Research Article

Monitoring of Insecticide Resistance in Anopheles culicifacies in Twelve Districts of Madhya Pradesh, Central India (2017–2019)

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Background. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are malaria vector control measures used in India, but the development of insecticide resistance poses major impediments for effective vector control strategies. As per the guidelines of the National Vector Borne Disease Control Programme (NVBDCP), the study was conducted in 12 districts of Madhya Pradesh to generate data on insecticide resistance in malaria vectors. Methods. The susceptibility tests were conducted on adult An. culicifacies as per the WHO standard technique with wild-caught mosquitoes. The blood-fed female mosquitoes were exposed in 3 to 4 replicates on each occasion to the impregnated papers with specified discriminating dosages of the insecticides (DDT: 4%, malathion: 5%, deltamethrin: 0.05%, and alphacypermethrin: 0.05%), for one hour, and mortality was recorded after 24-hour holding. Results. An. culicifacies was found resistant to DDT 4% in all the 12 districts and malathion in 11 districts. Resistance to alphacypermethrin was also observed in two districts, and possible resistance was found to alphacypermethrin in seven districts and to deltamethrin in eight districts, while the vector was found susceptible to both deltamethrin and alphacypermethrin in only 3 districts. Conclusion. An. culicifacies is resistant to DDT and malathion and has emerging resistance to pyrethroids, alphacypermethrin, and deltamethrin. Therefore, regular monitoring of insecticide susceptibility in malaria vectors is needed for implementing effective vector management strategies. However, studies to verify the impact of IRS with good coverage on the transmission of disease are required before deciding on the change of insecticide in conjunction with epidemiological data.

1. Introduction

Malaria is a major public health problem in India, contributing to about 89% of incidence from South East Asia [1]. Five Indian states are responsible for transmission of more than 70% of malaria in the country of which Madhya Pradesh is the fifth highly malarious state which contributes about 5% of total malaria cases [2]. Anopheles culicifacies is the main malaria vector in rural and periurban areas in India contributing to about 65% of annual malaria transmission [3]. Insecticide-based vector control interventions currently in use in India include indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) [4]. One of the major impediments for effective vector control is the development of resistance in vectors to the insecticides which are used in public health sprays. Presently, three insecticides, DDT (organochlorine), malathion (organophosphate), and mostly synthetic pyrethroids, are used in IRS and LLINs. An. culicifacies has shown resistance to DDT [5–7] and malathion [8, 9] and also reduced susceptibility to synthetic pyrethroids in a few areas including in Madhya Pradesh and Chhattisgarh [10–14]. This study was undertaken as a task force project under the aegis of the Indian Council of Medical Research. Based on epidemiological data and
geographic ecosystems, the National Vector Borne Disease Control Programme (NVBDCP) selected 12 districts of Madhya Pradesh to generate data on insecticide resistance in malaria vectors (Table 1).

There are 50 districts in Madhya Pradesh with a population of about 60 million, including 12 million tribal populations. The state consists of sparsely settled forested hills with a 31% forested area and serves as a reservoir for intense perennial malaria transmission [15]. In the present investigation, we monitored the insecticide susceptibility status of An. culicifacies in 12 districts of Madhya Pradesh against commonly used insecticides in the public health system.

2. Materials and Methods

2.1. Study Area. The susceptibility tests against An. culicifacies were carried out in 12 districts of Madhya Pradesh located in the northern, eastern, western and southern parts of the state, namely, districts Umaria, Singrauli, Anuppur, Panna, Tikamgarh, Hoshangabad, Khargone, Dhar, Alirajpur, Bhind, Datia, and Shivpuri, from July 2017 to July 2019. In the districts, three to seven villages in two to three CHCs (about 1% of total villages in the district) in different terrains, i.e., hilltop, plain, foothill, and forest terrains were selected for the studies (Table 2). Anuppur, Umaria, and Bhind were under DDT indoor spray and Singrauli, Panna, Hoshangabad, Khargone, Dhar, Alirajpur, and Shivpuri were under alphacypermethrin (synthetic pyrethroid) indoor spray. In districts Tikamgarh and Datia, there was no routine indoor spray for the last >20 years due to low malaria prevalence (<2 API). However, in the year 2016, 26 villages in the district of Tikamgarh received focal sprays of DDT. In five districts, viz., Panna, Anuppur, Singrauli, Alirajpur, and Dhar, long-lasting insecticide-treated nets were distributed (Table 2). However, all the districts were proposed for LLIN distribution by the year 2019.

2.2. Insecticide Susceptibility Tests. Susceptibility tests were conducted on adult An. culicifacies following essentially the WHO standard procedures using the kit and method [16]. Wild-caught mosquitoes were collected from different resting sites (indoors-human dwellings/cattle sheds and outdoors) and preferably blood-fed female mosquitoes [17] and identified based on morphological characters [18] in the selected villages of the districts in the different months from 2017 to 2019 (Table 2). The collected mosquitoes were brought to the laboratory for testing in cloth cages wrapped with wet towels. Female mosquitoes were exposed in 3 to 4 replicates on each occasion to the WHO impregnated papers with specified discriminating dosages of the insecticides (DDT: 4%, malathion: 5%, deltamethrin: 0.05%, and alphacypermethrin: 0.05%), with respective insecticide controls for comparison (two replicates) for one hour, and mortality was recorded after 24-hour holding. The tests were repeated within 2 or 3 days in different villages of different terrains in each district. Cartons with wet towels at the bottom were used to conduct the tests to maintain the ambient temperature of 26 ± 2°C and the RH of 70–80% [19]. Mortality after 24 hrs of holding period was recorded [20]. Percent mortality was calculated separately for the test and control replicates using the following formula: % observed mortality = number of dead mosquitoes × 100/number of mosquitoes tested.

If the mortality in control replicates is between 5% and 20%, the test mortality was corrected with the control mortality using Abbott’s formula [21]. In cases where the mortality in the controls exceeded 20%, the test was discarded: % corrected mortality = (% test mortality – % control mortality) × 100/(100 – % control mortality).

According to the WHO criteria [20], mosquito species that show on exposure to the diagnostic dosage of a given insecticide a mortality rate of 98 to 100% are designated as “susceptible,” <90% as “confirmed resistance,” and between 90% and 98% as “possible resistance.”

3. Results

Results of the susceptibility tests carried out in 12 districts are given in Table 3. An. culicifacies was found resistant to DDT in all the districts with a % mortality rate ranging from 7.6 to 60% and resistant to malathion in 11 districts (62 to 87%) (Figure 1).

In district Datia, species showed possible resistance to malathion, registering 93% mortality. Resistance to alphacypermethrin was observed in Dhar and Alirajpur districts where % mortality was 84.2 and 87.6, respectively, and tests were repeated after 6 months, and the mortality was 82.9 and 85.7% indicating no variation in mortality (Table 3).

An. culicifacies was reported susceptible to pyrethroids, viz., alphacypermethrin and deltamethrin in 3 districts, i.e., Anuppur, Panna, and Tikamgarh (98.1 to 100.0% mortality), while in Datia it was susceptible to deltamethrin (100% mortality).

An. culicifacies was possibly resistant to alphacypermethrin in 7 districts, viz., Singrauli, Umaria, Hoshangabad, Khargone, Datia, Shivpuri, and Bhind, with mortality ranging from 90.5 to 97.0%. However, to deltamethrin, possible resistance in An. culicifacies was observed in 8 districts, viz., Singrauli, Umaria, Hoshangabad, Khargone, Alirajpur, Dhar, Shivpuri, and Bhind where mortality registered was between 93.3 and 97% (Table 3).

The terrain-wise pooled data of 12 districts (Table 4) showed similar susceptibility status in all 4 terrains, i.e., plain, foothill, hilltop, and forest areas except for deltamethrin. The species was possibly resistant with registered mortality of 96.7%, 94.3%, and 96.4%, respectively, in plain, foothill, and hilltop terrains, whereas in forest terrain the species was susceptible to deltamethrin and with 98% mortality. However, the difference in observed mortalities was within a range of 2–4% indicating the population to be near susceptible or possibly resistant.

An. culicifacies showed resistance to DDT and malathion in all the terrains with the observed % mortality rate to DDT in the range of 14.5 to 23.4 and to malathion in the range of 67.0 to 74.4%. Possible resistance to alphacypermethrin was
observed in all 4 terrains with a mortality rate in the range of 91.3 to 95.4%.

Based on the spray history in last 10 years in different districts, districts were categorized into three groups: group A-IRS with pyrethroids, 7 districts, viz., Panna, Hoshangabad, Singrauli, Khargone, Dhar, Alirajpur, and Shivpuri; group B-IRS with DDT, 3 districts, viz., Anuppur, Umaria, and Bhind; and group C-without IRS, 2 districts, viz., Tikamgarh and Datia (Table 5). An. culicifacies was found resistant to DDT and malathion registering low % mortality rates for DDT of 16.2, 15.6, and 37.6% in groups A, B, and C, respectively, while increased % mortality rates were registered for malathion at 69.2, 68.3, and 88.8%, respectively. To pyrethroid alphacypermethrin, the species showed possible resistance in groups A and B with % mortality in the range of 90.2 and 96.2, respectively, but was susceptible in group C with 98.5% mortality. Statistical analysis of mortalities against alphacypermethrin between the sprayed group (A) and the no spray group (C) was highly significant (chi sq. = 15.36, p < 0.0001) and with the DDT sprayed group (B) (chi.

### Table 1: Epidemiological situation in the districts selected for the insecticide monitoring study.

| Districts | Year | Population | BSE | +VE | PF | ABER | API | SPR | PF% |
|-----------|------|------------|-----|-----|----|------|-----|-----|-----|
| Dhar      | 2015 | 2364759    | 348960 | 4328 | 1949 | 15.00 | 2.00 | 1.00 | 45.00 |
|           | 2016 | 2412054    | 320115 | 2100 | 765  | 13.00 | 1.00 | 1.00 | 36.00 |
| Hoshangabad| 2015| 1343272    | 197175 | 1672 | 665  | 14.68 | 1.24 | 0.85 | 39.0  |
|           | 2016 | 1372137    | 197608 | 1139 | 445  | 14.42 | 0.83 | 0.58 | 39.0  |
| Anuppur   | 2015 | 811306     | 79769  | 2007 | 1280 | 10.00 | 3.00 | 3.00 | 64.0  |
|           | 2016 | 776248     | 79504  | 1226 | 764  | 10.00 | 2.00 | 2.00 | 62.0  |
| Panna     | 2015 | 1078217    | 167850 | 1332 | 242  | 16.00 | 1.00 | 1.00 | 18.00 |
|           | 2016 | 1099781    | 13969  | 1582 | 479  | 13.00 | 1.00 | 1.00 | 30.00 |
| Tikamgarh | 2015| 1534632    | 152233 | 1336 | 18   | 9.07  | 0.87 | 0.88 | 1.35  |
|           | 2016 | 1564028    | 185300 | 1327 | 38   | 11.8  | 0.85 | 0.72 | 2.86  |
| Shivpuri  | 2015 | 1884582    | 220925 | 3858 | 576  | 12.00 | 2.00 | 2.00 | 15.00 |
|           | 2017 | 1922690    | 201781 | 1648 | 93   | 11.00 | 1.00 | 1.00 | 6.00  |
| Datia     | 2016 | 868232     | 90424  | 456  | 8    | 44.00 | 2.00 | 2.00 | 7.00  |
|           | 2017 | 885586     | 113899 | 506  | 3    | 10.00 | 0.50 | 0.50 | 2.00  |
| Alirajpur | 2016 | 800942     | 122482 | 1602 | 713  | 15    | 2    | 1    | 44.0  |
|           | 2017 | 810614     | 84331  | 704  | 302  | 10    | 1    | 1    | 43.0  |
| Bhind     | 2016 | 1880870    | 218174 | 3424 | 51   | 11.60 | 1.82 | 1.57 | 1.49  |
|           | 2017 | 1918488    | 191213 | 1925 | 11   | 9.97  | 1.00 | 1.01 | 0.57  |

Note. The epidemiological data of three districts (Singrauli, Khargone, and Umaria) were not available at the time of study. BSE: blood smear examination, +VE: number of malaria positive cases, PF: Plasmodium falciparum, ABER: annual blood examination rate, API: annual parasite incidence, SPR: slide positivity rate, and PF%: Plasmodium falciparum percentage.

### Table 2: Profile of study areas including the vector control measures and the period of study.

| S No. | Districts | Location | Insecticide used for IRS | LLIN distributed (yes or no) | No. of study villages | Ecotype of villages | Period of surveys |
|-------|-----------|----------|--------------------------|-------------------------------|-----------------------|---------------------|-------------------|
| 1     | Anuppur   | East     | DDT                      | Yes                           | 6                     | Plain, foothill, hilltop | Sept 2017         |
| 2     | Panna     | North    | SP                       | Yes                           | 6                     | Plain, foothill, hilltop | Oct 2017          |
| 3     | Tikamgarh | North    | No spray                 | No                            | 3                     | Plain, forest         | Oct 2017          |
| 4     | Singrauli | East     | SP                       | Yes                           | 6                     | Plain, foothill, hilltop | Oct 2017          |
| 5     | Umaria    | East     | DDT                      | No                            | 6                     | Plain, foothill, hilltop | July 2017         |
| 6     | Hoshangabad | South  | SP                       | Yes                           | 6                     | Plain, foothill, hilltop | Apr 2018          |
| 7     | Khargone  | South    | SP                       | No                            | 6                     | Plain, foothill, forest | Sept 2018         |
| 8     | Alirajpur | West     | SP                       | Yes                           | 6                     | Plain, foothill, forest | Dec 2018 and Jul 2019 |
| 9     | Dhar      | West     | SP                       | Yes                           | 6                     | Plain, foothill, forest | Dec 2018 and Jul 2019 |
| 10    | Shivpuri  | North    | SP                       | No                            | 7                     | Plain, forest          | Feb 2019          |
| 11    | Datia     | North    | No spray                 | No                            | 6                     | Plain, forest          | Feb 2019          |
| 12    | Bhind     | North    | DDT                      | No                            | 7                     | Plain, foothill         | Jul 2019          |

SP = synthetic pyrethroids: alphacypermethrin
The species showed possible resistance to deltamethrin with % mortality in the range of 95.2 and 97.1% in groups A and B, respectively, but was completely susceptible in group C.

Table 3: Susceptibility status of *An. culicifacies* to discriminatory dosages of DDT, malathion, alphacypermethrin, and deltamethrin in 12 districts of Madhya Pradesh.

| Insecticide-% | Districts | No. of mosquitoes exposed | Dead 24 hr | Mortality (%) | Susceptibility status** |
|---------------|-----------|---------------------------|------------|---------------|-------------------------|
|               |           | Exp | Control | Exp | Control |               |                     |
| DDT-4%        | Anuppur   | 105 | 60      | 17  | 1       | 16.2          | CR                  |
|               | Panna     | 105 | 45      | 13  | 1       | 12.4          | CR                  |
|               | Singrauli | 105 | 45      | 19  | 0       | 18.1          | CR                  |
|               | Tikamgarh | 105 | 30      | 17  | 0       | 16.2          | CR                  |
|               | Umaria    | 105 | 45      | 15  | 0       | 14.3          | CR                  |
|               | Hoshangabad | 105 | 60      | 8   | 2       | 7.6           | CR                  |
|               | Khargone  | 120 | 60      | 15  | 1       | 12.5          | CR                  |
|               | Alirajpur | 105 | 45      | 8   | 1       | 7.6           | CR                  |
|               | Dhar      | 120 | 45      | 21  | 0       | 17.5          | CR                  |
|               | Datia     | 100 | 45      | 60  | 1       | 60            | CR                  |
|               | Shivpuri  | 100 | 55      | 41  | 0       | 41            | CR                  |
|               | Bhind     | 105 | 45      | 17  | 0       | 16.2          | CR                  |
| Malathion-5%  | Anuppur   | 105 | 60      | 68  | 1       | 64.8          | CR                  |
|               | Panna     | 105 | 45      | 77  | 0       | 73.3          | CR                  |
|               | Singrauli | 105 | 45      | 74  | 2       | 70.5          | CR                  |
|               | Tikamgarh | 105 | 30      | 89  | 0       | 84.8          | CR                  |
|               | Umaria    | 105 | 45      | 80  | 0       | 76.2          | CR                  |
|               | Hoshangabad | 105 | 60      | 76  | 2       | 72.4          | CR                  |
|               | Khargone  | 120 | 60      | 78  | 0       | 65            | CR                  |
|               | Alirajpur | 105 | 45      | 61  | 0       | 58.1          | CR                  |
|               | Dhar      | 120 | 45      | 78  | 0       | 65            | CR                  |
|               | Datia     | 100 | 45      | 93  | 2       | 93            | PR                  |
|               | Shivpuri  | 100 | 55      | 87  | 2       | 87            | CR                  |
|               | Bhind     | 105 | 45      | 67  | 0       | 63.8          | CR                  |
| Alphacypermethrin-0.05% | Anuppur | 105 | 60      | 103 | 2     | 98.1          | S                   |
|               | Panna     | 105 | 45      | 104 | 0     | 99            | S                   |
|               | Singrauli | 105 | 45      | 95  | 1     | 90.5          | PR                  |
|               | Tikamgarh | 105 | 30      | 105 | 0     | 100           | S                   |
|               | Umaria    | 105 | 45      | 100 | 1     | 95.2          | PR                  |
|               | Hoshangabad | 105 | 60      | 101 | 0     | 96.2          | PR                  |
|               | Khargone  | 120 | 60      | 112 | 1     | 93.3          | PR                  |
|               | Alirajpur | 105 | 45      | 92  | 2     | 87.6          | CR                  |
|               | Dhar      | 120 | 45      | 101 | 1     | 84.2          | CR                  |
|               | Datia     | 100 | 45      | 97  | 2     | 97            | PR                  |
|               | Shivpuri  | 100 | 55      | 93  | 2     | 93            | PR                  |
|               | Bhind     | 105 | 45      | 100 | 0     | 95.2          | PR                  |
| Deltamethrin-0.05% | Anuppur | 105 | 60      | 103 | 2     | 98.1          | S                   |
|               | Panna     | 105 | 45      | 104 | 0     | 99            | S                   |
|               | Singrauli | 105 | 45      | 98  | 1     | 93.3          | PR                  |
|               | Tikamgarh | 105 | 30      | 105 | 0     | 100           | S                   |
|               | Umaria    | 105 | 45      | 101 | 1     | 96.2          | PR                  |
|               | Hoshangabad | 105 | 60      | 100 | 0     | 95.2          | PR                  |
|               | Khargone  | 120 | 60      | 116 | 1     | 96.7          | PR                  |
|               | Alirajpur | 105 | 45      | 101 | 2     | 96.2          | PR                  |
|               | Dhar      | 120 | 45      | 112 | 1     | 93.3          | PR                  |
|               | Datia     | 100 | 45      | 100 | 2     | 100           | S                   |
|               | Shivpuri  | 100 | 55      | 97  | 2     | 97            | PR                  |
|               | Bhind     | 105 | 45      | 102 | 0     | 97.1          | PR                  |

*The control mortality in all districts in all insecticides was either <5.0. ** CR = confirmed resistant, PR = possible resistant, and S = susceptible.

Sq. = 11.15, *p* = 0.001) and no significance was seen in alphacypermethrin mortality when compared with the no spray (C) and the DDT sprayed (B) group (chi. Sq. = 2.44, *p* = 0.118).
4. Discussion

Insecticide resistance is becoming a limiting factor for effective malaria vector control for national programmes worldwide, especially in view of the committed elimination of malaria in this decade by 2030. Presently, about 125 species of mosquitoes are documented to show resistance to one or more insecticides.

![Map of Madhya Pradesh showing study districts and insecticide susceptibility status of An. culicifacies.](image)

**Table 4:** Terrain-wise grouped insecticide susceptibility data in *An. culicifacies*.

| Type of terrain | Insecticide | No. of mosquitoes exposed | Mortality in 24 hr | Mortality (%) | Susceptibility status* |
|-----------------|-------------|---------------------------|--------------------|---------------|------------------------|
|                 | DDT         | Exp 285                   | Exp 116            | 19.3          | CR                     |
|                 | Malathion   | 600                        | 285                |               |                        |
|                 | Alphacypermethrin | 600 285          | 548                | 91.3          | PR                     |
|                 | Deltamethrin | 600 285                   | 580                | 96.7          | PR                     |
| Plain           | Malathion   | 295                        | 120                | 23.4          | CR                     |
|                 | Alphacypermethrin | 295 120          | 213                | 72.2          | CR                     |
|                 | Deltamethrin | 295 120                   | 274                | 92.9          | PR                     |
|                 | Malathion   | 400                        | 190                | 14.5          | CR                     |
|                 | Alphacypermethrin | 400 190          | 366                | 91.5          | PR                     |
|                 | Deltamethrin | 400 190                   | 377                | 94.3          | PR                     |
|                 | Malathion   | 195                        | 75                 | 20.5          | CR                     |
|                 | Alphacypermethrin | 195 75           | 145                | 74.4          | CR                     |
|                 | Deltamethrin | 195 75                    | 186                | 95.4          | PR                     |
|                 | Malathion   | 195                        | 75                 | 96.4          | PR                     |

*CR = confirmed resistance, PR = possible resistance, and S = susceptible.
Raghavendra et al. [22] reviewed the status of insecticide resistance among the major malaria vectors in India in the last quarter century (1991–2016) based on the available information from published and unpublished reports. Resistance to DDT in *An. culicifacies* is widespread in the country [5, 6], and resistance to malathion is widespread in the districts in the states of Maharashtra [8], Gujarat [23, 24], Andhra Pradesh [24], Uttar Pradesh [9], and Madhya Pradesh [13]. There are a few reports of resistance to synthetic pyrethroids in various parts of the country [10–14]. Resistance to malathion was detected in five districts of Andhra Pradesh, nine districts of Odisha, and possible resistance in two districts of Jharkhand, 4 districts of Odisha, and 4 districts of West Bengal. *An. culicifacies* was found susceptible to malathion in two districts of Jharkhand and six districts of Odisha, resistant to deltamethrin in four districts of Andhra Pradesh, with possible resistance in 10 districts of Odisha, and susceptible to deltamethrin in some districts of Odisha, Jharkhand, and West Bengal [25].

In the present study, in Madhya Pradesh, *An. culicifacies* the main malaria vector was found resistant to DDT 4% in all the 12 districts surveyed and resistant to malathion in 11 districts, except in Datia district where the species is reported possibly resistant. The species was reported resistant to alphacypermethrin in two districts Dhar and Alirajpur. This vector was found susceptible to both deltamethrin and alphacypermethrin in three districts, i.e., Anuppur, Panna, and Tikamgarh. Possible resistance was found to alphacypermethrin in seven districts, namely, Singrauli, Umaria, Hoshangabad, Datia, Shivpuri, Bhind, and Khargone, and to deltamethrin in eight districts, viz., Singrauli, Umaria, Hoshangabad, Khargone, Alirajpur, Dhar, Shivpuri, and Bhind. Thus, the species was resistant to DDT and malathion in all the districts while it was mostly possible resistant to pyrethroids.

It may be stated that DDT has been sprayed in these areas in surveyed districts since the inception of the national malaria control activities in the early 1950s. Decreased mortality in *An. culicifacies* to pyrethroids was found in areas that received alphacypermethrin IRS in the last 5–10 years. In all the areas, the species in different districts have shown resistance to DDT and malathion. However, in areas without pyrethroid indoor spray, the species registered possible resistance and were susceptible to pyrethroids, alphacypermethrin, and deltamethrin in the range of 96.2 to 100%. Malathion was not sprayed regularly in these areas and the observed resistance to malathion could be due to possible selection by its use in agriculture/forestry in the absence of its use in public health sprays but needs further investigation.

To date, DDT, malathion, deltamethrin, alphacypermethrin, and lambda cyhalothrin are the most commonly used insecticides for vector control in public health in India, and other pyrethroid insecticides, namely, cyfluthrin and bifenthrin, are also recommended for use in antimalaria spraying [26]. Deltamethrin and alphacypermethrin impregnated LLINs are in extensive use in India in different states of the country, and Madhya Pradesh is receiving the LLINs in 2019 in all endemic districts.

The resistance in mosquitoes may develop due to changes in their enzyme systems resulting in more rapid detoxification or sequestration of the insecticide or due to mutations in the target site preventing the insecticide target site interaction [27]. Spraying of insecticides without proper understanding of the prevailing resistance mechanism may lead to increased vector resistance and failure of vector control intervention. In India, end-point replacement of insecticides is practiced after failure of control of a given class of insecticide resulting in multiple resistance in malaria vectors [28].

### 5. Conclusion

Results of the present study in 12 districts of Madhya Pradesh indicate that *An. culicifacies* is reported resistant in all the districts to DDT and to malathion, while to pyrethroids, alphacypermethrin, and deltamethrin the species is reported mostly possible resistant. Owing to the dynamics of

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**Table 5: Susceptibility status of *An. culicifacies* in the districts grouped under different categories based on IRS.**

| Villages with different insecticide sprays | Insecticide | No. of mosquitoes exposed | No. of mosquitoes dead | % mortality | Susceptibility status* |
|------------------------------------------|-------------|--------------------------|------------------------|------------|------------------------|
|                                           |             | Test                     | Control                |            |                        |
| Group A—pyrethroid IRS since last 5–10 years and earlier with DDT IRS-7 districts (Singrauli, Panna, Hoshangabad, Khargone, Dhar, Shivpuri, and Alirajpur) | DDT         | 970                      | 445                    | 157        | 5                      | 16.2 | CR                       |
|                                           | Malathion   | 970                      | 445                    | 671        | 7                      | 69.2 | CR                       |
|                                           | Alphacypermethrin | 970                 | 445                    | 875        | 9                      | 90.2 | PR                       |
|                                           | Deltamethrin| 970                      | 445                    | 923        | 9                      | 95.2 | PR                       |
| Group B—DDT spray since last 5–10 years—3 districts (Anuppur, Bhind, and Umaria) | DDT         | 315                      | 105                    | 49         | 1                      | 15.6 | CR                       |
|                                           | Malathion   | 315                      | 105                    | 215        | 1                      | 68.3 | CR                       |
|                                           | Alphacypermethrin | 315              | 105                    | 303        | 3                      | 96.2 | PR                       |
|                                           | Deltamethrin| 315                      | 105                    | 306        | 3                      | 97.1 | PR                       |
| Group C—no spray since last 20 years—2 districts (Tikamgarh and Datia) | DDT         | 205                      | 75                     | 77         | 1                      | 37.6 | CR                       |
|                                           | Malathion   | 205                      | 75                     | 182        | 2                      | 88.8 | CR                       |
|                                           | Alphacypermethrin | 205           | 75                     | 202        | 2                      | 98.5 | S                        |
|                                           | Deltamethrin| 205                      | 75                     | 205        | 2                      | 100.0| S                        |

*C = confirmed resistant, PR = possible resistant, and S = susceptible.*
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