Susceptibility Status of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* against Various Insecticides in Lahore: Pakistan

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Introduction

• Mosquitoes are members of the family *culicidae*.
• Various species are dangerous because they suck the blood from their hosts (vertebrates) and transmit different diseases to them.
• Over 3,500 species are present around the world. They are vectors of various diseases of humans.
Lahore Environment excellent for mosquito breeding

- The environment of a city provides favorable conditions for dispersal, reproduction and development of these mosquitoes.
- The stagnant and polluted water present in marshes, swamps, lakes and ponds all around the city are preeminent places available for their breeding.
- Green lush lawns and forests spread in whole of the city provide excellent spots for their resting.
- The humid environment, accumulation of water bodies in human dwellings, lifestyle and deforestation could be the reason of mosquito prevalence in and around the city.
STUDY AREA

- Allama Iqbal Town
- Aziz Bhatti Town
- Data Ganj Baksh Town
- Gulberg Town
- Nishtar Town
- Ravi Town
- Samanabad Town
- Shalimar Town
- Wagah Town
- Lahore Cantonment
Total number of mosquitoes collected monthly from ten (10) towns of Lahore

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<th>Anopheles annularis</th>
<th>Anopheles pulcherrimus</th>
<th>Anopheles subpictus</th>
<th>Anopheles nigerimus</th>
<th>Anopheles stephensi</th>
<th>Anopheles culcifacies</th>
<th>Culex sitiens</th>
<th>Culex ppiiens</th>
<th>Culex tritaeniorhynchus</th>
<th>Culex vangans</th>
<th>Culex vishnui</th>
<th>Culex theleri</th>
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RESULTS

Anopheles species

1) Anopheles annularis
2) Anopheles pulcherrimus
3) Anopheles stephensi
4) Anopheles nigerrimus
5) Anopheles subpictus
6) Anopheles culcifacies

Culex Species

1. Culex vagans
2. Culex pipiens
3. Culex sitiens
4. Culex tritaeniorhychus
5. Culex vishnui
6. Culex theleri

Aedes species

1) Aedes albopictus
2) Aedes aegyptii

Mansonina species

Mansonia uniformis
## Relationship of Number of mosquitoes and Temperature

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<th>Temperature</th>
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\[ R^2 = 0.2121 \]
Relationship between number of mosquitoes and humidity

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R² = 0.0574

Graph showing the relationship between the number of mosquitoes and humidity, with a linear trend line represented by the equation y = 0.0574x + b.
Relationship between number of mosquitoes and rainfall

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This study shows that the number of *Culex* mosquitoes were higher as compared to the *Anopheles* mosquitoes in the same town. The reason of this difference may be the major changes in the climate, abrupt use of insecticides, decrease in the freshwater habitat and increased industrial development etc.

The number of *Aedes* mosquito is found to be very high in all the towns which gives the evidence of increase in Dengue cases in Lahore.

The data of *Aedes* mosquito collected is very much correlated with the data of dengue patients collected from different hospitals of Lahore. Therefore this study will help in future, to take effective control measures for vector borne diseases.
• Mosquitoes are responsible for public health hazard and serves as a vector for transmitting various diseases to humans such as:
  • Dengue fever
  • Lymphatic filariasis
  • West Nile Virus
  • Malaria etc
Aedes aegypti

Eggs of *Aedes aegypti*.  
*Source*: commons.wikimedia.org

Larva of *Aedes aegypti*.  
*Source*: medicites.com

Adult of *Aedes aegypti*.  
*Source*: nopests.com
Culex quinquefasciatus

Eggs of *Culex quinquefasciatus*.  
*Source*: ento.okstate.edu

Larva of *Culex quinquefasciatus*.  
*Source*: entnemdept.ufl.edu

Adult of *Culex quinquefasciatus*.  
*Source*: phsource.us
Anopheles stephensi

Eggs of *Anopheles spp.*
*Source:* en.impact-malaria.com

Larva of *Anopheles spp.*
*Source:* bugguide.net

Adult of *Anopheles spp.*
*Source:* votebits.com
• Dengue ("breakbone") fever, a viral disease transmitted to humans by the bite of an infected *Aedes* mosquito.

• World’s most important insect-transmitted viral disease.

• Every year about 100 million people get infected.
Four classes of synthetic insecticides

1. **Organochlorines:** contain carbon, hydrogen, and chlorine, *chlorinated hydrocarbons*. e.g DDT, Chlordane, Aldrin, Dieldrin, and Endrin.

2. **OPs:** contain phosphorus, e.g. Temephos.

3. **Carbamates:** derivatives of carbamic acid, e.g Aldicarb, Bendiocarb.

4. **Pyrethroids:** pyrethrum plants, e.g. Cypermethrin, Allethrin and Deltamethrin.
APPLICATION OF INSECTICIDE
- Active Ingredients

PENETRATION
- Integument of an Insect

REACHING
- Target Sites

BINDING
- The Action Sites

THRESHOLD CONCENTRATION
- Cause the Death of Insect
Insecticide Resistance

• A variety of resistance developed in insects and pests.

• **Metabolic Resistance** or **Target-site Resistance**.

• **Metabolic resistance** changes the enzymatic system of mosquitoes that result in the rapid detoxification of any applied insecticide and it goes least effective to the insect.
• **Target site resistance** the protein receptor targeted by the insecticide is altered by mutation and insecticide cannot bind with this site of receptor, as the result the insect is unaffected.

• **Knock down Resistance** (kdr) where gene is altered.

• **Cross- Resistance** occurs between different insecticides that share the same mode of action, like the insects resistant to pyrethroids would also be resistant to DDT because they have kdr gene.
Resistance in Mosquitoes

• Mosquitoes become resistant by various insecticides. Resistance may occur against insecticides and can be passed from immature to adult stages of mosquitoes.

• The reason of the resistance development is the frequent and extensive use of insecticides in agriculture and in households.
Aims and Objectives

• This study aims to:
• Determine the current status of susceptibility and resistance of mosquitoes against various WHO recommended and market available insecticides.
• Identify the efficacy of insecticides for dengue control and to facilitate selection of insecticides with the greatest promise to minimize vector density.
• Recommend strategies for controlling dengue vectors and to reduce risks to human health.
Work plan

Step 1: Rearing of mosquitoes

Step 2: Susceptibility test

Step 3: Bioassays
  - Larval bioassay
  - Cone test
  - Adult bioassay

Step 4: Percentage mortality

Step 5: Results, statistical analysis and computation of data
Work plan and Methodology

• Experimental sites:
The research was conducted in Entomology research laboratory, Lahore College for Women University, Lahore and National Institute of Malaria Research and Training (NIMRT), Lahore.

• Experimental specimen: Larvae and adults of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*.
Susceptibility tests

• All the test were conducted by using WHO recommended insecticides (Tmephos and Deltamethrin) and the market available insecticides (K-Orthine, Bifenthrin and Imidacloprid).

• Tests were conducted according to WHO guidelines against larvae and adults of mosquitoes.
Larval Bioassays

- Larvicide temephos granular 1% and temephos 50EC.
- Larvae of late third instar to early fourth instar.
- Three replicates and one control, containing 25 larvae each.
- Exposed to the diagnostic concentrations of temephos according to the WHO protocols.
- %age mortality after 24 hours.
Adult Bioassays

• Adulticides like deltamethrin liquid 1.5 EC and deltamethrin 5%WP, K-Orthine 15EC, Bifenthrin 3.6% and Imidacloprid 5%SC were used.

• Susceptibility kit test and Cone test were carried out to check the efficacy of all these adulticides.
Susceptibility kit Test

- Filter papers were impregnated with insecticides.
- Then were adjusted in the kit.
- Three replicates of treatment and one control.
- 15 adult female mosquitoes in each tube and were exposed for 60 minutes.
- Mortality was observed after 24 hours.
Susceptibility kit Apparatus
Cone Test

• Insecticides were sprayed on the wall at the area of 1m²
• WHO plastic cones, Batches of 10 non-blood-fed mosquitoes, were released in to the cone, exposed for 10 min.
• The mosquitoes were removed and placed in plastic cups after the exposure.
• Mortality was recorded after 24 hours.
Cone Test Apparatus
RESULTS

WHO recommended insecticides

- Results showed that >95% mortality was found in all three mosquito strains against two formulations of larvicide Temphos.

- A variation in mortality was observed when treated with different concentrations of commercial available insecticides (K-Othrine 15EC, Bifenthrin 3.6% and Imidaclorpid 5% SC) in both cone and susceptibility kit bioassays.

- Deltamethrin showed significantly positive results against these mosquitoes in adult bioassays with >75% mortality. *Cu. quinquefasciatus* was found to be the most resistant mosquito strain.
K-Othrine 15EC

• When adults of *Ae. aegypti* were exposed to four different concentrations of K-Othrine 0.04, 0.05, 0.06 and 0.075µl/ml the percentage mortality was 13%, 33%, 86% and 93% respectively in Kit test and 25%, 33%, 92% and 100% in Cone test.

• In control sets the percentage mortalities were 0.00% and 0.01% in Kit and Cone test respectively.

• Analysis of variance indicated that significant positive trends were observed between different concentrations of K-Othrine against *Ae. aegypti* in Kit test (F= 176.45; d.f= 10; P < 0.05) and in Cone test (F=102.04; d.f=11; P< 0.05).
• Adults of *Cu. quinquefasciatus* were exposed to four different concentrations of K-Othrine 0.04, 0.05, 0.06 and 0.075 µl/ml the percentage mortality was 8%, 20%, 40% and 60% respectively in Kit test and 6%, 30%, 58% and 70% in Cone test.

• In control sets the percentage mortalities were 0.00% and 0.00% in Kit and Cone test respectively. Analysis of variance indicated that significant positive trends were observed between different concentrations of K-Othrine against *Cu. quinquefasciatus* in Kit test ($F = 94.89; \text{d.f} = 11; P < 0.05$) and in Cone test ($F = 55.56; \text{d.f} = 11; P < 0.05$).
• Adults of *An. stephensi* were exposed to four different concentrations of K-Othrine 0.04, 0.05, 0.06 and 0.075µl/ml the percentage mortality was 20%, 73%, 80% and 100% respectively in Kit test and 30%, 50%, 100% and 100% in Cone test.

• In control sets the percentage mortalities were 0.00% and 0.01% in Kit and Cone test respectively.

• Analysis of variance indicated that significant positive trends were observed between different concentrations of K-Othrine against *An. stephensi* in Kit test (F= 26.56; d.f= 11; P < 0.05) and Cone test (F= 63.67; d.f= 11; P < 0.05).
Bifenthrin 3.6%

- When adults of *Ae. aegypti* were exposed to four different concentrations of Bifenthrin 0.001, 0.005, 0.01 and 0.02µl/ml the percentage mortality was 6%, 33%, 34% and 40% respectively in Kit test and 0%, 13%, 26% and 50% in Cone test.

- Analysis of variance indicated that significant positive trends were observed between different concentrations of Bifenthrin against *Ae. aegypti* in Kit test (F= 22.25; d.f= 10; P < 0.05) and in Cone test (F=4.27; d.f=11; P< 0.05).

- Adults of *Cu. quinquefasciatus* were exposed to four different concentrations of Bifenthrin 0.001, 0.005, 0.01 and 0.02µl/ml the percentage mortality was 0%, 0%, 6% and 40% respectively in Kit test and 0%, 0%, 10% and 50% in Cone test.
• Analysis of variance indicated that significant positive trends were observed between different concentrations of Bifenthrin against *Cu. quinquefasciatus* in Kit test (F= 63.67; d.f= 10; P < 0.05) and in Cone test (F=22.25; d.f=11; P< 0.05).

• Adults of *An. stephensi* were exposed to four different concentrations of Bifenthrin 0.001, 0.005, 0.01 and 0.02µl/ml the percentage mortality was 20%, 35%, 46% and 66% respectively in Kit test and 20%, 33%, 40% and 70% in Cone test.
Imidacloprid 5% SC

• When adults of *Ae. aegypti* were exposed to four different concentrations of Imidacloprid 0.005, 0.01, 0.02 and 0.03μl/ml the percentage mortality was 26%, 46%, 62% and 88% respectively in Kit test and 30%, 40%, 70% and 90% in Cone test.

• Analysis of variance indicated that significant positive trends were observed between different concentrations of Imidacloprid against *Ae. aegypti* in Kit test (F= 22.68; d.f= 10; P < 0.05) and in Cone test (F=90.00; d.f=11; P< 0.05).

• Adults of *Cu. quinquefasciatus* were exposed to four different concentrations of Imidacloprid 0.005, 0.01, 0.02 and 0.03μl/ml the percentage mortality was 6%, 20%, 33% and 55% respectively in Kit test and 0%, 20%, 30% and 60% in Cone test.
Results

Larval Bioassay:

• When larvae were exposed to WHO recommended larvicides, maximum mortality (96% and 100%) was observed after 24 hours.

• No larval mortality was observed in control group.
Adult bioassays

• WHO recommended adulticides provide efficient results by showing the mortality (80-100%) in all the three species of mosquitoes in Kit and Cone tests.

• Non-WHO recommended adulticides shows the minimum mortality at their lowest concentration and maximum mortality at highest concentrations.
Regression line of toxicity of K-Orthine with *Ae. aegypti* (after 24 hours treatment)

\[ y = 4.5x - 4.5 \quad R^2 = 0.9854 \]

\[ y = 2.8x - 0.5 \quad R^2 = 0.9561 \]
Regression line of toxicity of K-Orthine with *Cu. fasciatus* (after 24 hours treatment)

\[ y = 2.7x - 2 \]
\[ R^2 = 0.9918 \]

\[ y = 2.4x - 2 \]
\[ R^2 = 0.96 \]
Regression line of toxicity of K-Orthine with *An. stephensi* (after 24 hours treatment)

\[ y = 4.1x - 1 \]
\[ R^2 = 0.9917 \]

\[ y = 2.6x + 0.5 \]
\[ R^2 = 0.8895 \]
Regression line of toxicity of Bifenthrin with Ae. aegypti (after 24 hours treatment)

\[ y = 1.5x - 0.5 \quad R^2 = 0.8824 \]

\[ y = 1.7x - 2 \quad R^2 = 0.9797 \]
Regression line of toxicity of Bifinthurin with *Cu. fasciatus* (after 24 hours treatment)

\[
y = 1.3x - 2 \\
R^2 = 0.786
\]

\[
y = 1.6x - 2.5 \\
R^2 = 0.7529
\]
Regression line of toxicity of Bifenthrin with An. stephensi (after 24 hours treatment)

\[
y = 2.3x + 0.5 \\
R^2 = 0.9888
\]

\[
y = 1.6x \\
R^2 = 0.9143
\]
Regression line of toxicity of Imidacloprid with *Ae. aegypti* (after 24 hours treatment)

- **Doğrusal (Kit test)**
  - $y = 2.9x + 1$
  - $R^2 = 0.9836$

- **Doğrusal (Cone test)**
  - $y = 2.1x + 0.5$
  - $R^2 = 0.9692$

- **Kit test**
- **Cone test**

**Mean mortality**

**Concentration (µl/ml)**
Regression line of toxicity of Imidacloprid with *Cu. fasciatus* (after 24 hours treatment)

\[ y = 2.3x - 1.5 \]
\[ R^2 = 0.9888 \]

\[ y = 1.9x - 2 \]
\[ R^2 = 0.9627 \]
Regression line of toxicity of Imidacloprid with *An. stephensi* (after 24 hours treatment)

- **Kit test**
  - $y = 3.3x$
  - $R^2 = 0.9595$
- **Cone test**
  - $y = 2x + 0.5$
  - $R^2 = 0.9524$

![Graph](image)
LC$_{50}$ and LC$_{95}$ values of K-Othrine, Bifenthrin and Imidacloprid against Ae. aegypti, Cu. quinquefasciatus and An. stephensi.

- LC$_{50}$ values recorded in Kit test for Ae. aegypti against K-Othrine, Bifenthrin and Imidacloprid were 0.15, 0.20 and 0.46µl/ml and in Cone test the values recorded were 0.13, 0.16 and 0.35µl/ml.

- LC$_{95}$ values recorded in Kit test for Ae. aegypti against these insecticides were 0.36, 0.45 and 0.43µl/ml and in Cone test.

- LC$_{50}$ values recorded in Kit test for Cu. quinquefasciatus against K-Othrine, Bifenthrin and Imidacloprid were 0.15, 0.20 and 0.35µl/ml and in Cone test the values recorded were 0.14, 0.7 and 0.15µl/ml.
• LC\textsubscript{95} values recorded in Kit test for \textit{Cu. quinquefasciatus} against these insecticides were 3173, 1.98 and 7618\,\mu l/ml and in Cone test the values recorded were 2.03, 0.42 and 4901\,\mu l/ml.

• LC\textsubscript{50} values recorded in Kit test for \textit{An. stephensi} against K-Othrine, Bifenthrin and Imidacloprid were 0.13, 3.91 and 0.31\,\mu l/ml and in Cone test the values recorded were 0.14, 3.31 and 0.44\,\mu l/ml.

• LC\textsubscript{95} values recorded in Kit test for \textit{An. stephensi} against these insecticides were 49.2, 9.00 and 19.3\,\mu l/ml and in Cone test the values recorded were 28.7, 1.47 and 43.07\,\mu l/ml.
Conclusion

• WHO recommended insecticides provide satisfactory results as compared to the Non-WHO recommended insecticides.

• *Culex* species was found to be the most resistant as compared to the *Anopheles* and *Aedes* species.

• The reason of the resistance development is the frequent and extensive use of insecticides in agriculture and in households.
CONCLUSIONS

• The monitoring of insecticide resistance should be increased in consistency, periodicity, and geographic coverage and should be an integral part of any vector/public health program.

• The investigation of cross resistance to the same, similar or related synthetic compounds should also be included in any insecticide evaluation program.

• Knowledge of vector/pest susceptibility to pesticides, changing trends of resistance and their operational implications are basic requirements to guide pesticide use in vector-borne disease and pest control programs.
Recommendations

• Different classes of insecticides including insect growth regulators should be used to avoid resistance development in mosquito species.

• There should be periodic inspections of possible breeding sites of mosquitoes.

• There is a need to study the possible factors which contribute in the development of resistance and mechanism at molecular level.
Thank You
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