Susceptibility of malaria vectors to insecticides in Gadchiroli district (Maharashtra), India

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Malaria is hyperendemic in central parts of India with 95% of the population at risk of infection1. In Maharashtra state, Gadchiroli district is one of the high malaria endemic districts where 13,553 malaria positive cases were reported in the year 2010, out of which Plasmodium falciparum accounted for 82.4%. Slide positivity rate (SPR) in Gadchiroli district has been ranging from as low as 1.43 to as high as 2.5% between the years 2006 and 20102. But it has been substantially higher (2.5%) in 2010.

Geographical area of Gadchiroli district is 14,412 km² and it is located on the north-eastern side of the state of Maharashtra. It is situated at 18.43' to 21.50' north latitude and 79.45' to 80.53' east longitude and has uneven terrain with hills, valleys and forests at different altitudes and having total population of 10,71,795 (as of census 2011)3. The average rainfall is 1743.5 mm, temperature minimum 11.3 and maximum 47.7°C.

Since 1958, the district has been receiving two rounds of sprays of DDT every year. An. culicifacies has developed resistance to DDT, dieldrin and malathion (triple resistance) in about 30 districts of Maharashtra. Presently, this area is receiving two rounds of spray with a synthetic pyrethroid, alpha-cypermethrin. In spite of these control measures, malaria remains a major public health problem. The primary vectors of malaria in Gadchiroli district are An. culicifacies and An. fluviatilis4,5.

In India, An. culicifacies Giles alone contributes about 65% of the total cases of malaria annually and is the widespread malaria vector of rural and peri-urban areas. However, this species has developed resistance to DDT in 286 districts, to malathion in 81 and to pyrethroids in 2 districts in India6–8. In order to find out the rationale about using alpha-cypermethrin in vector control, this study was carried out on insecticide susceptibility in 10 endemic villages of PHC, Murumgaon, Gadchiroli district of Maharashtra state.

Insecticides susceptibility tests were carried out in the the year 2010 by using WHO procedure against various insecticides9–10. In the morning hours (0600 to 0800 hrs) with the help of suction tube, An. culicifacies Giles, An. fluviatilis James and An. annularis Van der Wulp were collected from cattlesheds, human dwellings and mixed dwellings of different villages of Murumgaon PHC. The collected female mosquitoes were provided with 10% glucose solution soaked in cotton pads and transported in caged cloth to the field laboratories at the PHC of Murumgaon, Gadchiroli district. Insecticide-impregnated papers with different diagnostic doses received from University Sans Malaysia were used for detection of resistance to DDT (4%), malathion (5%) and deltamethrin (0.05%).

Only full-fed and semi-gravid mosquitoes were used to determine the susceptibility status. Mosquitoes were exposed against the diagnostic doses of insecticides for an hour, as per standard WHO technique9,10. Three to four replicates of each vector species containing 15–25 female mosquitoes were taken simultaneously for each insecticide. Two replicates for control were also held parallel to each test. The mosquitoes were exposed for 1 h and kept for observation for 24 h. The percent mortalities were scored after 24 h of recovery period and if the control mortality remained within 5–20%, test mortality was corrected using Abbott’s formula11 and expressed as corrected percent mortality. If the mortality is >20%, the tests were discarded.

Results of susceptibility tests on An. culicifacies, An. fluviatilis and An. annularis against DDT (4%), malathion (5%) and deltamethrin (0.05%) are given in Table 1. The corrected percent mortality of adult An. culicifacies, An. fluviatilis and An. annularis to DDT (4%) ranged between 21 and 36.66%; to malathion (5%) between 90.52 and 96%; and to deltamethrin (0.05%) between 94 and 96.42%. These results indicate that the tested mosquito species are resistant to DDT but tolerant to malathion and deltamethrin.

In India, resistance in malaria vectors to DDT at sev-
eral places has been reported\textsuperscript{6–8}. First indication of resistance to DDT in \textit{An. culicifacies} malaria vectors was found in Maharashtra state by Rao and Bhatia\textsuperscript{12} in the year 1957. However, first confirmed report on DDT resistance was published by Rahman \textit{et al}\textsuperscript{13} in 1959. Later, Krishnamurthy and Singh\textsuperscript{14} recorded resistance in \textit{An. culicifacies} to DDT in a village of Uttar Pradesh in the year 1962. Double or triple resistance to DDT, dieldrin and malathion was recorded in \textit{An. culicifacies} from 30 districts of Maharashtra\textsuperscript{12,15–18}. Resistance in \textit{An. culicifacies} to malathion was first time observed from the adjoining state of Gujarat by Rajagopal \textit{et al}\textsuperscript{19} in the year 1977. Raghavendra \textit{et al}\textsuperscript{20} in the year 1991 reported malathion resistance in adjoining state of Andhra Pradesh. Dhiman \textit{et al}\textsuperscript{21} in the year 2005 reported resistance to DDT in \textit{An. culicifacies} from Dhanora taluka in Gadchiroli district, but it was found susceptible to other insecticides. Our study confirmed this finding in Murumgaon PHC area of District Gadchiroli, where \textit{An. culicifacies}, as well as \textit{An. fluviatilis} and \textit{An. annularis} were found resistant to DDT only, while tolerant to other insecticides tested.

\textit{Anopheles fluviatilis} has been reported as a primary vector of malaria in the forests of adjoining States of Chhattisgarh including Maharashtra\textsuperscript{4,5}. \textit{Anopheles fluviatilis} is found to transmit malaria throughout the year in these areas, whereas \textit{An. culicifacies} transmit malaria in post-monsoon months between July and September. \textit{Anopheles fluviatilis} is mainly distributed in hilly tract villages and the preferential breeding habitat is stream\textsuperscript{22,23}, where the chances of exposure to the agriculture pesticides are relatively less. \textit{Anopheles fluviatilis} is resistance to DDT in 11 districts from 8 states including Maharashtra\textsuperscript{7}. Resistance to DDT in adult \textit{An. fluviatilis} has also been reported from Orissa (now Odisha)\textsuperscript{24}. However, Sharma \textit{et al}\textsuperscript{25} reported that \textit{An. fluviatilis} was found susceptible to DDT, malathion and deltamethrin in seven districts of Odisha. Singh \textit{et al}\textsuperscript{26} recently reported resistance in \textit{An. culicifacies}, \textit{An. fluviatilis} and \textit{An. annularis} to DDT from Jharkhand in the year 2010\textsuperscript{27}. In our study, \textit{An. fluviatilis} was found resistant to DDT only, while they are tolerant to other insecticides tested. Resistance to DDT in \textit{An. fluviatilis} may be due to prolonged use of DDT in indoor residual spray since 1958. However, presently this area is receiving two rounds of IRS with synthetic pyrethroids to which these species are in tolerant stages (verification required).

For the first time, \textit{An. annularis} resistance to DDT was detected in the year 1962 from Meerut district of Uttar Pradesh\textsuperscript{14}. Later on resistance was reported from various parts of the country\textsuperscript{6–8, 24–28}. \textit{Anopheles annularis} has developed double resistance to DDT and dieldrin from Assam\textsuperscript{7}. In our study, \textit{An. annularis} was found resistant to DDT only, while they are at verification required stage to malathion and deltamethrin.

Present study indicated that \textit{An. culicifacies}, \textit{An. fluviatilis} and \textit{An. annularis} are resistant to DDT while tolerant in case of other insecticides tested. Malathion resistance has been reported in \textit{An. culicifacies} and \textit{An. annularis} in earlier studies in Maharashtra, but our study showed no indication of malathion resistance in any of the tested mosquitoes species in this study area. High endemicity of malaria in the study area, which is receiving two rounds of IRS with synthetic pyrethroids, warrants that synthetic pyrethroids use should be undertaken as per norms of the National Vector Borne Disease Control Programme, where, there is a need to educate community for enhanced spray coverage of houses and rooms. There is also need to strictly monitor whether two rounds of IRS activities are done during coverage of houses. To delay the precipitation of resistance to synthetic pyrethroid, other insecticides can be used in rotation with synthetic pyrethroids. District-wise susceptibility status of mosquitoes

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Insecticide (% conc. tested)</th>
<th>No. of mosquitoes exposed</th>
<th>No. of mosquitoes dead</th>
<th>Corrected mortality (%)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>\textit{An. culicifacies}</td>
<td>DDT (4)</td>
<td>100</td>
<td>40</td>
<td>29</td>
<td>3</td>
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<tr>
<td></td>
<td>Malathion (5)</td>
<td>100</td>
<td>40</td>
<td>96</td>
<td>0</td>
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<tr>
<td></td>
<td>Deltamethrin (0.05)</td>
<td>100</td>
<td>40</td>
<td>94</td>
<td>1</td>
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<tr>
<td>\textit{An. fluviatilis}</td>
<td>DDT (4)</td>
<td>60</td>
<td>30</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Malathion (5)</td>
<td>60</td>
<td>30</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin (0.05)</td>
<td>60</td>
<td>30</td>
<td>58</td>
<td>2</td>
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<tr>
<td>\textit{An. annularis}</td>
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<td>40</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Malathion (5)</td>
<td>100</td>
<td>40</td>
<td>91</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin (0.05)</td>
<td>100</td>
<td>40</td>
<td>95</td>
<td>1</td>
</tr>
</tbody>
</table>

R—Resistant; VR—Verification required (tolerant).
to the insecticides being used in the programme should be monitored for judicious use of appropriate insecticides for malaria control in these areas.

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