INSECTICIDE RESISTANCE STATUS OF ANOPHELES SUBPICTUS FROM DISTRICT KASUR, PUNJAB, PAKISTAN.

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ABSTRACT*: The current study was carried out to evaluate the insecticide susceptibility status of Anopheles subpictus from district Kasur, Punjab. Field collected adult blood fed females of An. subpictuswerereared in the insectary for F1 generation. Two to three days old non engorged adults of An. subpictus were evaluated by using WHO susceptibility bioassays. Both male and female mosquitoes shown resistance against DDT, deltamethrin and permethrin. Percentage mortalities recorded were DDT 29.47 %, deltamethrin 51.65 % and permethrin 76.28 %.*

Key words: Mosquito, Anopheles, bioassay, small ruminants, DDT, resistance.

INTRODUCTION

Mosquito borne diseases like malaria, filariasis, dengue fever, yellow fever and Japanese encephalitis have significant detrimental impact on global economics and public health [1-5] Pakistan has been placed among tropical countries where multifactorial conditions like climate changes, increasing human population densities, presence of vast agricultural lands, open networks of irrigation channels, rivers and several water dams for power generation are conducive to the proliferation of arthropod vectors in the country [6, 7].

Mosquitoes act as arthropod vectors for many human and animal diseases. Among these, mosquitoes of genus Anopheles are mainly responsible for transmission of human malaria[8]. In Pakistan about 24 Anopheline species are reported among which *Anophelesculicifacies*and *Anopheles stephensi*are confirmed vectors for malarial transmission [8]. *Anopheles subpictus* is a relatively more abundant specie in Pakistan and is newly emerging malaria vector in oriental region [9, 10]. This multiple host feeding mosquito is believed to transmit malaria, some helminthes and arboviruses causing high morbidity and mortality in oriental and Australian zone [9, 11].

Use of insecticides is one of the integral component of vector control strategy to reduce the burden of mosquito borne diseases [12]. About 12 insecticides belonging to four main groups i.e. Carbamates, Organochlorines, Organophosphates and Pyrethroids are currently recommended by WHO for this purpose. In developing countries Pyrethroids group has been successful so far in mosquito abatement programs [13].

Evolution of resistance against various classes of insecticides is an important threat for malaria control programs [14-17]. The irrational use of insecticides has greatly increased the selection pressure leading to emergence of resistance in mosquitoes [18-22]. Evaluation of the resistance status against particular insecticide is an effective and necessary step in controlling vectors [15]. Several methods have been reported to detect insecticide susceptibility status of mosquitoes among which "WHO susceptibility test" is a primary tool. It is used to assess mosquito mortality against known concentrations of a given insecticide by giving direct exposure [23]

There is no earlier record present regarding susceptibility status of *Anopheles subpictus* in Punjab, Pakistan. The present study was aimed to detect the insecticide susceptibility status of *Anopheles subpictus* in the area of District Kasur, Punjab, Pakistan. This was done by using WHO susceptibility test against standard concentrations of insecticides. It will help in providing data for the implementation of alternate vector control strategies on local as well as national level.

MATERIALS AND METHODS

Mosquito collection site

The study was conducted from September, 2012 to February, 2013 on mosquito population in district Kasur, Punjab, Pakistan. It is situated at 31. 12º North Latitude and 74.45º East Longitude in the South of Lahore with about 201 m sea elevation. This is a semi-arid and subtropical zone with intense summer heat and cold weather. After the approved consent of the people various localities of this region were selected for the mosquito collection on the basis of mosquito relative abundance and feasibility of work.

Mosquito collection and identification

Mosquitoes were collected almost fortnightly in every month. Various indoors and outdoors resting places from cattle stalls, animal sheds and house bedrooms were selected for collection. Mainly the collection was made during dawn timings. Mosquitoes were collected with the help of mouth and mechanical aspirators, along with CDC sweeper where dense populations were present [24]. After collection, mosquitoes were transferred into labeled paper cups provided with 10% sugar solutions soaked in cotton buds and were transported to insectary for species identification and further processing. For identification, field collected mosquitoes were subjected to CO_{22} anesthesia and distinguished on the basis of their morphological features as described byAmerasinghe et al.[25]. Adult blood fed/ gravid females of *Anopheles subpictus* were transferred into large cages for oviposition.

Rearing of mosquitoes

The mosquitoes were reared in the insectary to take F1 generation under standard conditions i.e. 80% (± 10%) relative humidity, temperature (25±2 °C) and 12hL: 12hD photoperiod. For providing regulated heat and humidity, a small electric light bulb and a wet towel draped over the cage was used. For rearing of larvae, enameled pans (30 x 19 x 5 cm high) filled with about a liter of tap water wereused. Larvae were fed with a diet of finely ground liver powder. The pupas were transferred into emerging cages for adult emergence within 1-2 days. Anopheline mosquitoes were colonized in large cages provided with cotton pads soaked in 10% sugar solution.

WHO Susceptibility Tests

Insecticide susceptibility status of *Anopheles subpictus* against DDT, Deltamethrin and Permethrin was detected by using "WHO standard susceptibility test method [23]. Two to three days old non blood fed adults app (n=100) of F1 generation were tested against each insecticide by using impregnated papers of discriminating dosage i.e. DDT 4%, deltamethrin 0.05% and permethrin 0.75%. Minimum four replicates of each insecticide with 20-25 mosquitoes were exposed to each paper for about 1 hour to calculate the knockdown time. Percentage mortality was recorded after withholding period of 24 hours. The rate of knock down of mosquitoes was observed after 10, 20, 30, 40, 50 and 60 minutes of exposure. Final mortality of mosquitoes was checked after 24 hours. For control mosquitoes were exposed to plane papers in the same manner.

Data interpretation of susceptibility test

According to WHO criteria, resistance status was evaluated as mentioned below;

- Mortality rate > 98%: fully susceptible population
- Mortality rate ranged 80-97%: suspected resistance in population further evaluation requires
- Mortality rate <80: resistantpopulation

Abbott's formula was used to correct the percentage mortality when mortality in control was between 5-20%.

$$\text{Corrected mortality} = \frac{(\%\text{mortality after treatment}) - (\%\text{mortality in control})}{(100 - \%\text{mortality in control})} \times 100$$

Statistical analysis

Bioassay data was analyzed with the help of log-time Probit model using Ldp line software. Fifty percent and ninety five percent knockdown time was estimated by using this software.

RESULTS

A total app (n=650) mosquitoes belonging to three genera i.e. Aedes, Anopheles and Culex were identified. For present study *Anopheles subpictus* was further processed. It was found relatively highly abundant specie among Anopheline species. This result is in accordance with the results of one another study, in which they also reported that it is highly abundant not only in peripheral areas of Lahore and Kasur but almost in all provinces of Pakistan.

The insecticide susceptibility status of *Anopheles subpictus* was evaluated by using WHO criteria [23]. Percentage mortality against DDT, deltamethrin and permethrin was ranged from 29.47% to 75.03%. *Anopheles subpictus* showed resistance against all tested insecticides with highest mortality against Permethrin (75.03 %) followed by deltamethrin (51.65 %) and DDT (29.47 %).

Knockdown time (KT_{50} and KT_{95}) of mosquitoes was estimated using Ldp line software by means of time-response model. KT_{50} and KT_{95} of *Anopheles subpictus* using DDT 4% were at 209 minutes and 1871 minutes respectively which is highest one. The confidence limit at 50% mortality was 107.78 - 3154.46 and at 95% this was 424 - 918552 with regression coefficient of 1.7307 ± 0.5433 S.E. This showed little bit higher resistance against DDT. Similarly KT_{50} and KT_{95} of deltamethrin and permethrin were 78.11, 237 minutes and 47.97, 1661 minutes respectively. At 50% and 95% mortality due to deltamethrin, confidence limit was 65.35-107.10 and 154.75-53217 estimated with regression coefficient 3.4126 ± 0.549. Confidence limit was calculated at 50% and 95% mortality of mosquitoes against permethrin; 43.08-55.49 and 117.29-275.22 with regression slope of 3.1246 ± 0.4175 respectively. Detailed results of WHO susceptibility bioassay are shown in table below.

Diagnostic Insecticide dose	No. of mosquitoes tested (n)	% Corrected mortality after 24 hrs	KT_{50}* (lower-upper limit) (min)	KT_{95}* (lower-upper limit) (min)	Regression slope ± SE	Z² (d.f)
DDT 4%	95	29.47	209.78 (107.78-3154.46)	1871 (424-918552)	1.7307 ± 0.5433	0.364 (6)
Deltamethrin 0.05%	97	51.65	78.11 (65.35-107.10)	237 (154.75-53217)	3.4126 ± 0.549	0.230 (6)
Permethrin 0.75%	97	75.03	47.97 (43.08-55.49)	1661.22 (117.29-275.22)	3.1246 ± 0.4175	0.238 (6)

DISCUSSION

Anopheles subpictus is found to be an abundant mosquito species in district Kasur, Punjab during this study. The vectorial capacity of *Anopheles subpictus* in malarial transmission has been confirmed in different areas of oriental region. This species is incriminated to be significant primary malaria vector in Bangladesh, India and Srilanka and minor vector in Indonesia and Malaysia [11, 26-30].

In Pakistan various insecticide regimes had been implemented in last several decades to control mosquitoes [31]. DDT was introduced in early 1960s followed by benzene hexachloride (BHC) but emergence of resistance against DDT and BHC in malaria vectors results in increase of transmission rates in Pakistan. Earlier studies reported organophosphate resistance in malaria vectors [32]. Afterwards pyrethroids group has replaced all these insecticide for indoor residual spraying [31].

Although *Anopheles subpictus* is abundantly present in Pakistan but surveillance data regarding insecticide resistance is meager. Only one old study reported DDT resistance in *Anopheles subpictus* in Lahore and West Pakistan [33]. However, several studies regarding insecticide susceptibility status of *Anopheles subpictus* have been conducted in India, Srilanka and Malaysia. Sharma and Krishnamourthy (1957) first outlined the pronounced DDT resistance towards *Anopheles subpictus* in India. Another study reported resistance in *Anopheles subpictus* against

DDT and malathion (with mortality range 40.62- 70.83% and 36-75% respectively) [34]. Same status of insecticide resistance had been found for this species from Bikaner, India [35]. In Srilanka high levels of DDT and malathion resistance in *Anopheles subpictus* with adult mortality of 14-47% and 23-49% was reported [36]. Our findings have also shown that *Anopheles subpictus* is resistant to all three insecticides tested.

CONCLUSION

With context to integrated pest management, grasping the information of insecticide resistance in mosquitoes plays an important role in controlling the resumption of malaria and several other mosquito borne diseases. Selection of rational insecticide has been becoming a limiting factor to curtail malaria vectors due to documentation of insecticide resistance and rapidly eroding the number of suitable insecticide. There is need to explore more efficient toxic chemicals with additional control tactics necessary with substantial increasing population and preservation of species diversity.

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