



Research Article

Insecticides Susceptibility Status of Malaria Vectors in a High Malaria Endemic Tribal District Gadchiroli (Maharashtra) of India

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A B S T R A C T

Background and Objective: The current study was undertaken to determine insecticide susceptibility of malaria vectors in various villages of high malaria endemic PHCs of Gadchiroli district of Maharashtra.

Methods: Adult malaria vectors were collected from the human dwellings/ cattle sheds of 156 villages of 18 malaria endemic PHCs. Susceptibility tests were carried out for different insecticides against *An. culicifacies* and *An. fluviatilis* mosquitoes as per the World Health Organization (WHO) procedure. Cone bioassays were also done to assess the quality and efficacy of indoor residual spray.

Results: *An. fluviatilis* could be collected from 23 villages only and all the populations were fully susceptible to synthetic pyrethroid (deltamethrin) while being tolerant to organophosphorous (malathion). Susceptibility of *An. culicifacies* from 156 villages indicated that only 3 populations of *An. culicifacies* were resistant to deltamethrin while 57 populations were fully susceptible and other 96 populations were tolerant to deltamethrin. Resistance was recorded in 25 populations of *An. culicifacies* against malathion and 30 populations were tolerant to malathion insecticide. Remaining populations of *An. fluviatilis* and *An. culicifacies* were highly resistant to organochlorine. Results of cone bioassay revealed the mortality ranged from 32.5-51.1% on cemented and 27.5-43.3% on the mud wall sprayed with lambda cyhalothrin.

Conclusion: The current study indicates that resistance has developed to synthetic pyrethroids in the major malaria vector *An. culicifacies*. Therefore, there is an urgent need for the evaluation of new insecticide molecules for better control of malaria vectors.

Keywords: Malaria Vector, Insecticide Susceptibility, Gadchiroli District of Maharashtra, India



Introduction

Malaria is the most common vector-borne disease and it is curable if effective treatment is started early. Delay in the treatment of malaria may cause serious health problems and may even lead to death. Many people are being affected by the disease in tropical and sub-tropical countries. 228 million people are at the risk of being infected with malaria and nearly four lac people died in 2018 from this disease globally.¹ Malaria is one of the oldest and highly endemic diseases throughout India, and it is a major cause of mortality and morbidity among human population with 429928 cases and 96 deaths reported during the year 2018.² The central part of the country is a high malaria endemic region where about 90% of the population is at risk of infection of malaria.³ In Maharashtra, 10757 malaria cases and 13 deaths were reported in 2018 by National Vector Borne Disease Control Programme (NVBDCP),² and Vector Borne Disease Control Programme of the state was benefited from the enhanced malaria control project (EMCP) funded by the World Bank since 1997.⁴

Gadchiroli district of Maharashtra is a tribal district in the central part of the country and one of the high malaria endemic districts, where 34206 malaria positive cases were reported in the year 2015, out of which *Plasmodium falciparum* (PF) accounted for 79.6%. Slide positivity rate (SPR) in Gadchiroli has been ranging from as low as 2.57% to as high as 4.57% between the years 2011 and 2015.⁵

Malaria disease is caused by a protozoan parasite of genus *Plasmodium* in humans. Four species of malaria parasites of genus *Plasmodium*, namely *Plasmodium vivax*, *P. falciparum*, *P. malariae* and *P. ovale* are responsible for the disease, of which *P. vivax* and *P. falciparum* are both the common malarial parasites responsible for global mortality including in India.⁶⁻⁹ In India, malaria is transmitted by bites of female *Anopheles* mosquitoes of *An. culicifacies*, *An. stephensi*, *An. fluviatilis*, *An. minimus*, *An. dirus*, and *An. sundanicus*, the six primary vectors, and *An. varuna*, *An. annularis*, *An. philippinensis* and *An. jeyporiensis* the four secondary vectors of malaria. *An. culicifacies* alone contributes more than 65% of the total cases of malaria annually and is found widely in rural and peri-urban areas.⁸ *An. culicifacies* and *An. fluviatilis* are the main malaria vectors in India. *An. culicifacies* is the common primary malaria vector found throughout the year while *An. fluviatilis* is found mostly in the winter season.

The use of insecticides is an important tool for reducing the density of adult *Anopheles* malaria vector for malaria control. Vector control is a major part of the strategy of Vector Borne Disease Control Programme and two types of vector control methods, indoor residual spray (IRS) and insecticide-treated bed nets (ITBNs)/ long-lasting insecticide treated nets (LLINs) are used to control malaria transmission.⁶⁻⁸ In spite

of these control measures, malaria remains a major public health problem in India. IRS with synthetic insecticides like DDT and BHC was used to control malaria vectors under malaria control programme in India since 1958, but due to continued use of synthetic insecticides, *An. culicifacies* has developed resistance against DDT, dieldrin, and malathion in few districts of Maharashtra such as Gadchiroli, and in Gujarat.¹⁰⁻¹¹ Synthetic pyrethroid (deltamethrin) was introduced in IRS in 1998 in Gadchiroli district.^{5,12,13} Later in 2009, other synthetic pyrethroids as alphacypermethrin, cyfluthrin and lambda cyhalothrin were used for malaria control and ITBNs/ LLINs were distributed in Gadchiroli district. Synthetic pyrethroids are used in IRS, impregnation of bed nets and making LLINs to control malaria. More than fifteen years ago, the resistance in *An. culicifacies* against synthetic pyrethroids (deltamethrin) was reported from Surat district of Gujarat¹⁴ and malaria vectors also reported tolerant/ partial resistance to synthetic pyrethroids used in IRS. However, malaria vectors have developed resistance to DDT in 286 districts, to malathion in 81 and to pyrethroids in 2 districts in India due to selection pressure.⁶⁻⁸ Presently, Gadchiroli district has been receiving two rounds of IRS with synthetic pyrethroids and regular use of ITBNs/ LLINs every year.^{5,12,13}

In order to find out the current status of insecticide resistance in malaria vectors of Gadchiroli, the current study was undertaken. An in-depth monitoring of insecticide susceptibility of *An. culicifacies* and *An. fluviatilis* malaria vectors against various insecticides like lambda cyhalothrin, permethrin, cyfluthrin, deltamethrin, malathion and DDT was done in 156 endemic villages of various PHCs of Gadchiroli district. This information may be very useful to finalise the strategy for malaria prevention and elimination of malaria from Gadchiroli district of Maharashtra state.

Materials and Methods

Study Site

The Gadchiroli district of Maharashtra was created in 1982 from Chandrapur district. It is geographically a large district with a geographical area of 14412 km.² It is situated at 18.43' to 21.50' N latitude and 79.45' to 80.53' E longitude and has uneven terrain with hills, valleys, and forests at different altitudes. It has a total population of 1072942 as of census 2011.¹⁵ The average rainfall is 1743.5 mm, minimum temperature 11.3°C and maximum 47.7°C. Gadchiroli is a tribal dominant district and most of the people are Gond tribals. Their houses have mud-plastered walls with thatched/ tiled roofs and all the cattle sheds are near human dwellings. These types of houses are more suitable for malaria vectors to rest and bite the host, thereby spreading malaria rapidly. In tribal and forest areas, malaria control is a very difficult task due to various topographical, socio-economical and ecological factors. Water reservoirs with

streams, drains and springs and water lodged in the downhill are the main sources of immature stages of mosquitoes.

Analysis of Malaria Epidemiological Data

The Primary Health Centre (PHC) wise malaria epidemiological data were collected for five years, i.e. from 2011-15, from

the District Malaria Officer (DMO), Gadchiroli district of Maharashtra state. The data were also analysed to obtain various parameters like annual blood examination rate (ABER), slide positivity rate (SPR), slide falciparum rate (SFR), Pf percent and number of deaths (Table 1).

Table 1. Epidemiological Data of Malaria in Gadchiroli District of Maharashtra (2011-2015)

Year	Population	BSC&E	+ve	PF	PV	ABER	API	SPR	SFR (%)	PF (%)	Death
2011	1158947	529846	13670	10581	3089	45.72	11.80	2.57	1.99	77.40	6
2012	1177242	520258	6596	4656	1940	44.19	5.60	1.26	0.89	70.58	4
2013	1209496	536341	6436	4919	1517	44.34	5.32	1.19	0.91	76.42	6
2014	1187784	692277	24469	21410	3059	57.23	20.23	3.53	3.09	87.49	18
2015	1204101	747113	42062	7228	6978	62.7	28.40	4.57	3.64	79.60	11

Table 2. List of Villages and PHCs in Gadchiroli (Maharashtra) selected for Insecticide Susceptibility Study undertaken on Malaria Vectors

Name of PHCs	Name of Sub Centres	Name of Study Villages
Kotgul	Sonpur	Sonpur, Bhimankozi
	Gyarahpatti	Gyarahpatti, Pitesur gaon & tola, Nehalgad
	Kosami 2	Kosami 2
Malewada	Devsara	Devsara, Mendha, Murmadi, Khatitola
	Khobramenda	Khobramenda, Huryal dand, Badbada, Yeduskuhi
	Jaysing Tola	Wagha bhumi, Mange wada, Jaysing tola, Khamtala
Gatta	Jhanbia	Jhanbia gaon & tola, Andenge, Nander
	Hedari	Hedari gaon, Mallam pahari, Surjagad
	Gatta	Gatta gaon & tola, Mohndi gaon, Tadguda
Kasansur	Kasansur	Venasur, Chokhe wada, Jhuri
	Kotami	Kotami
	Kondawahi	Etawahi, Kondawahi
	Tadguda	Tadguda
	Ghotsur	Ghotsur, Jaweli
Todsa	Ekrakhurd	Ekrakhurd, Ekrabhurj
	Aalinga	Aalinga gaon & tola, Zarewada, Alandi
	Tadhpalli	Tadhpalli, Lanji, Karampalli gaon & tola
	Todsa	Petha gaon
	Devada	Devada
Aarewada	Kiyar	Hemal kasha, Karampalli gaon & tola
	Aarewada	Aarewada, Medpalli
	Bhamaragad	Dubba guda
	Tadgaon	Tadgaon, Dhulepalli, Erakdumme
Laheri	Dhondraj	Dhondraj, Ranipudar
	Laheri	Laheri, Murangal, Aaldandi, Gundenoor
	Malum padur	Kuka menda, Malum padur, Bhoose wada
	Beena gunda	Beena gunda

Manne Rajaram	Manne Rajaram	Mokela
	Chichoda	Chichoda, Jijgaon, Bhade nagar
	Yechali	Yechali, Basa guda, Rela, Rai guda, Maram palli, Lakan duga
Godalwahi	Godalwahi	Godalwahi gaon, Godalwahi tola, Michgaon
	Sallebhatti	Sallebhatti, Lekha, Kannartola, Mendha
	Girola	Udegaon
Pendhari	Pendhari	Pulkal, Dhorgatta, Pekinmudza
	Durgapur	Durgapur, Khargi
	Chichoda	Chichoda, Sinsur
	Kaneli	Kaneli, Paidi
	Gatta	Gota gaon & tola, Jhawal zora, Pather gatta
Karwafa	Kondawahi	Kondawahi gaon & tola, Fulbodi, Zari
	Fulbodi	Kanartola
	Pustola	Pustola, Kopagoda
	Karwafa	Talodhi, Navegaon
	Sakhera	Sakhera, Jamblitola
	Chatgaon	Katezari
	Mendhatola	Mendhatola, Khutgaon, Michgaon
Murumgaon	Savargaon	Morchul, Savar gaon, Markagaon & tola, Gajamendi, Kangadi, Makdand
	Khambada	Tadegaon, Dongarhud
	Sursundi	Sursundi, Eurup dodari, Saygaon, Bhojghata, Daranchi
	Kulbhatti	Kulbhatti, Khedegaon, Fulkoda, Tumdi kasa
	Muska	Muska
	Devsura	Kosami, Devsura
	Pannemara	Pannemara, Ampayali
	Yerkad	Muzal gondi, Kanhartola, Tavetola, Sindesur
Rangi	Nimgaon	Borigaon, Nimanwada, Shivgatta
	Mohali	Kanargaon
	Jangada	Jangada gaon & Tola
Regadi	Regadi	Regadi, Garnjigaon & Tola, Vegnoor
	Chapalwara	Potepalli
Potegaon	Guruwada	Rakhagaon & Tola
	Maroda	Maroda, Savela
	Yewali	Kurpada
Amirza	Kursa	Kursa
	Murmadi	Murmadi
	Usegaon	Usegaon
	Maregaon	Maregaon & Tola
Bodali	Bhagwan pur	Bhagwan pur
Mahagaon	Mukhtav pur	Mukhtav pur, Chintal pade, Allapalli, Muktapur

Mosquito Collection

Mosquitoes were collected from various villages of high malaria endemic PHCs of Gadchiroli district of Maharashtra in years 2015-19 to know the susceptibility status of malaria vectors as per the World Health Organization (WHO) procedure.¹⁶ The selection of study villages of malaria-endemic PHCs was based on the prevalence of malaria cases during the years 2011-15 and the availability of vector mosquitoes. Adult mosquitoes were collected from 156 villages of 18 high malaria endemic PHCs out of a total of 47 PHCs of Gadchiroli (Table 2) by using an aspirator and flashlight from human dwellings/ cattle sheds in the morning from 6-9 am including mosquito larvae from different breeding habitats.¹⁷ *An. culicifacies* and *An. fluviatilis* collected were provided 10% glucose solution soaked in cotton pads and transported in caged cloth to the field laboratory at the PHC and district level. Similarly, mosquito larvae were collected from different breeding sources and reared till emergence for proper identification.^{18,19} During mosquito collection, maximum number of *An. culicifacies* and *An. fluviatilis* were recorded in cattle sheds as compared to human dwellings in the surveyed villages.

Bioassay Test

Bioassay test kits and insecticide-impregnated papers at diagnostic concentrations of various insecticides were received from University Sains Malaysia (vector control unit of WHO). Susceptibility tests were conducted by using the bioassay test kit for lambda cyhalothrin (0.05%), permethrin (0.75%), cyfluthrin (0.15%), deltamethrin (0.05%), malathion (5.0%) and DDT (4.0%) against *An. culicifacies* and *An. fluviatilis* as per WHO procedure.^{16,17} Only full-fed and semi-gravid female mosquitoes were exposed against diagnostic concentrations of different insecticides for one hour as per the WHO procedure. Three to five replicates of 10-25 female mosquitoes were exposed against diagnostic concentrations of each insecticide. Control replicates were also run parallel to each test of insecticide. After exposure, the holding tubes were kept for recovery in dark and cool places immediately under controlled temperature and humidity conditions. Cotton pads soaked in glucose solution were given as supplementary food to the tested mosquitoes during the recovery period for 24 hours.

Interpretation of Results

The mortality was calculated by counting the number of dead and live mosquitoes after the post-exposure period in both the test and control tubes. The results of this study were made by following criteria, the strain was considered resistant if the mortality was < 80%, verification required/ tolerant/ possible resistant, if mortality ranged between 80-97.99%, and susceptible in case of 98-100% mortality. If the control mortality was above 5% but less than 20%,

then the observed mortality was corrected by using the following Abbot's formula:²⁰

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

$$\text{Per cent corrected mortality} = \frac{\% \text{ observed mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Cone Bioassay Test

Cone bioassay tests were carried out in 20 sprayed villages of 4 PHCs of Gadchiroli to assess the residual action of insecticide spray on wall surfaces available in the study area by using the WHO bioassay test kit as per WHO procedure.²¹ The houses sprayed with the insecticide on different surfaces were selected for cone bioassay tests. Before starting these tests, the wild-caught full-fed/ semi-gravid female adults of *An. culicifacies* were collected from unsprayed villages and their F1 generation was used to determine the efficacy of insecticide. WHO Plastic bioassay cones were attached with self-adhesive packing on sprayed wall surfaces of houses in each village and full-fed/ semi-gravid 10-20 female mosquitoes were introduced in each plastic bioassay cone for 30 minutes. Each test was done with three replicates with control for a standard period of IRS during morning hours. After 30 minutes of exposure, all the mosquitoes were removed from the bioassay cones and were held in recovery cages at maintained temperature and humidity. Cotton pads soaked in glucose solution were given as supplementary food to the tested mosquitoes during the recovery period for 24 hours and mortality was calculated after 24 hours. This study indicates the efficacy of the insecticide over a period on different wall surfaces.

Results

Insecticide susceptibility of *An. fluviatilis* collected from 23 villages showed that all the populations were fully susceptible (98.3-100.0% mortality) to synthetic pyrethroids (deltamethrin) and resistant (73.8-79.5% mortality) to organophosphorous malathion (Table 3). Susceptibility study of *An. culicifacies* collected from 156 villages showed that 57 populations were fully susceptible to deltamethrin with a mortality range of 98.0% to 100.0% and 96 populations were tolerant to deltamethrin with 80.0% to 97.5% mortality. Only three populations of *An. culicifacies* were resistant to deltamethrin with 77.5% to 78.0% mortality (Table 4 and Figure 1). In addition, *An. culicifacies* collected from 12 same villages was tested against lambda cyhalothrin, out of which 6 populations were found susceptible with from 98.6 to 100.0% and 6 populations were found tolerant to lambda cyhalothrin with from 85.0 to 97.6% mortality. Similarly, 18 populations were tested against cyfluthrin, out of which 9 populations were fully susceptible with 100.0% mortality and 9 populations were tolerant (88.3-96.2% mortality).

Further 9 populations were tested against permethrin, out of which 8 populations were fully susceptible with mortality varying from 88.3% to 100.0% and only one population was tolerant to permethrin with 97.5% mortality (Table 4). Resistant was recorded in 25 populations of *An. culicifacies* to malathion with mortality ranging from 60.0% - 78.75%

and 30 populations were found to be tolerant to malathion with 80.0% to 96.2% mortality (Table 4 and Figure 2). All the remaining populations of both species of *An. culicifacies* and *An. fluviatilis* were highly resistant to organochlorine (DDT 4.0%) with mortality range from 20.0% to 58.3% and 43.3% to 51.2% respectively as shown in Tables 3 and 4.

Table 3. Insecticide Susceptibility of *An. fluviatilis* in Gadchiroli district of Maharashtra

Insecticides Tested & Dose	Name of PHC & Name of Villages surveyed in that PHC	No. of Mosquitoes Exposed		No. of Mosquitoes Dead in 24 Hours		% Mortality		Corrected Mortality (%)
		Exp	Cont	Exp	Cont	Exp	Cont	
DDT 4.0	Kotgul (Sonpur, Bhimankozi, Gyarahpatti, Pitesur gaon & tola, Nehal gad)	30	10	14	00	46.66	00	46.66
Malathion 5.0		45	15	34	00	75.55	6.66	73.8
Deltamethrin 0.05		178	60	178	00	100	3.33	100
DDT 4.0	Malewada (Mendha, Khobramenda, Waghabhumi)	30	10	15	00	50.00	00	50.00
Malathion 5.0		44	15	35	00	79.54	00	79.54
Deltamethrin 0.05		180	60	178	00	98.88	5	98.88
DDT 4.0	Kasansur (Kondawahi)	30	10	13	00	43.33	00	43.33
Deltamethrin 0.05		60	20	59	00	98.33	00	98.33
Deltamethrin 0.05	Todsa (Tadhpalii)	55	20	55	00	100	5	100
Malathion 5.0	Aarewada (Medpalii)	60	20	47	00	78.33	00	78.33
Deltamethrin 0.05		60	20	60	00	100	00	100
Deltamethrin 0.05	Laheeri (Murangal)	60	20	60	00	100	5	100
DDT 4.0	Pendhari (Pekinmudza, Khargi, Paidi, Gota gaon & tola, Jhawal zora)	30	10	15	00	50.00	00	50.00
Malathion 5.0		45	15	35	00	77.77	6.66	76.18
Deltamethrin 0.05		265	100	264	00	99.66	3	99.66
DDT 4.0	Murumgaon (Markagaon & tola, Makdand, Dongarhud, Devsura, Fulkodo)	45	15	26	00	57.77	13.3	51.29
Malathion 5.0		45	15	34	00	75.55	00	75.55
Deltamethrin 0.05		290	100	288	00	99.33	4	99.33
DDT 4.0	Karwafa (Kondawahi gaon & tola)	30	10	13	00	43.33	00	43.33
Deltamethrin 0.05		60	20	60	00	100	00	100

The mortality ranges were grouped as: < 80% (resistant), 80-97.99% (tolerant), and 98-100% (susceptible).

Table 4. Insecticide Susceptibility of *An. culicifacies* in Gadchiroli district of Maharashtra

Name of PHC	Name of Village	Observed Corrected Mortality (%)					
		DDT 4	Mala 5	Delta 0.05	LCM 0.05	Cyflu 0.15	Perm 0.75
Kotgul	Sonpur	-	73.75	98.75	-	-	-
	Bhimankozi	27.5	80.00	99.00	-	-	-
	Gyarahpatti	35.00	62.5	98.97	-	-	-
	Pitesur gaon & tola	-	76.25	99.00	-	100	-
	Nehalgad	-	-	100	-	-	-
	Kosami 2	-	-	100	-	-	-

Malewada	Devsara	-	81.66	90.00	85.00	100	-
	Mendha	26.25	73.07	98.75	-	100	-
	Murmadi	-	95.00	95.00	-	-	-
	Khatitola	-	-	100	-	-	-
	Khobramenda	31.00	70.00	99.00	-	-	-
	Huryaldand	-	82.5	98.75	-	-	-
	Badbada	48.86	78.75	98.75	100	100	-
	Yeduskuhi	-	-	98.75	100	100	100
	Waghabhumi	-	-	78.00	-	-	-
	Mangewada	-	-	83.33	-	-	-
	Jaysing tola	-	-	88.00	-	91.11	-
	Khamtala	-	-	98.00	100	-	-
Gatta	Jhanbia gaon & tola	-	-	83.75	-	-	-
	Andenge	45.00	81.11	98.88	-	-	-
	Nander	28.33	-	90.00	-	-	-
	Hedari gaon	-	-	88.00	-	-	-
	Mallam pahari	-	80.00	100	-	-	-
	Surjagad	-	-	89.00	-	-	-
	Gattagaon & tola	-	-	95.00	-	-	-
	Mohndi gaon	-	-	87.5	-	-	-
Kasansur	Tadguda	-	-	97.00	-	-	-
	Kotami	-	-	90.00	-	93.75	97.5
	Etawahi	-	-	93.00	-	-	-
	Kondawahi	-	91.66	100	-	-	100
	Tadguda	-	76.25	99.00	-	-	-
	Ghotsur	-	70.00	92.5	-	-	-
	Jaweli	28.33	-	96.00	-	-	-
	Venasur	-	-	100	-	-	-
	Chokhewada	-	-	89.00	-	-	-
Jhuri	40.00	90.00	99.00	98.6	95.00	100	
Todsia	Ekrakhurd	-	93.75	100	-	-	-
	Ekrabhurj	-	-	98.75	-	-	-
	Aalinga gaon & tola	33.33	-	99.00	-	-	-
	Zarewada	-	-	100	-	-	-
	Alandi	-	-	100	-	-	-
	Tadhpalii	-	65.00	91.25	-	-	-
	Lanji	-	-	92.00	100	-	-
	Karampalli gaon & tola	-	-	96.92	-	-	-
	Pethagaon	-	-	92.5	-	-	-
	Devada	33.33	-	100	-	-	-
Aarewada	Hemal kasha	26.66	75.00	100	-	-	-
	Karampalli gaon & tola	-	-	100	-	-	-

	Aarewada	-	-	96.28	-	-	-
	Medpalli	38.33	93.33	100	-	100	100
	Dubbaguda	-	-	95.00	-	-	-
	Tadgaon	-	-	93.75	-	-	-
	Dhulepalli	-	-	100	-	-	-
	Erakdumme	-	-	87.5	-	-	-
7. Laheri	Dhondraj	-	-	92.5	-	-	-
	Ranipudar	35.00	91.66	92.5	-	-	-
	Kukamenda	-	95.00	100	-	-	-
	Malumpadur	-	-	100		100	
	Bhoosewada	-	-	96.66	-	-	-
	Laheri	-	87.5	89.23	-	-	-
	Murangal	-	-	88.33	-	-	-
	Aaldandi	31.66	-	95.00	-	-	-
	Gundenoor	-	-	93.75	-	-	-
	Beena gunda	-	90.00	99.00	-	-	-
8. Manne Rajaram	Mokela	-	-	100	-	100	-
	Chichoda	-	-	90.00	-	-	-
	Jijgaon	-	-	93.75	-	-	-
	Bhadenagar	-	-	100	-	-	-
	Yechali	-	-	91.66	-	-	-
	Basa guda	26.66	88.33	98.33	-	-	-
	Rela	21.11	96.25	100	-	-	-
	Rai guda	-	-	91.66	-	-	-
	Marampalli	-	-	83.80	-	-	-
	Lakan duga	-	-	96.25	-	-	-
Godalwahi	Godalwahi gaon	31.66	80.00	93.75	-	-	-
	Godalwahi tola	-	80.00	77.5	-	-	-
	Michgaon	-	-	96.25	-	-	-
	Sallebhatti	-	83,75	98.75	91.25	-	-
	Lekha	30.00	85.00	100	-	-	-
	Kannartola	-	-	98.75	-	-	-
	Mendha	-	-	95.38	-	-	-
	Udegaon	-	-	83.33	-	-	-
Pendhari	Pulkal	-	81.16	96.25	-	-	-
	Dhorgatta	-	-	96.25	85.00	-	-
	Pekinmudza	-	-	95.00	-	-	-
	Durgapur	31.66	-	97.5	90.00	-	-
	Khargi	33.33	83.75	100	100	-	-
	Chichoda	-	-	95.00	-	-	-
	Sinsur	-	-	88.75	-	-	-
	Kaneli	-	-	98.75	-	-	-

	Paidi	-	68.33	100	-	-	-
	Gotagaon & tola	-	60.00	77.5	-	91.66	100
	Jhawal zora	24.00	71.66	98.75	-	-	-
	Pather gatta	-	71.66	86.25	-	90.00	-
Karwafa	Kondawahi gaon & tola	26.66	76.66	97.5	-	-	-
	Fulbodi	-	-	88.75	-	-	-
	Zari	-	-	87.5	-	88.33	-
	Kanartola	-	-	91.25	-	-	-
	Sakhera	-	-	89.13	-	-	-
	Jambli tola	-	-	94.19	-	-	-
	Pustola	-	-	94.66	-	-	-
	Kopagoda	-	-	95.00	-	-	-
	Katezari	-	-	95.83	-	-	-
	Mendha tola	-	-	89.33	-	-	-
	Khutgaon	-	-	90.66	-	-	-
	Michgaon	-	-	93.75	-	-	-
	Talodhi	-	-	90.00	-	-	-
	Navegaon	36.66	84.12	98.75	97.46	-	-
Murumgaon	Morchul	-	-	93.47	91.66	-	-
	Savar gaon	-	-	96.25	-	-	-
	Markagaon & tola	58.33	73.75	98.75	-	-	-
	Gajamendi	-	-	97.5	-	100	-
	Kangadi	-	-	93.75	-	-	-
	Makdand	-	-	96.25	-	-	-
	Tadegaon	-	-	98.75	-	-	-
	Dongarhud	-	-	100	-	-	-
	Sursundi	38.33	82.5	93.75	-	-	-
	Eurup dodari	-	-	92.5	-	-	-
	Saygaon	-	-	100	-	-	-
	Bhojghata	-	-	100	-	-	-
	Daranchi	-	-	100	-	-	-
	Kosami	-	-	93.00	-	-	-
	Devsura	-	85.00	96.25	-	-	-
	Kulbhatti	50.00	75.00	97.09	-	-	-
	Khedegaon	-	78.75	92.5	-	-	-
	Fulkoda	23.63	78.33	95.23	-	92.85	98.33
	Tumdi kasa	-	75.00	94.56	-	-	-
	Pannemara	33.33	-	85.00	-	-	-
Ampayali	-	-	91.25	-	-	-	
Muzalgondi	26.66	-	90.00	-	93.33	100	
Kanhartola	-	-	81.25	-	-	-	
Tavetola	-	72.5	97.5	-	-	-	

	Sindesur	-	-	96.25	-	-	-
	Muska	-	-	94.44	-	-	-
Rangi	Borigaon	28.33	71.66	83.75	-	-	-
	Niman wada	-	-	85.00	-	-	-
	Shivgatta	-	-	88.33	-	-	-
	Kanar gaon	-	-	88.75	-	-	-
	Jangada gaon & tola	50.00	75.00	90.00	-	-	-
	Regadi	21.66	-	91.25	-	-	-
Regadi	Garnji gaon & tola	-	72.5	87.5	-	-	-
	Vegnoor	-	-	95.38	-	-	-
	Potepalli	55.00	80.00	90.00	-	-	-
	Rakha gaon & tola	30.00	80.00	100	-	96.25	100
Potegaon	Maroda	-	-	93.75	-	-	-
	Savela	-	-	96.66	-	-	-
	Kurpada	-	-	98.75	-	-	-
	Kursa	20.00	86.75	96.25	-	-	-
Amirza	Murmadi	-	-	95.00	-	-	-
	Usegaon	32.00	87.5	100	-	-	-
	Maregaon & tola	-	-	97.33	-	-	-
	Bodali	Bhagwan pur	25.00	66.66	99.00	-	-
Mahagaon	Mukhtav pur	25.00	86.66	96.66	-	-	-
	Chintal pade	-	-	98.66	-	-	-
	Allapalli	-	-	100	-	-	-
	Muktapur	-	-	98.75	-	-	-

The mortality ranges were grouped as: < 80% (resistant), 80-97.99% (tolerant), and 98-100% (susceptible).

The PHC-wise susceptibility of *An. culicifacies* was analysed and observed. The corrected mortality range in different PHCs varied from 18.00% to 58.38% for DDT, 60.00% to 96.25% for malathion, 77.5% to 100.00% for deltamethrin, 85.00% to 100.00% for lambda cyhalothrin, 88.33% to 100.00% for cyfluthrin and 97.5% to 100.00% for permethrin (Table 5). The population of *An. culicifacies* was fully susceptible to deltamethrin in Kotgul and Bodali PHC, tolerant in 16 other PHCs surveyed, and resistant to deltamethrin in only three villages of three PHCs of Gadchiroli (Figure 1). Results of the present study showed that the main malaria

vector *An. culicifacies* was highly resistant to DDT in all the PHCs of Gadchiroli (Figure 3). Resistance to malathion in *An. culicifacies* was recorded in 11 PHCs and tolerance to malathion was recorded in 7 PHCs in the study area. In addition, *An. culicifacies* species was not found resistant to other synthetic pyrethroids (lambda cyhalothrin, cyfluthrin and permethrin) in any PHC of Gadchiroli, but the species was found tolerant to lambda cyhalothrin and cyfluthrin in 6 PHCs, and tolerant to permethrin in only one village of one PHC, Kasansur.

Table 5. Primary Health Centre-wise Insecticide Susceptibility of *An. culicifacies* in Gadchiroli district of Maharashtra

Insecticides tested & dose (%)	Name of PHC (No. of Villages surveyed in that PHC)	No. of Mosquitoes Exposed		No. of Mosquitoes Dead in 24 Hours		Mortality (%)		Corrected Mortality (%)	% Corrected Mortality Range in PHC
		Exp	Cont	Exp	Cont	Exp	Cont		
DDT 4.0	Kotgul (6)	80	40	50	01	62.5	2.5	62.5	27.5-35.00
Malathion 5.0		160	40	111	00	69.37	00	69.37	62.5-80.00

Deltamethrin 0.05		560	135	556	05	99.25	3.70	99.25	98.75-100	
Cyfluthrin 0.15		80	20	80	00	100	00	100	100	
DDT 4.0	Malewada (12)	270	70	97	03	36.92	4.28	36.92	26.25-48.86	
Malathion 5.0		400	110	307	06	76.75	5.45	75.35	73.07-82.5	
Deltamethrin 0.05		1030	225	960	06	93.20	2.66	93.20	78.00-100	
Lambda cyhalothrin 0.05		380	95	367	01	96.57	1.05	96.57	85.00-100	
Cyfluthrin 0.15		415	105	397	03	95.66	2.85	95.66	91.11-100	
Permethrin 0.75		90	20	90	00	100	00	100	100	
DDT 4.0		Gatta (9)	140	40	53	00	37.85	00	37.85	28.33-45.00
Malathion 5.0			180	40	137	01	97.85	2.5	97.85	80.00-81.11
Deltamethrin 0.05	830		205	767	04	92.40	1.95	92.40	83.75-100	
DDT 4.0	Kasansur (9)	120	40	39	00	32.5	00	32.5	28.33-40.00	
Malathion 5.0		280	80	226	00	80.71	00	80.71	70.00-90.00	
Deltamethrin 0.05		840	210	802	06	95.47	2.85	95.47	89.00-100	
Lambda cyhalothrin 0.05		75	25	74	01	98.66	4.00	98.66	98.6	
Cyfluthrin 0.15		160	45	152	01	95.00	2.22	95.00	95.00-95.00	
Permethrin 0.75		220	60	218	00	99.09	00	99.09	97.5-100	
DDT 4.0		Todsa (10)	120	40	35	00	29.16	00	29.16	33.33-33.33
Malathion 5.0	240		65	206	01	85.83	1.53	85.83	65.00-93.75	
Deltamethrin 0.05	805		230	788	06	97.88	2.6	97.88	91.25-100	
DDT 4.0	Aarewada (8)	120	40	39	00	32.5	00	32.5	26.66-38.33	
Malathion 5.0		140	40	126	00	90.00	00	90.00	75.00-93.33	
Deltamethrin 0.05		620	170	599	04	96.61	2.35	96.61	87.5-100	
Cyfluthrin 0.15		80	20	80	00	100	00	100	100	
Permethrin 0.75		80	20	80	00	100	00	100	100	
DDT 4.0		Laheri (10)	120	40	40	00	33.33	00	33.33	31.66-35.00
Malathion 5.0	280		80	254	02	90.71	2.5	90.71	87.5-95.00	
Deltamethrin 0.05	745		205	711	04	95.43	1.95	95.43	88.33-100	
Cyfluthrin 0.15	80		20	80	00	100	00	100	100	
DDT 4.0	Manne Rajaram (10)	120	40	27	02	22.5	5.00	22.5	21.11-26.66	
Malathion 5.0		140	40	130	00	92.85	00	92.85	88.33-96.25	
Deltamethrin 0.05		695	200	671	04	96.54	2.00	96.54	83.80-100	
Cyfluthrin 0.15		60	20	60	00	100	00	100	100	
DDT 4.0	Godalwahi (8)	120	40	35	00	29.16	00	29.16	30.00-31.66	
Malathion 5.0		320	80	263	01	82.18	1.25	82.18	80.00-85.00	
Deltamethrin 0.05		600	150	552	02	92.00	1.33	92.00	77.5-100	
Lambda cyhalothrin 0.05		80	20	74	00	92.5	00	92.5	91.25	
DDT 4.0	Pendhari (12)	195	65	57	01	29.23	1.53	29.23	18.00-33.33	
Malathion 5.0		400	120	291	03	72.75	2.5	72.75	60.00-83.75	
Deltamethrin 0.05		860	220	798	06	92.79	2.72	92.79	77.5-100	

Lambda cyhalothrin 0.05		198	60	183	00	92.42	00	92.42	85.00-100	
Cyfluthrin 0.15		60	20	55	00	91.66	00	91.66	90.00-100	
Permethrin 0.75		70	20	70	00	100	00	100	100	
DDT 4.0	Karwafa (14)	120	40	38	00	31.66	00	31.66	26.66-36.33	
Malathion 5.0		203	60	165	01	81.28	1.66	81.28	76.66-84.75	
Deltamethrin 0.05		1100	295	1019	06	9263	2.03	9263	87.5-98.75	
Lambda cyhalothrin 0.05		79	20	77	00	97.46	00	97.46	97.46	
Cyfluthrin 0.15		60	20	53	00	88.33	00	88.33	88.33	
DDT 4.0		Murumgaon (26)	360	125	140	00	38.88	00	38.88	23.63-58.38
Malathion 5.0			620	165	483	02	77.99	1.21	77.99	73.75-85.00
Deltamethrin 0.05	2080		545	2043	14	98.22	2.56	98.22	81.25-100	
Lambda cyhalothrin 0.05	60		20	55	00	91.66	00	91.66	91.66	
Cyfluthrin 0.15	212		60	205	00	96.69	00	96.69	92.85-100	
Permethrin 0.75	120		40	119	00	99.16	00	99.16	98.33-100	
DDT 4.0	Rangi (5)	120	40	47	00	39.16	00	39.16	28.33-50.00	
Malathion 5.0		120	40	89	00	74.16	00	74.16	71.66-90.00	
Deltamethrin 0.05		380	100	333	02	87.63	2.00	87.63	83.75-90.00	
DDT 4.0	Regadi (4)	120	40	46	00	38.33	00	38.33	21.66-55.00	
Malathion 5.0		140	40	110	00	78.57	00	78.57	77.5-80.00	
Deltamethrin 0.05		310	80	282	02	90.96	2.5	90.96	87.5-95.38	
DDT 4.0	Potegaon (4)	60	20	18	00	30.00	00	30.00	30.00	
Malathion 5.0		60	20	48	00	80.00	00	80.00	80.00	
Deltamethrin 0.05		330	85	321	02	97.27	2.35	97.27	93.75-100	
Cyfluthrin 0.15		80	20	78	00	97.5	00	97.5	96.25	
Permethrin 0.75		80	20	80	00	100	00	100	100	
DDT 4.0		Amirza (4)	110	40	30	00	27.27	00	27.27	20.32
Malathion 5.0	160		40	139	00	86.87	00	86.87	86.75-100	
Deltamethrin 0.05	315		85	306	01	97.14	1.17	97.14	95.00-100	
DDT 4.0	Bodali (1)	60	20	15	00	25.00	00	25.00	25.00	
Malathion 5.0		60	20	40	00	66.66	00	66.66	66.66	
Deltamethrin 0.05		100	20	99	00	99.00	00	99.00	98.75	
DDT 4.0	Mahagaon (4)	60	20	15	00	25.00	00	25.00	25.00	
Malathion 5.0		60	20	52	00	86.66	00	86.66	86.66	
Deltamethrin 0.05		275	85	271	01	98.54	1.17	98.54	86.66-100	

The mortality ranges were grouped as: < 80 (resistant), 80-97.99% (tolerant), and 98-100% (susceptible).

Table 6. Cone Bioassay Tests of *An. culicifacies* on different types of Wall Surfaces sprayed with Lambda cyhalothrin in Study Villages of Malaria Endemic PHCs of Gadchiroli district, Maharashtra

Sr. No.	Name of Village and PHC	Week	% Mortality of <i>An. culicifacies</i> in Control	% Mortality of <i>An. culicifacies</i> on Wall Surfaces of Sprayed Houses in Study Villages against Lambda cyhalothrin 0.05%	
				Cemented wall	Mud wall
1.	Kotami (Kasansur)	2	0.00 (10)	46.66 (30)	-
2.	Ghotsur (Kasansur)	1	0.00 (10)	-	40.00 (30)
3.	Kondawahi (Kasansur)	4	0.00 (15)	-	28.88 (45)
4.	Venasur (Kasansur)	3	0.00 (10)	-	36.66 (30)
5.	Chokhewada (Kasansur)	2	0.00 (10)	-	33.33 (30)
6.	Katezari (Murumgaon)	1	6.66 (15)	51.11 (45)	-
7.	Murumgaon (Murumgaon)	1	6.66 (15)	48.88 (45)	-
8.	Savargaon (Murumgaon)	2	0.00 (15)	46.66 (30)	43.33 (30)
9.	Markagaon (Murumgaon)	1	0.00 (15)	-	27.5 (40)
10.	Gajamendi (Murumgaon)	1	0.00 (10)	-	40.00 (30)
11.	Morchul (Murumgaon)	2	0.00 (15)	-	31.11 (45)
12.	Dhorgatta (Pendhari)	1	6.66 (15)	-	42.5 (40)
13.	Pekinmudza (Pendhari)	1	0.00 (10)	-	43.33 (30)
14.	Durgapur (Pendhari)	2	0.00 (10)	43.33 (30)	-
15.	Khargi (Pendhari)	3	0.00 (15)	43.33 (30)	30.00 (30)
16.	Paidi (Pendhari)	2	5.00 (20)	48.88 (45)	33.33 (30)
17.	Devsara (Malewada)	1	6.66 (15)	46.66 (30)	27.5 (40)
18.	Khobra menda (Malewada)	2	5.00 (20)	43.33 (30)	30.00 (30)
19.	Jaysing tola (Malewada)	3	0.00 (10)	32.5 (40)	-
20.	Mange wada (Malewada)	2	0.00 (10)	-	30.00 (30)
	Mortality range		5.00-6.66	51.11-32.5	43.33-27.5

Note: Figures in brackets represent names of PHCs of Gadchiroli district, Maharashtra and the total number of *An. culicifacies* tested.

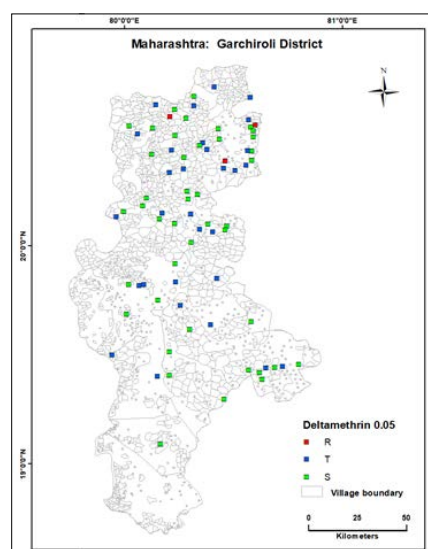


Figure 1. Map of Gadchiroli District showing Susceptibility Status of Malaria Vector to Deltamethrin in Study Villages of Gadchiroli, Maharashtra

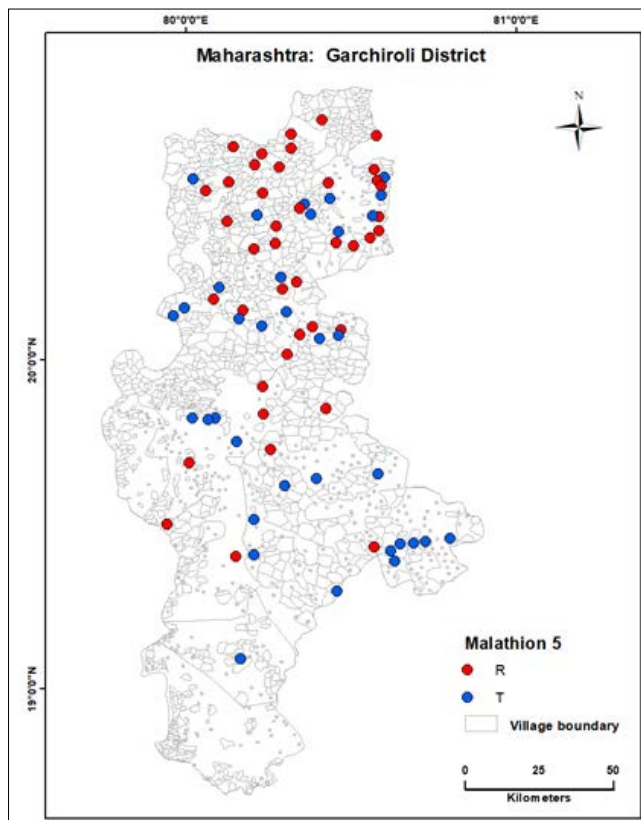


Figure 2. Map of Garchiroli District showing Malathion Resistance & Tolerance of Malaria Vector in Study Villages of Garchiroli, Maharashtra

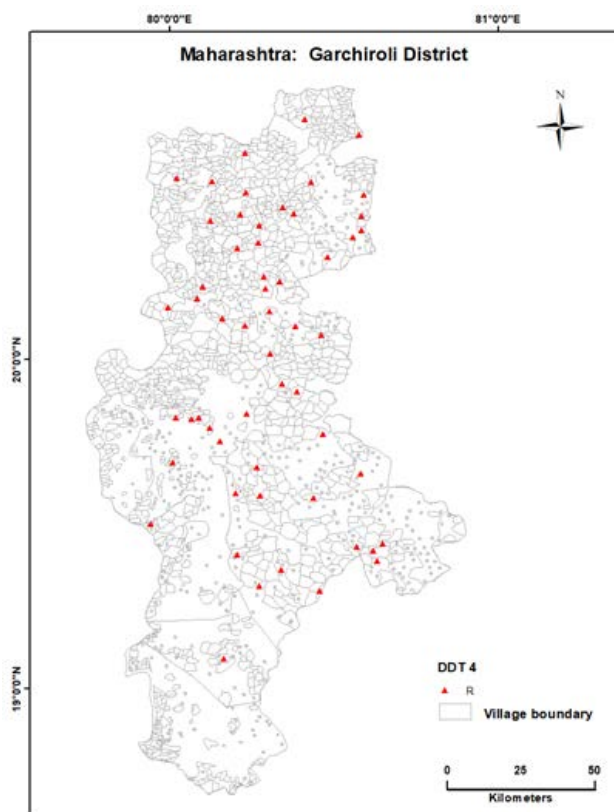


Figure 3. Map of Garchiroli District showing DDT Resistance to Malaria Vector in Study Villages of Garchiroli, Maharashtra

Residual action of sprayed insecticide was determined by using cone bioassays kits on different sprayed surfaces available in the study area. Results of the cone bioassay tests revealed that IRS was found satisfactory in few sites in terms of effectiveness in the control of *An. culicifacies* on the wall surfaces tested in 20 study villages of 4 PHCs of Gadchiroli. Mortality range was from 51.11% to 32.5% on cemented or 43.33% to 27.5% on the mud wall sprayed with lambda cyhalothrin. Lambda cyhalothrin was found more effective on cemented walls as compared to mud walls of the houses. Detailed information regarding the results of cone bioassays is given in Table 6. Quality and coverage of IRS were not found appropriate during the study because IRS coverage was patchy, not uniform, and incomplete on all walls of the houses. Moreover, the spray was found only on upper portion of the outer wall of the houses in some villages.

Discussion

Malaria control in the country is undertaken by following the guidelines provided by NVBDCP and management of malaria vector is the main tool of the malaria control programme. To manage the malaria vectors, insecticide based intervention measures like IRS and ITBNs/ LLINs are the important components of the Indian malaria control programme,⁶ but the development of insecticide resistance in malaria vectors is one of the major problems in malaria control. IRS with synthetic insecticides like DDT and BHC was used to control malaria vectors for interrupted transmission of malaria in Gadchiroli since 1958. This mode of vector control observed huge success in the beginning, but later development of insecticide resistance in malaria vectors was recorded in 1959 after more than one and a half decades of its use due to continuous use of insecticides under the programme.

An. culicifacies is the common primary vector of malaria responsible for most of malaria epidemics and perennial malaria transmission in India. In Gadchiroli district of Maharashtra, the prevalent vectors of malaria are *An. culicifacies* and *An. fluviatilis*, of which *An. culicifacies* is prevalent widely while *An. fluviatilis* is prevalent only in few pockets. The findings are in accordance with the studies undertaken from Godda and Gumla districts of Jharkhand,^{22,23} and are also from Murumgaon PHC area of Gadchiroli district.²⁴ Dhiman et al. reported *An. culicifacies*, *An. fluviatilis*, and *An. annularis* vectors of malaria from Dhanora taluka of Gadchiroli district and also observed that there was no evidence of outdoor resting of *An. culicifacies* except in one village of Dhanora PHC in 2005, where the malaria vector was found during hand collections from tree holes near human dwellings. However, *An. culicifacies* could not be collected in light trap collections.²⁵ The finding has given an indication of more possibilities of outdoor

malaria transmission as tribal people go to the forest for their earning in early morning hours and come back late. It may play a great role in malaria control.

The resistance in *An. culicifacies* malaria vector against DDT was first time found in Maharashtra, 2627 and confirmatory report on DDT resistance in *An. culicifacies* was published by Rahman et al. in the year 1959.¹⁰ Similarly, DDT resistance in *An. culicifacies* and *An. annularis* was reported from a village of Meerut city, Uttar Pradesh in 1962.²⁸ Multiple resistance in *An. culicifacies* to DDT, HCH, and malathion was reported from Thane district of Maharashtra,²⁹ and *An. culicifacies* resistance to BHC and dieldrin was reported from adjoining areas of Gujarat and Maharashtra,³⁰ and other states by earlier research workers.³¹⁻³² Malathion was introduced in 1969 in IRS for vector control, and resistance in *An. culicifacies* against malathion was found very quickly from Gujarat within four years of usage of insecticide¹¹; and also from Maharashtra²⁸ and other states like Andhra Pradesh, Orissa, Madhya Pradesh, Chhattisgarh and Jharkh and^{22,31-36} Later on, deltamethrin was introduced in IRS in 1996 to control malaria vectors due to their safety for humans at low dosage, excito-repellent properties, and knock-down, killing effects. Deltamethrin was introduced in IRS to control malaria vectors on the pilot basis in Gadchiroli district in 1998, and later in 2008, the insecticide was widely used in IRS activities under malaria control programme, impregnation of bed nets, and making LLINs to prevent malaria.^{12,13}

In earlier studies, all the species of *An. culicifacies*, *An. fluviatilis*, and *An. annularis* mosquitoes were found tolerant to deltamethrin and malathion and highly resistant against DDT in Murumgaon PHC of Gadchiroli in the year 2012²⁴ and later on, Gyan Chand et al. (2017) reported resistance to DDT, malathion and other synthetic pyrethroids as lambda cyhalothrin, deltamethrin, cyfluthrin, and tolerance to permethrin in *An. culicifacies*.³⁷ However, *An. culicifacies* species has developed resistance to DDT in 286 districts, to Malathion in 81, and to pyrethroids in 2 districts in India.⁶⁻⁸ *Anopheles fluviatilis* is a secondary malaria vector in Gadchiroli district with less prevalence limited to a few villages. However, it has been reported as a primary vector of malaria in the adjoining areas of the neighbouring states of Chhattisgarh and Maharashtra.^{9,30,38}

Anopheles fluviatilis plays a role in malaria transmission throughout the year and is mainly found in forest hilly tract villages near a stream. Presently *An. fluviatilis* is resistant to DDT in 11 districts from 8 states including Maharashtra due to exposure to the pesticides used in agriculture. Resistance in *An. fluviatilis* against DDT was found from Odisha³⁹ and other studies also reported resistance in *An. fluviatilis* to DDT in areas like Jharkhand and Maharashtra.^{22,24,31} These findings have also been confirmed recently from Gadchiroli

by Gyan Chand et al.³⁷ Similar results have been reported earlier by different researchers from abroad on multiple resistances in *An. gambiae*, *An. funestus*, *An. arabiensis*, and *An. coluzzi* from different parts of Africa and other countries. But, Sharma et al. (2004) reported that this species was found susceptible to DDT and other insecticides like malathion and deltamethrin in 7 districts of Odisha.³² The resistance developed in *An. fluviatilis* against DDT may be due to the regular use of DDT in IRS since 1958. Later on, the tolerance or resistance of *An. fluviatilis* to Malathion was found from different districts and states including Maharashtra.^{22,24,25,31,32}

The results of the present study indicated that both *An. culicifacies* and *An. fluviatilis* were highly resistant to DDT in all the PHCs while moderately resistant to malathion and tolerant to deltamethrin. Similar observations were made by earlier researchers from Madhya Pradesh, central India in the year 2012.³⁵ *An. culicifacies* was found resistant to deltamethrin in a few villages only but susceptible to synthetic pyrethroids in most of the villages. Moreover, it was resistant to malathion in most of the villages of PHCs surveyed and highly resistant to DDT in all the villages. *Anopheles fluviatilis* was resistant to DDT only, tolerant to malathion and fully susceptible to deltamethrin in all the villages of Gadchiroli district. Cone bioassay tests were done for the determination of residual action in 20 villages of 4 malaria endemic PHCs on the wall surface of the houses sprayed with lambda cyhalothrin insecticide after 1 to 4 weeks of indoor residual spray (IRS). Results of this study revealed that IRS was found satisfactory in terms of effectiveness in the control of *An. culicifacies* on the wall surfaces tested in 20 study villages of 4 PHCs of Gadchiroli. Lambda cyhalothrin was found more effective on cemented walls as compared to mud walls of the houses and IRS was found satisfactory in a few villages. IRS was not uniform and was found incomplete or patchy on walls of the houses and the quality of IRS was poor in some houses. Similar findings were observed by previous research workers from Odisha and Bhojpur PHC of Moradabad District of Uttar Pradesh by Gunasekaran and Shukla et al. and also from Aurangabad town of Maharashtra state by Vittal et al.⁴⁵⁻⁴⁷

Suitable Strategy for Control of Malaria Vectors in Gadchiroli District

The district of Gadchiroli has been receiving two rounds of IRS with lambda cyhalothrin and cyfluthrin synthetic pyrethroid along with the use of LLINs containing permethrin (Olyset brand) to effectively control malaria. In spite of these control measures, malaria remains a major public health problem in this district. There is an urgent need of an increased emphasis on regular and effective IRS in high-risk PHCs for better control of malaria vectors. PHC wise IRS activities should be done with more susceptible

insecticides. The results of the present study may be made use of regarding the selection of the insecticide as malaria vector was resistant to deltamethrin only in few villages. Introduction of newly developed LLINs (with added synergists like PBO) may lead to effective control of the resistant population of mosquito vectors. Moreover, the use of available mosquito repellents should be encouraged in the community to minimise the outdoor biting of malaria vectors since there may be more possibilities of outdoor malaria transmission as most of the tribal people/villagers go to forests early in the morning to earn their livelihood and come back late. It may play a great role in the control of malaria in the district. There is also a need to find out alternate insecticides for IRS activities and insecticide susceptibility needs to be tested regarding other insecticides of synthetic pyrethroids like bifenthrin & alpha-cypermethrin as approved by NVBDCP. IRS for these insecticides has not been used before in Gadchiroli district of Maharashtra and it may be a useful option after indication of cross-resistance. Special IEC activities should be taken up in the local language to increase the community's knowledge and awareness and to ensure a change of behaviour at the ground level to eliminate malaria from Gadchiroli district of Maharashtra.

Conclusion

In conclusion, the finding of this study indicates that resistance has developed to synthetic pyrethroids in the major malaria vector *An. culicifacies*. As synthetic pyrethroids are used in malaria control programmes for IRS and impregnation of bed nets, it is an adverse indication to achieve the goal of eliminating malaria by 2030. Therefore, there is an urgent need for the evaluation of new insecticide molecules for better control of malaria vectors. In addition, there is also a need for study on insecticide susceptibility of malaria vectors from the region to confirm this finding.

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References

1. World Health Organization [Internet]. World Malaria Report 2018; [cited 2020 Nov 20]. Available from: <http://apps.who.int/iris/bitstream/hand>

- le/10665/275867/9789241565653-eng.pdf?ua=1
2. National Vector Borne Disease Control Programme [Internet]. Malaria situation in India, Delhi; [cited 2020 Nov 20]. Available from: <https://nvbdcp.gov.in/WriteReadData/l892s/78341804791628840488.pdf>
 3. Kumar A, Valecha N, Jain T, Dash AP. Burden of Malaria in India: retrospective and prospective view. *Am J Trop Med Hyg.* 2007;77(6 Suppl):69-78. [PubMed]
 4. Dhingra N, Joshi RD, Dhillon GP, Lal S. Enhanced Malaria Control Project for World Bank support under National Malaria Eradication Programme (NMEP). *J Commun Dis.* 1997;29(3):201-8. [PubMed] [Google Scholar]
 5. Office of District Malaria Office, Gadchiroli, Maharashtra state. <https://scholar.google.com/scholar?q=Office+of+District+Malaria+Office+,+Gadchiroli,+Maharashtra+>
 6. Sharma VP. Continuing challenge of malaria in India. *Curr Sci.* 2012;102(5):678-82. [Google Scholar]
 7. Singh RK, Kumar G, Mittal PK. Insecticide susceptibility status of malaria vectors in India: A review. *Int J Mosq Res.* 2014;1(1):5-9. [Google Scholar]
 8. Sharma VP, Dev V. Biology & control of *Anopheles culicifacies* Giles 1901. *Ind J Med Res.* 2015;141(5):525-36. [PubMed] [Google Scholar]
 9. Sharma VP. Fighting malaria in India. *Curr Sci.* 1998;75:1127-40. [Google Scholar]
 10. Rahman J, Roy ML, Singh K. Development of increased tolerance to DDT in *Anopheles culicifacies* Giles, in the Panch Mahal district of Bombay state, India. *Indian J Malariol.* 1959;13(2/3):125-30. [Google Scholar]
 11. Rajagopal R. Malathion resistance in *Anopheles culicifacies* in Gujarat. *Indian J Med Res.* 1977;66:27-8. [PubMed] [Google Scholar]
 12. Dhingra N, Joshi RD, Dhillan GP, Lal S. Enhanced Malaria Control Project by World Bank support under National Malaria Eradication Program (NMEP). *J Commun Dis.* 1997; 29:201-208.
 13. Government of Maharashtra Public Health Department, Directorate of Health Services, [cited 2020 Nov 1]. Available from: <https://arogya.maharashtra.gov.in/Site/Form/DiseaseContent.aspx?CategoryDetailsID=gWjOzP09lf8=>
 14. Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *An. culicifacies* in Surat district, Gujarat, west India. *Curr Sci.* 2002;82:547-50. [Google Scholar]
 15. Office of the Registrar General & Census Commissioner, India [Internet]. Census of India. 2011. Available from: <https://www.censusindia.gov.in> (accessed on 26.11.2021)
 16. World Health Organization [Internet]. Instructions for determining the susceptibility or resistance of adult mosquito to organo-chlorine organophosphate and carbonate insecticides – Diagnostic test. WHO/VBC/1981-806.
 17. World Health Organization. Manual on practical entomology in malaria part II. Methods and techniques. Geneva: World Health Organization; 1975. 191 p.
 18. Wattal BL, Kalra NL. Regionwise pictorial keys to the female Indian *Anopheles*. *Bull Nat Soc Mal Other Mosq Borne Dis.* 1961;9:85-138. [Google Scholar]
 19. Das BP, Rajagopal R, Akiyama J. Pictorial key to the species of Indian Anopheline mosquitoes. *J Pure Appl Zool.* 1990;2:131-62. [Google Scholar]
 20. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 1925;18:265-7.
 21. WHO. Instructions for the bioassay of insecticidal deposits on wall surfaces. VBC/81.5 (WHO/VBC/81.812). Geneva: World Health Organization; 1981.
 22. Singh RK, Dhiman RC, Mittal PK, Das MK. Susceptibility of Malaria Vectors to insecticides in Gumla district, Jharkhand state, India. *J Vector Borne Dis.* 2010;47(2):116-8. [PubMed] [Google Scholar]
 23. Singh RK, Dhiman RC, Das MK. Situation analysis of malaria in Godda district of Jharkhand during malaria epidemic. *J Commun Dis.* 2011;43(2):135-42. [PubMed] [Google Scholar]
 24. Singh RK, Mittal PK, Gourshettiwar MP, Pande SJ, Dhiman RC. Susceptibility of malaria vectors to insecticides in Gadchiroli district (Maharashtra), India. *J Vector Borne Dis.* 2012;49(1):42-4. [PubMed] [Google Scholar]
 25. Dhiman RC, Shahi B, Sharma SN, Nanda N, Khargiwarkar VN, Subbarao SK. Persistence of malaria transmission in a tribal area in Maharashtra, India. *Curr Sci.* 2005;88(3):475-8. [Google Scholar]
 26. Rao TR, Bhatia SC. A note on the degree of susceptibility of *Anopheles culicifacies* to DDT in some parts of Bombay state. *Indian J Malariol.* 1957;11(3):261-9. [PubMed] [Google Scholar]
 27. Deobhankar RB, Palkar ND. Magnitude of DDT resistance in *Anopheles culicifacies* in Maharashtra state. *J Commun Dis.* 1990;22:77. [PubMed] [Google Scholar]
 28. Krishnamurthy BS, Singh NN. DDT resistance in *Anopheles culicifacies* Giles, 1901 and *Anopheles annularis* Van der Wulp 1884 in a village of Meerut district, U.P. *Indian J Malariol.* 1962;16:375-7. [Google Scholar]
 29. Vittal M, Deshpande LB. Development of Malathion resistance in a DDT, HCH resistant *Anopheles culicifacies* population in Thane district (Maharashtra). *J Commun Dis.* 1983;15:144-5. [PubMed] [Google Scholar]
 30. Sharma MID, Samnotra KG. A note on gamma BHC and dieldren resistance in *Anopheles culicifacies* Giles in adjoining areas of Gujarat and Maharashtra states. *Bull Natl Soc India Malar Mosq Dis.* 1962;10:151-7.

31. Singh RK, Dhiman RC, Kumar G, Sinha ATS, Dua VK. Susceptibility status of malaria vectors to insecticides in Koderma, Jharkhand. J Commun Dis. 2011;43:273-6. [PubMed] [Google Scholar]
32. Sharma SK, Upadhyay AK, Haque MA, Singh OP, Adak T, Subbarao SK. Insecticide susceptibility status of malaria vectors in some hyper-endemic tribal districts of Orissa. Curr Sci. 2004;87(12):1722-6.
33. Sahu SS, Gunasekaran K, Vijayakumar T, Jambulingam P. Triple insecticide resistance in *Anopheles culicifacies*: a practical impediment for malaria control in Odisha State, India. Indian J Med Res. 2015 Dec;142(Suppl 1):S59-63. [PubMed] [Google Scholar]
34. Raghavendra K, Vasantha K, Subbarao SK, Pillai MK, Sharma VP. Resistance in *Anopheles culicifacies* sibling species B and C to Malathion in Andhra Pradesh and Gujarat states, India. J Am Mosq Control Assoc. 1991;7(2):255-9. [PubMed] [Google Scholar]
35. Mishra AK, Chand SK, Barik TK, Dua VK, Raghavendra K. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, central India. J Vector Borne Dis. 2012;49(1):39-41. [PubMed] [Google Scholar]
36. Bhatt RM, Sharma SN, Barik TK, Raghavendra K. Status of insecticide resistance in malaria vector, *Anopheles culicifacies* in Chhattisgarh state, India. J Vector Borne Dis. 2012;49(1):36-8. [PubMed] [Google Scholar]
37. Chand G, Behera P, Bang A, Singh N. Status of insecticide resistance in *An. culicifacies* in Gadchiroli (Maharashtra) India. Pathog Glob Health. 2017;111(7):362-6. [PubMed] [Google Scholar]
38. Singh N, Khare KK. Forest malaria in Madhya Pradesh: changing scenario of disease and its vectors. Indian J Parasit Dis. 1999;23:105-12.
39. Sahu SS, Patra KP. A study on insecticides resistance in *Anopheles fluviatilis* and *An. culicifacies* to HCH and DDT in the Malkangiri district of Orissa. Indian J Malariol. 1995;32(3):112-8. [PubMed] [Google Scholar]
40. Koekemoer LL, Spellings BL, Christian RN, Lo TC, Kaiser ML, Norton RA, Oliver SV, Choi KS, Brooke BD, Hunt RH, Coetzee M. Multiple insecticide resistance in *Anopheles gambiae* (Diptera: Culicidae) from Pointe Noire, Republic of the Congo. Vector Borne Zoonotic Dis. 2011;11:1193-200. [PubMed] [Google Scholar]
41. Dabiré KR, Diabaté A, Djogbenou L, Ouari A, N'Guessan R, Ouedraogo JB, Hougard JM, Chandre F, Baldet T. Dynamics of multiple insecticide resistance in the malaria vector *Anopheles gambiae* in a rice growing area in South-Western Burkina Faso. Malar J. 2008;7(1):188. [PubMed] [Google Scholar]
42. Djouaka RJ, Atoyebi SM, Tchigossou GM, Riveron JM, Irving H, Akoton R, Kusimo MO, Bakare AA, Wondji CS. Evidence of a multiple insecticide resistance in the malaria vector *Anopheles funestus* in South West Nigeria. Malar J. 2016;15(1):565. [PubMed] [Google Scholar]
43. Nardini L, Christian RN, Coetzer N, Koekemoer LL. DDT and pyrethroid resistance in *Anopheles arabiensis* from South Africa. Parasit Vectors. 2013;6(1):229. [PubMed] [Google Scholar]
44. Toé KH, N'Falé S, Dabiré RK, Ranson H, Jones CM. The recent escalation in strength of pyrethroid resistance in *Anopheles coluzzi* in West Africa is linked to increased expression of multiple gene families. BMC Genomics. 2015;16:146. [PubMed] [Google Scholar]
45. Gunasekaran K, Sahu SS, Jambulingam P, Das PK. DDT Indoor Residual spray, still an effective tool to control *Anopheles fluviatilis*-transmitted *Plasmodium falciparum* malaria in India. Trop Med Int Health. 2005;10:160-8. [PubMed] [Google Scholar]
46. Shukla RP, Sharma SN, Bhat SK. Malaria outbreak in Bhojpur PHC of District Moradabad, Uttar Pradesh, India. J Commun Dis. 2002;34:118-23. [PubMed] [Google Scholar]
47. Vittal M, Bhat MR. Bioassay tests on the effectiveness of malathion spraying on *Anopheles culicifacies* resting on different wall surfaces in Aurangabad town, Maharashtra. Indian J Malariol. 1981;18:124-5.