INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 2, p. 18-23, 2019

RESEARCH PAPER OPEN ACCESS

Determination of insecticidal resistance in *Culex* mosquitoes against temephos, cypermethrin and deltamethrin

Sadia Abbas, Shabab Nasir*, Muhammad Kashif Zahoor, Muhammad Asrar

Department of Zoology, Government College University, Faisalabad, Pakistan

Key words: Enzymes, Insecticidal resistance, Mosquitoes, Punjab, Pakistan.

http://dx.doi.org/10.12692/ijb/14.2.18-23 Article published on February 12, 20199

Abstract

Pakistan is an agricultural country, so farmers use huge amount of insecticides without discrimination to save their crops. In the mean while they also use those pesticides in their homes for the control of house hold pests. So, this study was carried out to know the status of resistance of temephos and pyrethroids in mosquitoes. For this purpose, mosquitoes were collected, identified and reared in laboratory for bioassay. Larvae were treated in beakers while adults with impregnated papers. In case of temephos, LC_{50} values from 0.009 to 0.327 ppm were noted and highest value was found from Lahore mosquitoes that were about 34 fold resistant than susceptible strain. In case of adulticides, cypermethrin showed up to 17.67 fold variation in susceptibility level across all populations while deltamethrin showed up to 33.88 fold variation. The results showed that Lahore mosquitoes were more resistant followed by Rawalpindi and least in case of Faisalabad. Higher level of activity in case of different enzymes like esterase, mixed-function oxidase, glutathione S-transferase and acetyl-cholinesterase were found in resistant populations as compared to susceptible strain. We should use selective chemicals with new mode of action to minimise this problem.

* **Corresponding Author:** Sadia Abbas, Shabab Nasir ✉ flourenceshabab@yahoo.com

Introduction

Mosquitoes are the commonest disease carriers for a host of parasites such as bacteria and viruses in man (Samidurai *et al.,* 2009). Mosquitoes are tiny creatures belonging to order Diptera and family Culicidae. This family has three medically important genera (Samidurai *et al.,* 2009); *Anopheles* (vector of malaria in man), *Culex* (vector of filarial worms, West Nile virus) (Kilpatrick, 2011) and *Aedes* (responsible for the spread of dengue, yellow fever, chikungunya, filarial diseases and Zika virus) (Lambrechts *et al*., 2010). The only solution is to control the mosquitoes. Although the widely used method to control mosquitoes is the chemical control but the main drawback of this method is the development of resistance in vector species. Due to development of resistance, the vector-borne diseases are spreading and causing more problems for the world population especially in Africa and South Asia (Strode *et al*., 2014). Resistance has been reported from every group of insecticides (Soko *et al*., 2015). No doubt, insecticidal resistance is a big issue but other issues like availability of non registered insecticides in market and black marketing of registered insecticides also contribute towards the failure of vector control (Owusu *et al*., 2015). Insecticidal resistance can be determined and monitored in vector population through bioassay test, biochemical assays or molecular assays (Brogdon and McAllister, 1998; Ranson *et al*., 2011). Bioassays are either time mortality or dose mortality bioassay and first one is more sensitive in determining insecticidal resistance than second one. Time-mortality bioassays are easy and simple to perform and provide more information about limited pool of mosquitoes. The standard technique used for these bioassays in mosquito species is WHO susceptibility test (WHO, 2006).

Majority of people (more than 75%) in Pakistan depend up on agriculture for their livelihood. They are using insecticides blindly to protect their crops even they are unaware of the dose and potency of insecticide (Habib, 1996). They use these pesticides on crops as well as for house hold pests like mosquitoes with over and under doses. Moreover, after dengue epidemics during 2010, insecticides in huge amount with high doses were sprayed in major cities of the Punjab to control mosquito. Although *Culex* mosquitoes are not involved in spreading of any disease in Pakistan but they create great nuisance and itching due to biting. So, its rearing is easy and is without any fear of disease, so we used this mosquito to know the status of insecticidal resistance of commonly used insecticides against *C. quinquefasciatus.*

Materials and methods

Mosquito larvae were collected with the help of dipper from urban, agricultural and industrial areas of Lahore, Rawalpindi, Sialkot and Faisalabad cities.

These localities were selected due to high insecticide use for agriculture and household pest management. Collected mosquito populations were brought to the Entomology laboratory, department of Zoology, Government College University, Faisalabad for identification and rearing in plastic trays under laboratory conditions (26 ± 2 °C and 60 ± 5% RH) (Ahmad *et al*., 2017). Third instar larvae (20 larvae for each treatment) were used for the larval bioassay and adult females (20 females for each treatment with insecticide-impregnated papers) used for the adult bioassay. The susceptible strain was collected from remote areas and then reared under laboratory conditions for more than 20 generations without any exposure to insecticides.

The insecticides along with their trade names and the formulations used in this study were temephos (Abate 1 SG, BASF S.A., Brazil), cypermethrin (Bulletin 10% EC, Ali Akbar Group, Pakistan) and deltamethrin (Decis 2.5% EC, Bayer Pakistan (Pvt.) Ltd.). These insecticides were used for bioassay with concentrations as used by the farmers in field as temephos (1, 0.5, 0.25, 0.125, 0.06 and 0.03 ppm), cypermethrin (250, 125, 62.5, 31.25, 15.5 and 7.8) and deltamethrin (62.5, 31.25, 15.6, 7.8, 3.9 and 1.95 ppm). All the treatments in triplicate along with control group (by using tap water for larvae and water treated filter paper for adults) were carried out under

laboratory conditions (Abbas *et al.*, 2019).

Mortality data was recorded after 24 hours and was corrected using Abbott's formula and then analysed with probit analysis (Finney, 1971) using statistical software Mini tab 17. The mosquito populations showing the highest resistance factors were selected for the enzyme studies. The following scale was used to categorize the populations on the basis of their resistance factors (RFs): Low <5, Moderate >5 and <10, High 10-50 and Extremely High >51 (Lima *et al.*, 2011).

Biochemical analysis

For biochemical analysis, 30 mosquito larvae were washed with dist. H_2O and dried with bloating paper. These larvae were homogenized with ice-cold sodium phosphate buffer (20mM. pH 7.0) with the help of Teflon hand homogenizer. Then, the homogenate was centrifuged at 8000×g and 4°C for 20 minutes and supernatant was used for the estimation of Esterases or Phosphatases. Solutions and glassware used for homogenization were kept at 4°C prior to use, and the homogenates were held on ice until used for various assays. Different enzymes like acetylcholinesterase (AChE), mixed function oxidase (MFO), esterases and glutathione S-transferase (GSTs) were assessed from this homogenate (Li *et al.*, 2007).

Statistical analysis

Mortality data was recorded after 24 hours and was corrected using Abbott's formula and then analysed with probit analysis (Finney, 1971) using statistical software Mini tab 17. Resistance ratios were also calculated with respect to the corresponding susceptible populations.

Results and discussion

Larval bioassay

The data (Table 1) showed that Temephos LC_{50} of *C. quinquefasciatus* susceptible population (SS) and field populations of Faisalabad (FSD), Sialkot (SKT), Rawalpindi (RWP) and Lahore (LHR). Thirty 3rd instar larvae of *Cx. quinquefasciatus* were subjected to temephos, and lethal concentrations (LC_{50}) of 0.009 to 0.327 ppm was noted after 24 h in susceptible and field strains, such as Lahore, Rawalpindi, Sialkot and Faisalabad.

Table 1. Lethal concentration (LC_{50}) for susceptible and field population of *C. quinquefasciatus* larvae to Temephos.

Population	LC_{50}	Fiducial limit	Equation	χ^2	RR	P value
SS	0.009	(0.002 – 0.017) a	0.40x + 1.88	3.32	1	0.52
FSD	0.028	(0.010 – 0.050) ab	0.26x + 0.93	2.38	3.11	0.66
SKT	0.049	(0.020 – 0.081) bc	0.23x + 0.71	2.60	9.8	0.62
RWP	0.143	(0.061 – 0.281) cd	0.16x + 0.31	0.09	15.88	0.99
LHR	0.327	(0.178 – 0.937) de	0.16x + 0.18	0.11	34.44	0.99

Different letters in the same column indicate significant differences due to non-overlapping basis of 95% CI.

The fiducial limits ranged between 0.002 – 0.178 and 0.017 – 0.937 among all populations, and these populations showed up to 34.44-fold variation. The result clearly exhibited that LC_{50} of susceptible population (SS) was very less in comparison with other population.

These results also indicated that LHR population was found highly resistant (34.44 fold) followed by RWP (15.88 fold), SKT (9.8 fold) and FSD population (3.11 fold).These results indicate that the LHR mosquito populations were more resistant than the others due to the greater and longer (approximately 8 years) use of temephos in the LHR city, followed by RWP (5 years of application) (Arslan *et al.*, 2015).

Adult bioassay

The data in Table 2 showed cypermethrin lethal concentration (LC_{50}) for 50 percent mortality of *Cx. quinquefasciatus* susceptible (SS) compared with field populations of Faisalabad (FSD), Sialkot (SKT), Rawalpindi (RWP) and Lahore (LHR).

Table 2. Lethal concentration (LC_{50}) for susceptible and field population of *Cx. quinquefasciatus* adults to Cypermethrin.

Population	LC_{50}	Fiducial limit	Equation	χ^2	RR	P value
SS	2.236	(0.668 – 4.235) a	0.36x - 0.31	3.16	1	0.58
FSD	5.368	(3.459 – 8.143) ab	0.20x + 0.02	1.26	2.4	0.86
SKT	10.833	(6.552 – 14.227) bc	0.16x + 0.07	1.89	4.84	0.75
RWP	17.117	(4.963 – 31.587) d	0.17x – 0.48	0.83	7.65	0.93
LHR	39.505	(20.826 – 70.596) de	0.18x – 0.68	1.70	17.67	0.79

Different letters in the same column indicate significant differences due to non-overlapping basis of 95% CI.

The result showed that lethal concentration ranged from 2.236 to 39.505 µg ml^{-1} with fiducial limit range from (0.668 – 20.826 to 4.235 – 70.596) for susceptible and field populations and showed up to 17.67-fold variation in susceptibility level across all populations. The results also indicated that LHR population was found more resistant (17.67 fold) followed by RWP (7.65 fold), SKT (4.84 fold) and FSD population (2.4 fold).

Table 3. Lethal concentration (LC_{50}) for susceptible and field population of *Cx. quinquefasciatus* adults to deltamethrin.

Population	LC_{50}	Fiducial limit	Equation	χ^2	RR	P value
SS	0.379	(0.080 – 0.846) a	0.36x + 0.35	3.03	1	0.60
FSD	0.761	(0.148 – 2.015) ab	0.14x + 0.48	0.56	2	0.96
SKT	2.253	(1.06 – 6.349) abc	0.16x + 0.23	2.61	5.94	0.62
RWP	5.224	(1.637 – 9.678) d	0.16x – 0.27	1.02	13.78	0.90
LHR	12.814	(7.667 – 22.673) de	0.20x – 0.52	3.56	33.88	0.46

Different letters in the same column indicate significant differences due to non-overlapping basis of 95% CI.

The data in Table 3 showed deltamethrin lethal concentration (LC_{50}) for 50 percent mortality of *C. quinquefasciatus* susceptible (SS) compared with field populations of Faisalabad (FSD), Sialkot (SKT), Rawalpindi (RWP) and Lahore (LHR).

The result showed that lethal concentration ranged from 0.379 to 12.814 µg ml^{-1} with fiducial limit range from (0.080 – 7.667 to 0.846 – 22.673) for susceptible and field populations and showed up to 33.88-fold variation in susceptibility level across all populations. The results also indicated that LHR population was found more resistant (33.88 fold) followed by RWP (13.78 fold), SKT (5.94 fold) and FSD population (2 fold).

The results (Table 4) showed that the areas from where insecticidal resistance was reported in mosquitoes, there was increased activity of different enzymes as compared to susceptible strain. The highest amount of acetyl-cholinesterase enzyme was found in mosquitoes that were caught from Sialkot and Rawalpindi followed by Faisalabad and Lahore mosquitoes and least activity was observed in susceptible strain. The activity of other enzymes (esterases, mixed function oxidases and glutathione S transferase) from resistant and susceptible mosquito populations was shown in Table 4.

These changes in enzyme concentration were also noted by other workers in variety of mosquito species (Liu, 2015). Some other researchers showed that when we used same group of insecticide for longer period then resistance developed in mosquitoes (Pimsamarn *et al.*, 2009; Owusu *et al.*, 2015). If different groups of pesticides were used then this problem of resistance can be minimized (Alsheikh *et al.*, 2016).

Table 4. Activities of different enzymes in *C. Quinquefasciatus*.

Population	Esterase	Mixed function Oxidases	Glutathione S transferase	Acetyl-cholinesterase
SS	0.19 ± 0.02	0.47 ± 0.01	0.10 ± 0.01	0.04 ± 0.01
FSD	0.25 ± 0.01	0.53± 0.03	0.12± 0.01	0.08± 0.01
SKT	0.28 ± 0.01	0.57± 0.02	0.11± 0.02	0.09± 0.01
RWP	0.34 ± 0.01	0.59± 0.01	0.13± 0.03	0.09± 0.02
LHR	0.42 ± 0.01	0.61± 0.02	0.13± 0.01	0.08± 0.00

Different works *in situ* and *in vitro* showed insecticidal resistance in mosquitoes against pyrethroid insecticides present in the market (Thomas and Read, 2016). These findings are in line with our results because we also recorded insecticidal resistance against pyrethroids (cypermethrin and deltamethrin). In the light of present study, we should use new products with different mode of action to minimize this problem.

Conclusion

It is concluded that resistance in mosquitoes is developed due to indiscriminate use of insecticides of same group and can be managed by using insecticides having new mode of action.

References

Abbas S, Nasir S, Fakhar-e-Alam M, Saadullah M. 2019. Toxicity of different groups of insecticides and determination of resistance in Aedes aegypti from different habitats. Pakistan Journal of Agricultural Sciences **55**, (in press). http://dx.doi.org/10.21162/PAKJAS/19.7778.

Ahmad T, Nasir S, Nasir I, Nawaz T, Rafique A, Yousaf I. 2017. Response surface modeling for West Nile viral encephalitis mosquito control experiments. Pakistan Veterinary Journal **37(4),** 465469.

Alsheikh AA, Mohammed WS, Noureldin EM, Daffalla OM, Shrwani YA, Hobani KJ, Alsheikh FA, Alzahrani MH, Binsaeed AA. 2016. Studies on Aedes aegypti resistance to some insecticides in the Jazan district, Saudi Arabia. Journal of Egyptian Society of Parasitology **46(1),** 209-216. http://dx.doi.org/10.12816/0026166.

Arslan A, Mukhtar MU, Mushtaq S, Zakki AB, Hammad M, Bhatti A. 2015. Comparison of Susceptibility Status of laboratory and field populations of Aedes aegypti against Temephos in Rawalpindi. Journal of Entomology and Zoological Studies **3,** 374-378.

Brogdon WG, McAllister JC. 1998. Insecticide resistance and vector control. Emerging Infectious Diseases **4,** 605. http://dx.doi.org/10.3201/eid0404.980410

Finney DJ. 1971. Probit analysis. Cambridge University Press, Cambridge, UK.

Habib N. 1996. Invisible Farmers: A Study on the Role of Woman in Agriculture and Impact of Pesticides on Them. Khoj Research and Publication Centre, Lahore, Pakistan, p 129.

Kilpatrick AM. 2011. Globalization, land use, and the invasion of West Nile virus. Science **334,** 323–327. http://dx.doi.org/10.1126/science.1201010.

Lambrechts L, Scott TW, Gubler DJ. 2010. Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. PLoS Neglected Tropical Diseases **4,** e646. http://dx.doi.org/10.1371/journal.pntd.0000646.

Li X, Schuler MA, Berenbaum MR. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology **52,** 231-53. http://dx.doi.org/10.1146/annurev.ento.51.110104.15110.4

Lima EP, Paiva MHS, de Araújo AP, da Silva ÉVG, da Silva UM, de Oliveira LN, Santana AEG, Barbosa CN, de Paivaneto CC, Goulart MOF, Wilding CS, Ayres CFJ, Santos MAVDM. 2011. Insecticide resistance in Aedes aegypti populations from Ceará, Brazil. Parasites & Vectors **4(5),** 1-12. http://dx.doi.org/10.1186/1756-3305-4-5

Liu N. 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual Review of Entomology **60,** 537-559. http://dx.doi.org/10.1146/annurev-ento-010814-020828.

Owusu HN, Jancaryova D, Malone D, Müller P. 2015. Comparability between insecticide resistance bioassays for mosquito vectors: time to review current methodology? Parasites & Vectors **8,** 357. http://dx.doi.org/10.1186/s13071-015-0971-6.

Pimsamarn S, Sornpeng W, Akksilp S, Paeporn P, Limpawitthayakul M. 2009. Detection of insecticide resistance in Aedes aegypti to organophosphate and synthetic pyrethroid compounds in the north-east of Thailand. Dengue Bulletin **33,** 194-202.

Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends in Parasitology **27,** 91–8. http://dx.doi.org/10.1016/j.pt.2010.08.004.

Samidurai K, Jebanesan A, Saravanakumar A, Govindarajan M, Pushpanathan T. 2009. Larvicidal, ovicidal and repellent activities of Pemphis acidula Forst. (Lythraceae) against filarial and Dengue vector mosquitoes. Academic Journal of Entomology **2,** 62-66.

Soko W, Chimbari MJ, Mukaratirwa S. 2015. Insecticide resistance in malaria-transmitting mosquitoes in Zimbabwe: a review. Infectious Diseases of Poverty **4,** 1-12. http://dx.doi.org/10.1186/s40249-015-0076-7.

Strode C, Donegan S, Garner P, Enayati AA, Hemingway J. 2014. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. PLoS Medical **11,** e1001619. http://dx.doi.org/10.1371/journal.pmed.1001619

Thomas MB, Read AF. 2016. The threat (or not) of insecticide resistance for malaria control. Proceeding of National Academy of Science **113,** 8900-8902. http://dx.doi.org/10.1073/pnas.1609889113

WHO. 2006. World Malaria Report 2006, WHO Press, World Health Organization, Geneva Switzerland.