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Structure Activity Relationship of 4-((2-hydroxybenzylidene) amino) benzoic acid and 4-((4 methoxybenzylidene) amino) benzoic acid as Potential Larvicidal Agents against *Aedes aegypti* Larvae

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ABSTRACT: The widespread application of conventional synthetic pesticides has raised major environmental issues because of their persistence, bioaccumulation, and biomagnification. The urgent need to address these risks calls for the development of more eco-friendly alternatives in pest management. Consequently, the objective of this paper was to investigate the structure activity relationship of 4-((2-hydroxybenzylidene) amino) benzoic acid [A1] and 4-((4 methoxybenzylidene) amino) benzoic acid [A2] as potential larvicidal agents against Aedes aegypti larvae using appropriate standard procedures. Data obtained reveals that percentage mortality was determined using Abbott's formula. Both compounds demonstrated larvicidal activity in a dose-dependent manner. At 2.5 mg/mL, the Schiff bases achieved 95.5 and 99 % mortality for A1 and A2 respectively. At lower concentrations of 0.5 mg/ml, the ligands showed slightly high activity. These findings indicate that these Schiff bases have promising larvicidal activity, suggesting their potential as safer and more environmentally friendly pest management alternatives. The results highlight the possibility of these compounds supporting environmental biodiversity

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Pesticides are a category of chemical compounds designed to control or eradicate pests, encompassing a range of organisms such as insects, weeds, and fungi that threaten human life and the quality of agricultural output. While pesticides are crucial in protecting life, their extensive use has led to significant environmental concerns (Verdadero *et al*., 2025). Pesticides can contaminate water, soil, and even air, adversely affecting non-target organisms, including beneficial insects like bees, aquatic organisms, and even humans. The persistence of certain pesticides in the environment can result in bioaccumulation and biomagnification, posing long-term ecological risks (Pathak *et al*., 2022; Dad *et al.*, 2022). The World Health Organization (WHO) categorizes pesticides based on toxicity, focusing on

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the need for careful management to mitigate their harmful effects on public health and the environment (WHO, 2019; Zhou *et al*., 2025). The extensive use of conventional pesticides in both small- and large-scale has become a significant concern because of their detrimental effects on the environment and human health. These pesticides, while effective at controlling pests, often persist in the environment long after application, leading to bioaccumulation and biomagnification, in which toxic substances build up in living organisms and become more concentrated as they move up the food chain. This process disrupts entire ecosystems, affecting not only the targeted pests but also beneficial organisms such as pollinators, soil microbes, and aquatic life. The adverse effects extend to human health, with exposure to pesticide residues linked to neurological disorders, reproductive problems, and cancers (Ahmad *et al*., 2024; Zhou *et al*., 2025). Recent research in Schiff base chemistry has highlighted their potential as eco-friendly alternatives to conventional pesticides, owing to their biodegradability, low toxicity, and effectiveness in pest control (Bhavsar, 2023). Schiff bases are organic compounds characterized by the presence of an imine or azomethine group (-C=N-). They are typically synthesized through the condensation reaction between primary amines and carbonyl compounds. Schiff bases have garnered significant attention in various fields, including medicinal chemistry, catalysis, and material science, due to their versatile chemical properties and ease of synthesis (Kumari, 2024; Bhavsar, 2023; Ullah and Bukhari, 2024). One of the key advantages of Schiff bases in green chemistry approach is their synthetic methods, biodegradability and low toxicity to non-target organisms, which make them attractive alternatives to conventional pesticides (Nagar *et al*., 2023; Mondal *et al*., 2024; Islam *et al*., 2024). The biodegradability of Schiff bases is attributed to their chemical structure, which allows them to undergo hydrolysis and other degradation natural processes in the presence of environmental factors such as moisture and microbial activity, reducing their environmental footprint (Mondal *et al*., 2024; Bhavsar, 2023). Studies have shown that Schiff bases derived from natural sources exhibit minimal toxicity to humans and wildlife, further supporting their use in sustainable environment (Mondal *et al*., 2024). These approaches align with the broader principles of environmental sustainability, ensuring that pest management strategies are both effective and environmentally responsible.

Schiff bases exert their pesticidal effects through various mechanisms depending on their chemical structure and the target pest. One common mechanism involves the inhibition of key enzymes in the pest's metabolic pathways, leading to the disruption of vital physiological processes. Schiff bases have been shown to inhibit acetylcholinesterase, an enzyme essential for nerve function in insects, resulting in paralysis and death of the pest (Salga *et al*., 2011). Also, generation of reactive oxygen species (ROS) within the pest organism, causing oxidative stress and cellular damage, particularly effective against fungal pathogens (Rocha *et al*., 2021; Mondal *et al.,* 2024). The presence of electron donating or electron withdrawing substituent groups significantly affect pesticidal activity as these can modify electron density on the active sites and lipophilicity, which determines how the pesticides bind to the target pest receptor (Wany and Song, 2026). Structure activity relationship of Schiff bases containing *p*-tosyloxy benzaldehyde, indole and quinoxaline derivatives against *Spodoptera Frugiperda* has been reported (Alkorbi *et al*., 2024).

The integration of these compounds into pest management strategies can promote human health, protect non-target organisms, reduce chemical residues in food and water sources, support environmental balance, thereby providing a feasible solution to the urgent demand for eco-friendly pesticides.

Consequently, the objective of this paper was to investigate the structure activity relationship of 4-((2-hydroxybenzylidene) amino) benzoic acid [A1] and 4-((4-methoxybenzylidene) amino) benzoic acid [A2] as potential larvicidal agent against Aedes aegypti larvae.

MATERIALS AND METHODS

Materials: All chemicals and reagents used were purchased from Sigma-Aldrich Chemical Co. Ltd. and used without further purification; 4-aminobenzoic acid, salicylaldehyde, 4-methoxybenzaldehyde, dimethyl sulfoxide (DMSO), ethanol, ethyl acetate, n-hexane, and distilled water. The Schiff bases 4-((2-hydroxybenzylidene) amino) benzoic acid, 4-((4-methoxybenzylidene)amino) benzoic acid have previously been synthesized and reported. *Aedes aegypti* specie, Aedes aegypti larval instar were obtained from the Insectary of Department of Zoology, University of Lagos Nigeria, while the holding cups were purchased from the University of Lagos Campus mall. Distilled water was obtained from the Department of Chemistry, University of Lagos.

Synthesis of Schiff bases: Synthesis of 4-((2-hydroxybenzylidene) amino) benzoic acid **(**A1**):** 4-aminobenzoic acid (0.343 g, 2.5 mmol) and salicylaldehyde (0.2 mL, 2.5 mmol) were dissolved in ethanol (20 mL). The reaction mixture was kept under reflux for 6 h at 70^0C and monitored by TLC using (3:2) *n*-hexane and ethyl acetate solvent system. The mixture obtained was cooled to room temperature and the product formed was collected by filtration and recrystallized using ethanol (Ejiah *et al*., 2025).

Synthesis of 4-((4-methoxybenzylidene)amino) benzoic acid **(**A2**)** 4-aminobenzoic acid (0.343 g, 2.5 mmol) and 4-methoxybenzaldehyde (0.34 g, 2.5 mmol) were dissolved in ethanol (80 mL). The reaction mixture was kept under reflux for 6 h at 70^0C and monitored by TLC using *n*-hexane and ethyl acetate solvent system in the ratio 1:1. The mixture obtained was cooled to room temperature and the product formed was collected by filtration and recrystallized using ethanol (Ejiah *et al*., 2025).

Pesticidal Activity

Sample collection and treatment: Aedes aegypti mosquitoes (3^{rd}-4^{th} instar larvae) used for this study were sourced from the Insectary at the Zoological Garden, University of Lagos, Nigeria. A total of 1,200 2^{nd}-3^{rd} instar was collected at intervals and transferred into holding containers (20 cm^3) half-filled with deionized water and acclimatized for at least two days prior to larvicidal exposure. Larvae were fed with finely dissolved granules of coppens fish meal once before exposure to test compounds (Imam *et al*., 2014).

Sample analysis methodologies: The pesticidal activity of the synthesized Schiff base ligands was evaluated against 3^{rd}-4^{th} instar larvae of *Aedes aegypti.* Stock solutions (10 mg/mL) of each Schiff base were prepared by dissolving 0.1 g of each sample in 10 mL ethanol. Serial dilutions of 0.5 mg/mL, 0.75 mg/mL, 1.0 mg/mL, 2.0 mg/mL, and 2.5 mg/mL were prepared for bioassay. One hundred 3^{rd}-4^{th} larval instars of *Aedes aegypti* were placed in glass containers filled with 200 ml deionized water pre-mixed with the afore-mentioned variant concentrations of the two test compounds. A control group of same number of Aedes aegypti larval instar without the test compounds was also maintained throughout the study. Each treatment was performed in duplicates and maintained at 27 ± 2°C and 65 ± 5% relative humidity. Mortality of mosquito larvae was recorded 24 hours post-exposure to test compounds (Perumalsamy *et al*., 2015).

Statistical Data Analysis: Data pooled from the study were analyses using SPSS version 27. Lethal concentration (LC_{50}) values for the test compounds against *Aedes aegypti* larvae were analyzed using probit/regression analysis. Mean mortality values of *Aedes* larvae exposed to test compounds were analyzed using a two-way analysis of variance (ANOVA), and separated using Post hoc c Tukey's Post Hoc test for significant differences within groups ($p \leq 0.05$) with Bonferroni adjustment for confidence intervals. The mean mortality was calculated from the replicate counts, and percentage mortality was obtained using Abbott's formula (Perumalsamy *et al*., 2015) equation 1:

$$\text{Percentage mortality } \% = (A\text{-}B/100\text{-}B) * 100 \qquad (1)$$

Where: A = percentage mortality of larvae in treatment; B = percentage mortality of larvae in control

RESULTS AND DISCUSSIONS

Synthesis and Characterization of Schiff Bases: The synthesis and characterization of Schiff bases, 4-((2-hydroxybenzylidene)amino)benzoic acid A1 (Fig. 1) with hydroxyl substituent and 4-((4-methoxybenzylidene)amino)benzoic acid A2 (Fig. 2) with methoxy substituent have been reported (Ejiah *et al*., 2025). The compounds were characterized using FTIR, ^{1}H NMR, ESI MS and elemental analysis. The structures of both Schiff bases are presented below. This paper reports only on the potential larvicidal activity.

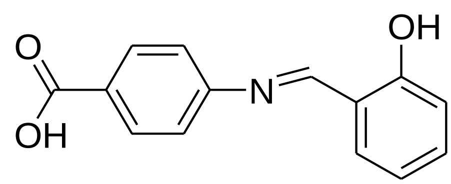

Fig. 1: Structure of 4-((2-hydroxybenzylidene)amino)benzoic acid A1

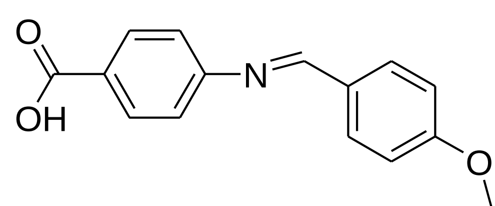

Fig. 2: Structure of 4-((4-methoxybenzylidene)amino)benzoic acid A2

The ESI MS and elemental analysis for A1 and A2 which has previously been reported confirmed the structure of the synthesized compound as the calculated and found were in agreement; ESI-MS calculated $C_{14}H_{11}NO_3$ $[M+1H]^{+1}$ m/z = 242.07, found

242.06 for A1 and elemental analysis calculated $C_{15}H_{13}NO_3$: C, 70.28; H, 5.11; N, 5.33. Found: C, 70.58; H, 5.09; N, 5.49 for A2 respectively (Ejiah *et al*., 2025).

The infrared spectra of the Schiff base ligands displayed a strong absorption band at 3069 cm^{-1}, 1678 cm^{-1} 1622 cm^{-1} and 1175 cm^{-1} attributed to O–H, C=O, C=N, and C-O for A1, 3089 cm^{-1}, 1670 cm^{-1}, 1629 cm^{-1} and 1162 cm^{-1}, corresponding to O-H, C=O, C=N and C–O stretching vibrations for A2 respectively (Ejiah *et al*., 2025).

^{1}H NMR spectra revealed resonated protons at the expected range, 8.87 δ [HC=N, s, 1H], 7.01-8.14 δ [Ar-H], 10.10 δ [OH, s, 1H] for A1 and 8.88 δ, 7.57-8.38 δ, and 3.33 δ, attributed to the imine, aromatic, and methoxy protons for A2 respectively (Ejiah *et al*., 2025).

Pesticidal Activity: Probit analysis (Fig. 3 and 4) showed that both Schiff base derivatives were acute toxicants against Aedes aegypti, and had similar LC_{50} values. For example, 4-((4-methoxybenzylidene)amino)benzoic acid had an LC_{50} of 0.44 mg/ml and 4-((2-hydroxybenzylidene)amino)benzoic acid had an LC_{50} of 0.47 mg/ml (Table 1). Both compounds have relatively low values. But however, the higher dose response to both compounds (as indicated by their LC_{90} values) shows some variability. The 4-((4-methoxybenzylidene)amino)benzoic acid was slightly more toxic than the hydroxy derivative with toxicity factor of 1.09 and 1.00 respectively (Table 1). The homogeneity test also showed the assumption of equal variances was correct, with Levene's statistic being found not significant (p = 0.129) (Table 2). This confirms that ANOVA can be used to compare mortality rates among the various concentration levels. The two-way ANOVA showed that compound type and concentration had significant effects on mosquitoes' mortality (Table 3). There was negligible variance attributable to any other factors (Partial Eta Squared = 0.995) except the compound type (p = 0.012). No interaction between type of compound and concentration was significant (p = 0.147), indicating that there are no synergistic or antagonistic effects between the two derivatives and that both have similar dose-response relationships. Mortality was compared with concentration (Fig. 5), using Tukey's HSD post hoc comparisons; mortality was consistently higher with increasing concentration, though significant differences were found between most concentrations (Table 4).

Table 1: Lethal concentration of Schiff bases acute toxicity against *Aedes aegypti*

Schiff Bases	LC_{50} (mg/ml) (95% CL)	LC_{90} (mg/ml) (95% CL)	Df	Probit line of equation	TF
4-((2-hydroxybenzylidene) amino) benzoic acid	0.47(0.02 - 0.76)	1.87(1.16- 43.33)	3	y=0.70+x2.15	1.00
4-((4-methoxybenzylidene) amino) benzoic acid	0.44(0.00 - 0.80)	2.94(1.49 - 44.04)	3	y=0.56+x1.55	1.09

Note: DF= degree of freedom, TF= Toxicity factor; CL = Confidence limits; TF =Toxicity factor

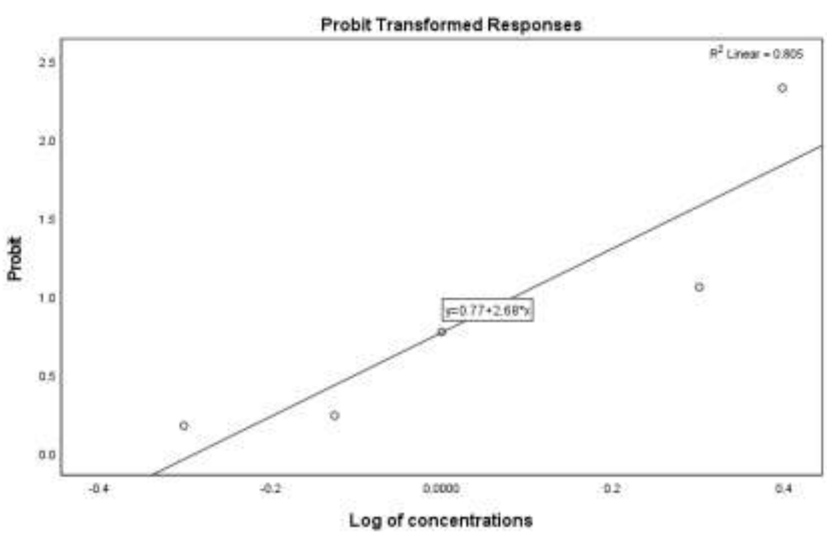

Fig. 3: 4-((2-hydroxybenzylidene)amino)benzoic acid effect curve against *Ae. egypti*

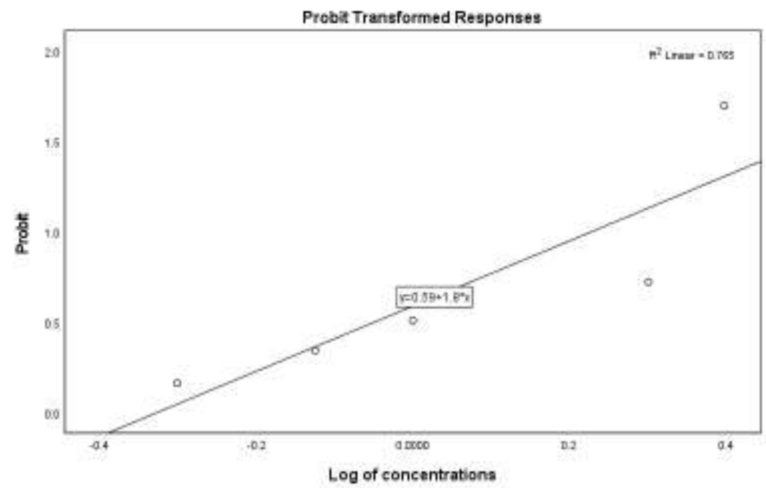

Fig. 4: 4-((4-methoxybenzylidene)amino)benzoic acid effect curve against *Ae. egypti*

Table 2: Homogeneity test of equal assumption

Levene's Test of Equality of Error Variances[a,b]					
		Levene Statistic	df1	df2	Sig.
Mortality rate	Based on Median and with adjusted df	7.135	10	2	0.129

Df=degree of freedom

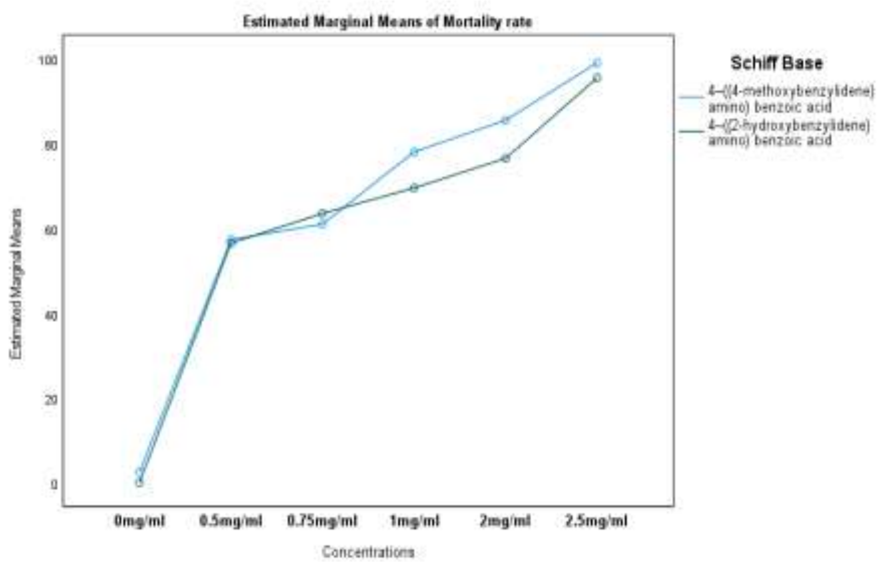

Fig. 5: The concentration effect of the Schiff bases and their mortality rate

Table 3: Analysis of variance between the Schiff bases and different level of concentrations against *Ae. aegypt*

Tests of Between-Subjects Effects					
Dependent Variable: Mortality rate					
Source	*Type III Sum of Squares*	*df*	*Mean Square*	*Sig.*	*Partial Eta Squared*
Corrected Model	22031.667a	11	2002.879	0.001	0.995
Intercept	87596.32	1	87596.32	0.001	0.999
Schiff bases	75.268	1	75.268	0.012	0.419
Concentrations	21852.63	5	4370.526	0.001	0.995
Schiff bases & Concentrations	87.882	5	17.576	0.147	0.458
Error	104.167	12	8.681		
Total	114144	24			
Corrected Total	22135.83	23			
a R Squared = .995 (Adjusted R Squared = .991)					

Table 4: Comparison analysis of the Schiff bases effect at different concentration levels

Multiple Comparisons Tukey HSD					
Dependent Variable:	**Mortality rate**				
Concentrations	0.5mg/ml	0.75mg/ml	1mg/ml	2mg/ml	2.5mg/ml
0mg/ml	-55.75*	-61.42*	-72.50*	-79.75*	-96.00*
	p=0.001	*p=0.001*	*p=0.001*	*p=0.001*	*p=0.001*
0.5mg/ml	55.75*	-5.67	-16.75*	-24.00*	-40.25*
	p=0.001	*p=0.001*	*p=0.001*	*p=0.001*	*p=0.001*
0.75mg/ml	61.42*	5.67	-11.08*	-18.33*	-34.58*
	p=0.001	*p=0.162*	*p=0.004*	*p=0.001*	*p=0.001*
1mg/ml	72.50*	16.75*	11.08*	-7.25*	-23.50*
	p=0.001	*p=0.001*	*p=0.004*	*p=0.041*	*p=0.001*
2mg/ml	79.75*	24.00*	18.33*	7.25*	-16.25*
	p=0.001	*p=0.001*	*p=0.001*	*p=0.041*	*p=0.001*
2.5mg/ml	96.00*	40.25*	34.58*	23.50*	16.25*
	p=0.001	*p=0.001*	*p=0.001*	*p=0.001*	*p=0.001*

** The mean difference is significant at the 0.05 level.*

The larvicidal activity of 4-((2-hydroxybenzylidene)amino)benzoic acid A1 and 4-((4-methoxybenzylidene)amino)benzoic acid A2 was evaluated against *Aedes aegypti* larvae at concentrations of 0.5, 0.75, 1.0, 2.0, and 2.5 mg/mL. The 24-hour mortality rates for the Schiff base ligands are presented in (Tables 5 and 6) respectively, while (Table 7) presents a direct comparison of the percentage mortality rates of both compounds across all tested concentrations. It was observed that the methoxy substituted ligands A2 exhibited increased larvicidal activity when compared to the hydroxyl substituent A1. This corroborates report on the role of methoxy group in drug discovery (Gładkowski *et al*., 2019; Beč *et al*., 2024; Chiodi and Ishihara, 2024). Methoxy group has also been reported to be present in approved drugs from a visual inspection of Njardarson's "Top Pharmaceuticals Posters"..

Table 5: 24-hour mortality rate of 4–((2-hydroxybenzylidene) amino) benzoic acid on *Aedes aegypti* larvae.

		Mortality		**Mean Mortality**	**Corrected Mortality**		**%Mortality**
Conc (mg/ml)	Total Exposed	i	ii	I + ii /2	A-B=Y	Y/(100-B)	Z*100
Control	200	0	0	0			
0.5	200	55	58	56.5	56.5	0.565	56.5
0.75	200	62	65	63.5	63.5	0.635	63.5
1	200	71	68	69.5	69.5	0.695	69.5
2	200	78	75	76.5	76.5	0.765	76.5
2.5	200	91	100	95.5	95.5	0.955	95.5

Table 6: 24-hour mortality rate of 4–((4-methoxybenzylidene) amino) benzoic acid on Aedes aegypti larvae.

		Mortality		**Mean Mortality**	**Corrected Mortality**		**%Mortality**
Conc (mg/ml)	Total Exposed	i	ii	I + ii /2	A-B=Y	Y/(100-B)	Z*100
Control	200	0	5	2.5			
0.5	200	55	59	57	54.5	0.544	54.5
0.75	200	58	61	59.5	57	0.57	57
1	200	75	81	78	75.5	0.755	75.5
2	200	84	87	85.5	83	0.83	83
2.5	200	98	100	99	96.5	0.965	96.5

Table 7: Comparison of % mortality rate of the Schiff base A1 and A2

Conc. (mg/ml)	**Schiff base %Mortality rate A1**	**Schiff base % Mortality rate A2**
0.5	56.5	57
0.75	63.5	59.5
1	69.5	78
2	76.5	85.5
2.5	95.5	99

Evidently, presence of methoxy group on ligand has been associated with protein binding, conformation, and metabolism as the OCH_3 moiety contains hydrophilic oxygen (O) and hydrophobic methyl (CH_3) group (Chiodi and Ishihara, 2024).

Overall, both compounds demonstrate strong larvicidal potential, though appear to be more effective at higher concentrations, making it a promising eco-friendly pesticide candidate. This comparison underscores the importance of dose optimization and highlights the potential of Schiff base compounds in sustainable vector control strategies

Conclusion: The study successfully revealed the structure activity relationship of 4-((2-hydroxybenzylidene) amino) benzoic acid A1 and 4-((4-methoxybenzylidene)amino)benzoic acid A2, that can be employed in development of larvicides. This study established a significant correlation in the dose-response of the test compounds on *Aedes larvae*. Methoxy-substituted Schiff base was slightly more toxic. Since *Aedes aegypti* has been implicated as the major vector for arboviral infections such as yellow fever, and vector control being the most viable control option, results from this study imply that an adoption of new eco-friendly active compounds could mitigate the increase in vector population and disease burden, offering a significant solution towards achieving SDG 3. The study had constraints in terms of larval population size, and comparison of test compounds with a referenced WHO-approved larvicide. Future studies of these compounds should consider investigating their mode of action on test organisms using physiological and biochemical endpoints, and more diverse mosquito species.

DECLARATIONS

Ethical Approval: This study did not involve human participants or animal experimentation and therefore did not require formal ethical approval. All procedures involving data handling and analysis were conducted responsibly and in accordance with standard academic and institutional guidelines.

Disclaimer on Artificial Intelligence usage: Author(s) hereby declare that no generative Artificial Intelligence (AI) technologies such as Large Language Models (ChatGPT, COPILOT, XLNet, Ernie 3.0 Titan etc) and text-to-image generators have been used during writing or editing of this manuscript.

Declaration of conflict of interest: The authors declare that no known conflict of interest or personal relationships that have influence the work reported in this paper.

Data availability statement: The data that are used in this research are available upon request from the corresponding author.

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