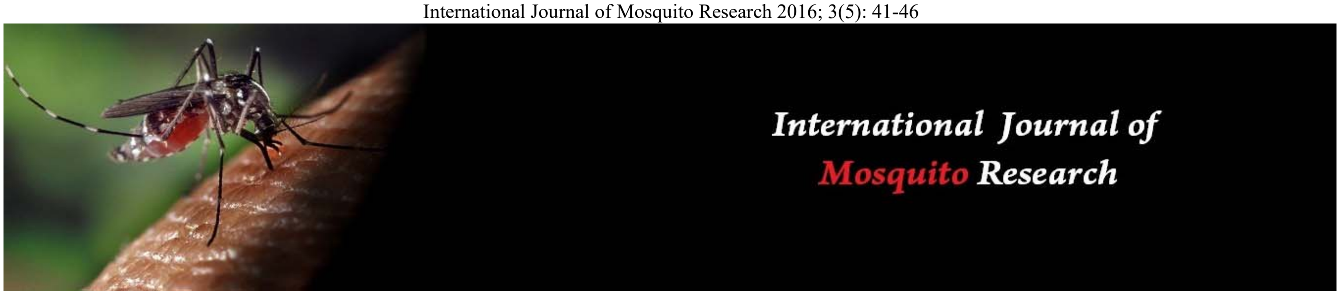

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Susceptibility status of *Aedes aegypti and Aedes albopictus* against insecticides at eastern Punjab, Pakistan

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Abstract
Susceptibility status of *Ae. albopictus* and *Ae. aegypti* was evaluated in different localities of four towns namely Samanabad, Nishtar, Ravi and Iqbal town in Lahore district, Eastern Punjab, Pakistan. Susceptibility test using WHO test kits was applied on adult females (12-24) hrs. Post emergence (non-blood fed) live mosquitoes under laboratory conditions at 27-29 ^{0}C temperature and 85-95% humidity. *Ae. aegypti* and *Ae. Albopictus* were found resistant to 4% DDT with < 36.28% mortality. Both species were also found resistant against 0.05% deltamethrin, 0.05%, lambdacyhalothrin and 0.75% permethrin at all localities with <75.00%, <87.00% and <81.05% mortalities respectively. *Ae. albopictus* was found resistant to 0.1% bendiocarb at Samanabad and Iqbal towns, while resistance possible to be confirmed at Nishtar and Ravi towns. *Ae. aegypti* found susceptible to 0.1% bendiocarb at Samanabad town, resistant at Ravi town and resistance possible to be confirmed (incipient) at Nishtar and Iqbal towns with 92.23% and 94.00% mortalities. Malathion was found most suitable insecticide with 100% mortality rate in *Ae. albopictus and Ae. aegypti* at Lahore district. Alternative use of malathion and bendiocarb may be the best option to reduce dengue vector density.

Keywords: *Aedes, albopictus, aegypti,* mosquito, dengue, insecticide, resistance, control, Lahore, Pakistan

1. Introduction

Dengue fever and Dengue hemorrhagic fever (DHF) are arboviral vector-borne diseases which is endemic in more than 100 countries in the WHO regions of Africa, the Americas, the Eastern Mediterranean, Western Pacific and the South-East Asia [1]. Increasingly the growing emergence and resurgence of *Aedes*-related dengue and neglected tropical diseases have been documented in new and old areas since 2009 according World Health Organization (WHO) report [1]. The unprecedented evolving dengue and Zika threats increased the global community concern and require concerted local and international commitment in proactive and innovative approaches and tools in tackling the global scourge. Specifically, dengue has experienced a 30-fold increase in incidence over the past 50 years that shows no sign of slowing down [2]. The Western Pacific regions and South-East Asia are the most seriously affected [1]. Recent estimates are that approximately 390-400 million people are infected each year and 96 million manifest with clinically apparent disease [2].

In Pakistan, *Ae. aegypti* and *Ae. albopictus* are suspected vectors of dengue, which are responsible for the first infection of dengue hemorrhagic fever (DHF), reported from Karachi in 1994 and so on [3, 4]. In 2006, about 5,522 dengue cases were reported by Health Directorate of Pakistan. In 2010, unprecedented flooding increased the breeding sites for mosquitoes and resulted in dengue epidemic resurgence with documented 9,000 cases and 38 deaths [5]. Moreover, 2011 witnessed the worst dengue epidemic disaster with 22,562 confirmed dengue cases and 363 deaths in Pakistan [5]. Overall from 2011-2014, more than 48,000 laboratories confirmed dengue cases were reported across the country [6].

Integrated efforts in tackling this evolving public health burden have been relying on recommended global and national WHO pesticide evaluation scheme and other approaches and strategies. Since the introduction of organic insecticides in 1940s, chemical control has become the most important method in mosquito control [1, 7].

Chemical insecticides belonging to different classes including organochlorines, pyrethroids, carbamates and organophosphates have been successfully used for the control and elimination of mosquito vectors in the past 2-3 decades in the world [7, 8]. From 2000- 2009, about 394 tons of organophosphates and 154 tons of pyrethroids have been used, against mosquito vectors [7]. About 53 countries documented the types of insecticide used in 2014, in which 43 countries sprayed only pyrethroids, which impart the extensive use of these chemicals worldwide [9].

The time-bound vector control implementation in Pakistan showed that dichlorodiphenyltrichloroethane (DDT) was used as most effective insecticide for mosquito control in early 1969 to 1979, after that it was not effective and was also banned. Benzene hexachloride (BHC) was used from 1972 to 1983 [3]. But from 1984 to1996, the main insecticide used for the control of malaria vector was Malathion [3]. Since last 15 years pyrethroids like deltamethrin 1.5% EC, permethrin 2.5% EC, deltamethrin 5% WP and lambdacialotrina 1.5% are being used in vector control programs at Punjab especially in Lahore [10], but previously there was no any proper documented report, mentioning the susceptibility status of *Ae. aegypti* and *Ae. albopictus* against different insecticides groups from Pakistan [3, 8].

Little contextual insecticides trend and nature on mosquito vectors abundance and susceptibility data and information is documented on vulnerability to dengue public health burden in Lahore, Pakistan. However, previous studies showed that only *Ae. aegypti* which was tested before, for resistance to deltamethrin and cypermethrin, collected from only specific slum area of Misri Shah, Lahore, Pakistan [3, 11].

This study aims at assessing of *Ae. aegypti* and *Ae. albopictus* vectors susceptibility status against various insecticides in different geographical and ecological localities to inform effective vector control strategy (IVM) and to minimize resistance development and spread in Pakistan and the region over time.

2. Materials and Methods

2.1 Study sites and mosquito surveillance

The geography of Lahore comprises the various topographies relating to the land and climate of Lahore, Pakistan. Lying between 31°15′-31°45′ N and 74°01′-74°39′ E, Lahore is bounded on the north and west by the Sheikhupura District, on the east by Wagah, and on the South by Kasur District. The Ravi River flows on the northern side of Lahore. Lahore city covers a total land area of 1014 km² and is still growing. Lahore is the capital of Pakistan's largest province, Punjab; with a population exceeding 10 million, it is a megacity and ranked as the country's second largest metropolis after Karachi [12]. Collectively, it is also the fifth largest city in South Asia and the 26th largest city in the world in terms of population. As a major urban center of Pakistan, it was graded in 2008 as a city with high sufficiency to become a Gamma world city. The climate of the district can see extremes, with a summer maximum temperature 50 °C (122 °F) and a winter temperature of −1 °C (30.2 °F). May, June and July are the hottest months [12] (Figure 1).

In the current study, *Aedes* species larvae were collected from the natural breeding habitats (tiers, plastic water tanks, room coolers, flower, bird's pots and discarded junks) as well as from the ovi-traps (Dark plastic containers, 15 cm diameter; 12 cm depth) [13], set in residential areas at different localities of four towns, Samanabad town, Nishtar town, Ravi town and Iqbal town at Lahore district, Pakistan. Collected *Aedes* larvae and pupae were harvested to adults under standard conditions (28 ^{0}C temperature, 85% relative humidity and 12:12 hours light: dark photoperiod) [14], in the entomological laboratory at Lahore. Taxonomical identification of *Ae. aegypti* and *Ae. albopictus* was done using *Aedes* mosquito key identifiers [15]. The ethical clearance was submitted and approved by the ethical review board, Health Department at Punjab local government, Lahore, Pakistan.

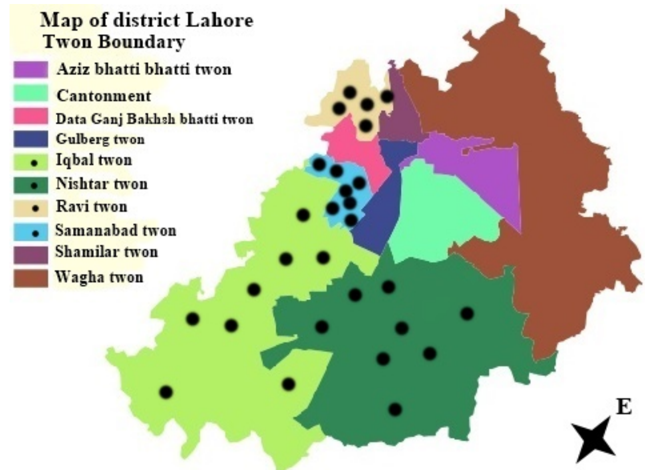

Fig 1: Map of Lahore. The *Aedes* mosquito collection sites are shown in dots, with their corresponding towns like Iqbal town, Nishtar town, Ravi town and Samanabad town (anonymous).

2.2 WHO bioassays testing performance

Aedes species, adult females (12-24) hours post emergence (non-blood fed) mosquitoes were exposed for 1-h to the discriminating doses of WHO recommended test papers impregnated with 0.05% lambda-cyhalothrin, 0.75% permethrin, 0.05% deltamethrin, 4% DDT, 0.1% bendiocarb and 5% malathion, under laboratory condition at 27-29 ^{0}C temperature and 85-95% humidity. After 24-h holding period mortality was observed. Specific controls were run in all cases. All WHO test kits, insecticide impregnated test papers and their appropriate controls were provided by Health Services Academy, Pakistan. Approximately 100 female mosquitoes were tested against apiece insecticide at every locality**.** Moist cotton pads and water was provided during the 24-h holding period of each test replicate, and at the end mortality was calculated. Normally, no mortalities were observed in the controls, but where 5–20% mortalities were observed, Abbott's formula was applied to correct the percentage of mortalities [16]. The calculated percentages of mortalities were used to establish the susceptible and resistant status of these vector populations.

2.3 Data processing and analysis

Interpretation of the susceptibility test data were based upon the following arbitrary criteria [16]. Resistance (R) = 0–89%, Resistance Possible to be Confirmed (RPC) = 90–97% Susceptible (S) = 98–100%. Chi-square test (X^2) was applied to compare populations collected from different locations for heterogeneity. If P value is less than 0.05, then population is heterogeneous and significant, where P value was more than 0.05 it showed population was homogeneous and non-significant [17].

3. Results

3.1 Variability in susceptibility status of *Aedes aegypti* to varied insecticides

Ae. aegypti exposed to various diagnostic doses of pyrethroids (deltamethrin, lambda-cyhalothrin, and permethrin), organophosphate (Malathion), chlorinated hydrocarbon (DDT), and carbamate (bendiocarb), showed a wide range of physiological response at different localities (towns) of Lahore district. (Table 1)

Ae. aegypti was observed resistance against deltamethrin at all localities including Samanabad, Nishtar, Ravi and Iqbal towns with 69.44%, 80.39%, 78.64% and 77.00% mortalities respectively (X^2=4.087, P=0.252). *Ae. aegypti* was again found resistance to 0.05% Lambdacyhalothrin at all localities including Samanabad, Nishtar, Ravi and Iqbal towns recorded 82.41%, 68.63%, 78.30% and 70.58% mortalities respectively (X^2=5.81, P=0.121). Similarly, the resistance against 0.05% permethrin was evaluated at all localities Samanabad (58.18%), Nishtar (70.59%), Ravi (81.05%) and Iqbal (79.41%) respectively (X^2=17.43, P=0.001).

Ae. aegypti was observed resistance against 4% DDT at all localities including Samanabad, Nishtar, Ravi and Iqbal towns 14.54%, 07.84%, 22.86% and 17.64% mortalities (X^2=11.77, P=0.008). *Ae. aegypti* was observed susceptible against malathion at all localities including Samanabad, Nishtar, Ravi and Iqbal towns with 100% mortality. *Ae. aegypti* was found susceptible to 0.1% bendiocarb with maximum mortality 98.01% at Samanabad town. This specie was found resistant with 87.50% mortality at Ravi town, in contrast to possibly resistance to be confirmed (incipient resistance) at Nishtar and Iqbal towns with 92.23% and 94.00% mortalities (X^2=8.855, P=0.03)1 (Table 1 & Figure 2).

Table 1: Summary of results on susceptibility tests applied *Ae. aegypti* with 0.05% Lambda-cyhalothrin, 0.05% Deltamethrin, 0.75% Permethrin, 4% DDT, 0.1% Bendiocarb and 5% Malathion at different localities of district Lahore

	Deltamethrin			Lambdacyhalothrin			Permethrin			DDT			Malathion			Bendiocarb		
Localities	No. of females tested	Corrected Mortality %	Status**	No. of females tested	Corrected Mortality %	Status**	No. of females tested	Corrected Mortality %	Status**	No. of females tested	Corrected Mortality %	Status**	No. of females tested	Corrected Mortality %	Status**	No. of females tested	Corrected Mortality %	Status**
Smanbd twn	108	69.44	R	108	82.41	R	110	58.18	R	110	14.54	R	107	100.00	S	101	98.01	S
Nishtar twn	102	80.39	R	102	68.63	R	102	70.59	R	102	07.84	R	100	100.00	S	103	92.23	RPC
Ravi twn	103	78.64	R	106	78.30	R	105	81.05	R	105	22.86	R	100	100.00	S	104	87.50	R
Iqbal town	100	77.00	R	102	70.58	R	102	79.41	R	102	17.64	R	102	100.00	S	100	94.00	RPC
Chi squared*		X^2=4.087 df=3 P=0.252			X^2=5.81 df=3 P=0.121			X^2=17.43 df=3 P=0.001			X^2=11.77 df=3 P=0.008						X^2=8.855 df=3 P=0.031	

Twn= town, Susceptibility S= 98-100% Resistance possible to be confirmed RPC = 90-97% Resistance R= 0-89%
Level of Significance P<0.05, Non- Significance P>0.05

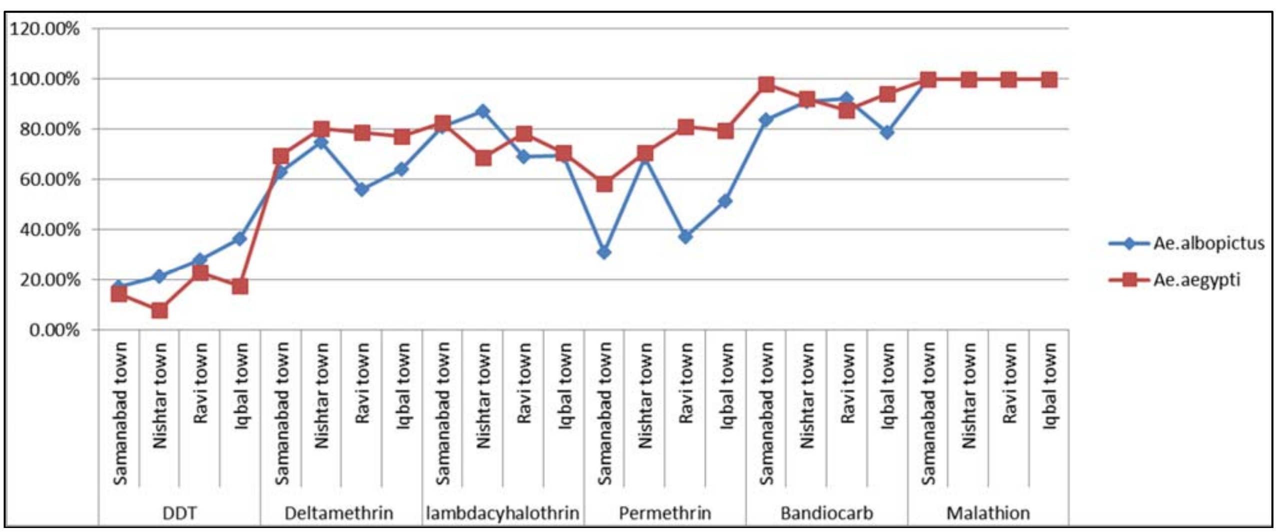

Fig 2: Showing the susceptibility test result of *Ae. Aegypti* and *Ae. albopictus* against various chemical at different localities at Lahore.

3.2 Variability in susceptibility status of *Aedes albopictus* to varied insecticides

Ae. albopictus species were exposed to diagnostic doses of various insecticides showed a wide range of physiological response at different localities of Lahore. *Ae. albopictus* was observed resistance against deltamethrin, lambda-cyhalothrin, and DDT at all localities including Samanabad, Nishtar, Ravi and Iqbal towns (Table 2)

Against deltamethrin 62.75%, 75.00%, 55.88% and 64.00% mortalities in *Aedes aegypti* were observed at Samanabad, Nishtar, Ravi and Iqbal towns respectively (X^2=7.813, P=0.05). Per cent mortality in *Ae. Albopictus* against 0.05%

Lambdacyhalothrin at Samanabad, Nishtar, Ravi and Iqbal towns were 81.00%, 87.00%, 69.23% and 69.64% respectively (X^2=13.22, P=0.004). Mortalities against permethrin at all localities including Samanabad, Nishtar, Ravi and Iqbal towns were 31.00%, 68.51%, 37.00% and 51.32% (X^2=35.15, P=0.000). Against 4% DDT at Samanabad, Nishtar, Ravi and Iqbal towns were 17.00%, 21.30%, 28.00% and 36.28% respectively (X^2=11.90, P=0.008).

Ae. albopictus was observed susceptible against malathion at all localities including Samanabad, Nishtar, Ravi and Iqbal towns with 100% mortality. *Ae. albopictus* was found resistant to 0.1% bendiocarb with 98.01% mortality at Samanabad town and 78.84% mortality at Iqbal town. *Ae. Albopictus* showed resistance possible to be confirmed or incipient resistance status at Nishtar and Ravi towns with 91.00% and 92.07% mortalities respectively (X^2=7.139, P=0.068 (Table 2 & Figure 2).

Table 2: Summary of results on susceptibility tests applied *Ae. albopictus* with 0.05% Lambda-cyhalothrin, 0.05% Deltamethrin, 0.75% Permethrin, 4% DDT, 0.1% Bendiocarb and 5% Malathion at different localities of district Lahore

	Deltamethrin			**Lambdacyhalothrin**			**Permethrin**			**DDT**			**Malathion**			**Bendiocarb**		
Localities	**No. of females tested**	**Corrected Mortality %**	**Status****	**No. of females tested**	**Corrected Mortality %**	**Status****	**No. of females tested**	**Corrected Mortality %**	**Status****	**No. of females tested**	**Corrected Mortality %**	**Status****	**No. of females tested**	**Corrected Mortality %**	**Status****	**No. of females tested**	**Corrected Mortality %**	**Status****
Smanbd twn	102	62.75	R	100	81.00	R	100	31.00	R	100	17.00	R	108	100.00	S	103	83.50	R
Nishtar twn	104	75.00	R	100	87.00	R	108	68.51	R	108	21.30	R	101	100.00	S	100	91.00	RPC
Ravi twn	102	55.88	R	104	69.23	R	100	37.00	R	100	28.00	R	100	100.00	S	101	92.07	RPC
Iqbal town	100	64.00	R	112	69.64	R	113	51.32	R	113	36.28	R	110	100.00	S	104	78.84	R
Chi squared*		X^2=7.813 df=3 P =0.05			X^2=13.22 df=3 **P=0.004**			X^2=35.15 df=3 **P=0.000**			X^2=11.90 df=3 **P=0.008**						X^2=7.139 df=3 P=0.068	

Susceptibility S= 98-100% Resistance possible to be confirmed RPC = 90-97% Resistance R= 0-89%

Level of Significance *P*<0.05, Non- Significance *P*>0.05

4. Discussion

Susceptibility tests applied on two species, *Ae. aegypti* and *Ae. albopictus* of different localities of district Lahore showed that both species were resistant to 4% DDT, 0.05% Deltamethrin, 0.05%, Lambdacyhalothrin and 0.75% Permethrin at all towns. Similar results were observed in studies conducted on insecticide resistance in *Aedes aegypti* which were collected from slum area of Misri Shah, Lahore and tested for resistance to deltamethrin and cypermethrin [3]. This resistance factor may be the result of irrational use of pyrethroids through untrained pesticides applicators since last 15 years in Punjab especially in Lahore, because proper dengue control program carrying trained human resource just started few years back [10]. Similarly, Pyrethroids were also found resistant in *Ae. albopictus* collected from agricultural areas of Lahore, Sargodha and Faisalabad [23]. One important threat that may compromise future control efforts is the potential for cross resistance between organophosphates and pyrethroids [24]. In current study *Ae. aegypti* & *Ae. albopictus* showed high degree of resistance against DDT. Similarly, *Ae. albopictus* & *Ae. aegypti* were also reported resistant against DDT in India (Koderma), Benin, Cameroon and Malaysia [25-28, 11]. Whereas in Santiago, Mexico *Ae. aegypti* was also reported resistant to deltamethrin [29, 30]. Correspondingly, moderate to high resistance was also reported in *Ae. aegypti* populations against lambda-cyhalothrin and permethrin from Colombia [14]. In Nigeria and Thailand, *Ae. aegypti* also exhibited resistance or incipient to deltamethrin and permethrin [31-33]. Resistance to deltamethrin was also suspected in *Ae. albopictus* from Yaoundé with 83% mortality and found with incipient resistance (97 - 80% mortality) to permethrin [27]. Similar results were found in Malaysia, to Permethrin with mortality < 80% [28, 11].

Insecticide susceptibility of *Ae. aegypti* populations from Cape Verde Archipelago and Senegal, the Dakar strain was resistant to 0.05% deltamethrin and 0.05% lambda-cyhalothrin with mortality 81% and % 94.5% respectively but susceptible only to 0.75% Permethrin [34]. In Jharkhand (Koderma), India *Ae. aegypti* was resistant to deltamethrin ranged from 98.26 to 100% and lambda-cyhalothrin ranged from 97.33% to 100% [25]. So this world wide resistance development against pyrethroids may be the result of its massive use in vector control programs and cross resistance of DDT [7, 24].

Our findings showed that *Ae. aegypti* is susceptible to 0.1% bendiocarb with maximum mortality (98.01%) while *Ae. Albopictus* is developing resistance to 0.1% bendiocarb (carbamate) with 83.50% to 92.07% mortalities range. In similar study, *Aedes albopictus* collected from agricultural areas of Lahore, Sargodha and Faisalabad exhibited moderate level of resistance to carbamates [23]. Resistance development in *Ae. albopictus* compared to *Ae. aegypti* may be due to its exophilic behaviour [23] Although bendiocarb was found resistance in *Ae. aegypti* from Thailand [11], but *Ae. aegypti* populations in five small towns surrounding the city of Merida, Mexico was found susceptible against bendiocarb [29].

In current study both species (*Ae. aegypti* and *Ae. albopictus*) were found susceptible against malathion with 100% mortality at all surveyed localities (towns) of Lahore. While before *Aedes albopictus* collected from agricultural areas of Lahore, Sargodha and Faisalabad tested for resistance revealed high level of resistance to Organophosphates [23]. This reversal of resistance phenomena needs more study to find root cause, but one of major factor might be that malathion is not being used about last 20 years in study area [10]. Aedes populations in two suburban residential areas in Kampar town, Perak, Malaysia and Santiago were also observed susceptible to malathion [35, 30].

5. Conclusions
Our findings showed *Ae. aegypti and Ae. albopictus* have developed resistance against pyrethroids (0.05% deltamethrin, 0.05%, lambdacyhalothrin and 0.75% Permethrin) in Lahore Pakistan. Malathion 5% was found most effective and suitable insecticide against dengue vectors (*Aedes aegypti and Aedes albopictus*) with 100% mortality. Usefulness of Malathion and Bendiocarb insecticide alternative choice coupled with integrated innovative mosquito surveillance and other vector control management (IVM) programs and interventions should be established and sustained to prevent, control potential arboviral disease threats and outbreaks over time.

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