Revista Eletrônica
Acervo Saúde
Electronic Journal Collection Health ISSN 2178-2091

Molecular protein targets in *Aedes aegypti* for insecticide development

Alvos proteicos moleculares em *Aedes aegypti* para o desenvolvimento de inseticidas

Objetivos proteicos moleculares en *Aedes aegypti* para el desarrollo de insecticidas

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ABSTRACT

Objective: To identify the main proteins of *Aedes aegypti* investigated in the development of insecticides. **Methods:** A review of the scientific literature published between 2014 and 2024 was conducted using PubMed, ScienceDirect, and Scopus databases, with the descriptors "*Aedes aegypti*," "protein targets," and "mode of action." Peer-reviewed experimental articles published in English were included. The selected studies were analyzed according to the types of target proteins investigated and the methodological approaches used (*in silico*, *in vitro*, and *in vivo*). **Results:** Of the 335 articles initially retrieved, 17 met the inclusion criteria. Glutathione S-transferase (GST) was the most frequently investigated detoxification target, followed by several GST isoforms. Key metabolic targets included odor-binding protein 1 (OBP1), 3-hydroxykynurenine transaminase (HKT), acetylcholinesterase (AChE), sterol carrier protein-2 (SCP-2), and carbonic anhydrase (CA). Neurotransmission-related targets comprised the dopaminergic receptor AaDOP2 and the chloride-cation cotransporters CCC2 and CCC3. **Conclusion:** The results highlight protein targets as a promising strategy for *A. aegypti* control and emphasize the need to identify new molecular targets for more effective and sustainable insecticides.

Keywords: *Aedes aegypti*, Protein targets, Insecticide development, Molecular targets.

RESUMO

Objetivo: Identificar as principais proteínas de *Aedes aegypti* investigadas no desenvolvimento de inseticidas. **Métodos:** Foi realizada uma revisão da literatura científica publicada entre 2014 e 2024 utilizando as bases de dados Pubmed, ScienceDirect e Scopus, com os descritores "*Aedes aegypti*", "proteínas-alvo" e "modo de ação". Foram incluídos artigos experimentais revisados por pares e publicados em inglês. Os estudos selecionados foram analisados de acordo com os tipos de proteínas-alvo investigadas e as abordagens metodológicas utilizadas (*in silico*, *in vitro* e *in vivo*). **Resultados:** Dos 335 artigos inicialmente recuperados, 17 atenderam aos critérios de inclusão. A glutationa S-transferase (GST) foi o alvo de detoxificação mais frequentemente investigado, seguida por diversas isoformas de GST. Os principais alvos metabólicos incluíram a proteína de ligação ao odor 1 (OBP1), a 3-hidroxiquinurenina transaminase (HKT), a acetilcolinesterase (AChE), a proteína transportadora de esteróis 2 (SCP-2) e a anidrase carbônica (CA). Os alvos relacionados à neurotransmissão compreenderam o receptor dopaminérgico AaDOP2 e os cotransportadores de cloreto-cátion CCC2 e CCC3. **Conclusão:** Os resultados destacam os alvos proteicos como uma estratégia promissora para o controle de *A. aegypti* e

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SUBMETIDO EM: 3/2026 | **ACEITO EM: 4/2026** | **PUBLICADO EM: 7/2026**

enfatizam a necessidade de identificar novos alvos moleculares para inseticidas mais eficazes e sustentáveis.

Palavras-chave: *Aedes aegypti*, Alvos proteicos, Desenvolvimento de inseticidas, Alvos moleculares.

RESUMEN

Objetivo: Identificar las principales proteínas de *Aedes aegypti* investigadas en el desarrollo de insecticidas. **Métodos:** Se realizó una revisión de la literatura científica publicada entre 2014 y 2024 utilizando las bases de datos PubMed, ScienceDirect y Scopus, con los descriptores "*Aedes aegypti*", "protein targets" y "mode of action". Se incluyeron artículos experimentales revisados por pares y publicados en inglés. Los estudios seleccionados se analizaron según los tipos de proteínas diana investigadas y los enfoques metodológicos utilizados (*in silico*, *in vitro* e *in vivo*). **Resultados:** De los 335 artículos recuperados inicialmente, 17 cumplieron los criterios de inclusión. El glutatión S-transferasa (GST) fue la diana de desintoxicación investigada con mayor frecuencia, seguida de varias isoformas de GST. Las dianas metabólicas clave incluyeron la proteína fijadora de olores 1 (OBP1), la 3-hidroxiquinurenina transaminasa (HKT), la acetilcolinesterasa (AChE), la proteína transportadora de esteroles 2 (SCP-2) y la anhidrasa carbónica (CA). Las dianas relacionadas con la neurotransmisión incluyeron el receptor dopaminérgico AaDOP2 y los cotransportadores de cationes cloruro CCC2 y CCC3. **Conclusión:** Los resultados destacan las dianas proteicas como una estrategia prometedora para el control de *A. aegypti* y enfatizan la necesidad de identificar nuevas dianas moleculares para insecticidas más eficaces y sostenibles.

Palabras clave: *Aedes aegypti*, Dianas proteicas, Desarrollo de insecticidas, Dianas moleculares.

INTRODUCTION

Dengue virus (DENV) is a flavivirus belonging to the Flaviviridae family and is transmitted mainly by *Aedes aegypti* mosquitoes. This viral family comprises more than 70 virusescapable of infecting humans and is associated with high morbidity and mortality rates worldwide. Currently, four antigenically distinct serotypes of dengue virus are recognized (DENV-1, DENV-2, DENV-3, and DENV-4) (KOK BH, et al., 2023).

DENV infection can cause a broad spectrum of clinical manifestations, ranging from asymptomatic infection or mild dengue fever to severe conditions such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It is estimated that approximately 3.6 billion people are at risk of infection worldwide, with about 390 million infections occurring annually, of which approximately 96 million are clinically apparent (AGUIAR GRF, et al., 2024).

From a structural perspective, the dengue virus exhibits a spherical morphology and is an enveloped virus with a diameter of approximately 40–50 nm. Its genome consists of single-stranded positive-sense RNA, which can be directly translated by the host cell. This genetic material is associated with an icosahedral nucleocapsid formed by the capsid protein. Externally, the virus is surrounded by a lipid bilayer containing the membrane (M) and envelope (E) proteins, with the latter being essential for host cell receptor recognition and viral entry (POURZANGIABADI M, et al., 2024).

According to the World Health Organization (WHO), in 2023, approximately 4.1 million suspected dengue cases were reported across 42 countries in the Americas, resulting in 2,049 deaths, corresponding to a case fatality rate of 0.05% (WHO, 2023; SANTOS LLM, et al., 2023). In Brazil, the epidemiological scenario is particularly concerning. In 2023, the country recorded 2,376,522 dengue cases, representing the highest number of cases in the Americas. The simultaneous circulation of the four viral serotypes, combined with the increasing resistance of *A. aegypti* to conventional insecticides, has compromised the effectiveness of traditional control strategies (SANTOS LLM, et al., 2023).

Dengue fever is considered one of the most important arboviral diseases worldwide, mainly due to the high adaptability of *A. aegypti* to domestic environments in densely populated urban areas of tropical and subtropical regions, together with the coexistence of the four viral serotypes (LIMA-CÂMARA TN, et al., 2016). In recent decades, several prevention and health-promotion measures have been implemented in

Brazil to reduce disease transmission, including public awareness campaigns, community-based breeding-site elimination programs, governmental epidemiological surveillance actions, and public policies focused on vector control, as well as the recent introduction of vaccines as a complementary strategy.

Among the strategies used to control *A. aegypti*, biological, chemical, mechanical, and biotechnological approaches stand out, which can be applied either individually or in an integrated manner. In this context, chemical control has been widely adopted due to its high effectiveness and relatively low cost, particularly considering that mosquito larvae predominantly develop in small domestic water containers, which reduces the risk of widespread environmental contamination (POURZANGIABADI M, et al., 2024). However, many of the insecticides currently used exhibit high toxicity and adverse effects on non-target organisms, limiting their long-term and continuous use.

In addition, the development of insecticide resistance in mosquito populations, along with toxic effects reported in aquatic organisms such as fish and crustaceans, reinforces the urgent need for alternative control approaches. Most insecticides currently available target the nervous system of the vector, interfering with essential physiological processes, such as nerve impulse conduction, by inhibiting ion channels or key enzymes involved in neurotransmission, including acetylcholinesterase (MUTUNGA JM, et al., 2019). Given this scenario, the present study aims to identify and analyze the main protein targets investigated in *A. aegypti* for insecticide development, with emphasis on their biological roles and potential application in innovative vector control strategies.

METHODS

This study adopted a qualitative and quantitative methodological approach, based on an integrative review of scientific literature focusing on the identification of protein targets in *A. aegypti* associated with the development of insecticidal compounds.The bibliographic search was conducted in the PubMed, Scopus, and ScienceDirect databases, using the descriptors "*Aedes aegypti*," "protein targets," and "mode of action," combined using operators when applicable.

After study identification, the data were analyzed using qualitative and quantitative approaches, considering the following inclusion criteria: (i) peer-reviewed experimental studies, with no geographical restriction; (ii) articles published in English between January 2014 and December 2024; and (iii) studies that investigated molecular targets in *A. aegypti* with potential application in insecticide development.

A total of 23 molecular target occurrences were identified across the selected studies. Duplicate records referring to the same molecular target, whether from experimental or *in silico* approaches, were consolidated, and protein families represented by multiple isoforms were grouped into a single category. This procedure included, in particular, grouping glutathione S-transferase isoforms and integrating repeated targets such as acetylcholinesterase, odorant-binding protein 1, and voltage-gated sodium channels. After this consolidation process, the final dataset comprised 14 unique molecular targets, which were used as the basis for percentage calculations.

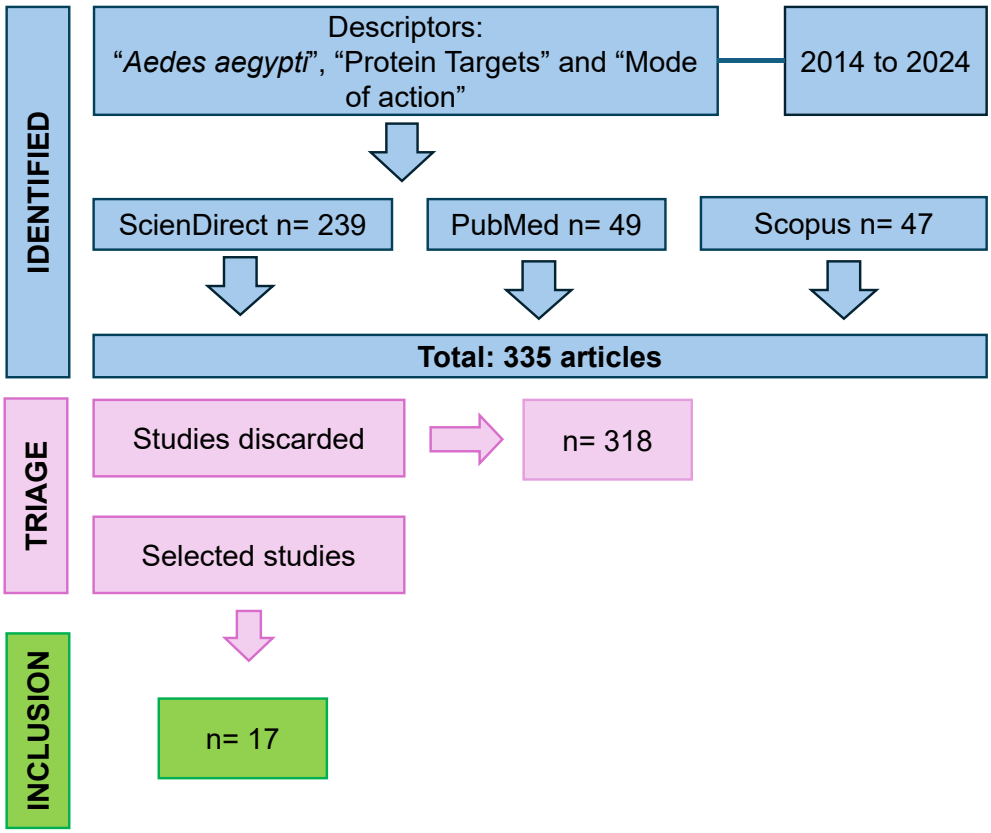
The identified molecular targets were subsequently categorized by target type, cellular localization, physiological function, amino acid sequence, molecular weight (MW), identification code (ID), assay type, and the bibliographic reference in which the protein was described as a molecular target. The molecular targets discussed below were selected based on their biological relevance, frequency of investigation, representation across the analyzed studies, and functional importance in key physiological processes of *A. aegypti*. Subsequently, a search for their corresponding entries was performed in the UniProt database (https://www.uniprot.org) to obtain relevant molecular and functional information for subsequent analyses. In addition, structural analyses of the selected proteins were performed using PyMOL, enabling three-dimensional visualization and detailed assessment of key structural features.

RESULTS AND DISCUSSION

Initially, a search was conducted in the ScienceDirect database (**Figure 1**), retrieving 239 articles. After screening, 232 records were excluded for not meeting the predefined inclusion criteria, leaving seven studies included. In PubMed, 49 articles were retrieved, of which 2 were included. In Scopus, 47 articles were

identified, of which 8 met the inclusion criteria. Therefore, after screening and selection, a total of 17 studies were included in the review.

Figure 1 - Flowchart illustrating the identification, screening, and inclusion of studies in the integrative literature review, based on searches conducted in the ScienceDirect, PubMed, and Scopus databases using the descriptors "*Aedes aegypti*", "protein targets", and "mode of action", covering the period from 2014 to 2024.



Source: Silva CW, et al., 2026.

Molecular Targets in Aedes aegypti

In this review, 14 unique molecular targets associated with insecticidal activity in *Aedes aegypti* were identified after consolidating duplicate records and grouping protein isoforms into single functional categories. A total of 23 target occurrences were recorded across experimental and *in silico* studies, indicating differences in the frequency with which specific proteins have been investigated. These targets encompass a broad spectrum of physiological functions, reflecting the diversity of molecularsystems explored for the development of novel vector control strategies.

Glutathione S-transferase (GST), including its isoforms grouped as a single family, was the most frequently investigated target, accounting for 31% of the total occurrences. This was followed by acetylcholinesterase (AChE), odorant-binding protein 1 (OBP1), and the voltage-gated sodium channel (VGSC), each representing 9% of the reported targets.

Targets with intermediate frequency included the inward-rectifierpotassium channel AeKir1 and odorant-binding protein 22 (OBP22), each accounting for 5% of total occurrences. The remaining molecular targets were less frequently reported, each representing 4%, including sterol carrier protein-2 (SCP-2), 3-hydroxykynurenine transaminase (HKT), the D1-like dopaminergic receptor AaDOP2, α-esterase, carbonic anhydrase (CA), histone deacetylase 2 (HDAC2), and the sodium-coupled cation–chloride cotransporters CCC2 and CCC3, as summarized in **Tables 1** and **2**.

Table 1 - Molecular targets identified in *Aedes aegypti* with experimentally validated biological activity *in vitro* and *in vivo* assays.

Molecular Targets	Location	Physiological Function	Sequence of residues	Molecular weight (Daltons)	UniProt code	Type of test	References
Inward rectifier potassium channel(AeKir1)	Malpighian tubules	Transport of potassium ions (K^+) and regulation of fluid excretion.	486	55,24	L0EQS9	*in vitro* (LC_{50})	ROUHIER MF, et al., 2014
							PIERMARINI PM, et al., 2017
						in vivo (LC_{50})	BEYENBACH KW, et al., 2015
							SWALE DR, et al., 2016
Acetylcholinesterase (AChE)	Nervous system	Hydrolysis of acetylcholine	702	78,19	Q6A2E2	*in vitro* (LC_{50})	SILVA AS, et al., 2023
						in vivo (LC_{50})	JOHNSON EJ, et al., 2023
Odor-Binding Protein22 (OBP22)	Antennae and sensory organs - olfactory sensilla of the antennae (subcellular)	Detection of volatile chemical compounds	138	15,86	Q1HRL7	*in vitro* (LC_{50})	ALBUQUERQUE BNL, et al., 2022
Odor-Binding Protein1 (OBP1)			143	16,36	Q6Y2R8	*in vitro* (LC_{50})	
Voltage-Gated Sodium Channel (VGSC)	Nervous system	Conduction of nerve impulses	2.140	240,2	C3RTM2	*in vivo* (LC_{50})	FARIA-REIS A, et al., 2023
D1-like dopamine receptor (AaDOP2)	Plasma membrane	G protein-coupled receptor (GPCR), activated by dopamine.	476	5,33	H6D5H2	*in vivo* (LC_{50})	CONLEY JM, et al., 2015
3-hydroxykynurenine transaminase (HKT)	Cytoplasm	It catalyzes the transamination Reaction of 3-hydroxykynurenine (3-HK)	400	44,69	Q0IG34	*in vitro* (LC_{50})	MACIEL LG, et al., 2020
							MACIEL LG, et al., 2021

Source: Silva CW, et al., 2026.

Table 2 - Molecular targets of *Aedes aegypti* investigated using *in silico* approaches.

Molecular Targets	Location	Physiological Function	Sequence of residues	Molecular weight (Daltons)	UniProt code	References
Acetylcholinesterase (AChE)	Nervous system	Hydrolysis of acetylcholine	702	78,19	Q6A2E2	SANTOS ELVS, et al., 2024 SHAHID M,et al., 2023
Sterol Carrier Protein-2 (SCP-2)	Cytoplasm	Intracellular transport of lipids	110	12,27	Q86PR3	MOURÃO EDS, et al., 2021
Glutathione-S-transferase (GST)		Cytoplasmic detoxification enzymes	218	24,06	Q95W09	SETLURAS, et al., 2022
α-esterase	Nervous tissues	Hydrolysis of neurotransmitters	556	62,453	Q17G39	
Voltage-Gated Sodium Channels(VGSC)	Nervous system	Conduction of nerve impulses	2.140	240,2	C3RTM2	
Odor-Binding Protein (OBP1)	Antennae and sensory organs	Detection of volatile chemical compounds	143	16,36	Q6Y2R8	RANA A, et al., 2024
Carbonic anhydrase (CA)	Food channel	It plays a role in bicarbonate metabolism, pH regulation, and ion transport	276	31,3	Q17BL7	ARAÚJO MO, et al., 2020
Histone deacetylase 2 (HDAC2)	Nuclear (in cells in general)	It deacetylates histones, regulating gene expression and chromosomal stability	487	55,24	Q17CF0	
Sodium-coupled chloride cation cotransporter 2 (CCC2)	Cell membrane	Ion transporter (Na^+/Cl^-) associated with osmotic balance and cell signaling	1.063	117,3	Q16UI3	
Sodium-coupled chloride cation cotransporter 3 (CCC3)		Like CCC2, it acts in ion transport, but its exact mechanism is not yet clear	1.027	113,4	Q16UI2	
Glutathione transferase 2 (GSTE2)	Cytoplasm	Cytoplasmic detoxification enzymes	222	24,69	Q170D0	
Glutathione transferase 4 (GSTE4)			224	25,04	Q170D1	
Glutathione transferase D2 (GSTD2)			290	33,04	Q17MB0	
Glutathione transferase T1 (GSTT1)			229	26,83	Q5PY76	
Glutathione transferase X2 (GSTX2)			218	24,06	Q16SS9	
Glutathione transferase T2 (GSTT2)			227	26,46	Q16X19	

Source: Silva CW, et al., 2026.

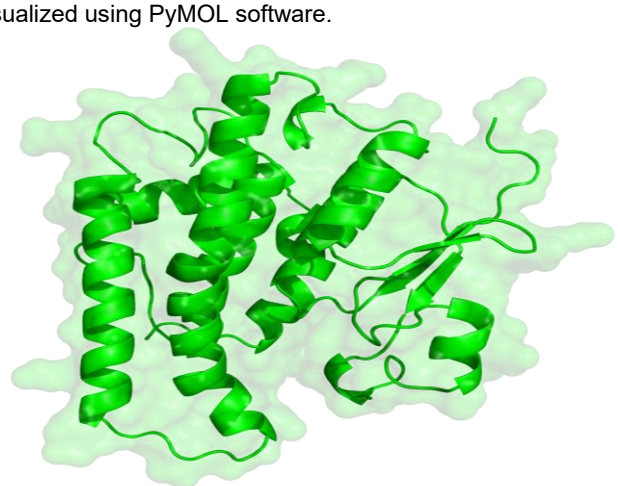
Glutathione-S-transferase (GST)

Glutathione S-transferases (GSTs) are phase II detoxification enzymes widely distributed across most organisms and play a central role in cellular defense mechanisms. In insects, these enzymes are fundamental to metabolic detoxification, acting primarily by conjugating reducedglutathione (GSH) to xenobiotics and toxic byproducts generated during oxidative stress. In mosquitoes, elevated GST activity has been consistently associated with increased resistance to insecticides, as these enzymes catalyze the nucleophilic attack of GSH on electrophilic compounds, thereby reducing their toxicity and facilitating their elimination (SETLUR AS, et al., 2022). In line with this, glutathione S-transferases have been widely recognized as key molecular targets in arthropods, as their inhibition compromises detoxification capacity and can enhance susceptibility to xenobiotics, including acaricides and insecticides (BEZERRA WAS, et al. 2026).

Within this context, the characterized glutathione S-transferase corresponds to a cytosolic GST from *Aedes aegypti*, the main vector of dengue and yellow fever. Evidence indicates that this isoform plays an active role in detoxification pathways and oxidative stress responses, particularly following blood feeding, a physiological event that promotes heme release and thereby increases reactive oxygen species production. Oliveira JHM, et al. (2020) demonstrated that distinct GST isoforms exhibit differential expression in mosquito tissues exposed to heme, reinforcing previous observations regarding the functional relevance of this cytosolic GST. The association with heme metabolism highlights the biological and physiological importance of this enzyme in the mosquito lifecycle.

From a structural perspective, cytosolic GSTs of *A. aegypti* (**Figure 2**) are composed of two subunits that can assemble as homodimers or heterodimers, a feature that contributes to their functional versatility. Each subunit comprises approximately 210–240 amino acids, with an average molecular mass of around 25 kDa. Structurally, GST subunits contain a conserved binding site for reduced glutathione, the G-site, which is adjacent to a more variable hydrophobic pocket, the H-site. The G-site, located in the N-terminal region, shows high conservation across GST isoforms, reflecting its essential catalytic role. In contrast, the H-site is predominantly formed by residues in the C-terminal region and displays considerable structural diversity, which is directly associated with the differences in substrate specificity observed among GSTs (MANNERVIK B, 1976; RANSON H, et al., 1998).

Figure 2 - Three-dimensional structure of glutathione S-transferase (GST), corresponding to the UniProt accession code Q16X19, obtained from the UniProt database and visualized using PyMOL software.



Source: Silva CW, et al., 2026. Data extracted UniProt database (https://www.uniprot.org), visualization generated using PyMOL.

Recent studies have investigated the role of the glutathione S-transferase Noppera-bo (Nobo) in *A. aegypti*, demonstrating that specific flavonoids can inhibit enzymatic activity. Using integrated biochemical assays, structural analyses, and larvicidal tests, the authors demonstrated that inhibition of GST Nobo

disrupts ecdysteroid biosynthesis, hormones essential for insect growth and metamorphosis, resulting in delayed larval development and increased mortality. The relevance of GSTs in the insecticidal context lies in their dual role: they are involved not only in the detoxification of xenobiotic compounds but also in critical physiological processes in insects. Therefore, targeting GSTs, particularly the Nobo isoform, as molecular targets represents a promising strategy for the development of more selective insecticides, effective against *A. aegypti*, with reduced environmental impact, and constituting an alternative approach to mitigate the increasing resistance to conventional insecticides (EBIHARA K and NIWA R, 2023).

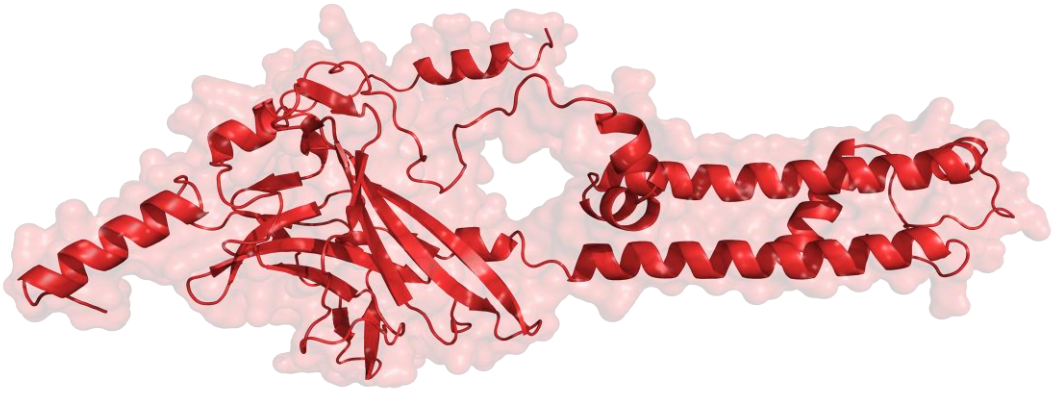
In addition to their role as direct insecticide targets, insect GSTs are strongly associated with mechanisms of insecticide resistance, acting through the direct metabolic degradation of insecticides, the sequestration of toxic molecules, or the protection against secondary toxic effects, such as enhanced lipid peroxidation induced by insecticide exposure. Several studies have demonstrated that GST-mediated detoxification occurs across multiple insecticide classes, including organophosphates and other chemical groups, highlighting the broad involvement of these enzymes in neutralizing diverse insecticidal compounds. In the case of organophosphate insecticides, GSTs catalyze O-dealkylation and O-dearylation reactions, which significantly reduce the toxicity of these molecules (KOSTAROPOULOS I, et al., 2001).

Potassium channel input rectifier (AeKir1)

The AeKir1 channel, identified in *A. aegypti*, belongs to the family of inwardly rectifying potassium (Kir) channels, which are responsible for preferentially allowing the influx of K^+ ions into the cell. In insects, Kir channels play a crucial ro/le in the regulation of membrane potential and ionic homeostasis, with AeKir1 being one of the most highly expressed channels in the Malpighian tubules, a tissue that is essential for maintaining osmotic balance in mosquitoes (ROUHIER MF, et al., 2014; BEYENBACH KW, et al., 2015). The inward rectification property characteristic of Kir channels results from the blockage of outward K^+ currents by intracellular polyamines and Mg^{2+} ions, thereby ensuring electrical stability of the cell membrane (HIBINO H, et al., 2010).

From a structural perspective, Kir channels are composed of two transmembrane helices (M1 and M2), a pore-forming loop containing a highly conserved selectivity filter, and extensive cytoplasmic N- and C-terminal domains. These subunits are assembled into a tetrameric complex, which constitutes the functional ion channel (HIBINO H, et al., 2010). Comparative structural analyses indicate that AeKir1 conserves all of these fundamental architectural elements, which accounts for its high selectivity for K^+ ions. The tetrameric arrangement is essential for the precise alignment of the selectivity filter, a feature that is conserved among both vertebrate and hematophagous insect Kir channels (ROUHIER MF, et al., 2014; BEYENBACH KW, et al., 2015; SWALE DR, et al., 2016) (**Figure 3**).

Figure 3 - Three-dimensional structure of the inward rectifier potassium channel (AeKir1), corresponding to the UniProt accession code L0EQS9, obtained from the UniProt database and visualized using PyMOL software.



Source: Silva CW, et al., 2026. Data extracted UniProt database (https://www.uniprot.org), visualization generated using PyMOL.

Beyond its basic architecture, the cytoplasmic domains of AeKir1 play a pivotal role in regulating channel activity. Studies have demonstrated that these regions interact directly with phosphatidylinositol-4,5-bisphosphate (PIP_2), a membrane lipid that is essential for channel opening and for maintaining Kir channels in a conductive state (HIBINO H, et al., 2010; PIERMARINI PM, et al., 2015). The binding of PIP_2 stabilizes the channel's open conformation, whereas disruption of this interaction may reduce ionic conductance or lead to functional pore closure, ultimately impairing channel activity.

From a physiological standpoint, the high expression of AeKir1 in the Malpighian tubules underscores its critical role in primary urine formation. The K^+ flux mediated by Kir channels generates the electrochemical driving force required for the secondary transport of other ions and water, thereby supporting the rapid excretion of excess fluid following a blood meal (PIERMARINI PM, et al., 2015). Interference with this process severely compromises the mosquito's ability to maintain osmotic homeostasis, leading to profound physiological dysfunctions that are incompatible with survival.

Taken together, the physiological relevance, structural specificity, and pharmacological accessibility of AeKir1 highlight this channel as a highly promising molecular target for the development of selective insecticides.

Compounds that inhibit AeKir1 induce functional collapse of the insect excretory system, leading to mortality without direct interference with the central nervous system. This mode of action reduces the likelihood of cross-resistance with conventional neurotoxic insecticides (RAPHEMOT R, et al., 2013; PIERMARINI PM, et al., 2015). Therefore, an in-depth investigation of the structure and function of AeKir1 not only advances understanding of *A. aegypti* physiology but also supports the development of innovative, environmentally sustainable strategies for vector control.

Acetylcholinesterase

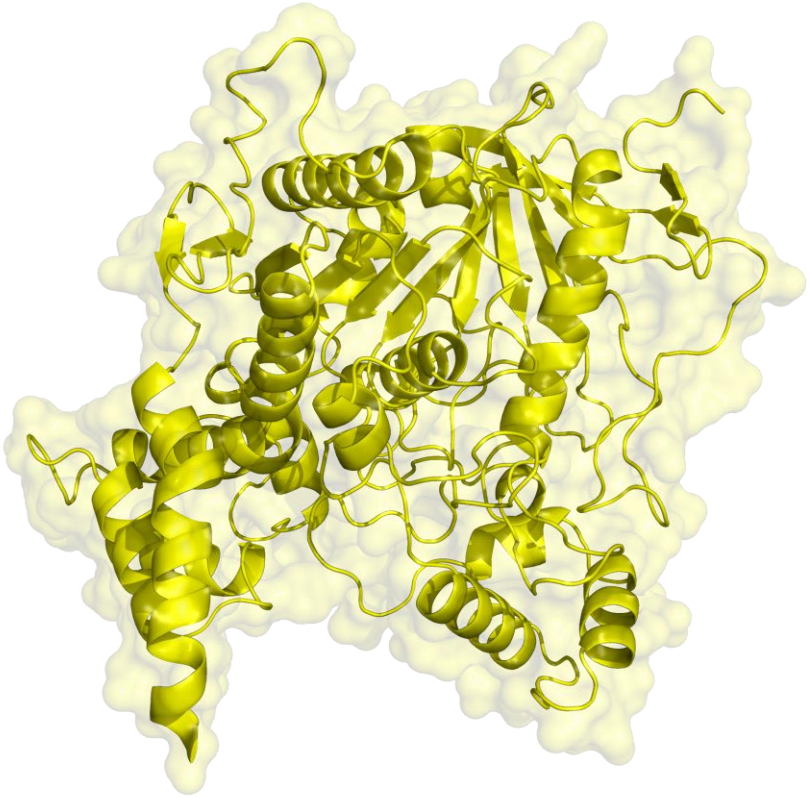
Acetylcholinesterase (AChE) is a key enzyme in the nervous system of *A. aegypti*, responsible for the rapid hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. This enzyme is one of the principal molecular targets of organophosphate and carbamate insecticides, which have been widely employed in vector control programs. At synapses, AChE terminates nerve signal transmission by rapidly degrading released acetylcholine, thereby ensuring precise and orderly communication between neurons. Cholinergic synapses are broadly distributed throughout both the central and peripheral nervous systems, where they play essential roles in motor coordination, sensory processing, and other vital physiological functions of the insect (JOHNSON EJ, et al., 2023; SILVA AS, et al., 2023).

When AChE activity is reduced or inhibited, acetylcholine accumulates in the synaptic cleft, leading to prolonged postsynaptic receptor stimulation. This condition results in severe disruption of neuronal signaling, causing impairment of motor and sensory functions, followed by neuromuscular paralysis and, ultimately, insect death (JOHNSON EJ, et al., 2023; SANTOS ELVS, et al., 2024). This critical role in neurotransmission explains why AChE remains one of the most effective and widely exploited targets in the development of chemical insecticides.

Acetylcholinesterase belongs to the serine hydrolase family and is highly conserved across both vertebrates and invertebrates, reflecting the evolutionary importance of its catalytic function. The enzyme exhibits a three-dimensional structure that underlies its remarkable catalytic efficiency. Structurally, AChE adopts a characteristic α/β fold, consisting of a central β-sheet core surrounded by α-helical elements.

One of the most distinctive structural features of AChE is the presence of a deep, narrow catalytic gorge that extends from the protein surface to the active site. Within this gorge lies the catalytic triad,composed of serine, histidine, and glutamic acid residues, which is responsible for the rapid hydrolysis of acetylcholine. This architectural arrangement promotes optimal substrate orientation and efficient transition-state stabilization, thereby contributing to the high catalytic specificity and turnover rate of the enzyme (SUSSMAN JL, et al., 1991) (**Figure 4**).

Figure 4 - Three-dimensional structure of acetylcholinesterase (AChE), corresponding to the UniProt accession code Q6A2E2, obtained from the UniProt database and visualized using PyMOL software.



Source: Silva CW, et al., 2026. Data extracted UniProt database (https://www.uniprot.org), visualization generated using PyMOL.

In addition to the catalytic site, AChE contains a peripheral anionic site (PAS) near the entrance to the catalytic gorge, which plays a key role in the initial recognition and binding of the substrate, as well as in modulating enzymatic activity. This site also contributes to interactions with inhibitors, including organophosphate and carbamate insecticides. In insects, subtle structural variations in AChE, particularly within the catalytic gorge and active-site regions, can significantly alter enzyme–inhibitor affinity and are directly associated with the emergence of insecticide resistance. Therefore, detailed structural knowledge of AChE is essential for the rational design of more selective and effective insecticides (SANTOS ELVS, et al., 2024; SHAHID M, et al., 2023).

Acetylcholinesterase is the primary molecular target of organophosphate and carbamate insecticides, which exert their toxic effects by inhibiting catalytic activity, leading to acetylcholine accumulation at synapses, followed by continuous neuronal stimulation, paralysis, and insect death. However, reduced AChE sensitivity to inhibition has been widely reported in insect populations.Recent evidence indicates that resistance is multifactorial, involving structural alterations in the active site of the enzyme, often combined with the overexpression of genes associated with detoxification pathways (JOHNSON EJ, et al., 2023; SILVA AS, et al., 2023).

In recent years, population-based and molecular studies have reported decreased susceptibility of *A. aegypti* populations to organophosphate insecticides, such as temephos, across different regions of Brazil and other parts of the world. This phenomenon has been associated with changes in AChE activity and genetic polymorphisms in the ace-1 gene (JOHNSON EJ, et al., 2023; SHAHID et al., 2023; SILVA AS, et al., 2023). Although classical resistance-associated mutations, such as the G119S substitution, are rare in *A. aegypti*, regional genetic variability in ace-1 suggests distinct evolutionary trajectories, emphasizing the need for continuous surveillance. Thus, a comprehensive understanding of the structure, function, and genetic

variation of AChE in *A. aegypti* is fundamental for the development of new chemical and biotechnological control strategies, supporting the rational management of insecticide resistance (SPADARA, et al., 2024).

Within this framework, Santos ELVS, et al. (2024) conducted an *in silico* study to evaluate the interaction between constituents of *Ocimum basilicum* essential oil and *A. aegypti* acetylcholinesterase (UniProt: Q6A2E2), a central target in insecticide toxicity mechanisms.

The authors performed molecular docking analyses, comparing plant-derived compounds with the reference larvicide temephos. The results showed that certain compounds, such as α-selinene, exhibited high binding affinity to AChE (−9.1 kcal/mol), exceeding that of the positive control (temephos, −4.7 kcal/mol), thereby indicating strong inhibitory potential. Other constituents, including eugenol, also displayed relevant binding interactions, reinforcing the potential of natural products as promising sources of new, selective insecticides.

Odorant-binding protein 22 (OBP22) and D1-like dopamine receptor (AaDOP2)

The proteins OBP22 and AaDOP2 in *A. aegypti* represent two key molecular systems that regulate mosquito behavior, physiology, and vector competence. OBP22 (Odorant-Binding Protein 22) belongs to the odorant-binding protein (OBP) family, whose members are responsible for transporting hydrophobic molecules, such as volatile odorants and lipids, to sensory receptors.

According to Wang J, et al. (2020), AeOBP22 exhibits high affinity for fatty acids and undergoes ligand-induced conformational changes, indicating that its function extends beyond odor capture alone. Functional studies have demonstrated that loss or silencing of OBP22 leads to pronounced behavioral alterations, revealing pleiotropic effects that impact feeding behavior, reproductive performance, and viral transmission capacity (DONG S, et al., 2021).

The involvement of OBP22 in host-seeking behavior is well established. As highlighted by Pelosi P, et al. (2018) and Albuquerque BNL, et al. (2022), OBPs facilitate olfactory perception by transporting hydrophobic ligands through the sensillar lymph, thereby enabling efficient activation of olfactory receptors.

This mechanism is particularly critical in hematophagous insects such as *A. aegypti*, which rely heavily on olfactory cues to locate hosts. In the specific case of OBP22, its additional association with immune and reproductive processes further underscores its potential as a strategic molecular target, especially for interventions aimed at reducing mosquito–human contact and disease transmission.

From a structural standpoint, OBP22 belongs to the class of classic odorant-binding proteins, characterized by low molecular weight (approximately 14–16 kDa) and a highly conserved architecture across insects.

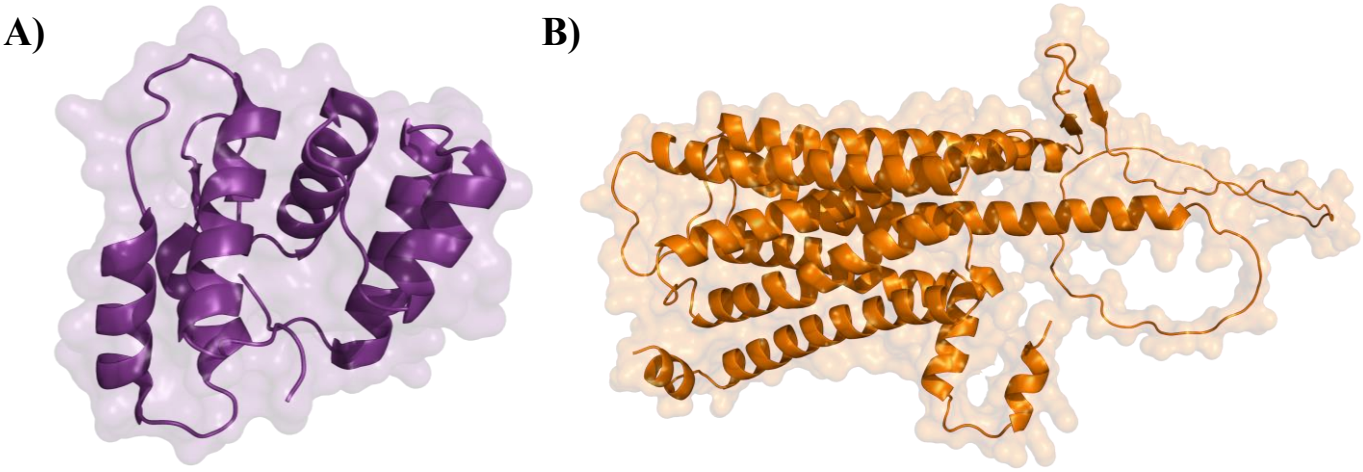
The protein adopts a predominantly α-helical conformation, stabilized by six conserved cysteine residues that form three intramolecular disulfide bridges. These disulfide bonds confer high structural stability and are essential for maintaining the internal hydrophobic binding pocket, which accommodates fatty acids and volatile compounds (PELOSI P, et al., 2018; ALBUQUERQUE BNL, et al., 2022).

Structural analyses indicate that this binding cavity undergoes ligand- and pH-dependent conformational changes, allowing controlled release of odorant molecules near olfactory receptors (ALBUQUERQUE BNL, et al., 2022).

Furthermore, *in silico* and functional studies have revealed that OBP22 displays notable structural flexibility, particularly in its terminal regions, which enhances its ability to interact with chemically diverse ligands. Wang J, et al. (2020) demonstrated that fatty acid binding induces reorganization of the ligand-binding site, suggesting an adaptive structural mechanism.

This conformational plasticity is closely linked to the pleiotropic phenotypes observed upon OBP22 disruption, reinforcing that its three-dimensional structure is critical not only for olfactory signaling but also for metabolic, reproductive, and immunological functions in *A. aegypti* (DONG S, et al., 2021).

Figure 5 - A) Three-dimensional structure of Odorant-Binding Protein 22 (OBP22), corresponding to the UniProt accession code Q1HRL7, obtained from the UniProt database and visualized in violet/purple.
B) Three-dimensional structure of the D1-like dopamine receptor (AaDOP2), corresponding to the UniProt accession code H6D5H2, obtained from the UniProt database and visualized in orange. All images were generated using PyMOL.



Source: Silva CW, et al., 2026. Data extracted UniProt database (https://www.uniprot.org), visualization generated using PyMOL.

In contrast, AaDOP2 is a D1-like dopaminergic receptor that belongs to the G protein–coupled receptor (GPCR) superfamily. These receptors regulate key neurological processes in insects, including locomotion, development, and behavioral modulation. Meyer JM, et al. (2012) demonstrated that the D1-like dopaminergic receptors AaDOP1 and AaDOP2 in *A. aegypti* exhibit the canonical GPCR architecture, characterized by seven transmembrane helices, and are capable of activating cAMP-dependent signaling pathways in response to dopamine, indicating their involvement in multiple physiological functions of the insect.

From a structural perspective, AaDOP2 is composed of seven α-helical transmembrane segments (TM I–VII), which are connected by extracellular and intracellular loops, with the N-terminus oriented toward the extracellular space and the C-terminus located in the cytoplasm. This highly conserved organization is essential for ligand recognition and for activation of the associated G protein, ultimately leading to stimulation of the cAMP signaling pathway (MEYER JM, et al., 2012). Specific structural regions, particularly transmembrane domains III, V, and VI, contribute directly to the formation of the dopamine-binding pocket, whereas the intracellular loops are critical for efficient G protein coupling and signal transduction.

Pharmacological evidence indicates that even subtle structural modifications in these key regions can markedly alter receptor affinity for agonists and antagonists, which helps explain the efficacy of selective compounds capable of inducing larval mortality through AaDOP2 blockade (MEYER JM, et al., 2012). In this context, inhibition of AaDOP2 has emerged as a promising strategy for developing innovative insecticides, as it targets neuromodulatory signaling pathways distinct from classical neurotoxic mechanisms, thereby reducing the likelihood of cross-resistance with conventional insecticides.

Taken together, OBP22 and AaDOP2 represent two complementary dimensions of the biological functioning of *A. aegypti*. OBP22 is primarily associated with chemical ecology, host-seeking behavior, and mosquito–host interactions, whereas AaDOP2 is linked to neuromodulatory signaling and behavioral regulation.

Both proteins contribute directly to the ecological success of the mosquito as a disease vector, and their in-depth characterization has revealed how targeted molecular disruptions can impair survival, feeding behavior, and pathogen transmission. These findings reinforce the potential of OBP22 and AaDOP2 as strategic molecular targets for the development of innovative and sustainable vector control approaches.

Metabolic and enzymatic targets involved in the physiological homeostasis of Aedes aegypti

In addition to targets associated with olfaction and neurotransmission, several metabolic enzymes and proteins play essential roles in maintaining the physiological homeostasis of *A. aegypti*, representing promising targets for vector control strategies. 3-hydroxykynurenine transaminase (HKT) participates in the tryptophan metabolism pathway by preventing the accumulation of toxic metabolites, and disruptions in its activity are associated with impaired development and reduced survival. Sterol carrier protein 2 (SCP-2) is crucial for lipid metabolism, mediating intracellular sterol transport required for membrane synthesis and hormonal processes; its inhibition negatively affects growth, reproduction, and larval viability and may influence viral replication (MACIEL LG, et al., 2023).

Carbonic anhydrase (CA) plays a key role in acid–base balance, ion transport, and osmoregulation, being highly expressed in the midgut and Malpighian tubules. Inhibition of this enzyme causes a severe physiological imbalance, compromising excretion and survival. Within detoxification mechanisms, α-esterases contribute to metabolic resistance by hydrolyzing or sequestering insecticides, particularly organophosphates and carbamates, and are frequently overexpressed in resistant mosquito populations. Finally, sodium-coupled cation–chloride cotransporters (CCC2 and CCC3) are involved in transcellular ion transport and electrolyte balance. Disruption of their activity leads to ionic homeostasis failure and severe physiological dysfunction, reinforcing their potential as molecular targets for innovative vector control approaches (ARAÚJO MO, et al., 2020).

CONCLUSION

This review examines protein targets in *Aedes aegypti* associated with insecticidal activity, highlighting the diversity of physiological and biochemical mechanisms involved in mosquito survival and vector competence. The findings indicate that, beyond traditional neurotoxic targets such as acetylcholinesterase and voltage-gated sodium channels, detoxification enzymes, particularly glutathione S-transferases, play a central role in vector control strategies. The frequent identification of these targets underscores their relevance and reinforces their potential for developing more effective, environmentally sustainable insecticides for *A. aegypti* management.

ACKNOWLEDGMENT

We acknowledge the Universidade Estadual do Maranhão (UEMA), Zé Doca - MA, for institutional support, and we also thank the First Steps in Science Program for awarding a fellowship toW. C. Silva.

DECLARATION OF ARTIFICIAL INTELLIGENCE (AI) AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to enhance the cohesion of an initial version of the manuscript. After using this tool/service, the authors reviewed and edited the text as needed and take full responsibility for the content of the publication.

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