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# Studies on the efficacy of selected insecticides against *Anopheles* mosquitoes of village Goth Bhoorji (Sindh) Pakistan

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**Abstract**
*Anopheles subpictus* mosquitoes were exposed to various diagnostic doses of three pyrethroids (Deltamethrin, Lambda-cyhalothrin, and Permethrin), one organophosphate (Malathion), and one chlorinated hydrocarbon (DDT), showed a range of mortalities in village Goth Bhoorji of district Mirpur Khas. *Anopheles subpictus* was resistant to two out of five insecticides in village Goth Bhoorji. Results of test done on *Anopheles subpictus*, against diagnostic dose of 4% DDT shown percentage mortality 100%. *Anopheles subpictus*, shown no resistance against 5% Malathion with mortality rate of 100%. Test results against 0.05% Deltamethrin diagnostic dose showed susceptibility in *Anopheles subpictus* with percentage mortality 100%. Wild caught female *Anopheles subpictus* showed percentage mortality 67.79% against 0.05% Lambda-cyhalothrin, while against 0.75% Permethrin the species were resistant with percentage mortality 67.54%.

**Keywords:** *Anopheles subpictus,* Pyrethroids Deltamethrin, Lambda-cyhalothrin, Permethrin, Organophosphate Malathion, One chlorinated hydrocarbon, DDT.

## 1. Introduction

Malarial disease is caused by female *Anopheles* mosquitoes. It is preventable and treatable, yet it kills millions of people every year [1]. Approximately half of the world's population is at risk of malaria and most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected. In 2011, 99 countries had ongoing malaria transmission. According to the latest estimates, there were about 219 million cases of malaria in 2010 and an estimated 660 000 deaths. Most deaths occur among children living in Africa where a child dies every minute from malaria [2]. In 2010, four countries accounted for 97% of the confirmed cases which were Sudan (58%), Pakistan (22%), Yemen (10%) and Afghanistan (6%) [3]. Malaria is the second most prevalent and devastating disease in Pakistan. Malaria has a tendency for epidemic outbreaks over larger area, particularly in Balochistan, NWFP and Sindh province. However, the disease is now emerging as a prominent health problem in FATA particularly along the international border with Iran and Afghanistan. In 2011, the total number of confirmed malaria cases in Pakistan (public sector), reported from all the districts were 319,592 [4].

About 380 species of *Anopheles* occur around the world. Some 60 species are sufficiently attracted to humans to act as vectors of malaria [5]. In Pakistan, out of total 24 Anophelines, there are two major vector species named *Anopheles culicifacies* and *Anopheles subpictus.* Recently two new species *An. fluviatilis* and *An. annularis* have been identified from Baluchistan province. Previously these two species have been considered confirmed malaria vectors in Iran and Afghanistan. However, their role as malaria vector needs to be confirmed through systematic operational research [4]. Vector control is an important part of the global malaria control strategy. Vector control is highly dependent on the use of pyrethroids, which are the only class of insecticides currently recommended for ITNs or LLINs. In recent years, mosquito resistance to pyrethroids has emerged in many countries. In some areas, resistance to all four classes of insecticides used for public health has been detected. Sub-Saharan Africa and India are characterized by high levels of malaria transmission and widespread reports of Insecticide resistance. The development of new insecticides for use on bed nets is a particular priority. Detection of insecticide resistance should be an essential component of all national

malaria control efforts to ensure that the most effective vector control methods are being used. The choice of insecticide for IRS should always be informed by recent, local data on the susceptibility target vectors [2].

Malaria vectors have also acquired widespread resistance to many of the currently used insecticides, including synthetic pyrethroids. Hence, there is an urgent need to develop alternative insecticides for effective management of insecticide resistance in malaria vectors [6].

The two primary malaria vectors in Pakistan *An. subpictus and An. Culicifacies* have developed resistance to insecticide of chlorinated hydrocarbon group such as DDT and dieldrin in neighboring countries such as Iran, Afghanistan, Iraq, Saudi Arabia, India and these species developed resistance to malathion in Iran. In Pakistan resistance to previously used organo-chlorides (DDT, dieldrin), carbamates (propoxur) and organophosphates (Malathion, fenitrothion) has been well documented. The Malaria Control Program has used pyrethroids for both indoor residual spraying and long-lasting insecticide-treated nets since 1992, and the efficacy of this group of insecticides needs further validation [1]. Resistance/Susceptibility status of *Anopheles* mosquitoes has not yet established in Mirpur Khas district so this study will provide baseline data on Resistance/Susceptibility status of *Anopheles* mosquitoes against different groups of insecticides. This study aims the establishment of susceptibility/ Resistance status in *Anopheles* mosquitoes against different groups of insecticides (OC, O Pand Pyrethroid) of district Mirpur Khas.

## 2. Materials and Methods

### 2.1 Study Area

The study area is village Goth Bhoorji, district Mirpur Khas in province Sindh, Pakistan.

### 2.2 Study Design

It was a cross sectional descriptive study. The study was done during August, 2014. It was a pre-defined sampling. Mosquitoes were collected from animal and human dwelling in the morning from time 06.00 am - 8.30 am. Minimum of 100 mosquitoes were used in 4-5 replicates with 15-25 females per tube / replicate for each insecticide concentration / dosage. Two controls were used for each test (According to WHO test procedures). Village Goth Bhoorji in district Mirpur Khas was selected with high density of *Anopheles* mosquito's population. Wild-caught females were collected from four different locations so as to ensure a broadly representative sample of the local population. Live female blood fed, adult mosquitoes were preferred but semi gravid mosquitoes were also used for test. Those adult female mosquitoes that were got damaged before performing tests were not used for test. CDC sweeper, Stereo microscope, Paper cups, WHO test kit, Insecticide impregnated papers.

### 2.3 Data Entry

Data was entered in SPSS_16 software and analyzed to find the chi-square and p. value to find the level of significance of tests and homogenicity in mosquito populations from different localities.

### 2.4 Plan of Analysis

The Percentage mortality of test sample was calculated by summing the number of dead mosquitoes across all four exposure replicates and expressing this as a percentage of the total number of exposed mosquitoes:

$$\textbf{Observed mortality} = \frac{\text{Total number of dead mosquitoes} \times 100}{\text{Total sample size}}$$

Percentage mortality was recorded after 24 hours recovery period on the standard form. When control mortality was greater than 5% but less than 20%, then the observed mortality was corrected using Abbots formula, as follows:

$$\textbf{Abbots formula} = \frac{(\text{\% observed mortality} - \text{\% control mortality}) \times 100}{(100 - \text{\% control mortality})}$$

### 2.5 Test Procedure

Goth Bhoorji a sentinel site in district Mirpur Khas was selected with high density of vector population. Mosquitoes were collected by mouth aspirators, CDC sweeper from animal and human dwellings in the morning from 06.00 am to 8.30 am from pre-defined areas. According to availability of *Anopheles* mosquitoes, live adult female fed, half fed, semi gravid *Anopheles* mosquitoes for each concentration of insecticides were used. They were exposed to discriminating doses which are recommended by WHO (DDT 4%, Malathion 5%, Deltamethrin 0.05% and Lambda-cyhalothrin 0.05% Permethrin 0.75%). At the end of recovery period which is after 24 hours, mosquitoes which were killed by different insecticide impregnated papers and also those killed in the control tube were counted. (A mosquito is classified as dead if it is immobile or unable to stand or fly in a coordinated way). These all test were made under field conditions [7] [9].Three kinds of susceptibility tests were performed on adult stages:

a) Wild-caught fed females were tested under field conditions
b) Progeny of the females that survived the diagnostic doses in the field were tested under laboratory conditions
c) Testing of the progeny of the wild-caught females not tested in the field [8].

## 3. Results

| | |
|---|---|
| Date: 20/08/14 | Locality: Goth Bhoorji |
| Species: *An. subpictus* | Temperature: 33°C |
| District: Mirpurkhas | Humidity: 70% |

**Table 1:** Susceptibility/resistance tests results on mosquitoes of Genus *Anopheles* against 4 % DDT.

| Species | No of replicates treated | After 1 hour exposure | | | | After 24 hour holding | | | | status | Chi square |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Dead | Total | % Mortality | Alive | Dead | Total | % Mortality | | |
| *An. subpictus* | R1 | 4 | 17 | 21 | 81.03% | 0 | 21 | 21 | 100% | R | P=.927 df= 4 $\chi^2$=.883 |
| | R2 | 5 | 19 | 24 | | 0 | 24 | 24 | | | |
| | R3 | 5 | 20 | 25 | | 0 | 25 | 25 | | | |
| | R4 | 3 | 22 | 25 | | 0 | 25 | 25 | | | |
| | R5 | 5 | 16 | 21 | | 0 | 21 | 21 | | | |
| | Total | 22 | 94 | 116 | | 0 | 116 | 116 | | | |
| | C1 | 20 | 0 | 20 | 0% | 20 | 0 | 20 | 0% | | |
| | C2 | 21 | 0 | 21 | 0% | 21 | 0 | 21 | 0% | | |

S= Susceptible if 98-100% observed mortality
? = 90-97 % observed mortality suggests the possibility of resistance that needs to be further confirmed.
R= Resistant if < 90% observed mortality
R1-R5= Replicates, C1-C2= Control
Level of Significance $p<0.05$; non-significance $p>0.05$, df= Degree of freedom, $\chi^2$= Chi Square Value

The WHO susceptibility/resistance tests were conducted in district Mirpurkhas at village Goth Bhoorji, under optimal temperature and humidity, with sample size of 116 female mosquitoes, under five replicates by using WHO insecticide impregnated papers. On the basis of results it is concluded that mortality of *An. subpictus* 100 % against 4% DDT which shows susceptibility.

Date: 20/08/14 Locality: Goth Bhoorji
Species: *An. subpictus* Temperature: 33C
District: Mirpurkhas Humidity: 70%

**Table 2:** Susceptibility/resistance tests results on mosquitoes of Genus *Anopheles* against 5% Malathion.

| Species | No of replicates treated | After 1 hour exposure | | | | After 24 hour holding | | | | status | Chi square |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Dead | Total | % Mortality | Alive | Dead | Total | % Mortality | | |
| *An. subpictus* | R1 | 3 | 22 | 25 | 90.68% | 0 | 25 | 25 | 100% | **S** | P=.871 df= 4 $\chi^2$=.241 |
| | R2 | 1 | 24 | 25 | | 0 | 25 | 25 | | | |
| | R3 | 1 | 23 | 24 | | 0 | 24 | 24 | | | |
| | R4 | 2 | 21 | 23 | | 0 | 23 | 23 | | | |
| | R5 | 4 | 17 | 21 | | 0 | 21 | 21 | | | |
| | Total | 11 | 107 | 118 | | 0 | 118 | 118 | | | |
| | C1 | 20 | 0 | 20 | 0% | 20 | 0 | 20 | 0% | | |
| | C2 | 20 | 0 | 20 | 0% | 20 | 0 | 20 | 0% | | |

S= Susceptible if 98-100% observed mortality
? = 90-97 % observed mortality suggests the possibility of resistance that needs to be further confirmed.
R= Resistant if < 90% observed mortality
R1-R5= Replicates, C1-C2= Control
Level of Significance $p<0.05$; non-significance $p>0.05$, df= Degree of freedom, $\chi^2$= Chi Square Value

The WHO susceptibility/resistance tests were conducted in district Mirpurkhas at village Goth Bhoorji, under optimal temperature and humidity, with sample size of 118 female mosquitoes, under five replicates by using WHO insecticide impregnated papers. On the basis of results it is concluded that mortality of *An. subpictus* 100% against 5% Malathion which shows susceptibility.

Date: 20/8/14 Locality: Goth Bhoorji
Species: *An. subpictus* Temperature: 33C
District: Mirpurkhas Humidity: 70%

**Table 3:** Susceptibility/resistance tests results on mosquitoes of Genus *Anopheles* against 0.05 % Deltamethrin.

| Species | No of replicates treated | After 1 hour exposure | | | | After24 hour holding | | | | status | Chi square |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Dead | Total | %Mortality | Alive | Dead | Total | %Mortality | | |
| *An. subpictus* | R1 | 5 | 19 | 24 | 72.27% | 0 | 24 | 24 | 100% | R | p=.933 df= 4 $\chi^2$=.841 |
| | R2 | 9 | 16 | 25 | | 0 | 25 | 25 | | | |
| | R3 | 8 | 17 | 25 | | 0 | 25 | 25 | | | |
| | R4 | 6 | 19 | 25 | | 0 | 25 | 25 | | | |
| | R5 | 5 | 15 | 20 | | 0 | 20 | 20 | | | |
| | Total | 33 | 86 | 119 | | 0 | 119 | 119 | | | |
| | C1 | 21 | 0 | 21 | 0% | 21 | 0 | 21 | 0% | | |
| | C2 | 20 | 0 | 20 | 0% | 20 | 0 | 20 | 0% | | |

S= Susceptible if 98-100% observed mortality
? = 90-97 % observed mortality suggests the possibility of resistance that needs to be further confirmed.
R= Resistant if < 90% observed mortality
R1-R5= Replicates, C1-C2= Control
Level of Significance $p<0.05$; non-significance $p>0.05$, df= Degree of freedom, $\chi^2$= Chi Square Value

The WHO susceptibility/resistance tests were conducted in district Mirpurkhas at village Goth Bhoorji under optimal temperature and humidity, with sample size of 119 female mosquitoes, under five replicates by using WHO insecticide impregnated papers. On the basis of results it is concluded that mortality of *An. subpictus* 100% against 0.05% Deltamethrin which indicates the presence of susceptibility.

Date: 20/8/14 Locality: Goth Bhoorji
Species: *An. subpictus* Temperature: 33C
District: Mirpurkhas Humidity: 70%

**Table 4:** Susceptibility/resistance tests results on mosquitoes of Genus *Anopheles* against 0.05% Lambda-cylothrin.

| Species | No of replicates treated | After 1 hour exposure | | | | After24 hour holding | | | | status | Chi square |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Dead | Total | %Mortality | Alive | Dead | Total | %Mortality | | |
| *An. subpictus* | R1 | 17 | 6 | 23 | 33.89% | 8 | 15 | 23 | 67.79% | R | p=.729 df= 4 $x^2$= 2.036[a] |
| | R2 | 13 | 12 | 25 | | 7 | 18 | 25 | | | |
| | R3 | 17 | 8 | 25 | | 8 | 17 | 25 | | | |
| | R4 | 15 | 7 | 22 | | 8 | 14 | 22 | | | |
| | R5 | 16 | 7 | 23 | | 7 | 16 | 23 | | | |
| | Total | 78 | 40 | 118 | | 38 | 80 | 118 | | | |
| | C1 | 20 | 0 | 20 | 0% | 20 | 0 | 20 | 0% | | |
| | C2 | 21 | 0 | 21 | 0% | 21 | 0 | 21 | 0% | | |

S= Susceptible if 98-100% observed mortality
? = 90-97 % observed mortality suggests the possibility of resistance that needs to be further confirmed.
R= Resistant if < 90% observed mortality
R1-R5= Replicates, C1-C2= Control
Level of Significance $p<0.05$; non-significance $p>0.05$, df= Degree of freedom, $\chi^2$= Chi Square Value

The WHO susceptibility/resistance tests were conducted in district Mirpurkhas at village Goth Bhoorji, under optimal temperature and humidity, with sample size of 118 female mosquitoes, in five replicates by using WHO insecticide impregnated papers. On the basis of results it is concluded that % mortality of *An. subpictus* 67.79% against 0.05% Lambda cyhalothrin which indicates the presence of resistance.

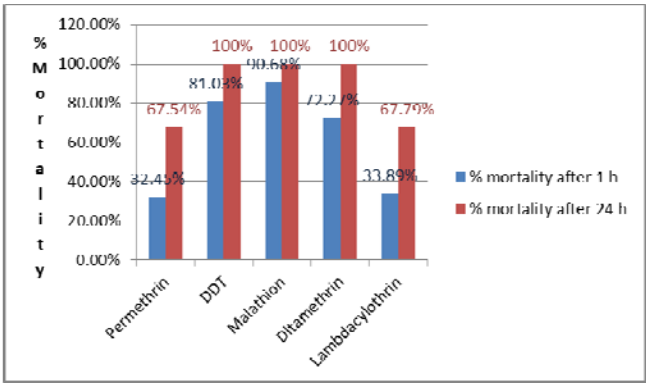
Date: 20/8/14 Locality: Goth Bhoorji
Species: *An. subpictus* Temperature: 33C
District: Mirpurkhas Humidity: 70%

**Table 5:** Susceptibility/resistance tests results on mosquitoes of Genus *Anopheles* against 0.75 % Permethrin.

| Species | No of replicates treated | After 1 hour exposure | | | | After24 hour holding | | | | status | Chi square |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Dead | Total | %Mortality | Alive | Dead | Total | %Mortality | | |
| *An. subpictus* | R1 | 14 | 7 | 21 | 32.45% | 7 | 14 | 21 | 67.54% | R | p=.729 df= 4 $\chi^2$=.036 |
| | R2 | 17 | 8 | 25 | | 6 | 19 | 25 | | | |
| | R3 | 16 | 7 | 23 | | 9 | 14 | 23 | | | |
| | R4 | 16 | 9 | 25 | | 7 | 18 | 25 | | | |
| | R5 | 14 | 6 | 20 | | 8 | 12 | 20 | | | |
| | Total | 77 | 37 | 114 | | 37 | 77 | 114 | | | |
| | C1 | 21 | 0 | 21 | 0% | 21 | 0 | 21 | 0% | | |
| | C2 | 21 | 0 | 21 | 0% | 21 | 0 | 21 | 0% | | |

S= Susceptible if 98-100% observed mortality
? = 90-97 % observed mortality suggests the possibility of resistance that needs to be further confirmed.
R= Resistant if < 90% observed mortality
R1-R5= Replicates, C1-C2= Control
Level of Significance $p<0.05$; non-significance $p>0.05$, df= Degree of freedom, $\chi^2$= Chi Square Value

The WHO susceptibility/resistance tests were conducted in district Mirpurkhas at village Goth Bhoorji, under optimal temperature and humidity, with sample size of 114 female mosquitoes, in five replicates by using WHO insecticide impregnated papers. On the basis of results it is concluded that % mortality of *An. subpictus* 67.54% against 0.75% Permethrin which indicates the presence of resistance.




**Graph 1**

## 4. Discussion

*Anopheles subpictus* was exposed to various diagnostic doses of three pyrethroids (deltamethrin, lambda-cyhalothrin, and permethrin), one organophosphate (malathion), and one chlorinated hydrocarbon (DDT), showed a range of mortalities in village Goth Bhoorji of district Mirpur Khas (Sindh). *Anopheles subpictus* was resistant to two out of five insecticides.

The results of the present study were compared with the results of previous studies in which resistance to DDT and malathion was first recorded 32 years ago in Sindh Province [10][11][12][13]. In the present study we noted that in district Mirpur Khas, *A. subpictus* remained resistant to DDT and Malathion, and there was no sign of reversal of resistance. This was despite the fact that the use of both pesticides for malaria vector control has been discontinued for nearly two decades. The evidence for the disuse of both DDT and Malathion for malaria vector control is provided by the official report of the directorate of malaria control of Pakistan (ministry of health 2009). According to the report the use of DDT for malaria vector control in Pakistan started in 1961 and stopped in 1979. Malathion was used from the early 1980s to mid-1990. Due to the development of resistance to Malathion, the use of Malathion was discontinued in 1996, and it was replaced by deltamethrin, which is still being used. This shows that the end of the use of DDT and Malathion for malaria vector control was stopped 33 and 16 years ago, respectively. Normally it is expected that discontinuation of the use of a pesticide may result in reduction of insecticidal selection pressure on the vector mosquitoes, and may lead to the reversal of resistance. However, resistance to some pyrethroids is slowly developing in some districts, for example to permethrin, lambda-cyhalothrin, and deltamethrin.

## 5. Conclusion and Recommendations

This is the preliminary survey of insecticide resistance/susceptibility of *Anopheles* species in village Goth Bhoorji, district Mirpur Khas. Insecticide resistance was observed in malaria vector species *Anopheles subpictus* against 4% DDT, 5% malathion 0.05% lambda-cyhalothrin 0.05% deltamethrin and 0.75% permethrin according to WHO interpretation. This survey generates record of different species of *Anopheles* in the district Mirpur Khas. It provide evidence base data on insecticide resistance, which enable to follow trends in susceptibility status in this area and will also serve as a base for resistance management interventions. In view of the present status of the resistance in disease vector, the development and implementation of comparatively new strategies for integrated vector management (IVM), needs to be planned in light of the data of this study. In order to manage, prevent, or slow the development of resistance to the presently used effective insecticides, a strategic approach for the judicious use of pesticides is essential. This approach requires efficient and regular monitoring of the susceptibility status of disease vectors as an important component of IVM. Unfortunately, pesticide resistance monitoring and surveillance is extremely inadequate in Pakistan. An effective resistance management policy is not possible without the strong evidence obtained from monitoring and surveillance. Further research is needed to confirm the role of *Anopheles* species in malaria transmission and to monitor the trend of insecticide resistance in malaria vectors. Genetic/molecular studies required for the isolation of resistant genes in prevalent vectors is also recommended to confirm the findings of this survey.

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