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Diminished reproductive fitness associated with the deltamethrin resistance in an Indian strain of dengue vector mosquito, *Aedes aegypti* L.

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Abstract. The susceptible (SS) and resistant (DLR) strains of *Aedes aegypti* selected with deltamethrin and combination of deltamethrin and PBO (1:5) at the larval/adult stage were studied in the laboratory for their reproductive fitness in terms of fecundity, hatchability and longevity of gonotrophic cycles. The DLR strains exhibited 73-88% reduction in the duration of gonotrophic cycles as compared to their SS counterparts. There was a considerable decrease in egg production and hatchability rates in the selected strains of *Ae. aegypti*, as compared to that of the SS strain. Data indicate deltamethrin being an effective insecticide against *Ae. aegypti* and a possible correlation between the deltamethrin resistance and disadvantages during reproduction. The most drastic and significant effect was observed in DLR1b strains exhibiting 36.7% decrease in fecundity and 32.4% reduction in hatchability. Another important observation was diminished reproductive fitness in DLR2 strains. This suggests the usefulness of synergized deltamethrin selections in reducing the frequency of resistant individuals. A significant finding was to observe the reproductive disadvantage in adult-selected strains having negligible resistance to deltamethrin implicating the efficacy of deltamethrin as an adulticide rather than as a larvicide. Various probable reasons for the reduction in the reproductive potential and the possible resistance-management strategies of *Ae. aegypti* are discussed.

INTRODUCTION

One of the most important vector borne diseases currently prevailing in most parts of the Tropics is dengue and the primary vector is *Aedes aegypti* L. In the Indian sub-continent, cases of dengue fever are on the rise and, therefore, the control of dengue vector needs immediate attention (WHO, 1999). The various measures for the control of this mosquito have been unable to control this, thus posing a serious threat to human beings.

It has been observed that resistant and susceptible strains of insects frequently differ in fitness components, including longevity, fecundity and fertility, in addition to their susceptibility to insecticides (Arnaud *et al.*, 2002). In general, resistant strains exhibit a reproductive disadvantage in the absence of insecticides (Roush & Plapp, 1982; Kono, 1987; Argentine *et al.*, 1989; Li *et al.*, 2002), as a result of which, the frequency of resistant individuals may reduce over time (Roush & McKenzie, 1987; Arnaud & Haubruge, 2002). However, in certain cases, the resistant strains may have a fitness advantage and their frequency remains stable (Arnaud *et al.*, 2002). A few researchers have reported that various insecticides reduce the fecundity of the treated *Ae aegypti* as compared to their susceptible counterparts. The adults of *Ae. aegypti* were found to lay few number of eggs after exposure to sublethal concentration doses of dieldrin (Duncan, 1963), d-phenothrin and d-allethrin (Liu *et al.*, 1986) and abate (Reyes-Villanueva *et al.*, 1990).

The efficacy of deltamethrin against the life stages of *Ae. aegypti*, as one of the most potent insecticides has been well documented (Sahgal & Pillai, 1993; Kumar *et al.*, 2002). Yet, few studies have compared reproductive fitness components between field and resistant strains of *Ae. aegypti*.

Preliminary laboratory studies were thus carried out on the field-collected and deltamethrin and synergized deltamethrin-selected strains of *Ae. aegypti*, to study the sublethal effects of deltamethrin and the development of deltamethrin resistance on the reproductive fitness of these strains. The studies also demonstrated the effects of reversed deltamethrin resistance caused by the synergist on the reproductive fitness of mosquito vector. These included duration of gonotrophic cycles, fecundity and egg hatchability in the non-selected and selected strains of dengue vector. Information of these sublethal effects of insecticides is important in planning and implementing dengue vector control programs.

MATERIALS AND METHODS

Insect stock culture

Present investigations used the larvae and adults of *Ae. aegypti* originated from field-collected engorged female adults (Base-line Strain) from Delhi. The colony was maintained in an insectary at 28 ± 1 ºC and 80 + 5 %RH with a photoperiod of 14h and 10h darkness (Kumar *et al.*, 2002).

Chemicals

Technical grade deltamethrin with a purity of 98.8% and the synergist, piperonyl butoxide (PBO) were obtained from Roussel Uclaf, India. Insecticide impregnated papers of deltamethrin (0.025%) were procured from World Health Organization, Geneva. The papers were stored at 4 ºC and were not used more than 3 times.

Strains of *Ae. aegypti* selected for studies

The Base-Line or the Parent strain was exposed to the deltamethrin and synergized deltamethrin (deltamethrin + PBO, 1:5) at the larval/adult stage and their LC_{50} and LC_{90} values were calculated. Each strain was thereafter selected at LC_{90} levels, separately for successive generations (Kumar *et al.*, 2002) to obtain the resistant strains (R-Line) of *Ae. aegypti*. The various strains selected for studies are as follows:

(i) Base-Line: PS Strain (Parental strain); Field-collected strain.

(ii) R-Line:

(a) DLR1 Strain; Deltamethrin larval-selected strain:

- DLR1a Strain: PS Strain selected with deltamethrin at larval stage for 20 generations.
- DLR1b Strain: DLR1a Strain selected with deltamethrin till 40 generations at larval stage.

(b) DLR2 Strain; Deltamethrin + PBO (1:5) larval-selected strain:

- DLR2a Strain: PS Strain selected with synergized deltamethrin at larval stage for 20 generations.
- DLR2b Strain: DLR1b Strain subjected to selection pressure of synergized deltamethrin at larval stage of 24th generation till 40 generation.

(c) DLR3 Strain: Deltamethrin adult-selected strain:

- DLR3a Strain: PS Strain selected with deltamethrin at adult stage for 20 generations.
- DLR3b Strain: DLR3a Strain exerted with deltamethrin selection pressure at adult stage for next 20 generations.

The reproductive fitness of the mosquito of each strain with LC_{50} values tabulated in Table 1 was estimated by scoring the fecundity and hatchability during three consecutive gonotrophic cycles.

Table 1. Larval LC_{50} levels and increase in levels of tolerance to deltamethrin in different strains of *Aedes aegypti*

Strain	Larval LC_{50} to deltamethrin (ppm)	Fold increase in tolerance to deltamethrin
PS	0.000118 (0.000095-0.000147)*	–
DLR1a	0.004585 (0.003899-0.005390)	38.8
DLR1b	0.082965 (0.06827-0.100812)	703.0
DLR2a	0.001824 (0.001503-0.002214)	16.7
DLR2b	0.007810 (0.006422-0.009499)	66.2
DLR3a	0.000583 (0.000392-0.000824)	4.2
DLR3b	0.000618 (0.000524-0.000728)	5.3

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

Gonotrophic cycle

Batches of freshly emerged 100 mosquitoes, 50 males and 50 females of each strain, were released in the respective cages. Following three days of emergence, adults were provided with blood meal marking the beginning of first gonotrophic cycle. Unfed males and dead mosquitoes were removed from cages and surviving blood-fed females were counted. After first round of egg laying remaining females were counted again and after 24h of starvation, a second blood meal was provided. The period from the beginning of one blood meal to the next blood meal was recorded as one gonotrophic cycle (Detinova, 1962).

Second blood meal initiated the 2nd gonotrophic cycle. Numbers of blood-fed females were recorded again and allowed to lay eggs. Another blood meal was given to the surviving females after the completion of second round of oviposition and the observations were repeated. The duration of three consecutive cycles was accounted in different strains of *Ae. aegypti*.

Fecundity

For fecundity studies, the eggs laid by blood-fed females were collected each day on a filter paper in an ovitrap kept in the cage and were counted daily. A fresh ovitrap was kept daily in the cage until no further eggs were laid for at least 48h. The number of eggs was counted using a dissecting microscope at the magnification of 40X. The eggs counted everyday after each blood meal were documented separately.

Hatchability

The eggs collected on the filter paper during each gonotrophic cycle were submerged in a tray filled with dechlorinated water and were allowed to hatch separately. The hatched larvae were counted carefully and the hatch proportion was calculated in each instance by taking a ratio of the number of hatched larvae to the number of eggs laid.

Comparison of Biotic Performances

For each gonotrophic cycle, fecundity rate per female was calculated based on the number of females present in each cycle. The fecundity of PS strain was compared with the fecundity of DLR strains. The fecundity of each strain during three cycles was also compared. In a similar way, the hatch proportion of PS strain was compared with the hatch proportion of DLR strains. The total duration of oviposition in each gonotrophic cycle was also recorded.

RESULTS

Table 1 shows the LC_{50} and tolerance levels of S-line and various DLR strains of R-line of *Ae. aegypti* to deltamethrin, when selected at larval or adult stage. In the R-line, DLR1b strain developed highest resistance levels followed by DLR2b strain whereas DLR3 strains exhibited almost negligible levels of resistance to deltamethrin.

Reproductive fitness studies of the DLR strains of *Ae. aegypti* revealed 73-88% reduction in the duration of three consecutive gonotrophic cycles as compared to the PS strain (Table 2). The DLR2b and DLR1b strain exhibited the gonotrophic cycles of shortest duration as compared to the others; though the difference in the duration was not significant.

Our investigations clearly demonstrated significant reduction in fecundity of DLR strains during first gonotrophic cycle (Table 3). The most drastic and significant effect was evident in the DLR1b strain with 36.7% reduction in fecundity and DLR3b strain with 23.5% reduced fecundity. In other insecticide-selected DLR strains, the decrease in fecundity ranged from 8-17% (Table 3). Also, the PS adults took longer duration to complete their oviposition than the selected strains. These results indicate that deltamethrin resistance caused reproductive disadvantage in the selected strains as compared to the non-selected strain.

The studies on the hatchability during 1st cycle confirm the fact that the eggs of DLR strains were significantly less fertile in comparison to their parent counterpart (Table 3). DLR1b strain exhibited maximum (30.7%) reduced egg hatchability in contrast to the parent strain while other strains showed 18 to 23% decreased hatchability. Only 57% of the eggs laid by DLR1b strain hatched as against 82% of the eggs in PS strain.

The second gonotrophic cycle displayed reduced fecundity rates as against the first cycle in all the strains of *Ae. aegypti*. The decrease was more pronounced in the DLR strains (Table 4) with DLR2b strain exhibiting the maximum and significant reduction of 25%. However, the fecundity of

Table 2. Duration (in days) of the three consecutive gonotrophic cycles of parent and insecticide-selected strains of *Aedes aegypti*

Strain	First Gonotrophic Cycle	Second Gonotrophic Cycle	Third Gonotrophic Cycle	Total Duration
PS	9.0	8.0	8.0	25.0
DLR1a	7.0	7.0	6.0	20.0
DLR1b	6.0	6.33	6.0	18.33
DLR2a	7.0	6.0	6.67	19.67
DLR2b	6.0	6.0	6.0	18.0
DLR3a	7.0	6.0	6.0	19.0
DLR3b	8.0	6.67	6.0	20.67

Table 3. Effect of the deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Aedes aegypti* in the first gonotrophic cycle

Strain	No. of females Blood-fed	Days of Oviposition	Total No. of Eggs Laid	No. of Eggs per female	Relative* Fecundity	Total Hatch	Hatch Per Female	% Hatch**
PS	36	5	3906	108.50	–	3222	89.5	82.5
DLR1a	34	3	3383	99.50	91.7	2295	67.5	67.8
DLR1b	35	2	2405	68.71	63.3	1375	39.28	57.2
DLR2a	33	3	2969	89.97	82.9	1974	59.82	66.5
DLR2b	34	2	3179	93.50	86.2	2159	63.50	67.9
DLR3a	35	3	3340	95.42	87.9	2219	63.40	66.4
DLR3b	40	4	3320	83.00	76.5	2110	52.75	63.6

* Fecundity calculated with respect to PS Strain
** Calculated by dividing total hatch from total no. of eggs laid

Table 4. Effect of the deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Aedes aegypti* in the second gonotrophic cycle

Strain	No. of females Blood-fed	Days of Oviposition	Total No. of Eggs Laid	No. of Eggs per Female	Relative* Fecundity	Total Hatch	Hatch Per Female	% Hatch**
PS	32	4	3344	104.50	–	2592	81.00	77.5
DLR1a	30	3	2850	95.00	90.9	1890	63.00	66.3
DLR1b	30	2	1970	65.67	62.8	960	32.00	48.7
DLR2a	32	2	2205	68.91	65.9	1420	44.38	64.4
DLR2b	29	2	2030	70.00	67.6	1131	39.00	55.7
DLR3a	32	3	2512	78.50	75.1	1616	50.50	64.3
DLR3b	31	3	2110	68.06	65.1	1372	44.26	65.0

* Fecundity calculated with respect to PS Strain
** Calculated by dividing total hatch from total no. of eggs laid

Table 5. Effect of the deltamethrin and synergized deltamethrin selections on the fecundity and hatchability of *Aedes aegypti* in the third gonotrophic cycle

Strain	No. of females Blood-fed	Days of Oviposition	Total No. of Eggs Laid	No. of Eggs per Female	Relative* Fecundity	Total Hatch	Hatch Per Female	% Hatch**
PS	24	3	2380	99.17	–	1966	81.92	82.6
DLR1a	24	2	2184	91.00	91.8	1344	56.00	61.5
DLR1b	28	2	1785	63.75	64.3	1032	36.86	57.8
DLR2a	33	2	1500	45.45	45.8	825	25.00	55.0
DLR2b	34	2	1392	58.00	58.5	600	17.65	43.1
DLR3a	35	2	1716	71.50	72.1	1008	28.80	58.7
DLR3b	40	2	1773	65.66	66.2	1035	25.88	58.4

* Fecundity calculated with respect to PS Strain
** Calculated by dividing total hatch from total no. of eggs laid

DLR1 strains diminished only 4.5% in the second cycle. Further, during the 2nd cycle, the pattern of reduction was similar to the first one with maximum reduction of 37% recorded in DLR1b strain and 32% reduction in DLR3b strain. Other strains exhibited a range of 9 to 17% reduction. The hatchability rates also followed an identical pattern like that of 1st gonotrophic cycle. The DLR1b strain appeared to be the least fertile with only 48.7% larval hatch, though other selected strains also showed an appreciable reduction in the per cent hatch. Also, as compared to the parental population, the DLR1b strain exhibited 37% reduced hatchability and other DLR strains displayed 14-28% lower hatchability potential (Table 4). Again, the PS strain had longer oviposition period than the selected strains.

Further reduction in the egg laying capacity was noticed in third consecutive gonotrophic cycle in all the strains of *Ae. aegypti*. The maximum reduced fecundity was observed in DLR2a strain which laid 34% less eggs as compared to the second cycle (Table 5). Again DLR1 strains did not show reduced fecundity as compared to the earlier cycle. Further, 54% reduced fecundity was noticed in DLR2a strain as compared to the PS strain. Similar trend was recorded in the hatchability rates of various strains though the maximum decrease in fertility was observed in DLR2b strain, the reduced larval hatch proportion ranging from 25-48% (Table 5). No marked difference in the oviposition durations was seen in third cycle.

DISCUSSION

Deltamethrin is known to have very high insecticidal activity against various species of *Aedes* (Das & Kalyanasundaram, 1984; Kumar *et al.*, 2002). It has already been shown by Kumar *et al.* (2002) that field-collected strain of *Ae. aegypti* could develop appreciable levels of deltamethrin resistance on subjection to continuous deltamethrin selection pressure, which could be reversed while subjected to synergized deltamethrin selection pressure for successive generations.

The present studies demonstrated that successive selection of field-collected parent strain with deltamethrin/synergized deltamethrin also changed biotic performance of *Ae. aegypti*. It confirms the reproductive disadvantage in deltamethrin-resistant strains of *Ae. aegypti* with reduced reproductive fitness after continuous selection pressure. It has been reported that insecticide resistance is due to changes in the genetic constitution that, in turn, results in the changes in the biochemical and physiological properties (Hemingway *et al.*, 1998; Andreev *et al.*, 1999).

The duration of the successive gonotrophic cycles was found to be higher in the PS strain compared to the DLR strains. On the contrary, Priyalakshmi *et al.* (1999) recorded longer life cycle in an Indian strain of *Anopheles stephensi* treated with deltamethrin, when compared with control strain. No evidence for any change in the duration of the gonotrophic cycles of *Anopheles gambiae* was found in relation to the permethrin-treated bed nets (Quinones *et al.*, 1997).

Our investigations revealed deltamethrin-resistant DLR1b strain exhibiting much lower fecundity as compared to PS strain, though there was a significant reduction in the egg production of all the strains in successive gonotrophic cycles. In a Chinese strain of *Culex pipiens pallens* with 617-fold deltamethrin resistance, Li *et al.* (2002) reported 44.8% reduced fecundity after 12 generations of deltamethrin selection. In an Indian strain of *An. stephensi*, Priyalakshmi *et al.* (1999) reported 32-40% decrease in fecundity rates when treated with sublethal doses of deltamethrin at the larval stage. In the same year, Mohapatra *et al.* (1999) had reported significant decrease in the fecundity rates of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* after treatments with cyfluthrin and fenfluthrin. Earlier, Verma (1986) also recorded reduced fecundity of 43.3%, 67.2% and 40.6% after selection pressures of pyrethroids in *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*, respectively. On the contrary, an increased fecundity of 33% was reported in *Ae. aegypti* after subjection to the low doses of d-phenothrin which then reversed to the normal levels in next generation (Lui *et al.*, 1986). Whereas, Abedi & Brown (1960) observed an initial reduction of reproductive capability in North American populations of *Ae. aegypti* after prolonged selection pressure of DDT but regaining thereafter. Also, Inwang (1968) had confirmed lower oviposition rates in a malathion-resistant strain of *Ae. aegypti*.

A direct relationship among the size, amount of blood ingested and egg production was found in *Aedes albopictus* (Blackmore & Lord, 2000) and *Aedes nigromaculis* after DDT treatments (Miura & Takahashi, 1972). In *Culex pipiens*, Gaaboub & Dawood (1973) reported 40-50% reduction of fecundity on exposure to DDT and malathion selection pressure, separately, at the larval stage for 5 generations. Identical decrease in the size and fecundity was recorded, when subjected to DDT treatments, in *Culex gelidus* (Thomas, 1962) and in *Cx. quinquefasciatus* (Kerdpikule *et al.*, 1981).

Our studies also reveal similar patterns in hatchability like that of the fecundity rate. In the successive gonotrophic cycles, the parental populations exhibited maximum hatch in contrast to the insecticide-selected strains; further reducing gradually in later cycles. During first two cycles, DLR1b strain proved to be least fertile, whereas in the third cycle DLR1b strain was observed with lowest hatchability. The larval treatment of an Indian strain of *An. stephensi* with sublethal doses of deltamethrin exhibited 24% decrease in the fertility rates (Priyalakshmi *et al.*, 1999). Significant reduction in fertility rates of Indian strains of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* were recorded after exposure to cyfluthrin and fenfluthrin (Mohapatra *et al.*, 1999). However, Okoye *et al.* (2007) reported that 81.5% larval hatch from the eggs laid by the females of pyrethroid-resistant African strain of *Anopheles funestus* as compared to 66.9% hatch recorded in the susceptible strain. Earlier, Aiku *et al.* (2006) had observed that 2% pyriproxyfen on bed-nets has no effects on the fecundity of *An. stephensi*, but it reduced the fertility of eggs significantly. Rao & Shetty (1992) reported reduced hatch and sex ratio distortion towards males in *An. stephensi* when subjected to organophosphorous compounds at sublethal doses. Later, Ferrari & Georghiou (1981) reported adverse effects of temephos selection pressure on fertility of *Cx. quinquefasciatus*. Similar increase in the number of basal follicles in *Cx. pipiens* with reduced number of offspring had

In the present investigations the probable reason for reduced fecundity after selection pressure of insecticides may be the penetration of insecticides into the ova imparting toxic effects on the egg growth resulting in reduced fecundity rates. According to Inwang (1968) the low fecundity in malathion-resistant, *Ae. aegypti* was due to the long-term effects of organophosphates on follicle growth possibly causing inhibition of synthesis and transport of egg yolk proteins. Earlier, the egg mortality in insecticide-selected strains of different insects had been suggested to be due to the penetration of insecticide into the egg during embryogenesis (Grosscurt, 1977; Broadbent & Pree, 1984).

Our earlier laboratory studies have confirmed the development of significant levels of deltamethrin resistance in different strains of *Ae. aegypti* employed in the present investigations (Kumar *et al.*, 2002). There seems to be a direct correlation between the level of insecticide resistance and hatchability rate of *Ae. aegypti*. The DLR1b strain with highest level of deltamethrin resistance (703-fold) in the larvae proved to be the least fertile exhibiting lowest hatchability rate as compared to the other strains. The possible cause may be the selection pressure of insecticides at higher dosage as compared to the dosage subjected to the less resistant strains. Further, the potential of deltamethrin exerting delayed toxic effects, once penetrated may have resulted in reduced egg hatchability. Also, deltamethrin has been reported to have delayed ovicidal effects in mosquitoes when subjected to deltamethrin and permethrin selection pressure at larval stage (Sahgal & Pillai, 1993).

It is generally considered that a trade-off between fitness and insecticide resistance may result from pleiotropic effects of the genes involved in resistance (McKenzie & Batterham, 1994; Arnaud *et al.*, 2002). However, in some cases, the strains may differ in fitness for reasons independent of resistance (Roush & McKenzie, 1987). Okoye *et al.*, (2007) showed that pyrethroid resistance in southern African *An. funestus* does not incur any loss of fitness under laboratory conditions. They suggested that the removal of pyrethroid insecticide selection pressure may not lead to a regression of resistance alleles in pyrethroid resistant *An. funestus* populations in southern Africa.

The present studies make it clear that DLR3 adult-selected strains with negligible levels of deltamethrin resistance exhibit much lower reproductive fitness as compared to PS strain. Kumar *et al.* (2002) have earlier shown that the development of deltamethrin resistance *Ae. aegypti* is stage-specific and the surviving adults from the deltamethrin-selected larvae do not develop resistance to deltamethrin. The failure of larval/adult selection to induce the development of deltamethrin resistance in the adults of *Ae. aegypti* but success in causing reproductive disadvantage encourages the effectiveness and long-term use of deltamethrin as an adulticide against dengue vector.

Another remarkable observation in the present studies was the low fertility rates in DLR2 strains. It shows that larval selection of *Ae. aegypti* with synergized deltamethrin not only retards or reverses the deltamethrin resistance (Kumar *et al*., 2002) but it also provides reproductive disadvantage to the strain and reduces its reproductive fitness. Thus the use of PBO along with deltamethrin may help in reducing the frequency of resistant individuals with time with regression of resistance alleles. These observations could be useful in planning and implementing strategies for mosquito control.

Our investigations emphasize the fact that deltamethrin is an effective insecticide against the control of dengue vector mosquito. Despite causing the development of significant levels of larval resistance to deltamethrin after continuous subjection of selection pressure in *Ae. aegypti*, deltamethrin holds great promise as a control agent due to its efficacy as an adulticide and its ability to reduce reproductive potential of adult mosquito almost to half. Thus, the use of deltamethrin as an adulticide rather than as a larvicide, against *Ae. aegypti* is a more effective strategy from a resistance-management perspective.

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