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Monitoring of resistance status in dengue vector *Aedes albopictus* (Skuse) (Culicidae: Diptera) to currently used public health insecticides in selected districts of Khyber Pakhtunkhwa-Pakistan

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Abstract
Monitoring of insecticides susceptibility is one of the most important tools for determination of success or failure of vector control programs. The study indicates the susceptibility status in the field population of dengue vector; *Aedes albopictus.* Larvae/adults samples of *Aedes albopictus* were collected from various habitats at various locations with history of insecticides usage from four districts of Khyber Pakhtunkhwa viz., Peshawar, Nowshera, Mardan and Charsadda districts and were tested *in Vitro* against commonly used public health insecticides, viz., chlorpyrifos **(**50%EC), deltamethrin (0.05% EC), lambdacyhalothrin (0.05% EC) and temephos (2%SG) as per WHO protocols. The toxicity range of insecticides trend observed was found in the order of temephos (97.31) > lambdacyhalothrin (93.70%) > deltamethrin (92.31%) > chloropyrifos and (86.48%) that highlights the mosquitoes' resistance in areas with high selection pressure of agricultural and public health pesticides.

Keywords: Susceptibility, *Aedes albopictus*, insecticides, susceptibility, Khyber Pakhtunkhwa-Pakistan

1. Introduction
Dengue fever is the most serious and a major resurgent tropical diseases worldwide [1]. It is the most common health problem globally, the initial contagion by this viral disease was estimated 2.5 to 3 billion people resulting 50 million dengue infections annually [2, 3]. The vector mosquitoes spread disease symptoms in humans beings by the appearance of high fever, severe headache; muscular and eye pain, stringent joint, nausea and vomiting [4]. In general, *Aedes* species larvae breed and grow in clean water and irrigation channels in and around the houses. A huge population of mosquitoes can take place under favorable environmental conditions. The spread of dengue vector is kept limited using different methods. Source reduction and application of insecticides are the major control strategies for dengue vector suppression. Control of adult mosquitoes using a variety of chemical means is encumbered with complications including high cost, slow operational response, ineffective timing of application, low efficacy and evolution of resistance to insecticides. However, the reduction of vector densities alone has often proven insufficient; insecticides continue to play a crucial role in helping to reduce the vector mosquito's population. Various recommended formulations of deltamethrin and temephos are available to control *Aedes* and other mosquitoes populations successfully. Various researchers as Thipwara [5], Rapeeporn [6], Kamgang [7], Marcombe [8] and Polson [9]; they also reported the toxicity and levels of resistance of *Aedes* species against public health insecticides in the field populations as compared to the susceptible labortary strain.

In spite of the importance of development of resistance to insecticides naturally in dengue vectors in Pakistan and elsewhere, there is no effective monitoring system and protocols in order to prevent the problem Suleman [10] *et al*., So, there is a dire need to undertake the situation seriously and initiate appropriate monitoring tools for planning efficient vector management techniques to control the dengue transmission throughout Pakistan.

Also complete vulnerability and monitoring of dengue vectors to currently used insecticides should be a necessary constituent of resistance in addition to disease control activities Thipwara [5]. Therefore, the aim of this research is to develop monitoring tool for working out the resistance level in planning the long term sustainable control strategies for the dengue vector in Pakistan.

2. Materials and Methods

Table 2.1: Description of study area

Location	Site	Mosquito's habitats
Peshawar	NIFA, Hayatabad	Irrigation water channel, old tyres, temporary water containers
Nowshera	Military farm Khaishk, Azakhel Park	
Mardan	SCRI, Toru	
Charsadda	Naguman, Malkadher	

2.1 Study design
Aedes albopictus larvae and pupae were collected at eight sites from four locations (districts) Khyber Pakhtunkhwa, Pakistan. The experiment was conducted during 2016-2017 with completely randomized design (CRD).

2.2 Insect Rearing
A laboratory colony was established by collecting the larvae from the different selected breeding habitats. Larval and pupal collections were made with 0.5 liter standard iron dippers. The collected larvae were brought to laboratory for rearing using ventilated plastic bottle (2L) and cooler during transportation. Albino rats were provided as blood source to the adult females. The culture was established following the standard mosquitoes rearing procedures Khan [11] *et al*, Identification to the species level was made with the help of available taxonomic keys. Susceptible strains were developed in the laboratory by rearing the colony up to 17 filial generation.

2.3 Bioassays
Larvae of mosquitoes collected from the selected sites under study were tested for their susceptibility to different groups of insecticides viz., chlorpyrifos (50%EC), deltamethrin (0.05% EC), lambdacyhalothrin (0.05% EC) and temephos (2%SG) that are used for vector control in the province. History of the pesticides usage or otherwise was taken into consideration while collecting the mosquitoes species from the sites (following the information provided by the agriculture and public health departments). Collection and identification methods were same as described above in the insect rearing portion. Bioassay tests were made following standard techniques of WHO [3].

2.4 Adult's bioassays
For testing the susceptibility or otherwise condition (resistance level) of adult's female mosquitoes, blotting paper was used as test media during the bioassays. Blank Ultra Low Volume (ULV) spray of tap water was used as determining tool for calculating the volume of water on the target measured area of blotting paper. The concentration required for the optimum mortality was optimized by series of tests starting from the lowest possible doses of the insecticides within range 10-50ul/tested kit. The recommended doses for each tested insecticides were formulated as per manufacturer prescription and were also kept into consideration during the trials. As testing media the WHO standard vials kits comprising of 2 units separated by movable net were replaced by simple plastic bottles designed in the same fashion for simplification of the tests. The bottles were cut on the both sides and covered with nylon cloth after inserting the treated w/v or v/v paper at different concentrations 10-50ul/tested kit of the chemicals under study. Proper care was taken during the handling of adults mosquitoes using locally designed kits to avoid the escape during the experiment. Physically healthy 30 adults mosquitoes were selected from the established colony of medical entomology NIFA, Peshawar by naked eyes for the bioassays tests. 10 percent sugar solution was provided during and after the study period. The kits were used only once in the replicated trials. The whole testes were repeated twice under similar laboratory conditions for maximizing the chances for precision and accuracy. The percent adults mortaliy were recorded after 01, 24 and 48 hour period.

2.5 Data analysis
Data on the mortality were recorded as per exposure period and compared by using ANOVA. Statistix-8.1 software was used for plotting the dosage and mortality by log transformation. The data was used as criteria for the resistance or susceptibility level of the respective field strains of the mosquitoes from the selected sites.

3. Results
Mosquitoes larvae were collected from different sites and tested for their susceptibility or resistance status. Bioassay tests were made for 01, 24 and 48 hour revelation period as per methods described above. The samples of *Aedes albopictus* collected from various sites showed significant differences tested with Chlorpyrifos for mortality level (Table 2). The adult mosquitoes of this species collected from NIFA Tarnab showed low level of mortality (45.28%) and thus high level of resistance. The highest percent mortality (86.48%) was noted after 48 hours exposure period nearest to laboratory susceptible strain (control). Highest percent mortality (100%) was noted in adult mosquitoes collected from Military farm Khaishk, Azakhel Park and Naguman after 24 and 48 hours exposure period. The use of Chloropyrifos during time post exposure from 01 to 48 hour increased the level of mortality from 30% (NIFA) to 100% (Military farm Khaishk, Azakhel Park and Naguman).

Table 2: Efficacy of Chloropyrifos against adult mosquitoes of *Aedes albopictus.*

Site	Mortality (%) after			Overall Mean
	01 hr	24 hrs	48 hrs	
NIFA	30.00^{m}	45.83^{jk}	60.00^{ef}	45.28^{e}
Hayatabad	42.50^{kl}	65.00^{de}	86.67^{b}	64.72^{b}
Military Farm Khaishk	50.00^{ij}	100.0^{a}	100.0^{a}	83.33^{a}
Azakhel Park	53.33^{ghi}	100.0^{a}	100.0^{a}	84.44^{a}
SCRI	36.67^{l}	58.33^{fgh}	80.00^{c}	58.33^{c}
Toru	38.33^{l}	50.83^{ij}	69.17^{d}	52.78^{d}
Naguman	52.50^{hi}	100.0^{a}	100.0^{a}	84.17^{a}
Malakadher	41.67^{kl}	63.33^{def}	82.50^{bc}	62.50^{b}
Control	59.17^{efg}	100.0^{a}	100.0^{a}	86.39^{a}
Overall Mean	44.91^{c}	75.93^{b}	86.48^{a}	-

Standard deviation = 24.23
Standard error = 2.33
LSD value at 0.05% for site = 3.45
LSD value at 0.05% for duration = 1.99
LSD value at 0.05% for interaction = 5.97
Means in columns/rows followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

The insect samples of *Ae. albopictus* collected from various sites showed considerable variations where, Lambdacialotrina was used as fighting agent (Table 3). Adult mosquitoes showed medium mortality (61.11%) collected from Toru District Mardan. Highest percent mortality (85.56%) was noted in mosquitoes collected from Military farm Khaishksimilar to laboratory susceptible strain (86.39%). Highest percent mortality (100%) was noted in adult mosquitoes collected from Military farm Khaishk, Azakhel Park and Naguman after 24 and 48 hours exposure period comparable to laboratory susceptible strain (control). The application of Lambdacialotrina during time post exposure from 01 to 48 hour increased the level of mortality from 36.67% at Sugar Crops Research Institute (SCRI) to 100% atMilitary farm Khaishk, Azakhel Park and Naguman areas.

Table 3: Efficacy of lambdacyhalothrin against mosquitoes of *Aedes albopictus*.

Site	Mortality (%) after			Overall Mean
	01 hr	24 hrs	48 hrs	
NIFA	40.00^{ij}	62.50^{de}	90.00^{b}	64.17^{c}
Hayatabad	49.17^{g}	79.17^{c}	92.50^{b}	73.61^{b}
Military Farm Khaishk	56.67^{f}	100.0^{a}	100.0^{a}	85.56^{a}
Azakhel Park	55.83^{f}	100.0^{a}	100.0^{a}	85.28^{a}
SCRI	36.67^{j}	62.50^{de}	89.17^{b}	62.78^{cd}
Toru	43.33^{hi}	50.83^{g}	89.17^{b}	61.11^{d}
Naguman	55.83^{f}	100.0^{a}	100.0^{a}	85.28^{a}
Malakadher	46.67^{gh}	65.00^{d}	82.50^{c}	64.72^{c}
Control	59.17^{ef}	100.0^{a}	100.0^{a}	86.39^{a}
Overall Mean	49.26^{c}	80.00^{b}	93.70^{a}	-

Standard deviation = 22.61
Standard error = 2.18
LSD value at 0.05% for site = 2.52
LSD value at 0.05% for duration = 1.45
LSD value at 0.05% for interaction = 4.36
Means in columns/rows followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

The exploitation of Deltamethrin insecticide applied against adult females of *Aedes albopictus* of different selected sites showed significant differences in mortality (Table 4). The adult mosquitoes collected from NIFA Tarnab showed medium level of mortality (56.11%). Highest percent mortality (87.78%) was noted in mosquitoes collected from Hayatabad. Moderate mortality (50.65%) was noted after 01 hour exposure period which increased to 80.37% and 92.31% after 24 and 48 hours, respectively. Highest percent mortality (100%) was noted in adult mosquitoes collected from Hayatabad, Military farm Khaishk, Azakhel Park and Naguman after 24 and 48 hours exposure period similar to laboratory susceptible strain. The application of Deltamethrin during time post exposure from 01 to 48 hour increased the level of resistance from 33.33% (NIFA) to 100% in Hayatabad, Military farm Khaishk, Azakhel Park and Naguman. Results showed high mortality (100%) when treated with Deltamethrin confirming the divergent trend of susceptibility in these mosquitoes to this group of insecticides.

Table 4: Efficacy of Deltamethrin against *Aedes albopictus* mosquitoes

Site	Mortality (%) after			Overall Mean
	01 hr	24 hrs	48 hrs	
NIFA	33.33^{l}	57.50^{fgh}	77.50^{d}	56.11^{e}
Hayatabad	63.33^{e}	100.0^{a}	100.0^{a}	87.78^{a}
Military Farm Khaishk	55.00^{h}	100.0^{a}	100.0^{a}	85.00^{b}
Azakhel Park	60.00^{efg}	100.0^{a}	100.0^{a}	86.67^{ab}
SCRI	50.00^{i}	59.17^{fg}	82.50^{c}	63.89^{c}
Toru	36.67^{l}	45.83^{j}	88.33^{b}	56.94^{e}
Naguman	56.67^{gh}	100.0^{a}	100.0^{a}	85.56^{b}
Malakadher	41.67^{k}	60.83^{ef}	82.50^{c}	61.67^{d}
Control	59.17^{fg}	100.0^{a}	100.0^{a}	86.39^{ab}
Overall Mean	50.65^{c}	80.37^{b}	92.31^{a}	-

Standard deviation = 23.34
Standard error = 2.25
LSD value at 0.05% for site = 2.11
LSD value at 0.05% for duration = 1.22
LSD value at 0.05% for interaction = 3.65
Means in columns/rows followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

Adult mosquitoes of *Aedes* spp. collected from various sites showed considerable variations where Temephos was used as insecticide (Table 5). The samples of *Aedes* spp collected from NIFA, Tarnabshowed moderate mortality (61.94%). Highest percent mortality (88.06%) was noted. in mosquitoes collected from Naguman. Medium mortality (50.93%) was

recorded after 01 hour exposure period which increased to 90.93% and 97.31% after 24 and 48 hours, respectively. Highest percent mortality (up to 100%) was noted in mosquitoes collected from all sites except NIFA and Hayatabad after 24 and 48 hours exposure period similar to laboratory vulnerable strain (control). The application of Temephos insecticide during time post exposure from 01 to 48 hour increased the level of mortality from 37.50% to 100%.

Table 5: Efficacy of Temephos against *Aedes albopictus* mosquitoes.

Site	Mortality (%) after			Overall Mean
	01 hr	24 hs	48 hrs	
NIFA	46.67^{h}	56.67^{f}	82.50^{c}	61.94^{f}
Hayatabad	42.50^{i}	61.67^{e}	93.33^{b}	65.83^{e}
Military Farm Khaishk	50.83^{g}	100.0^{a}	100.0^{a}	83.61^{bc}
Azakhel Park	54.17^{fg}	100.0^{a}	100.0^{a}	84.72^{b}
SCRI	37.50^{j}	100.0^{a}	100.0^{a}	79.17^{d}
Toru	50.83^{g}	100.0^{a}	100.0^{a}	83.61^{bc}
Naguman	64.17^{de}	100.0^{a}	100.0^{a}	88.06^{a}
Malakadher	45.00^{hi}	100.0^{a}	100.0^{a}	81.67^{c}
Control	66.67^{d}	100.0^{a}	100.0^{a}	88.89^{a}
Overall Mean	50.93^{c}	90.93^{b}	97.31^{a}	-
Standard deviation			=	23.81
Standard error			=	2.29
LSD value at 0.05% for site			=	2.28
LSD value at 0.05% for duration			=	1.31
LSD value at 0.05% for interaction			=	3.94

Means in columns/rows followed by similar letters are not significantly different at 0.05% level of probability (LSD test).

4. Discussion

Monitoring of resistance status of insect pests play a significant role in any vector control program in addition to the knowledge of this status assists significantly in devising a long term sustainable control vector population. Insecticides are the most useful method for the management of mosquitoes since long, but the irrational use of insecticides poses selection pressure that result in resistance development in the mosquito's populations Brogdon and Mcallister [12] therefore; it has become a serious concern in many groups of vector mosquitoes. The extended exposure of mosquito populations to insecticides consequently leads to the appearance of cross resistant strains also.

In the Present work, we commonly used public health insecticides for monitoring of resistance status in the field strains of dengue vector; *Aedes albopictus* in selected areas of Khyber Pakhtunkhwa-Pakistan.

Chloropyrifos treatment yielded significantly different mortalities in *Ae. albopictus* collected from various sites. The adult *Aedes albopictus* collected from NIFA, Tarnab showed low mortality, whereas highest mortality (100%) was recorded in adults collected from Military Farm Khaishk, Azakhel Park and Naguman after 24 and 48 hours exposure period, which was similar to control. It is evident that application of Chloropyrifos requires more time to kill this species of mosquitoes. Feng [13] reported high resistance in *Culex* spp. and *Aedes* spp. To four classes of insecticides, organochlorines, organophosphates, carbamates and pyrethroids. Thus our results of evidence of insecticide resistance at NIFA, Tarnab were in conformation of the previous reports at international level. The present findings are strongly correlated with the results of Khan [11] *et al,* they reported the level of resistance to agrochemicals in field populations of *Aedes albopictus* from moderate to high level for the first time in Khyber Pakhtunkhwa Pakistan. The resistance at the specific site (NIFA, Tarnab) may be attributed to high selection pressure due to continuous use of agro-chemicals including Chloropyrifos in the vicinity i.e. agricultural field.

Highest mortality in *Ae. albopictus* collected from the selected sites, 24 hours after Lambdacialotrina treatment might be due to its high toxicity against it. Hidayati [14] reported that *Aedes* spp. adults were highly resistant to DDT, moderately resistant to Propoxur and tolerant to permethrin. According to Thipwara [5] *Aedes species* adults were highly susceptible to permethrin, deltamethrin and lambdacyhalothrin.

Some earlier researchers had reported toxicity results contrary to the present one, e.g. Rapeeporn [6] reported low levels of resistance in *Ae.* spp. against deltamethrin as compared to the susceptible strain. Kamgang [7] found field *Ae. spp*. populations susceptible to deltamethrin. However, Marcombe [8] observed high mortality rates of susceptible sentinel *Ae.* spp treated with deltamethrin similar to our findings. Polson [9] reported 80-98% resistance in *Aedes species* to deltamethrin. Thus the susceptibility of this species of mosquitoes in case of Lambdacialotrina Lambdacialotrina can be utilized effectively in devising the vector control program.

Higher mortalities (100%) in *Aedes* spp. collected from in almost all studied sites except NIFA and Hayat Abad after 24 hours exposure of Temephos showed its high toxicity against the Aedes mosquitoes. Our results were similar to that of Jahan and Amna [15] reports, where 100% mortality in field collected *Aedes* species using different doses of Temephos after one hour exposure. Karen [16] also reported 80-98% mortality in Temephos and fenthion against *Aedes* spp. The amplified resistance in NIFA, Tarnab population of *Aedes* spp. may be due to regular use of Agricultural chemicals in the nearby Agriculture fields of Tarnab Farms. While Hayat Abad being the hub of high up of the society in Khyber Pakhtunkhwa province is mainly kept de-pest by using pesticides in the area on regular basis by public health, which might be the reason for high level of resistance in *Aedes* species.

5. Conclusion

The toxicity range of insecticides observed was Temephos > Lambdacyhalothrin > Deltamethrin > Chloropyrifos. It is suggested that Temephos and Deltamethrin insecticides may be used to control insect population of *Ae. species.* Furthermore an effective long term resistance management plan is needed in order to sustain insecticides susceptibility in nature and this is a target to be achieved in Pakistan and elsewhere to control dengue vectors or vector borne diseases.

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7. Competing interest

The authors declare that they have no competing interests.

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