Susceptibility of Aedes Aegypti Against Various Insecticides in Lahore, Pakistan

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Abstract:
Dengue fever is an arbo-viral infection, widespread all over the world. In 21st Century, there is no safe affordable and effective vaccine accessible yet; vector control is that most effective method for the control of the disease.

Objective: To determine the susceptibility status of wild and laboratory strains larvae and adults of Aedes aegypti against different group of insecticides in Lahore city.

Method: From Lahore sites, larvae were collected where insecticides used for wild strain at high frequency and quantity. The Insectary of National Institute of Malaria Research and Training (NIMRT), Lahore, Pakistan, adults and larvae were collected for laboratory strain. The laboratory strains for larvae bioassays were used. The mosquitoes populations indoor and outdoor collected in 2009, hatched from larvae into adults insectary in Lahore, Pakistan. During this study, four major insecticides groups are used which include Pyrethroids (Deltamethrine 2.5% SC), Neonicotenoids (Imidacloprid 5% SC), Phenyl-pyrazoles (Fipronil 2.5% EC) and Organophosphates dichlorvos (DDVP 50% EC). For data analysis, Minitab statistical software (Version 13.20) used for data expressed as mean ± S.E.M from bioassays. By using EPA Probit, LC₅₀ was estimated with 95% confidence. The statistically significant p value was <= 0.05. For comparing the concentrations of insecticides, Duncan's multiple range tests was used with significant difference (5% level) using at New Co stat.

Results:
Different location of Lahore samples, Imidacloprid the most toxic to Aedes aegypti's wild strains on the other hand while Fipronil was also active for wild larval samples. Deltamethrine showed least activity against both adults and larval strains. The susceptibility of the field strains was lower than laboratory strains; resistant ratio varies from insecticide to insecticide. In reporting results, mosquitoes' population was resistance because of infrequent and incomplete coverage.

Conclusions:
This study concluded that Pyrethroids and agriculture pest control play role in indirect growth of insecticides' classes. Based on this study it is suggested that by using new strategies to prevent and delay in growth of insecticides will helpful in Lahore, city, Pakistan.

Key words: Aedes aegypti, Insecticides, Neonicotenoids, Phenyl-pyrazoles, Pyrethroids, Organophosphates, larval and adult toxicity, LC₅₀

Introduction:
Dengue fever is an arbo-viral infection, widespread all over the world. In 21st Century, there is no safe affordable and effective vaccine accessible yet; vector control is that most effective method for the control of the disease.¹²

Dengue vector has not been identified in Pakistan before 1994 when it was first reported in port city of Karachi. In the later years, it was found to spread the disease regularly in different...
parts of the country, especially in districts like Swat, Haripur, Abbottabad in KPK (Khyber Pakhtunkhwa) and Lahore, Rawapindi in Punjab province. The first widespread epidemic was observed in 2011 when more than 27,000 cases of dengue were reported in the Punjab with more than 315 deaths. In Lahore alone, more than 279 deaths reported due to dengue. Since then increased cases of this disease has been noted after every 2-3 years. By using neurotoxic insecticides both neuro inhibitory and neuro excitatory mechanism can be used to kill the dengue vector both immature and mature. There is difference between behavioral effects of excitation on organism. In neuro excitation, organism becomes hyperactivity, rigid and tremors. On the other hand neuro inhibition on organism is lower immobility and flaccid paralysis happens. The energy depletion with neuromuscular fatigue is reason of mortality. In Pakistan, major strategy to prevent and control vector borne diseases like dengue fever is use of insecticides. Past years, many studies reported that in insecticides high level of resistance was commonly used. But in their studies research on resistance level for Aedes population at Lahore was missing. By World Health Organization (WHO) rules and control programs, insecticide resistance monitoring continuous needed. The study was aimed to determine the susceptibility status of wild and laboratory strains larvae and adults of Aedes aegypti against different group of insecticides in Lahore city.

**Method:**

**Mosquito Collection Sites**

From Lahore sites, larvae were collected where insecticides used for wild strain at high frequency and quantity. The Insectary of National Institute of Malaria Research and Training (NIMRT), Lahore, Pakistan, adults and larvae were collected for laboratory strain.

**Mosquitoes**

The first star larvae for wild strain of Ae. Aegypti were kept under controlled temperature (25°C ± 2) and relative humidity (70% ± 5) condition inlaboratory along with constant photoperiod (light: dark, 14h:10h). The mixture of dried yeast, biscuits and fat free milk were fed to these larvae. The adult bioassays used as these wild larvae hatched into adults after three to five days. The laboratory strains for larvae bioassays were used. The mosquitoes populations indoor and outdoor collected in 2009, hatched from larvae into adults insectary in Lahore, Pakistan.

**Insecticides**

During this study, four major insecticides groups are used which include Pyrethroids (Deltamethrine 2.5% SC), Neonicotenoids (Imidacloprid 5% SC), Phenyl-pyrazoles (Fipronil 2.5% EC) and Organophosphates dichlorvos (DDVP 50% EC). In distilled water preparing, DDVP, Deltamethrine, Imidacloprid and Fipronil four insecticides were used.

**Larval bioassay**

With minor modifications in World Health Organization procedure (WHO) 1981, bioassays of larval were conducted. In 100 ml of water fourth instars larvae (batches of 20) were introduced for the larval bioassay containing different concentrations if insecticides in a plastic cups. For fee larvae, 0.02 g of powered mixture was used in each cup. For determine lethal concentrations, insecticide ranging from 0.001 to 0.5µl with five different concentration were used. To get valid results, three replicates were done in bioassays for each concentration. Distilled water was used in control test. After 24th hours, numbers of dead larvae were recorded in plastic cups. After 48 hours, Larval mortality for Deltamethrine, Imidacloprid, DDVP was finalized and Fipronil for 72 hours. Moribund larvae were considered dead in bioassay.

**Adult Bioassay**

With some modification in World Health Organization procedure (WHO 2016) Adult bioassays were conducted. About 3 to 5 days old adults, in a glass tube, Whatman number 1 filter paper treated with 0.05% Imidacloprid, 0.5% Deltamethrine, 0.5% Dichlorvos (DDVP)
and 0.01% Fipronil were exposed as for larvae under the same laboratory. No treatments were given in control to filter paper. Then, after 24 hours the mortality was observed. For data analysis, Minitab statistical software (Version 13.20) used for data expressed as mean ± S.E.M from bioassays. By using EPA Probit, LC₅₀ was estimated with 95% confidence. The statistically significant p value was <= 0.05. For comparing the concentrations of insecticides, Duncan's multiple range tests was used with significant difference (5% level) using at NewCo stat. For insecticides in larval and adult bioassays, lines from log concentration-probability regression were drawn. By following the WHO guidelines, the resistance status was determined.

Results:
The results of susceptibility of *Ae. aegypti* larvae (lab and field strains) and adults against various insecticides are shown in Table 1 and Table 2, respectively.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Diagnostic dosages/ mg/µl</th>
<th>No. of mosquitoes exposed</th>
<th>Mortality</th>
<th>Susceptibility status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid 5% SC</td>
<td>0.05</td>
<td>100</td>
<td>85</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Fipronil 2.5% EC</td>
<td>0.03</td>
<td>100</td>
<td>90</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Deltamethrine 2.5% SC</td>
<td>0.7</td>
<td>100</td>
<td>75</td>
<td>Resistant</td>
</tr>
<tr>
<td>DDVP 50% EC</td>
<td>0.5</td>
<td>100</td>
<td>80</td>
<td>Tolerant</td>
</tr>
</tbody>
</table>

Table 1: Susceptibility of *Ae. aegypti* larvae (lab and field strains) against various insecticides: Three replicates, 20 larvae each; control mortalities ranged from 0.0% -3.0%. a: $R_{50}^a$: = LC₅₀ of field strain / LC₅₀ of the lab strain.
Outstanding performance of Fipronil was in agreement with Pridgeon et al., who reported relative potency of 19 insecticides against female Ae. aegypti. However, in contrast to present study, they use six concentrations to estimate 0-100% mortality. Similar results were also reported by Sandra who prepared toxic baits by using active ingredients of five insecticidal groups (macro cyclic lactones, neonicotinoids, phenylpyroles, pyrethrroids and pyrroles) in sucrose solution. These toxic baits were used against Anopheles quadrimaculatus, Ae.taeniorhynchus and Culex quinquefasciatus. In Ae. taeniorhynchus, imidacloprid was considered to be most toxic insecticide in present study.

This study emphasized the susceptibility of Ae. aegypti to commonly used (DDVP and Deltametntrine) insecticides and newly introduced Fipronil and Imidacloprid in Lahore city. Results of this study is in agreement with the studies carried out by Sareehari Uragayala et al (2015) who reported that that insecticide resistant strains of mosquito species tested showed more susceptibility to the three neonicotinoids including imidacloprid.

Present study also revealed an increase in the level of tolerance to operational usage of larvicides and adulticides after dengue outbreaks in Lahore. Sunin Dhiman et al (2015) have also reported that St. albopicta showed resistance to DDT and reduced sensitivity to Deltamethrine and malathion in similar situations. Development of resistance in vectors against insecticides was the main reason for this particular dengue outbreak. Main reason of resistance development in mosquitoes to these insecticides especially Deltamethrine (Pyrethroids) was household use of insecticidal products such as liquid, mat, coil and cream formulations having ingredients of Pyrethroids. These products play an important role in development of resistance in Ae. aegypti.

Frequent use of insecticides with same modes of action can accelerate the development of resistance.

The use of low concentrations of Pyrethroids and Organophosphates for mosquitoes control was effective and safe as revealed by present study (Table 2). Chareonviriyaphap et al. 1999 and Somboon et al. 2003 had reported similar findings in their studies. So it can be said that insecticides has played an imperative role in vector control. Reiter and Gubler 1997 have also reported that vector borne disease control principal methods by applications of adulticides and larvicides.

Resistance in mosquitos' population is an important issue. Main reasons of this is incomplete and frequent spraying of insecticides spray practices in many areas. From the results of the present study, it is quite clear that Ae. aegypti mosquitoes did not exhibit marked resistance against insecticides used except Deltamethrine. As this study showed that mortality percentage varies from 75% to 90% but in Deltamethrine mortalities percentage ranges (15-85% at 0.2-0.65µl) in the laboratory strain and for field strain (10-80% at 0.3-0.7) µl it decreases. This is in contrast to studies conducted by different researchers who reported high level of resistance in Aedes mosquitoes against majority of insecticides used. In addition to this, the extensive application of pyrethroids for mosquito and agriculture pest control caused indirect contribution for development of resistance to these classes of insecticides.

Based on this study it is suggested that by using new strategies to prevent and delay in growth of insecticides will helpful in Lahore, city, Pakistan.

**Conclusions:**

This study concluded that Pyrethroids and agriculture pest control play role in indirect growth of insecticides’ classes. Based on this study it is suggested that by using new strategies to prevent and delay in growth of insecticides will helpful in Lahore, city, Pakistan.
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