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Research Article

Insecticides Resistance in Dengue Mosquitoes from District Rawalpindi, Pakistan

Syeda Hina Bukhari*, Farid Asif Shaheen*, Talha Habib*, Muhammad Waseem Akram*, Nida Zahra*, Usama Afzal* and Ahsan Irshad*

**Pir Mehr Ali Shah Arid Agriculture University, 46300-Rawalpindi, Pakistan*

**Corresponding Author Email: shaheen@uaar.edu.pk*

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Abstract – In Pakistan, application of pyrethroids insecticides and temephos is on peak. The present research is designed to detect current level of the temephos and pyrethroids resistance and identifying the presence of knockdown resistance. LC_{50} and RR_{50} values from Pothohar town and Rawal town were (0.245 ppm, 7.17) and (0.032 ppm, 8.2). LC_{95} and RR_{95} values from pothohar town and Rawal town were (0.413, 8.4) and (0.245, 5).LC_{50} values of deltamethrin from Pothohar and Rawal Town were 0.016 and 0.015 % with resistance ratio 6.4 and 6, respectively. LC_{50} value of cypermethrin from Pothowar and Rawal Town were 0.017 and 0.019 % with resistance ratio 5.6 and 6, respectively.

Keywords –Insecticides, Resistance, Dengue Mosquitoes, Cypermethrin, Mortality.

I. INTRODUCTION

Mosquitoes are the most important vector of human diseases like zika, dengue, chikungunya, malaria, filariasis and yellow fever (WHO, 2016a).The public health impact of dengue is enormous and around 2.5 billion people in more than 125 countries of the world live in dengue endemic areas (WHO, 2012a; 2016b). In Asia the first eruption of dengue fever was reported in Thailand and Philippines in 1950s (Khan*et al.,* 2010). Both *A. aegypti* and *A.albopictus* are commonly present in Pakistan and causing dengue (Jahan, 2011).The outburst of dengue fever was first reported in 1994 from Karachi. In 2010, 3305 cases of dengue fever were reported from Karachi (Shamim, 2010). Dengue epidemic is recorded form Rawalpindi for having maximum number of cases after Lahore. Rawalpindi has border areas with Swat district of KPK and Islamabad. It is facing more threat due to recent outburst of dengue in Swat and also due to the huge breeding places and increased number of reported cases in Islamabad. The cases reported from Rawalpindi district in 2013 and 2014 were 1100 and 1406, respectively (PITB, 2015).

Due to non-availibity of vaccine, vector control is the best option (Flores *et al.,* 2013). Chemical control becomes most important with organic insecticides introduction in 1940s (Yap *et al.,* 2003). Chemical larvicides are the most widely used method to target the larval population of *A.*mosquito in their breeding sites (Ranson *et al.,* 2010). According to WHO, In Pakistan, the most important and commonly use pesticides to control malaria and dengue vectors are organophosphate and pyrethroids (Zaim and Guillet, 2002). Temephos is commonly used to control the immature of *A. aegypti* (chavasse and Yap, 1997).). Due to frequent use of insecticides, field population of *A.* mosquitoes has developed widespread resistance to these chemical insecticides in Pakistan (Khan *et al.,* 2011).In mosquitoes, target site resistance and metabolic based resistance are the two distinct insecticide resistance mechanisms

(Hemingway *et al.,* 2004). Mutations have been detected in VGSC codons from different countries. Mutants like V1016G, V1016I and F1534C are linked with *kdr* (Brengues *et al.,* 2003). Many studies also revealed no *kdr* mutations inspite of resistance population like In the Indian *A. albopictus* population no *kdr* were observed (Kushwah *et al.,* 2015). In this study we investigated the temephos resistance against larval population and the pyrethroids (Deltamethrin, cypermethrin) resistance and examined *kdr* mutation in *A. aegypti*in two locations in Rawalpindi.

II. MATERIALS AND METHOD

Collection and identification of mosquitoes

Adult and immature stages of mosquitoes were collected from two localities of district Rawalpindi based on preceding dengue prevalent areas and also having selection pressure against insecticides. These localities include Rawal Town and Pothowar Town.

Collection of immature stages (larval/pupal) of mosquitoes

All kinds of potentially suitable aquatic habitats for mosquito breeding were visited to collect the larval/pupal stages. For collecting mosquito's larvae/pupae dipper were used. The visited aquatic habitats included irrigation channels, tap catch basins, tree holes, shallow pool, ponds, rock pools, wells, waste water houses, construction pools, water leakage points and different containers (empty tins, drums, pitchers) etc. Larvae were reared for identification and further experiments.

i) Indoor-resting mosquitoes were collected with mouth operated and battery operated aspirator.
ii) Adult mosquitoes were collected with the help of hand net and light traps.

Aedes mosquitoes identification

Identification of *A. aegypti* mosquitoes were carried out by observing different morphological features of female's mosquitoes as described by Faran (1980) and Faran and Linthicum (1981).

Insecticide bioassay on field collected population collection of mosquitoes

Rearing of mosquitoes

A. aegypti were reared following the method as described by (Clemons *et al.,* 2010). *A. aegypti* culture was reared in cages under controlled conditions with 25°C and 75% relative humidity. The photoperiod was maintained at 16 h L: 8 h D. *A. aegypti* larvae were reared in plastic trays. Pupae were separated and were shifted to mosquito cages before adult emergence. Adults were kept in culture cages. A diet of 12-15% sucrose solution was provided constantly to adults. Further, females were provided with blood meals once a week. The blood meal stimulated egg production in *A. aegypti*. Larvae were reared to adult stages to carry out bioassays.

Insecticides

Temephos 50% E.C Temeguard was used in the larval bioassay.Pure deltamethrin and cypermethrin 97-98% technical ingredient were the insecticides used in adult bioassay.

Larval bioassay

Larval bioassay was conducted for *A. aegypti*. Serial concentrations were prepared with five replication (10 larvae per replicate). 2ppm stock solution was prepared and subsequent serial dilution (0.5, 0.125, 0.03 and 0.007) was made from it. Mortality was recorded after 24 hours.

Insecticide required is calculated by the formula:

$$\textit{Insecticide required (ppm)} = \frac{Concentration\ level\ to\ be\ made \times solvent\ (ml)}{concentration\ of\ the\ insecticide \times 10}$$

Adult bioassay (WHO bioassay)

Stock solution

To produce 1% stock solution of each insecticide 20mg of deltamethrin and cypermethrin were mixed with 20ml of Acetone-silicon solvent. Further concentrations (0.05%, 0.025%, 0.005%, 0.0025% and 0.0005%) were made from it. To prevent the crystallization of insecticide and for the spread of compound across filter paper, silicon was added.

Experiment

Around eighty mosquitoes were used for each above mentioned insecticide for each site. For the control concentration, mosquitoes were released on only solvent impregnated papers. All the bioassays were done with the help of five tubes, four of them carrying treatment concentrations, 20 mosquitoes per tube and one for the control concentration. The four replicates of mosquitoes containing 20 female mosquitoes per replicate were set up simultaneously for each insecticide. After one hour mosquitoes were transferred to another tube for recovery. At this recovery time period the tubes were placed in cool, dark place and sugar solution is provided for its diet. The percentage mortality was recorded by calculating the quantitative data of dead and alive mosquitoes after 24 hours.

Data analysis

By probit analysis, data were analyzed using POLO-PC software (Leora,1987) and (Finney, 1971). LC_{50}values for field collected mosquitoes population were compared with the LC_{50} value of susceptible mosquito population and resistance ratios (RR) were obtained using following equation:

$$\textit{Resistance Ratio (RR)} = \frac{LC50\ of\ field\ collected\ mosquito\ population}{LC50\ of\ susceptible\ mosquito\ population}$$

III. RESULTS

Estimation of susceptibility and resistance ratios of *A. aegypti* against temephos

Percentage mortality of field population of Rawal Town, Pothowar Town and laboratory population of *A. aegypti* at five concentrations after 24 hours exposure was assessed. LC_{50} value of the laboratory strain after 24 hours exposure was recorded as 0.0039ppm, while the LC_{50}values for Rawal and Pothowar Town were recorded as 0.032 ppm and 0.028ppm, respectively. LC_{95} value of the laboratory strain after 24 hours exposure was recorded as 0.049ppm while the LC_{95} values for Rawal and PothowarTown were recorded as 0.245 ppm and 0.413 ppm, respectively (Table 1 & 2).

Table 1 Lethal toxicity of temephos against *A. aegypti* susceptible population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{50} (FL at 95%)	0.0039 (0.000-0.006)
RR_{50}	-
LC_{95} (FL at 95%)	0.049 (0.029-0.189)
RR_{95}	-
Slope ± SE	1.334±0.376
Df	3
n+Control	300

n = (control + number of insect exposed)
df= (degree of freedom)
SE= (Standard Error)
FL= Fiducial limits (95% level)
LC_{50}= Lethal concentration (95% level)
LC_{95}= lethal concentration(95% level)
RR_{50}= Resistance ratio 50%: (LC_{50} field strain/LC_{50} lab strain)
RR_{95}= Resistance ratio 95%:(LC_{50} field strain/LC_{95} lab strain

Figure 1 revealed the percent mortality of susceptible, Pothowar Town and Rawal Town population against temephos. Percent mortality was recorded highest at 2ppm, while the least mortality was observed at the lowest dose 0.007.86 % and 90% mortality was observed at 0.125ppm from PothowarTown and Rawal Town, respectively.

The LC_{50}values were further used to estimate resistance ratio RR_{50}. Resistance ratio for Rawal was 8.2 while resistance ratio for Pothowar Town was recorded as 7.17 (Table 3). The LC_{95} values were further used to estimate resistance ratio RR_{95}. Resistance ratio for Rawal Town was 5 while resistance ratio for Pothowar Town was recorded as 8.4 (Table 4)

Figure 2 revealed the moderate to high resistance against temephos in Pothowar and Rawal Town of Rawalpindi according to criteria of Mazzari and Georgiou, 1995.Highest resistance ratio was RR_{95} (8.4) found from Pothowar Town, while the RR_{95} for Rawal Town was 5.

The present results were almost similar with study of (Arslan *et al.*, 2015) who reported the Moderate to high resistance in Rawal, Pothowar and Cantonment area of Rawalpindi, Pakistan. LC_{50} value for Rawal, Pothowar and Cantonment areas were 0.024, 0.019, 0.025 ppm with RR_{50} 6.32, 5, 6.58, respectively. While LC_{95} value for Rawal, Pothowar and Cantonment areas with RR_{95} were 0.26, 0.23 and 0.25 with RR_{95} 9.29, 8.21, 8.93. According to Arslan *et al.* (2016) incipient level of resistance was found from larval population with 81.25 % mortality from Rawal Town and 83.96 mortality from Pothowar Town Rawalpindi.

Table 2 Lethal toxicity of temephos against *A. aegypti* Rawal Town population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{50} (FL at 95%)	0.032 (0.024-0.042)
RR_{50}	8.2
LC_{95} (FL at 95%)	0.245 (0.158-0.475)
RR_{95}	5
Slope ± SE	1.858±0.223
Df	3
n+Control	300

Table 3 Lethal toxicity of temephos against *A. aegypti* Pothowar Town population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{50} (FL at 95%)	0.028 (0.019-0.039)
RR_{50}	7.17
LC_{95} (FL at 95%)	0.413 (0.244-0.923)
RR_{95}	8.4
Slope ± SE	1.404±0.173
Df	3
n+Control	300

Table 4 Lethal toxicity of Deltamethrin against *A. aegypti* Susceptible population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{1o} (FL at 95%)	0.00089 (0.0001-0.001)
RR_{10}	-
LC_{50} (FL at 95%)	0.0025 (0.002-0.011)
RR_{50}	-
LC_{90} (FL at 95%)	0.012 (0.010-0.0146)
RR_{90}	-
Slope ± SE	1.680±0.137
Df	2
n+Control	480

The results were differ from (Goindin *et al.*, 2017) who reported high level of resistance RR_{50} ranged from 8.9 to 33.1 fold. Our finding lethal concentrations LC_{50} of laboratory samples (LC_{50}: 0.0039) were almost like the susceptible Rockefeller strain (LC_{50} of 0.0037) observed by Marcombe*et al.* (2011). The results of field population LC_{50} were also slightly higher than field population observed by (Singh, 2014).

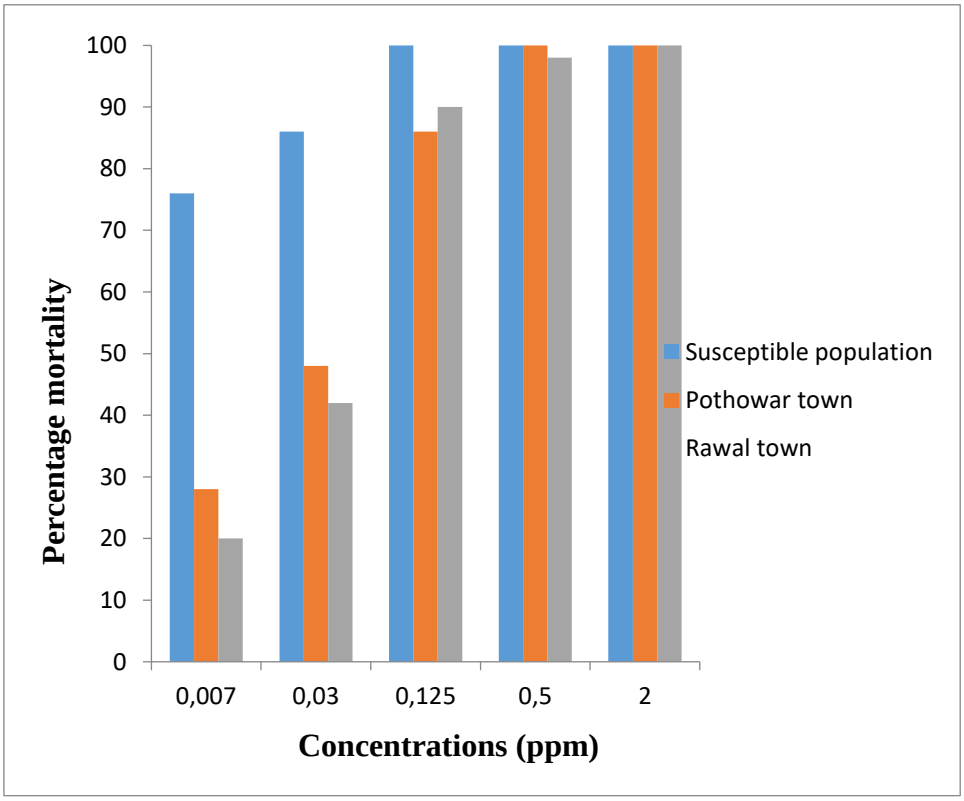

Figure 1 Percent mortality of larval population of *Aedes aegypti* against temephos at various concentrations

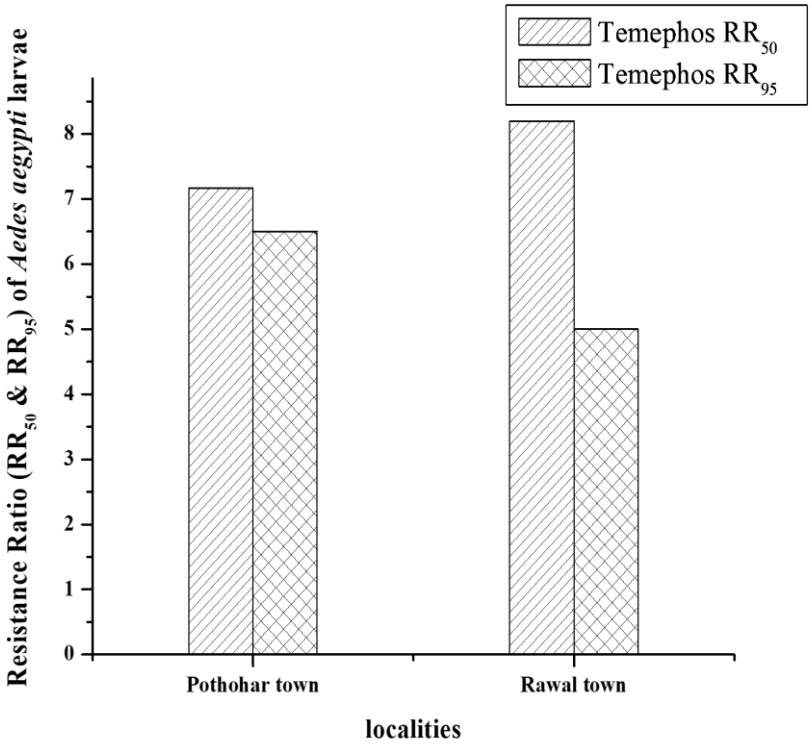

Figure 2 Resistance Ratios (RR_{50}& RR_{95}) of *Aedes aegypti* population against temephos collected from different localities of District Rawalpindi

Estimation of susceptibility and resistance ratios status of *A. aegypti* against two pyrethroids insecticides

Toxicity of Deltamethrin

On Adult mosquito, observations were also recorded after 24 hours of exposure to deltamethrin and cypermethrin insecticide. The $LC_{10,}$ $LC_{50}$and LC_{90}values of deltamethrin after 24 hours of exposure against susceptible population of*A. aegypti* were 0.00089, 0.0025 and 0.012 %, respectively (Table 4.4). The $LC_{10,}$ $LC_{50}$and LC_{90}values of deltamethrin after 24 hours of exposure against *A. aegypti*collected from PothowarTown were 0.005, 0.016 and 0.055 %(Table 4.5). The $LC_{10,}$ $LC_{50}$and LC_{90}values of deltamethrin after 24 hours of exposure against *A. aegypti* collected from RawalTown were 0.005, 0.015 and 0.047 %, respectively. The LC values were further used to estimate resistance ratio. RR_{10}, RR_{50} and RR_{90} values form PothowarTownwere 5.7, 6.4 and 4.9, respectively. Resistance ratios from Rawal Townwere RR_{10}= 5.7, RR_{50}=6 and RR_{90}=4 (Table 5).

Figure 3 showed that the percent mortality of deltamethrin against *A. aegypti* from PothowarTown, Rawal Town and susceptible population. Mortality at 0.05% was84%, 88% and 100%, respectively, while the percent mortality at 0.025% was 75%, 77% and 97%, respectively. Percent mortalities from PothowarTown at 0.005, 0.0025, and 0.0005 were 38%, 35%, 18%, respectively. Percent mortalities from Rawal Town at 0.005, 0.0025, and 0.0005 were 42%, 33%, 20%, respectively. Percent mortalities for susceptible population at 0.005, 0.0025, and 0.0005 were 56%, 45% and 17%, respectively.

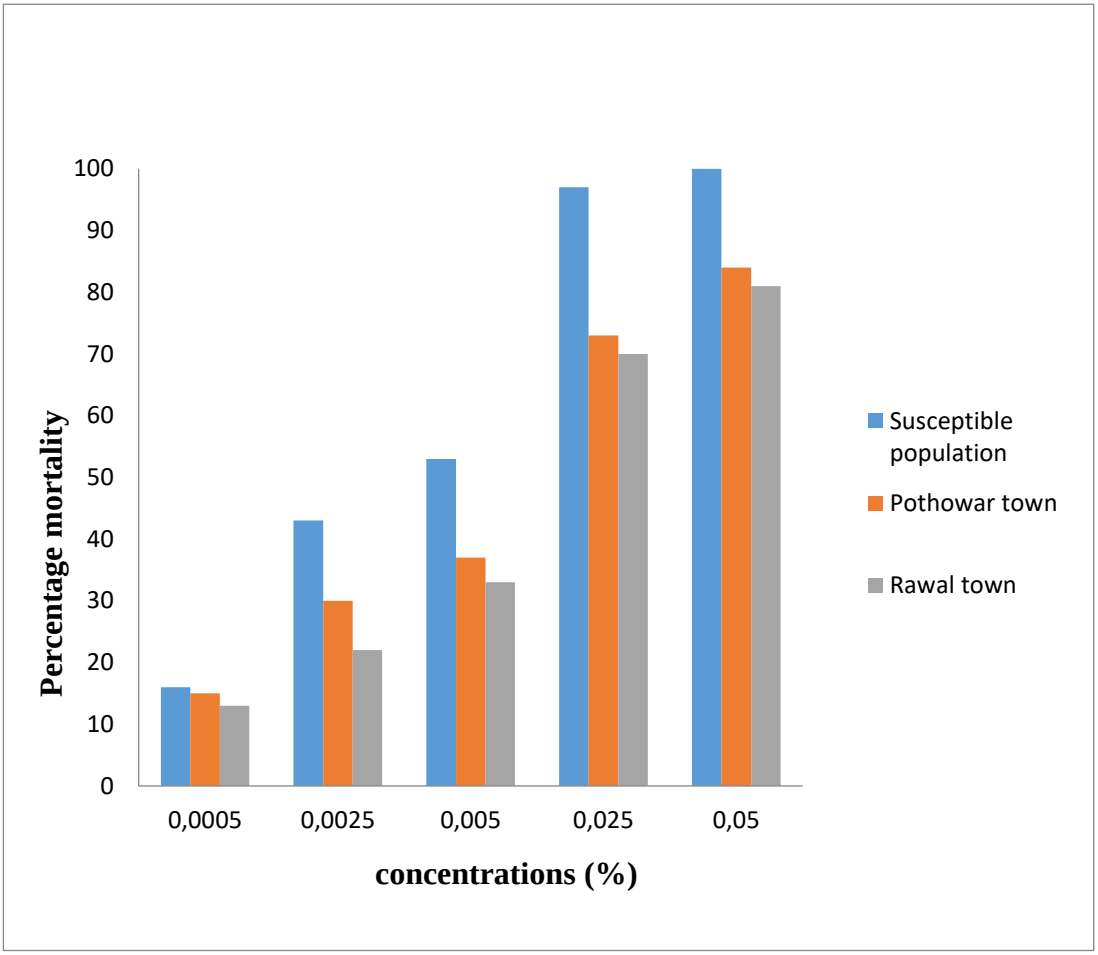

Figure 3 Percent mortality of adult*Aedes aegypti* against deltamethrin at various concentrations

Table 5 Lethal toxicity of Deltamethrin against *A. aegypti* Pothowar Town population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{1o} (FL at 95%)	0.005 (0.000-0.008)
RR_{10}	5.7
LC_{50} (FL at 95%)	0.016 (0.012 to 0.028)
RR_{50}	6.4
LC_{90} (FL at 95%)	0.055 (0.030 to 0.689)
RR_{90}	4.9
Slope ± SE	2.382±0.257
Df	3
n+Control	480

Toxicity of Cypermethrin

The $LC_{10,}$ $LC_{50}$and LC_{90}values of cypermethrin after 24 hours of exposure against susceptible population of *A. aegypti* were 0.0009, 0.0032 and 0.016 %, respectively (Table 6). The LC_{10}, LC_{50} and LC_{90}values of cypermethrin after 24 hours of exposure against *A. aegypti* collected from Pothowar Town were 0.005, 0.017 and 0.057 %, respectively (Table 7). The $LC_{10,}$ $LC_{50}$and LC_{90}values of cypermethrin after 24 hours of exposure against *A. aegypti* collected from RawalTown were 0.0055, 0.019 and 0.062, respectively. The LC values were further used to estimate resistance ratio. RR_{10}, RR_{50} and RR_{90} values

from PothowarTown were 5.5, 5.3, and 3.6, respectively. RR_{10}, $RR_{50}$and RR_{90} from Rawal Town were 6.1, 5.9 and 3.8, respectively (Table 8).

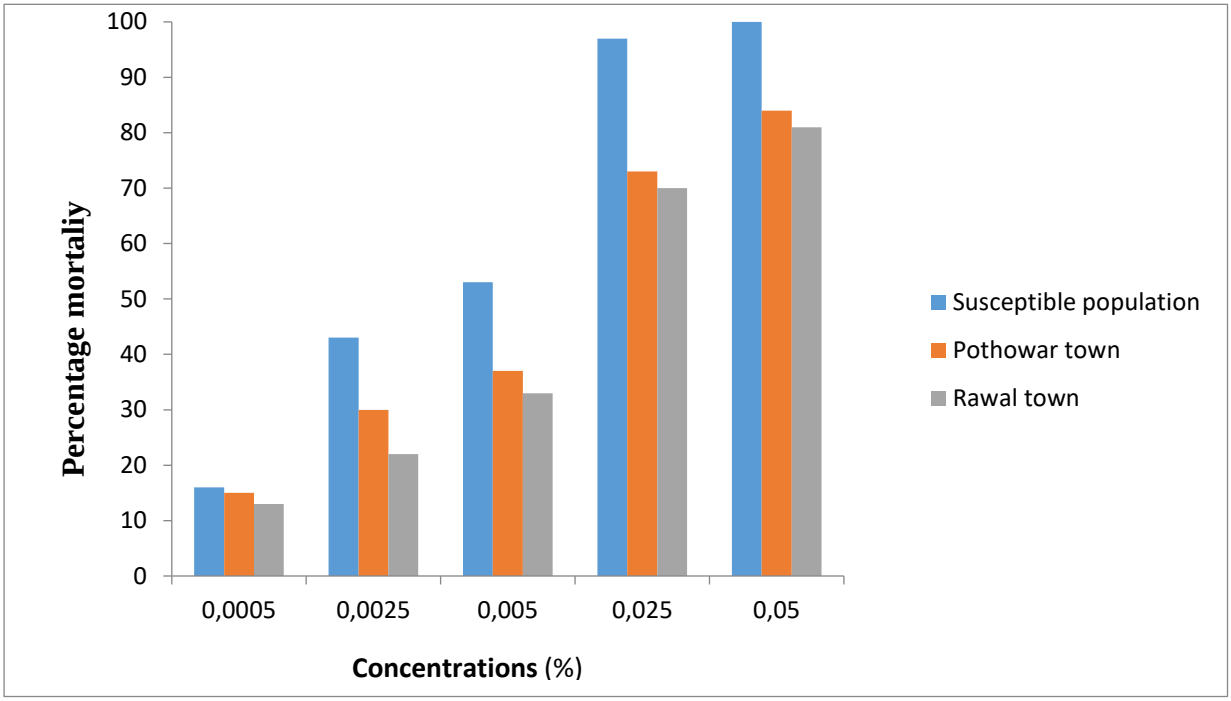

Figure 4 Percent mortality of adult*Aedes aegypti* against cypermethrin at various concentrations

Table 6 Lethal toxicity of Deltamethrin against *A. aegypti* Rawal Town population assessed after exposure time of 24 hours

Parameters	**Required values**
LC_{1o} (FL at 95%)	0.005 (0.001-0.008)
RR_{10}	5.7
LC_{50} (FL at 95%)	0.015 (0.009-0.024)
RR_{50}	6
LC_{90} (FL at 95%)	0.047 (0.027-0.405)
RR_{90}	4
Slope ± SE	2.583±0.27
Df	3
n+Control	480

Figure 4 showed the percent mortality of Cypermethrin against *A. aegypti* from Pothowar Town, Rawal Townand susceptible population at 0.05% were 84%, 81% and 100%, respectively, while the percent mortality at 0.025% was 72%, 70% and 97%, respectively. Percent mortalities from Pothowar Town at 0.005%, 0.0025%, and 0.0005% were 33%, 22%, 13%, respectively. Percent mortalities from Rawal Town at 0.005%, 0.0025%, and 0.0005%were 37%, 30%, 15%,respectively. Percent mortalities for susceptible population at 0.005%, 0.0025% and 0.0005% for susceptible population were 53%, 43% and 16%.

Table 7 Lethal toxicity of Cypermethrin against *A. aegypti* susceptible population assessed afterexposure time of 24 hours

Parameters	**Required values**
LC_{1o} (FL at 95%)	0.0009 (0.0005-0.001)
RR_{10}	-
LC_{50} (FL at 95%)	0.0032 (0.001-0.005)
RR_{50}	-
LC_{90} (FL at 95%)	0.016 (0.009-0.029)
RR_{90}	-
Slope ± SE	1.706±0.135
Df	2
n+Control	480

Table 8 Lethal toxicity of Cypermethrin against *A. aegypti* PothowarTown population assessed afterexposure time of 24 hours

Parameters	Required values
LC1o (FL at 95%)	0.005 (0.000-0.009)
RR_{10}	5.5
LC_{50} (FL at 95%)	0.017 (0.012-0.034)
RR_{50}	5.3
LC_{90} (FL at 95%)	0.057 (0.031-1.176)
RR_{90}	3.6
Slope ± SE	2.437±0.255
Df	3
n+Control	480

The present study also revealed that pyrethroid resistance was observed in the Rawalpindi district. High pyrethroid resistance was observed in PothowarTown with highest resistance ratio (RR) values. Insecticide resistance in *A.* mosquitoes has been reported from many countries such as Latin America, South East Asia and Caribbean (Jirakanjanakit *et al.*, 2007a, b; Rodriguez *et al.*, 2005). Figure 5 revealed the LC_{50} value for deltamethrin after 24 hours of exposure against *A. aegypti* collected from Pothowar Town was recorded highest as compared with Rawal Town, while LC_{50} value for cypermethrin after 24 hours of exposure against *A. aegypti* collected from Rawal Town was recorded highest as compared with Rawal Town.

Figure 6 showed that the resistance ratios (RR value) of cypermethrin and deltamethrin against *A. aegypti*. The RR_{10} value of cypermethrin after 24 hours of exposure against *A. aegypti* collected from Rawal Town was highest, while RR_{50}value for deltamethrin from PothowarTown was recorded highest. RR_{90} value for deltamethrin from PothowarTown was also recorded highest.

Positive correlation was found between concentration of deltamethrin and cypermethrin and 24 hours of mortality, as the percent mortality were increased with the increasing concentrations of insecticides. The highest mortality was observed at 0.05%, while at 0.0005% least mortality was observed. The mortality was less than 50% for concentrations 0.005% and less. Least mortality was observed at 0.0025% and 0.0005% which showed that at certain doses*A. aegypti* were unaffected by cypermethrin and deltamethrin.

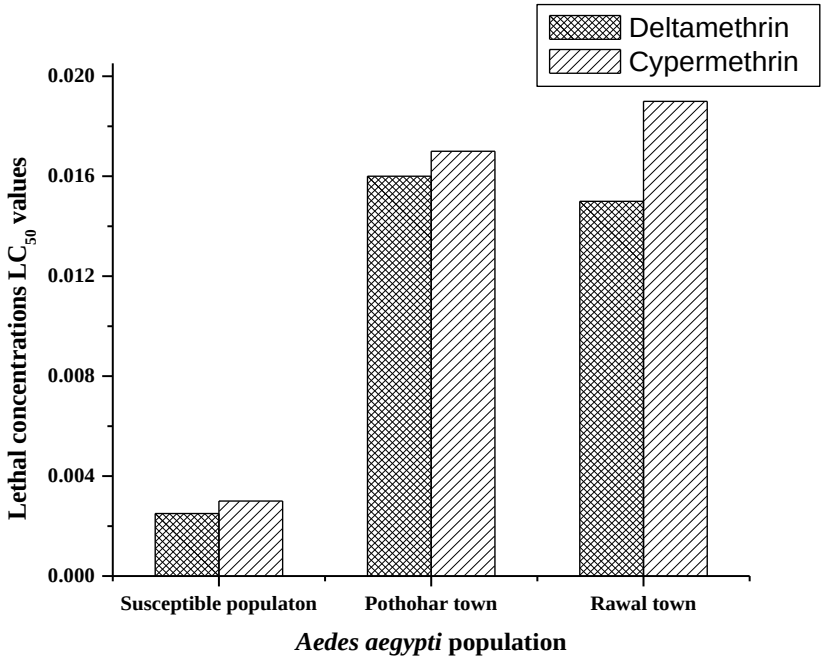

Figure 5 Lethal Concentration (LC_{50}) values of deltamethrin and cypermethrin against *Aedes aegypti* population collected from different areas of District Rawalpindi

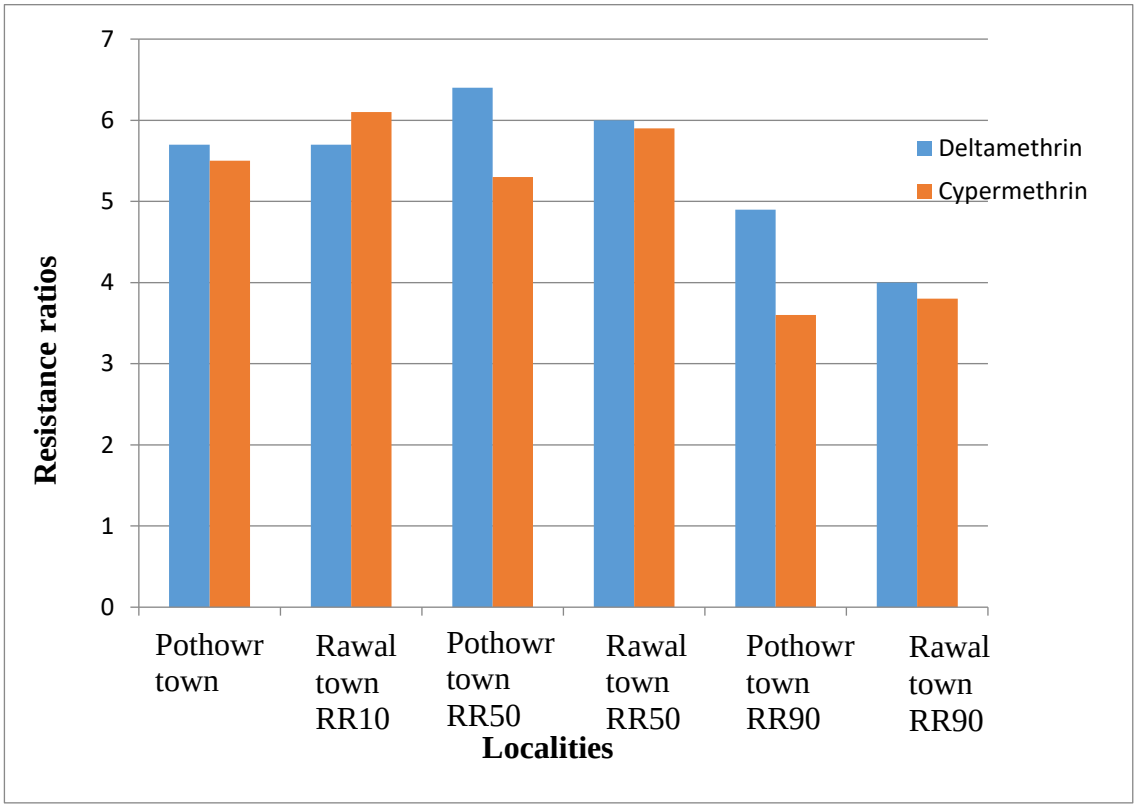

Figure 6 Resistance Ratios (RR_{10}, RR_{50} and RR_{90}) of *Aedes aegypti* population against Deltamethrin and Cypermethrin collected from different localities of District Rawalpindi

IV. DISCUSSION

The present study revealed the moderate to high resistance against temephos in Pothowar and Rawal town of Rawalpindi according to criteria of Mazzari and Georgiou, 1995.The present results were almost similar with study of (Arslan *et al.,* 2015) who reported the Moderate to high resistance in Rawal, Pothowar and Cantonment area of Rawalpindi, Pakistan. The results were differ from (Goindin *et al.,* 2017) who reported high level of resistance RR_{50} ranged from 8.9 to 33.1 fold. The results of field population LC_{50} were also slightly higher than field population observed by (Singh, 2014). Our finding lethal concentrations LC_{50} of laboratory samples (LC_{50}: 0.0039) were almost like the susceptible Rockefeller strain (LC_{50} of 0.0037) observed by Marcombe *et al.* (2011).

The present study also revealed that pyrethroid resistance was observed in the Rawalpindi district. High pyrethroid resistance was observed in Pothowar town with highest resistance ratio (RR) values. Our results are in affirmative with the results obtained by Marcombe *et al*. (2009; 2012); Faucon *et al*. (2015) and Ishak *et al*. (2016). Although *kdr* type resistance is known to cause resistance to pyrethroids in *A. aegypti*, many studies also showed that metabolic resistance might cause pyrethroids resistance in *A. aegypti* (Ahmad *et al.,* 2007; Strode *et al.,* 2008).Similar results were observed for the *kdr* mutation in India, where Kushwah *et el.* (2015) did not find any *Kdr* mutation in *A.* mosquitoes collected from different localities of India. Further studies are needed to observe the other mechanism of pyrethroids resistance in *A.* mosquitoes collected from Rawalpindi district.

V. CONCLUSION

Present study showed that the low-moderate level of insecticides resistance in larval and adult population of *A. aegypti*. Further, amplification of target site showed non-involvement of kdr in the resistant population. However, there exists possibility that other mechanisms were present and acting to confer the resistance.

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