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Efficacy of a commercial liquid vaporiser (Transfluthrin 0.88% (w/v)) under various room sizes against *Culex quinquefasciatus* Say.

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ABSTRACT
The standard protocol (WHOPES) efficacy testing on flying insect is under Peet Grady Chamber (PGC) of size 6m^3 in closed condition. However, in the real scenario, the efficacy is bound to vary with the room size, ventilation and mode of activity of the vaporising machine etc. To know the role of room size in the efficacy of vaporisers, efficacy studies were carried out with a commercial liquid vaporiser with Transfluthrin 0.88% w/v as active. The heating device used had two different modes of operation *viz.*, low setting and high setting. At low setting, the liquid is expected to emanate slowly compared to high setting. Evaluation was carried out at different settings under various room size *viz.*, 6 m^3, 20 m^3 and 40 m^3 and the results are compared. All the experiments were conducted without ventilation, so that all the chamber ports remained closed throughout the experiment. The time required to knock down 50% and 95% of test population (KT_{50} and KT_{95}), and mortality at 24 hours after exposure were analysed and compared. The results revealed that experiments in 6m^3 at high emanation mode recorded minimum KT_{50} value (10.61 min.) followed by experiments at high emanation mode in 20m^3 room size (15.06 min.). Evaluation at low emanation mode in PGC recorded faster knock than in higher volume rooms with high emanation rate. Efficacy was found to decrease with the increase in room size which was reflected in increase in KT_{50} and KT_{95} values and decrease in mortality at 24 hours of exposure indicating the importance of room volume for the product performance. It is concluded from the present investigation that performance of the products with varying room size should be considered as criteria for efficacy as it reflects the real scenario and to avoid the failure of any household products under field conditions.

Keywords: Efficacy, Liquid vaporiser, Room size, Transfluthrin, *Culex quinquefasciatus*.

1. Introduction
Evaluation of household insecticide formulation such as coils, vaporisers, ambient emanators and aerosol under unventilated room condition of standard size is generally recommended by World Health organisation. Regulatory authorities across the globe follow some specific protocol, where the efficacy is assessed under room conditions to arrive the knockdown time for 50 and 95% of test population within a specific time period, generally within 60 minutes. Normally these formulations are used for minimum of eight hours under household conditions. Moreover, the actual usage pattern of these formulations normally is not in consensus to the condition they are evaluated for regulatory purpose. This leads to failure of performance with the formulation registered successfully. Though the variation in the species susceptibility to the active plays a crucial role in determining the efficacy of the product, the present study is focussed on *Culex quinquefasciatus*, which is quite prevalent in tropics and sub tropics of Asian continent. Though the various factors such as ventilation, airflow, active, temperature and species susceptibility plays role in determining the efficacy of household formulations, this study is designed to demonstrate the role of room size in the determining the efficacy of Liquid vaporiser which is the common household formulation in urban areas. A commercial liquid vaporiser containing Transfluthrin 0.88% w/v was used in rooms of different sizes to assess the variation in its performance.

2. Materials and Methods
2.1 Mosquito Culture: The common brown mosquito, *Culex quinquefasciatus* larvae were reared in the laboratory at the department of Entomology, IIBAT. Dry bread powder and dry fish powder

were used as larval feed. Mosquitoes were maintained at temperature of 28±2 °C and 50–80% relative humidity with 10% sugar solution and water soaked resins as adult food.

2.2 Efficacy Testing

Unblood fed, 3-5 days old adult female mosquitoes were aspirated from breeding cage into a small ventilated plastic container. Later, they were anaesthetized using CO_2 and females were separated in another plastic container and allowed to recover for a minimum of 1 hour before being used for testing. Studies were conducted under three different room size *viz.,* 6 m^3 made of glass, 20 m^3 and 40 m^3 rooms made with aluminum walls and steel floor. A number of 25 female mosquitoes were released in to a cage made of coarse mesh material and the same was used for the test at 6m^3 room size. Four cages containing mosquito species were exposed for single replication. Similarly, 300 (12 cages) and 600 (24 cages) mosquitoes were exposed for room size 20 m^3 and 40 m^3 respectively. The cages containing mosquitoes were hung up in the four corners of the room for study in 6 m^3. For the studies in 20 m^3 and 40 m^3 the cages were hung on the ceiling in such a way that there was air circulation around the cages. All the experiments were conducted under unventilated condition to compare the results from identical situation. Liquid vaporiser were activated outside the room for an hour and then introduced in to the test room.

Fan with 30 cm diameter was used in the room to have uniform distribution of active in the room. Mosquitoes were assessed for knockdown at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, and 60 minutes after the activation of treatment in the room. After 60 minutes, mosquitoes were collected from the cages (both knocked and alive) in a plastic container with sugar solution as food and kept in a room to observe the recovery, if any after 24 hours of treatment.

Experiments were conducted at both low and high emanation modes of the liquid vaporising machines. Weight of the liquid emanated during the experiment was assessed by weighing the liquid vaporiser before and after the experiment. The temperature and relative humidity during experiment in the test rooms ranged from 26.8 to 28.1, 26.0 to 27.8 and 25.8 to 27.1 °C for 6, 20 and 40 m^3 rooms, respectively. Similarly the relative humidity was ranged from 54 to 69, 53 to 72 and 54 to 73%.

The mean amount of emanation was assessed based on the difference between the pre and post weight of the device.

2.3 Statistical Analysis

KT_{50} and KT_{95} were assessed for the knocked down data. The knockdown at each assessment point, KT_{50}, KT_{95} and mortality data was subjected to Analysis of variance to compare the performance using SAS 9.3.

3. Results

The knockdown at each assessment points revealed that there was no knockdown upto 5 minutes after exposure. During 6th and 7th minute of assessment, knockdown was observed only in 6 m^3 room. In 8th minute assessment, knockdown was observed in 20 m^3 (high setting). Treatments 20 m^3 (low setting) and 40 m^3 (low setting) did not record any knockdown till 10 minutes after exposure. At 10 minutes, knockdown in the three room sizes varied from 0.11 to 39.67%. However, maximum knockdown was recorded in 6 m^3 (high setting).

In 20 minutes assessment, 100% knockdown was observed in 6 m^3 (high setting) and 86.33% 20 m^3 (high setting). Treatments 20 m^3 (low setting) and 40 m^3 (low setting) had just started to record knockdown (<3%) at 20 minutes after exposure. At 30 minutes assessment, 20 m^3 (high setting) recorded maximum knockdown (98.8%) followed by 6 m^3 (low setting), 74.67%. During 40 minutes, 100% knockdown was observed in treatment 20 m^3 (High setting) followed by 6m^3 (Low setting), 99%. Treatments 20 and 40 m^3 (Low setting) did not record 100% knockdown within 60 minutes exposure (Table 2, Fig. 1).

Recovery was noticed in knocked down mosquitoes, when observed at 24 hours after treatment. Maximum recovery was observed in treatments 20 m^3 (low setting) and 40 m^3 (low setting). Treatment, 6 m^3 (high setting) recorded the least recovery (6%) (Fig 2).

Analysis of variance revealed that all the treatments were statistically on par upto 6 minutes after treatment. Upto 20 minutes, treatment 6m^3 (High setting) was significantly different from the other treatments. At 30 minutes 6m^3 (High setting) and 20m^3 (high setting) were on par statistically. At 40, 50 and 60 minutes assessment, treatments, 6m^3 (low and high settings), 20 m^3 (high setting) and 40 m^3 (High setting) was on par followed by 20 m^3 (low setting) and 40 m^3 (low setting) (Table 2).

The data were subjected to probit analysis to arrive at KT_{50} and KT_{95} values. Treatment, 6 m^3 (high setting) exhibited the least KT_{50} value (10.61 min.) followed by 20m^3 at high setting (15.06 min.). However 40 m^3 (high setting) recorded KT_{50} of 24.98 min. The KT_{50} value for all the other treatments varied from 24.41 to 80.17 min. Similar trend was observed with KT_{95}. Minimum KT_{95} value of 15.86 min. was exhibited by 6 m^3 (high setting) followed by 20 m^3 at high setting (23.59 min.). The KT_{95} values of all other treatments varied from 37.20 to 217.52 min (Table 1). The mean amount of emanation was 0.070, 0.247, 0.112, 0.244, 0.078, and 0.268 for 6 m^3 (low setting), 6 m^3 (high setting), 20 m^3 (low setting), 20 m^3 (high setting), 40 m^3 (low setting) and 40 m^3 (high setting) respectively.

4. Discussion

The results of the present study reveal the relation between the efficacy of the liquid vaporiser against mosquito and different room sizes. As the vaporiser works as inhalation poison, the different surfaces of the chamber would not have interfered with the knockdown. The rate of knockdown and mortality of mosquitoes is largely dependent on release and degradation rates of active, initial loading dose on substrate and environmental conditions [1]. In the present experiment, though there was continuous dispersion of active emanated by the vaporiser by fan, the knockdown pattern was not similar under the conditions tested. The knockdown got initiated first in the lower size room under higher emanation mode, followed by smaller room under low emanation mode. This underlines the fact that the amount of active present in the room was sufficient enough to cause knockdown in a room of smaller size. Efficacy studies on mosquitoes with various types of devices and revealed that the emanating devices also play a major role for efficacy determination [6]. In the present study, same type of device was used for all the test rooms and hence there were no possibility of device oriented effect on the efficacy. However, the amount of active emanated was found to vary with settings. Variation in the amount released at same setting was also observed in certain treatments which were marginal.

The spatial activity of airborne insecticide is dependent on airflow (i.e., air exchange), wind speed, temperature and humidity within the treated space [2]. Similar observation was made in the present study. Knockdown was higher in smaller room, as the active emanation distributed uniformly by the fan. Whereas in the larger room, though there was uniform active distribution by the fan, the dilution in active owing to room size has reduced the knockdown rate. However greater the air current, greater the insecticidal dispersion over a specified area followed by reduced insecticide

concentration accompanied by dilution of chemical attractants from the human thus reduced host attack by mosquitoes [5].

The emanation rate was almost uniform with respective modes of emanation, irrespective of the room size. The amount of active present in unit area was sufficient in smaller rooms when compared to larger room as there is dilution of active. Caged mosquitoes placed immediately near metofluthrin treated paper strips showed 100% knockdown within 30 minutes and 100% mortality 24-hours post-exposure, while mosquitoes placed 1.5 m away from the strip had slower knockdown and 70% mortality and mosquitoes placed 5m away were unaffected [2]. Studies proved that that high temperature, increases evaporation rate of active ingredient which may improve efficacy but can also lead to faster loss of actives followed by reduced efficacy over time [3]. Similar explanation could be attributed to the post recovery after 24 hours of exposure. Maximum mortality was obtained with smaller rooms with higher mode emanation of liquid vaporiser. The amount of active inhaled should of more concentrated than in the rooms of larger size, where even knockdown was delayed. Minimum mortality was experienced with larger room size.

The higher mortality in smaller room (lower emanation) than the larger room (higher emanation) reveals the importance of the room size. The household formulations are expected to give faster knockdown and hence the knockdown at initial time points (1 to 10 minutes) decides the efficacy of the formulation. In the present experiment, within 10 minutes none of the larger rooms (even with high emanation) recorded knockdown. Cage size and repellent effects are inversely proportional [7]. Findings of the present study concurs with the above as increased efficacy was observed with decrease in room size.

It is necessary to determine the rate at which chemical actives are released from coils and emanators under different environmental conditions in order to determine how much repellent active ingredient will be required for efficacy over time [1]. But the efficacy of household formulations is decided based on the efficacy exhibited by them under unventilated conditions, in a standard room of volume 6 m^3, where the active concentration keeps on increasing with time as there is no ventilation. This is followed under the ambit of registration as well. Therefore household insecticide formulation especially spatial/ ambient emanators miserably fail when put into actual use in the field where household possess larger rooms with ventilation than the 6 m^3, where the products were tested for efficacy. Hence, a data from field studies would be more appropriate than the laboratory data to assess the efficacy of household formulations.

Table 1: KT_{50} and KT_{95} Estimates of Brown House Mosquito, *Culex quinquefasciatus*

Chamber size (m^3)	Device Setting	KT_{50} and KT_{95} Estimates of *Culex quinquefasciatus*		LSD (P = 0.05)
		Knockdown Times	Estimate (Minutes)	
6	Low	50.0	24.41 [b]	8.88
	High	50.0	10.61 [a]	
20	Low	50.0	44.49 [c]	
	High	50.0	15.06 [a]	
40	Low	50.0	80.17 [d]	
	High	50.0	24.98 [b]	
6	Low	95.0	37.20 [a]	23.43
	High	95.0	15.86 [a]	
20	Low	95.0	71.40 [b]	
	High	95.0	23.59 [a]	
40	Low	95.0	217.49 [c]	
	High	95.0	38.55 [a]	

KT_{50} & KT_{95} = Time in minutes for 50% and 95% mortality of Mosquitoes

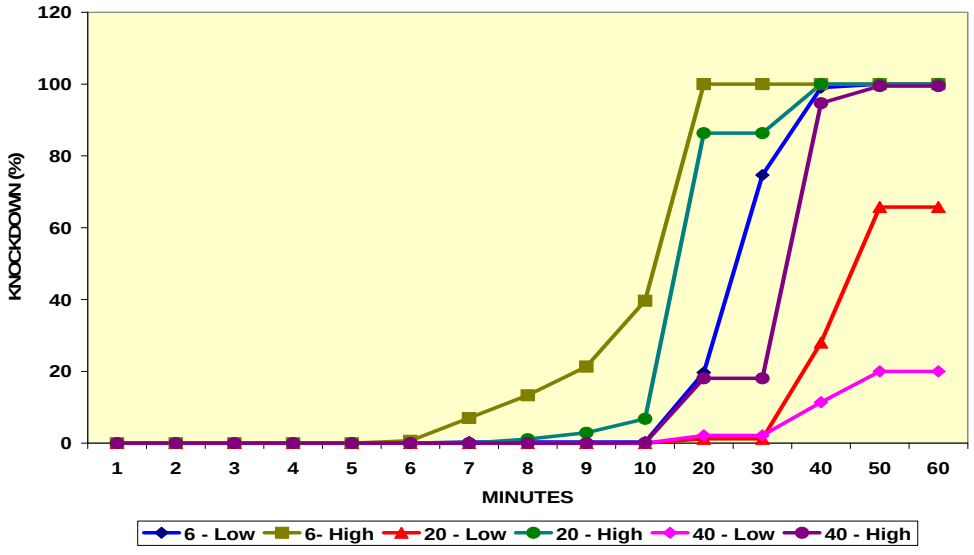

Fig 1: Knockdown pattern of Transfluthrin 0.88% Liquid vaporiser with different room size against *Culex quinquefasciatus*

Table 2: Data on Knockdown and Mortality of Transfluthrin 0.88% Liquid vaporiser against *Culex quinquefasciatus*

Chamber size (m^3)	Device Setting	Mean Knocked Down and Mortality (%) #											
		Upto 5 MAE	6 MAE	7 MAE	8 MAE	9 MAE	10 MAE	20 MAE	30 MAE	40 MAE	50 MAE	60 MAE	Mortality at 24h
6	Low	0	0 [a]	0.33 [b]	0.33 [b]	0.33 [c]	0.33 [c]	19.67 [c]	74.67 [b]	99 [a]	100 [a]	100 [a]	52.33 [c]
	High	0	0.67[a]	7.0 [a]	13.33 [a]	21.33 [a]	39.67 [a]	100 [a]	100 [a]	100 [a]	100 [a]	100 [a]	94.0 [a]
20	Low	0	0[a]	0 [b]	0 [b]	0 [c]	0 [c]	1.22 [d]	10.33 [c]	28.11 [b]	65.78 [b]	90.22 [b]	30.89 [d]
	High	0	0[a]	0 [b]	1.11 [b]	2.89 [b]	6.78 [b]	86.33 [b]	98.78 [a]	100 [a]	100 [a]	100 [a]	77.55 [b]
40	Low	0	0[a]	0 [b]	0 [b]	0 [c]	0 [c]	2.16[d]	6.22 [c]	11.39 [c]	20 [c]	35.78 [c]	29 [d]
	High	0	0 [a]	0 b	0 [b]	0 [c]	0.11 [c]	18.05 [c]	79.61 [b]	94.67 [a]	99.45 [a]	100 [a]	33.50 [d]
Control*	-	0	0 [a]	0 [b]	0 [b]	0 [c]	0 [c]	0 [d]	0 [c]	0 [d]	0 [d]	0 [d]	4.82 [e]
F Probability	-	-	0.46	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD 5 %	-	-	0.76	0.38	1.44	2.17	2.17	4.54	14.36	6.26	13.43	5.74	6.58

Mean of three replicates, * Mean of control from all the three chambers, MAE = Minutes after Exposure, Values followed by similar alphabet are on par statistically

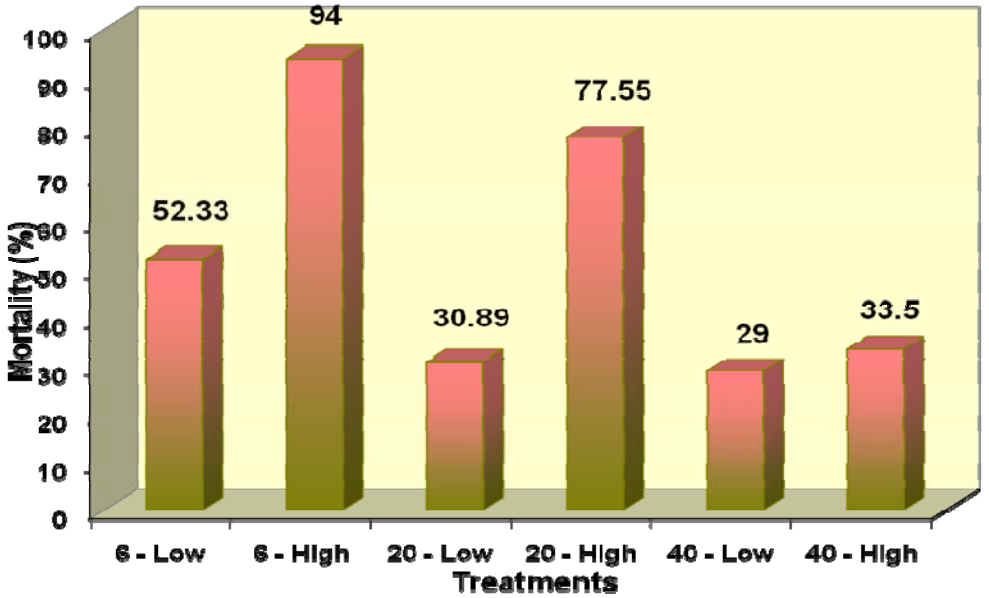

Fig 2: Mortality of Transfluthrin 0.88% Liquid vaporiser in different room size against *Culex quinquefasciatus*

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