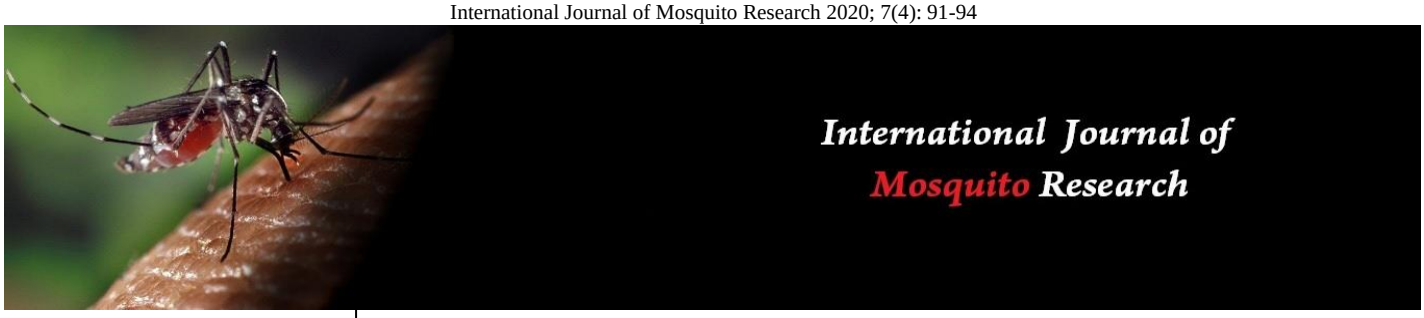

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Synthetic pyrethroid resistance/susceptibility status of *Anopheles culicifacies* Giles and *Anopheles subpictus* Grassi in Telga Aarapur Village, Bastar District, Chhattisgarh

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Abstract
Background & Objectives: One of the major malaria vectors in Bastar District is *Anopheles culicifacies* Giles and the most abundant species of Anopheles is *Anopheles subpictus* Grassi. Indoor residual spraying and long lasting insecticide treated nets are the major methods used for controlling malaria transmission. In Chhattisgarh State, the control of malaria vectors has become dependent on synthetic pyrethroids, which are used for treatment of all approved long-lasting insecticidal nets (LLINs). The vast use of just one class of insecticide has led to the problem of resistance to insecticides in malaria vectors. The aim of this study was to determine the resistance/susceptibility status of *An. culicifacies* and *An. subpictus* to alpha-cypermethrin and deltamethrin, the commonly using synthetic pyrethroids.
Methods: Adult mosquitoes were collected during May 2019 to February 2020 from TelgaAarapur village in Bastar District. The blood-fed wild caught females were exposed to the diagnostic dosage of alpha-cypermethrin (0.05%) and deltamethrin (0.05%) for one hour. Mortality was recorded at 24 h after the exposure.
Results: Results of present study indicated that *An. subpictus* was resistant to both pyrethroid insecticides used in the malaria control programme and *An. culicifacies* resistant towards deltamethrin and moderately resistant to alpha-cypermethrin.
Interpretation & Conclusions: Development of pyrethroid resistance in *An. culicifacies* is alarming and it would be a major problem in the malaria control programme. Resistance management strategy by appropriate rotation of different groups of insecticides should be considered for the control of malaria vectors. Periodical monitoring of susceptibility/resistance status of malaria vectors to different insecticide classes need to be carried out to find out the efficacy of control measures.

Keywords: *Anopheles culicifacies*, *Anopheles subpictus*, insecticide resistance, malaria, alpha-cypermethrin, deltarmethrin

Introduction
In Bastar district of Chhattisgarh state, the dominant vector of malaria was found to be *Anopheles culicifacies* Giles also *An.fluviatilis, An.annularis, An.subpictus, An.stephensi, An.minimus and An.Varuna* were found. Kulkarni, reported the presence of sporozoites in the salivary gland of *An. Subpictus* [1]. The main strategy to control *An. culicifacies*, the major malaria vectorin rural areas is indoor spraying of residual insecticides and usage of insecticide impregnated nets. In 1950's the spraying of DDT and HCH under the National malaria control programme was introduced in India. After few years of implementation of these insecticides, *An. culicifacies* developed resistance towards DDT, dieldrin and BHC [2-4]. As a result, malathion was introduced in Gujarat and Maharashtra in 1969 and synthetic pyrethroids in 1990's for IRS in high risk areas and the species developed resistance to these insecticides as well [5, 6].
The most effective way to prevent malaria transmission is to avoid human-vector contact and vector control plays a major role during this. In malaria endemic regions, mosquito resistance can increase rapidly following the implementation of vector control. In Bastar, the malaria control is reliant on indoor residual spraying (IRS) with alphacypermethrin, combined with distribution of LLINs mostly Perma Net and Dura Net. The real effectiveness of the vector control methods, depend upon the proper utilization.

Field studies indicate that bed nets weren't employed by all relations in all seasons and the nets were alternatively used for fishing, covering crops, to guard saplings etc. The efficacy of IRS depends upon social factors like cooperation of the residents; most of the villagers won't allow doing IRS altogether the rooms. Therefore the incomplete IRS with right formulation and insecticide also will cause selection of resistance mosquitoes within the field. Long-term intensive insecticide use to control agricultural pests and disease vectors has resulted in the selection of resistance in many insect species.

Accurate information on the underlying resistance mechanisms and their intensity or frequency in malaria vectors can then inform vector control programmes and ensure timely management of insecticide resistance. The present study tried to find out the susceptibility/ resistance status of *An. culicifacies*, the major malaria vector, along with *An. subpictus*, the abundant mosquito in Telga Aarapur village, Bastar district towards the commonly using synthetic pyrethroids in IRS and LLINs. Area was selected for the collection of vectors according to the availability of adequate number of vectors, usage of insecticide impregnated nets, and regularity of indoor residual spraying.

Materials and Methods

Study Duration: The study was conducted from May 2019 to February 2020in Telga Aarpur village of Bastar District.

Study Area: Telga Aarapur village is located in Tokapal Tehsil of Bastar district in Chhattisgarh, India. It is situated 4km away from sub-district headquarter Tokapal and 24km away from district headquarter Jagdalpur. The area is surrounded by paddy fields. Seasonal vegetable cultivation and cattle farming is also noticed as source of income.

Fig 1: Telga Aarapur

Bioassay: Adult mosquitoes were collected during 05.00 am to 07.00am using oral aspirator and torch. Insecticide susceptibility assay carried out using alpha-cypermethrin (0.05%) and deltamethrin (0.05%) impregnated papers provided in WHO insecticide susceptibility evaluation test kits and protocol [7]. The control tests were performed by using pre-impregnated silicone paper along with each set of insecticide bioassay. After the treatment period of 1hour the mosquitoes were transferred to holding tube and % mortality was recorded after 24hours [7]. The mortality observed in the mosquito species were corrected using the Abbot's formula [8].

Inclusion Criteria: *An. culicifacies*, the principal malaria vector and *An. subpictus*, the most abundant mosquito of the area were used as test organisms, the insecticides used for IRS and LLIN were used for the susceptibility assay.

Exclusion Criteria: Other species of *Anopheles* mosquitoes and insecticides were excluded.

Results and Discussion

Table 1 shows data regarding the % mortality after 24 hours of time duration and susceptibility/resistance status of *An. culicifacies* and *An. subpictus* collected from Telga Aarapur area of Bastar. 150 and more than 150 mosquitoes were exposed for each insecticide. The death recorded using the control papers were less than 5%. Mortality was more in *An. culicifacies*, treated with alpha-cypermethrin. Less mortality recorded when An. subpictus treated with deltammethrin.

Table 1: Data regarding % mortality after 24hours and susceptibility/ resistance status

Insecticide used	Number of mosquitoes exposed	Number of mosquitoes died	%mortality (corrected)	Status
Anopheles culicifacies				
Alpha cypermethrin	180	166	92.22%	MR
Deltamethrin	150	134	89.3%	R
Anopheles subpictus				
Alpha cypermethrin	150	111	74%	R
Deltamethrin	150	90	60%	R
R- resistant, MR- moderately resistant				

According to WHO, mortalities below 90% represents high resistance, where as mortalities ranging from 90 to 98% represents moderate degrees of resistance and mortality above 98% represents susceptible [7]. In the present study *An. subpictus* had shown resistance towards both alpha-cypermethrin and deltamethrin. But in the case of *An.culicifacies*, resistance observed towards deltamethrin and moderately resistant towards alpha-cypermethrin.

Resistance to DDT and malathion in *An. culicifacies* was reported in most parts of India [9]. Synthetic pyrethroids are now being used to tackle DDT- and malathion-resistant mosquitoes either in the form of IRS or as impregnated mosquito nets. It was reported that *An. culicifacies* had shown resistance towards deltamethrin in Jagdalpur, Bastar District [10]. In a study conducted at Chilkuty area of Bastar, 87.5% and 85% mortality was shown by *An. culicifacies* against alpha-cypermethrin and deltamethrin respectively and highly resistant towards DDT, Bendiocarb and Malathion [11].

An. Subpictus breeds in a variety of breeding habitats which includes wells, burrow pits, channels, lake margins, ponds, cemented tanks, ground pools, fallow and freshly flooded rice fields and cisterns etc. [12]. Chatterjee and Chandra [13] got 41% human blood positive mosquitoes out of 480 indoor resting *An. subpictus*. It was the first *Anopheline* species to be reported resistant to DDT [12]. Resistance towards organochlorine insecticide, DDT from various parts of India was reported [14-21]. Resistance against another organochlorine compound, dieldrin, has been detected in *An. subpictus* from Rajasthan and Pondicherry [18-20]. Resistance to organophosphate malathion and tolerance to synthetic pyrethroid was also reported in one study carried out in arid and semi arid parts of India. In the aquatic stages of this species, tolerance to DDT, chlorpyriphos, fenthion, malathion, bifenthrin and carbofuran has been detected [20, 22]. *An. subpictus* of Chilkuty, Bastar had shown resistance towards alpha-cypermethrin, deltamethrin, malathion and bendiocarb [11].

One of the major reasons for the development of pyrethroid resistance in *Anopheles* mosquitoes of the study area found to be, the wide usage of pyrethroid pesticides in agriculture, mostly in paddy fields and vegetable cultivation lands. Both *An. culicifacies* and *An. subpictus* preferred to breed in paddy fields and nearby ponds and rivers. Presence of paddy fields and river near in the study area acts as the main source for breeding of mosquitoes. Usage of pyrethroids both in IRS and LLINs was found to be another reason for the fast development of insecticide resistance in *Anophelines*. IRS has become less effective and operationally difficult on account of poor acceptance by communities. The coverage of insecticides seems to be less as villagers denied to spray the insecticides in all the rooms. Almost 99% houses were provided with adequate number of mosquito nets, by Chhattisgarh Government in Collaboration with NVBDCP, NCDC and Department of Forestry. But its usage is also limited season wise. High temperature in night and less mosquito prevalence were the major reasons given by the people for the low use rate of mosquito nets during the summer season. This will also lead to the selection of resistant mosquitoes. Therefore, through an appropriate IEC package, the community needs to be made cognizant of the benefit of IRS and LLINs use in the prevention of both malaria scourgeand development of insecticide resistance in mosquitoes.

Conclusion

The development of insecticide resistance would be a major setback to the national malaria control programme due to the unavailability of alternative insecticides, which are safe and cost effective. In the point of view of safety and effectiveness, pyrethroids are the best insecticides ever developed for public health use. It is therefore essential to use this very important group of insecticides judiciously and cautiously, with regular monitoring of the status of insecticide resistance in vector populations for an effective vector control programme. In conclusion, the present study results indicated development of resistance to pyrethroids in *An. culicifacies* is of great concern to Indian malaria control programme as it is the only viable choice in vector control programmes for IRS and for impregnation of bed nets. There is a need for regular monitoring to assess insecticide susceptibility to formulate effective and alter vector control strategies like by insecticide rotation, mosaic application, and integrating bio-environmental approaches.

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