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Repellent Action of Neem Cream against Mosquitoes

VIRENDRA K. DUA, B.N. NAGPAL[a] and V.P. SHARMA[a]

Neem cream was used as mosquito repellent to provide protection against *Aedes albopictus*, *Ae. aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies* and *An. subpictus* mosquitoes. The application of neem cream on exposed body parts @ 2.0 gm/person showed 78 (range 65-95), 89 (range 66-100) and 94.4 (range 66-100) per cent protection against *Aedes*, *Culex* and *Anopheles* mosquitoes respectively. Significant difference was observed between neem cream treated and untreated group of population for *Aedes* mosquitoes ($p<0.001$). Application of neem cream was found to be a safe and suitable alternative to insecticide impregnated coils for personal protection against mosquitoes and one application was 68% effective for four hours.

Keywords: Neem, Cream, Mosquito, Repellent

INTRODUCTION

The repellent properties of some of the plants to mosquitoes and other pest insects are well-known before the advent of synthetic chemicals[1]. A review on the use of botanical derivatives against mosquito has been reported recently[2]. Coils containing synthetic pyrethroids[3] have become very popular however, prolonged use of insecticide impregnated coils and mats may be harmful to human health[4]. Neem (*Azadirachtha indica*) occupies an important place due to its strong action in inducing toxicity through inhibition of growth and reproduction of pest insects. On the basis of selective proper-

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ties from ecological perspectives, neem became a promising phytochemical in mosquito control. Recently, mats impregnated with neem oil are found very effective in repelling mosquitoes[5]. We report the repellent action of neem cream against different mosquito species.

MATERIALS AND METHODS

The study was conducted during July to October 1994 within the campus of Bharat Heavy Electricals Limited (BHEL), Hardwar for *Ae. albopictus* and *Ae. aegypti* mosquitoes, because of their extensive breeding in iron scraps, discarded drums and tyres and in the vicinity of Indian Oil Corporation (IOC), Mathura and National Thermal Power Corporation (NTPC), Shaktinagar for *Cx. quinquefasciatus*, *An. culicifacies* and *An. subpictus* mosquitoes.

Neem cream (5% neem oil in vanishing cream base)– a product of Malaria Research Centre (ICMR), Delhi was used throughout the study as repellent cream, while vanishing cream base was used as control.

Fifteen human bait collections were carried out in the month of July to October 1994 in evening between 1500 to 1730 hrs in the factory area of BHEL, Hardwar to study the repellency of neem cream against *Aedes* mosquitoes. Two gm neem cream was applied on each human bait uniformly over arms, legs and other exposed body parts, while control human bait was treated only with base cream. Similarly, 13 human bait whole night (1800-0600 hrs) collections in the vicinity of IOC, Mathura and 9 human bait whole night collections (1900-0600 hrs) in the vicinity of NTPC, Shaktinagar were carried out to find the repellent action of neem cream against *Cx. quinquefasciatus*, *An. culicifacies* and *An. subpictus* mosquitoes.

Landing mosquitoes were collected on the baits with the help of a suction tube and a flash light. Insect collectors and baits were rotated to avoid sampling errors due to bias. Mosquitoes collected were identified, pooled separately and tabulated for each collection.

Per cent protection was calculated by the following formula:

$$\% \text{ prot.} = \frac{\text{Nos. collected (Cont.-Expt.)}}{\text{Control}} \times 100$$

The significance of the difference between the number of mosquitoes caught under two categories was evaluated by student's *t*-test, while protection time for *Aedes* mosquitoes was determined as reported earlier[6].

RESULTS AND DISCUSSION

The results of repellent action of neem cream on human volunteers against *Aedes* mosquito are given in Table 1. Out of 1583 *Aedes*, 1538 (97%) were

Table 1. Repellent action of neem cream against *Ae. albopictus* and *Ae. aegypti* on human volunteers*

Mosquitoes		Total	Mean (range)	% reduction
Ae. albopictus	Control	1262	84.73 (29-141)	78.1
	Experimental	276	18.4 (3-38)	
Ae. aegypti	Control	39	2.6 (0-15)	84
	Experimental	6	0.40 (0-2)	
Total *Aedes*	Control	1301	86.73 (29-141)	78.3
	Experimental	282	18.8 (2-38)	

*Total collection of 15 h in 15 days.

identified as *Ae. albopictus* and 45 (3%) *Ae. aegypti*. Results clearly show that application of neem cream gave 78% (range 65-95) protection against the bite of *Aedes* mosquito. Statistical analysis of the data from experimental and control group showed highly significant difference ($p < 0.001$). It is observed that neem cream has similar repellent effect against *Ae. albopictus* and *Ae. aegypti* mosquitoes. The protection time varied from 1.5 to 3 hours which is low as compared to synthetic compound diethyl tolumide (DEET)[7]. It is noticed that 85, 80, 73 and 68% protection were recorded after 1, 2, 3 and 4 hours respectively of one application of neem cream on human volunteers from possible bite of *Aedes* mosquitoes.

Thirteen whole night collections were carried out on human volunteers at IOC, Mathura to see the effect of neem cream against *Cx. quinquefasciatus* and *Anopheles* mosquitoes and the results are given in Table 2. A total of 473 *Cx. quinquefasciatus* mosquitoes were collected from indoor and outdoor collections. Similarly, 102 *Anopheles* were also collected, out of which 31 (30%) were *An. subpictus* and 71 (70%) were *An. culicifacies*. Results revealed that the application of neem cream gave 89.87% (range 66-100) and 89.3% (range 71-100) protection against *Cx. quinquefasciatus* mosquito during indoor and outdoor collections respectively. Similarly, application of neem cream showed 94.87% (range 80-100) and 95.7% (range 66-100) protection against *Anopheles* mosquitoes during indoor and outdoor collections respectively. It is noticed that peak biting hours for *Anopheles* were between 2100 and 0200 hrs of the collection.

Nine whole night collections were also performed at NTPC, Shaktinagar to confirm the repellent action of neem cream observed at IOC, Mathura against *Culex* and *Anopheles* mosquitoes. These collections were aimed to find variation due to different topographic conditions on the action of neem cream. The results are shown in Table 3. A total of 269 *Cx. quinquefasciatus* were caught.

Table 2. Results of whole night collection (n = 13) at IOC, Mathura

Time (hrs)	Indoor						Outdoor					
	Experiment		Control		% protection		Experiment		Control		% protection	
	An.	*Cx.*	*An.**	*Cx.***	*An.*	*Cx.*	*An.*	*Cx.*	*An.*	*Cx.*	*An.*	*Cx.*
1800-1900	–	–	–	–	–	–	–	–	–	–	–	–
1900-2000	–	4	–	22	–	81.81	–	1	–	7	–	85.71
2000-2100	–	1	6	62	100.00	98.38	–	1	1	16	100.00	93.75
2100-2200	–	8	17	56	100.00	85.71	1	3	3	35	66.00	91.42
2200-2300	1	8	18	83	94.44	90.36	–	4	3	14	100.00	71.42
2300-2400	2	9	14	50	85.71	82.00	–	1	5	12	100.00	91.66
2400-0100	–	2	13	24	100.00	91.66	–	1	3	10	100.00	90.00
0100-0200	1	–	5	14	80.00	100.00	–	–	2	7	100.00	100.00
0200-0300	–	–	4	6	100.00	100.00	–	–	1	1	100.00	100.00
0300-0400	–	–	–	5	–	100.00	–	–	1	1	100.00	100.00
0400-0500	–	1	1	3	100.00	66.66	–	–	–	–	–	–
0500-0600	–	–	–	–	–	–	–	–	–	–	–	–
Total	4	33	78	326	94.87	89.87	1	1	19	103	95.7	89.32

**An.* were mainly *An. culicifacies* (70%) followed by *An. subpictus* (30%); ***Cx.* were identified as *Cx. quinquefasciatus*.

Table 3. Results of whole night collection (n = 9) at NTPC, Shaktinagar

Time (hrs)	Indoor						Outdoor					
	Experiment		Control		% protection		Experiment		Control		% protection	
	An.	*Cx.*	*An.**	*Cx.***	*An.*	*Cx.*	*An.*	*Cx.*	*An.*	*Cx.*	*An.*	*Cx.*
1800-1900	–	–	–	2	–	100.00	–	–	–	–	–	–
1900-2000	–	–	–	4	–	100.00	–	–	–	6	–	100.00
2000-2100	–	–	–	16	–	100.00	–	–	–	7	–	100.00
2100-2200	–	3	4	20	100.00	85.00	–	–	–	19	–	100.00
2200-2300	–	2	4	32	100.00	93.75	–	3	–	31	–	90.32
2300-2400	–	2	3	25	100.00	92.00	–	2	2	29	100.00	93.10
2400-0100	–	1	2	17	100.00	94.11	–	2	1	17	100.00	88.23
0100-0200	–	1	3	8	100.00	87.50	–	–	–	8	–	100.00
0200-0300	–	–	–	6	–	100.00	–	–	–	1	–	100.00
0300-0400	–	–	–	3	–	100.00	–	–	–	–	–	–
0400-0500	–	–	–	2	–	100.00	–	–	–	–	–	–
0500-0600	–	–	–	–	–	–	–	–	–	–	–	–
Total	–	9	16	135	100.00	93.33	–	7	3	118	100.00	94.06

**An.* were mainly *An. culicifacies* (75%) followed by *An. subpictus*; ***Cx.* were identified as *Cx. quinquefasciatus*.

Similarly, 19 *Anopheles* were caught during indoor and outdoor collections. Out of which 16 were *An. culicifacies* and 3 were *An. subpictus*. Results revealed that the application of neem cream on human volunteers showed 93.3% protection against *Cx. quinquefasciatus* and 100% protection against *Anopheles* in indoor collections (Table 3). Outdoor collections showed same results. Maximum number of mosquitoes were caught between 2100 and 0200 hrs of the collection.

A results of the efficacy of neem cream at IOC, Mathura and NTPC, Shaktinagar implies that the neem cream had similar repellent effect in both the areas and slight variations may be recorded due to change in climatic condition and the nature of the mosquito species.

Neem occupies an important place because of its strong action in inducing toxicity in pest insects through inhibition of growth and reproduction[2]. Zebitz[8] suggested that azadirachtin the main compound of neem acts as an anti-ecdysteroid or effects the neuroendocrine control of the ecdysteroids thereby emerging as a promising phytochemical in malaria control. All parts of neem plant have been reported as larvicidal and growth inhibitors[8-10]. Repellent action of neem oil against mosquitoes has been recently reported by Sharma *et al.*[5] and showed that neem oil impregnated mats produced better results as compared to commercially available allethrin mats. In the present study we have shown the use of neem cream for the personal protection against the bites of *Ae. albopictus*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies* and *An. subpictus* mosquitoes. Effective protection time varied from 1.5-3.0 hours and one application gave up to 68% protection after 4 hours of its application. It may be noted that no side effects on the skin or other parts of the human volunteers were observed during and after 3 months of the applications. Therefore, use of neem cream may be a suitable and safe alternative to insecticide for personal protection against *Ae. albopictus*, *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies* and *An. subpictus* mosquitoes.

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Anopheline Fauna of Ajodhya Hills, District Purulia, West Bengal

N. TANDON, B. BASAK and S. DAS[a]

Anopheline fauna of cattlesheds (CS) in Ajodhya hills, District Purulia, consisted of 12 species during summer, monsoon and 10 species during winter. Of the six vector species encountered in CS, *Anopheles culicifacies*, *An. annularis*, *An. subpictus*, *An. maculatus* and *An. fluviatilis* were found in all the three seasons. *An. philippinensis* was encountered in monsoon only and constituted 0.61% of the total anopheline catch made during the season. *An. culicifacies* was the most predominant species during summer (MHD 30.38) and monsoon (MHD 11) but ranked third (MHD 9.64) to *An. annularis* (MHD 16.28) during winter; *An. subpictus* being second (MHD 10.28) in order of predominance. *An. annularis* was the second most prevalent vector species in summer (MHD 21.38) and monsoon (MHD 9.92). The population density of the remaining three vector species was extremely low in the two seasons. In human dwelling (HD), of the five anopheline species encountered (*An. subpictus*, *An. annularis*, *An. culicifacies*, *An. vagus* and *An. splendidus*), the former three were found in all the three seasons. MHD of *An. annularis* in HD in summer, monsoon and winter was 3.7, 1.1 and 1.9 respectively, while MHD of *An. culicifacies* in the corresponding seasons was 5.01, 2.09 and 1.31 respectively.

Keywords: Anopheline fauna, Malaria vector, West Bengal

INTRODUCTION

Purulia is one of the three notoriously malaria endemic districts of West Bengal. The disease poses a serious health problem in Ajodhya hills of the district with transmission of the disease occurring throughout the year,

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and a peak being attained in monsoon.

Increase in the prevalence and incidence of malaria, specially in Ajodhya hills, Purulia district has been reported earlier (Data obtained from Deputy Director, Malaria, Deptt. of Health, Government of West Bengal).

Recent information on the anopheline fauna and vectorial competence of individual anopheline species in Ajodhya hills, is lacking. In view of paucity of literature with respect to the above and increase in the prevalence of the disease, a preliminary survey of anophelines of Ajodhya hills (a highly *P. falciparum* endemic pocket of the district) was carried out during 1993-94. The data on anopheline fauna of Ajodhya hills and results of dissection are presented in this paper.

Study area

Ajodhya hills is situated at Bagmundi block of Purulia district about 360 km northwest of Calcutta. It lies between Subarnarekha and Kansabati or Kasai valleys and is an elongated hilly area about 20 km long and 10 km wide. Its sides at some places rise steeply 200 to 300 m and the highest point Gorgaburu is 677 m high. Most of the land area is covered with forests interspersed with many streams. There are a number of small springs and fissures in rocks through which water comes out throughout the year. The western border of Ajodhya hills extends up to the river Subarnarekha and the northern side up to Gowai, a tributary of river Damodar. The houses are mud-built with thatched roofs. Rooms are dark and poorly ventilated, domestic animals and birds are usually kept inside the living rooms.

MATERIALS AND METHODS

Anopheline survey was conducted in Ajodhya hills, District Purulia during March 1993 to February 1994. Collection of adult anophelines was done from fixed number of cattlesheds and some human dwellings, entry into which was obtained with great difficulty. The survey was conducted during each of the three seasons, i.e. summer (March-June 1993), monsoon (July-October 1993), and winter (November 1993-January 1994); by two trained insect collectors for a fixed time in each habitat, during the early hours of the morning, by hand capture method using test tubes and torches. Aspirators were used to collect mosqutoes from inaccessible sites. The mosquitoes were identified[1] and data analysed. Gut and salivary glands of female *An. culicifacies* and *An. annularis* collected from human dwellings were examined for the presence of oocysts and sporozoites. Temperature and humidity at the study sites was recorded on each visit in all the three seasons.

RESULTS

Anopheline survey conducted in Ajodhya hills revealed the presence of 12

species (unidentical) during summer, monsoon and 10 species during winter of which six species are known to transmit malaria in different parts of the country. The anopheline density during the present survey was highest during summer and lowest in monsoon, contrary to expectations, but can possibly be attributed to extremely inclement weather, heavy rains and frequent northwesters during the survey period.

Five of the six vector species, i.e. *An. annularis, An. culicifacies, An. subpictus, An. maculatus* and *An. fluviatilis* were encountered in all the three seasons. *An. philippinensis* was found only in monsoon, and that too in negligible proportions.

The data also reveals that *An. culicifacies* was the most predominant species during summer (MHD 30.38) and monsoon (MHD 11). *An. annularis* ranked second (MHD 21.38) and third (MHD 9.92) in order of predominance in the two seasons respectively (Table 1). The population density of the three remaining vector species, i.e. *An. subpictus, An. maculatus* and *An. fluviatilis* was extremely low in summer and monsoons.

Table 1. Man hour density (MHD) of anophelines in cattlesheds of Ajodhya hills, District Purulia, West Bengal

Species	Man hour density		
	Summer (Mar-Jun 93)	Monsoon (Jul-Oct 93)	Winter (Nov 93-Feb 94)
An. vagus	6.05	10.76	4.5
An. annularis	21.38	9.92	16.28
An. philippinensis	–	0.23	–
An. culicifacies	30.38	11	9.64
An. nigerrimus	0.5	–	0.85
An. theobaldi	2.16	–	1.28
An. pulcherrimus	0.27	–	–
An. subpictus	0.66	1.76	10.28
An. splendidus	2.72	0.15	–
An. maculatus	0.27	0.07	0.64
An. fluviatilis	0.16	2	8.78
An. jeyporiensis	0.38	0.23	1.71
An. aconitus	–	0.15	–
An. jamesii	0.16	–	–
An. moghulensis	–	0.07	–
An. barbirostris	–	1.23	1.07

In winter, preponderance of *An. annularis* (MHD 16.28), followed by *An. subpictus* (MHD 10.28), *An. culicifacies* (MHD 9.64), *An. fluviatilis* (MHD 8.78) and *An. maculatus* (MHD 0.64) respectively was observed.

In human dwellings, only five anopheline species, namely *An. vagus, An. subpictus, An. annularis, An. culicifacies* and *An. splendidus* were encountered, the former four in all the three seasons, whereas, *An. splendidus* was not found in winters. Relative prevalence of the vector species in HD was significantly less than in CS in the corresponding seasons. MHD of *An. annularis* in summer, monsoon and winter was 3.1, 1.1 and 1.9 and of *An. culicifacies* 5.01, 2.09 and 1.31 respectively in the corresponding seasons.

Gut and salivary glands of *An. annularis* and *An. culicifacies* collected from human dwellings so far did not reveal any malarial infection.

The average temperature and humidity in summer was 31°C, RH 55%; in monsoon 28°C, RH 68% and in winter 16.5°C, RH 47% respectively.

DISCUSSION

Investigations on the anopheline fauna of Ajodhya hills, District Purulia, one of the three problem districts of West Bengal with respect to malaria, revealed the occurrence of six vector species in the study area; amongst which *An. culicifacies* and *An. annularis* were found in sufficiently high density in cattlesheds during summer and monsoons. While *An. subpictus, An. maculatus* and *An. fluviatilis* contributed very little to the composition of anopheline fauna during the two seasons, *An. philippinensis* was found in negligible proportions in monsoons only. During winters, *An. annularis* (MHD 16.28) predominated, followed by *An. subpictus* (MHD 10.28), *An. culicifacies* (MHD 9.64), and *An. fluviatilis* (MHD 8.78). The population density of *An. maculatus* (MHD 0.64) however, remained low (Table 1).

As mentioned earlier, only a few human dwellings could be searched for the presence of anophelines; *An. annularis, An. subpictus* and *An. culicifacies* were the only three vector species found, and that too in small numbers.

Perusal of literature reveals that *An. culicifacies* and *An. fluviatilis* were incriminated as vectors of malaria in Purulia, the former being more common at the foothills and the latter at higher regions[2].

During the present investigations, *An. culicifacies* was found to be the most common species in CS of Ajodhya hills. The prevalence of *An. fluviatilis* in CS was relatively low in summer and monsoon. In winters, however, a considerable increase in the density of the species was noticed (Table 1).

Rajagopalan *et al.*[3] and Reisen and Aslam Khan[4] observed *An. culicifacies* to be an indoor resting species and reported that more adults were collected from cattlesheds than from human dwellings. Similar observations were made in the present study. *An. fluviatilis* was observed to be an outdoor rester by Rao[5], whereas, Issaris *et al.*[6] observed otherwise.

Collection of *An. philippinensis* during the present study in significant specially when claims of its disappearance have been made[7]. The species was however, reported having been found in certain pockets of West Bengal[8].

As stated earlier, perennial transmission of malaria occurs in Ajodhya hills. Presence of at least two vector species, i.e. *An. culicifacies* and *An. annularis* in sufficiently high density throughout the year probably explains the phenomenon to certain extent.

Information on the relative prevalence of the four sibling species constituting *An. culicifacies* population in CS and HD of Ajodhya hills is necessary to reveal the ratio between the vector and non-vector species in the two biotopes and further studies on vector competance of each sibling species are also called for.

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ABO Blood Groups among Malaria Cases from District Mandla, Madhya Pradesh

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A total of 2095 patients with fever were tested for malaria and classified according to ABO blood groups. Only 696 cases were malaria positive. While blood group A, B and O were equally susceptible to malaria infection, AB blood group had less number of persons with malaria parasites. A significantly lower frequency of *Plasmodium falciparum* was observed among individuals with blood groups A and O. In other two blood groups B and AB, no difference in *P. vivax* and *P. falciparum* proportions were observed. A two-year study showed that the frequency of repeated attacks between all blood groups was similar.

Keywords: ABO blood groups, Malaria, *P. falciparum*, *P. vivax*

INTRODUCTION

Madhya Pradesh is a tribal belt and the tribal people are known by their specific genetic characters. The association of genetic markers with malaria has been the subject of many investigations in past. Since malaria has re-emerged as a major health problem in Madhya Pradesh during the past few years[1,2], a study has been initiated to know the relationship between blood groups and malaria infection among tribal population of District Mandla, Madhya Pradesh.

A few reports[3,4] of higher susceptibility to malaria in patients of blood group A, as compared with blood groups B and O are available. Athreya and Coriell[5]

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reported that blood group B may have an advantage in a malarious region. There are also reports that ABO blood groups may not have any relationship with malarial infection[6-9]. In order to resolve the relationship between the two, we have initiated this study in a malaria endemic area.

MATERIALS AND METHODS

Study site and population

A two-year study was carried out in a tribal dominating PHC, Bizadandi (consisting of 80% Gonds) which is known to be endemic for malaria. Annual parasite incidence and *Pf* per cent in this PHC were 76 and 45 in 1991, and 149 and 50 in 1992 respectively. This investigation was carried out on patients with fever who came to MRC clinic for blood test. A relevent history was taken from every patient including name, age, duration and nature of fever and medication taken so far. In every case, thick and thin blood smears were prepared, stained and examined for malaria parasites under 1000x magnification. ABO grouping was done by agglutination slide test[10]. Patients who had patent parasitaemia at the time of investigation were followed up to two years. On each monthly visit, blood smear was examined for malaria parasite if patient had fever. All those who were positive for malaria parasite were given radical treatment (chloroquine and primaquine). The dose given was according to the species of the parasite and age of the patient. This practice continued throughout the study. All the patients were advised not to take antimalarials other than those prescribed by MRC workers during febrile attacks. The baseline distribution of ABO blood groups in the normal population (afebrile cases) was established by taking blood samples from local inhabitants of the same PHC (control).

RESULTS AND DISCUSSION

A total of 2095 patients were tested out of which 696 were found to have patent parasitaemia. Table 1 summarizes the distribution of different blood groups in febrile individuals with and without fever at the time of collection of blood and in control group. Both the groups had similar socio-economic status and were living under similar ecological conditions. Using chi-square analysis the proportion of each blood group under febrile and non-febrile population was analysed. There was no significant variation in the frequency distribution of blood groups between the febrile and non-febrile population. The chi-square values for blood groups A, B, AB and O was 0.69, 0.31, 0.12 and 2.68 respectively. The frequencies of A, B, O and AB were comparable with the earlier reports from Madhya Pradesh[11].

P. vivax was the predominant infection being present in 368 cases (53%) out of a total of 696. Twenty-one cases (3%) had mixed infection of *P. vivax* and *P. falciparum*. Using chi-square analysis the total positivity observed in each blood group was analysed. While blood

Table 1. Distribution of ABO blood groups in fever cases with and without malaria in normal population control of Mandla

Blood groups	No examined (%)	Study group (Febrile)				Control group	
		Malaria positive			% positive	No. examined	%
		Pv	*Pf*	Mix (*Pv*+*Pf*)			
A	604 (28.83)	121	85	2	34.44	101	25
B	697 (33.27)	105	125	6	33.86	135	34
AB	245 (11.6)	38	26	2	26.94	32	8
O	549 (26.1)	104	71	11	33.88	132	33

groups A, B and O were equally susceptible to malaria infection, analysis showed that AB was significantly less prone to infection ($\chi^2 = 4.63$, $p<0.05$). In blood groups A and O the proportions of *Pv* was significantly higher than *Pf* (A 7.47, $p<0.01$; O 6.74, $p<0.01$). In other two blood groups B and AB, no difference in *Pv* and *Pf* proportion were observed.

A record of reinfection of malaria (*Pv* and *Pf*) in 2 yrs showed (Table 2) that 20.6, 14.8, 16.2 and 1.67% persons of A, B, O and AB blood groups respectively had two attacks of malaria, 6.9, 7.9, 4.7 and 6.6% had 3 attacks of malaria, and 1.2, 1.0, 1.67 and 2.0% had experienced four attacks. At least one or two per cent from each group (except AB) had experienced 5 to 7 attacks of malaria. Whether these cases were relapses, reinfection, recrudescence or drug failure etc. were not determined. Using one way analysis of variance the frequency of repeated at-

Table 2. Follow-up of malaria cases* in different blood groups

Blood group	No. of attacks reported							
	1	2	3	4	5	6	7	8
A	160	33	11	2	1	1	–	–
B	189	28	15	2	–	1	–	1
AB	60	1	4	1	–	–	–	–
O	148	24	7	3	2	1	1	–
Total	557	86	37	8	3	3	1	1

*Both *P. vivax* and *P. falciparum* are included in this study.

tacks between all blood groups were found to be similar.

The association of genetic markers with malaria has been the subject of numerous investigations. Sickle-cell anaemia and sickle-cell trait were found in high frequencies in the tribal population of Madhya Pradesh[12,13]. Evidences are available that HbS mutation confers a protective value against *P. falciparum* infection[14,15]. During our study more *Pv* cases were recorded from blood groups A and O. Therefore, screening for duffy antigens is very essential for *P. vivax* studies, so that the knowledge gained could be utilized for a better understanding of malaria dynamics in the area.

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Control of Mosquito Breeding using Wood Scrapings Treated with Neem Oil

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Wood scrapings were given shape of a ball and soaked in 5, 10 and 20% neem (*Azadirachta indica*) oil diluted in acetone. Control of *Anopheles stephensi* and *Aedes aegypti* breeding in water storage overhead tanks (OHTs) with the application of these balls was achieved for 45 days. Two balls soaked in 5% neem oil produced the best results among other concentrations tested.

Keywords: Dengue, Malaria, Mosquito, Neem oil, Vector control

INTRODUCTION

Anopheles stephensi and *Aedes aegypti*, are the well-known vectors of urban malaria and dengue fever (DF) respectively. Both vectors breed in domestic water containers such as overhead water tanks (OHTs), water storage tanks, earthen pots, used and unused wells, desert coolers, tyre dumps, masonry tanks and curing tanks etc.[1-4].

In urban areas malaria and dengue have emerged as major problems. At the time of implementation of NMEP in the country, urban malaria was a marginal problem but today it contributes to nearly 12% of the total reported cases in the country with many focal outbreaks. *Ae. aegypti* has caused 40 dengue outbreaks till date in different parts of the country[5-7].

To control vector breeding a simple method of balls made of wood scrapings, soaked in neem oil solution was developed. These balls were introduced in water tanks with heavy breeding of *An.*

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stephensi and *Ae. aegypti*. Results of the impact of neem oil treated balls on mosquito control are given in this paper.

MATERIALS AND METHODS

A residential colony in south Delhi namely Ayurvigyan Nagar was selected for field trials. This colony has about 300 cement overhead water storage tanks (OHTs) of 40 cm radius and 1 m height (0.50 cubic m water capacity) which are connected to the municipal water supply. The tanks were generally half filled (0.25 cu m) with water which is used only for household chores and not for drinking. In October 1993, a survey of mosquito breeding revealed that 24 OHTs out of 100 were positive for mosquito breeding (20 OHTs with *An. stephensi* and 4 with *Ae. aegypti* + *An. stephensi*). Out of 24 breeding OHTs, 18 were with neem oil balls and two were held as untreated control.

The balls were hand-made from wood scrapings. In India wood scraping is used to prepare pads for desert coolers. The scraping weighing 250 gms were mixed in 250 ml acetone containing 5% neem oil thoroughly for 10 to 15 min to ensure uniformity. Precautions were taken to avoid any losses of neem oil. 25 balls were made out of 250 gms of scraping, each weighing about 10 gms. The balls were held in shape with the help of rubber bands. Similarly balls with 10 and 20% neem oil were also prepared.

One week after preparation of neem oil balls 18 positive tanks were treated with 2, 4 and 6 balls dipped in 5, 10 and 20% neem oil. For each concentration and number of balls two replicates were used. In a separate experiment density of aquatic stages of mosquitoes in tanks with balls treated with only acetone were compared with tanks without any balls, and the difference found was statistically insignificant. Hence, tanks without balls were kept as control. Before the introduction of neem oil treated balls average immature density was measured in each of the experimental and control OHT, by taking 5 dips using a dipper of 500 ml capacity. In each dip, number of larvae and pupae were counted for estimation of average density per dip.

To observe the impact of neem oil treated balls on the density of immatures, first observation was recorded after 24 h of treatment by taking five dips in each of the experimental and control OHT and then up to 49 days continuously at an interval of 2 to 8 days. All the immatures encountered in dips were reintroduced in the same tank after counting. Care was taken to ensure no mortality during handling.

RESULTS AND DISCUSSION

A total of 18 OHTs were treated with 5, 10 and 20% of neem oil balls and 2 OHTs were held as control. Out of 18 OHTs 16 were positive for *An. stephensi* and two for *Ae. aegypti* and *An. ste-*

Table 1. Impact of balls made of wood scrapings* and soaked in neem oil on the control of mosquto breeding in Ayurvigyan Nagar

Days	Concentration of neem oil																		Control	
	5%						10%						20%						L	P
	2 balls		4 balls		6 balls		2 balls		4 balls		6 balls		2 balls		4 balls		6 balls			
	L	P	L	P	L	P	L	P	L	P	L	P	L	P	L	P	L	P		
0#	7.8	0.7	3.0	0.1	4.3	0.4	4.5	0.6	3.0	0.0	3.3	0.0	4.0	0.3	3.0	0.6	3.7	0.3	2.62	0.37
1	5.6	0.0	1.2	0.0	1.9	0.0	1.4	0.0	1.5	0.0	2.6	0.5	2.6	0.3	2.6	0.4	3.5	0.2	2.18	0.33
7	0.9	0.0	1.5	0.7	1.2	0.0	0.7	0.3	1.9	0.0	4.4	0.5	0.9	0.0	1.1	0.4	3.2	0.0	2.6	0.09
9	0.6	0.0	1.2	0.0	1.7	0.1	0.9	0.0	0.6	0.0	2.2	0.0	1.4	0.0	1.1	0.2	2.6	0.6	1.91	0.09
13	0.3	0.0	1.2	0.0	0.9	0.0	0.8	0.0	0.2	0.1	0.7	0.1	2.0	0.0	0.9	0.2	0.4	0.0	8.63	0.25
17	0.8	0.0	0.7	0.2	1.3	0.0	1.5	0.0	0.8	0.6	1.2	0.1	3.0	0.1	0.8	0.5	0.0	0.0	6.12	0.08
21	1.2	0.0	0.8	0.0	1.4	0.2	0.9	0.0	0.6	0.0	0.6	0.3	0.4	0.5	0.6	0.1	0.0	0.0	2.15	0.17
28	0.9	0.0	1.3	0.0	1.0	0.0	0.8	0.0	0.9	0.0	0.0	0.0	0.1	0.4	1.0	0.0	0.0	0.0	1.35	0.3
36	0.7	0.0	1.4	0.0	1.2	0.1	0.8	0.0	0.92	0.0	0.0	0.0	0.2	0.3	1.1	0.0	0.0	0.0	1.8	0.39
44	0.9	0.0	0.3	0.4	0.0	0.2	0.0	0.0	1.1	0.0	2.3	0.1	0.5	0.3	0.4	0.5	0.2	0.4	1.45	0.4
48	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.2

*Weight of one ball is 10 gms; #Pre-treated larvae/dip, pupae/dip; L – Larval density (I-IV instar); P – Pupal density; The density of larvae and pupae/dip is an average of 10 dips, 5 in each replicate.

phensi both. Control OHTs were breeding for *An. stephensi* and *Ae. aegypti*. The impact of neem oil treated balls on the mosquito breeding is given in Table 1. The average density of larvae before treatment ranged from 4.0- 7.8 in case of 2 balls, 3.0 in 4 balls and 3.3-4.3 in tanks with 6 balls. The average pupal density in the case of tanks with 2 balls was about 0.6, 4 balls 0.0-0.6 and 6 balls 0.0-0.4. In control OHTs, the average larval and pupal density was 2.5 and 0.37 respectively. Table 1 also reveals that the application of neem oil treated balls reduced both the larval and pupal density as compared to control (untreated tanks), and in OHTs treated with 2 balls of 5% neem oil pupal density reduced from 0.7 to 0 and the same was maintained for 45 days.

The effect of different concentrations of neem oil in OHTs treated with 2, 4 and 6 balls was compared separately using one way ANOVA and no significant difference was found in larval densities ($p>0.21$) except in case of pupal density in the tanks treated with 2 balls ($p<0.02$). Similarly, the effect of the number of balls on larval densities was compared for each concentration and no significant difference was observed ($p>0.15$). Each combination of number of balls and neem oil concentration was compared with control separately using *t*-test and the difference found was highly significant in each case ($p<0.01$).

Any increase in the number of balls and oil concentration had no significant difference in reduction of the larval densities. However, per dip larval densities showed that 2 balls soaked in 5% neem oil in 0.25 cu m water produced the best results. No explanation could be found as to why higher concentration or higher number of balls/ OHT were less effective, may be acetone facilitates partitioning of oil from wood into water. Fig. 1 (a and b) shows reduction in larval density from third day onwards till 45th day and pupal production was also successfully controlled till Day 45. It was also observed that various concentrations of neem oil or number of balls introduced in tanks to control mosquito breeding does not prevent breeding of *An. stephensi* and *Ae. aegypti* but arrests pupal production.

This technique of vector control can be effectively applied to any non-potable water storage tanks, curing tanks in construction sites, OHTs, desert coolers, tyre dumps etc. In India desert coolers support heavy *Ae. aegypti* breeding and one such application would be sufficient to control breeding for the entire rainy season. Toxicological studies have shown that neem oil is safe for human[8]. Neem preparations are also used in the treatment of diseases particularly skin ailments and it is found to have antibiotic properties[9].

In Delhi there are about 0.5 million OHTs, and 0.7 million coolers[10]. To control breeding of *An. stephensi* and *Ae. aegypti* in these inaccessible sites is always a difficult task. Complete dry-

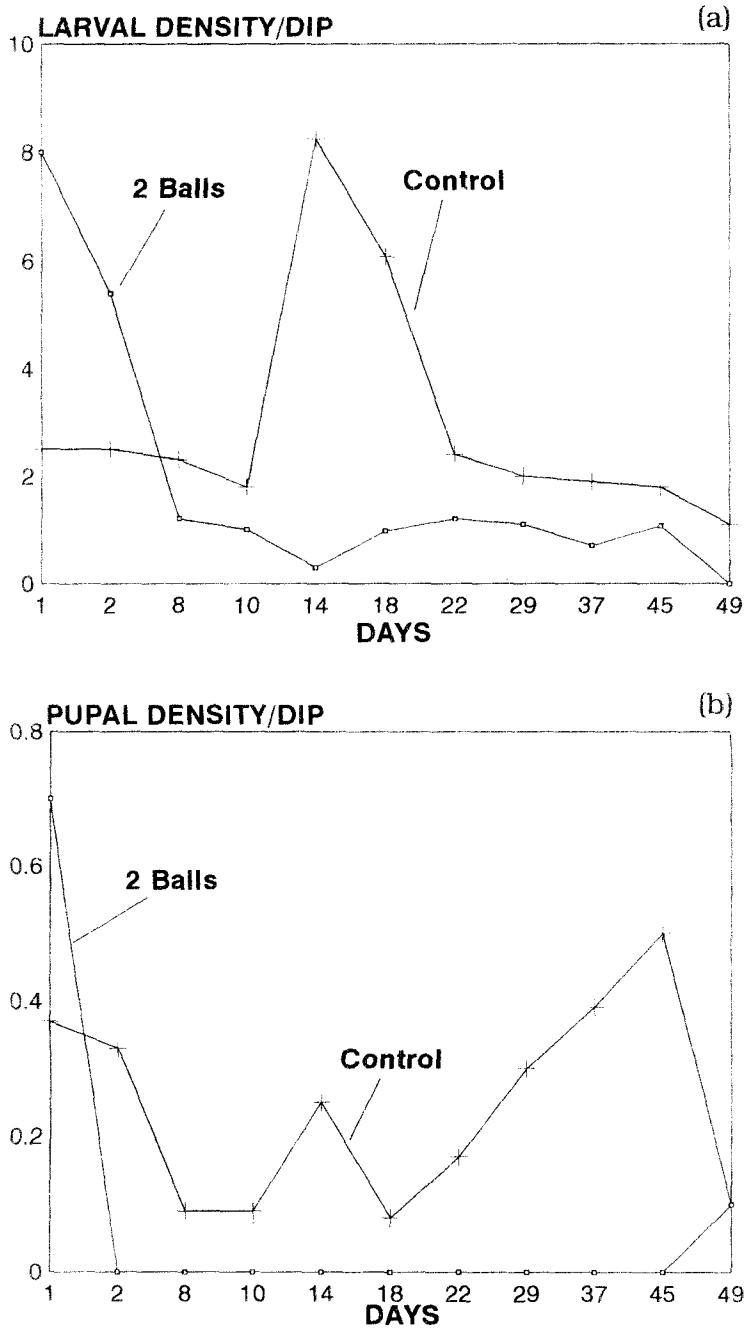

Fig. 1: Effect of two wood scrapping balls dipped in 5% neem oil on *An. stephensi* and *Ae. aegypti* (a) Larval development, and (b) Pupal production in overhead water storage tanks

ing of these sites on a weekly basis and chemical control are quite cumbersome and unacceptable to the owners. Therefore, application of neem oil treated balls made of wood scrapings is a simple, non-toxic and cost-effective technique for controlling mosquito breeding in stored water.

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Cerebral Malaria in Jabalpur, India

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A total of 1783 patients were admitted in Govt. Medical College Hospital, Jabalpur with fever in 1993. Out of these 152 (8.5%) patients had cerebral malaria, of which 39 (25.6%) patients died. Age and sex-wise break-up indicated that males suffered more ($p<0.01$) from malaria and majority of patients belonged to 16-40 yrs age-group. Mortality was significantly higher in patients with hyperparasitaemia, hypoglycaemia and delayed diagnosis and treatment. Comatose condition was the main determinant of death.

Keywords: Cerebral malaria, Chloroquine resistance, *Plasmodium falciparum*

INTRODUCTION

In Madhya Pradesh (MP) malaria is still a major cause of morbidity and mortality. It is the largest state in the country (52.2 million population of which 12 million are tribals) and a reservoir of intense perennial malaria transmission (0.25-0.30 million cases annually with 40-55% *Pf* cases)[1]. Both *Plasmodium vivax* and *P. falciparum* are prevalent[2] with a high degree of chloroquine resistance in *P. falciparum*[3,4].

We have carried out a hospital-based study on mortality due to malaria in patients admitted in the Govt. Medical College, Jabalpur with the main objective to see the incidence of cerebral malaria cases and their management problems in a government hospital at

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the district level. Results of this study are reported in this paper.

MATERIALS AND METHODS

Study area

District Jabalpur is one of the most important city of MP with 101.6 thousand sq km area and 2.6 million population. The district is divided into 15 primary health centres (PHC). Government Medical College is the largest medical facility in the district and serves both as a hospital for local people and referral hospital for the division comprising six districts. Slums are common in urban areas. Rural areas are mostly rocky, undulating and some areas are inaccessible during rainy season (July-September) with predominantly tribal population settled in the forest. *Anopheles culicifacies, An. fluviatilis* are the vectors in rural areas and *An. stephensi* in urban areas.

Study population

All patients with high fever were admitted in Govt. Medical College Hospital and administered antimalarial therapy, principally intravenous chloroquine on the basis of clinical symptoms. Peripheral blood smear is not made routinely before giving chloroquine. A case sheet giving patients name, age, sex, address and diagnosis is prepared by the physician. Patients who responded quickly to the antimalarials were discharged, whereas those with not much improvement after 12-24 h were referred to Malaria Research Centre for malaria diagnosis. All patients who were hospitalized in Medicine and Paediatrics Department between January to December 1993 were included in the study. Pregnant women were excluded from the study because they were further referred from Medicine to Obstetrics and Gynaecology Department and complications and mortality in pregnant women are influenced by a number of factors which may not be common with otherwise normal adults. On admission histroy was obtained and physical examination was performed.

Diagnostic criteria

Degree of coma was determined using Glasgow-coma scale[5]. High parasitaemia was defined as more than 50 per 1000 red blood cells (5%) were infected by the malaria parasite in a thin blood smear[6]. Severe anaemia was defined as a haemoglobin level less than 7 gm %. The blood glucose was ascertained on admission and hypoglycaemia was defined as a blood glucose level less than 45 mg %. Serum bilirubin was determined for jaundice and blood urea for renal function. Lumber puncture was performed to exclude meningitis. Post-mortem examination was not performed.

Treatment

Due to non-availability of quinine, only few patients were treated with loading dose of intervenous quinine dihydrochloride 20 mg salt/kg body weight over

4 h, followed by maintenance dose of 10 mg salt/kg 8 hourly. Oral therapy was substituted as soon as the patient was able to take the drug. Remaining patients were given chloroquine at dose of 5 mg base/kg body weight 6 hourly until oral therapy was possible. Vital signs (pulse, blood pressure, respiratory rate) and temperature were recorded 6 hourly. Intravenous fluids were given to maintain hydration and patent I/V line. One or two units of blood was given to some patients with high parasitaemia (>10%) as whole blood exchange was not possible. In addition, symptomatic treatment for hypoglycaemia, severe anaemia and fever were also given. Drug-resistance could not be monitored as generally patients leave hospital for economic reasons and do not return for follow-up. *Z*-test was used to compare the difference between sample mean.

RESULTS AND DISCUSSION

Table 1 summarizes the result of one-year admission of cerebral malaria cases. A total of 1783 patients with fever (1053 adults and 733 children) were hospitalized in the medicine and paediatric wards, of these 564 patients were admitted with malaria (401 adults and 163 children). Among these 152 were cerebral malaria cases (95 adults and 47 children). Significantly more malaria cases were recorded in adults (71%) than in children (29%, $p<0.01$), however, number of cerebral malaria cases were more or less similar in both the age-groups. Males suffered more ($p<0.01$) and majority of the patients were in the age-group of 16-40 yrs. This may be because of the outdoor activities and mobility of majority of male population of this age-group for economic reasons. Of the 152 cases,

Table 1. Age and sex-wise distribution of malaria and cerebral malaria cases in Govt. Medical College, Jabalpur

Age-groups	Fever cases	Total malaria cases		Cerebral malaria	
		Male	Female	Male	Female
> 1	83	1	0	0	0
1-4	320	58	30	15 (3)	6
5-15	433	111	47	33 (3)	15 (2)
16-40	452	136	57	21 (10)	16 (9)
> 40	495	73	51	33 (7)	13 (5)
Total	1783	379	185	102 (23)	50 (16)

Figures in parentheses indicate the number of fatal cases.

47 patients (31%) were referred from nearby districts. A total of 39 patients (31 adults and 8 children) with cerebral malaria died, of which 15 were referred cases. Most of these patients were from urban slums settled on the bank of Narmada. Of these 7 cases could not be given antimalarials and they died within 12 h of admission. In children, in addition to 8 deaths, there were 28 deaths due to meningitis of which 10 were having meningitis with cerebral malaria.

Table 2 shows the clinical and laboratory findings in cerebral malaria patients. All patients with cerebral malaria were febrile with and without any periodicity of fever. Fifteen patients (8 adults and 7 children) had hypoglycaemia (one adult had less than 20 mg % blood glucose level). Ten patients (4 adults and 6 children) had jaundice (highest serum bilirubin 13.2 mg gm %. Oligourea was recorded in 13 cases (8 adults and 5 children) the highest blood urea level being 147 mg % in one adult. Twelve patients (all adults) had more than 20% parasitaemia and 15 (10 adults and 5 children) had parasitaemia between 10-15%. Blood transfusion (1 or 2 units) was given to 31 patients (7 adults and 24 children). Quinine (I.V.) was given to only 37 cases (32 adults and 5 children). The initial assessment of the patient's mental state revealed that 9 (5 adults and 4 children) were confused, 21 (10 adults and 11 children) were semiconscious and not answering questions, while majority of them were in deep coma. Analysis of data revealed that severe anaemia, hyperparasitaemia, convulsions and deep coma were significantly higher in patients who died than those who improved ($p<0.05$). The time of death after admission in hospital ranged from 2 h to 4 days. Sixteen patients (12 adults and 4 children) died within first 24 h, 11 (8 adults and 3 children) within 48 h, eight patients (7 adults and 1 child) in 72 h and 4 adults died within 4 days.

Table 2. Clinical and laboratory findings in survivors and non-survivors with cerebral malaria

Symptoms/ clinical findings	Patients	
	Improved (n=113)	Died (n=39)
Fever	113	39
Headache	16	24
Chills/rigor	91	34
Vomiting	43	12
Convulsions	7	28
Bodyache	50	25
Abdominal pain	5	4
Diarrhoea	25	10
Bleeding	1	4
Severe anaemia	14	32
Oligurea/Renal failure	5	8
Jaundice	3	7
Hypoglycaemia	6	9
Hyperparasitaemia (> 10%)	6	21
Unconsciousness/ Deep coma	82	39

Persual of case sheets for residential address revealed that case fatality rate was higher (32%) in referred cases in comparison to local cases (23%) and often these patients were administered various antibiotics along with inadequate antimalarials by medical practitioners or other health functionaries. These patients were in critical condition as in seeking better facilities they lost crucial time. Chances of survival were less in patients who were unconscious on reaching the hospital than those who were restless, psychotic and drowsy.

Information on malaria morbidity and mortality was not availbale from majority of hospitals and clinics in Jabalpur. In one private hospital, Jabalpur Research Centre, there were 93 cerebral malaria cases with 22 deaths (23.6%) during the same period. From these results it can be stated that cerebral malaria is common in Jabalpur. A mortality rate of 30% was reported in cerebral malaria patients from Rourkela Steel Plant, Orissa[7]. Mortality due to cerebral malaria was 20% in Thailand[8], 33% in Zimbabwe[9] and 57.7% from Ethiopia[10].

Even though resistance in *P. falciparum* to chloroquine has been reported from this region[4,11] quinine was not available in hospitals. Quinine dihydrochloride is the drug of choice in patients with severe malaria[12,13]. During post-monsoon season (September-November), the number of malaria cases were so high in the region that often quinine is in short supply in the open market as well. In majority of cases (25) the patients were given I.V. quinine (1800 mg) for two-three days and then switched over to I.V. chloroquine or oral chloroquine.

This study has shown that cerebral malaria is one of the common complications of severe *P. falciparum* with high mortality. Some aspects of the problem requiring immediate attention are (i) facilities to manage serious cases of malaria are scarce with lack of clinicians suitably trained in the management of malaria. Further, different types of treatment employed by medical practitioners together with the choice of drugs not only create confusion but pose serious problems in the management of cases. This problem is aggravated by the shortage of antimalarials and prevalence of chloroquine resistance, and (ii) there is an urgent need to provide suitable guidelines and training to clinicians regarding the choice of drug, dosage schedule of therapy for improved management of cases.

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Efficacy of Two Flowable Formulations of *Bacillus sphaericus* against Larvae of Mosquitoes

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Laboratory evaluation revealed that the Spherimos and Vectolex formulations of *Bacillus sphaericus* produced 97 and 100% larval mortality respectively in *Culex quinquefasciatus* at a dose as low as 0.008 ml/sq m as against 93 and 97% mortality respectively at 1 ml/sq m in *Anopheles stephensi*. However, in *An. culicifacies* similar level of mortality was not observed even at 10 ml/sq m of these formulations. Field evaluation revealed 100% reduction of *Cx. quinquefasciatus* larvae for 2–3 weeks in pools and wells with single application of Spherimos @ 2 ml/sq m as against 95.4% reduction @ 10 ml/sq m in irrigation channel for one week. Vectolex @ 10 ml/sq m provided 99–100% reduction of *Cx. quinquefasciatus* larvae up to 9 weeks in wells and 1 week in channels.

Keywords: *Anopheles culicifacies, Bacillus sphaericus, Culex quinquefasciatus*

INTRODUCTION

In view of the global interest to exploit the potential of Biolarvicides in the development of Integrated Disease Vector Control Programme as outlined in the global control strategy, several *Bacillus sphaericus* formulations have been evaluated to know the relative efficacy of candidate compounds against mosquito species in different habitats[1–4]. The present communication reports the efficacy of two new formulations, viz. Vectolex 2.5 AS (ABG 6262) and

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Spherimos against vectors of malaria and filariasis in Delhi.

MATERIALS AND METHODS

The two water-based flowable formulations of *Bacillus sphaericus* 2362, viz. Spherimos and Vectolex 2.5 AS (ABG 6262) were supplied by WHO for evaluation against vector species of mosquitoes in India under the Tropical Disease Research Programme.

Laboratory evaluation: Formulations were tested against larvae of *An. culicifacies*, *An. stephensi*, the main vectors of malaria in rural and urban areas respectively and *Cx. quinquefasciatus* the vector of filariasis in India. Tests were conducted under simulated conditions in enamel trays (15x20 cms) containing 500 ml of water in which twenty-five late III or early IV instar laboratory reared larvae were exposed to different dosages of biolarvicides. Larval food which consists of a mixture of yeast and dog biscuit powder was given in each tray. Each test was replicated three times and corrected per cent mortality was calculated from the concurrent control mortality by using Abbott's formula.

Field evaluation: Small-scale field trials were carried out during September–October 1989 in small pools and pits, irrigation channels, drains and unused wells in Dehra village, Dhaulana Primary Health Centre, District Ghaziabad, Uttar Pradesh. The water-based suspensions of two flowable concentrates of *B. sphaericus* were made after dilution with water and sprayed with the help of a compression pump at the pre-calculated dosage rate on the water surface area basis in pools, pits, wells and irrigated unlined channels with stagnant water. The larval density in untreated and treated habitats was determined by dipping method and estimated as density of III+IV instar larvae per dips. The per cent reduction in the post-treatment larval density was calculated using Mulla's formula[5], which is as follows.

$$\% \text{ reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100$$

where C_1 and T_1 are the pre-treatment density and C_2 and T_2 are the post-treatment density of III+IV instar larvae per dip in the control and treated habitat respectively.

RESULTS AND DISCUSSION

Laboratory evaluation: Results of laboratory evaluation of two flowable formulations of *B. sphaericus* 2362, viz. Spherimos and Vectolex against larvae of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* are shown in Table 1. It is evident from the table that larvicidal activity of the two formulations was more or less same against different species of mosquitoes. Culicine larvae were found more susceptible in comparison to anophelines. Spherimos and Vectolex 2.5 AS produced 97 and 100% mortality in *Cx. quinquefasciatus* larvae at a dose as low as 0.008 ml/sq m, while 1 ml/sq m was required to

Table 1. Laboratory evaluation of *Bacillus sphaericus* 2362 flowable formulations against larvae of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus*

Formulation	Dosage (ml/m^2)	Corrected % mortality after 48 h exposure		
		An. culicifacies	*An. stephensi*	*Cx. quinquefasciatus*
Vectolex 2.5 AS (ABG-6262)	10	58	100	100
	5	51	100	100
	1	19	97	100
	0.008	–	–	100
Spherimos	10	73	100	100
	5	43	100	100
	1	16	93	100
	0.008	–	–	97

produce 93 to 97% mortality in *An. stephensi* larvae. The larvicidal activity of both the formulations was found quite low against immatures of *An. culicifacies* even at a dose as high as 10 ml/sq m. The mortality obtained at this dosage was only 58% with Vectolex 2.5 AS and 73% with Spherimos. Earlier studies have also reported variable degrees of susceptibility between culicine and anophelines[6] and also between *An. stephensi* and *An. culicifacies*[7] with different formulations of *B. sphaericus*. Dosages for field evaluation were selected on the basis of laboratory tests.

Field evaluation: Table 2 shows the results of field evaluation of Spherimos formulation against larvae of *Anopheles* species in different habitats. Results revealed 100% reduction of anopheline larvae for one week when Spherimos was applied @ 2 ml/sq m in pools, pits and wells where breeding of *An. subpictus* was predominantly observed. However, similar level of control was not obtained in same habitat where *An. culicifacies* was breeding predominantly. Even a higher dosage, i.e. 10 ml/sq m failed to produce more than 74.8% mortality after two days of application. In irrigated channels the initial kill was low, i.e. 37.8–58.7% but 97.1 and 88.6% reduction was obtained after first and second weeks of post treatment period respectively where mixed breeding of anopheline was observed. Enhancement of dose (10 ml/sq m) did increase the per cent reduction of immature density in irrigation channel where predominant breeding of *An. subpictus* was observed.

Results of field evaluation of Spherimos against *Cx. quinquefasciatus* larvae are

presented in Table 3. It produced 100% reduction of culicine larvae in pools treated @ 2 and 10 ml/sq m for a period of 1 and 3 weeks respectively. In wells 98.4, 96.7 and 99.04% reduction was observed @ 2 ml/sq m in first, second and third weeks respectively during post-treatment period. However, higher dose, i.e. 10 ml/sq m was required to control the breeding of the same species in irrigation channels.

Table 4 shows the effect of Vectolex 2.5 AS formulation on anopheline larvae. Results revealed that Vectolex formulation was highly effective against anopheline larvae @ 1 ml/sq m in pools, 2 ml/sq m in wells and 5 ml/sq m in irrigation channels. The residual activity of biolarvicide was evident for 4–5 weeks in pools and wells and only one week in irrigation channels, where mainly *An. subpictus* breeding was re-

Table 2. Evaluation of Spherimos formulation of *Bacillus sphaericus* 2362 against III+IV instar larvae of Anopheles* spp. under field conditions

Days/Weeks	Habitats, dosage and larval density/dip					
	Pool 1 (2ml/m^2)	Pool 2 (2ml/m^2)	Pool 3 (10ml/m^2)	Well 1 (2ml/m^2)	Well 2 (2ml/m^2)	Channel (10ml/m^2)
0 Day (Pre-treatment)	22.2	4.5	22.6	4.9	13.0	16.7
1 Day (Post-treatment)	0 (100)	1.8 (79.7)	14.6 (56.3)	1.0 (76.6)	4.4 (39.8)	9.4 (95.4)
2 Days	0 (100)	2.6 (35.2)	7.8 (74.8)	0.1 (99.1)	6.7 (58.7)	0 (100)
3 Days	0 (100)	–	9.6 (67.1)	0 (100)	3.7 (77.2)	–
1 Week	0 (100)	1.2 (72.9)	12.4 (35.9)	0 (100)	2.6 (97.1)	0.1 (92.1)
2 Weeks	1.4 (90.8)	0 Dried	13.2 (49.5)	1.3 (46.9)	11.8 (88.6)	0 (100)
3 Weeks	Discontinued		27.8 (+)	13.8 (39.1)	Discontinued	
4 Weeks			11.4 (+)			

Figures in parentheses indicate per cent reduction in larval density based on untreated control; *Anopheles breeding in control was eliminated; Breeding predominantly consisted: Pool 1 (21.1 sq m) – *An. subpictus* ; Pool 2 (22.7 sq m) – *An. culicifacies*; Pool 3 (8.0 sq m) – *An. annularis* and *An. culicifacies*; Well 1 (1.4 sq m) – *An. subpictus*; Well 2 (50 sq m) – *An. culicifacies, An. subpictus* and *An. annularis*; and Channel (10 sq m) – *An. subpictus*; (+) No reduction.

Table 3. Evaluation of Spherimos formulation of *Bacillus sphaericus* 2362 against III + IV instar larvae of Culex* spp. under field conditions

Days/Weeks	Habitats, dosage and larval density/dip			
	Pool 1 (2ml/m^2)	Pool 2 (10ml/m^2)	Well (2ml/m^2)	Channel (10ml/m^2)
0 Day (Pre-treatment)	12.2	7.2	382.2	12.5
1 Day (Post-treatment)	0 (100)	1.6 (78.5)	29.2 (89.9)	1.7 (100)
2 Days	0 (100)	0 (100)	3.0 (98.9)	0 (100)
3 Days	0 (100)	0 (100)	1.2 (99.6)	0 (100)
1 Week	0 (100)	0 (100)	3.2 (98.4)	0.7 (95.4)
2 Weeks	12.2 (82.74)	0 (100)	4.0 (96.7)	3.2 (54.3)
3 Weeks	14.6 (17.8)	0 (100)	3.0 (99.04)	5.7 (76.3)
4 Weeks		0.4 (70.3)		28.6 (72.8)

Figures in parentheses indicate per cent reduction in larval density based on untreated control; *Predominant breeding of *Cx. quinquefasciatus* was observed; Pool 1 – 21.1 sq m area; Pool 2 – 8.0 sq m area; Well – 2.0 sq m area; and Channel – 10 sq m area.

corded. Inconsistent results were obtained with higher dosages (5–10 ml), where mixed breeding of *An. culicifacies*, *An. annularis* and *An. subpictus* was observed in wells, pools and irrigation channels. This clearly indicate that efficacy of biocide formulations depends upon the habitat, species and the dosage used.

Table 5 shows the effect of Vectolex 2.5 AS formulation against culicine larvae in different habitats. Effective control of *Cx. quinquefasciatus* larvae was observed in pools and wells. The effect was highly pronounced at 5 ml and 10 ml/sq m dosage in these habitats and larvicidal activity was observed for 4 weeks in pools and up to 10 weeks in wells. In irrigated channels treated @ 5 ml/sq m the reduction was observed only for two days and, thereafter the larval population started reappearing in this habitat because of flowing water.

The results of field trials clearly indicate that the two formulations of *B.*

Table 4. Evaluation of *Bacillus sphaericus* 2362 Vectolex (ABG 6262) flowable concentrate against III+IV instar larvae of *Anopheles* spp. under field conditions

Days/Weeks	Habitats, dosage and larval density/dip						
	Pool 1 (1ml/m^2)	Pool 2 (2ml/m^2)	Pool 3 (5ml/m^2)	Pool 4 (10ml/m^2)	Well 1 (5ml/m^2)	Well 2 (10ml/m^2)	Channels (5ml/m^2)
0 Day (Pre-treatment)	45.6	15.3	18.04	35.2	7.6	7.3	11.1
1 Day (Post-treatment)	0.05 (99.9)	0.3 (98.8)	0.14 (99.45)	1.4 (96.12)	0 (100)	0 (100)	0.4 (94.4)
2 Days	0 (99.9)	0 (100)	3.1 (99.7)	0 (92.35)	0 (100)	0 (100)	0 (100)
3 Days	0.05 (100)	0 (100)	0.4 (99.8)	3.4 (87.93)	0 (100)	0 (100)	-
1 Week	6.9 (87.8)	1.4 (91.4)	0.12 (99.4)	4.6 (78.12)	0 (100)	0 (100)	0 (100)
2 Weeks	3.2 (88.2)	15.5 (+)	0 (100)	8.1 (62.54)	0.4 (97.8)	0 (100)	0.4 (57.6)
3 Weeks	1.15 (92.2)	0.3 (+)	Dried	1.4 (90.61)	0.8 (57.3)	0 (100)	0.3 (+)
4 Weeks	2.9 (80.9)	-	-	2.5 (61.0)	1.0 (+)	0 (100)	-
5 Weeks	11.45 (7.8)	-	-	-	12.0 (+)	0.06 (98.3)	-

Figures in parentheses indicate per cent reduction in larval density based on untreated control; Breeding mainly consists: Pool 1 (113 sq m) - *An. subpictus*; Pool 2 (35 sq m) - *An. subpictus* and *An. culicifacies*; Pool 3 (35 sq m) - *An. subpictus* ; Pool 4 (50 sq m) - *An. culicifacies*, *An. annularis* and *An. subpictus*; Well 1 (1.8 sq m) - *An. annularis* ; Well 2 (1.7 sq m) - *An. subpictus* ; and Channel (26.25 sq m) - *An. subpictus*; (+) No reduction.

Table 5. Evaluation of *Bacillus sphaericus* Vectolex (ABG 6262) flowable concentrate against III + IV instar larvae of *Culex spp. under field conditions**

Days/Weeks	Habitats, dosage and larval density/dip					
	Pool 1 (1ml/m^2)	Pool 2 (2ml/m^2)	Pool 3 (5ml/m^2)	Well 1 (5ml/m^2)	Well 2 (10ml/m^2)	Channel (5ml/m^2)
0 Day (Pre-treatment)	26.46	48.2	16.8	15.8	86.4	11.4
1 Day (Post-treatment)	0.53 (98.9)	1.73 (97.9)	0.33 (98.9)	0.07 (99.7)	10.0 (94.5)	1.7 (80.04)
2 Days	0.33 (98.7)	0.73 (98.9)	0.06 (99.6)	0 (100)	0 (100)	0 (100)
3 Days	0.03 (99.9)	0.2 (99.7)	0.06 (99.7)	0 (100)	0 (100)	–
1 Week	4.36 (91.4)	2.1 (95.7)	2.4 (92.6)	0 (100)	0 (100)	0.8 (94.2)
2 Weeks	14.43 (+)	3.5 (65.4)	0.3 (96.5)	0 (100)	0 (100)	5.3 (17.04)
3 Weeks	8.13 (+)	0.2 (98.8)	0.1 (99.03)	0 (100)	0 (100)	8.0 (63.5)
4 Weeks	3.86 (+)	0 (100)	0 (100)	0.4 (94.9)	0 (100)	40.2 (58.2)
5 Weeks	Dried	Dried	0.3 (+)	1.11 (72.05)	0 (100)	30.0 (64.4)
6 Weeks	——— Discontinued ———			0.7 (83.6)	0 (100)	Discontinued
7 Weeks				1.11 (91.9)	0.2 (99.9)	
8 Weeks				2.4 (76.3)	2.6 (97.8)	
9 Weeks				0.4 (96.6)	1.4 (99.0)	
10 Weeks				0.4 (93.7)	8.0 (89.2)	

Figures in parentheses indicate per cent reduction in the larval density based on untreated control; *Predominant breeding of *Cx. quinquefasciatus* was observed; Pool 1 – Area 109 sq m; Pool 2 – Area 35 sq m; Pool 3 – Area 27 sq m; Well 1 – Area 1.75 sq m; Well 2 – Area 0.85 sq m; and Channel – Area 26 sq m; (+) No reduction.

sphaericus were highly effective in controlling the breeding of *Cx. quinquefasciatus* but their efficacy against anophelines varied from habitat-to-habitat depending on the composition of anopheline breeding in particular habitat. The biocide formulations were more effective in habitats where *An. subpictus* was breeding profusely but they were less effective in habitats where profuse breeding of *An. culicifacies* or *An. annularis* breeding was observed. Similar results have also been reported earlier[4]. Though the two formulations of *B. sphaericus* had high initial efficacy in most of the habitats, their residual activity lasted for varying duration in different habitats depending upon the type of habitat, the dosages of biocides and also other environmental factors[8]. The prolonged larvicidal activity of flowable formulation of *B. sphaericus* particularly at higher dosages have been shown due to the low settling rate of *B. sphaericus* spores in stagnant water habitats and thereby retaining the larvicidal toxins in the feeding zone of larvae for a longer time[9].

The two formulations produced longer duration of control in deep located habitats such as disused wells than in shallow pools, probably due to lesser exposure to direct sunlight which may result in the degradation of larvicidal toxin. The prolonged larvicidal action of *B. sphaericus* flowable formulations may either be due to the persistence of large number of spores of *B. sphaericus* in the feeding zone of mosquito larvae or it may be due to recycling of spores in the larval habitats of mosquitoes[10].

In general the two biocide formulations evaluated in this study can be successfully utilized in controlling the breeding of *Cx. quinquefasciatus* and a non-vector *An. subpictus* in small and stagnant water habitats but their role in controlling the breeding of *An. culicifacies*, a major vector of rural malaria in India is very limited. Nevertheless these formulations can be incorporated in a scheme of bioenvironmental control where source reduction is an expensive proposition and also in polluted water bodies which supports profuse breeding of *Cx. quinquefasciatus*.

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SHORT NOTE

Indian Journal of Malariology
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A Study on the Mosquito Emergence from the Underground Sewerage System in some Areas of Delhi

C.P. BATRA, P.K. MITTAL and T. ADAK

Keywords: *Culex quinquefasciatus*, Emergence, Mosquito

Increased mosquito nuisance in most urban areas is mainly because of *Culex quinquefasciatus* which is directly associated with waste-water disposal. *Cx. quinquefasciatus* breeds profusely in all stagnant and slow-moving polluted water habitats, making maximum use of the larval food to be found in the urban environment[1]. In 1990 during a mosquito survey in Delhi, though the density of adult mosquitoes was very high in certain localities, the corresponding density of immature mosquitoes in surface breeding habitats was very low[2]. With this in view we undertook a study to determine the mosquitogenic potential of underground sewerage system in various developed and partially developed localities in Delhi. Although there are various methods to monitor the mosquitogenic potential of different open-water habitats[3], there is no

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standard method for estimating the mosquitogenic potential of underground sewerage system.

To estimate the mosquito emergence from the sewer lines observations were made during May-September 1990. Nylon-net cages were used to trap mosquitoes from the sewer man-holes which had partially covered or broken lids. the nylon-net cages measuring 60x60x60 cms were fixed over the man-hole in such a way that mosquitoes emerging from it did not escape. Initially a whole night collection was made on an hourly basis but since almost 80 per cent emergence was noticed only during evening, i.e. 1900 to 2000 hrs, thus all subsequent collections were made only during this hour. The mosquitoes thus trapped were kept in separate bowls for identification, counting and checking their abdominal conditions in the laboratory.

Average mosquito emergence per trap ranged from 5 to 1461 in different localities. Maximum emergence was recorded from Ghazipur Dairy in east (1461), followed by Radio Colony in North (1257), Rohini Sector VI in west (1305) and Sadiq Nagar in south Delhi (50). While minimum emergence in the four directions were recorded from Trilokpuri in the east (12), Timarpur in the north (35), Paschim Vihar in the west (99) and R.K. Puram in the south (5) (Table 1). Average emergence per trap in all 21 localities was 328 mosquitoes. As evident from the data mosquito population constituted only *Culex* species. About 2% of the collected mosquitoes were dissected for parity status and all were found nulliparous, indicating that the mosquitoes were freshly emerged. There was no gravid or fully-fed mosquito which further indicated that the mosquitoes caught were freshly emerged and not the resting population.

Emergence of high mosquito density from the sewer man-holes in certain localities could be related to the poor maintenance of sewer system, viz. broken or partially covered lids of the man-holes, leakage and run-off of the surface breeding into the sewerage system and blockages due to silt, clay, waste construction materials, refuse etc. Organic pollution of water depletes the oxygen content of water and thus most *Anopheles* species can not breed in such water, but certain culicine species breed freely in water polluted with organic matter[4].

High CO_2 content of polluted water also acts as an attractant for culicine mosquitoes for oviposition. In India, at present the sewerage system is only in big cities and that too is partially laid. Since sewerage treatment and disposal is essential to check environmental decay as well as to maintain the healthy living conditions, more and more towns in future will be covered with sewerage system. However, poor maintenance and faulty sewerage systems would create high mosquitogenic conditions re-

Table 1. Emergence of mosquitoes from sewer man-hole during May to August 1990

Locality	Month of collection	No. of traps	*Culex quinquefasciatus*			Av. nos./ trap
			Male	Female	Total	
Radio Colony	May	4	3002	2024	5026	1257
Ghazipur Dairy	May	4	1525	4317	5842	1461
Jang Pura	May	2	17	34	51	25.5
Sarai Kalekhan	May	2	19	37	56	28.0
Seelampur III (Welcome Colony)	May	3	790	1340	2130	71.0
Mansarover Park	May	3	1610	2150	3760	1253
Paschim Vihar	May	4	169	227	396	99
Madhuvan	May	2	124	386	510	255
Shalimar Bagh	May	4	3082	2044	5126	1281
Timar Pur	May	7	115	131	246	35.14
Pitampura	June	5	327	229	556	111.2
Rohini (Sector V)	July	4	234	246	480	120
Rohini (Sector VI)	July	4	254	268	522	1305
Sadiq Nagar	July	4	36	164	200	50.0
Pushp Vihar	July	4	9	41	50	12.5
Trilok Puri	July	4	18	29	47	11.75
Chirag Delhi	Aug	4	49	40	89	22.25
Saket	Aug	4	30	18	48	12.0
R.K. Puram	Aug	2	2	8	10	5.0
Ghazipur village	Aug	4	25	121	146	36.5
Nand Nagri	Aug	4	40	233	273	68.25
Total (21 localities)		78	11,477	14,087	25,564	328

sulting in prevalence of diseases transmitted by these species. Environmental management by engineering methods can provide long-term solutions to the problem of mosquitoes in the sewerage system. Since the problem is related to sanitation so if mosquito control programme in urban areas is linked with sanitary services under the direct supervision of a Public Health Engineer, mosquito control can be achieved in a cost-effective manner with the least trained man-power. For effective control all iron covers of man-holes should be replaced with heavy cemented material to avoid thefts and to seal them

hermatically because even a small gap in the man-hole cover is enough for the penetration of mosquitoes and subsequent breeding. In addition to this all vent pipes of the system should be closed with msoquito net or wire gauze to stop entry and exist of mosquitoes.

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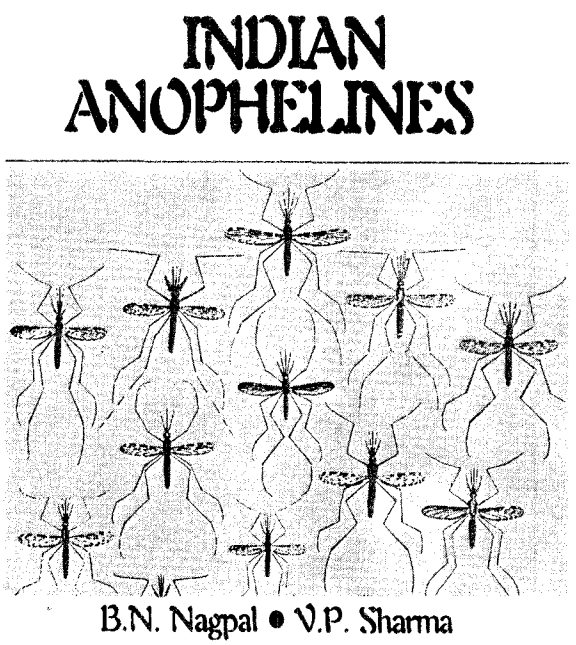

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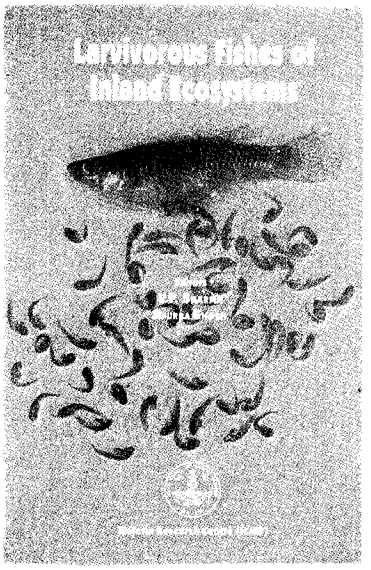

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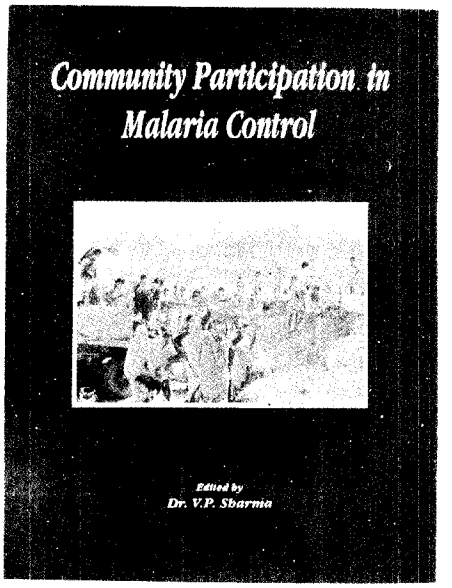

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