

Research Article

Density and Susceptibility Status of Malaria Vectors of Chilkuti Area, Bastar, Chhattisgarh

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A B S T R A C T

Introduction: The study was carried out in Chilkuti, a village 22 km from Jagdalpur in Bastar Division of Chhattisgarh State, India. The malaria incidence of this area was declined with distribution of insecticide treated LLINs to the villagers. The present study aims to find out the vector density and insecticide susceptibility status of vector mosquitoes of the area.

Methods: Adult mosquito collections were done from both cattle shed and human dwelling by hand catch method and per man hour density calculated to compare the density of mosquitoes. The susceptibility/resistance status against the conventional insecticides as per NVBDCP guidelines was evaluated by WHO susceptibility kit. Correlation of climatic factors and vector density was evaluated by SPSS version 20. Knock-down time (KDT50 and KDT 99) along with slope and 95% confidence interval (CI) was determined using Ldp Line computer programme.

Result: The vector density was evaluated regularly and found to be the density of *Anopheles culicifacies* Giles, the major vector of malaria in the area getting reduced and in the meantime the density of *Anopheles subpictus* Grassi, is increasing. Though the *An. culicifacies* density found reducing and the cases of malaria were reported from the area indicating the possibility of another vector may be playing the role in parasitic transmission in the area. As per the previous study from NCDC Jagdalpur, the presence of sporozoites was confirmed in *An. subpictus* and anthropophilic index indicated the chance of its vectorial capacity in Jagdalpur area, Bastar division So the present study focused on both these mosquitoes. The susceptibility tests shown that there is variation in the susceptibility/resistant status of both the mosquitoes against the different classes of insecticides.

Conclusion: The results of the study exhibit the need of further exploratory research studies confirming the vectorial capacity of *An. subpictus* and alternate measures to control malaria transmission.

Keywords: Malaria, *Anopheles culicifacies*, *Anopheles subpictus*, Insecticide Resistance

Introduction

Malaria is a major health problem in Chhattisgarh and a main cause of mortality and morbidity among the peoples of tribal dominated districts. This disease is caused by female anopheles mosquito transmitting *Plasmodium* parasites. In India, *Anopheles culicifacies*, *An. stephensi*, *An. fluviatilis*, *An. minimus*, *An. dirus* and *An. sundaicus* are six primary and *An. varuna*, *An. annularis*, *An. philippinensis* and *An. jeyporiensis* are four secondary vectors of malaria.¹ In addition to these, *An. subpictus* has also been reported as a potential malaria vector.² Occurrence of oocyst and sporozoites in *An. subpictus* in India was first reported by Russel et al., in 1939 from Tamil Nadu.³ As per the reports of Kulkarni, sporozoites were detected in the salivary gland of *An. subpictus* from Bastar District in 1982.⁴

For the control of vector mosquitoes, indoor residual spraying with DDT was used initially, but gradually *An. Culicifacies* has developed resistance against DDT and now *An. culicifacies* is resistant to DDT in almost all parts of the country.⁵ *An. subpictus* also had shown resistance towards DDT.⁶ In 1969, malathion was introduced in mosquito control programme in DDT resistant areas.⁷ *An. culicifacies* and *An. Subpictus* has developed varying degree of resistance to malathion in the country.⁸⁻¹¹ In 1990's synthetic pyrethroids like deltamethrin were introduced in national vector borne disease control programme (NVBDCP). Mittal et al.,¹² Singh et al.¹³ and Sharma et al.^{8,14} reported reduced susceptibility to deltamethrin in *An. culicifacies*. The continuous and over usage of these synthetic pyrethroids leads to the development of resistance in *Anopheles* mosquitoes¹⁵ then also, synthetic pyrethroids are still used in various parts of the country as an effective insecticide for mosquito control.^{16,17}

In the present study, Chilkuti, the study area has the number of malaria cases declined as compared with previous years after the implementation of vector control methods like IRS and LLINs. The Chhattisgarh Government supplied nets were treated with deltamethrin and NCDC supplied LLINs were with alphacypermethrin with free of cost and ratio of one bed net per two persons. The real effectiveness depends upon the proper usage. Usage of bed nets has strong relation with seasonality, mainly using in rainy season. Field studies indicate that bed nets were not used by all family members and the nets were alternatively used as fishing nets, used for covering crops, to protect saplings etc. The efficacy of IRS depends upon social factors like cooperation of the residents; most of the villagers won't allow doing IRS in all the rooms. So, the incomplete IRS with right formulation and insecticide will also lead to selection of resistance mosquitoes in the field. Insecticide spraying for mosquito control and LLINs were common methods for vector control regularly; however, resistance levels in malaria vectors has not been monitored for any insecticides in the area. So, the

present study aims to find out the insecticide susceptibility/ resistance status of *An. culicifacies*, (major vector) and *An. subpictus*, (most abundant *Anopheline* mosquito) in the Chilkuti area.

Methods

The study was conducted from October 2017 to August 2019 in Chilkuti a small Village/ hamlet in Jagdalpur Tehsil in Bastar District of Chhattisgarh State, India. It comes under Kurandi Panchayath. It is located 22 KM towards South from District headquarters Jagdalpur. Gonds, Halwa, Bhadra and Sundi are the major tribes residing in this area. The main base of the economy of this area is agriculture and people depend on forest for wood, bamboo mahua, sal seeds and leaves. Both male and female works in agricultural field. Primarily they prefer natural medicines rather than allopathic medicines and treatment.

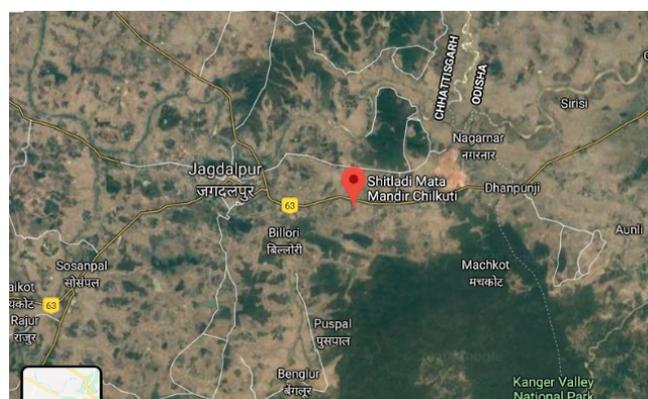


Figure I. Map of Chilkuti (Study Area)

Collection of Mosquitoes and Calculation of Vector Density

Adult mosquitoes were collected from cattle sheds using oral aspirator.

Inclusion Criteria

An. culicifacies and *An. subpictus* were sorted out from the collected mosquitoes and included in the study for susceptibility assay.

Exclusion Criteria

Other genus of mosquitoes and subspecies of *Anopheline* mosquitoes were excluded.

Vector Density Calculated using the Formula

Per man hour density = No. of mosquitoes collected / No. of collectors x time spent in search per hour.

Data on Climatic factors sought from the website of world weather online. Correlation of climatic factors and vector density was evaluated by SPSS version 20.

Insecticide susceptibility assay done using Alphacypermethrin (0.5%), Deltamethrin (0.05%), DDT (4%), Malathion (5%)

and Bendiocarb (0.1%) treated papers provided in WHO insecticide susceptibility evaluation test kits and protocol.¹⁸ The control tests were performed using pre-impregnated paper with silicone oil (Deltamethrin and Alphacypermethrin Control), risella oil (DDT control) and olive oil (malathion control) along with each set of insecticide bioassay. 20 mosquitoes were used in the test for 1 hour and cumulative knock-down time was recorded after an interval of 10 minutes up to 1hour. Knock-down time (KDT50 and KDT99) along with slope and 95% Confidence Interval (CI) was determined using Ldp Line computer programme. After 1 hour the mosquitoes were transferred in to holding tube and % Mortality was recorded after 24 hours holding period. The mortality observed in the mosquito species was corrected using Abbott's formula.¹⁹

Statistical Analysis

SPSS version 20 and Ldp Line computer programme were used for the interpretation of data.

Result and Discussion

Figure 2, depicts the per man hour density of *An. culicifacies* and *An. subpictus* collected from the cattle sheds of Chilkuti area. The mosquito collection done on October-2017, January, April, July-2018 and April, July, August-2019. *An. Culicifacies* density was very less as compared with *An. subpictus* in all the time. In 2019 July and August, the density of both species seems to be very high. The rainfall rate was very high as compared with the previous years, and that may be a reason for the hike in vector density. In October 17, January & April-2018, March & April-2019, the PMHD of *An. culicifacies* was found to be zero. But *An. subpictus* collections were got in every catch.

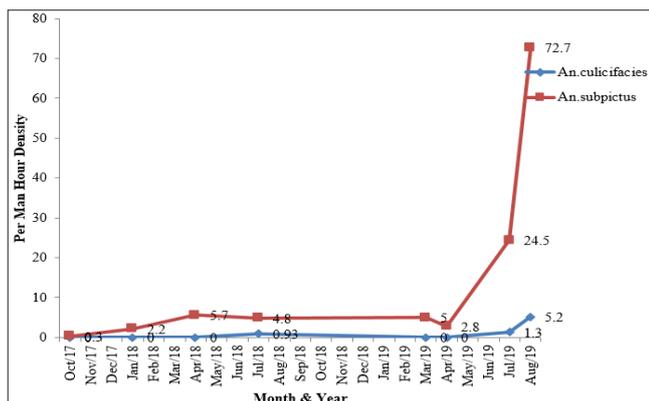


Figure 2. PMHD of *An. culicifacies* and *An. subpictus*

Figure 3 and 4, depicts climatic factors like temperature, humidity and rainfall in the Chilkuti area from January 2017 to October 2019 and the correlation of mosquito density with the climatic factors. Rainfall was high in the year 2019 as compared with previous years. In the present study mosquito density is significantly correlated with rainfall with r-value more than 0.8. It is proved changes in climatic

factors influence malaria directly by modifying the behaviour, geographical distribution and density of vectors.²⁰ Limited studies on relationship between the climatic factors and malaria were carried out in malarial endemic areas Dehradun of Uttaranchal,²¹ Sonitpur²² and Kokrajhar of Assam²³ and found to be the density and cases were related with climatic factors.

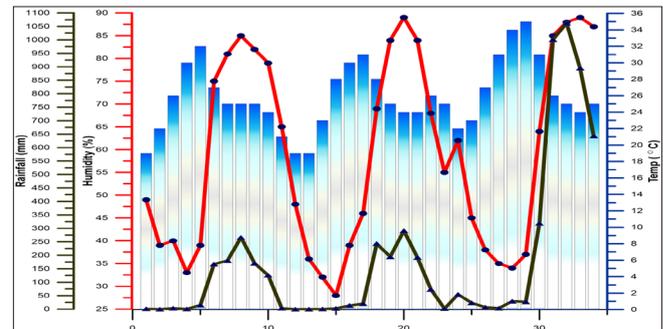
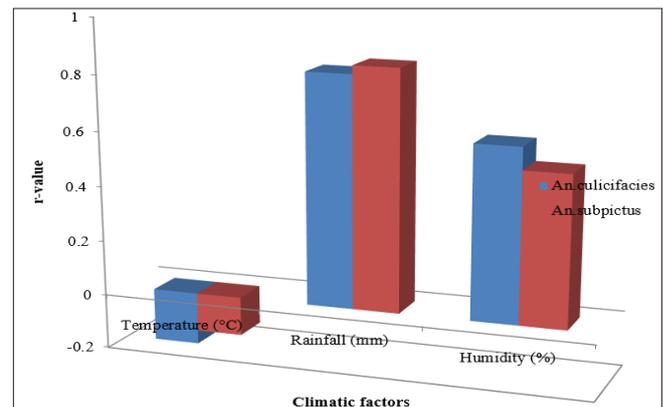


Figure 3. Climatic conditions of Chilkuti Area from January 2017 to October 2019



r > 0.8 = highly correlated, + r = positive linear relationship, - r = negative linear relationship

Figure 4. Correlation of mosquito density with temperature, rainfall and humidity

Table 1, show data regarding the knock down % in 1 hour, mortality % after 24 hours and susceptibility/resistance status of *An. culicifacies* and *An. subpictus* against DDT, bendiocarb, malathion, deltamethrin and alphacypermethrin. According to WHO guidelines, 98-100% mortality indicates susceptibility, 80-98% indicates the possibility of resistance that needs to be confirmed and <80% indicates resistance. In the present study, *An. subpictus* had shown percentage mortality value less than 80% and proved its resistance towards all the conventionally used insecticides. The percentage mortality rate was very less in the test with DDT and its shows high resistance towards DDT. Higher levels of malathion resistance could be due to agricultural pesticides used for paddy pest control where *An. subpictus* breeds and it also used in vector control.²³ This species has been reported to be resistant to DDT and dieldrin/ HCH in Gujarat.²⁴

Table I. Knock-down time and susceptibility/ resistant status of *An. culicifacies* and *An. subpictus* in Chilkuti against Alphacypermethrin, Deltamethrin, Malathion, DDT and Bendiocarb

Mosquito species	Insecticide	%KD 1h (N)	KDT50 (95% CI)	KDT99 (95% CI)	Slope±SD	χ^2 (p)	r	%CM 24 h	S/R status
<i>An. culicifacies</i>	Alpha cypermethrin	87.5 (40)	25.3 (23.9-26.7)	144.0 (124.8-170.9)	3.1±0.2	4.0 (0.9)	1	87.5	VR
	Deltamethrin	85 (40)	31.4 (29.6-32.4)	148.2 (130.7-170.1)	3.4±0.2	0.3 (1.0)	1	85	VR
	Malathion	62.5 (40)	51.4 (43.2-73.6)	384.8 (341.8-1583.2)	2.6±0.2	14.7 (0.05)		62.5	R
	Bendiocarb	50 (40)	71.2 (53.0-83.3)	484.7 (441.7-883.1)	2.7±0.2	11.5 (0.05)	1	50	R
	DDT	35 (40)	160.2 (100.2-421.3)	2831.6 (1444.8-7437.6)	1.0±0.2	4.1 (0.28)	1	35	R
<i>An. subpictus</i>	Alpha cypermethrin	78.3 (120)	32.2 (24.9-40.9)	152.5 (103.4-466.9)	2.9±0.2	10.6 (0.02)	1	78.3	R
	Deltamethrin	75 (120)	37.7 (35.4-40.3)	170.8 (139.6-222.6)	3.5±0.3	6.6 (0.2)	1	75	R
	Malathion	65 (120)	48.2 (44.0-75.8)	380.8 (336.6-1580.2)	2.5±0.2	12.6 (0.05)	1	65	R
	Bendiocarb	55 (100)	64.5 (42.8-86.1)	416.7 (299.6-542.2)	3.4±0.2	1.8 (0.8)	1	55	R
	DDT	37 (100)	91.4 (79.8-109.3)	1357.3 (841.5-2592.5)	2.0±0.2	3.1 (0.5)	1	37	R

% KD-knock down % in 1h; N-number exposed; KDT-knock-down time in minutes; CI-confidence interval; SD-standard deviation; r-correlation; Coefficient % CM-corrected mortality after 24 h; S-Susceptible (mortality > 98%); R-Resistant (mortality <80%) and VR-Tolerant (mortality 81-97%).

An. culicifacies shown resistance towards DDT, malathion and bendiocarb, with percentage mortality values 35, 62.5 and 50% respectively. While using alphacypermethrin and deltamethrin the % mortality values were 87.5 and 85% respectively, it indicates the possibility of development of resistance towards these insecticides. Further studies are needed for the confirmation of the status; hence the sample size was less as compared with *An. subpictus*. *An. Culicifacies* is resistant to DDT in almost all parts of the country²⁵ and the resistance towards DDT, malathion and deltamethrin in Chhattisgarh.²⁶ Synthetic pyrethroids are being used in public health programs to control multiple-resistant vectors and tackle epidemic outbreaks. Also, this is the only group of insecticides currently used for bed-net impregnation for malaria control. In addition, commonly synthetic pyrethroids are used for vector control via mosquito coils, mats and liquid vaporizers. Currently, the national program has no alternative insecticide for effective vector control or for insecticide resistance management.¹⁷ In India, synthetic pyrethroids and organophosphate pesticides are currently being used, not only for vector control, but also

in the agricultural sector, mainly for control of lepidopteron pests. The continuous and over usage of these synthetic pyrethroids leads to the development of resistance in *Anopheles* mosquitoes.¹⁵

Conclusion

Variation in insecticide resistance mainly depends upon the class and frequency of insecticide use in the field. Excessive and unwanted usage of insecticides not only increases vector resistance, but also results in cross resistance to other insecticides and it affects the proper vector control measures. In the present study, *An. culicifacies* and *An. subpictus* had shown high resistance to DDT irrespective of its withdrawal from IRS and replaced with other insecticide spray. This could be due to increased genetic stability of DDT resistance gene as was observed in both species.

An. Subpictus had shown resistance towards all the insecticides used for the study. And that may be a reason for its high density in the study area. But in the case of *An. culicifacies*, the mortality rates indicate the chances of developing resistance towards pyrethroid insecticides

and resistance towards all other classes of insecticides. It requires further confirmation because the sample size was less as compared with *An. subpictus*.

In conclusion, the present data indicated the chances of development of resistance to pyrethroids in *An. Culicifacies* and resistance to pyrethroids in *An. subpictus* of great concern to Indian malaria control programme as it is in extensive use in public health programmes for IRS and for impregnation of bed nets. Since there are limited numbers of insecticides available for vector control, an approach focused on the rotational use of insecticides or a mosaic strategy can be adopted to delay development of resistance in malaria vectors. Effective resistance management mainly depends upon early detection of the status of resistance; therefore, there is a need of monitoring to assess insecticide susceptibility at regular intervals and necessary to formulate effective vector control strategies.

Conflict of Interest: None

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