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Effect of lambda cyhalothrin and temephos on detoxification enzyme systems in *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract

Mosquitoes serve as vector for transmitting diseases. Among mosquitoes, *Culex quinquefasciatus* transmits lymphatic filariasis, yellow fever Japanese encephalitis etc. Application of chemical insecticides is still the best option for vector control programmes. Continuous use of these chemicals on mosquito reduces its effects. The present study determined the baseline susceptibility of *Cx. quinquefasciatus* in response to λ-cyhalothrin and temephos treatments. In addition, the biochemical mechanisms and zymogram analysis involved in insecticide detoxification among larval mosquitoes were studied. The larval bioassay indicated high LC_{50} value for λ-cyhalothrin (0.1484ppm) as compared to temephos (0.01092ppm). While AChE assay showed increased activity in temephos treatments, glutathione reductase (GR) and esterase levels were increased at both the treatments. Esterase quantitative analysis revealed the expression of three bands at 43kDa, 67kDa and 245kDa. The findings suggest that insensitivity of AChE, esterase and high GR activity may play an important role in developing resistance to synthetic pyrethroid and organophosphate insecticides in *Cx. quinquefasciatus* population.

Key words

Culex quinquefasciatus, Detoxification enzymes, Insecticide Resistance, Synthetic pyrethriod

Introduction

Mosquitoes play an important role in transmitting diseases. Among mosquitoes, *Culex quinquefasciatus* act as vector for lymphatic filariasis, yellow fever and Japanese encephalitis. Over 120 million people from 83 countries are physically disabled by lymphatic filariasis caused by *Culex* mosquitoes (WHO, 1995). WHO (2009) has suggested various methods to control mosquito vectors. Chemical insecticides are still the main part of vector control programme (Devine and Ogusuku, 2009). Synthetic pyrethroids are extensively being used for veterinary and public health purposes because of their quick knockdown effect, neuro toxic effect and relatively low mammalian toxicity at operational doses (Guessan *et al.,* 2007). However, extensive use of pyrethroid, organophosphate and carbamates have led to development of resistance in many insect species (Tikar *et al.,* 2008; Hemingway and Ranson, 2000). Among various synthetic pyrethroids, type-II pyrethroids like, cypermethrin, lambda-cyhalothrin has been suggested to be use instead of permethrin pyrethroid insecticides, to prevent novel mutations that occurr in mosquito as a result of selection with permethrin insecticide (Yanola *et al.,* 2011). Effective management of insecticide resistance is possible only if the mechanisms underpinning the phenotype are understood. In general, a metabolic resistance mechanism is an important mechanism for contributing insecticide resistance (Pourpardin *et al.*, 2008).

Three major enzyme families, the cytochrome (P450) monooxygenases, glutathione transferases (GST) and carboxy/ cholinesterases (CCE) are mainly involved in insecticide metabolism. Each of these enzymes catalyzes a wide range of detoxification reactions. They were the primary enzymatic defenses against xenobiotic, responsible for the removal of many byproducts of metabolism (Feyereisen, 2005; Oakeshott *et al.*, 2005). In mosquitoes, esterases are the primary mechanism involved in organophosphate, carbamates and pyrethriod resistance (Polson *et al.*, 2011 and Marcombe *et al.*, 2012). Several studies have shown that glutathione S-transferase (GST) enzymes are involved in conferring resistance to these classes of insecticides (Ranson

and Hemingway, 2005). Acetylcholinesterase (AChE), a serine esterase found in nerve synapses is the target site for OP and carbamate insecticides and decreased insensitivity to these insecticide is due to mutation in AChE (Osta *et al*., 2012), thereby these enzymes are used as reliable marker to assess the impact of many toxic compound on a range of test organisms (Fourcy *et al.,* 2002; Smirle *et al*., 2010). Many studies have been conducted throughout the world to understand the mechanism of pyrethroid resistance in mosquitoes. Studies on Cuban population of *Culex quinquefasciatus* have identified resistance mechanisms as elevated nonspecific esterases and insensitive acetylcholinesterase (AChE) which confer resistance against OPs (Bisset *et al*., 1990).

The present study was undertaken to find out the baseline susceptibility and mechanism of insecticide detoxification in *Cx. quinquefasciatus* exposed to λ-cyhalothrin and temephos insecticides.

Materials and Methods

Maintenance of mosquito larvae : Two population of *Cx. quinquefasciatus* was used for study. The laboratory susceptible (control) and a field population. The susceptible population was originally collected from Salem town, Tamil Nadu, India and were maintained in the laboratory for 10 generations without exposure to any chemical insecticides. Egg masses were collected from the same location during November 2011 in ground water and reared for the first generation. Both colonies were fed daily with yeast, dog biscuits in the separate trays and maintained at 25ºC ± 2ºC with relative humidity of 80ºC. Adults were fed with 10% sucrose solution.

Larval bioassay : The larval bioassay was conducted according to the standard protocol of WHO (World Health Organization, 2005). Twenty-five early fourth (4^{th}) instar larvae were introduced into 250 ml of test solution containing lambda-cyhalothrin and water in 250 ml plastic cups for 24 hr. Different test concentrations ranging from 0.001-1ppm were made by diluting with commercial formulation of lambda-cyhalothrin (5% a.i w/v) and temephos (10% a.i w/v) with distilled water. Distilled water served as control. The experiment was replicated three times per concentration. The mortality of larvae was observed after 24hr and the larvae were considered dead if they sank to the bottom of the multi well cups and failed to move or float after being probed (Hardstone *et al.,* 2009). The bioassay data was analyzed using probit method (Finney,1977).

Biochemical analysis : Batches of 100 late 3^{rd} instar larvae were homogenized in 1.5ml of ice-cold phosphate buffer (0.1M; pH 7.2), using pre-chilled mortar and pestle. The homogenates were centrifuged at 8000g for 4°C for 10 min at 4ºC. The supernatant was used as enzyme samples. Quantification of total protein of the homogenate was done according to the standard procedure of Lowry *et al*. (1951). Bovine serum albumin (BSA) was used as standard protein.

Acetylcholinesterase (AChE), nonspecific esterase, glutathione S-transferase and glutathione reductase assay in larval populations were performed following the method previously described by Kranthi *et al.* (2005) with some modification. Acetylcholinesterase (AChE) activity was carried out in the reaction mixture containing 0.075 M acetylthiocholine-iodide and 0.01M dithio-bis-2-nitrobenzoic acid (DTNB), reaction rates were monitored at 412 nm for 5 min. Non specific esterase activity was assayed by 20-min incubation of larval homogenate in each tube with 5m of 30mM α napthyl acetate at room temperature. The reaction was further incubated for 5 min with 1 ml of staining solution (5% SDS and 1% fast blue BB [Base N-(4-Amino-2, 5 diethoxyphenyl) benzamide] salt dissolved in 16 ml of sodium phosphate buffer pH 6.8) and the OD value was measured at 590 nm.

Glutathione S-transferase activity was measured in the reaction mixture containing 0.05M reduced glutathione and 50mM 1-chloro-2, 4-dinitro benzene (CDNB). The reaction rates were measured at 340 nm for 5 min, and the activity was expressed in nmoles CDNB conjugated min^{-1} mg^{-1} protein, using extinction coefficient of 9.6 mM^{-1} cm^{-1}. Glutathione reductase was assayed with 50mM CDNB, GSH and 4.3 mM of NADPH. The reaction was incubated for 2-3 min at 20ºC. The OD was read at 412 nm. All the specific enzyme activities were calculated and compared with the susceptible population by analysis of variance (ANOVA) using SPSS statistical programme.

Native polyacrylamide gel electrophoresis : Non-denaturing PAGE was carried out to detect the esterase profile of the *Cx. quinquefasciatus* following the procedure of Davis (1964). A small vertical slab (10×11cm) gel electrophoresis was used for this study. Individual larval samples were homogenized using 30µl of 30 % sucrose solution and spun at 2400g for 5 min at 4ºC. An equal volume of supernatant sample was loaded to each well. The gels were run at 40 V for nearly 25 min and that increased to 60V for about 3 hr. The separated gel was immersed in staining solution containing 2% α-napthyl acetate and 1% (w/v) fast blue BB salt to visualize the isoenzyme banding pattern. The gel was washed with distilled water and stored in 7% acetic acid solution for further analysis.

Results and Discussion

The result obtained from the bioassay with temephos and λ-cyhalothrin treatment on 4^{th} instar *Cx. quinquefasciatus* larvae revealed that temephos was more toxic (LC_{50}-1.092 ppm; $p<0.05$) than λ-cyhalothrin (LC_{50} - 1.884ppm). Jamal *et al*. (2011) reported that *Cx. quinquefasciatus* was resistant to malathion, λ –cyhalothrin and permethrin in field population, as compared to temephos treatment.

AChE activity increased in temephos treatment from 0.1 to 1ppm in a dose dependent manner (Fig.1a), while in λ-cyhalothrin treatment, the activity was found significantly lower

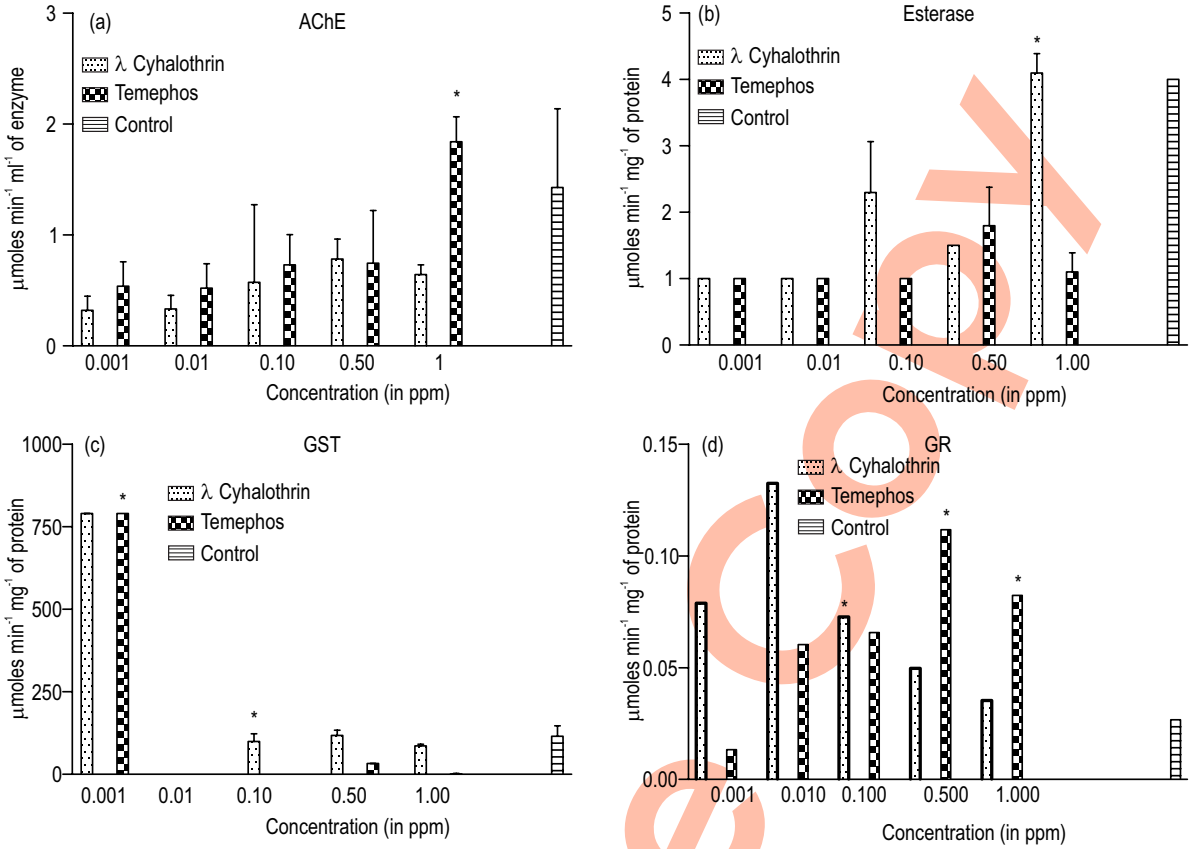

Fig 1 (a-d): Biochemical enzyme profile of 4th instar *Cx.quinquefaciatus* larvae exposed to formulations of temephos and λ-cyhalothrin. Enzyme activities are represented as Mean ±SD value; *Asterisk indicates significant difference among treatments with respect to control (susceptible) One Way ANOVA (Bonferronii post-hoc test) (P<0.05)

as compared to control activity (p<0.05) revealing organophosphate resistance and metabolic degradation of pyrethriod in *Culex quinquefasciatus* population. Similar studies on Cuban populations of *Culex quinquefasciatus* mosquitoes have identified OP resistance mechanisms mediated by insensitive acetyl cholinesterase (AChE) (Bisset *et al*., 1997). Sarar (2010) reported that OP resistance and detoxification mechanism in *Cx. pipiens*, results due to insensitivity of AChE to OP. In addition to the elevated levels of detoxification enzymes resulting in resistance to insecticides, alterations in the insecticide target site can also cause target site insensitivity, leading to resistance. A decrease in the sensitivity of AChE to inhibition by OP and carbamates, leading to resistance has been demonstrated in a broad range of insects (Coleman and Hemingway, 2007). A common resistance mechanism observed in several species of arthropods is due to the selection of a modified AChE, less sensitive to these insecticides (Kady *et al*., 2008). Esterase activity increased from 1 µ mole min^{-1} protein to 4µ $mole^{-1}$ min^{-1} mg^{-1} protein in λ-cyhalothrin treatment from lower concentration to highest concentration (Fig. 1b). But in temephos treatment, the activity decreased significantly (3-fold) as compared to control activity (p<0.05). It has been suggested that esterase detoxification may be the most common resistance mechanisms in *Culex quinquefasciatus.* Esterases act by cleaving phosphotriester bonds and have been found to be important in resistance to OP and carbamates insecticides, and pyrethroids to a lesser extent (Achaleke *et al*., 2009).

Elevated esterase activity was associated with temephos resistance in Brazilian populations of *Ae. aegypti* (Montella *et al*., 2007), but the present study revealed esterase mediated pyrethriod resistance in the population studied. In the present study, GST activity increased in temephos and λ-cyhalothrin treatment at low dose; where as decreased significantly (p<0.05) at high concentrations (Fig. 1c) suggest that slow activation of metabolic degradation of insecticides in this pest. Increased GST activity has been associated with OP resistance to houseflies including mosquitoes (Hemingway *et al*., 2004). Studies on metabolic mechanisms of pyrethroid detoxification in insects revealed that glutathione S-transferase (GST) is involved in

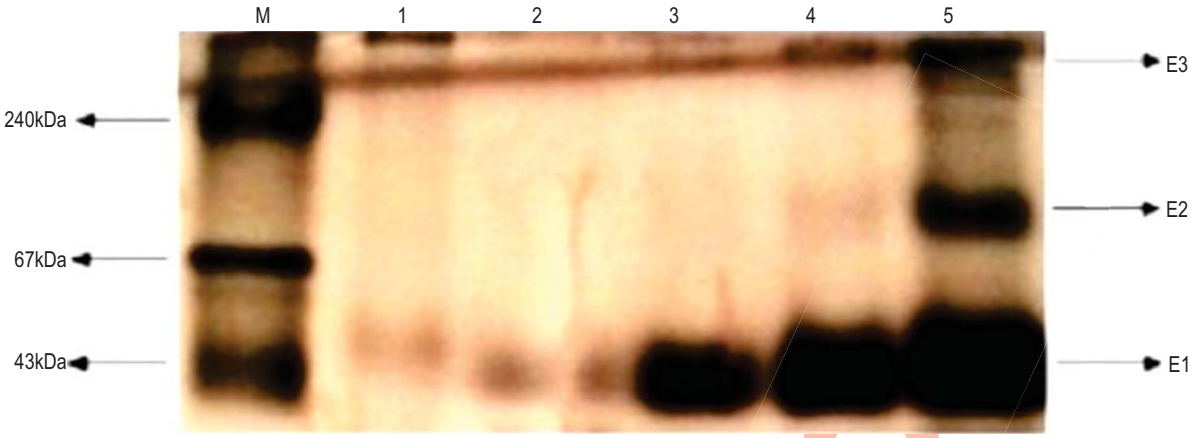

Fig. 2 : Native PAGE of esterase isoenzyme from *Cx.quinquefasciatus* stained with α-napthyl acetate M=Molecular Marker (Catalase 240kDa, bovine serum albumin 67kDa and ovalbumin 43kDa); lane 1and 2 control (susceptible), lane3, and lane4, Temephos treated samples, lane 5-λ-Cyhlothrin treated sample

insecticide detoxification mechanism in many insects (Graffton *et al*., 2004). The activity of glutathione reductase increased in temephos and λ-cyhalothrin treatment (Fig. 1d) as compared to control activity ($p<0.05$). Which suggest that free radical damage caused by insecticides were scavenged by GR antioxidant enzyme system, which may lead to detoxification mechanism. Similar result was reported earlier by Suman *et al.* (2009) where GR activity involved in reducing free radical mediated damage in resistance lepidopteron insects.

Native PAGE electrophoresis revealed a total of three esterase bands designated as E1-E3 in selected population at 240 kDa, 70 kDa and 40 kDa (λ-cyhalothrin treatment), whereas only one esterase band was observed at 40 kDa in the susceptible population (control) (Fig. 2), which supports our biochemical studies that increased activity of esterase in λ-cyhalothrin treatment may confer esterase mediated resistance to pyrethriod insecticide. Similar studies reported that elevated esterase activity in *Cx.quinquefasciatus* population revealed increased resistance to malathion insecticides (Gopalan *et al*., 1997). Karunaratne and Hemingway (2001) reported that elevated esterase activity was associated with malathion resistance in *Cx. quinquefasciatus* population. While the present study revealed that high expression of esterase bands and activity may be responsible for pyrethroid resistance in *Cx.quinquefasciatus*. The data of the present study showed that *Culex quinquefasciatus* detoxifies pyrethroid insecticide through esterase and GR enzyme complex, while organophosphate detoxification occurs by cholinesterase enzyme. From this study it can be concluded that *Culex quinquefasciatus* uses a different detoxifying enzymes for metabolizing pyrethroid and organophosphate insecticides.

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References

Achaleke, J., T. Martin, R.T, Ghogomu, M. Vaissayre and T. Brévault Esterase-mediated resistance to pyrethroids in field populations of Helicoverpa armigera (Lepidoptera: Noctuidae) from Central Africa. *Pest. Manag. Sci.*, **65**, 1147–1154 (2009).

Bisset, J.A., M. Rodriguez, C. Diaz, E. Ortiz and M.C. Marquette: The mechanisms of organophosphate and carbamate resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from Cuba. *Bull. Entomol. Res.,* **80**, 245–250(1990).

Bisset, J.A., M.M. Rodriguez, A. Soca, N. Pasteur and M. Raymond: Cross resistance to pyrethroids and Organophosphorous insecticides in the southern house mosquito (Diptera: Culicidae) from Cuba. *J. Med. Entomol.,* **34**, 244–246 (1997).

Coleman, M., and J. Hemingway: Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. *J. Pestic. Sci.,* **32**, 69–76 (2007).

Davis, B.J.: Disc electrophoresis II Method and application to human serum proteins. *Ann. N. Y.Acad. Sci.,* **121**, 404-427 (1964).

Feyereisen, R.: Insect cytochrome P450. In: Gilbert, L.I., Latrou, K., Gill, S.S. (Eds.), *Comprehensive Molecular Insect Science* Elsevier, Oxford, p. 1–77 (2005).

Finney, D.J.: Probit analysis: A statistical treatment of the sigmoid response curve, Cambridge University press, London, New York, Melbourne. p. 333 (1977).

Fourcy, D., A. Jumel, M. Heydorff and L. Lagadic: Esterases as biomarkers in *Nereis* (Hediste) *diversicolor* exposed to temephos and *Bacillus thuringiensis* var. *israelensis* used for mosquito control in coastal wetlands for Morbihan (Brittany, France). *Marine Environ. Res.,* **54**, 755–759 (2002).

Gopalan, N., B.K. Bhattacharya and K.M. Prakash Rao: Characterization of Carboxylesterases from malathion-resistant *Culex quinquefasciatus* Say (Diptera: Culicidae) Mosquitoes. *Pestic. Biochem. Physiol.,* **57**, 99–10 (1997).

Graffton-Cardwell, E.E., Y. Yuyong, R.A. Striggow, J.A. Christiansen and

C.S. Black: The role of esterase enzyme in monitoring for resistance to California red scale, *Aonidiella aurantii* (Homoptera: Diaspidiae), to organophosphate and carbamate insecticide. *J. Econ. Entomol.,* **97**, 606-613 (2004).

Hardstone, M.C., C.A. Leichter and J.G. Scott: Multiplicative interaction between the two major mechanisms of permethrin resistance, kdr and cytochrome P450-monooxygenase detoxification, in mosquitoes. *J. Evol. Biol*. **22**, 416–423 (2009).

Hemingway, J., N. Hawkes, L. McCarroll and H. Ranson: The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol. Biol.,* **34**, 653-665 (2004).

Hemingway, J. and H. Ranson: Insecticide's resistance in insect vectors of human disease. *Ann. Rev. Entomol.,* **45,** 371-391(2000).

Kady, G.A., N.H. Kamel, Y.Y. Mosleh and I.M. Bahght: Comparative toxicity of two bio-insecticides (Spinotoram and Vertemic) compared with Methomyl against Culex *pipiens* and *Anopheles multicolor*. *World J. Agric. Sci.,* **4,** 198–205 (2008).

Karunaratne, J. and Hemingway: Malathion resistance and prevalence of the Malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. *Bull. World Heal. Org.,* **79**, 1060-1064 (2001).

Kranthi, K.R.: Insecticide resistance monitoring, mechanism management manual. *Cent. Ins. Cot. Res.*, India, p. 78-82 (2005).

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall: Protein measurement with the Folins phenol reagent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Miller, T.A.: Mechanisms of resistance to pyrethroid insecticides. *Parasitol. Today.,* **4**, 3–7 (1998).

Montella, I.R., A.J. Martins, P.F. Viana-Medeiros, J.B.P. Lima, I.A. Braga and D. Valle: Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004. *Ame. J. Trop. Med. Hyg.,* **77**, 467–477 (2007).

Marcombe, S., R.B. Mathieu, N. Pocquet, M.A. Riaz and R. Poupardin: Insecticide resistance in the dengue vector Aedes aegypti from Martinique: distribution, mechanisms and relations with environmental factors. *PLoS One.*, **7**, e30989 (2012).

Oakeshott, J.G., C. Claudianos, P.M. Campbell, R.D. Newcomb and R.J. Russell: Biochemical genetics and genomics of insect esterases. In: Gilbert, L.I., K. Latrou and S.S. Gill: (Eds.), *Comprehensive Mol. Ins. Sci. Pharm.,* **5**, p. 309–381 (2005).

Osta, M.A., J.Z. Rizk, P. Labbe, M. Weill and K. Knio: Insecticide resistance to organophosphates in Culex pipiens complex from Lebanon. *Parasites and Vectors*., **5**, 132 (2012).

Polson, K.A., S.C. Rawlins, W.G. Brogdon and D.D. Chadee: Characterization of DDT and pyrethroid resistance in Trinidad and Tobago populations of Aedes aegypti. *Bull. Entomol. Res. 101*, 435-441(2011).

Pourpardin, R., S. Reynaud, C. Strode, H. Ranson, J. Vontas and J.P. David: Cross–induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti:* Impact on larval tolerance to chemical insecticides. *Ins. Biochem. Mol. Biol.,* **38**, 540-551 (2008).

Ranson, H. and J. Hemingway: Glutathione Transferases. In: *Comprehensive Molecular Insect Sci. Pharmacol.,* 5, 383-402 (2005).

Sarar, A.S.: Insecticide resistance of Culex pipiens (L.) populations (Diptera: Culicidae) from Riyadh city, Saudi Arabia: Status and overcome. *Saudi J. Biol. Sci*., **17**, 95-100 (2010).

Smirle, M.J., C.L. Zurowski, D.T. Lowery and R.G. Foottit: Relationship of insecticide tolerance to esterase enzyme activity in *Aphis pomi* and *Aphis spiraecola*. *J. Econ. Entomol.,* **103**, 374–378 (2010).

Suman, S., R. Kumar, S. Seth and S. Chandna: Mitochondrial antioxidant defence in radio-resistant *Lepidopteran* insect cells. *Bioinformation* **4**, 19-23 (2009).

Weill, M., G. Lutfalla, K. Mogensen, F. Chandre, A. Berthomieu, C., Berticat, N. Pasteur, A. Phillips, P., Fort and M. Raymond: Comparative genomics: insecticide resistance in mosquito vectors. *Nat.,* **423**, 136–137 (2003).

World Health Organization. Bridging the Gaps World Health Report. Geneva, Switzerland (1995).

World Health Organization: Guidelines for laboratory and field testing of mosquito larvicides. DocumentWHO/CDS/WHOPES/GCDPP/13, Geneva, Switzerland (2005).

World Health Organization: Dengue guidelines for diagnosis, treatment, prevention and control: new edition. Geneva. pp. 147 (2009).

Yanola, J., P. Somboon, C. Walton, W. Nachaiwieng, P. Somwang and L.A. Prapanthadara: 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. *Trop. Med. Int. Health.,* **16,** 501-509 (2011).