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Dr. Ali Arslan
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Dr. Muhammad Uzair Mukhtar
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Shumaila Mushtaq
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Arqam Bakhtiyar Zakki
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Muhammad Hammad
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Adil Bhatti
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Correspondence:
Dr. Ali Arslan
Medical Entomology & Disease Vector Control (MEDVC), Health Services Academy, Islamabad, Pakistan.

Comparison of Susceptibility Status of laboratory and field populations of *Aedes aegypti* against Temephos in Rawalpindi

Ali Arslan, Muhammad Uzair Mukhtar, Shumaila Mushtaq, Arqam Bakhtiyar Zakki, Muhammad Hammad, Adil Bhatti

Abstract
In Pakistan, application of the larvicide temephos to the aquatic breeding sites of *Aedes aegypti* is a key part of the dengue control strategy. Throughout Pakistan no data is available on the susceptibility status of *Ae. aegypti* to temephos. The aim of the study was to examine the susceptibility status of *Ae. aegypti* against temephos and compare and estimate the resistance ratios of both laboratory and field populations on the basis of median lethal concentration LC_{50} and LC_{95}. Larval bioassays were carried out according to WHO method on the field population of *Ae. aegypti* from three different localities (Rawal Town, Pothohar Town and Cantonment) of Rawalpindi. The laboratory reared susceptible strain of *Ae. aegypti* provided by the insectarium of Medical Entomology and Disease Vector Control (MEDVC) department was used as reference for comparison. The LC_{50} values of all field populations of *Ae. aegypti* were recorded as 0.024 mg/L for Rawal Town, 0.019 mg/L for Pothohar Town and 0.025 mg/L for Cantonement showing resistance ratios RR_{50} of 6.32, 5 and 6.58 respectively. Similarly LC_{95} values were recorded as 0.26 mg/L for Rawal Town, 0.23 mg/L for Pothohar Town and 0.25 mg/L for Cantonement showing resistance ratios RR_{95} of 9.29, 8.21 and 8.93 respectively. Our results indicated that all the field populations of *Ae. aegypti* were resistant to temephos, compromising its field effectiveness. This comparative data of resistance ratios RR_{50} and RR_{95} on the temephos susceptibility of *Ae. aegypti* could help public health services to design more effective vector control measures.

Keywords: *Aedes aegypti*, Temephos, Susceptibility, Larvicide resistance

1. Introduction
Dengue is an arboviral disease. It is caused by any one of the four serotypes of Dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4). These four distinct serotypes are closely related to each other [1]. It is transmitted by the bites of mosquitoes of the genus *Aedes* and mostly by the species *Aedes aegypti* and *Ae. albopictus*. In the last 50 years, there is a 30 fold increase in the incidence of this disease. In the past decade, it has expanded from urban to rural areas [2].
According to World Health Organization (WHO), there are currently 50-100 million cases of dengue every year. Case fatality rate of dengue is about 2.5% [1].
The first confirmed outbreak of dengue fever (DF) was reported from Karachi in 1994. The epidemics occurred continuously since 2005 in Karachi [3]. There were about 3305 confirmed cases of DF in Karachi in 2010 [4]. Since 1994 the disease remained endemic in the southern parts of Sindh and Baluchistan however from 2004-5, dengue has spread across the country [5]. Pakistan is now in endemic situation for DF in Khyber Pakhtun Khaw (KPK), Punjab and Sindh [3]. The Swat district of KPK province in Pakistan has experienced an outbreak of DF in 2013. Since 7 August 2013, suspected cases reached to a level of 6376 which also included 23 deaths having the death rate of about 0.36% [6]. The outbreaks of DF continued to increase after the floods in 2010 [7]. This epidemic situation has resulted in the 16580 confirmed cases of DF with 257 deaths in Lahore. In rest of the country there were approximately 5000 cases and 60 deaths [3]. In 2011, there were about 252935 suspected and 17057 laboratory confirmed cases with 219 deaths in Pakistan [6]. Rawalpindi is facing an epidemic of dengue having the highest number of cases after Lahore in Punjab. It has the border areas with Islamabad and the Swat district of Khyber Pakhtun Khaw (KPK). It is facing more threat to dengue due to huge breeding places and increased number of cases in Islamabad and the recent outbreak of dengue in the Swat district of KPK.

Ae. aegypti is highly anthropophilic with markedly endophilic and endophagic behaviors; these characteristics are directly related to its high efficiency as a disease vector [8, 9]. In the absence of a vaccine or effective therapeutic medications, the best strategy to control and prevent the disease is to control the vector populations [9]. Control strategies are based on community participation which includes the removal of water from the containers in domestic and peri-domestic areas and environmental sanitation. These actions may also be supplemented with other vector control interventions such as the chemical control. Many dengue vector control interventions target the immature stages of the mosquito, which breed in artificial containers in close proximity to human dwellings. The most widely used method for controlling immature *Ae. aegypti* is the periodic treatment of actual and potential breeding sites with chemical larvicides. The organophosphate (OP) insecticide temephos is commonly used to control immature dengue vectors due to its cost-effectiveness and community acceptance [8, 10, and 11]. The application of chemical insecticides is a major tool in minimizing the transmission of DF in most Latin American countries [12, 13]. Resistance to this temephos has also been recorded in *Ae. aegypti* in Asia including Cambodia [14], Thailand [15, 16], and Malaysia [17] and South America [18, 19].

Temephos is the most widely used larvicide in our country and in Rawalpindi. However, its use is strictly subjected to available information of resistance to the local *Ae. aegypti* populations. This changing trend of resistance in target vector species needs to be properly monitored and assessed against the most widely used larvicide temephos. Current study was carried out to examine the susceptibility status of *Ae. aegypti* mosquito larvae against temephos in field populations of Rawalpindi and compare and estimate the resistance ratios of both laboratory and field populations on the basis of median lethal concentration LC_{50} and LC_{95}.

2. Materials and Methods

2.1 Study Time Period

The study time period was from June 2014 to December 2014.

2.2 Mosquito Sampling

The study was conducted in three different localities of Rawalpindi i.e. Rawal Town, Pothohar Town and Cantonment, based on the previous dengue endemic areas and areas having the insecticidal selection pressure. The larvae were collected from potential breeding sites of *Ae.* mosquito. For each sampling site, larvae from random breeding places were collected and stored in plastic boxes. These field collected larvae were then transferred to Medical Entomology and Disease Vector Control (MEDVC) insectarium of Health Services Academy, Islamabad for rearing to the adult stage. Adult female mosquitoes of *Ae. aegypti* were identified by using the pictorial keys of Leopoldo M. Rueda [20].

2.3 Mosquito Rearing

Field collected mosquitoes were reared to generation F1 for carrying out the larval bioassays. Mosquito populations were maintained at insectaries conditions (27°C $\pm$ 2°C; relative humidity 80% $\pm$ 10%) and females were fed on albino mice to complete their gonotrophic cycle [21]. While the susceptible reference strain of *Ae. aegypti* were provided by the Medical Entomology and Disease Vector Control (MEDVC) insectarium of Health Services Academy.

2.4 Preparation of Stock Solution

1% stock solution was prepared by mixing 200 mg of active ingredient of temephos (0.4 ml of 50% E.C Temeguard^R) in 20 ml distilled water. Subsequent serial dilutions were made as per WHO guidelines. Wide range of concentrations (0.0001-0.5 ppm) was made as per WHO guidelines. A total of 100 larvae were exposed to each concentration of temephos [22].

2.5 Larval Bioassays

Larval bioassay was conducted separately on both laboratory reared and field collected larvae. Batches of 25 late third or early fourth instar larvae were transferred by means of droppers to small disposable test cups, each containing 99 ml of water and 1 ml of temephos concentration. Small, unhealthy or damaged larvae were removed and replaced [21]. Each bioassay included a control group which received 1 mL of ethanol. Tests were run at 27°C ± 2°C, and mortality was assessed after 24 h of insecticide exposure [22, 23].

Data from all the replicates was pooled and subjected to computer software programs (Minitab v 16.0) for analysis. LC_{50} and LC_{95} values were calculated from a log dosage–probit mortality regression line. Bioassays were repeated three times, using new solutions or suspensions and different batches of larvae each time. Resistance ratios (RR_{50} and RR_{95}) of each population compared to the laboratory strain of *Ae. aegypti* (used as reference) were calculated as follows: RR_{50} = LC_{50} assay/LC_{50} reference; RR_{95} = LC_{95} assay/LC_{95} reference [23]. If the control mortality is between 5% and 20%, the mortalities of treated groups are corrected according to Abbott's formula [24].

A mosquito population was considered susceptible when RR_{50} was less than 2, less resistant when RR_{50} was between 2 and 5, moderately resistant when RR_{50} was between 5 to 10 and highly resistant when RR_{50} was over 10 [25].

3. Results

Percentage mortality of laboratory and field populations of *Ae. aegypti* larvae at various concentrations (mg/L) after 24 hours exposure is shown in table 1. The LC_{50} value of laboratory reference strain was recorded as 0.0038mg/L while the LC_{50} values from field populations of Rawal Town, Pothohar Town and Cantonment were recorded as 0.024 mg/L, 0.019 mg/L and 0.025 mg/L respectively. The LC_{50} values from the field populations of Rawal Town, Pothohar Town and Cantonement were far more than the LC_{50} value of laboratory susceptible reference strain. The LC_{50} values of both laboratory and field populations were pooled for analysis to estimate the resistance ratios RR_{50}. The resistance ratio RR_{50} was recorded as 6.32 for Rawal Town, 5 for Pothohar Town and 6.58 for Cantonement showing moderate resistance according to the criteria of Mazzari & Georghiou [25].

The LC_{95} value of laboratory strain was recorded as 0.028 mg/L while the LC_{95} values from field populations of Rawal Town, Pothohar Town and Cantonement were recorded as 0.26 mg/L, 0.23 mg/L and 0.25 mg/L respectively. The LC_{95} values from the field populations of Rawal Town, Pothohar Town and Cantonement were far more than the LC_{95} value of laboratory reference strain. The LC_{95} values of both laboratory and field populations were pooled for analysis to estimate the resistance ratios RR_{95}. The resistance ratio RR_{95} was recorded as 9.29 for Rawal Town, 8.21 for Pothohar Town and 8.93 for Cantonement showing moderate resistance according to the criteria of Mazzari & Georghiou [25].

The estimated LC_{50} and LC_{95} of temephos for the laboratory susceptible reference strain and the three field populations of *Ae. aegypti* are presented in Table 2 where data of temephos lethal concentrations and resistance ratios are estimated. By analyzing the lethal concentrations, all the three field

populations differed from the laboratory susceptible reference strain and also among each other. The results suggested that all the three field populations were moderately resistant to temephos.

Table 1: Percentage mortality of laboratory and field populations of *Aedes aegypti* larvae at various concentrations (mg/L) after 24 hours exposure:

Sample		No. of dead larvae at various conc. (mg/L) post exposure 24 hours							
	Control	0.0001	0.0005	0.001	0.005	0.01	0.05	0.1	0.5
Laboratory Population	13/600	0	0	59	114	172	225	225	225
Mortality (%)	2.17	0	0	26.22	50.67	76.44	100	100	100
Rawal Town Population	8/600	0	0	11	32	62	99	225	225
Mortality (%)	1.33	0	0	4.89	14.22	27.55	44	100	100
Pothohar Town Population	5/600	0	0	14	41	80	116	225	225
Mortality (%)	0.83	0	0	6.22	18.22	35.55	51.55	100	100
Cantonement Population	7/600	0	0	59	114	172	225	225	225
Mortality (%)	1.17	0	0	4	12.44	25.78	44	100	100

Table 2: Estimated lethal concentrations of temephos (Confidence interval) and resistance ratios based on the laboratory susceptible reference strain

Sample	Regression line	Pearson χ^2 goodness of fit	LC_{50} (95% CI)	RR_{50}	LC_{95} (95% CI)	RR_{95}
Aedes aegypti						
Laboratory Population	0.82±0.037	53.02	0.0038 (0.0034-0.0042)	1	0.028 (0.023-0.035)	1
Rawal Town Population	0.69±0.029	136.34	0.024 (0.021-0.027)	6.32	0.26 (0.21-0.34)	9.29
Pothohar Town Population	0.66±0.028	111.7	0.019 (0.017-0.022)	5	0.23 (0.18-0.30)	8.21
Cantonment Population	0.72±0.031	132.33	0.025 (0.022-0.028)	6.58	0.25 (0.20-0.32)	8.93

LC_{50}: Lethal concentration which kills 50% of population expressed in mg/L
LC_{95}: Lethal concentration which kills 95% of population expressed in mg/L
CI: Confidence Interval
RR: Resistance ratio
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
$P > 0.05$ suggests a well-fitting model
$P < 0.05$ suggests an invalid model population

4. Discussion

This study was carried out to examine the susceptibility status of field populations of *Ae. aegypti* larvae against temephos in three different localities of Rawalpindi city i.e. Rawal Town, Pothohar Town and Cantonement. The larvicide temephos is the most commonly used chemical in Rawalpindi for the suppression of *Ae. aegypti* larvae in dengue control programs. These programs have often been implemented without information on the resistance selection risk posed by temephos in the field. The lack of data on *Ae. aegypti* susceptibility to temephos is a limiting factor for the success of control programs. For this reason, evaluations are often performed using the susceptibility of laboratory colonies as a reference, which do not necessarily reflect the natural variations that can be found among field populations, which has been demonstrated in other studies [26]. The widespread use of temephos in controlling the *Ae. aegypti* larval population has led to resistance in many Latin American countries including Cuba [27], El Salvador [28], Argentina [29], Bolivia [30], Venezuela [31], Peru [32] and Colombia [33]. It is believed that the extent of temephos resistance is underestimated due to under-reporting and lack of surveillance [11].

Our estimations of lethal concentrations LC_{50} of laboratory reference strain of *Ae. aegypti* were almost equal with the lethal concentrations of susceptible Rockefeller strain observed by Marcombe *et al.* [34] (LC_{50} of 0.0037 mg/L); Rodriguez *et al.* [35] (LC50 of 0.00364 mg/L). Our estimations of lethal concentrations LC_{50} of laboratory reference strain of *Ae. aegypti* were lower than the lethal concentrations of susceptible Rockefeller strain observed by Uthai *et al.* [36] (LC_{50} of 0.0060 ppm); Ponlawat *et al.* [37] (LC_{50} of 0.0054 mg/L); and higher than the LC_{50} observed by Andrighetti *et al.* [38] (LC_{50} of 0.0025 ppm); Prophiro *et al.* [39] (LC_{50} of 0.00167 mg/L); Bisset *et al.* [40] (LC_{50} of 0.0012 mg/L); Gomez *et al.* [41] (LC_{50} of 0.003058 mg/L). Our estimations of lethal concentrations LC_{95} were slightly higher than the lethal concentrations observed by Araujo *et al.* [42] (LC_{95} of 0.011 mg/L); Lima *et al.* [43] (LC_{95} of 0.017 mg/L). Our estimations of lethal concentrations LC_{50} of field populations were slightly higher than the LC_{50} of field populations observed by Singh, R. K in Delhi, India [44]. The resistance ratios in all the field populations of Rawalpindi showed moderate to high resistance according to the Criteria of Mazzari & Georghiou [25]. This high resistance pattern observed in the *Ae. aegypti* field populations of Rawalpindi may be associated with the prolonged use of temephos in each locality with different intensities or application frequencies thus subjecting them to different selection pressures with consequent increase in resistance mechanisms in each population.

5. Conclusion

The results of the present study confirm the detection of resistance in field population of *Ae. aegypti* larvae to temephos in Rawalpindi. The findings of temephos resistant larvae of *Ae. aegypti* field populations in Rawalpindi confirm the need for monitoring the susceptibility of these populations as one of the strategies for ensuring the efficacy of control programs for this vector in the area. Detection of resistance could help public health personnel to formulate appropriate alternative steps to ensure the effectiveness of control effort that may accompany with the emerging problems of insecticide resistance. Furthermore, cross resistance or resistance as a result of agricultural uses of insecticides may evolve and adversely impact the options to switch to an alternative method or insecticides for disease control. Our observations could help to guide insecticide-based strategies, although broader monitoring of insecticide resistance in *Aedes* mosquitoes is needed.

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