

Investigation of P-glycoprotein (P-gp) ATPase gene variation in temephos resistant *Aedes albopictus* (Skuse, 1894), Chidambaram, India

Soliang Manyu^{1*}, C. Elanchezhiyan² and P. Basker³

¹Department of Zoology, Indira Gandhi Government College, Tezu 792001, Arunachal Pradesh, India.

²Department of Zoology, Annamalai University, Chidambaram 608002, Tamil Nadu, India

³Advance Research Institute (ARI), Dr. MGR Educational Research Institute, Maduravoil, Chennai 600107, Tamil Nadu, India.

Email: soliangmanyu95.53.49@gmail.com*

ABSTRACT: The study investigated the role of P-glycoprotein (P-gp) ATPase gene expression in mediating resistance to the organophosphate larvicide temephos in *Aedes albopictus*. Larval populations were subsequently classified into control, susceptible, and resistant groups based on their differential response to temephos following laboratory-induced selection pressure. Total RNA was isolated, and relative gene expression was quantified using RT-PCR, with β -actin serving as the endogenous control for normalization. An upregulation of P-gp expression was observed in resistant larvae compared to susceptible and control groups. Densitometric analysis revealed P-gp expression levels of 35,863.67 AU in resistant larvae, 30,761.16 AU in susceptible larvae, and 33,786.40 AU in control larvae, whereas β -actin expression remained consistent across all groups. The elevated expression of P-gp indicates its involvement in temephos resistance, likely through enhanced xenobiotic efflux. The finding underscores the potential of ATP-binding cassette (ABC) transporters as molecular targets for improving vector control strategies.

© 2026 Association for Advancement of Entomology

KEY WORDS: Vector, arbovirus, insecticide resistance, RT-PCR, ABC transporter

INTRODUCTION

Insecticide resistance in medically significant vectors present a critical obstacle to vector control strategies (WHO, 2022; Ibrahim *et al.*, 2024). Principal resistance mechanisms include the upregulation of detoxification enzymes and target-site mutations (Han *et al.*, 2024; Wang *et al.*, 2024). However, contemporary studies implicated permeability glycoprotein (P-gp), a plasma membrane-associated ATP-binding cassette (ABC)

transporter, in conferring multidrug resistance (Rani *et al.*, 2024; Bharathiraja *et al.*, 2024; Lespine *et al.*, 2024; Elbahnsi *et al.*, 2024). Structurally, P-gp is composed of two repeating units, each containing a nucleotide-binding region and transmembrane helices that together form the functional transporter (Allikmets *et al.*, 1996; Ayrton and Morgan, 2001; Buss and Callaghan, 2008). The ATP-driven expulsion of various substrates from cells is primarily mediated by P-glycoprotein (See *et al.*, 1974; Guiral *et al.*, 1994; Schlemmer and Sirotnak,

* Author for correspondence

1994), contributing to reduced intracellular accumulation and therapeutic failure (Lanning *et al.*, 1996). It is ubiquitously expressed across cell types in diverse organisms, including insects, where it protects against toxic compounds (Podsiadlowski *et al.*, 1998; Xu *et al.*, 1998; Buss and Callaghan, 2008). Notably, presence of P-gps hamper entry of xenobiotics into protected compartments complicating treatment efficacy (Abu-Qare *et al.*, 2003; Giacomini *et al.*, 2010; Kido *et al.*, 2024; Johnston *et al.*, 2024; Huang *et al.*, 2024).” Functional inhibition or silencing of P-gp in resistant insect species, including *Aedes aegypti* and *Helicoverpa armigera*, has been shown to increase susceptibility to insecticides, underscoring its role in resistance mechanisms (Figueira-Mansur *et al.*, 2013). The present study investigates P-gp gene expression in temephos-resistant *A. albopictus* larvae to elucidate its contribution to resistance and inform molecular strategies for vector control.

MATERIALS AND METHODS

For the present study, temephos-resistant *A. albopictus* populations were derived from a susceptible (Manyu *et al.*, 2022), through cyclic mass rearing under temephos pressure in a controlled insectary environment in accordance to protocols prescribed by the International Atomic Energy Agency (IAEA, 2017). Samples of resistant larvae (RL), susceptible larvae (SL) and control larvae (CL) were chosen. Total RNA was isolated from each sample (RL, SL, and CL) using the TRIzol method and centrifuged in RNase-free tubes at 5000 rpm for 10 minutes to obtain cell pellets (1×10^7 cells). Each pellet was lysed with 700 μ L of TRIzol, and the lysate was mixed with 300 μ L of chloroform. It was then centrifuged (12,000 rpm) for 20 minutes at 4°C, to collect the RNA. RNA precipitation was done by adding isopropanol (700 μ L), followed by centrifugation to obtain a visible pellet. The RNA pellet was washed with 70 per cent ethanol and dried. The RNA was diluted in 30 μ L of double-distilled, autoclaved water, stored at 80 °C, and quantified with Labman UV–V is spectrophotometer (Labman, India).

To remove DNA contamination, RNA samples were treated with DNase-I in a 20 μ L reaction volume at 37°C for 30-45 minutes. Following addition of 2 μ L of 20 μ M EGTA, the solution was incubated for 10 minutes at 66°C. RNA was precipitated by adding sodium acetate (1/10 volume) and absolute ethanol (2 volumes), incubated for 60 minutes at -20°C and centrifuged for 20 minutes in 4°C at 12,000 rpm. RNA pellet was extracted and ringed with 500 μ L 75 **per cent** ethanol. Air dried the sample and 20 μ L double-autoclaved nuclease-free Milli-Q water was added.

First-strand complementary DNA (cDNA) was synthesized from 1.5 μ g of total RNA using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT). Reverse transcription was performed at 25°C for 10 min, followed by 42°C for 59 min. The reaction was terminated by incubation at 99°C for 5s to denature RNA–cDNA hybrids and inactivate the reverse transcriptase enzyme, followed by holding at 4°C. The synthesized cDNA was stored at “20°C until use.

Analysis of P-glycoprotein (P-gp) gene expression (Table 1): The synthesized cDNA was used as a template for amplification of the target P-glycoprotein ATPase (P-gp ATPase) gene by reverse transcription polymerase chain reaction (RT-PCR). Amplifications were performed using 2 $\times$ Taq PCR Master Mix (BioLit, India) according to the manufacturer’s recommendations. Gene expression levels were normalized against the endogenous reference gene β -actin, which served as the internal control. PCR amplification of the P-gp ATPase gene was carried out under the following thermal cycling conditions: an initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30s, annealing at 56°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min and a hold at 4°C. Amplification of the β -actin gene was performed using an initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min and a hold at 4°C.

Agarose gel electrophoresis and densitometric analysis: PCR amplicons were resolved by electrophoresis on a 1 per cent agarose gel prepared in 1× Tris-acetate-EDTA (TAE) buffer. Agarose (0.3g) was dissolved in 30mL of 1× TAE buffer, heated until completely solubilized, cooled to approximately 50°C, and stained with ethidium bromide (1.5µL). The gel was cast using an appropriate comb and allowed to solidify at room temperature. Electrophoresis was performed in 1× TAE running buffer. PCR products (3µL) mixed with an equal volume of loading dye were loaded into the wells and electrophoresed at 70 V for 15–20 min. Amplified DNA fragments were visualized under ultraviolet illumination using a gel documentation system. Band intensities were quantified by densitometric analysis using ImageJ software (National Institutes of Health, USA). Relative expression of the P-gp ATPase gene was determined after normalization against the corresponding β-actin expression levels.

Relative expression analysis: Semi-quantitative assessment of P-gp ATPase gene expression was performed by comparing the densitometric intensity of the target gene amplicons with that of the endogenous β-actin control. Relative expression levels were calculated from normalized band intensities obtained using ImageJ software and expressed as fold change relative to the control group.

RESULTS AND DISCUSSION

P-glycoprotein (P-gp) gene expression: There was a significant upregulation of P-gp gene expression in temephos-resistant *A. albopictus* (Figs. 1, 2). Densitometric analysis indicated overexpression, with levels recorded as,

35,863.67AU in RL, 30,761.16AU in SL, and 33,786.40AU in CL. The findings are consistent with those of Figueira *et al.* (2013), who reported similar evidence in resistant *Aedes aegypti*. Identical overexpression of P-gp is reported in *Anopheles gambiae* (Roth *et al.*, 2003), *Heliothis virescens* and *Helicoverpa armigera* (Lanning and Carter, 2006), *Drosophila melanogaster* (Grailles *et al.*, 2003), *Teleogryllus commodus* (Leader and O'Donnell, 2005), *Rhodnius prolixus* (Rheault *et al.*, 2006), *Coptis japonica* (Buss and Callaghan, 2008), and *Aedes caspius* (Porretta *et al.*, 2008). The elevation of P-gp is correlated to insecticide resistance (Bourguinat *et al.*, 2007; Aurade *et al.*, 2012).

A comparative analysis of β-actin gene expression (Fig. 1), alongside P-gp gene expression (Fig. 3), indicated minimal variation, confirming its suitability as a reference gene. Expression values were 49,340.06AU (RL), 49,340.97AU (SL), and 49,342.35AU (CL), demonstrating consistent expression across all groups. P-glycoprotein (P-gp), first reported by Juliano and Ling (Gottesman and Ling, 2006), is known to actively efflux xenobiotics from cells using ATP hydrolysis (Buss and Callaghan, 2008; Aurade *et al.*, 2012; Figueira-Mansur *et al.*, 2013; Thevenod and Lee, 2024; Devi *et al.*, 2024), that results in reduced drug delivery efficacy (Abu-Qare *et al.*, 2003; Veider *et al.*, 2024). Empirical studies have demonstrated its role in conferring resistance to multiple classes of insecticides (Buss and Callaghan, 2008; Szpila *et al.*, 2008; Xu *et al.*, 2023; Tseng *et al.*, 2024). The P-glycoprotein is a potential molecular target to enhance insecticide efficacy and manage resistance (Akhtar *et al.*, 2011; Ma *et al.*, 2013; Parida *et al.*, 2024).

Table 1. Primers for P-glycoprotein (P-gp) gene

Genes	Direction	Sequence (5' – 3')
P-gp	Forward	52 - TAAAATGAAGTTGAACGTATCGT-3'
	Reverse	52 - AGATGGTCAGCGGTGATTTC-3'
β-actin	Forward	52 - ATGGTCGGTATGGGGCAGAAGGACTC-3'
	Reverse	52 - TCGCACTTCATGATAGAGTTGTA-3'

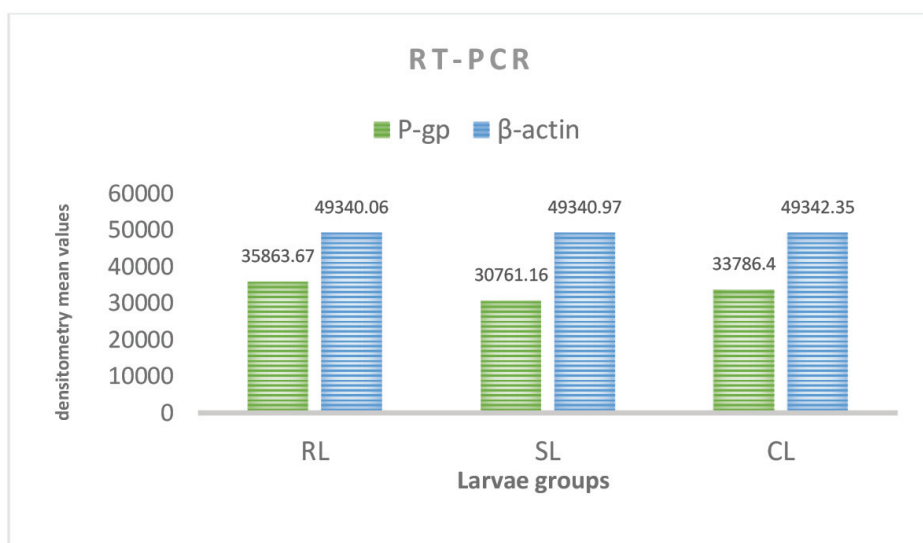


Fig 1. Densitometric analysis of P-gp and β -actin gene expression values in control and experimental *Aedes albopictus* larvae.

Densitometric analysis: All the data are expressed as the mean $\pm$ S.D measured by RT-PCR. The values are statistically significant and different at $p < 0.05$. β -actin was used as loading control; RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae

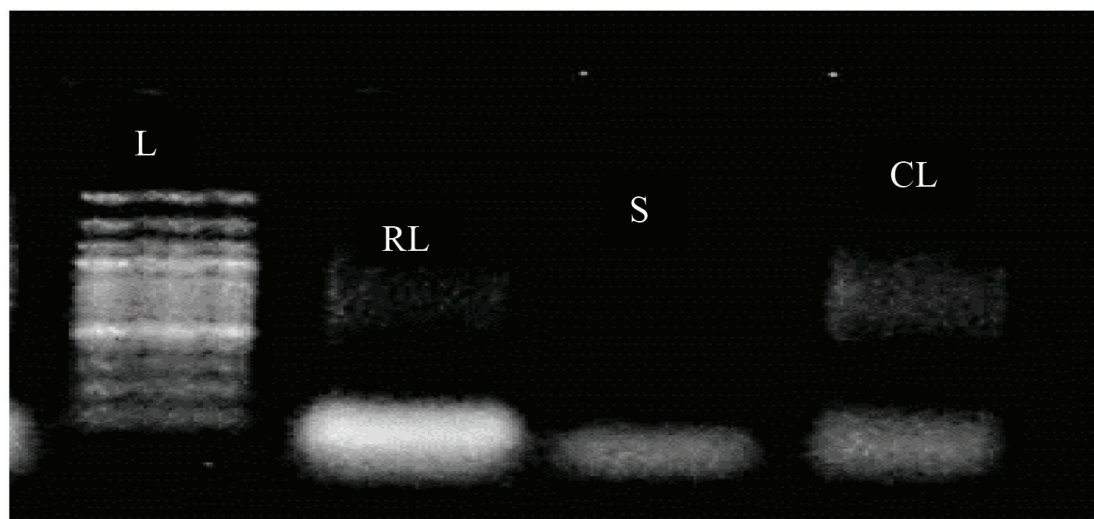


Fig 2. Gene expression profile of P-gp using SDS electrophoresis in control and experimental *Aedes albopictus* larvae. RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae; L: ladder-100 bp

Reports have been necessitated the investigation of P-glycoprotein inhibitors to improve the efficacy of therapeutic agents and pesticides (Shukla *et al.*, 2008). Documented P-gp inhibitors include curcumin, flavonoids, kaempferol, quercetin, tariquidar, verapamil, cyclosporin A, quinidine, reserpine, and tamoxifen (Xu *et al.*, 1998; Akhtar

et al., 2011; Lima *et al.*, 2014; Sankar and Kumar, 2023). Among these, verapamil has demonstrated a significant increase in mortality rates through effective inhibition of P-gp activity (Kundu *et al.*, 2024). This strategy may also be relevant for addressing insecticide resistance by facilitating improved drug delivery and directly targeting

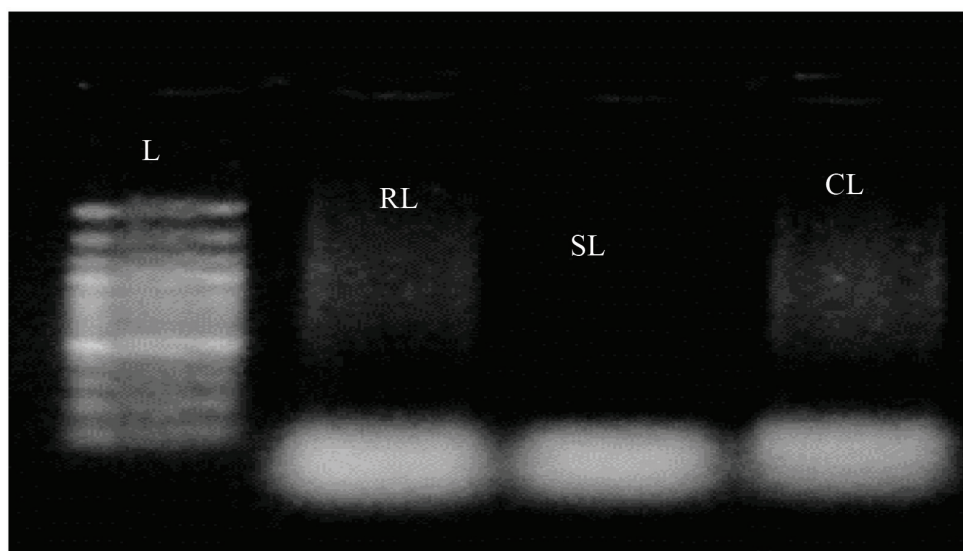


Fig 3. Gene expression profile of α -actin using SDS electrophoresis in control and experimental *Aedes albopictus* larvae. RL: Resistant larvae; SL: Susceptible larvae; CL: Control larvae; L: ladder-100 bp

resistance mechanisms (Tapadia and Lakhota, 2005). Elucidation of such molecular study in insecticide resistance is highly significant to formulate effective management strategies (Akbar *et al.*, 2014). Analysis of α -actin gene expression indicated its suitability as a reference gene. The elevated expression of P-gp indicates its involvement in temephos resistance, likely through enhanced xenobiotic efflux. The finding underscores the potential of ATP-binding cassette (ABC) transporters as molecular targets for improving vector control strategies.

REFERENCES

- Abu-Qare A., Elmasry E. and Abou-Donia M. (2003) A role for P-glycoprotein in environmental toxicology. *Journal of Toxicology and Environmental Health Part B* 6(3): 279-288.
- Akbar S.M., Aurade R.M., Sharma H.C. and Sreeramulu K. (2014) Mitochondrial P-glycoprotein ATPase contributes to insecticide resistance in the cotton bollworm, *Helicoverpa armigera* (Noctuidae: Lepidoptera). *Cell biochemistry and biophysics* 70: 651-660.
- Akhtar N., Ahad A., Khar R.K., Jaggi M., Aqil M., Iqbal Z., Ahmad F.J. and Talegaonkar S. (2011) The emerging role of P-glycoprotein inhibitors in drug delivery: a patent review. *Expert opinion on therapeutic patents* 21(4): 561-576.
- Allikmets R., Gerrard B., Hutchinson A. and Dean M. (1996) Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. *Human molecular genetics* 5(10): 1649-1655.
- Aurade R.M., Jayalakshmi S.K., Udikeri S.S. and Sreeramulu K. (2012) Modulation of p glycoprotein atpase of *helicoverpa armigera* by cholesterol: effects on atpase activity and interaction of insecticides. *Archives of Insect Biochemistry and Physiology* 79(1): 47-60.
- Ayrton A. and Morgan P. (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica* 31(9): 469-497.
- Bharathiraja P., Balamurugan K., Govindasamy C., Prasad N.R. and Pore P.M. (2024) Solasodine targets NF- κ B signalling to overcome P-glycoprotein mediated multidrug resistance in cancer. *Experimental Cell Research* 441(1): 114153.
- Bourguinat C., Ardelli B.F., Pion S.D., Kamgno J., Gardon J., Duke B.O., Boussinesq M. and Prichard R.K. (2007) P-glycoprotein-like protein, a possible genetic marker for ivermectin resistance selection in *Onchocerca volvulus*. *Molecular and Biochemical Parasitology* 2: 101-111.

- Buss D.S. and Callaghan A. (2008) Interaction of pesticides with p-glycoprotein and other ABC proteins: A survey of the possible importance to insecticide, herbicide and fungicide resistance. *Pesticide biochemistry and physiology* 90(3): 141–153.
- Devi R., Goyal P., Verma B., Hussain S., Chowdhary F., Arora P. and Gupta S. (2024) A transcriptome-wide identification of ATP-binding cassette (ABC) transporters revealed participation of ABCB subfamily in abiotic stress management of *Glycyrrhiza glabra* L. *BMC genomics* 25(1): 315.
- Elbahnsi A., Dudas B., Cisternino S., Declèves X. and Miteva M.A. (2024) Mechanistic insights into P-glycoprotein ligand transport and inhibition revealed by enhanced molecular dynamics simulations. *Computational and Structural Biotechnology Journal* 23: 2548–2564.
- Figueira Mansur J., Ferreira Pereira A., Mansur J.F., Franco T.A., Alvarenga E.S.L., Sorgine M.H.F., ... and Moreira M.F. (2013) Silencing of P glycoprotein increases mortality in temephos treated *Aedes aegypti* larvae. *Insect molecular biology* 22(6): 648–658.
- Giacomini K.M., Huang S.M., Tweedie D.J., Benet L.Z., Brouwer K.L.R., Chu X., Dahlin A., Evers R., Fischer V., Hillgren K.M., Hoffmaster K.A., Ishikawa T., Keppler D., Kim R.B., Lee C.A., Niemi M., Polli J.W., Sugiyama Y., Swaan P.W., Ware J.A., Wright S.H., Yee S.W., Zamek-Gliszczynski M.J. and Zhang L. (2010) Membrane transporters in drug development. *Nature Reviews Drug Discovery* 9(3): 215–236.
- Gottesman M.M. and Ling V. (2006) The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. *FEBS letters* 580(4): 998–1009.
- Grailles M., Brey P.T. and Roth C.W. (2003) The *Drosophila melanogaster* multidrug-resistance protein 1 (MRP1) homolog has a novel gene structure containing two variable internal exons. *Gene* 307(1): 41–50.
- Guiral M., Viratelle O., Westerhoff H.V. and Lankelma J. (1994) Cooperative P-glycoprotein mediated daunorubicin transport into DNA-loaded plasma membrane vesicles. *FEBS letters* 346(2-3): 141–145. doi: 10.1016/0014-5793(94)00447-1.
- Han Y., Gao H., Sun Y., Wang Y., Yan C., Ma H., Liu X and Huang Z. (2024) Target gene overexpression and enhanced metabolism confer resistance to nicosulfuron in *Eriochloa villosa* (Thunb.). *Pesticide Biochemistry and Physiology* 202: 105946. doi: 10.1016/j.pestbp.2024.105946.
- Huang Y., Chen Z., Lan J., Zhang L., Chen H., Jiang L., Yu H., Liu N., Liao C. and Han Q. (2024) MDR49 coding for both P-glycoprotein and TMOF transporter functions in ivermectin resistance, trypsin activity inhibition, and fertility in the yellow fever mosquito, *Aedes aegypti*. *Pesticide Biochemistry and Physiology* 201: 105899. doi: 10.1016/j.pestbp.2024.105899.
- IAEA (2017) Guidelines for Routine Colony Maintenance of *Aedes* Mosquito Species. Food and Agriculture Organization of the United Nations, International Atomic Energy Agency, Vienna. 18pp.
- Ibrahim S.S., Kouamo M.F., Muhammad A., Irving H., Riveron J.M., Tchouakui M. and Wondji C.S. (2024) Functional Validation of Endogenous Redox Partner Cytochrome P450 Reductase Reveals the Key P450s CYP6P9a/-b as Broad Substrate Metabolizers Conferring Cross-Resistance to Different Insecticide Classes in *Anopheles funestus*. *International Journal of Molecular Sciences* 25(15): 8092.
- Johnston C.U. and Kennedy C.J. (2024) A Review of P-Glycoprotein Function and Regulation in Fish. *Fishes* 9(2): 51.
- Kido Y., Nanchi I., Matsuzaki T., Watari R., Kiyohara H., Seki N. and Okuda T. (2024) Prediction of drug-drug interaction risk of P-glycoprotein substrate in drug discovery. *Drug Metabolism and Pharmacokinetics* 56: 101008.
- Kundu A., Ghosh P. and Bishayi B. (2024) Vitexin along with verapamil downregulates efflux pump P-glycoprotein in macrophages and potentiate M1 to M2 switching via TLR4-NF- κ B-TNFR2 pathway in lipopolysaccharide treated mice. *Immunobiology* 229(1): 152767.
- Lanning C.L., Fine R.L., Corcoran J.J., Ayad H.M., Rose R.L. and Abou-Donia M.B. (1996) Tobacco budworm P-glycoprotein: biochemical characterization and its involvement in pesticide resistance. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1291(2): 155–162.
- Lanning N.J. and Carter-Su C. (2006) Recent advances in growth hormone signalling. *Reviews in Endocrine and Metabolic Disorders* 7: 225–235.
- Leader J.P. and O'Donnell M.J. (2005) Transepithelial

- transport of fluorescent p-glycoprotein and MRP2 substrates by insect Malpighian tubules: confocal microscopic analysis of secreted fluid droplets. *Journal of Experimental Biology* 208(23): 4363–4376.
- Lespine A., Blancfuney C., Prichard R. and Alberich M. (2024) P-glycoproteins in anthelmintic safety, efficacy, and resistance. *Trends in Parasitology* 40(10): 896–913. doi: 10.1016/j.pt.2024.07.008.
- Lima E.P., Goulart M.O.F. and Rolim Neto M.L. (2014) Evaluation of the role of ATP-binding cassette transporters as a defence mechanism against temephos in populations of *Aedes aegypti*. *Memórias do Instituto Oswaldo Cruz* 109(7): 961–963.
- Ma A., Wang C., Chen Y. and Yuan W. (2013) P-glycoprotein alters blood & amp; ndash; brain barrier penetration of antiepileptic drugs in rats with medically intractable epilepsy. *Drug Design Development and Therapy* 1447. doi: 10.2147/dddt.s52533.
- Manyu S., Elanchezhian C., Sivasankaran K. and Basker P. (2022) Susceptibility of *Aedes albopictus* (Skuse, 1894) against the organophosphorus insecticide temephos, in Chidambaram, Tamil Nadu, India. *ENTOMON* 47(4): 383–390. doi: 10.33307/entomon.v47i4.791.
- Parida K.K., Lahiri M., Ghosh M., Dalal A. and Kalia N.P. (2024) P-glycoprotein inhibitors as an adjunct therapy for TB. *Drug Discovery Today* 29(9): 104108. doi: 10.1016/j.drudis.2024.104108.
- Podsiadlowski L., Matha V. and Vilcinskis A. (1998) Detection of a P-glycoprotein related pump in Chironomus larvae and its inhibition by verapamil and cyclosporin A. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 121(4): 443–450.
- Porretta D., Gargani M., Bellini R., Medici A., Punelli F. and Urbanelli S. (2008) Defence mechanisms against insecticides temephos and diflubenzuron in the mosquito *Aedes caspius*: the P glycoprotein efflux pumps. *Medical and veterinary entomology* 22(1): 48–54.
- Rani P., Mandal P., Rajak B.K. and Singh D.V. (2024) A review on dynamics of permeability-glycoprotein in efflux of chemotherapeutic drugs. *Frontiers in Drug Discovery* 4: 1363364.
- Rheault M.R., Plaumann J.S. and O'Donnell M.J. (2006) Tetraethylammonium and nicotine transport by the Malpighian tubules of insects. *Journal of insect physiology* 52(5): 487–498.
- Roth C.W., Holm I., Graille M., Dehoux P., Rzhetsky A., Wincker P., Weissenbach J. and Brey P.T. (2003) Identification of the *Anopheles gambiae* ATP-binding cassette transporter superfamily genes. *Molecules and Cells* 15(2): 150–158.
- Sankar M. and Kumar S. (2023) A systematic review on the eco-safe management of mosquitoes with diflubenzuron: an effective growth regulatory agent. *Acta Ecologica Sinica* 43(1): 11–19.
- Schlemmer S.R. and Sirotinak F.M. (1994) Functional studies of P-glycoprotein in inside-out plasma membrane vesicles derived from murine erythroleukemia cells overexpressing MDR 3. Properties and kinetics of the interaction of vinblastine with P-glycoprotein and evidence for its active mediated transport. *Journal of Biological Chemistry* 269(49): 31059–31066.
- See Y.P., Carlsen S.A., Till J.E. and Ling V. (1974) Increased drug permeability in Chinese hamster ovary cells in the presence of cyanide. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 373(2): 242–252.
- Shukla S., Wu C.P. and Ambudkar S.V. (2008) Development of inhibitors of ATP-binding cassette drug transporters—present status and challenges. *Expert opinion on drug metabolism & toxicology* 4(2): 205–223.
- Szpila K., Pape T. and Rusinek A. (2008) Morphology of the first instar of *Calliphora vicina*, *Phormia regina* and *Lucilia illustris* (Diptera, Calliphoridae). *Medical and Veterinary Entomology* 22(1): 16–25.
- Tapadia M.G. and Lakhota S.C. (2005) Expression of *mdr49* and *mdr65* multidrug resistance genes in larval tissues of *Drosophila melanogaster* under normal and stress conditions. *Cell stress & chaperones* 10(1): 7.
- Thévenod F. and Lee W.K. (2024) Cadmium transport by mammalian ATP-binding cassette transporters. *Biometals* 1–23.
- Tseng S.P., Lee S.H., Choe D.H. and Lee C.Y. (2024) Overexpression of cytochrome P450 gene CYP6K1 is associated with pyrethroid resistance in German cockroaches (Blattodea: Ectobiidae) from California. *Journal of Economic Entomology* 117(3): 1071–1076.
- Veider F., Haddadzadegan S., Armengol E.S., Laffleur F., Kali G. and Bernkop-Schnürch A. (2024) Inhibition of P-glycoprotein-mediated efflux by thiolated

- cyclodextrins. *Carbohydrate Polymers* 327: 121648.
- Wang A., Zhang Y., Liu S., Xue C., Zhao Y., Zhao M., Yang Y. and Zhang J. (2024) Molecular mechanisms of cytochrome P450-mediated detoxification of tetraniliprole, spinetoram, and emamectin benzoate in the fall armyworm, *Spodoptera frugiperda* (JE Smith). *Bulletin of Entomological Research* 114(2): 159–171. doi: 10.1017/S000748532300038X.
- WHO (2022) World Health Organization guidelines for malaria, 3 June 2022 (No. WHO/UCN/GMP/2022.01 Rev. 2).
- Xu J., Zheng J., Zhang R., Wang H., Du J., Li J., ... and Shen B. (2023) Identification and functional analysis of ABC transporter genes related to deltamethrin resistance in *Culex pipiens pallens*. *Pest Management Science* 79(10): 3642–3655.
- Xu M., Molento M., Blackhall W., Ribeiro P., Beech R. and Prichard R. (1998) Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Molecular and biochemical parasitology* 91(2): 327–335.

(Received March 21, 2026; revised ms accepted July 113, 2026; published September 30, 2026)