



# Reversion of CYP450 monooxygenase-mediated acetamiprid larval resistance in dengue fever mosquito, *Aedes aegypti* L.

Roopa Rani Samal , Kungreilu Panmei, P. Lanbiliu and Sarita Kumar 

Department of Zoology, Acharya Narendra Dev College, University of Delhi, Kalkaji, New Delhi 110019, India

## Research Paper

**Cite this article:** Samal RR, Panmei K, Lanbiliu P, Kumar S (2022). Reversion of CYP450 monooxygenase-mediated acetamiprid larval resistance in dengue fever mosquito, *Aedes aegypti* L.. *Bulletin of Entomological Research* **112**, 557–566. <https://doi.org/10.1017/S0007485321001140>

Received: 25 June 2021  
Revised: 26 November 2021  
Accepted: 6 December 2021  
First published online: 24 February 2022

### Keywords:

Acetamiprid; *Aedes aegypti*; monooxygenase; PBO; resistance; synergism

### Author for correspondence:

Sarita Kumar, Email: [sarita.sanjay90@gmail.com](mailto:sarita.sanjay90@gmail.com); [saritakumar@andc.du.ac.in](mailto:saritakumar@andc.du.ac.in)

### Abstract

*Aedes*-borne diseases are on the rampant rise despite continued application of chemical insecticide-based interventions. The appearance of high degree of insecticide resistance in *Aedes* species and noxious effects on environment and non-targets have raised further concerns. Among new chemical interventions, neonicotinoids are considered a safe and effective approach. The present study investigated the control potency of acetamiprid and development of resistance in *Aedes aegypti* larvae; and the involvement of CYP450 monooxygenases in inducing resistance. The early fourth instars of *Ae. aegypti* parent susceptible strain (PS) were selected with acetamiprid for 15 generations (ACSF strain) increasing the resistance to 19.74-fold in ACSF-10 and 36.71-fold in ACSF-15. The ACSF-10 larvae were assayed with acetamiprid combined with piperonyl butoxide (PBO) in three different ratios (1:1, 1:5 and 1:10) and selected for next five generations with 1:10 combination. Selection with synergized acetamiprid (APSF strains) reversed as well as reduced the rate of resistance development resulting in only 1.35-fold resistance in APSF-15. The APSF strains showed %monooxygenase dependency ranging from 86.71 to 96.72%. The estimation of the monooxygenases levels in parent and selected larvae showed increased monooxygenase level in the ACSF strains by 2.42–2.87-fold. The APSF-15 strains exhibited 57.95% lower enzyme production than ACSF-15 strain. The reduction and reversion of resistance by using PBO and the elevated levels of monooxygenases in ACSF and reduction in APSF strains recommend the involvement of CYP450-mediated mechanism in the development of acetamiprid resistance in *Ae. aegypti*. These studies could help in devising resistance management strategies in order to preserve the efficiency of pre-existing insecticides.

## Introduction

*Aedes aegypti* L. is a widespread mosquito responsible for the transmission of ever-increasing infections causing extensive though variable degree of health hazards in the world, especially in tropical and sub-tropical regions. In several countries, *Aedes*-borne disease, dengue, has become a principal health concern due to worrisome rise to 390 million annual dengue infections with 96 million clinical manifestations (Bhatt *et al.*, 2013). India has recorded a total of 39,419 dengue cases and 56 deaths in year 2020 and 39,419 suspected cases of Chikungunya (NVBDCP, 2021a, 2021b).

In the absence of vaccines and adequate medication, mosquito-borne diseases are primarily kept under check *via* mosquito management, at larval as well as adult stage. The traditional ways of interventions, such as use of mosquito bed nets, window screens, etc., are still in practice widely. Yet, application of chemical-based measures is on rampant rise to manage complicated-resistant mosquitoes quickly and effectually (Liu *et al.*, 2006; Kumar *et al.*, 2009). Various groups of chemical toxicants have been used against mosquitoes; however, the negative impact of these on the surroundings, and non-target organisms along with the appearance of high insecticide resistance levels among mosquitoes has caused concerns (Bonner *et al.*, 2007; Moore *et al.*, 2009). Several countries have reported insecticide resistance in *Ae. aegypti*, including India (Kushwah *et al.*, 2015), Brazil (Lima *et al.*, 2011), China (Li *et al.*, 2015), Colombia (Fonseca-González *et al.*, 2011), Malaysia (Ishak *et al.*, 2015) and Thailand (Yanola *et al.*, 2011); and revealed metabolic detoxification and decreased sensitivity of insecticide-target proteins as the prime cause for the resistance (Bansal *et al.*, 2012; Yang and Liu, 2014).

Among the new approaches and chemical interventions, neonicotinoids, synthetic derivatives of nicotine, are one of the fastest-growing insecticides and considered a safe replacement of the conventional insecticides currently used in the mosquito management. These chemicals induce toxicity in the target insect pest by interacting with nicotinic acetylcholine receptors (nAChRs) of the insect nervous system mediating fast cholinergic transmission (Li *et al.*, 2012). Acetamiprid, a neonicotinoid, reacts with nAChRs located in the post-synaptic neural

© The Author(s), 2022. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

dendrites of central nervous system, ganglia and muscular junctions imparting contact as well as stomach toxicity (Jian-chu *et al.*, 2002; Kimura-Kuroda *et al.*, 2012; Sanche-Bayo, 2012). It has been reported that acetamiprid is selectively toxic to the insects, does not bio-accumulate in the sediments and fish, and is safer to the environment relative to the other insecticides in use (Ambrose, 2003). Despite a few reports of neonicotinoid resistance and cross-resistance in *Bemisia tabaci* (Gennadius) (Horowitz *et al.*, 2004), *Musca domestica* (Kristensen and Jespersen, 2008), *Frankliniella occidentalis* (Pergande) (Gao *et al.*, 2014) and *Leptinotarsa decemlineata* (Say) (Mota-Sanchez *et al.*, 2006); reports of such resistance are negligible in *Ae. aegypti*. Yet, all insects including mosquitoes have the capability to develop resistance to any toxicant, sooner or later. Therefore, it becomes important to understand the mechanism of resistance to a particular insecticide based on which resistance management strategies can be devised.

A key module of resistance management is based on the use of synergists which may reduce as well as reverse the development of resistance development in insects. Piperonyl butoxide (PBO) is a well-known insecticide synergist which impedes the cytochrome P450-mediated metabolism of an insecticide and enhances its toxicity. Several insecticides, majorly synthetic pyrethroids, used against agricultural or public health pests contain PBO as an active ingredient (Cetin *et al.*, 2019). Yet effective use of PBO as a synergist to other insecticide groups is being attempted to develop successful resistance management programme (Khan *et al.*, 2014).

Current study investigates the development of acetamiprid resistance in *Ae. aegypti* larvae and possible use of PBO as a synergist of acetamiprid to reduce or reverse the speed of development of resistance. Since PBO is an inhibitor of cytochrome P450 monooxygenases, the study will help to elucidate the involvement of metabolic enzyme (CYP450) in the development of acetamiprid resistance in *Ae. aegypti* and design an effective management strategy.

## Materials and methods

### Culture of *Aedes aegypti* L.

Pure line of *Ae. aegypti* has been maintained in the Rearing Unit of Acharya Narendra Dev College, New Delhi, India since last 10 years; without subjection to any insecticide selection pressure. The rearing conditions have been set at  $28 \pm 1^\circ\text{C}$  temperature,  $80 \pm 5\%$  relative humidity and 12 h:12 h (light:dark) photo-regime to ensure optimal growth, feeding, mating and oviposition (Warikoo *et al.*, 2012; Samal and Kumar, 2018). The pure line of *Ae. aegypti*, marked as the Base-Line, was considered as the parent susceptible strain (PS) (Samal *et al.*, 2020). General hygiene and sterility during rearing has been ensured to prevent infections, pest attack and infestations by potential predators and parasites, roaches and book lice, and to protect egg stocks and other stages (Zheng *et al.*, 2015).

Adult *Ae. aegypti* were kept in the  $45 \times 40 \times 40$  cm clothed cages and fed on sugary juice of deseeded water-soaked raisins. Female mosquitoes were given blood meals on alternate days, for at least an hour, to ensure adequate egg maturation. Eggs were collected in an ovitrap; consisting of a small enamel/plastic bowl lined with Whatman filter paper strips and filled two-third with dechlorinated water. The egg strips were then transferred into enamel/plastic trays ( $25 \times 30 \times 5$  cm) filled with at least 1.5–2.0 litres of dechlorinated water. A total of 200 larvae were

reared in each tray and were provided with an artificial diet (15 mg) of powdered dog biscuits and active yeast in a ratio of 3:1 by weight (Warikoo *et al.*, 2012). Water was changed every day to avoid the formation of any froth on its surface.

### Chemicals used

Technical grades of acetamiprid (99.9% purity) and PBO (99% purity) were procured from M/s Sigma-Aldrich, India. Desired concentrations were prepared in ethanol (eMerck) and stored at  $4^\circ\text{C}$ .

### Larvicidal efficacy and larval selection with acetamiprid

The toxic level of acetamiprid was assessed against early fourth instars of the PS of *Ae. aegypti*, based on standard WHO protocol (Samal and Kumar, 2021).

The larval selection of the PS strain was carried out at early fourth instar stage by imparting the selection pressure of acetamiprid at  $\text{LC}_{90}$  level as described in our earlier reports (Samal and Kumar, 2021). Five batches of healthy instars, each batch containing 200 larvae, were subjected to the insecticide pressure for a day. Survived larvae were strained, cleaned and reared to adults, the generation marked as acetamiprid-selected strain (ACSF – Acetamiprid Larval-Selected Filial).

The acetamiprid selection was continued till 15 successive generations (ACSF-1 to ACSF-15). The resistance level to acetamiprid induced was estimated in each generation according to equation 1 (Kumar *et al.*, 2002; Samal and Kumar, 2021).

$$\text{Resistance ratio} = \frac{\text{LC}_{50} \text{ value of acetamiprid against ACSF strain}}{\text{LC}_{50} \text{ value of acetamiprid against PS strain}} \quad (1)$$

### Larvicidal efficacy and larval selection with acetamiprid synergized with PBO

Three different combinations of acetamiprid and PBO (1:1, 1:5 and 1:10) were evaluated for their larvicidal efficacy against ACSF-10 strain of *Ae. aegypti*. The bioassays were run as per the protocol described in section 'Larvicidal efficacy and larval selection with acetamiprid'. Based on the efficacy and aim to use less toxic component in the combination, the acetamiprid added with PBO in 1:10 ratio was selected for further studies.

The early fourth instars of ACSF-10 population were selected with synergized acetamiprid (1:10) at  $\text{LC}_{90}$  value and the resultant strain was marked as APSF (Acetamiprid + PBO Larval-Selected Filial). The selection pressure was continued for next five successive generations to obtain APSF-15 strain.

The synergistic efficacy of PBO when combined with acetamiprid was evaluated by calculating the synergistic ratio and per cent suppression of acetamiprid resistance in each selected generation (equation 2).

Synergistic ratio (SR)

$$= \frac{\text{LC}_{50} \text{ value of Acetamiprid against ACSF-10 strain}}{\text{LC}_{50} \text{ value of Acetamiprid + PBO (1:10) against APSF strain}} \quad (2)$$

SR > 1 denotes synergistic effect; SR < 1 shows antagonistic effect; SR = 1 signifies additive effect.

### Estimation of CYP450 monooxygenase level in selected strains

Larvae of the following strains were selected for the estimation of the level of cytochrome P450 monooxygenase.

- (a) PS: Parent susceptible strain
- (b) ACSF-5: PS strain selected with acetamiprid at larval stage for five successive generations
- (c) ACSF-10: PS strain selected with acetamiprid at larval stage for ten successive generations
- (d) ACSF-15: PS strain selected with acetamiprid at larval stage for 15 successive generations
- (e) APSF-15: ACSF-10 strain selected with acetamiprid + PBO (1:10) at larval stage for five successive generations

### Synergistic ratios-based estimation of CYP450 monooxygenase level in selected strains

The SR-based monooxygenase levels *in vivo* were estimated in the strains according to the methodology of Osman and Brindley (1981) as adopted by Kumar *et al.* (1991) using synergistic difference (SD) and per cent dependency of mosquitoes on monooxygenase (%D) as the parameters. The observed synergistic differences (OSD) were calculated as per equation 3.

$$\text{OSD} = (\text{Unsynergised LC}_{50}\text{value}) - (\text{Synergised LC}_{50}\text{value}) \quad (3)$$

The expected SD (ESD) value being different from the observed SD value was calculated from a regression line expressing the linear relationship between LC<sub>50</sub> value of acetamiprid and synergistic difference (Osman and Brindley, 1981). The calculation was made according to the following equation 4:

$$\text{Log}(\text{Unsynergised LC}_{50}) = 1.014 \text{log(ESD)} - 0.01 \quad (4)$$

The calculated ESD value indicates the measurement which would have been if the mosquitoes were primarily dependent upon the monooxygenase system. Hence, this deviation expresses the relative dependency of mosquitoes upon monooxygenases and was calculated as follows (equation 5):

$$\text{Per cent dependency (\%D)} = \frac{\text{Observed synergistic difference (OSD)}}{\text{Expected synergistic difference (ESD)}} \quad (5)$$

### Biochemical estimation of CYP450 monooxygenase levels in selected strains

A total of newly emerged early fourth instars (100 larvae in five batches; each batch of 20 replicates) from PS, ACSF-5, ACSF-10, ACSF-15 and APSF-15 were selected for the CYP450 monooxygenase level estimation to assess the correlation between enzyme and acetamiprid-resistance level. The methodology of Brogdon *et al.* (1997) and WHO (1998) modified by Kona *et al.* (2018) was adopted for the assay. Each larva was homogenized in 200 µl of ice-cold autoclaved water with the help of a micro-homogenizer. The homogenate was spun at 17,000 × g for 30 s in a refrigerated microfuge (Hanil Science Smart R17 micro refrigerated centrifuge) and supernatant was used for monooxygenase estimation. The volume of 20 µl of the supernatant of each larval homogenate was pipetted out in separate microtiter

plate wells. To each well, 80 µl of 0.625 M potassium phosphate buffer was added to establish a reaction system. The mixture was supplemented with 200 µl of solution comprising one part of 8 mM methanolic solution of tetramethyl benzidine and three parts of 0.25 M sodium acetate buffer (pH 5.0) followed by addition of 25 µl of 0.88 M hydrogen peroxide (Brogdon *et al.*, 1997; WHO, 1998; Kona *et al.*, 2018). The final solution was incubated at room temperature for 10–15 min (Kona *et al.*, 2018). The absorbance was measured at 620 nm to estimate the concentration of monooxygenase in each larval strain.

## Results

### Larvicidal efficacy and larval selection with acetamiprid

The selection of *Ae. aegypti* with acetamiprid for 15 successive generations, at early fourth instar stage resulted into a continued decrease in susceptibility to acetamiprid. Out of 1000 larvae tested (200 larvae in five batches), only 157 larvae survived the selection pressure. The larvae showed reduced susceptibility by 94.93% in ACSF-10 and by 97.28% in ACSF-15 as compared to the PS larvae. The tolerance level of the larvae increased to 8.83-fold in ACSF-5 ( $P < 0.05$ ) and 19.74-fold in ACSF-10 ( $P < 0.05$ ) (reported earlier in Samal and Kumar, 2021) which drastically rose to 36.71-fold in ACSF-15 ( $P < 0.05$ ) (table 1). The gradual right shift of the d-m-r (dosage mortality regression) lines of ACSF strains denoting the speed of acetamiprid resistance development in larvae shows maximum shift in last five generations indicating the rapid development of resistance (fig. 1).

### Larvicidal efficacy and larval selection with acetamiprid synergized with PBO

#### Larvicidal bioassay with synergized acetamiprid

Larvicidal bioassay of ACSF-10 early fourth instars with acetamiprid combined with PBO in three different ratios (1:1, 1:5 and 1:10) enhanced the toxic effects of acetamiprid and reduced the developed resistance. The maximum synergistic effect was evident with acetamiprid + PBO (1:10) drastically depleting the acetamiprid resistance in ACSF-10 larvae from 19.74- to 1.24-fold (table 2). Other two combinations of synergized acetamiprid, 1:1 and 1:5, decreased the resistance ratio by 6.22- and 1.72-fold, respectively ( $P < 0.05$ ) (table 2). Since the synergized acetamiprid (1:10) imparted 1.39 times higher synergistic effects than the 1:5 combination and 5.02 times more than 1:1 ratio, further selection of ACSF-10, for acetamiprid resistance management, was held with acetamiprid and PBO in 1:10 ratio.

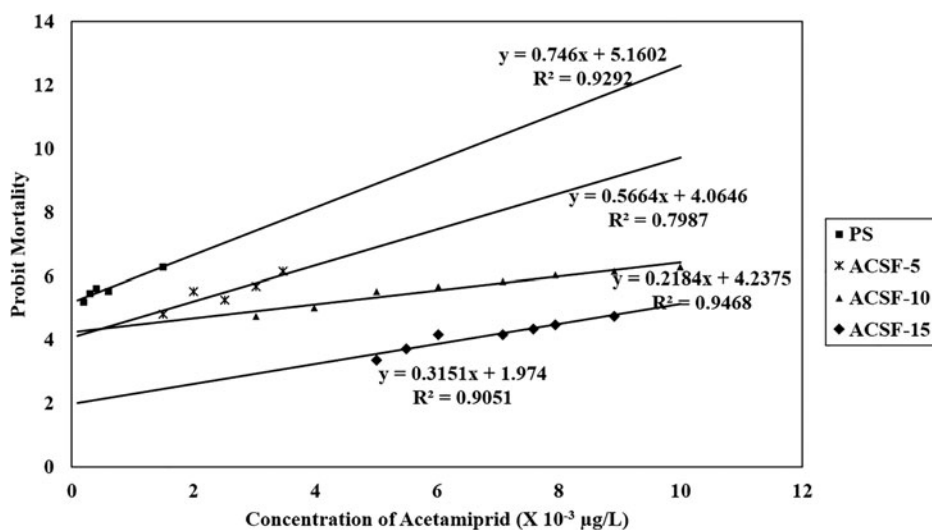
#### Selection of ACSF-10 larvae with acetamiprid + PBO (1:10)

A reversion in the acetamiprid resistance levels as well as reduction in the rate of resistance development was observed on continuous selection of the early fourth instar of ACSF-10 with acetamiprid and PBO (1:10). The APSF population developed insignificant levels of acetamiprid resistance, just 1.24-fold in APSF-11 and 1.72-fold in APSF-12, as against 16.22- and 23.66-fold on selection with acetamiprid alone ( $P < 0.05$ ). Further selection with synergized acetamiprid reversed the resistance to 1.35-fold in APSF-15 (table 3). Notably, the selections with synergized acetamiprid led to 86.71–96.33% suppression in acetamiprid resistance (table 3, fig. 2).

**Table 1.** LC<sub>50</sub> and LC<sub>90</sub> (in mg litre<sup>-1</sup>) values of acetamiprid against early fourth instars of *Aedes aegypti* when selected with acetamiprid for 15 successive generations

Strain	Larval LC <sub>50</sub> to acetamiprid (mg litre <sup>-1</sup> ) ± SEM	Larval LC <sub>90</sub> to acetamiprid (mg litre <sup>-1</sup> ) ± SEM	χ <sup>2</sup> (df)	Slope ± SEM	Fold increase in tolerance LC <sub>50</sub> (RR)	Fold increase in tolerance LC <sub>90</sub> (RR)
PS	0.18799 ± 0.03415 a	1.31547 ± 0.20707 a	3.395 (3)	1.52 ± 0.29	–	–
ACSF-5	1.65916 ± 0.05907 b	4.50887 ± 1.00328 b	2.285 (3)	2.95 ± 0.15	8.83	3.43
ACSF-10	3.71057 ± 0.11183 c	10.08811 ± 0.91644 c	0.959 (6)	2.97 ± 0.19	19.74	7.67
ACSF-15	6.90180 ± 0.22743 d	14.5258 ± 0.91349 d	0.829 (7)	7.14 ± 0.05	36.71	11.04

PS, Parental Strain; ACSF, Acetamiprid Larval-Selected Filial; RR, resistance ratio; LC<sub>50</sub>, lethal concentration at which 50% larvae are killed; LC<sub>90</sub>, lethal concentration at which 90% larvae are killed; SEM, standard error of mean; df, degree of freedom; RR, resistance ratio; SR, synergistic ratio; LC values in each column followed by different letters are significantly different  $P < 0.05$ ; one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

**Figure 1.** Dosage-mortality regression lines on selection of *Aedes aegypti* early fourth instars with acetamiprid for successive generations. PS, parent susceptible strain; ACSF-5, Acetamiprid Larval-Selected Filial-5; ACSF-10, Acetamiprid Larval-Selected Filial-10; ACSF-15, Acetamiprid Larval-Selected Filial-15.**Table 2.** Larval LC<sub>50</sub> and LC<sub>90</sub> (in mg ml<sup>-1</sup>) of ACSF-10 strain of *Aedes aegypti* when assayed with acetamiprid combined with PBO in different ratios

Strain	Acetamiprid + PBO	LC <sub>50</sub> in mg litre <sup>-1</sup> ± SEM	LC <sub>90</sub> in mg litre <sup>-1</sup> ± SEM	χ <sup>2</sup> (df)	Slope ± SEM	RR	SR
ACSF-10	–	3.71057 ± 0.11183 a	10.08811 ± 0.91644 a	0.959 (6)	2.97 ± 0.19	19.74	–
ACSF-10 ACE + PBO	1:1	1.16934 ± 0.18562 b	2.31934 ± 0.56982 b	1.389 (3)	4.31 ± 1.13	6.22	3.17
ACSF-10 ACE + PBO	1:5	0.32404 ± 0.03569 c	0.41827 ± 0.06598 c	0.151 (4)	11.56 ± 4.05	1.72	11.45
ACSF-10 ACE + PBO	1:10	0.23311 ± 0.01844 d	0.86004 ± 0.02267 d	6.182 (6)	2.26 ± 0.09	1.24	15.92

ACSF, Acetamiprid Larval-Selected Filial generation; ACE, acetamiprid; PBO, piperonyl butoxide; LC<sub>50</sub>, lethal concentration at which 50% larvae are killed; LC<sub>90</sub>, lethal concentration at which 90% larvae are killed; SEM, standard error of mean; df, degree of freedom; RR, resistance ratio; SR, synergistic ratio. LC values in each column followed by different letters are significantly different  $P < 0.05$ ; one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

### Synergistic ratios-based estimation of CYP450 monooxygenase level in selected strains

The *Ae. aegypti* APSF strains showed %monooxygenase dependency ranging from 86.71 to 96.72%; the minimum dependency observed in APSF-13 (table 4). A positive correlation ( $r = 1.48$ – $3.06$ ) was recorded in the monooxygenase activity in the APSF strains and the respective LC<sub>50</sub> value.

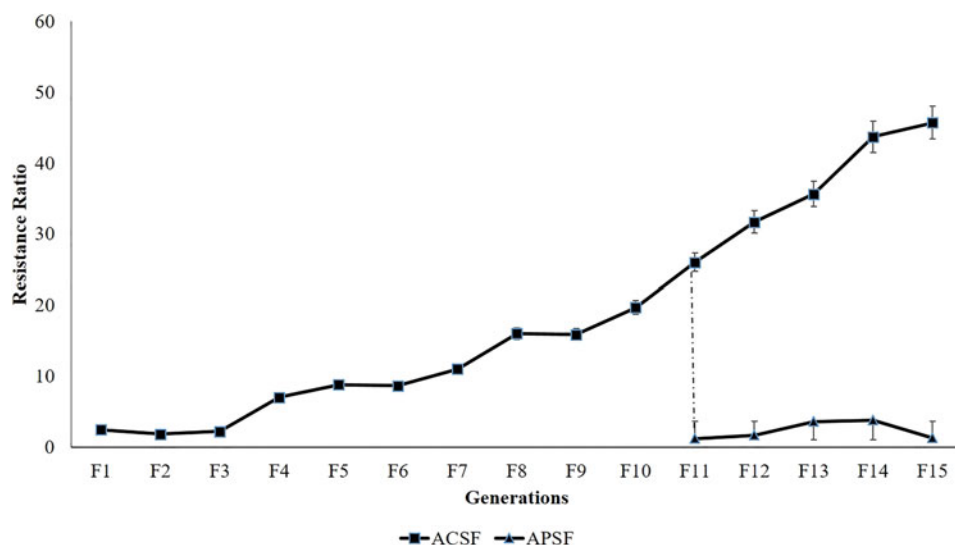
### Biochemical estimation of CYP450 monooxygenase levels in selected strains

The estimation of monooxygenases in PS revealed 0.0036 ( $\pm 0.0002$ ) mmoles mg<sup>-1</sup> of protein in different larval groups, while the total protein in the larval body was 3.8876  $\mu\text{g}\mu\text{l}^{-1}$  (table 5). In comparison, the ACSF larvae displayed upsurge in the enzyme levels, 2.42-fold in ACSF-5 ( $P < 0.05$ ) and 2.68-fold

**Table 3.** Larval LC<sub>50</sub> and LC<sub>90</sub> (in mg litre<sup>-1</sup>) of ACSF-10 strain of *Aedes aegypti* when selected with acetamiprid alone and acetamiprid + PBO (1:10) for five successive generations

Generations	F <sub>10</sub>	F <sub>11</sub>	F <sub>12</sub>	F <sub>13</sub>	F <sub>14</sub>	F <sub>15</sub>
Strain						
Acetamiprid larval-selected	ACSF-10	ACSF-11	ACSF-12	ACSF-13	ACSF-14	ACSF-15
LC <sub>50</sub> ± SEM	3.71057 ± 0.11183 a	3.04891 ± 0.05901 b	4.45069 ± 0.18105 c	5.17950 ± 0.28061 d	6.44338 ± 0.2069 e	6.90180 ± 0.22743 f
LC <sub>90</sub> ± SEM	10.08811 ± 0.91644 a	6.32572 ± 0.57076 b	8.93897 ± 0.56942 a	10.55190 ± 1.5007 a	13.40110 ± 1.09881 c	14.5258 ± 0.91349 c
χ <sup>2</sup> (df)	0.959 (6)	1.514 (6)	0.829 (6)	0.691 (6)	0.804 (6)	0.829 (7)
Slope ± SEM	2.97 ± 0.19	5.061 ± 0.086	4.527 ± 0.092	5.507 ± 0.069	6.565 ± 0.058	7.140 ± 0.051
RR	19.74	16.22	23.68	27.55	34.28	36.71
Acetamiprid + PBO (1:10) larval-selected	–	APSF-11	APSF-12	APSF-13	APSF-14	APSF-15
LC <sub>50</sub> ± SEM	–	0.23311 ± 0.01844 a	0.32374 ± 0.049 b	0.68829 ± 0.05054 c	0.72407 ± 0.05954 c	0.25306 ± 0.04085 a
LC <sub>90</sub> ± SEM	–	0.86004 ± 0.02267 a	0.93821 ± 0.16207 b	1.80764 ± 0.13043 c	2.28888 ± 0.27672 d	1.83977 ± 0.69312 d
χ <sup>2</sup> (df)	–	6.182 (6)	2.910 (6)	0.987 (3)	4.386 (5)	1.828 (4)
Slope ± SEM	–	2.26 ± 0.09	2.77 ± 0.09	3.06 ± 0.12	2.56 ± 0.10	1.48 ± 0.49
RR	–	1.24	1.72	3.66	3.85	1.35
RR wrt ACSF-10	–	15.92	11.46	5.39	5.12	14.66
SR	–	13.08	13.75	8.31	8.90	27.31
% Suppression	–	92.35	92.73	86.71	86.76	96.33

ACSF, Acetamiprid Larval-Selected Filial; APSF, Acetamiprid + PBO Larval-Selected Filial; LC<sub>50</sub>, lethal concentration at which 50% larvae are killed; LC<sub>90</sub>, lethal concentration at which 90% larvae are killed; SEM, standard error of mean; df, degree of freedom; RR, resistance ratio; SR, synergistic ratio; LC values in each row followed by different letters are significantly different *P* < 0.05; one-way ANOVA followed by Tukey's all pair wise multiple comparison test.



**Figure 2.** Resistance ratios in successive generations of *Aedes aegypti* larvae. ACSF, parent susceptible strain selected with acetamiprid alone (ACSF) for 15 generations; APSF, ACSF-10 strain selected with acetamiprid + PBO(1:10) for next five generations.

in ACSF-10 (*P* < 0.05). A similar elevation in the monooxygenase activity (2.87-fold) was observed in ACSF-15 which was 1.07-fold higher than that in PS and ACSF-10, respectively. Alternatively, a sudden and significant decline in the monooxygenase activity was observed in APSF-15 with respect to PS (9.28%) and ACSF-15 (57.95%) indicating the role of monooxygenases in imparting acetamiprid resistance to *Ae. aegypti* larvae. The box plot distribution of monooxygenase activity in all the five strains implied the heterogeneity in the population of each strain; more variation

observed in ACSF-5 and ACSF-10 in comparison to the ACSF-15 and APSF-15 (fig. 3).

The frequency distribution profiles of acetamiprid-selected larvae showed a drastic shift in the absorbance peak from 0.4 in PS to 1.0 in the selected larvae (ACSF-5) (fig. 4). In comparison to ACSF-5, 7% higher frequency of population in ACSF-10 and 20% more in ACSF-15 attained the peak. The profile also revealed the presence of high per cent of resistant individuals beyond the susceptible threshold in selected population, 80% in ACSF-5, 87% in

**Table 4.** Per cent dependency on monoxygenase in *Aedes aegypti* early fourth instar when selected with acetamidrid + PBO (1:10)

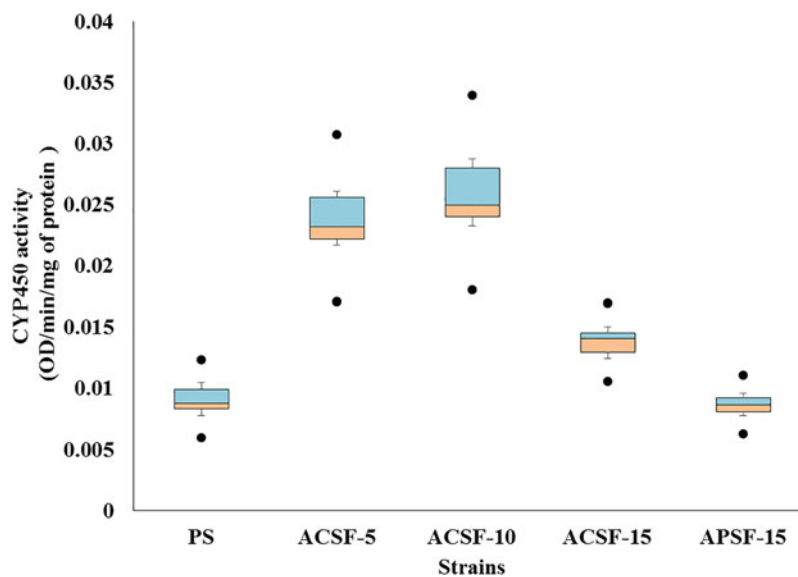
Strain	LC <sub>50</sub> (mg litre <sup>-1</sup> ) ± SEM	Regression coefficient ( <i>r</i> )	Synergistic ratio	OSD	ESD	% Dependency	
Un-synergized strain	ACSF-10	3.71057 ± 0.11183 a	2.97	–	–	–	
Synergized strains	APSF-11	0.23311 ± 0.01844 b	2.26	13.08	3.47746	3.72770	91.68
	APSF-12	0.32374 ± 0.04900 c	2.77	13.75	3.38683		92.53
	APSF-13	0.68829 ± 0.05054 d	3.06	8.31	3.02228		86.71
	APSF-14	0.72407 ± 0.05954 d	2.56	8.90	2.98650		89.03
	APSF-15	0.25306 ± 0.04085 b	1.48	27.31	3.45751		96.72

ACSF, Acetamidrid Larval-Selected Filial; APSF, Acetamidrid + PBO Larval-Selected Filial; OSD, observed synergistic ratio; ESD, expected synergistic ratio; LC<sub>50</sub>, lethal concentration at which 50% larvae are killed; SEM, standard error of mean. LC values followed by different letters are significantly different  $P < 0.05$ ; one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

**Table 5.** Level of monoxygenases in parent susceptible and selected strains of *Aedes aegypti*

Strains	Protein concentration (µg µl <sup>-1</sup> ) ± SEM	CYP450 Monoxygenase (mmoles mg <sup>-1</sup> of protein) ± SEM	CYP450 Monoxygenase (OD min <sup>-1</sup> mg <sup>-1</sup> ) ± SEM	CYP450 Monoxygenase (OD min <sup>-1</sup> mg <sup>-1</sup> ) fold increase in activity wrt PS
PS	3.8876 ± 0.1327 a	0.0036 ± 0.0002 a	0.0097 ± 0.0005 a	–
ACSF-5	3.8379 ± 0.1281 a	0.0088 ± 0.0002 b	0.0235 ± 0.0002 b	2.42
ACSF-10	4.0057 ± 0.1403 a	0.0097 ± 0.0003 c	0.0260 ± 0.0004 c	2.68
ACSF-15	4.4136 ± 0.1357 b	0.0104 ± 0.0004 c	0.0278 ± 0.0010 d	2.87
APSF-15	3.7318 ± 0.1533 a	0.0033 ± 0.0001 a	0.0088 ± 0.0003 e	0.91

PS, parental strain; ACSF, Acetamidrid Larval-Selected Filial; APSF, Acetamidrid + PBO Larval-Selected Filial; SEM, standard error of mean. Each strain had five replicates. Each replicate consisted of 20 larvae ( $N = 100$  larvae). Figures in each column followed by different letters are significantly different ( $P < 0.05$ ); one-way ANOVA followed by Tukey's all pair wise multiple comparison test.



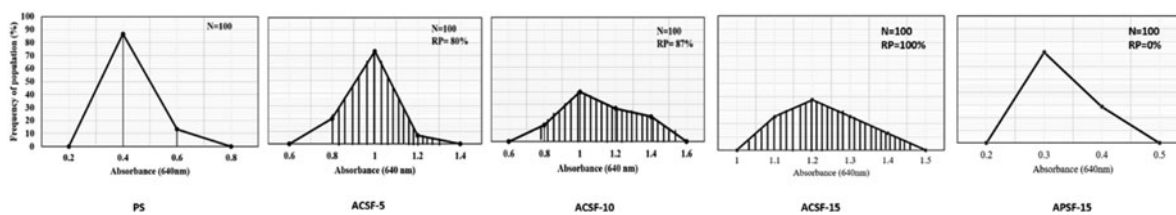
**Figure 3.** Box plot distribution of range of P450 monoxygenase (OD min<sup>-1</sup> µg<sup>-1</sup> of protein) in the larvae of PS, ACSF-5, ACSF-10, ACSF-15 and APSF-15 strains of *Aedes aegypti*. Middle line between the boxes represents the median; upper and lower boxes represent the 25 and 75 percentiles of the data; whiskers represent the standard error of the mean; the dots above and below the whiskers represent the outliers. PS, parent strain; ACSF-5, Acetamidrid Larval-Selected Filial-5; ACSF-10, Acetamidrid Larval-Selected Filial-10; ACSF-15, Acetamidrid Larval-Selected Filial-15; APSF-15, Acetamidrid + PBO Larval-Selected Filial-10.

ACSF-10 and 100% in ACSF-15. However, synergized acetamidrid-selected strain (APSF-15) did not show resistant individuals beyond the threshold level suggesting reversion of resistance (fig. 4).

## Discussion

Insecticide resistance, considered a pre-adaptive phenomenon, has emerged as the greatest problem to control all insect groups including disease vectors. The prolonged and frequent usage of

insecticides in various public health programmes, crop fields and domestic areas has eliminated susceptible individuals and selected resistant individuals resulting in the emergence of resistant strains (Uragayala *et al.*, 2015). It is proposed that prior to experiencing high insecticide exposure, a few organisms can survive the stress due to altered genome and get selected post-exposure (Faucon *et al.*, 2015). These immune organisms carry the genetic variance to the successive generation contributing to the resistance gene pool. Gradual and sequential selection through



**Figure 4.** Frequency distribution of absorbance values of P450 monoxygenase  $\text{mmol min}^{-1} \text{mg}^{-1}$  of protein in the PS, ACSF-5, ACSF-10, ACSF-15 and APSF-15 strains of *Aedes aegypti*. Susceptibility threshold based on maximum absorbance in PS strain. Shaded region represents the resistant population (beyond the threshold). N, number of larvae; RP, resistant population; PS, parent strain; ACSF-5, Acetamiprid Larval-Selected Filial-5; ACSF-10, Acetamiprid Larval-Selected Filial-10; ACSF-15, Acetamiprid Larval-Selected Filial-15; APSF-15, Acetamiprid + PBO Larval-Selected Filial-10.

inheritance increases the proportion of organisms possessing the resistance genes, alleles or polymorphisms. Ultimately, subjection to extended insecticide exposure outweighs the resistant organisms than the susceptible population.

Utilization of insecticides at a widespread scale resulting in resistance to that particular toxicant and cross-resistance to other insecticides has caused re-emergence of mosquito-borne disease in many parts of the world (Zaim and Guillet, 2002). Consequently, neonicotinoids, considered relatively safe chemicals, are under exploration as the possible and efficient control interventions (Hemingway *et al.*, 2002). Though, a few lepidopterans, hemipterans and coleopterans have been recorded with neonicotinoid resistance (Liu *et al.*, 2005; Qiong *et al.*, 2012), such resistance in mosquitoes, particularly to acetamiprid, is not yet cited in literature. Further, being approved by World Health Organization for its use in public health programmes (US-EPA, 2002), acetamiprid is being investigated as the probable control agent. The current study evaluated acetamiprid against *Ae. aegypti* larvae for imparting toxic effects. The possible development of acetamiprid resistance was assessed in *Ae. aegypti* and synergistic studies were carried out as a resistance management strategy.

Synergists, the inhibitors of the detoxifying enzymes, are known to inhibit the metabolic enzymes – primarily P450s and esterases; and enhance the toxicity of an insecticide (Lorini and Galley, 2000; Cetin *et al.*, 2019). These compounds thus have been frequently employed to combat resistance and effectively control target pest species (Lorini and Galley, 2000). In fact, comparative assessment of the toxic impact of synergized and unsynergized insecticides on target population can recognize and conclude the detoxification mechanisms involved in the development of resistance to that particular insecticide. Synergistic studies with the pyrethroid-resistant *Aedes*, *Anopheles* and *Culex* species have strongly backed the role of metabolic detoxification in imparting resistance (Brogdon *et al.*, 1999; Enayati *et al.*, 2003; Liu *et al.*, 2004; Cuamba *et al.*, 2010).

The commonly used insecticide synergists include PBO, S,S, S-tributyl phosphorotrithioate (DEF) and N-Octyl bicycloheptene dicarboximide (MGK-264). The combination of PBO with pyrethroids and organophosphates is recommended in insecticide formulations to reduce the insecticide concentration in vector control, thereby declining the risk of bioaccumulation. Consequently, many PBO-containing pesticide formulations have been used against a variety of vectors (Cetin *et al.*, 2010). However, till date and as per our knowledge, the efficacy of PBO on acetamiprid susceptibility against *Ae. aegypti* has not been evaluated though synergistic activity of PBO with imidacloprid has been reported against *Ae. aegypti* (Riaz *et al.*, 2013) and *Cx. pipiens* (Ahmed and Othman, 2020).

Thus, the present study evaluated the potential role of PBO in reducing and reversing the acetamiprid resistance and designing

an effective resistance management strategy in *Ae. aegypti*. After continuous laboratory selection for 15 consecutive generations with unsynergized acetamiprid, the larvae developed 36.71-fold resistance to acetamiprid. However, selection of ACSF-10 strain of *Ae. aegypti* with synergized insecticide (1:10) reduced and reversed the rate of resistance development significantly. This supports the overproduction of P450 monoxygenase in selected strains which was further advocated by the high per cent dependency on monoxygenases demonstrated by strains selected with synergized insecticides. Synergistic effects of PBO causing significant decline in the acetamiprid  $\text{LC}_{50}$  values of *Ae. aegypti* resistant strains suggest the role of CYP450s due to the involvement of monoxygenase-based metabolic detoxification mechanism in imparting acetamiprid resistance.

These results are in alignment with those obtained with a field strain of *Cx. pipiens*, 3.8–38.4-fold resistant to pyrethroids (Al-Sarar, 2010). Selecting the resistant larvae with pyrethroids synergized with PBO suppressed >90% pyrethroid resistance demonstrating the role of microsomal oxidases in reducing the pyrethroid toxicity. Similarly, larval treatment of deltamethrin-resistant (4–21-fold) strains of *Ae. aegypti*, *An. culicifacies*, *An. stephensi*, *An. vagus*, *Cx. tritaeniorhynchus* and *Cx. pipiens* with deltamethrin + PBO (1:6) suppressed the resistance by 75–95% (WHO, 2016).

A few studies have revealed the synergism between neonicotinoids and PBO against public health pests like house fly, whereas this relationship has not been confirmed in the mosquitoes yet. Ma *et al.* (2017) showed that 78-fold imidacloprid-resistant population of housefly registered 3.34-fold synergism with PBO. Resistance to imidacloprid and other neonicotinoid, thiamethoxam, has also been suppressed by PBO-synergized insecticides in Danish populations of *M. domestica* (Markussen and Kristensen, 2010). In thiamethoxam-resistant strains of *Musca* collected from Pakistan, Khan *et al.* (2015) reported a significant synergism with S,S,S-tri-butylphosphorotrithioate and PBO resulting in respective 2.94- and 5.00-fold reversion in resistance.

The larval selection of *Ae. aegypti* with acetamiprid alone for 15 generations caused an elevation in P450 monoxygenase level by 2–3-fold. Similar inhibitory effects of cytochrome P450 on the toxicity of imidacloprid in housefly were reported by Ma *et al.* (2017). The association of elevated P450 monoxygenase with pyrethroid resistance has been deduced in various mosquito species; *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Kumar *et al.*, 1991); and *An. stephensi* and *An. gambiae* (Hemingway *et al.*, 1991; Vulule *et al.*, 1994; Brogdon *et al.*, 1997). The pyrethroid-resistant South African strain of *An. funestus* showed upregulation of primarily P450 monoxygenase system (Brooke *et al.*, 2001; Wondji *et al.*, 2007, 2009; Amenyay *et al.*, 2008). High levels of permethrin resistance in *Cx. pipiens* has been solely conferred to P450-mediated detoxification

(Hardstone et al., 2009). Imidacloprid-resistant *Drosophila* showed increased expression of *CYP6G1*, indicating the involvement of CYP isozyme in detoxifying imidacloprid and perhaps other neonicotinoids (Daborn et al., 2001).

Formulation of novel insecticides with unique mode of action is difficult as well as expensive necessitating to devise resistance management strategies in order to preserve the efficiency of pre-existing insecticides. Since, elucidation of the mechanisms governing insecticide resistance could be a dynamic step towards the creation of more effective and safer interventions to combat resistance, bring resistant populations below threshold and ultimately reduce the incidences of mosquito-borne diseases; current studies are of extreme implications.

## Conclusion

Development of insecticide resistance in mosquitoes caused by continuous selection pressure has led to failure of vector control programmes necessitating employment of new chemicals in the fields. In the current study, selection pressure of acetamiprid on *Ae. aegypti* larvae for 15 generations caused development of considerable resistance which, however, could be reduced and reversed by selection with PBO synergized acetamiprid. It suggests the appearance of CYP450-mediated resistance in *Ae. aegypti* larvae which could be managed by synergistic approach. Use of synergized acetamiprid as a control intervention approach is recommended which is effective, safe and sustainable.

## Data

Not applicable.

**Acknowledgements.** The authors are highly grateful to the Council of Scientific Research (CSIR), New Delhi, India for providing financial assistance to carry out the present investigations. We are also highly grateful to the Principal, Acharya Narendra Dev College for providing infrastructure and research facilities.

**Author contributions.** RRS conceived the idea, conducted the experiments and wrote the manuscript. SK designed and guided the experiments. RRS, KP and PL analysed the results and SK helped in the analysis. RRS and SK were involved in the finalization of manuscript.

**Financial support.** This research was supported by the contingent grant from Council of Scientific and Industrial Research, New Delhi, India (Award No. 08/529(0003)/2015-EMR-I).

**Conflict of interest.** None.

**Ethical standards.** Not applicable.

**Consent for publication.** Not applicable.

**Code availability.** Not applicable.

## References

Ahmed MAI and Othman AAE (2020) Piperonyl butoxide enhances the insecticidal toxicity of nanoformulation of imidacloprid on *Culex pipiens* (Diptera: Culicidae) mosquito. *Vector-Borne and Zoonotic Diseases* **20**, 134–142.

Al-Sarar AS (2010) Insecticide resistance of *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: status and overcome. *Saudi Journal of Biological Sciences* **17**, 95–100.

Ambrose ML (2003) Characterization of the insecticidal properties of acetamiprid under field and laboratory conditions, Faculty of North Carolina State University, Raleigh, NC, US, 2003.

Amenya DA, Naguran R, Lo TC, Ranson H, Spillings BL, Wood OR, Brooke BD, Coetzee M and Koekemoer LL (2008) Over expression of a cytochrome P450 (CYP6P9) in a major African malaria vector, *Anopheles funestus*, resistant to pyrethroids. *Insect Molecular Biology* **17**, 19–25.

Bansal M, Barna B and Chaudhry A (2012) Pesticide effect on genetic components: a genotoxic study on *Culex quinquefasciatus* by applying dominant lethal test. *International Journal of Advanced Biological and Biomedical Research* **2**, 685.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, William Wint GR, Simmons CP, Scott TW, Farrar JJ and Hay SI (2013) The global distribution and burden of dengue. *Nature* **496**, 504–507.

Bonner MR, Coble J and Blair A (2007) Malathion exposure and the incidence of cancer in the agricultural health study. *American Journal of Epidemiology* **166**, 1023–1034.

Brogdon W, McAllister J and Vulule J (1997) Heme peroxidase activity measured in single mosquito identifies individuals expressing an elevated oxidase for insecticide. *Journal of the American Mosquito Control Association* **13**, 233–237.

Brogdon WG, McAllister JC, Corwin AM and Cordon-Rosales C (1999) Independent selection of multiple mechanisms for pyrethroid resistance in Guatemalan *Anopheles albimanus* (Diptera: Culicidae). *Journal of Economic Entomology* **92**, 298–302.

Brooke BD, Kloke G, Hunt RH, Koekemoer LL, Temu EA, Taylor ME, Small G, Hemmingway J and Coetzee M (2001) Bioassay and biochemical analyses of insecticide resistance in southern African *An. funestus* (Diptera: Culicidae). *Bulletin of Entomological Research* **91**, 265–272.

Cetin H, Demir E, Kocaoğlu S and Kaya B (2010) Insecticidal activity of some synthetic pyrethroids with different rates of piperonyl butoxide (PBO) combinations on *Drosophila melanogaster* (Diptera: Drosophilidae). *Ekoloji* **19**, 27–32.

Cetin H, Kocak O, Oz E, Koc S, Polat Y and Arıkan K (2019) Evaluation of some synthetic pyrethroid and piperonyl butoxide combinations against Turkish house fly (*Musca domestica* L.) populations. *Pakistan Journal of Zoology* **51**, 703–707.

Cuamba N, Morgan JC, Irving H, Steven A and Wondji CS (2010) High level of pyrethroid resistance in an *Anopheles funestus* population of the Chokwe District in Mozambique. *PLoS ONE* **5**, e11010.

Daborn P, Boundy S, Yen J and Pittendrigh B (2001) DDT resistance in *Drosophila* correlates with CYP6G1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid. *Molecular Genetics and Genomics* **266**, 556–563.

Enayati AA, Vatandoost H, Ladonni H, Townson H and Hemingway J (2003) Molecular evidence for a *kdr*-like pyrethroid resistance mechanism in the malaria vector mosquito *Anopheles stephensi*. *Medical and Veterinary Entomology* **17**, 138–144.

Faucou F, Dusfour I, Gaude T, Navratil V, Boyer F, Chandre F, Sirisopa P, Thanispong K, Juntarajumnong W, Poupardin R and Chareonviriyaphap T (2015) Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing. *Genome Research* **25**, 1347–1359.

Fonseca-González I, Quiñones ML, Lenhart A and Brogdon WG (2011) Insecticide resistance status of *Aedes aegypti* (L.) from Colombia. *Pest Management Science* **67**, 430–437.

Gao C, Ma S, Shan C and Wu S (2014) Thiamethoxam resistance selected in the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae): cross-resistance patterns, possible biochemical mechanisms and fitness costs analysis. *Pesticide Biochemistry and Physiology* **114**, 90–96.

Hardstone MC, Lazzaro BP and Scott JG (2009) The effect of three environmental conditions on the fitness of cytochrome P450 monooxygenase-mediated permethrin resistance in *Culex pipiens quinquefasciatus*. *BMC Evolutionary Biology* **9**, 42.

Hemingway J, Miyamoto J and Herath PRJ (1991) A possible novel link between organophosphorus and DDT insecticide resistance genes in *Anopheles*: supporting evidence from fenitrothion metabolism studies. *Pesticide Biochemistry and Physiology* **39**, 49–56.

Hemingway J, Field L and Vontas J (2002) An overview of insecticide resistance. *Science* **298**, 96–97.



- Horowitz AR, Kontsedalov S and Ishaaya I (2004) Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology* **97**, 2051–2056.
- Ishak IH, Jaal Z, Ranson H and Wondji CS (2015) Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasites & Vectors* **8**, 1–13.
- Jian-chu M, Tian-ci Y, Jia-an C and Xiao-gang S (2002) Lethal and sublethal effects of acetamiprid on the larvae of *Culex pipiens pallens*. *Insect Science* **9**, 45–49.
- Khan HAA, Akram W and Shad SA (2014) Genetics, cross-resistance and mechanism of resistance to spinosad in a field strain of *Musca domestica* L. (Diptera: Muscidae). *Acta Tropica* **130**, 148–154.
- Khan HAA, Akram W, Iqbal J and Naeem-Ullah U (2015) Thiamethoxam resistance in the house fly, *Musca domestica* L.: current status, resistance selection, cross-resistance potential and possible biochemical mechanisms. *PLoS ONE* **10**, e0125850.
- Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M and Kawano H (2012) Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats. *PLoS ONE* **7**, e32432.
- Kona MP, Kamaraju R, Donnelly MJ, Bhatt RM, Nanda N, Chourasia MK, Swain DK, Suman S, Urugayala S, Kleinschmidt I and Pandey V (2018) Characterization and monitoring of deltamethrin-resistance in *Anopheles culicifacies* in the presence of a long-lasting insecticide-treated net intervention. *Malaria Journal* **17**, 414.
- Kristensen M and Jespersen JB (2008) Susceptibility to thiamethoxam of *Musca domestica* from Danish livestock farms. *Pest Management Science* **64**, 126–132.
- Kumar S, Thomas A and Pillai MKK (1991) Involvement of monooxygenases as a major mechanism of deltamethrin resistance in larvae of three species of mosquitoes. *Indian Journal of Experimental Biology* **29**, 379–384.
- Kumar S, Thomas A, Sahgal A, Verma A, Samuel T and Pillai MKK (2002) Effect of the synergist, piperonyl butoxide, on the development of deltamethrin resistance in yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Archives of Insect Biochemistry and Physiology* **50**, 1–8.
- Kumar S, Thomas A, Samuel T, Sahgal A, Verma A and Pillai MKK (2009) Diminished reproductive fitness associated with the deltamethrin resistance in an Indian strain of dengue vector mosquito *Aedes aegypti* L. *Tropical Biomedicine* **26**, 55–64.
- Kushwah RBS, Dykes CL, Kapoor N, Adak T and Singh OP (2015) Pyrethroid resistance and presence of two knockdown resistance (kdr) mutations, F1534C and a novel mutation T1520I, in Indian *Aedes aegypti*. *PLoS Neglected Tropical Diseases* **9**, e3332.
- Li T, Zhang L, Reid WR, Xu Q, Dong K and Liu N (2012) Multiple mutations and combination combinations in the sodium channel of permethrin resistant mosquitoes, *Culex quinquefasciatus*. *Scientific Reports* **2**, 781.
- Li CX, Kaufman PE, Xue RD, Zhao MH, Wang G, Yan T, Guo XX, Zhang YM, Dong YD, Xing D and Zhang HD (2015) Relationship between insecticide resistance and kdr mutations in the dengue vector *Aedes aegypti* in Southern China. *Parasites & Vectors* **8**, 1–9.
- Lima EP, Paiva MHS, de Araújo AP, da Silva EVG, da Silva UM, de Oliveira LN, Santana AEG, Barbosa CN, de Paiva Neto CC, Goulart MO and Wilding CS (2011) Insecticide resistance in *Aedes aegypti* populations from Ceará, Brazil. *Parasites & Vectors* **4**, 5.
- Liu H, Cupp EW, Micher KM, Guo A and Liu N (2004) Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. *Journal of Medical Entomology* **41**, 408–413.
- Liu Z, Williamson MS, Landsdell SJ, Denholm I, Han Z and Millar NS (2005) A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *Proceedings of the National Academy of Sciences* **102**, 8420–8425.
- Liu N, Xu Q, Zhu F and Zhang LEE (2006) Pyrethroid resistance in mosquitoes. *Insect Science* **13**, 159–166.
- Lorini I and Galley DJ (2000) Effect of the synergists piperonyl butoxide and DEF in deltamethrin resistance on strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). *Anais da Sociedade Entomológica do Brasil* **29**, 749–755.
- Ma Z, Li J, Zhang Y, Shan C and Gao X (2017) Inheritance mode and mechanisms of resistance to imidacloprid in the house fly *Musca domestica* (Diptera: Muscidae) from China. *PLoS ONE* **12**, e0189343.
- Markussen MDK and Kristensen M (2010) Cytochrome P450 monooxygenase-mediated neonicotinoid resistance in field populations and selected laboratory strains of the house fly *Musca domestica* L. *Pesticide Biochemistry and Physiology* **98**, 50–58.
- Moore MT, Cooper CM, Smith Jr S, Cullum RF, Knight, SS, Locke MA and Bennett ER (2009) Mitigation of two pyrethroid insecticides in a Mississippi Delta constructed wetland. *Environmental Pollution* **157**, 250–256.
- Mota-Sanchez D, Hollingworth R, Grafius E and Moyer D (2006) Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Pest Management Science* **62**, 30–37.
- National Vector Borne Disease Control Programme (NVBDCP) (2021a) Dengue/DHF situation in India [Online]. Available at <https://nvbdcg.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715> (Accessed on June 24, 2021).
- National Vector Borne Disease Control Programme (NVBDCP) (2021b) Chikungunya situation in India [Online]. Available at <https://nvbdcg.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715> (Accessed on June 24, 2021).
- Osman DH and Brindley WA (1981) Estimating monooxygenase detoxification in field populations: toxicity and distribution of carbaryl in three species of *Labops grassbugs*. *Environmental Entomology* **10**, 676–680.
- Qiong RAO, Xu YH, Chen LUO, Zhang HY, Jones CM, Devine GJ, Gorman K and Denholm I (2012) Characterisation of neonicotinoid and pymetrozine resistance in strains of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China. *Journal of Integrative Agriculture* **11**, 321–326.
- Riaz MA, Chandor-Proust A, Dauphin-Villemant C, Poupardin R, Jones CM, Strode C, Régent-Kloekner M, David JP and Reynaud S (2013) Molecular mechanisms associated with increased tolerance to the neonicotinoid insecticide imidacloprid in the dengue vector *Aedes aegypti*. *Aquatic Toxicology* **126**, 326–337.
- Samal RR and Kumar S (2018) Susceptibility status of *Aedes aegypti* L. against different classes of insecticides in New Delhi, India to formulate mosquito control strategy in fields. *The Open Parasitology Journal* **6**, 52–62.
- Samal RR and Kumar S (2021) Cuticular thickening associated with insecticide resistance in dengue vector, *Aedes aegypti* L. *International Journal of Tropical Insect Science* **41**, 809–820.
- Samal RR, Panmei K, Lanbiliu P and Kumar S (2020) Biochemical characterization of acetamiprid resistance in laboratory-bred population of *Aedes aegypti* L. larvae. p. 169–176 in Proceedings of International Conference and the 10th Congress organized by the Entomological Society of Indonesia, Bali, 8th October–10th October 2019, Entomological Society of Indonesia.
- Sanchez-Bayo FP (2012) Insecticides mode of action in relation to their toxicity to non-target organisms. *Journal of Environmental & Analytical Toxicology* **5**, 4, 002.
- Urugayala S, Verma V, Natarajan E, Velamuri PS and Kamaraju R (2015) Adulticidal & larvicidal efficacy of three neonicotinoids against insecticide susceptible & resistant mosquito strains. *The Indian Journal of Medical Research* **142**, S64.
- US Environmental Protection Agency (US-EPA) Factsheet-Acetamiprid (2002) Available at [https://www3.epa.gov/pesticides/chem\\_search/reg\\_actions/registration/fs\\_PC-099050\\_15-Mar-02.pdf](https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-099050_15-Mar-02.pdf). pp23.
- Vulule JM, Beach RF, Atieli FK, Roberts JM, Mount DL and Mwangi RW (1994) Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bed-nets and curtains in Kenya. *Medical and Veterinary Entomology* **8**, 71–75.
- Warikoo R, Ray A, Sandhu JK, Samal R, Wahab N and Kumar S (2012) Larvicidal and irritant activities of hexane leaf extracts of *Citrus sinensis* against dengue vector *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine* **2**, 152–155.
- WHO (World Health Organization) (1998) Techniques to detect insecticide resistance mechanisms (field and laboratory manual). World Health Organization. Available at <https://apps.who.int/iris/handle/10665/83780>.
- WHO (World Health Organization) (2016) Monitoring and managing insecticide resistance in *Aedes* mosquito populations. Available at [http://apps.who.int/iris/bitstream/handle/10665/204588/WHO\\_ZIKV\\_VC\\_16\\_1\\_eng.pdf?sequence=2](http://apps.who.int/iris/bitstream/handle/10665/204588/WHO_ZIKV_VC_16_1_eng.pdf?sequence=2).

- Wondji CS, Morgan J, Coetzee M, Hunt RH, Steen K, Black WC, Hemingway J and Ranson H (2007) Mapping a quantitative trait locus (QTL) conferring pyrethroid resistance in the African malaria vector *Anopheles funestus*. *BMC Genomics* **8**, 34.
- Wondji CS, Irving H, Morgan J, Lobo NF, Collins FH, Hunt RH, Coetzee M, Hemingway J and Ranson H (2009) Two duplicated P450 genes are associated with pyrethroid resistance in *Anopheles funestus*, a major malaria vector. *Genome Research* **19**, 452–459.
- Yang T and Liu N (2014) Permethrin resistance variation and susceptible reference line isolation in a field population of the mosquito, *Culex quinquefasciatus* (Diptera: Culicidae). *Insect Science* **21**, 659–666.
- Yanola J, Somboon P, Walton C, Nachaiwieng W, Somwang P and Prapanthadara LA (2011) High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Tropical Medicine & International Health* **16**, 501–509.
- Zaim M and Guillet P (2002) Alternative insecticides: an urgent need. *Trends in Parasitology* **18**, 161–163.
- Zheng ML, Zhang DJ, Damiens DD, Yamada H and Gilles JR (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) – I – Egg quantification. *Parasites & Vectors* **8**, 42.