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Deltamethrin: Promising mosquito control agent against adult stage of *Aedes aegypti* L.

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ABSTRACT

Objective: To evaluate the effects of deltamethrin against field-collected adults of *Aedes aegypti L. (Ae. aegypti)*. **Methods:** The adults were selected with 0.025% deltamethrin for 40 successive generations. The selected adults were tested with 4% DDT and the emerging larvae were tested with various insecticides to study the cross-resistance spectrum. The knockdown and irritability studies were carried out in adult mosquitoes to investigate their behavioural response to deltamethrin. **Results:** Forty generations of selection with deltamethrin resulted in only 3.8-fold resistance in the adults of *Ae. aegypti*. The adults of parent (PS) and selected strains (DAS) exhibited only 0.8-fold cross resistance to 4% DDT. The larvae emerging from the PS and DAS strains did not develop appreciable levels of resistance to various insecticides tested. The knockdown studies revealed KT50 of 14.4 min in PS adults with no signs of recovery even after 24 h. The DAS strains could develop only 1.2 to 1.3-fold knockdown resistance (KDR). The knockdown response of DDT was though 5-6 times slower than deltamethrin but the continued response in deltamethrin-selected adults caused only 1.2-fold KDR. The PS and DAS strains exhibited significant irritability response towards deltamethrin and DDT. The DAS strains showed 5-6 fold increased irritability to deltamethrin as compared to the PS strain. **Conclusions:** The above results suggest the prolonged effective use of deltamethrin against *Ae. aegypti* as an adulticide.

1. Introduction

Pyrethroid resistance is envisioned to be a major problem for the vector control program, since at present there are no suitable chemical substitutes for pyrethroids. The emergence of pyrethroid resistance in most mosquito species of public health importance represents a threat for sustainable vector control programs implemented in the tropics[1,2]. Frequent usage of pyrethroids as residual domestic sprays, in mosquito mats, coils and impregnated bed nets is one of the major causes of development of pyrethroid resistance in mosquitoes. The other possible reasons may be the cross-resistance to other insecticides used in agriculture and domestic areas on a large scale and as spatial repellents leading to inheritance of pyrethroid resistance. Moreover, the cross-resistance conferred by pyrethroid resistance limits the number of effective alternatives suitable for vector control. Effective selection of resistance to pyrethroids has been reported in African strain of *Anopheles gambiae* as a result of their use as domestic insecticide and in bed nets[3].

Deltamethrin is among the six pyrethroid insecticides recommended by World Health Organization in the framework of WHO pesticide evaluation scheme for the treatment of mosquito nets[4]. Though, deltamethrin has been found an effective larvicide and adulticide against mosquitoes, various authors have reported the development of resistance against deltamethrin in these mosquitoes because of the involvement of mono-oxygenases, hydrolytic esterases or a knockdown resistance (*kdr*)-based mechanism[5,6].

The *kdr* gene, which confers cross-resistance to DDT

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and pyrethroids, is now widely prevalent in mosquitoes of public health importance[7-9]. Knockdown resistance is reported to be emerged from four amino acid substitutions in the sodium channel insecticide binding site in *Aedes aegypti (Ae. aegypti)* whereas by a single mutation in *Culex quinquefasciatus* and *Anopheles stephensi*[5,10]. These mutations have also been held responsible for cross-resistance against DDT and pyrethroid insecticides[9]. However, the effect of knockdown resistance on the vector control efficacy remains uncertain. In some countries, insecticide-treated nets can still provide individual protection against *kdr*-resistant mosquito populations[11,12] although more recently studies have shown reduced efficacy where the *kdr* mutation is high [13,14]. Most of the knockdown studies have been performed on the different species of *Anopheles*. The present studies were, thus aimed to assess development of resistance to deltamethrin in *Ae. aegypti* followed by their behavioural response to deltamethrin in terms of the knockdown and irritability. The studies also involved the evaluation of the cross-resistance and behavioural response to DDT in the adults, and cross-resistance in the emerging larvae to the insecticides of various groups. These studies are of extreme significance in designing effective strategies for vector control and implementing resistance management strategies.

2. Materials and methods

2.1. Mosquito population

The present investigations employ the larvae and adults of *Ae. aegypti* originated from field-collected engorged female adults from Delhi. The colony was maintained in an insectary without any insecticide exposure at (28±1) ℃, (80±5)% RH and 14L:10D photoperiod[15]. Adults were supplied with soaked deseeded raisins, while larvae were fed upon a mixture of yeast powder and grinded dog biscuits. Periodic blood meals were provided to female mosquitoes by keeping albino rats in the cages.

2.2. Chemicals

Insecticides used in the investigations; carbaryl, carbofuran, p,p′-DDT, deltamethrin, endosulphan, fenitrothion, fenthion, lindane, malathion, permethrin, propoxur and temephos; were of 84% (malathion) or 94%-99% purity. Insecticide-impregnated papers of deltamethrin (0.025%) and DDT (4%) were procured from World Health Organization, Geneva. The papers were stored at 4 ℃ and were used for a maximum of 3 times.

2.3. Adult susceptibility and selection studies

The adult susceptibility studies were carried out by the WHO procedure using standard WHO kits[16]. Freshly blood-fed 3-day old female adults of the field-collected strain (PS) were exposed in batches of 20 to 0.025% deltamethrin-impregnated papers. The mosquitoes unable to fly were recorded as dead. The observations were made after regular intervals and mortalities were scored. Three replicates were carried out and the data was subjected to regression analysis using SPSS 11.5 program to compute the LT_{50} and LT_{90} values. For selection studies, several batches of 20-25 fully blood-fed mated females were exposed to deltamethrin so as to induce 90% mortality in the adults. The surviving females were used for obtaining the next generation which was further assessed for their susceptibility status to deltamethrin and exposed to the deltamethrin selection pressure at LT_{90}. The selections were continued till 40th generation of selected adults was obtained (DAS_{40}).

2.4. Cross and stage-specific resistance studies

The adults emerged from parent and deltamethrin-adult selected strains at 20th ((DAS_{20}) and 40th generations (DAS_{40}) were tested with 4% DDT-impregnated papers respectively, to assess the development of cross-resistance levels. In addition, the larvae derived from parent and deltamethrin-adult selected strains were tested with deltamethrin, permethrin, DDT, lindane, endosulfan, malathion, fenthion, fenitrothion, carbaryl and propoxur; and their resistance levels to respective insecticide were computed.

2.5. Knockdown experiments

The knockdown studies were performed on the adult mosquitoes of *Ae. aegypti* according to the method of Kumar *et al*[17]. For each experiment 20 freshly blood-fed 3-day old adult females emerging from the parent and adult-selected populations at 20th and 40th generations were kept in control tubes for 1 hour to precondition them. Thereafter, they were released in the experimental tubes and exposed to 0.025% deltamethrin-impregnated papers and 4% DDT-impregnated papers, separately. Observations were recorded after regular intervals.

The mosquitoes unable to fly but alive were counted at regular intervals as knocked-down mosquitoes. The dead mosquitoes were recorded as mortalities. The observations were made till all the mosquitoes had been knocked-down. These mosquitoes were regularly removed down from the experimental tubes and transferred to the control tubes to observe any recovery.

The mortality data and knockdown data were subjected to regression analyses using computerized SPSS 11.5 Programme. The KT_{50} and KT_{90} values with 95% fiducial limits were calculated in each bioassay to measure difference between the test samples. The knockdown resistance ratio to deltamethrin and DDT was calculated by dividing the KT_{50} values of the selected strains by that of the parent population. The results obtained with different strains were analyzed using Student's *t*-test with statistical significance considered for $P<0.05$.

2.6. Irritability studies

Irritability responses in the adult female mosquitoes of *Ae. aegypti* were assayed using the modified WHO method[17]. A Perspex funnel with a hole on the top was kept inverted, separately over the insecticide-impregnated papers of deltamethrin and DDT on a glass plate. Tests were performed both on the parent and adult-selected strains.

The single adult was allowed to settle on the paper for three minutes after which the time taken for the first take-off was recorded. The experiment was continued for 15 minutes during which the total number of flights undertaken by each mosquito was scored. Parallel control tests were performed with silicone-oil impregnated papers. Each treatment had 25 replicates. Data was analyzed using Student's *t*-test.

The relative irritability was calculated by dividing mean number of take-offs stimulated by insecticide by that stimulated by control. The irritability ratio was determined by dividing relative irritability of adult-selected population by that of susceptible population.

3. Results

Deltamethrin was found to be highly effective against the parent adults of *Ae. aegypti* as 50% of the adult mortality was obtained in only 4.5 minutes. The selection pressure of deltamethrin exerted at the adult stage of *Ae. aegypti* caused only a slight increase in the levels of resistance, 2.6-fold in DAS_{20} strain and 3.8-fold in DAS_{40} strain after successive selections for forty generations (Table 1). When tested against 4% DDT, the PS and DAS adults exhibited LT_{50} of 63-74 minutes, thus exhibiting only 0.8-fold cross-resistance (Table 1).

When the selected adults were tested with deltamethrin at larval stage, they showed only 5-fold levels of resistance to deltamethrin. Further, these larvae did not exhibit any significant levels of cross-resistance to permethrin, different organochlorines, organophosphates and carbamates tested with, the larvae exhibiting highest cross-resistance level of 7.3-fold to DDT (Table 2).

The knockdown studies revealed that the adults of *Ae. aegypti* exhibited a pronounced knockdown response towards deltamethrin, as the PS strain took only 14 min exposure to deltamethrin to show 50% knockdown effect and 18 min to demonstrate 90% knockdown effect (Table 3). None of the mosquitoes recovered from the knocked-down state. The important observation was that the selection of mosquitoes at adult stage for forty generations resulted in only 1.3-fold knockdown resistance. Exposure of parent and deltamethrin-selected adults of *Ae. aegypti* to 4% DDT-impregnated paper elicited a 5-6 times slower knockdown response as compared to deltamethrin with PS strain taking 97 minutes whereas, adult-selected strains exhibiting KT_{50} values of 106-118 minutes (Table 3). However, the knocked-down mosquitoes did not recover on exposure to DDT also.

Deltamethrin also elicited a highly effectual irritability response in the adult *Ae. aegypti* resulting in 3.2 min to fly first in PS strain as against 5.5 min caused by the control paper. Adult selection with deltamethrin did not reduce the irritability response of the adults as the time elapsed before the first flight ranged from 3.5-3.8 min as compared to 3.1 min in the parent strain (Table 4). The mean number of take-offs recorded by adult *Ae. aegypti* also established the efficacy of deltamethrin as an irritant pyrethroid. Exposure to deltamethrin for 15 min caused 3-fold mean take-offs in PS and 15-17-fold in DAS strains as compared to that against control paper. Moreover, the selected strains showed 5-6 fold increased irritability as compared to the parent strain (Table 4).

The irritability response of adults *Ae. aegypti* to 4% DDT evinces the superiority of deltamethrin over DDT as an irritant insecticide as DDT exposure to PS adults caused almost double time before the first take-off as compared to deltamethrin (Table 4). The DAS strains also exhibited decreased irritability response to DDT as the adults took 1.4-1.8 times longer duration elapsed before the first flight take-off and 1.7-3.1 fold reduced number of flights as compared to deltamethrin exposure. However, as against the parent adults, the selected adults showed 5.5-fold more irritability to DDT-impregnated papers (Table 4) which is almost similar to that obtained when exposed to deltamethrin.

Table 1

Adult LT_{50} (min), adult LT_{90} (min), resistance ratios to 0.025% deltamethrin and 4% DDT in parent and adult-selected strains of *Ae. aegypti* (Values in parentheses indicate the lower and upper 95% fiducial limits).

Strain	0.025% Deltamethrin					4% DDT				
	LT_{50}	LT_{90}	Slope	Heterogeneity χ^2 (*df*)	RR to deltamethrin	LT_{50}	LT_{90}	Slope	Heterogeneity χ^2 (*df*)	RR to deltamethrin
PS	4.5 (3.8-5.1)	19.5 (13.8-26.9)	2.0± 0.2	2.98 (5)	-	73.8 (62.0-89.3)	179.8 (132.4-244.1)	3.3±0.5	6.95(3)	-
DAS_{20}	11.9 (9.2-15.6)	55.6 (33.9-91.1)	1.9±0.3	1.97(3)	2.6	64.4 (55.3-75.1)	143.7 (110.2-187.3)	3.7±0.6	1.09(3)	0.9
DAS_{40}	17.3 (13.1-22.9)	74.5 (48.5-114.5)	2.0±0.3	1.56(3)	3.8	63.4 (53.7-74.8)	192.0 (144.3-255.5)	2.7±0.3	7.82(4)	0.9

Table 2

LC_{50} and LC_{90} and cross-resistance ratios (CR) of the larvae derived from the parent and adult-selected strains of *Ae. aegypti* to various insecticides (μ g/mL).

Insecticide	PS		DAS_{20}			DAS_{40}		
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	CR	LC_{50}	LC_{90}	CR
Deltamethrin	0.0001	0.0006	0.0006	0.0019	4.9	0.0006	0.0018	5.2
Permethrin	0.0006	0.0027	0.0007	0.0022	1.1	0.0009	0.0038	1.3
DDT	0.0109	0.0323	0.0684	0.2257	6.3	0.0796	0.2524	7.3
Lindane	0.1314	0.5087	0.1627	0.6840	1.2	0.1597	0.8365	1.2
Endosulfan	0.3235	1.0802	0.3988	1.4978	1.2	0.4585	2.0855	1.4
Malathion	0.0248	0.1693	0.0741	0.2350	3.0	0.0729	0.2313	2.9
Fenthion	0.1279	0.3419	0.1446	0.4374	1.1	0.1687	0.5184	1.3
Fenitrothion	0.0060	0.0129	0.0034	0.0119	0.6	0.0037	0.0158	0.6
Temephos	0.0102	0.0390	0.0105	0.0412	1.0	0.0114	0.0444	1.1
Carbofuran	0.3452	0.7403	0.3930	0.9598	1.1	0.4482	1.3928	1.3
Carbaryl	0.4257	1.2448	0.9683	3.2101	2.3	1.2305	4.7300	2.9
Propoxur	0.4864	1.7439	0.5583	3.2237	1.1	0.6230	4.5357	1.3

Table 3

Adult KT_{50} and KT_{90} of the parent and insecticide-selected strains of *Ae. aegypti* on exposure to 0.025% deltamethrin and 4% DDT-impregnated papers (min).

Strain	0.025% Deltamethrin					4% DDT				
	KT_{50}	KT_{90}	Slope	Heterogeneity χ^2 (*df*)	RR to Deltamethrin	KT_{50}	KT_{90}	Slope	Heterogeneity χ^2 (*df*)	RR to Deltamethrin
PS	14.5 (14.0-14.8)	18.3 (17.4-19.2)	12.3± 1.3	7.36 (6)	–	96.6 (89.6-104.1)	135.8 (127.2-143.5)	8.6±2.3	3.64(5)	–
DAS_{20}	16.1 (15.2-17.0)	23.5 (21.6-25.6)	8.0±0.8	3.8(4)	1.3	106.6 (95.3-117.3)	152.6 (141.8-164.0)	9.2±3.2	0.58(4)	1.1
DAS_{40}	18.8 (18.0-19.7)	21.9 (20.5-23.3)	19.7±4.5	0.60(5)	1.3	118.7 (101.8-132.8)	161.2 (154.4-169.2)	7.8±1.4	2.34(5)	1.2

Values in parentheses indicate the lower and upper 95% fiducial limits.

Table 4

Response of 3-day old adult females of parent and deltamethrin-adult selected strains of *Ae. aegypti* in irritability tests when exposed to 0.025% deltamethrin and 4% DDT-impregnated paper.

	Strain	Mean time lapse before first take-off (in 15 min)		Mean number of take-offs for female (in 15 min)		Relative irritability	Irritability ratio
		Control	Experimental	Control	Experimental		
0.025% deltamethrin	PS	5.46±0.72 (3.10 – 18.00)	3.17±0.03 (3.00 – 3.50)	7.00±0.93 (0.00– 17.00)	20.73±2.01 (6 – 45)	2.96	–
	DAS_{20}	10.11±2.27 (3.15 – 18.00)	3.87±0.22 (3.10 – 8.20)	1.50±0.50 (0.00 – 5.00)	26.24±1.02 (17 – 41)	17.49	5.91
	DAS_{40}	12.32±1.80 (3.30 – 17.0)	3.50±0.12 (3.00 – 5.10)	2.60±0.91 (0.00 – 6.00)	38.08±1.04 (18.00 – 36.00)	14.60	4.93
4% DDT	PS	5.46±0.72 (3.10 – 18.00)	5.88±1.37 (3.08 – 15.17)	7.00±0.93 (0.00 – 17.00)	10.8±1.22 (6.00 – 18.00)	1.53	–
	DAS_{20}	10.11±2.27 (3.15 – 18.00)	5.37±0.89 (3.03 – 12.08)	1.50±0.50 (0.00 – 5.00)	12.40±1.24 (5.00 – 19.00)	8.27	5.40
	DAS_{40}	12.32±1.80 (3.30 – 17.00)	6.50±0.12 (3.12 – 8.13)	2.60±0.91 (0.00 – 6.00)	22.08±1.40 (9.00 – 20.00)	8.49	5.55

Figures in parentheses indicate the range.

4. Discussion

The insecticidal efficacy of deltamethrin against mosquitoes along with the knockdown and excito-repellent properties has been reported earlier[18]. Most of these studies have focused on the behavioral response of *Anopheles* species to insecticides, whereas comparatively little has been published on the avoidance behavior of *Ae. aegypti* exposed to test chemicals[19,20]. Therefore, the present studies were carried out on adult dengue vector to investigate the development of deltamethrin resistance and effect of the resistance on their behavioural response to deltamethrin in terms of the knockdown and irritability.

In the present studies the adult selection of *Ae. aegypti* with deltamethrin for forty generations caused just 3.8 - fold increase in the deltamethrin resistance in adults suggesting the effective use of deltamethrin as an adulticide. Similar results were reported by Kumar *et al*[17] in an Indian strain of *An. stephensi* where forty generations of adult selection caused only a six-fold increase in the resistance to deltamethrin in the adults. Earlier, Gayathri and Murthy[5] had reported that the adult selection of an Indian strain of *An. stephensi* with deltamethrin for 25 generations raised its tolerance to deltamethrin by 39-fold. The absence of resistance expression in adults may be significant in the management of deltamethrin resistance problem in *Ae. aegypti*, reported elsewhere, by using deltamethrin as an adulticide.

The cloning of voltage-gated sodium channel gene has allowed the development of PCR-based assays for the detection of nerve-insensitivity, a *kdr*-type resistance that is associated with DDT/pyrethroid cross-resistance[8]. Our investigations showed that though the parental adults of *Ae. aegypti* were resistant to DDT with LT_{50} of 68 min, yet after selections with deltamethrin for 40 generations they failed to develop any cross-resistance to DDT. The absence of cross-resistance between deltamethrin and DDT implicates the presence of a mechanism other than *kdr* factor in this species and suggests that the deltamethrin could be used effectively as an adulticide in the fields where low or even high levels of DDT resistance exist. Another remarkable observation was the development of negligible resistance to deltamethrin and cross-resistance to permethrin, various organochlorines, organophosphates and carbamates, except a 7.3-fold cross-resistance to DDT, in the larvae arising from the adult-selected strains. Lack of cross-resistance to many insecticides increases the potential for rotational and mosaic use of deltamethrin along with different groups of insecticide. Moreover, the failure of the expression of high larval resistance in the adult stage enables the use of deltamethrin as an adulticide from a resistance management perspective against *Ae. aegypti* as their larvae are reported to develop high levels of deltamethrin resistance when selected at the larval stage[5,21].

The present study also confirms deltamethrin as an efficient knockdown agent against the adults of *Ae. aegypti*, the parent susceptible adults taking only 14 min for 50% knockdown. In France, Pennetier *et al*[1] recorded KT_{50} value of 23.8 min and KT_{95} value of 38.3 min in a susceptible strain of *Ae. aegypti* when tested with 0.014% deltamethrin. Knockdown bioassays conducted by Gayathri and Murthy[5] on adults of an Indian strain of *An. stephensi* (selected with deltamethrin at the larval and adult stages for 25 generations with 151-fold and 39-fold resistance, respectively) against 0.05% deltamethrin-impregnated papers revealed that the adults selected at the adult stage were more resistant to deltamethrin and the other pyrethroids than those selected at the larval stage.

Knockdown time is a good indicator for an early detection of reduced susceptibility and a more sensitive method of incipient resistance. The spread of resistance genes in a treated region depends on the initial frequency of *kdr* gene, the degree of dominance of *kdr* allele and the importance of migration relative to the selection pressure. Present investigations showed only a slight increase in knockdown time in the adult-selected strains exhibiting 1.1-1.3-fold knockdown resistance to deltamethrin and DDT. This indicates the continued susceptibility in the adults of *Ae. aegypti* towards deltamethrin and DDT even after 40 generations of adult selections with deltamethrin. Raghavendra *et al*[22-28] showed a decrease in the KD_{50} values from 83 min to 32 min and KD_{90} values from 246 min to 48 min against deltamethrin in 2006 compared to 2001 indicating gradual increase in the susceptibility of the mosquito population to deltamethrin. Pereira da-Cunha *et al*[29] recorded 30%-46% recovery in the knocked-down adults of *Ae. aegypti* collected from various regions in Brazil and then selected with cypermethrin. However, in our study, the failure of the knocked-down mosquitoes to recover even after 24 h again emphasize and recommend the effective use of deltamethrin as an adulticide against dengue fever mosquito.

High irritability response of adults *Ae. aegypti* to deltamethrin further ascertains the efficiency of deltamethrin as an adulticide. The DAS strains exhibited little prolonged time for first take off than the PS strains against insecticide impregnated papers though the mean number of flights were higher. It is likely that the massive knockdown effect observed with the deltamethrin disrupted the time for first take off of mosquitoes, since knocked-down mosquitoes were unable to fly. The continued irritability response to deltamethrin and DDT in the deltamethrin-resistant mosquitoes indicates the effective and prolonged use of deltamethrin against the adults of *Ae. aegypti* as an irritant insecticide. In Iran, Vatandoost *et al*[18] reported that out of the different insecticides tested, permethrin had the maximum irritancy effect on *An. stephensi* and *An. dthali* while DDT and deltamethrin showed the least irritancy effect against *An. stephensi* with (0.42±0.08), and (0.77±0.12) take-offs/min/adult, respectively, however, lambdacyhalothrin had the least irritancy effect against *An. dthali* with (0.096 ±0.02) take-offs/min/adult. It has been argued that Indoor Residual Spraying exerts a much stronger selection pressure than insecticide-treated nets for insecticide resistance because resistant fed females would fly away from treated surfaces of sprayed houses while unfed females searching for a blood meal would have repeated and longer contacts on insecticide treated nets and would be killed as readily as susceptible ones[30].

Till now, much attention has been paid to the control of *Ae. aegypti* at the larval stage, very few reports are available on their behavioural response against the adults. Though the present investigations stress upon the efficacy of deltamethrin as an adulticide, the laboratory investigations cannot justify and ascertain the use of deltamethrin as an effective adulticide outdoors. Further research is needed to design and identify the resistance management strategies in the fields against dengue fever mosquito.

Conflict of interest statement

We declare that we have no conflict of interest.

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