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ABSTRACT

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), 4 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 8 In India, malaria is transmitted by bites of female Anopheles mosquitoes of An. culicifacies, An. stephensi, An. fluviatilis, An. minimus, An. dirus, and An. sundaicus, the six primary vectors, and An. varuna, An. annularis, An. philippensis and An. jeyporensis 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. 6 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 Gujarat 14 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-economic 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).

| Year | Population | BSC&E | +ve | PF | PV | ABER | API | SPR | SFR (%) | PF (%) | Death |
|------|------------|-------|-----|----|----|------|-----|-----|---------|--------|-------|
| 2011 | 1158947    | 529846| 1367| 10581| 3089| 45.72| 11.8 | 2.57| 1.99    | 77.40 | 6     |
| 2012 | 1177242    | 520258| 6596| 4656 | 1940| 44.19| 5.6  | 1.26| 0.89    | 70.58 | 4     |
| 2013 | 1209496    | 536341| 6436| 4919 | 1517| 44.34| 5.32| 1.19| 0.91    | 74.62 | 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    |

| Name of PHCs | Name of Sub Centres | Name of Study Villages |
|--------------|----------------------|------------------------|
| Kotgul       | Sonpur               | Sonpur, Bhimankozri    |
|              | Gyarahpatti          | Gyarahpatti, Pitesur gaon & tola, Nehalgad |
|              | Kosami 2             | Kosami 2               |
| Malewada     | Devsara              | Devsara, Mendha, Murmadi, Khattitola |
|              | 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, Surjugad |
|              | 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, Ekrbhurj    |
|              | 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          | Malum padur, Bhouse wada |
|              | Beena gunda          | Beena gunda            |
| Manne Rajaram | Manne Rajaram | Mokela |
|---------------|---------------|--------|
| Chichoda      | Chichoda      | Jijgaon, Bhade nagar |
| Yechali       | Yechali       | Basa guda, Rela, Rai guda, Maram pali, 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       | Karwafa       | Kondawahi gaon & tola, Fulbodi, Zari |
|---------------|---------------|-------------------------------------|
| Fulbodi       | Kanartola     |                                    |
| Pustola       | Pustola, Kopagoda |                                |
| Karwafa       | Talodhi, Navegaon |                                |
| Sakhera       | Sakhera, Jamblitol |                                |
| Chatgaon      | Katezari      |                                    |
| Mendhatola    | Mendhatola    | Khutgaon, Michgaon |

| Murumgaon     | Murumgaon     | Morchul, Savar gaon, Markagaon & tola, Gajamendi, Kangadi, Makdand |
|---------------|---------------|--------------------------------------------------------------------|
| Kambada       | 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         | Rangi         | Borigaon, Nimanwada, Shivgatta |
|---------------|---------------|--------------------------------|
| Mohali        | Kanargaon    |                                    |
| Jangada       | Jangada gaon & Tola |

| Regadi        | Regadi        | Regadi, Garnijigaon & Tola, Vegnoor |
|---------------|---------------|-------------------------------------|
| Chapalwara    | Potepalli     |                                    |

| Potegaon      | Potegaon      | Rakhagaon & Tola |
|---------------|---------------|--------------------|
| Maroda        | Maroda, Savela |                    |
| Yewali        | Kurpada       |                    |

| Amirza        | Amirza        |                    |
|---------------|---------------|--------------------|
| Kursa         | Kursa         |                    |
| Murmadi       | Murmadi       |                    |
| Usegaon       | Usegaon       |                    |
| Maregaon      | Maregaon & Tola |                    |

| Bodali        | Bodali        | Bhagwan pur |
|---------------|---------------|-------------|
| Mahagaon      | Mahagaon      | 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. 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. 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. 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. 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:

\[
\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times \frac{\% \text{ observed mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}}
\]

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. 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 |
|---------------------------|-----------------------------------------------|---------------------------|----------------------------------------------------------|
| DDT 4.0                   | Kotgul (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |
| DDT 4.0                   | Laosansur (Sonpur, Bhimankozhi, Gyarahpatti, Pitesur gaon & tola, Nehalgad) | 30 10 14 00 | 46.66 00 46.66 |
| Malathion 5.0             | Malewada (Mendha, Khobramenda, Waghabhumi)     | 30 10 15 00 | 50.00 00 50.00 |
| Deltamethrin 0.05         | Kasansur (Kondawahi)                           | 30 10 13 00 | 43.33 00 43.33 |

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 (%) |
|-------------|----------------|----------------------------------|
| Kotgul      | Sonpur         | DDT 4: 73.75  | Mala 5: 98.75 |
|             | Bhimankozhi    | Delta 0.05: 99.00 |
|             | Gyarahpatti    | LCM 0.05: - |
|             | Pitesur gaon & tola | Cyflu 0.15: - |
|             | Nehalgad       | Perm 0.75: - |
|             | Kosami 2       | -                  |

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| Village            | Malewada          | Gatta             | Kasansur        | Todsa            | Aarewada         |
|--------------------|-------------------|-------------------|-----------------|------------------|-----------------|
| Devsara            | -                 | -                 | -               | -                | 26.66           |
| Mendha             | 26.25             | 83.75             | 33.33           | 26.66            | 26.66           |
| Murmadi            | 73.07             | 70.00             | 70.00           | 75.00            | 75.00           |
| Khettola           | -                 | -                 | -               | -                | -               |
| Khobramenda        | 31.00             | 99.00             | -               | -                | -               |
| Huryaldand         | -                 | 98.75             | -               | -                | -               |
| Badbada            | 48.86             | 98.75             | 98.75           | 100              | 100             |
| Yeduskuhi          | -                 | 98.75             | 98.75           | 100              | 100             |
| Waghbhumti         | -                 | 83.33             | -               | -                | -               |
| Mangewada          | -                 | 98.00             | -               | -                | -               |
| Jaysing tola       | -                 | 88.00             | -               | 91.11            | -               |
| Khamtala           | -                 | 98.00             | 83.75           | -                | -               |
| Jhanbia gaon & tola| -                 | 83.75             | -               | -                | -               |
| Andenge            | 45.00             | 98.88             | -               | -                | -               |
| Nander             | 28.33             | 90.00             | -               | -                | -               |
| Hedari gaon        | -                 | 88.00             | -               | -                | -               |
| Mallam pahari      | -                 | 100               | -               | -                | -               |
| Sur jagad          | -                 | 89.00             | -               | -                | -               |
| Gattagaon & tola   | -                 | 95.00             | -               | -                | -               |
| Mohndi gaon        | -                 | 87.5              | 83.75           | -                | -               |
| Tadguda            | -                 | 97.00             | -               | -                | -               |
| Kotami             | -                 | 90.00             | 93.75           | -                | -               |
| Etawahi            | -                 | 93.00             | -               | -                | -               |
| Kondawahi          | -                 | 91.66             | 100             | -                | -               |
| Tadguda            | -                 | 76.25             | 99.00           | -                | -               |
| Ghotsur            | -                 | 70.00             | 92.5            | -                | -               |
| Jaweli             | 28.33             | 96.00             | -               | -                | -               |
| Venasur            | -                 | 100               | -               | -                | -               |
| Chokhewada         | -                 | 89.00             | -               | -                | -               |
| Jhuri              | 40.00             | 99.00             | 98.6            | 95.00            | 100             |
| Ekakhurdh          | -                 | 93.75             | 100             | -                | -               |
| Ekraburj           | -                 | 98.75             | -               | -                | -               |
| Aalinga gaon & tola| 33.33             | 99.00             | -               | -                | -               |
| Zarewada           | -                 | 100               | -               | -                | -               |
| Alandi             | -                 | 100               | -               | -                | -               |
| Tadhpalli          | -                 | 91.25             | -               | -                | -               |
| Lanji              | -                 | 92.00             | 100             | -                | -               |
| Karampalli gaon & tola | -          | 96.92             | -               | -                | -               |
| Pethagaon          | -                 | 92.5              | -               | -                | -               |
| Devada             | 33.33             | 100               | -               | -                | -               |
| Hemal kasha        | 26.66             | 75.00             | 100             | -                | -               |
| Karampalli gaon & tola | -          | 100               | -               | -                | -               |
| Village     | Dhar for Ca (kg/ha) | Dhar for Mg (kg/ha) | Dhar for Zn (kg/ha) | Dhar for P (kg/ha) | Dhar for K (kg/ha) | Dhar for Cu (kg/ha) | Dhar for Fe (kg/ha) |
|-------------|---------------------|---------------------|---------------------|-------------------|-------------------|---------------------|---------------------|
| Aarewada    |                     |                     |                     |                   |                   |                     |                     |
| Medpalli    | 38.33               | 93.33               | 100                 | -                 | -                 | -                   |                     |
| Dubbaguda   | -                   | -                   | 95.00               | -                 | -                 | -                   |                     |
| Tadgaon     | -                   | -                   | 93.75               | -                 | -                 | -                   |                     |
| Dhulepalli  | -                   | -                   | 100                 | -                 | -                 | -                   |                     |
| Erakdumme   | -                   | -                   | 87.5                | -                 | -                 | -                   |                     |
| Dhondraj    |                     |                     |                     |                   |                   |                     |                     |
| 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               | -                 | -                 | -                   |                     |
| Mokela      |                     |                     |                     |                   |                   |                     |                     |
| 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 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   | -                   | -                   | 95.00               | 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               | -                 | -                 | -                   |                     |
| Village          | Area (ha) | Productivity (kg/ha) | Yield (kg/ha) | 100% | 92.85 |
|------------------|-----------|----------------------|---------------|------|-------|
| Paidi            | 68.33     |                      | 100           |      |       |
| Gotagaon & tola | 60.00     | 77.5                 | 91.66         |      |       |
| Jhawal zora      | 24.00     | 71.66                | 98.75         |      |       |
| Pathar gatta     | 71.66     | 86.25                | 90.00         |      |       |
| Kondawahi gaon & tola | 26.66 | 76.66                | 97.5          |      |       |
| 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|       |
| 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                  |               |      |       |
| Bhoighata        | -         | 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                 |               |      |       |
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 100.0% 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   | 27.5-35.00     |
| Malathion 5.0                 |                                               | 160 | 40   | 111 | 00   | 69.37| 00    | 62.5-80.00     |
| Insecticide | Concentration | Test Site | Sample Size | Mortality (%) | Control Mortality (%) | Control (%) |
|-------------|---------------|-----------|-------------|---------------|-----------------------|-------------|
| Deltamethrin 0.05 | 560 135 556 05 | Malewada (12) | 99.25 | 99.25 | 98.75-100 |
| Cyfluthrin 0.15 | 80 20 80 00 | Gatta (9) | 3.70 | 100 | 100 |
| DDT 4.0 | 270 70 97 03 | Malathion 5.0 | 36.92 | 4.28 | 36.92 |
| Malathion 5.0 | 400 110 307 06 | Deltamethrin 0.05 | 76.75 | 5.45 | 75.35 |
| Lambda cyhalothrin 0.05 | 1030 225 960 06 | Kasansur (9) | 93.20 | 2.66 | 93.20 |
| Cyfluthrin 0.15 | 380 95 367 01 | DDT 4.0 | 96.57 | 1.05 | 96.57 |
| Permethrin 0.75 | 415 105 397 03 | Malewada (12) | 95.66 | 2.85 | 95.66 |
| DDT 4.0 | 90 20 90 00 | Malewada (12) | 100 | 100 | 100 |
| Malathion 5.0 | 140 40 53 00 | Malewada (12) | 37.85 | 00 | 37.85 |
| Deltamethrin 0.05 | 180 40 137 01 | Malewada (12) | 97.85 | 2.5 | 97.85 |
| DDT 4.0 | 830 205 767 04 | Malewada (12) | 92.40 | 1.95 | 92.40 |
| Malathion 5.0 | 120 40 39 00 | Malewada (12) | 32.5 | 00 | 32.5 |
| Deltamethrin 0.05 | 280 80 226 00 | Malewada (12) | 80.71 | 00 | 80.71 |
| Lambda cyhalothrin 0.05 | 840 210 802 06 | Malewada (12) | 95.47 | 2.85 | 95.47 |
| Cyfluthrin 0.15 | 75 25 74 01 | Malewada (12) | 98.66 | 4.00 | 98.66 |
| Permethrin 0.75 | 160 45 152 01 | Malewada (12) | 95.00 | 2.22 | 95.00 |
| DDT 4.0 | 220 60 218 00 | Malewada (12) | 99.09 | 00 | 99.09 |
| Malathion 5.0 | 120 40 35 00 | Malewada (12) | 29.16 | 00 | 29.16 |
| Lambda cyhalothrin 0.05 | 240 65 206 01 | Malewada (12) | 85.83 | 1.53 | 85.83 |
| Cyfluthrin 0.15 | 805 230 788 06 | Malewada (12) | 97.88 | 2.6 | 97.88 |
| Permethrin 0.75 | 620 170 599 04 | Malewada (12) | 90.00 | 00 | 90.00 |
| DDT 4.0 | 120 40 39 00 | Malewada (12) | 32.5 | 00 | 32.5 |
| Malathion 5.0 | 140 40 126 00 | Malewada (12) | 90.00 | 00 | 90.00 |
| Lambda cyhalothrin 0.05 | 805 230 788 06 | Malewada (12) | 97.88 | 2.6 | 97.88 |
| Cyfluthrin 0.15 | 620 170 599 04 | Malewada (12) | 96.61 | 2.35 | 96.61 |
| Permethrin 0.75 | 160 45 152 01 | Malewada (12) | 95.00 | 2.22 | 95.00 |
| DDT 4.0 | 220 60 218 00 | Malewada (12) | 99.09 | 00 | 99.09 |
| Malathion 5.0 | 120 40 35 00 | Malewada (12) | 29.16 | 00 | 29.16 |
| Lambda cyhalothrin 0.05 | 240 65 206 01 | Malewada (12) | 85.83 | 1.53 | 85.83 |
| Deltamethrin 0.05 | 805 230 788 06 | Malewada (12) | 97.88 | 2.6 | 97.88 |
| Cyfluthrin 0.15 | 120 40 39 00 | Malewada (12) | 32.5 | 00 | 32.5 |
| Permethrin 0.75 | 140 40 126 00 | Malewada (12) | 90.00 | 00 | 90.00 |
| DDT 4.0 | 120 40 40 00 | Malewada (12) | 33.33 | 00 | 33.33 |
| Malathion 5.0 | 140 40 130 00 | Malewada (12) | 92.85 | 00 | 92.85 |
| Lambda cyhalothrin 0.05 | 695 200 671 04 | Malewada (12) | 96.54 | 2.00 | 96.54 |
| Cyfluthrin 0.15 | 80 20 80 00 | Malewada (12) | 100 | 00 | 100 |
| DDT 4.0 | 80 20 80 00 | Malewada (12) | 100 | 00 | 100 |
| Malathion 5.0 | 120 40 40 00 | Malewada (12) | 33.33 | 00 | 33.33 |
| Lambda cyhalothrin 0.05 | 695 200 671 04 | Malewada (12) | 96.54 | 2.00 | 96.54 |
| Deltamethrin 0.05 | 80 20 80 00 | Malewada (12) | 100 | 00 | 100 |
| Cyfluthrin 0.15 | 120 40 40 00 | Malewada (12) | 33.33 | 00 | 33.33 |
| DDT 4.0 | 140 40 130 00 | Malewada (12) | 92.85 | 00 | 92.85 |
| Malathion 5.0 | 695 200 671 04 | Malewada (12) | 96.54 | 2.00 | 96.54 |
| Lambda cyhalothrin 0.05 | 80 20 80 00 | Malewada (12) | 100 | 00 | 100 |
| Deltamethrin 0.05 | 120 40 35 00 | Malewada (12) | 29.16 | 00 | 29.16 |
| Cyfluthrin 0.15 | 60 20 60 00 | Malewada (12) | 100 | 00 | 100 |
| DDT 4.0 | 120 40 35 00 | Malewada (12) | 29.16 | 00 | 29.16 |
| Malathion 5.0 | 320 80 263 01 | Malewada (12) | 82.18 | 1.25 | 82.18 |
| Lambda cyhalothrin 0.05 | 600 150 552 02 | Malewada (12) | 92.00 | 1.33 | 92.00 |
| Deltamethrin 0.05 | 80 20 80 00 | Malewada (12) | 92.5 | 00 | 92.5 |
| Cyfluthrin 0.15 | 195 65 57 01 | Malewada (12) | 29.23 | 1.53 | 29.23 |
| Deltamethrin 0.05 | 860 220 798 06 | Malewada (12) | 92.79 | 2.72 | 92.79 |
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 *An. culicifacies* in Control | % Mortality of *An. culicifacies* 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 Garchiroli District showing Susceptibility Status of Malaria Vector to Deltamethrin in Study Villages of Garchiroli, Maharashtra](image-url)
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, 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 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 Chhattisgarh and Maharashtra.9,30,38

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 insecticide; and also from Maharashtra40 and other states like Andhra Pradesh, Orissa, Madhya Pradesh, Chhattisgarh and Jharkhand and22,31-36 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.11,13

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. Similar results have been reported earlier by different researchers from abroad on multiple resistances in An. gambiae, An. funestus, An. arabiensis, and An. culicifacies 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. 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.

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. 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 Bhopur 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. 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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