–	–	–	0(2)	0(1)	0(1)	–	20(30)
Naula	–	–	–	–	–	–	–	0(2)	–	–	0(2)
Nawalapitiya	–	–	–	10(0)	–	–	–	–	–	–	10(0)
Padukka	–	–	–	–	4(0)	–	–	–	–	–	4(0)
Pannala	–	–	–	12(0)	–	–	–	–	–	0(1)	12(01)
Polgahawela	–	4(1)	–	4(0)	–	1(0)	–	–	–	–	9(1)
Polonnaruwa	50(27)	33(17)	9(7)	12(3)	5(5)	3(0)	52(20)	–	0(1)	–	164(80)
Puttalam	–	3(1)	16(1)	1(0)	–	–	–	–	–	–	20(2)
Rambukkana	–	–	–	–	–	–	–	–	–	3(7)	3(7)
Sandunpura	–	–	4(1)	–	–	–	–	–	–	–	4(1)
Thanamalwila	–	–	–	–	–	–	–	–	–	0(5)	0(5)
Thambuththe-gama	–	5(2)	25(5)	4(1)	–	–	–	1(0)	–	–	35(8)
Wariyapola	–	24(3)	30(4)	–	–	–	–	–	–	0(4)	54(11)
Wellarya	–	–	–	–	–	–	1(0)	–	–	–	1(0)
Total	173 (104)	326 (141)	376 (77)	246 (21)	141 (35)	36 (3)	106 (27)	55 (12)	16 (17)	4 (21)	479 (458)

Figures in parentheses indicate number identified on the basis of x-chromosome only .

lected from any area that is 923 m above sea level. Only 12 specimens were obtained between 308-923 m, 266 specimens were collected between 154-308 m elevation, and the rest (1659) from the lowlands.

DISCUSSION

During this study attempts were made to collect adequate samples of *An. culicifacies* throughout Sri Lanka. Specimens were collected from all administrative districts except Jaffana Peninsula, Mannar, Mulativu and Vavuniya. Relatively few specimens were obtained from the wet zone and southern part of the Island due to low density during the study period.

All specimen identified from various localities were of species B. Other chromosome arms-3, 4 and 5 were also compared whenever possible with those of the species B from India (photograph received from Dr. S.K. Subbarao) and no differences in the banding patterns were observed. These findings reveal that the species present in Sri Lanka is identical to the Indian sibling species B. The possibility that specimens of sibling species C may have existed among the material identified based on x-chromosomes alone cannot be ruled out. However, in view of the fact that not even a single specimen out of 1479 preparations identified on the basis of chromosome arm-2 characters belonged to sibling species B makes this possibility highly unlike. It is noteworthy that areas of south India nearest to Sri Lanka also contain *An. culicifacies* B as the dominant sibling species[7].

The present study conclusively establishes the presence of sibling species B on the Island. However, it cannot be considered a comprehensive survey of the status of the species throughout Sri Lanka. Several districts, particularly in the northern part of the Island, could not be studied because of civil unrest. In addition, information is lacking from several localities surveyed during the present study due to no collection of the species/non-availability of readable chromosomal preparations. Thus, the need for further field studies is required. Also, the identification of sibling species using other techniques such as DNA probes[11], karyotype identification by mitotic chromosomes[12-14] the cuticular hydrocarbon technique, and electrophoretic technique[15] are to be looked into.

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Operational Feasibility of Malaria Control by Burning Neem Oil in Kerosene Lamp in Beel Akbarpur Village, District Ghaziabad, India

M.A. ANSARI and R.K. RAZDAN

A field trial in Beel Akbarpur village, Dadri PHC, District Ghaziabad (U.P.) was carried out to test the impact of burning neem oil in kerosene lamp from dusk-to-dawn in living rooms on vector populations and incidence of malaria. Results revealed that burning 1% neem oil in kerosene lamps resulted in the deviation of *An. culicifacies* from living rooms to cattlesheds. This was also reflected when malaria incidence was compared in experimental and control villages. Cases/000 and *Pf*/000 were 1.03 and 0.0 in experimental village as against 9.6 and 4.3 in control village. Discontinuation of burning 1% neem oil in kerosene lamp resulted in recurrence of *An. culicifacies* in living rooms and increase in malaria incidence in experimental village.

Keywords: *An. culicifacies*, Malaria control, Neem oil

INTRODUCTION

In global malaria control strategy greater emphasis is given to personal protection measures to reduce dependence on insecticides in public health programme. To develop safer and indigenous methods of personal protection repellent action of neem oil (*Azadirachta indica*) against mosquito was seen on local application of oil alone and in combination with coconut oil[1]. Repellent action was also evident when 1% neem oil was burnt in combination with kerosene oil in a 100 ml capacity lamp from 1800-0600 hrs in living rooms[2]. In view of encouraging results field studies were carried

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Table 1. Entomological indices of Beel Akbarpur (E) and Anandpur (C) villages

Month/ Week	Av. MHD of *An. culicifacies*			
	Living room		Cattleshed	
	E	C	E	C
Pre-experimental period				
Apr 1992	79.7	105.5	74.8	131.0
May	50.0	69.0	45.2	74.5
Average	64.85	87.25	60.0	102.75
Post-experimental period				
Jun 1992	36.3	103.7	67.8	68.0
Jul	34.2	132.2	97.5	79.75
Aug	23.7	83.0	62.0	79.2
Sep	13.25	46.25	28.87	35.75
Oct	9.37	43.0	16.12	45.0
Nov	6.0	42.0	11.2	37.7
Dec	5.25	19.2	10.8	12.7
Jan 1993	3.25	21.0	12.0	22.7
Feb	4.2	29.2	15.2	28.7
Mar	5.1	30.7	16.2	32.2
Average	14.06	55.0	33.76	41.1
Follow-up				
Apr 1993	22.3	44.7	29.0	36.2
May	35.5	45.0	31.1	41.1
Jun	48.1	66.2	44.5	68.7
Jul	52.3	69.2	42.5	70.2
Aug	46.1	70.2	43.2	72.5
Sep	48.5	66.2	47.6	70.2
Oct	45.2	61.7	44.1	68.7
Nov	32.2	39.7	28.0	50.5
Dec	14.2	27.2	13.3	28.2
Average	38.26	54.4	32.33	56.25

Neem oil lamp was burnt from June 1992 to April 1993.

out in village Beel Akbarpur using neem oil in kerosene lamp. Results of this village-scale trial are presented in this paper.

MATERIALS AND METHODS

Beel Akbarpur (pop. 962) and Anandpur (pop. 1145) located in Dadri PHC, District Ghaziabad (U.P.) at 40 km south-east of Delhi were selected as experimental (E) and control (C) villages because of comparable vector population and incidence of malaria. *An. culicifacies* is the principal vector of malaria and breeds in irrigation channels, seepage water bodies and rain water collections. Both experimental and control villages were endemic for malaria. These villages were not electrified and kerosene lamps were used as the source of light. There were 462 human dwellings and 72 cattle-sheds in experimental village as against 510 and 19 in control village respectively. Neem oil procured from M/s. Unjha Ayurvedic Pharmacy, Gujarat, was used in the study. It was diluted in kerosene and stock solution of 1% neem in kerosene oil was distributed weekly to the head of the family to be used in the lamps from 1800-0600 hrs in living rooms (3.5 x 3.5 x 3.5 m). Tin lamp of 100 ml capacity with a wick (1 mm diam) and regulator was used throughout the study period. Used wicks and defective lamps were replaced with new ones. In control village only kerosene lamps were lit. The use of lamp was started in June 1992 and stopped in March 1993. Later follow-up studies were carried out for one year.

Early morning indoor resting density of *An. culicifacies* in living rooms and cattlesheds of experimental and control village was monitored fortnightly with the help of aspirator and flash light. Similarly, biting rate of *An. culicifacies* on human volunteer was monitored fortnightly in human dwellings. Blood smears were collected from fever cases during door-to-door surveillance and positive cases were given radical treatment.

RESULTS AND DISCUSSION

Entomological evaluation: Month-wise indoor average density of *An. culicifacies* in living rooms of experimental and control villages is presented in Table 1. Results revealed that the density of *An. culicifacies* in living room of experimental and control village was 64.8 and 87.2 respectively and more or less comparable during pre-experiment period (April-May). Burning of 1% neem oil in kerosene lamp resulted reduction in indoor resting density of *An. culicifacies* from June onwards in experimental village. The reduction was also reflected in subsequent months in comparison to control. The average indoor density of *An. culicifacies* was reduced from 64.8 to 14.06 in living rooms of experimental village. A reduction in density in living rooms of control village was also observed, where only kerosene lamps were used though it was less pronounced in comparison to experimental village.

Table 2. Impact of burning 1% neem oil in kerosene lamp on biting rate on human volunteers in Beel Akbarpur (E) and Anandpur (C) villages

Month	Av. mosquitoes collected/bait/night (1800 to 0600 hrs)											
	An. culicifacies			Total anophelines			*Culex*			Total mosquitoes		
	E	C	%p	E	C	%p	E	C	%p	E	C	%p
June 1992	0.0	3.37	100.0	0.0	17.37	100.0	6.0	25.37	76.3	6.0	42.75	85.9
Jul	0.87	7.75	88.7	8.75	51.5	83.0	17.37	55.5	69.5	26.12	107.0	75.5
Aug	0.25	6.5	96.1	2.25	23.0	90.0	12.25	34.0	63.9	14.5	57.0	74.5
Sep	0.0	6.25	100.0	1.25	12.25	89.7	3.5	11.5	69.5	4.75	23.75	80.0
Oct	0.25	2.75	90.9	0.87	5.62	84.4	2.5	10.25	75.6	3.37	15.87	78.7
Nov	0.0	2.25	100.0	0.5	4.25	88.2	1.0	4.0	75.0	1.5	8.25	81.8
Average	0.22	5.04	95.4	2.27	21.26	89.3	7.1	23.43	69.6	9.37	42.43	77.9

% p — Per cent protection; Twelve night collections.

In cattlesheds the density of *An. culicifacies* though increased initially in post-experimental period started declining from July onwards thereby showing either natural reduction or deviation to neighbouring villages. The same trend was seen in cattlesheds of the control village. The withdrawal of lamp resulted in gradual increase in the density of *An. culicifacies* in living rooms of both experimental and control villages suggesting thereby, that deviation was temporary in nature and reverted to normal on withdrawal of lamps. The average density was 14.06 in living rooms of experimental village during post-experimental period as against 38.26 in follow-up period. In control village, also marginal change was observed in density even after withdrawal of the kerosene lamps. This clearly shows that neem oil has repellent action and may be responsible for deviation of *An. culicifacies*. This can further be substantiated by comparing the data of biting rate on human volunteers in experimental and control villages.

The average female *An. culicifacies* collected/bait/night was 0.22 and 5.04 in experimental and control villages respectively during June–November, 1992. This shows 95.4% protection through neem oil burning with kerosene. Similarly, total anopheline females collected/bait/night were 2.27 in experimental and 21.26 in control villages and provided about 89.3% protection. The repellent action was more pronounced on anopheline as compared to culicines. Per cent protection against anophelines was ranged from 83-100, while it was 63-75 for culicines. Similarly, 7.1% *Cx. quinquefasciatus* females were collected/bait/night as against 23.4 in control village (Table 2). Similar results were obtained when esbiothrin-impregnated rope was allowed to smoulder in living rooms[3]. This clearly indicates the impact of burning 1% neem oil in kerosene lamp on entomological indices. The degree of protection varied considerably against various species of mosquitoes.

Epidemiological evaluation: Results of epidemiological evaluation are given in Table 3. Cases/000 during pre-experimental period (April-May 1992) were 3.1 and 2.6 in experimental and control villages and thus comparable. The impact of burning 1% neem oil in kerosene lamp was clear from July onwards as no fresh case of malaria was reported during the study period in the experimental village. Contrary to this, both vivax and falciparum cases were detected in control village. Cases/000 and *Pf*/000 were 1.03 and 0.0 in experimental village as against 9.6 and 4.3 respectively in control village. Similarly, SPR and SfR were 1.6 and 0.0 in experimental as compared to 12.2 and 5.5 respectively in control village.

The withdrawal of lamp resulted in increased malaria cases in experimental village. Both vivax and falciparum cases were recorded in experimental village suggesting resumption of transmission. Cases/000 and *Pf*/000 were

Table 3. Epidemiological indices of Beel Akbarpur (E) and Anandpur (C) villages, Distric Ghaziabad (U.P.)

Month	Epidemiological indices									
	Beel Akbarpur					Anandpur				
	BER	SPR	SfR	Cases per 1000	*Pf* per 1000	BER	SPR	SfR	Cases per 1000	*Pf* per 1000
Pre-experimental period										
Apr 1992	1.14	0	0	0	0	0.69	0	0	0	0
May	0.72	42.8	0	3.1	0	1.48	17.60	0	2.62	0
Average	1.87	16.6	0	3.1	0	2.8	12.0	0	2.62	0
Post-experimental period										
Jun 1992	0.72	14.2	0	1.03	0	0.87	20.0	0	1.74	0
Jul	0.83	0	0	0	0	1.74	5.0	0	0.87	0
Aug	0.51	0	0	0	0	0.69	0	0	0	0
Sep	1.66	0	0	0	0	1.22	21.42	14.28	2.62	1.74
Oct	0.51	0	0	0	0	0.78	11.1	0.0	0.87	0
Nov	0.41	0	0	0	0	0.34	50.0	25.0	1.74	0.87
Dec	0.31	0	0	0	0	0.43	20.0	20.0	0.87	0.87
Jan 1993	0.41	0	0	0	0	0.43	0	0	0	0
Feb	0.51	0	0	0	0	0.52	0	0	0	0
Mar	0.51	0	0	0	0	0.78	0.0	11.1	0.87	0.87
Average	6.44	1.6	0	1.03	0	7.86	12.2	5.5	9.60	4.3
Follow-up										
Apr 1993	0.72	0	0	0	0	0.52	0	0	0	0
May	0.41	0	0	0	0	0.26	0	0	0	0
Jun	0.51	0	0	0	0	0.34	0	0	0	0
Jul	0.41	0	0	0	0	0.26	0	0	0	0
Aug	1.97	5.2	0	1	0	0.78	11.1	0	0.87	0
Sep	2.18	4.7	0	1.0	0	0.52	33.3	16.6	1.74	0.87
Oct	2.18	9.5	9.5	2.0	2.0	0.52	0	0	0	0
Nov	1.35	0	0	0	0	0.69	0	0	0	0
Dec	1.14	0	0	0	0	0.61	0	0	0	0
Average	10.9	3.8	1.9	4.1	2.0	4.5	2.8	0.95	2.6	0.87

Neem oil lamp was burnt from June 1992 to April 1993.

4.1 and 2.0 respectively in experimental village as compared to 2.6 and 0.87 in control village. This indicates that smoke emitted from 1% neem oil in kerosene lamp has drastically reduced the man-mosquito contact and resulted in interruption of transmission of malaria in experimental village.

The study revealed that malaria transmission and mosquito nuisance can be largely reduced by using the indigenous technique. Kerosene lamps are commonly used for lighting houses in remote and inaccessible areas where electricity is not available, thus mixing of neem oil in kerosene could provide additional benefit of protection from mosquito bites and vector-borne diseases particularly malaria.

Neem oil used in the study was manufactured by a pharmaceutical company, so the cost was high. However, indigenous oil is priced approximately Rs. 35-50 per litre and for providing protection to 1000 people during transmission period of a year we may require about 50 litres of neem oil that will cost about approximately Rs. 2000. Per capita expenditure in a year will be Rs. 2.0 only provided the cost of kerosene is borne by the community. Kerosene consumption in a 100 ml tin lamp is only 70 ml for 12 h and if the lamp is lit for a month in a single room the total cost of kerosene would be Rs. 8.0 approximately per month and would be still cheaper method of protection in comparison to coil and impregnated mats[2]. It may also be right to point out that commercial devices do not provide more than 60-80% protection[4]. In view of this, neem oil or byproducts of neem could provide better and cheaper method of personal protection. Neem oil is indigenously available and considered safe in comparison to chemicals[5,6]. In future, neem oil could reduce our reliance on insecticides in public health programmes to a greater extent.

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SHORT NOTES

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First Report on Intraspecific Morphological Variations in Some Anophelines from District South 24-Parganas, West Bengal, India

NEELAM TANDON and BASAB BASAK

Keywords: Anophelines, Morphological variation, West Bengal

Morphological variations in the banding pattern of the palpi and hind tarsomere(s) of a few specimens belonging to five anopheline species namely, *An. theobaldi*, *An. tessellatus*, *An. aconitus*, *An. annularis* and *An. sundaicus* collected from District South 24-Parganas including the deltaic zone of West Bengal, India, were recorded for the first time.

Variations in the palpi were exhibited by *An. tessellatus*, *An. aconitus*, and *An. sundaicus* and both in palpi and hind tarsomeres in *An. annularis*. *An. theobaldi*, however, exhibited morphological variation in the hind tarsomere only.

Morphological variations in Indian anophelines in the form of ornamentation of palpal banding, tarsomere of hind legs and wings have been reported from time-to-time[1-11]. Since identification of anopheline species is mainly based on the morphological characters of hind tarsomere(s), palpi and wings, any variation from standard for type

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specimens cause confusion leading to erroneous identification.

Perusal of literature reveals that though morphological abnormalities in anophelines collected from U.P. Terai[2], Bihar[4], Andaman[6,7], Madhya Pradesh[11,12], and Gujarat[13] have been reported, occurrence of variations in anopheline collections from West Bengal have never been recorded so far.

Survey of the anopheline fauna in District South 24-Parganas during July 1991 to June 1992 revealed the presence of 16 species[14]. Out of these some specimens of *An. theobaldi*, *An. tessellatus*, *An. aconitus*, *An. sundaicus* and *An. annularis* exhibited peculiarities in the morphological characters of the hind legs and palpi, and differed from the description of the type specimen of the respective species. In view of the importance of *An. annularis* and *An. sundaicus* in transmission of malaria, in rural and estuarine regions of West Bengal respectively, it is considered worthwhile to describe the morphological variations encountered in specimens of each of the five species to avoid confusion in identification. Details of the variations are presented in Table 1 (Figs. 1-24).

It is worth pointing out that *An. theobaldi* was found in winters, *An. tessellatus* in monsoons, and *An. sundaicus* both in monsoons and winters, whereas, *An. aconitus* and *An. annularis* were encountered in all the three seasons in the study areas. Interestingly, none of the specimens of *An. aconitus* and *An. annularis* that were collected in summer exhibited any variations.

An. theobaldi exhibited four types of variations in the hind tarsi, which were never reported before. Morphologicai variations in the palpi have been reported from Rajasthan[2] and Orissa[10]. No palpal variation was, however, observed in the species during the present investigation.

While *An. tessellatus* (Fig. 7), *An. aconitus* (Figs. 8-11) and *An. sundaicus* (Figs. 16-24) exhibited morphological variations in palpi only, *An. annularis* showed variations both in hind tarsomeres (Figs. 5 and 6) and maxillary palpi (Figs. 12-15). Specimens of *An. tessellatus* exhibited only one type of variation in the palpi. Review of literature, however, reveals that morphological abnormalities in *An. tessellatus* have not been reported from any part of the country so far. *An. aconitus* collected during monsoons and winters showed four and two types of palpal variations respectively (Figs. 8-11), but those collected during summer did not exhibit any morphological abnormality.

Morphological variations in the palpi and wings of *An. aconitus* have also been reported[2], but the variations in the palpi observed during the present study differed from those observed earlier. Variations in the morphological characters of the wings, however, were

Table 1. Morphological variations in five anopheline species collected from District South 24-Parganas, West Bengal

Species		Description of variation	Description of type form
		Variation in hind leg	
An. theobaldi	(i)	Tarsomere-3 entirely dark except for pale interruptions which break the continuity of the dark area (Fig. 1)	Whole of tarsi-5 and 4 of hind legs white. Tarsomere-3 as shown in Fig. A .
	(ii)	III tarsomere entirely white with a narrow dark band near the proximal end (Fig. 2)	
	(iii)	Approximately 2/3 of distal part of III tarsomere white with two unequal dark bands proximally (Fig. 3).	
	(iv)	Tarsomere-3 white with a dark band almost in the centre (Fig. 4).	
An. annularis	(i)	Distal 1/3 of tarsal segment-3 dark (Fig. 5).	Tarsal segments 3, 4 and 5 completely white (Fig. B).
	(ii)	Distal half of tarsal segment 3 distinguishable into broad apical pale area followed by two narrow dark bands with an intervening pale area (Fig. 6).	
		Variation in the ornamentation of palpi	
	(i)	Apical and sub-apical pale bands and intervening dark band in left palp almost equal in length. Proximal part dark with a pale interruption. Apical and sub-apical pale band in right palp unequal, intervening dark band almost equal to sub-apical pale band. The proximal part of right palp entirely dark. Right palp slightly shorter (Fig. 12).	Three pale bands on the palp; apical pale band comparatively larger than two sub-apical pale bands (Fig. F).

contd...

Table 1. (*contd.*)

Species		Description of variation	Description of type form
	(ii)	Apical and sub-apical pale bands unequal; intervening dark area small. The apical pale band of left palp smaller and that of right palp broader than respective sub-apical pale bands. The proximal part of the palpi distinguishable into two broad unequal dark bands with a narrow pale interruption (Fig. 13).	
	(iii)	Apical and sub-apical pale bands unequal in one of the palpi; the latter being broader than former; the intervening dark band small. The proximal part of the palps with two unequal dark bands, the intervening pale area comparatively broader than type form (Fig. 14).	
	(iv)	Apical and sub-apical pale bands unequal; the apical pale band of left palp broader and that of right palp smaller than respective sub-apical pale bands. The proximal part of the palpi entirely dark (Fig. 15).	
		Variation in the ornamentation of palpi	
An. tessellatus	(i)	Right palp with 3 pale bands only. The apical and sub-apical bands unequal; the latter almost double the length of the former. Left palp resembles the palpi of type specimen (Fig. 7).	Four pale bands on the palp. The three distal bands are much broader than the proximal band that is narrow (Fig. D).
An. aconitus	(i)	The intervening dark band almost equal to the apical pale band. Right palp resembles that of type specimen (Fig. 8).	The two apical pale bands are equal or nearly equal in length and the intervening dark area is small (Fig. C).

contd...

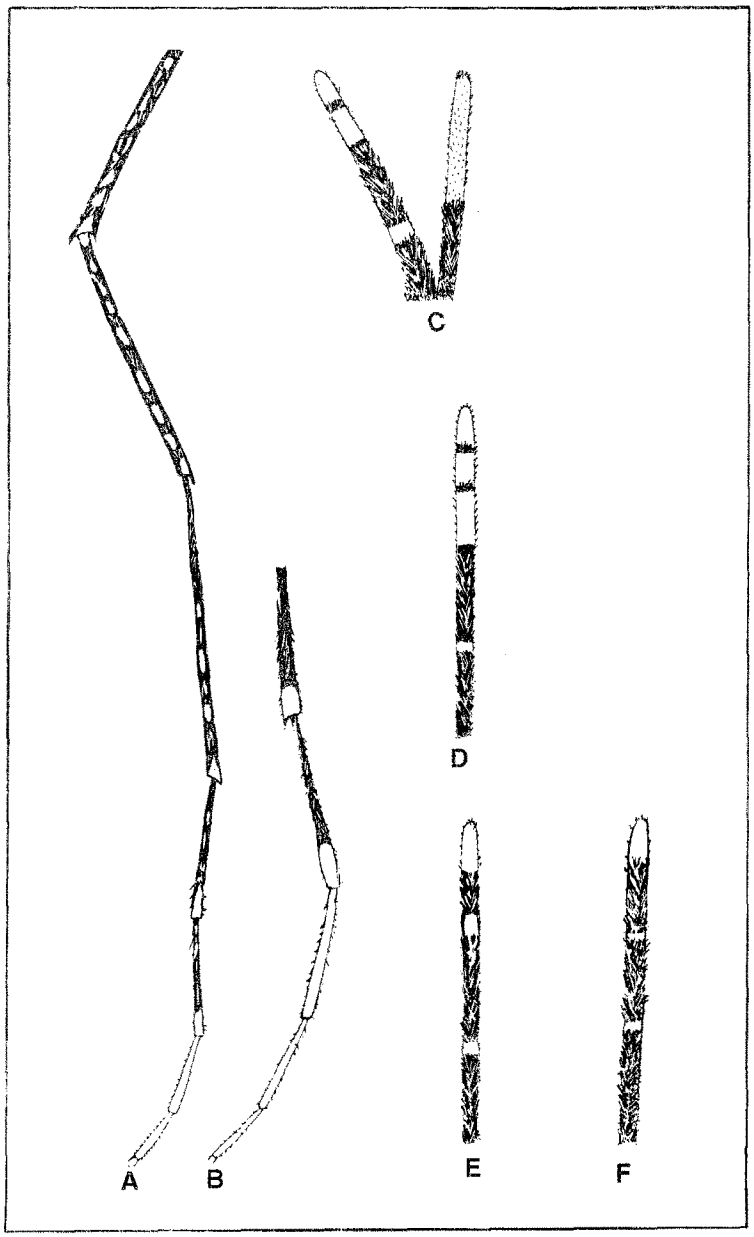
Table 1. (*contd.*)

Species		Description of variation	Description of type form
	(ii)	Apical and sub-apical pale bands of right palp unequal, intervening dark band almost equal to the sub-apical pale band. Left palp as in type specimen (Fig. 9).	
	(iii)	Apical and sub-apical pale band of left palp almost equal; intervening dark band slightly broader than both apical and sub-apical pale band. Right palp as in type specimen (Fig. 10).	
	(iv)	Apical pale band of left palp considerably smaller than sub-apical pale band (Fig. 11).	
An. sundaicus		*Variation in the ornamentation of palpi*	
	(i)	Three pale bands on the palp. The apical and sub-apical pale bands of each palp almost equal. The intervening dark area in left palp broader and in the right palp smaller than the pale bands. Left palp smaller in length (Fig. 16).	Three pale bands on the palpi. The apical and sub-apical pale bands unequal; the former being broader than the latter (Fig. E).
	(ii)	The apical and sub-apical pale bands and the intervening dark band equal or nearly equal in the left palp, while the apical and sub-apical pale bands in the right palp distinctly unequal; left palp smaller than right (Fig. 17).	
	(iii)	Apical and sub-apical pale bands unequal. Intervening dark band equal to the apical pale band in the left palp but small in the right palp (Fig. 18).	

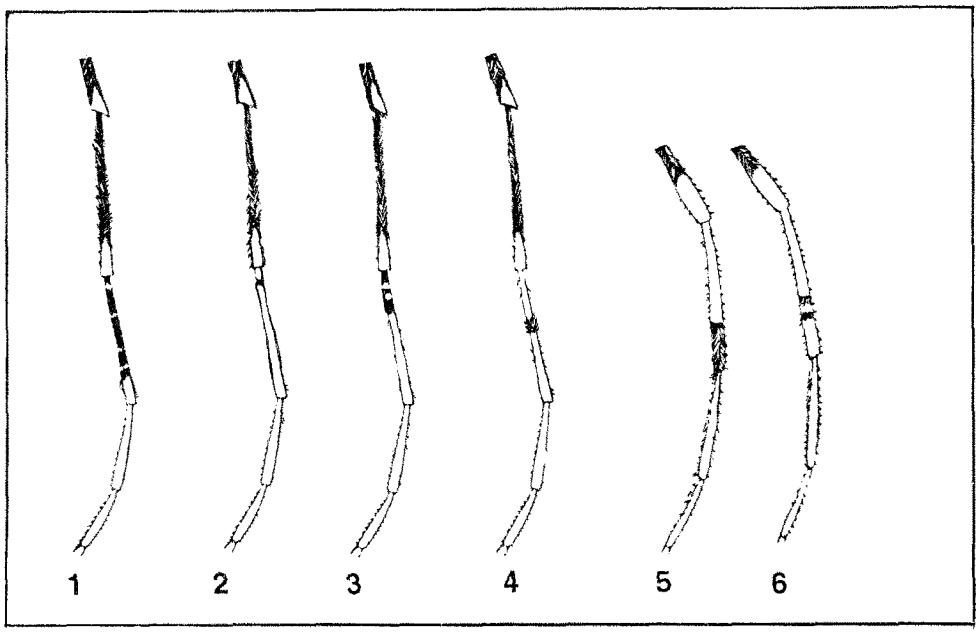
contd...

Table 1. (*contd.*)

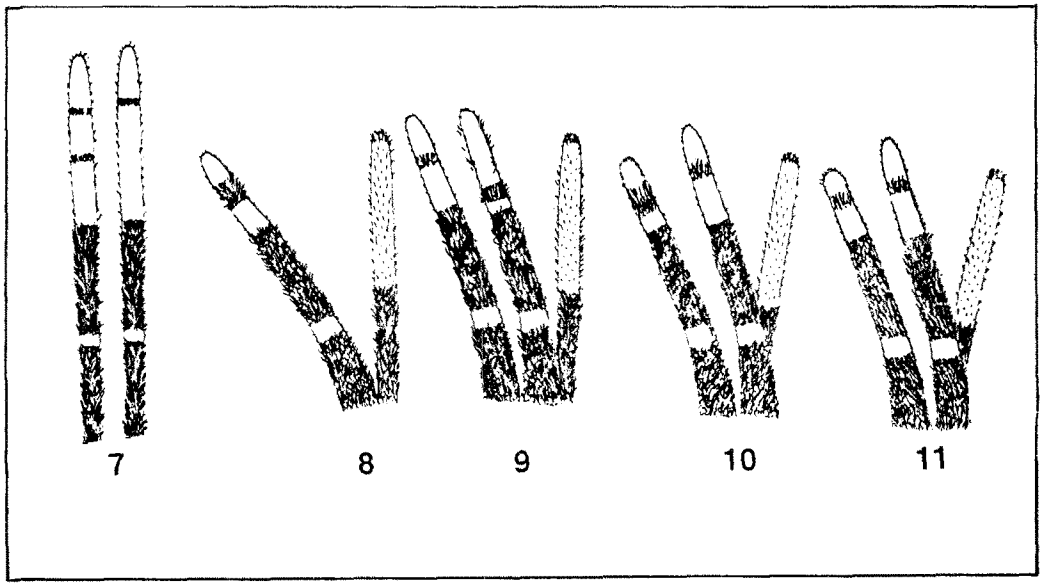
Species	Description of variation	Description of type form
	(iv) Three pale bands on left palp. The apical and sub-apical pale bands unequal the latter being broader than the former. A little less than anterior 1/3 of right palp entirely white. The proximal part of palp entirely dark. (Fig. 19).	
	(v) Apical and sub-apical pale bands unequal; the latter being broader than the former. Apical pale band of left palp with a narrow dark interruption (Fig. 20).	
	(vi) Two pale bands on the palpi; distal 1/3 of both the palpi entirely white (Fig. 21).	
	(vii) Left palp with three pale bands. The apical and sub-apical pale bands unequal; the latter being very much broader. Right palp with two pale bands. Anterior 1/3 of right palp entirely white (Fig. 22).	
	(viii) The apical and sub-apical pale bands unequal in one of the palpi; the former being broader than the latter; intervening dark band narrow (Fig. 23).	
	(ix) Left palp as in type specimen. In the right palp sub-apical pale band inconspicuously white (Fig. 24).	



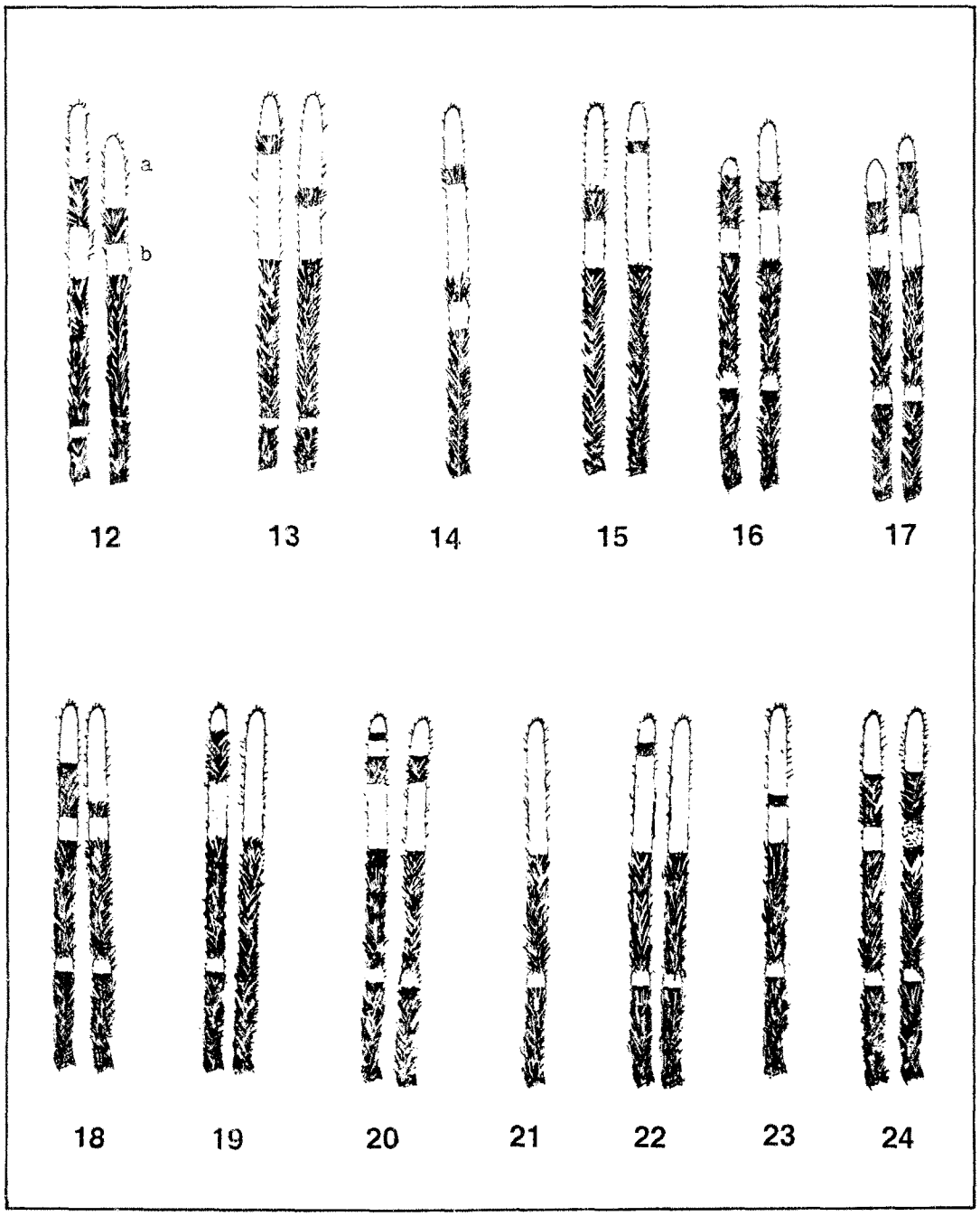
Figs. A-F: A and B — Hind legs of type specimens (A — *An. theobaldi*, B — *An. annularis*), C to F — Maxillary palpi of type specimens (C — *An. aconitus*, D — *An. tessellatus*, E — *An. sundaicus*, F — *An. annularis*)



Figs. 1-6: Variations in hind legs (1 to 4 — *An. theobaldi*, 5 and 6 — *An. annularis*)



Figs. 7-11: Variations in maxillary palpi (7 — *An. tessellatus*, 8 to 11 — *An. aconitus*)



Figs. 12-24: Variations in maxillary palpi (12 to 15 — *An. annularis*, 16 to 24 — *An. sundaicus*)

not observed during the present investigations.

An. annularis is one of the most prevalent species in District South 24-Parganas and was found throughout the year. Specimens showing variation in the banding pattern of hind tarsomeres (Figs. 5 and 6) and maxillary palpi (Figs. 12-15) were encountered during monsoons and winters only. Morphological variations in the hind tarsi, maxillary palpi and wings of *An. annularis* have been reported from time-to-time[2,4,5,8,12]. It is, however, to be pointed out that morphological variations in the palpi and hind tarsomeres as observed during the present investigation differed significantly from the observations made by earlier workers. Dash *et al.*[8,12] observed morphometrical variations in *An. annularis* collected from Orissa and Madhya Pradesh. Such morphometri-cal variations were, however, not ob-served during the present investigation. It is worth pointing out that occurrence of morphological variations in *An. annularis* collected from West Bengal are being reported for the first time. Since the vectorial status of *An. annu-laris* in rural West Bengal has been well established[15], acquaintance with the morphological variations in the species is necessary to avoid incorrect identification, specially in malarious areas.

An. sundaicus is one of the most important malaria carrying species in coastal areas of the country[16-18]. Disappearance of *An. sundaicus* from certain pockets of West Bengal had been reported[16]. In a recent survey conducted in District South 24-Parganas, the species was encountered from the deltaic blocks[14]. Nagpal and Sharma[7] recorded four types of palpal variations in *An. sundaicus* collected from Sipighat (south Andaman), but, none of them are in conformity with those reported in the present study. Morphological variations in collections of *An. sundaicus* from West Bengal are being reported for the first time. It is interesting to note that specimens exhibiting morphological abnormalities in the five anophelines species as observed during the present study, were found only in monsoons and winters and not in summer, a subject that needs to be investigated. Further studies are necessary to find out the influence of climatic and ecological conditions on the morphological characters of the species. Though occurrence of winter forms in *An. annularis* has been reported[1,8,12], morphological abnormalities in the species were encountered during summer also. Dash *et al.*[12] state that morphological variations may influence the behaviour and vectorial capacity of the species but it can not be claimed that the morphological characters and vectorial capacity of the anopheline species are governed by fluctuations in the seasonal parameters and ecological conditions.

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Use of Neem Cream as a Mosquito Repellent in Tribal Areas of Central India

NEERU SINGH, A.K. MISHRA and AJAY SAXENA

Keywords: Mandla, Mosquito, Neem (*Azadirachta indica*), Repellent

The repellent action of neem cream was evaluated against mosquitoes in two villages of District Mandla, Madhya Pradesh. About 2 g neem cream applied on the exposed body parts of a human volunteer provided 95% protection against *Anopheles culicifacies* throughout the night. Application of neem cream is safe and can be effectively used for protection against malaria.

The neem tree, *Azadirachta indica* Juss (Meliaceae) is known for its insecticidal properties[1]. Recently the repellent action of neem oil was tested in the field and laboratory against mosquitoes[2] and sandflies[3]. Results of field studies on the repellent action of neem cream (5% neem oil in vanishing cream) in tribal areas of District Mandla, central India are reported in this paper. This study was carried out between September to October 1994 for 25 nights only.

Study area includes two forest villages of Bizadandi PHC (23^0N latitude $80^010'$E longitude) of District Mandla. These are broken forest villages located in a valley traversed by perennial stream and inhabited by Gond tribe (90%). Baseline data collected during 1993 on the mosquito fauna of these villages revealed that there are 10 *Anopheles* species of which *An. culicifacies* Giles, 1901 and *An. fluviatilis* James, 1902 are known vectors of malaria. Among the anophelines, 85-90%

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of the specimens were *An. culicifacies* and prevalence of *An. fluviatilis* was rare (1-2%). Annual parasite incidence ranged between 200-250 with preponderance of *Plasmodium falciparum* (60-70%).

About 2 g neem cream was applied on exposed body parts of the human volunteers before 1800 hrs (experimental group) and simultaneously other volunteers (control group) applied ordinary vanishing cream for comparison. Collections were made from 1800-0600 hrs for 25 nights between September-October 1994, when the densities of all mosquito species were very high. Experimental and control volunteers occupied separate rooms and were allowed to lie on a cot. Mosquitoes landing on the exposed body parts were collected by an insect collector using a flashlight and suction tube. The experimental and control volunteers and their rooms were rotated to avoid bias resulting from the host/experimental

Table 1. Number of female mosquitoes collected on human baits using mosquito repellent cream*

Observation time (hrs)	No. of mosquitoes fed on volunteers					
	Neem cream**			Vanishing cream		
	An. culicifacies	Anophelines	Culicines	*An. culicifacies*	Anophelines	Culicines
1800-1900	0	0	0	0	0	1
1900-2000	0	0	1	8	9	7
2000-2100	1	1	1	12	13	6
2100-2200	1	2	2	14	18	9
2200-2300	1	3	0	14	18	8
2300-2400	0	0	0	8	9	4
2400-0100	1	1	0	7	8	4
0100-0200	0	0	1	6	6	0
0200-0300	0	0	0	3	3	0
0300-0400	0	0	0	1	3	3
0400-0500	0	0	0	2	2	5
0500-0600	0	0	0	0	0	4
Total	4	7	5	75	89	51

*5% neem oil in vanishing cream base; **2 g on exposed body parts; Apart from *An. culicifacies* eight other anopheline species, namely *An. subpictus, An. nigerrimus, An. splendidus, An. annularis, An. vagus, An. theobaldi* and *An. pallidus* were found in small numbers; Per cent protection for *An. culicifacies* : 94.66, Anophelines : 92.13, and Culicines : 91.20.

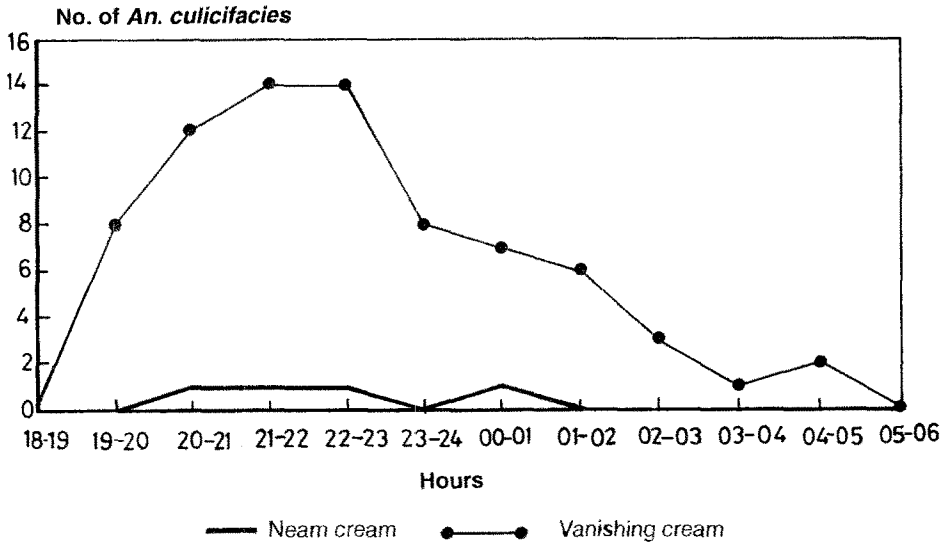

Fig. 1: Number of *An. culicifacies* collected on human baits using neem cream

room. The per cent protection was calculated as suggested by Sharma *et al*[2].

Neem cream produced a strong repellent action on anopheline and other nuisance creating mosquitoes (Table 1). Results revealed that protection provided for 12 h was 95% against *An. culicifacies* and 90% against culicine species (Fig. 1).

Malaria control in tribal areas by conventional methods of insecticidal spray as adopted under NMEP[4] is ineffective. Bioenvironmental methods for anopheline larval control are difficult to implement because of low population density and enormous breeding sites covered with dense vegetation. Fish can not be used in scattered temporary water-beds even with restocking. The management of streams become more difficult in summer when they form many river-bed pools and algal flora flourishes. Remote villages are inaccessible during rainy season. Use of insecticide-impregnated bednets that have proved very effective in north eastern states[5,6] is not feasible in this area because of outdoor life of tribals[7] and high population movement for collection of forest products.

Keeping in view aforesaid problems, 95% protection provided by neem cream is a promising solution to our fight against malaria.

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Feeding Behaviour of *Anopheles stephensi* in Calcutta

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Keywords: *An. stephensi*, Calcutta, Feeding behaviour

In a malarious area, the source of blood meal of the malaria vector has been considered as an important index[1,2]. The city of Calcutta is malarious since its inception and *An. stephensi* is the established vector of malaria[3,4]. Scanty information exist regarding the blood feeding spectra of *An. stephensi* in this metropolis[5], possibly due to paucity in the collection of wild adults from nature[5-9] as the daytime resting habitat was unknown. Discovery of its favourite daytime resting habitat has offered an opportunity for large-scale analysis of fresh blood meals of wild caught *An. stephensi*[4]. During the present study, fresh blood meals of 423 *An. stephensi* were analysed by treating with antisera of available hosts of the study area namely, human, cattle, dog and bird, followed by Ouchterlony gel diffusion technique[10,11]. A total of 423 *An. stephensi* were collected from indoor resting sites. Out of these, 225 and 198 specimens were collected from 66 human habitats and 29 cattlesheds respectively. The collection was made from four pockets of central Calcutta namely, Bowbazar, Central Avenue, Dharmatala and Sealdah in one year study period.

Out of 225 samples from human habitations, 213 (94.6%) and 2 (0.8%) were positive for human and bovine blood respectively. Ten (4.6%) samples were positive for mixed blood of human and cattle. Among 198 samples from

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cattlesheds (adjacent to human habitats), 109 (55.0%), 81 (40.9%) and 8 (4.1%) were positive for human, bovine and mixed blood (human and bovine) respectively. No blood sample was positive for canine and avian antisera. A high percentage of *An. stephensi* found resting in cattlesheds were positive for human blood. Probably, after having human blood from human habitats or from human hosts at outdoors, they come to rest undisturbed in the dark corners of cattlesheds. Combining both human habitat and cattlesheds, average human blood index was calculated to be 76.1. No significant ($p>0.05$) seasonal variations in HBI were noted during the present study. Roy *et al.*[5] carried out blood meal analysis of 172 *An. stephensi* for the first time from central Calcutta which showed that only 3.4% mosquitoes imbibed human blood and 95.5% bovine indicating a clear zoophagic tendency. So, in comparison to the previous findings human blood index has increased to a large extent at present, indicating an increasing trend of man-vector contact and thereby an increasing intensity of malaria transmission.

Human bovine ratio was not known during the present study. Recently due to removal of cattlesheds from central Calcutta there was reduction in cattle population. This might have changed the feeding behaviour of *An. stephensi*. Mukhopadhyay[12] eventually remarked that *An. stephensi* of Calcutta were probably losing their zoophagic characteristic. It is interesting that about 41% *An. stephensi*, collected from cattlesheds, imbibed bovine blood. According to Sweet and Rao[13] the type form of *An. stephensi* was more anthropophilic and var. *mysorensis* was zoophilic. So blood meal analysis indicates indirectly that both the forms of *An. stephensi* are inhabiting in Calcutta at present.

It is an established fact that the vectorial capacity and reproduction rate of malaria vectors vary as the square of the proportion of blood meals obtained from human host[1]. Therefore, high human blood index resulted in high malaria incidence in Calcutta which is 53% of the total cases recorded from West Bengal[14].

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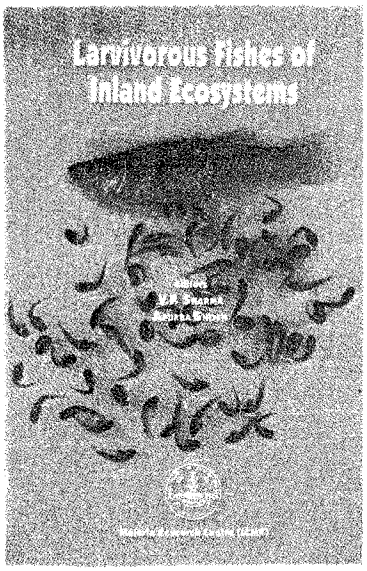

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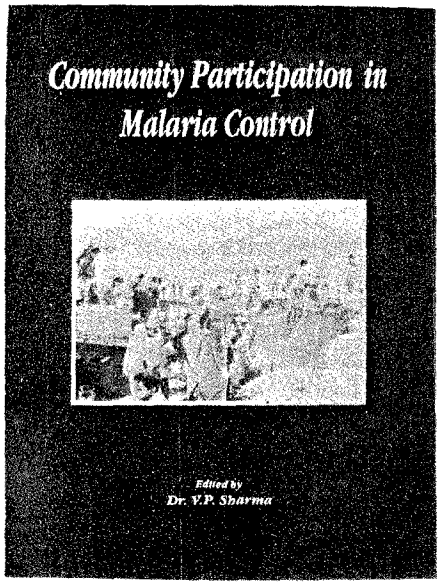

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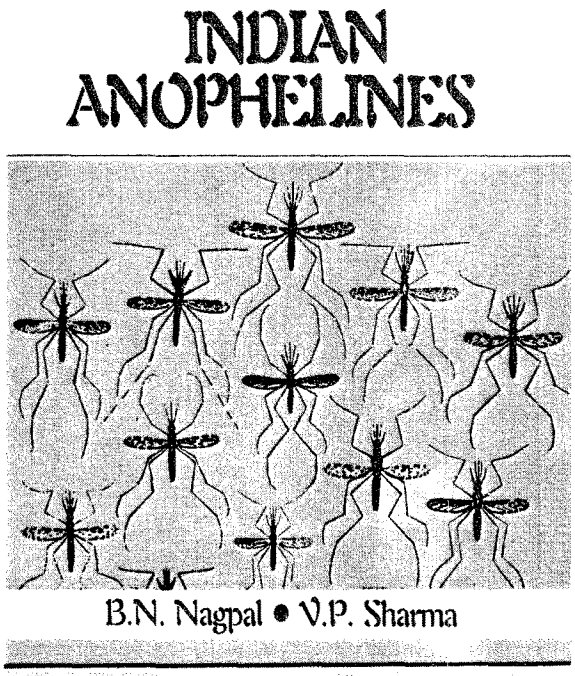

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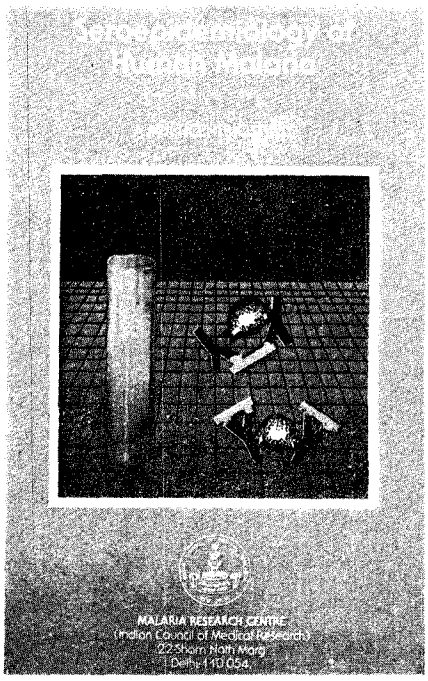

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