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LABORATORY STUDY OF PERSISTENCE AND RESIDUAL ACTIVITY OF PYRETHROID AGAINST *ANOPHELES STEPHENSI* AND *AEDES AEGYPTI* (DIPTERA: CULICIDAE) IN PAKISTAN

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Abstract Present study was carried out to test the residual activity of deltamethrin EC 1.5%, K-Othrine EC 1.5% and deltamethrin WP 5% under laboratory conditions on five different surfaces i.e. cement (concrete), mud, plaster, wood and filter paper. Four blocks were prepared for each surface, from which one block was not treated with insecticide to serve as control. The surfaces were attached to the wall and deltamethrin EC 1.5% , K-Othrine EC 1.5% and deltamethrin WP 5% were sprayed using standard Hudson pump. The bioassay test was carried out for evaluation of residual effect of insecticides using WHO standard cones. Result of bioassay test on *aedes aegypti* showed that deltamethrin 1.5% EC had best residual capacity on filter paper indicating 60.90% mortality and for *Anopheles stephensi* plaster surface had best residual effect showing 77.75% mortality after 2 months of Indoor Residual Spraying (IRS) application. For K-Othrine, filter paper had best residual capacity for both mosquito species. Deltamethrin WP 5% depicted different residual effects on different surfaces showing 58.33% mortality on filter paper against *An. stephensi* and *Ae.aegypti* showed 65.72% mortality on mud after 2 months of IRS application. The study also highlights proper knowledge of IRS and its management according to building material that may lead to best strategy, which might reduce vector population up to the desired level.

Key words Deltamethrin, K-Othrine, WP Deltamethrin, concrete, mud, plaster, *Ae aegypti, An stephensi,*

INTRODUCTION

Malarial vector mosquito *Anopheles* transmits malarial pathogen from one host to another by its bite. Globally, there are almost 380 known species of *Anopheles*. In Pakistan, among 24 reported species *An. culicifacies* and *An. stephensi* are two major malaria vectors (Druilhe et al*.,* 2008). Dengue is primarily an urban disease of the tropics, and the viruses that cause it are maintained in a cycle that involves humans. *Ae. aegypti,* a domestic, day-biting mosquito prefers to feed on human blood. Indoor Residual Spraying (IRS) is one of very good and efficient method for the control of different species of vector mosquitoes. The effectiveness and efficiency of this process depends on the application method, resistance of vector, killing ability of insecticide, properly maintained equipment, sufficient training of persons and good financial resources (Rasheed et al*.,* 2012). Pyrethroids are the most common insecticides being used for adult mosquito control. This is due to their short persistence in the environment (Antonious et al*.,* 1997). Present study was conducted to determine the residual effect of different insecticides against *Anopheles* and *Aedes* mosquitoes. The information generated will ensure the pattern of insecticide use for vector control. Data will also be helpful in future insecticide resistance management strategies targeted against malaria vectors in these regions (Tikkar et al*.,* 2011).

MATERIALS AND METHODS

Immature *Ae. aegypti* larvae were collected from breeding sites such as water storage tanks, fountains, pipe leakages, whereas *An. stephensi* larvae were collected mainly from muddy water, from ponds, stagnant water channel and rainwater collections also from artificial containers such as discarded jars, tyres, plastic tubs found in Misri Shah (slum area) in North of Lahore. All the collections were conducted between 17:00-18:30 pm from November 2015- January 2016. Deltamethrin EC 1.5% , K-Othrine EC1.5%, Deltamethrin WP 5% were sprayed by Hudson® X-Pert compression sprayer recommended by WHO for IRS at the rate of 755– 780 ml/min. The duration for spray was set for 30 minutes. The dried blocks were fixed on to the vertical surfaces of the wall. After spraying, all the blocks were collected and stored in the box for further bioassay tests. Five surfaces i.e. cement (concrete), mud, plaster, wood and filter paper were prepared in pans. The blocks were allowed to dry under laboratory conditions. All prepared samples were stored in wooden blocks in laboratory. Four blocks were prepared for each surface containing one cone on each tray, from which one block was untreated to serve as control. Three replicates of each treated surface were carried out. The filter paper (Whatman No.1) was attached to the sprayed surfaces normally and then removed and packed inside the kit for bioassay tests.

At least 12–15 sugar fed active female mosquitoes were aspirated from a mosquito cage into each cone through the hole at the vertical position. Once the mosquitoes have been transferred, the slide unit was closed and the cones were set in an upright position for one hour. At the end of this time, any damaged insects were removed. Mosquitoes were kept in the exposure cone, which were set in a vertical position, for a period of 30 minutes. At the end of the 30 minutes exposure period, the mosquitoes were transferred to the clean cup. Mosquitoes were maintained in the cones for 24 hours (the recovery period). During this time, it was important to keep the cones in a shady, sheltered place free from extremes of temperature (an insectary is ideal). Temperature and humidity were also recorded during the recovery period. At the end of recovery period (i.e. 24 hours post-exposure), the number of dead mosquitoes was counted and recorded. An adult mosquito was considered to be alive if it is able to fly, regardless of the number of legs remaining. Any knocked-down mosquitoes, whether or not they have lost legs or wings, were considered moribund and are counted as dead. Contact bioassay tests were carried out on day 1, 5, 15, 30, 45, and 60 after applications. Relative humidity and temperature of the laboratory were recorded during the bioassay experiments that were 75% and 27^0 C, respectively (WHO, 2010). Three replicates of the experiment were calculated for % mortality in treated and control cones. Percentage mortality was recorded after 24-hours recovery period on the standard form. Data were analyzed statistically for Mean, Standard deviations.

RESULTS

Table 1 shows the results of bioassay tests on *Ae. aegypti* and *An. stephensi*. For deltamethrin EC 1.5%, the best residual capacity is for filter paper after 60 days exposure showing 59.90% mortality followed by cement (concrete), mud and plaster with % mortality 52%, 49% and 45% against *Ae. aegypti*. Results of present study indicate that different insecticides have different results on different surfaces. K-Othrine is more effective on filter paper surface of *An. stephensi* and *Ae. aegypti* as compared to other surfaces and insecticides. The results of laboratory trials also revealed that deltamethrin WP 5% was more effective against *Ae. aegypti* as compared to *An. stephensi* on filter paper, plaster and mud surface. There was no significant difference between mortalities after first and second week after application ($p > 0.05$). There was also a significant difference between mortality after two months.

DISCUSSION

Pyrethroid resistance is very common in *Anopheles* and *Aedes* mosquitoes throughout the world. Rozilawati et al. (2005) studied residual effect of deltamethrin against the *Aedes* and found it effective up to almost 6 weeks. The study also showed that both *An. stephensi* and *Ae. aegypti* were more susceptible on cement (concrete) than wood. Etang et al. (2011) evaluated the efficacy of three different insecticides formulations on different surfaces. According to their study, it is very important to study the residual effects of insecticides. Results of present study indicate that there is variation in residual effects on different surfaces. K-Othrines is more effective on filter paper surface of *An. stephensi* and *Ae. aegypti* as compared to other surfaces and insecticides. The results of laboratory trials also indicate that deltamethrin was more effective against *Ae. aegypti* mosquitoes as compared to *An. stephensi* on filter paper surface instead of other surfaces like plaster, wood, cement (concrete) and mud surface. Deltamethrin (WP) was more effective on filter paper against *Ae. aegypti* and on plaster surface of *An. stephensi.* Among all these surfaces cement and wood have least performance. It was concluded that surfaces of filter paper and plaster are best in effectiveness against K-Othrin materials the residual effect according to building material should be kept in mind to effectively control disease vector population. The results of our study are also in accordance with previous investigations by different scientists. Rozilawati et al. (2005) studied residual effect of deltamethrin against the *Aedes* and found it effective up to almost 6 weeks.

Table1. Results of % mortality on different surfaces with deltamethrin EC 1.5%, K-Othrine EC 1.5% and deltamethrin WP 5% under laboratory conditions against *Ae. aegypti* and *An. stephensi*

		Aedes aegypti					***Anopheles***				
	Days	**Plaster**	**Mud**	**Cement**	**Wood**	**Filter paper**	**Plaster**	**Mud**	**Cement**	**Wood**	**Filter paper**
Deltamethrin	**1**	94.41 ±3.10	77.75 ±6.30	88.51 ± 6.24	74.29 ±5.80	91.18 ±3.8	83.33 ±5.47	73.54 ±5.50	59.72 ±7.22	68.55 ±6.90	74.29 ±7.55
	5	91.67 ±3.94	70.54 ±6.75	79.95 ± 5.93	77.12 ±6.00	79.95 ±5.99	80.5 ±6.1	72.16 ±4.98	62.81 ±6.66	68.83 ±6.65	73.54 ±5.54
	15	85.69 ±6.64	65.72 ±7.50	63.83 ± 7.01	71.37 ±6.8	86.08 ±6.2	71.37 ±6.8	62.81 ±6.66	69.41 ±7.4	51.41 ±7.15	80.5 ±5.40
	30	63.83 ±6.86	58.81 ±7.10	64.72 ±7.68	58.33 ±6.22	70.54 ±5.8	66.66 ±9.00	52.75 ±7.41	58.81 ±7.10	48.45 ±6.65	70.54 ±6.00
	45	54.54 ±6.22	65.72 ±7.50	55.45 ±6.10	55.5 ±6.80	69.42 ±6.75	60.54 ±7.20	61.72 ±7.22	58.33 ±7.00	47.17 ±6.52	61.08 ±7.22
	60	45.5 ±6.00	49.58 ±6.60	52.5 ±5.65	47.17 ±4.90	59.98 ±7.22	77.75 ±6.31	42.84 ±5.55	42.36 ±5.59	47.17 ±5.85	51.41 ±6.13

		Aedes aegypti					*Anopheles*				
	Days	**Plaster**	**Mud**	**Cement**	**Wood**	**Filter paper**	**Plaster**	**Mud**	**Cement**	**Wood**	**Filter paper**
K-Othrine	**1**	97.17 ±1.80	77.75 ±6.31	85.68 ±50.64	77.12 ±6.31	91.18 ±4.46	86.08 ±5.7	67.63 ±8.88	74.29 ±5.91	79.94 ±6.65	97.08 ±2.38
	5	88.83 ±4.89	70.54 ±6.75	79.94 ±6.1	68.55 ±7.5	79.94 ±4.33	88.83 ±1.32	66.66 ±7.68	65.72 ±7.68	66,66 ±9.00	91.18 ±4.46
	15	82.86 ±5.76	62.81 ±6.66	75 ±7.89	71.37 ±6.8	83.33 ±5.47	91.43 ±4.46	71.37 ±6.8	61.08 ±6.66	48.58 ±6.41	86.08 ±4.1
	30	75 ±5.89	47.08 ±5.12	64.72 ±7.68	47.17 ±6.87	82.36 ±5.76	77.75 ±4.10	63.83 ±6.20	67.63 ±8.75	51.45 ±7.41	76.45 ±5.89
	45	66.63 ±9	57.15 ±7.10	51.45 ±6.60	63.83 ±6.88	61.08 ±7.22	63.63 ±6.46	55.90 ±6.21	52.75 ±7.52	47.16 ±6.62	69.41 ±7.4
	60	58.33 ±5.88	48.58 ±6.48	47.17 ±6.59	50 ±6.62	59.98 ±5.22	58.33 ±7.00	55.5 ±6.21	52.75 ±5.65	47.16 ±6.65	63.83 ±6.64
5% WP Deltamethrin	**1**	91 ±2.90	80.5 ±5.93	82.86 ±5.50	79.94 ±6.88	91.18 ±3.88	88.83 ±6.24	79.36 ±5.93	88.51 ±6.24	74.29 ±5.91	91.43 ±3.26
	5	83 ±5.64	76.45 ±4.00	77.12 ±4.10	74.29 ±5.91	94.25 ±3.13	94.41 ±3.13	75 ±5.85	74.29 ±5.90	77.75 ±4.00	88.18 ±4.5
	15	77.12 ±6.31	65.72 ±7.68	72.16 ±6.10	62.81 ±6.66	83.33 ±5.51	82.86 ±5.76	68.55 ±7.50	58.33 ±7.89	59.98 ±7.45	66.66 ±6.99
	30	72.16 ±5.89	52.91 ±7.52	61.75 ±6.46	69.41 ±7.40	73.54 ±5.10	72.16 ±6.52	52.75 ±6.89	61.72 ±6.40	63.63 ±6.80	82.36 ±5.76
	45	57.54 ±6.52	52.91 ±7.52	54.54 ±7.89	61.08 ±6.30	66.66 ±7.80	63.63 ±6.70	58.81 ±6.69	69.41 ±7.5	55.5 ±6.21	63.83 ±7.68
	60	55.5 ±6.89	65.72 ±6.50	50 ±7.88	41.66 ±8.02	54.24 ±6.85	58.33 ±5.85	50 ±7.45	57.54 ±6.52	50± 7.41	54.24 ±6.86
	60	55.5 ±6.89	65.72 ±6.50	50 ±7.88	41.66 ±8.02	54.24 ±6.85	58.33 ±5.85	50 ±7.45	57.54 ±6.52	50± 7.41	54.24 ±6.86

The study also showed that both *An. stephensi* and *Ae. aegypti* were more susceptible on cement (concrete) than wood. Etang et al. (2011) evaluated the efficacy of three different insecticides formulations on different surfaces. According to their study the insecticides was more effective on mud surface. Mohammad et al. (2011) also studied the effectiveness of deltamethrin and evaluated the relationship between residual activity and persistence of it on different surfaces to control the malarial vectors. Deltamethrin showed its knockdown up to 120 days after its application on the walls used in the experiment against *Ae. aegypti*. Among all other tested surfaces, mud surface showed least performance. The whole study gave us fruitful findings that if we use insecticide for IRS on different building.

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