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Chlorfenapyr: Irritant effect compared to other insecticides and its intrinsic toxicity in multiple-insecticide-susceptible and -resistant *Anopheles stephensi* (Diptera: Culicidae)

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ABSTRACT

Background & objectives: For effective management of vector resistance there is a need for new insecticide molecules with novel modes of action. For desired toxic effect of an insecticide, apart from other behavioural aspects, toxicity and chemical nature of the molecule are important that may cause irritability in the mosquito to the insecticide affecting the uptake. In this study, a pyrrole class insecticide, chlorfenapyr (a late acting insecticide) was tested for its irritability against multiple-insecticide-susceptible and -resistant strains of *Anopheles stephensi* Liston 1901 (Diptera: Culicidae).

Methods: Studies were conducted to assess the irritability due to chlorfenapyr, DDT, malathion, deltamethrin and permethrin and intrinsic toxicity of chlorfenapyr in multiple-insecticide-susceptible and -resistant laboratory strains of *An. stephensi* following standard WHO methods.

Results: Chlorfenapyr molecule has shown least irritant effect against susceptible and resistant strains among all the insecticides tested allowing more landing time to the vector species on the impregnated surfaces to pick-up lethal dose.

Conclusion: Chlorfenapyr could be an ideal insecticide for management of multiple-insecticide-resistance including pyrethroids.

Key words *Anopheles stephensi;* chlorfenapyr; intrinsic toxicity; irritability

INTRODUCTION

An ideal residual insecticide for the control of mosquitoes should possess certain properties such as effective intrinsic toxicity, chemical and physical properties to facilitate good uptake of insecticide upon contact. Further, the formulations for application in the field should be toxic to target mosquito species at low dosages with ease of application, low volatility, stability of the sprayed residues, minimum irritability, and low mammalian toxicity (including to non-target species). However, the net mortality effect of insecticide on mosquitoes depends on the irritability and resting behaviour of mosquitoes.

Irritability in mosquitoes is a response to external stimulus, which produces discomfort resulting in changes in the resting and contact behaviour[1]. Thus, an insecticide molecule that is least irritant and allows sufficient contact of the mosquito on sprayed surface which will cause lethal effect is ideal for indoor residual spray (IRS).

Different species of mosquitoes exhibit different levels of irritation to residual insecticides[2]. Some species get irritated immediately after contact with the insecticide due to the intrinsic chemical properties. It is also possible that strains of the same species or of different species differ in irritability to some insecticide classes or insecticides of same class. Generally, pyrethroids exhibit high irritancy effect, especially to the type I pyrethroids[3]. According to WHO guidelines for testing and evaluation of insecticides[4], the irritant effect and intrinsic toxicity of an insecticide should be considered before its introduction to IRS, as irritant effect could vary the tarsal contact time with the treated surface affecting the uptake of the insecticide.

Previously, we have conducted a phase I study of chlorfenapyr for the determination of diagnostic dosage, assessment of residual activity on different substrates, cross-resistance to different classes of insecticides and potentiation studies using piperonyl butoxide against

Anopheles species[5]. The present study was conducted to assess the irritability effect of chlorfenapyr in comparison to other insecticides and its intrinsic toxicity using multiple-insecticide-susceptible and -resistant laboratory strains of *Anopheles stephensi* Liston 1901 (Diptera: Culicidae).

MATERIAL & METHODS

Mosquito strains

The following two strains of *An. stephensi* were used in this study.

Anopheles stephensi Nadiad: Laboratory-reared DDT-malathion-deltamethrin-susceptible strain collected from Nadiad, Gujarat, India established in 2009 (% mortality during the study DDT–99, malathion–100 and deltamethrin–100).

Anopheles stephensi Goa: Field-collected DDT-malathion-deltamethrin-resistant strain collected from Goa and established in 2009 (% mortality during the study DDT–21, malathion–46.5 and deltamethrin–79.4).

Above mosquito strains were colonized at the insectarium of National Institute of Malaria Research, New Delhi, India. Insecticide susceptibility status of different strains was ascertained quarterly following WHO method[4]. All the tests were conducted during May 2011 to October 2013.

Irritability studies were conducted by using WHO diagnostic dosage insecticide impregnated papers procured from Vector Control Research Unit, Universiti Sains Malaysia, Malaysia. Intrinsic toxicity of chlorfenapyr was determined using technical grade insecticide (99.3%), received gratis from M/s. BASF, Basel, Switzerland.

Irritability test

Irritability studies were conducted using the WHO cone method[4]. Insecticide-susceptible and -resistant laboratory strains of *An. stephensi* were exposed to DDT (4%), malathion (5%), deltamethrin (0.05%), chlorfenapyr (5%), and permethrin (0.75%) on impregnated papers and respective insecticide controls. Permethrin is considered as positive control for the irritability studies. Experiments were conducted in the laboratory maintained at 27±2°C temperature and 75±5% relative humidity. For each test 50, 3–5 day-old non-blood-fed female *An. stephensi* mosquitoes were used. Observations were made on individual mosquito by introducing single mosquito into WHO polyvinylchloride (PVC) cone placed on an insecticide impregnated paper on a modulated acrylic surface with an inclined plane of 45°. Simultaneously, tests were conducted for respective insecticide control paper. Mosquito was carefully introduced into the WHO cone with an aspirator through the orifice and was closed with PVC plug. After a settling period of 60 sec, observations were made to record the times for first landing (T1) and next take-off (T2) of the mosquito and the difference was recorded to represent the flight time (T2–T1). The data collected from all the exposed mosquitoes were then grouped by class-intervals of flight times (0–1, >1–2, >2–4…>16–32 min), and cumulative frequencies were used to calculate the time to take-off for 50% (FT_{50}) and 95% (FT_{95}) of the exposed mosquitoes by log-probit regression analysis using PASW statistics 18 (SPSS Inc., Chicago, USA). The FT_{50} and FT_{95} values were also determined for organochlorine control (OCC), organophosphate control (OPC) and pyrethroid control (PYC), to determine the mean of the maximum contact time with the paper. Data that registered no take-off at least once during the exposure to insecticide impregnated papers within the determined mean maximum contact time with control paper were excluded.

Topical application

The intrinsic toxicity was determined using the WHO topical application method[4]. Solutions of different concentrations of chlorfenapyr (0.5, 1, 2, 5, 10, 20 and 50 ppm) were prepared in acetone. A total of 50, 2–5 day-old sugar-fed female mosquitoes were weighed to determine the average weight. Two batches of 25 mosquitoes were used for each test. A batch of 25 female mosquitoes was anesthetized with regulated flow of CO_2 for 20 to 40 sec in an air tight plastic box and allowed 30 sec standby before deposition of insecticide. After anesthesia, mosquitoes were immediately transferred to a petri dish placed on a 4°C cold plate and 0.1 µl of the given concentrations of insecticide solution was deposited on ventral side of the thorax (pronotum) of each female mosquito. These were then transferred to a plastic bowl (~300 ml capacity) and covered with a nylon net fastened with elastic band, a cotton swab with glucose solution (10% in water) was placed on the net. These bowls were kept in climatic chamber for 24 h holding period maintained at 27±2°C temperature and 80±10% relative humidity. After 24 h of holding period percentage mortality was calculated. Mortality was corrected by applying Abbott's[6] formula if the mortality in control replicate were between 5 and 20%.

$$\% \text{ Corrected mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - (\% \text{ Control mortality})} \times 100$$

Mortality data were regressed against the dosages and lethal doses were determined that kill 50% (LD_{50}) and

95% (LD_{95}) of the exposed mosquitoes by log-probit regression analysis using statistical software PASW statistics 18, (SPSS Inc. Chicago, USA). The LD_{50} and LD_{95} were expressed as nanogram (ng) (a.i.)/mg body weight of female mosquito.

RESULTS & DISCUSSION

The results had shown variability in the irritancy levels against different classes of insecticides in insecticide-susceptible and -resistant *An. stephensi* strains (Table 1). The calculated FT_{50} values for susceptible strain ranged from 0.1 to 9.9 min, while for resistant strain it was 1 to 11.6 min. The FT_{95} values for susceptible strain ranged from 0.7 to 25 min, while for resistant strain it was 4.1 to 19.2 min. The FT_{50} values for permethrin (positive control) were respectively 0.1 min in susceptible strain and 1 min in resistant strain while FT_{95} values were 0.7 and 4.1 min respectively. These values for DDT, malathion, deltamethrin and permethrin were lower compared to values observed for control replicates. The FT_{50} and FT_{95} values for chlorfenapyr were almost similar to the FT_{50} and FT_{95} values in control replicates.

The observed FT_{50} values for chlorfenapyr were respectively 98 and 49 fold higher than the observed values for permethrin (0.1 min) and deltamethrin (0.2 min) respectively for susceptible strain. However, for resistant strain these were respectively 8.9 and 3.3 fold higher than the values observed for permethrin and deltamethrin. While, FT_{95} values of chlorfenapyr were respectively 35.7 and 16.7 fold higher for susceptible strain and 4.7 and 1.8 fold higher in resistant strain than that of the FT_{95} values for permethrin and deltamethrin respectively (Table 1). DDT exhibited expectedly lower values of FT_{50} and FT_{95} than malathion in both the susceptible and resistant strains, but were lower than those of chlorfenapyr (Table 1).

Multiple ANOVA was performed for flight times for chlorfenapyr and other insecticides. The flight time values were found highly significant against DDT, malathion, deltamethrin and permethrin, while for pyrethroid control and chlorfenapyr no significant difference between the susceptible and resistant strains of *An. stephensi* was observed (F=35.105; p >0.0).

The first flight times of the insecticide-susceptible and resistant *An. stephensi* mosquitoes exposed to WHO diagnostic dosages of different insecticides and respective controls are given in Figs. 1 and 2. Similar level of irritability to chlorfenapyr and pyrethroid control was observed indicating least irritability, increased irritancy was observed against permethrin followed by deltamethrin, malathion and DDT. The observed increased contact due to low irritancy of the mosquito to the chlorfenapyr may facilitate enhanced uptake of insecticide from sprayed surfaces in the field. However, the effectiveness of an insecticide in vec-

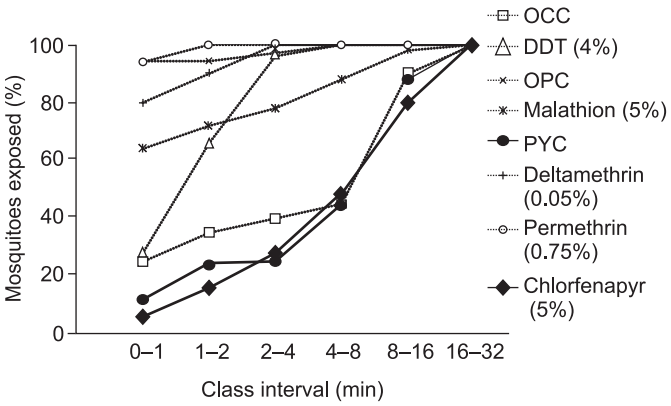

Fig. 1: Distribution of first flight times (class intervals 0–1 to 16–32 min) of multiple-insecticide-susceptible *An. stephensi* strain exposed to determine the irritability using diagnostic dosages WHO impregnated insecticide papers and respective controls; OCC—Organochlorine control; OPC—Organophosphate control; PYC—Pyrethroid control.

Table 1. Irritant effect in multiple-insecticide-susceptible and -resistant *An. stephensi* strains against different insecticide impregnated papers and respective controls

Control/ Insecticide	No. of mosquitoes exposed	FT_{50} (min)		FT_{95} (min)	
		Susceptible	Resistant	Susceptible	Resistant
OCC	20	7.5	6.5	11.5	15
DDT (4%)	50	1.1	2.1	2.4	6.7
OPC	20	4.2	2.1	13.4	12.2
Malathion (5%)	50	3.3	3.1	8.3	11.2
PYC	20	9.9	11.6	22	15.9
Deltamethrin (0.05%)	50	0.2	2.7	1.5	10.6
Permethrin (0.75%)	50	0.1	1	0.7	4.1
Chlorfenapyr (5%)	50	9.8	8.9	25	19.2

OCC—Organochlorine control; OPC—Organophosphate control; PYC—Pyrethroid control; FT_{50} and FT_{95}—Time to take-off for 50 and 95% of the exposed mosquitoes.

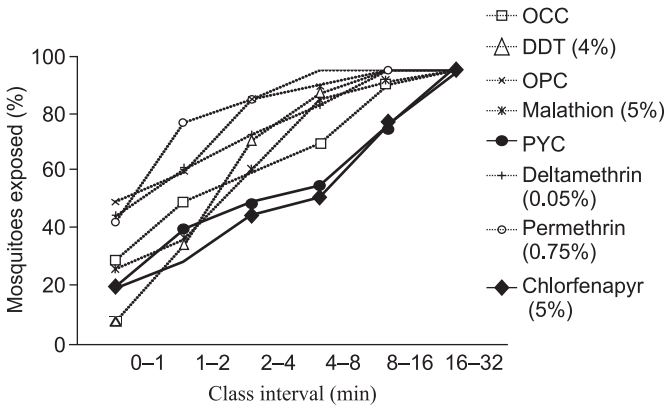

Fig. 2: Distribution of first flight times (class intervals 0–1 to 16–32 min) of multiple-insecticide-resistant *Anopheles stephensi* strain exposed to determine the irritability using diagnostic dosages WHO impregnated insecticide papers and respective controls; OCC—Organochlorine control; OPC—Organophosphate control; PYC—Pyrethroid control.

tor control depends on the level of irritability that facilitate uptake of insecticides and on the the intrinsic toxicity of the insecticide. Thus, there is a need for reviewing the criterion for discriminating susceptible from resistant individuals in the mosquito population based on the levels of mortality observed in WHO tube test for late acting insecticide molecules, *e.g.* chlorfenapyr.

Several studies showed that the extent of irritability may vary between different species and insecticides[7-8]. In a study carried out by Houghard *et al*[9], resistant strains of *An. gambiae* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) had different levels of irritability to pyrethroids. Similarly, permethrin showed different levels of irritability in *An. farauti, An. maculatus* (Diptera: Culicidae) and *Cx. quinquefasciatus*, in take-offs in the range of 8.7 to 33.4 in observation of 15 min/female[1]. In a study on *An. stephensi* by Vatandoost and Borhani[10], it was observed that lambdacyhalothrin was the most irritable insecticide followed by permethrin, cyfluthrin and deltamethrin and the take-offs/female/min values were respectively 1.69±0.35, 1.52±0.20, 1.385±0.25 and 0.946±0.13.

The intrinsic toxicity of chlorfenapyr was tested by topical application against multiple-insecticide-susceptible and -resistant strains of *An. stephensi* (Table 2). The observed LD_{50} and LD_{95} values determined from the mortality data after 24 h holding period for multiple-insecticide-susceptible *An. stephensi* laboratory strain were respectively 0.827 and 5.425 ng/female, while for resistant strain they were 0.674 and 3.401 ng/female respectively. This supports our earlier observation on the possibility of use of chlorfenapyr as candidate insecticide for IRS that registered 100% mortality in susceptibility test for managing DDT-malathion-bendiocarb-deltamethrin resistant mosquitoes[5].

Chlorfenapyr is a pro-insecticide and belongs to the pyrrole group. The suggested mechanism for chlorfenapyr metabolism is conversion of the pro-insecticide chlorfenapyr to toxic form CL30328 by monooxygenases and this toxic form inhibits ATP synthesis in the mitochondria leading to inhibition of oxidative phosphorylation and resulting in the death of the mosquito. This is a novel mechanism of resistance and different from the reported mechanisms in Indian anopheline strains and thus do not show cross-resistance to chlorfenapyr. Generally, involvement of multiple enzymes/proteins in conferring resistance delays the onset of resistance. It may be mentioned here that involvement of monooxygenases is known for conferring pyrethroid resistance in Indian malaria vectors. Thus, presence of elevated levels of monooxygenases due to pyrethroid resistance facilitates conversion of chlorfenapyr to toxic form (CL30328) and increased efficacy of chlorfenapyr and thus can be an ideal insecticide for management of pyrethroid resistance due to monooxygenases[5].

Report of studies on different anopheline species, *e.g. An. gambiae*[11], *An. funestus*[12] and *An. quadrimaculatus*[13] have also shown absence of cross-resistance to chlorfenapyr with other insecticides that are in use for vector control.

Table 2. Intrinsic toxicity of chlorfenapyr against insecticide-susceptible and -resistant *Anopheles stephensi* strains

Strains	Time (h)	LD_{50} (ng/mg)	Lower-upper limit at Cl 95%	LD_{95} (ng/mg)	Lower-upper limit at Cl 95%	Chi-square	*p*-value
An. stephensi (Susceptible to DDT, MLN and DM)	24	0.827	0.313–1.299	5.425	2.855–48.493	3.649	0.456
	48	0.616	0.217–0.983	3.874	2.149–22.729	4.014	0.404
	72	0.629	0.165–1.095	3.741	1.922–43.210	5.665	0.226
An. stephensi (Resistant to DDT, MLN and DM)	24	0.674	0.484–0.892	3.401	2.241–7.070	2.027	0.731
	48	0.683	0.535–0.819	2.134	1.654–3.293	1.029	0.905
	72	0.713	0.191–1.025	1.936	1.287–17.592	3.201	0.525

MLN—Malathion; DM—Deltamethrin; LD—Lethal doses that kill 50% (LD_{50}) and 95% (LD_{95}) of the exposed mosquitoes; CI—Confidence limit.

CONCLUSION

This study has brought out clearly the possible use of insecticides with novel mode of action and highlights on two important aspects of insecticides for successful vector control, irritability effect and intrinsic toxicity. Success of vector control mainly depends on use of effective insecticide and on the behaviour of the mosquitoes that prompt optimum contact with the sprayed surfaces to absorb sufficient dosage that could cause death. The low irritability may facilitate increased uptake of insecticide and may result in better efficacy on the disease vectors in conjunction with the intrinsic toxicity of insecticides on the mosquitoes. Further, it may be stated that the time lag between acquiring a malaria infection by mosquitoes and its transmission is few days and the criterion for use of such less irritable and long acting insecticides needs to be reviewed with respect to its bioefficacy criterion and impact on disease transmission in field.

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