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Research Article

Contemporary Resistance Status of *Anopheles Stephensi* against Insecticides in District Dungarpur, Rajasthan, India

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A B S T R A C T

Pyrethroids are powerful insecticides used in the vector control program with impregnated mesh, and residual indoor sprays. However, resistance to insecticide reduces the effectiveness. The present susceptibility study carried out against the *Anopheles* Stephensi to monitor the sensitivity conditions of *An. Stephensi* vector, which raises the need to understand the state of vector resistance in the Dungarpur region of Rajasthan, India in order to better report vector-based interventions. The sensitivity study was carried out by the WHO standard method using recommended diagnostic doses of DDT, alpha-cypermethrin, permethrin, and deltamethrin. *An. Stephensi* showed resistance to DDT from the entire study while sensitive to alpha-cypermethrin, permethrin, and deltamethrin. The study looks at the selection and circulation of the appropriate insecticide's molecule for a vector control program as insecticide need constant monitoring to develop effective vector control strategies such as improving insecticide by applying integrated biological and ecological methods.

Keywords: Insecticide Resistance, *Anopheles*, Mosquito Control, DDT, Alpha-cypermethrin, Permethrin, Deltamethrin

Introduction

Malaria is a comprehensive health problem and is a deadly disease that carries a 95% risk of infection by human parasites i.e., female *Anopheles* mosquitoes. In 2018, there are an estimated 228 million cases of malaria worldwide.[1] Globally, the 53% load of *Plasmodium vivax* is in the South-East Asia region, typically 47% in India. As of 2018, about 85% of global malaria deaths are concentrated in 20 countries, including Africa and India. Eighty-five percent of all malaria cases worldwide were in 19 countries, including India, and 18 in African countries. As estimated in 2018, more than 85% of cases of *vivax* malaria vector occur in just six countries, including India accounts for 47% of the *P. vivax* case.[2]

Many species of *Anopheles* are responsible for the transmission of malaria. *Anopheles Stephensi* Liston (Diptera, Culicidae) is the most frequently found in semi-arid and arid areas of Rajasthan. *An. Stephens* is a type of subtropical species and major vector in urban areas of India that spread across South Asia and the Middle East, which is accounting for about 12% of malaria cases each year. This species is a major transmitter of malaria, a significant vector in the arid regions of Rajasthan where it has an exclusive breeding ground for groundwater tanks spread across villages and urban areas.[3]

To prevent malaria, the WHO recommends preventing chemotherapy i.e., providing antibiotics or vector control i.e., reducing the likelihood of mosquito bites in certain groups of people i.e., children, pregnant women, and other high-risk groups, or such as complex crisis and termination. Basic interventions endorsed by the WHO to prevent mosquito bites under disinfectant-treated nets (ITN) and indoor residual spraying (IRS). In certain situations, ITN and IRS may be supplementary to larval resource management or other environmental improvement programs.[2]

The Insecticides used against the malaria parasite are organochlorine (OC) such as DDT; Organophosphates (OP) such as fenithrothione, tamephos, and malathion; Carbomate (C) such as propoxur and bendocarb; Pyrethroids (PY) such as alpha-cypermethrin, cyfluthrin, bifinthrin, cyfinothrin, cypermethrin, etophenprox, deltamethrin, permethrin, and lambda-cyhalothrin; Insect growth regulators (IGRs) such as diflubenzuron, novaluron, methoprene, and piproxifen.[4] Synthetic derivatives of pyrethroids are extensively exploits for residual indoor and/or space sprays, and insecticides impregnated bed nets. These methods are very effective if utilized appropriately, but development of resistance to these substances diminishes its effect.[5] However, resistance too many malaria vectors has developed worldwide due to the continued use of targeted insecticides. Many species of *An. culicifacies* in India are resistant to insecticides. Insecticides, the main vector of malaria in India, accounting for 60% to 70% cases of malaria, have been shown to be resistant against Malathion and DDT in India.[6]

The present study was conducted to estimate the status of susceptibility on *An. Stephensi* against WHO recommended insecticides such as DDT, alpha-cypermethrin, permethrin, and deltamethrin. The produced data will certify that the suitable insecticide samples required in these areas to avoid increasing use of insecticide that could jeopardize the sustainability of vector control strategies that lead to pest resistance. Therefore, to monitor the state of disinfection susceptibility compared to malaria carriers *An. Stephensi* in the Dungarpur district of Rajasthan will produce data, which will help in the pest control program and strategy aimed at fighting malaria.

Materials and Methods

Test Insects

The mosquitoes were sucked by tube method and kept in cages between 10:00 pm and 12:00 pm. *An. Stephensi* mosquitoes are given 10% glucose solution ad libitum released using a cotton swab.[7]

Insecticides

Insecticide susceptibility tests were performed on three insecticides i.e., DDT, alpha-cypermethrin, permethrin, and deltamethrin that used for indoor residual sprays in the malaria control program. DDT (4%), alphacypermethrin (0.05%), permethrin (0.75%) and deltamethrin (0.05%). Impregnated papers according to the diagnostic dose were used for insecticide susceptibility tests on stored malaria vector *An. Stephensi* species, as recommended by the WHO.

Insecticide Susceptibility Tests

The test for insecticide susceptibility was conducted between May/ June 2019 as per the standard procedure of WHO. *An. Stephensi* was confronted with insecticide-impregnated papers for an hour. A standard test to determine insecticide susceptibility in *An. Stephensi* mosquito against a diagnostic dose of insecticide was carried out by using mosquitoes that caught in the field with the help of a sucking tube. The WHO criteria were followed to take into account vector susceptibility if mortality > 98%, resistance if mortality < 80%, and intermediate resistance if mortality 80-98%. The deaths in the test subjects were subjected to correct using Schneider-Orelli's formula if the controlled deaths were considered to be 5-20%.[8]

Results and Discussion

Susceptibility status of the malaria vectors collected from various towns of Dungarpur in reference to insecticides i.e., DDT, alpha-cypermethrin, permethrin, and deltamethrin shown in Table 1. Outcomes of insecticide susceptibility tests show that DDT is resistant while alpha-cypermethrin, permethrin, and deltamethrin are susceptible in all eight towns of study. The above study will benefit the concerned sectors and agencies in deciding control strategy for malaria vector.

Table 1.Bioassay of DDT, Alpha-cypermethrin, Permethrin, and Deltamethrin to *An. Stephensi*

S. No.	Town	Insecticide (% concentration tested)	No. of mosquitoes exposed		No. of mosquitoes dead		Mortality (%)		Corrected mortality (%)	Susceptibility status
			Test	Control	Test	Control	Test	Control		
1.	Dungarpur	DDT (4%)	96	68	76	5	79.16	6.5	77.71%	Resistant
		Alpha-cypermethrin (0.05)	99	42	98	4	98.98	9.5	98.87%	Susceptible
		Permethrin (0.75%)	95	70	94	5	98.94	7.14	98.86	Susceptible

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		Deltamethrin (0.05)	93	47	92	4	98.92	8.51	98.82	Susceptible
2.	Bichhiwara	DDT (4%)	97	68	74	3	76.28	4	76.28%	Resistant
		Alpha-cypermethrin (0.05)	91	43	91	4	100	9.3	100%	Susceptible
		Permethrin (0.75%)	94	63	93	5	98.93	7.9	98.84	Susceptible
		Deltamethrin (0.05)	96	52	96	3	100	5.7	100	Susceptible
3.	Sagwara	DDT (4%)	99	67	75	5	75.75	7.4	73.81%	Resistant
		Alpha-cypermethrin (0.05)	98	49	98	4	100	8.1	100%	Susceptible
		Permethrin (0.75%)	93	65	92	5	98.92	7.69	98.83	Susceptible
		Deltamethrin (0.05)	91	48	90	6	98.90	12.5	98.74	Susceptible
4.	Galiyakot	DDT (4%)	101	69	79	3	78.21	4.34	78.21%	Resistant
		Alpha-cypermethrin (0.05)	95	51	95	5	100	9.8	100%	Susceptible
		Permethrin (0.75%)	96	69	95	5	98.95	7.24	98.87	Susceptible
		Deltamethrin (0.05)	92	48	92	3	100	6.25	100	Susceptible
5.	Simalwara	DDT (4%)	95	51	75	5	78.94	9.8	76.65%	Resistant
		Alpha-cypermethrin (0.05)	98	43	98	5	100	11.62	100%	Susceptible
		Permethrin (0.75%)	94	70	94	3	100	4.05	100	Susceptible
		Deltamethrin (0.05)	97	57	96	4	98.96	7.01	98.88	Susceptible
6.	Chikhli	DDT (4%)	102	65	75	5	73.53	7.69	71.32%	Resistant
		Alpha-cypermethrin (0.05)	92	59	92	3	100	5.08	100%	Susceptible
		Permethrin (0.75%)	103	64	103	6	100	9.37	100	Susceptible
		Deltamethrin (0.05)	94	52	94	4	100	7.69	100	Susceptible
7.	Aspur	DDT (4%)	93	67	74	3	79.56	4.41	79.56 %	Resistant
		Alpha-cypermethrin (0.05)	101	49	101	5	100	10.20	100	Susceptible
		Permethrin (0.75%)	97	72	97	4	100	5.55	100	Susceptible
		Deltamethrin (0.05)	91	48	91	3	100	6.25	100	Susceptible
8.	Sabla	DDT (4%)	104	69	83	5	79.8	6.5	78.40	Resistant
		Alpha-cypermethrin (0.05)	98	47	98	4	100	8.51	100	Susceptible
		Permethrin (0.75%)	103	73	103	3	100	4.1	100	Susceptible
		Deltamethrin (0.05)	99	44	98	2	98.98	4.54	98.98	Susceptible

An. Stephensi was resistant to DDT by 77.71% in Dungarpur, whereas 76.28% in Bichhiwara, 73.81% in Sagwara, 78.21% in Galiyakot, 76.65% in Simalwara, 71.32% in Chikhli, 79.56 % in Aspur, and 78.40% in Sabla whereas it was susceptible to Alpha-cypermethrin (98.87%), Permethrin (98.86%), and Deltamethrin (98.82%) in Dungarpur district. The study conducted in *An. Stephensi* was susceptible to Alpha-cypermethrin (100%), Permethrin (98.84%), and Deltamethrin (100%) in Bichhiwara town of Rajasthan. The susceptibility study conducted in Sagwara town of Rajasthan shows that *An. Stephensi* was susceptible to Alpha-cypermethrin by 100%, Permethrin by 98.83%, and Deltamethrin by 98.74%. A similar study conducted in the Galiyakot region of Rajasthan shows the susceptibility status of *An. Stephensi* against tested insecticides and found susceptible against tested insecticides i.e., Alpha-cypermethrin (100%), Permethrin (98.87%), and Deltamethrin (100%). Insecticides susceptibility status

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in Simalwara town also shows susceptibility against Alpha-cypermethrin (100%), Permethrin (100%), and Deltamethrin (98.88%). These three insecticides have shown 100% susceptibility against Alpha-cypermethrin, Permethrin, and Deltamethrin Chikhli town of Rajasthan. The susceptibility study conducted in Aspur town also shows 100% susceptibility against Alpha-cypermethrin, Permethrin, and Deltamethrin. Alpha-cypermethrin, and Permethrin shows 100% susceptibility in *An. Stephensi*, while 98.98% susceptibility against Deltamethrin.

In the contemporary study, DDT resistance at higher levels were detected in *An. Stephensi*, a similar type of studies conducted in Barmer, Jodhpur, and Jaisalmer area of the Thar desert observed resistance to DDT and susceptible to Deltamethrin in An. Stephensi .[9] Another study conducted in Goa revealed that *An. Stephensi* were shown resistant to DDT along with triple resistance against DDT, dieldrin, and Malathion in *An. Stephensi* .[10]

An. Stephensi found resistant to DDT in the study of insecticide susceptibility was led in Bikaner, Rajasthan.[11] Recently DDT resistance in An. Subpictus was also stated from many districts of Sri Lanka.[12] Insecticide susceptibility bioassay shows that *An. Stephensi* cumulated from various sites of semi-arid and arid zone of India were susceptible to DDT during 2005-07.[13]

The susceptibility status was evaluated at investigative doses of DDT, deltamethrin, and alphacypermethrin. Results have shown that *An. Stephensi* was found tolerant to deltamethrin and alphacypermethrin by 86.1%, 90.6% respectively, whereas susceptible to DDT by 98.1%.[14] *An. Stephensi* were resistant to deltamethrin insecticides and DDT in the study conducted in eastern Ethiopia.[15] *An. Stephensi* was highly susceptible to deltamethrin in Gujarat and Rajasthan, and also susceptible to lambda-cyhalothrin in Karnataka state.[16]

An. Stephensi, shows variable resistant to insecticides this resistance varies dramatically between populations that depending on the history of insecticide use and their selection.[17] Widespread resistance to malaria vectors was one of the factors that contribute to the ineffectiveness of DDT in the majority of regions of India.[18] The WHO has suggested that there would be no change in current recommendations for the safety of DDT for vector disease control, with constant monitoring of insecticide resistance and the selection of insecticide and their optimum use of management strategies.[19]

The study conducted in Nangarhar, Kunar, Badakhshan, Ghazni, and Laghman of Afghanistan shows resistance against deltamethrin and bendiocarb in both *An. Stephensi* and An. Culicifacies.[20] and in Kunar, Laghman, and Nangarhar provinces of Afghanistan exhibit resistance in *An. Stephensi* against deltamethrin, malathion, permethrin, and DDT while susceptible to bendiocarb.[21] The study was conducted in the Khanewal, Multan, Lodhran, and Bahawalpur region of Punjab province of Pakistan against λ-cyhalothrin, permethrin, cyfluthrin, deltamethrin, and cypermethrin in *An. Stephensi* exhibits resistance to cypermethrin, λ-cyhalothrin, and cyfluthrin in the region studied while susceptible to permethrin and deltamethrin except for Khanewal.[22] The study was conducted in southern Iran to assay the susceptibility status of *An. Stephensi* shows resistance against DDT, lambdacyhalothrin, deltamethrin, permethrin, cyfluthrin, and etophenprox.[23] The study was carried out in Chabahar city of South-eastern Iran to check the susceptibility status of *An. Stephensi* exhibits resistant against DDT, cyfluthrin, and lambdacyhalothrin, and susceptible against permethrin and etophenprox.[24] An. culicifacies show resistance against DDT, Malathion, lambda-cyhalothrin, and alpha-cypermethrin in the Upper Krishna Project catchment area of Karnataka state.[25] *An. Stephensi* and An. culicifacies show resistance against DDT and Malathion in southern districts of the Punjab province in Pakistan.[26]

Conclusion

The development of resistance could be a major obstacle to the national malaria control program due to the unavailability of alternative, safe, and low-cost pesticides. From the point of view of safety and efficacy, pyrethroids groups of insecticides are the best alternatives used against malaria vector. In the present study Resistance against DDT in *An. Stephensi* is an important finding because it has never been studied in the Dungarpur district of Rajasthan, and alpha-cypermethrin, Permethrin, and deltamethrin are the most susceptible in *An. Stephensi* . It is therefore important to use this group of pesticides wisely and carefully, by constantly monitoring the state of pesticide resistance in vectors to find an effective vector control program.

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