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# Larvicidal Effects of New Organophosphorus Schiff base compounds against Dengue Fever Vector *Aedes aegypti* (Diptera; Culicidae)

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**ABSTRACT**: Mosquitoes are the most important medical insect, that transmits diseases, such as dengue fever and malaria. The biological effects of new Organophosphorus Schiff base compounds (2-Furalidenediphenylphosphate-1-phenylsemicarbazide **OPSL$_1$**) and 2-methoxybenzalidenediphenyl phosphate -1-phenylsemicarbazide **OPSL$_2$**) against the mosquito larvae of *Aedes aegypti* was evaluated at different concentrations using WHO standard susceptibility test with some modifications. Late third or early fourth instar larvae were exposed to various concentrations of the investigated chemicals, ranging from 150 ppm to 1200 ppm, in a laboratory bioassay to evaluate the larvicidal efficacy. It was shown that mortality rates rose as concentrations rose. The results showed that **OPSL$_2$** revealed significantly high activity against *Ae. Aegypti* with ($LC_{50}$ = 600.6 ppm) compared to the **OPSL$_1$**, with $LC_{50}$ = 642.9 ppm respectively. Further investigations would be required to evaluate the new active Organophosphorus compounds responsible for larvicidal efficacy in the future.

CONTENTS



## 1. INTRODUCTION

*Aedes aegypti* Linnaeus, 1762 belongs to the family Culicidae and the order Diptera [1]. It is the main chikungunya vector [2], Zika [3-5], yellow fever [6], and dengue [7,8] in most parts of the world. The importance of *Aedes aegypti* stems

from its close association with humans. Females bite humans rather than other vertebrates and lay eggs in man-made containers in the vicinity of human dwellings, which are generally breeding sites that are not completely covered with few organic materials for larval feeding. Uncontrolled urbanization, rising temperatures, and a lack of effective long-term vector control tools have aided *Aedes aegypti* adaptation to urban environments throughout the world. [9-12].

In the past 30 years, dengue fever outbreaks have multiplied ten times across several tropical and subtropical nations [13,14]. More than 3 billion individuals worldwide have been infected with the dengue virus, and more than 20,000 people die from it every year. Around 128 nations are in danger [15-17].

Dengue fever was first identified in Yemen during a pandemic outbreak Hirsch recorded between 1870 and 1873 [18]. After that, the pandemic dengue fever that followed struck the Alhodeidah governorate in 1954 and infected 98% of the population and the traveler returning from Yemen was proven to carry dengue antibodies in 1984 [19]. Shabwah governorate received the first report of dengue illness and it was later confirmed there [20, 21].

The highest governorate hit by dengue fever in 2014 was Aden, followed by Alhodeidah and Almukalla [21]. But even so, the highest governorates were Aden, Alhodeidah, and Taiz, the Dengue virus was isolated from a viral hemorrhagic fever outbreak in Al-Mukalla city. Dengue-suspected cases increased in 2020 compared to 2019 since the outbreak began in Yemen and during the operation timeframe.

According to the Yemen Health Cluster [22], the number of suspected cases by the end of 2020 was seven times that of 2019, and six times that of 2018. Synthetic substances with carbon-phosphorus bonds are known as organophosphorus compounds. The related field of science known as "organophosphorus science" investigates the characteristics and reactivity of organophosphorus compounds [23].

Numerous organophosphorus chemicals have been made over time and utilized extensively in agriculture, including insecticides [24], herbicides later on [25], organocatalysis [25], medicinal chemists [26], and antimalarials [27]. This work was carried out to evaluate the potential larvicidal activities of two Organophosphorus Schiff base compounds against the larval stage of Dengue Fever Vector *Ae. aegypti*. under laboratory conditions using a standard WHO susceptibility test at varying concentrations [28].

## 2. MATERIALS & METHODS

### 2.1 Mosquito strain source and rearing

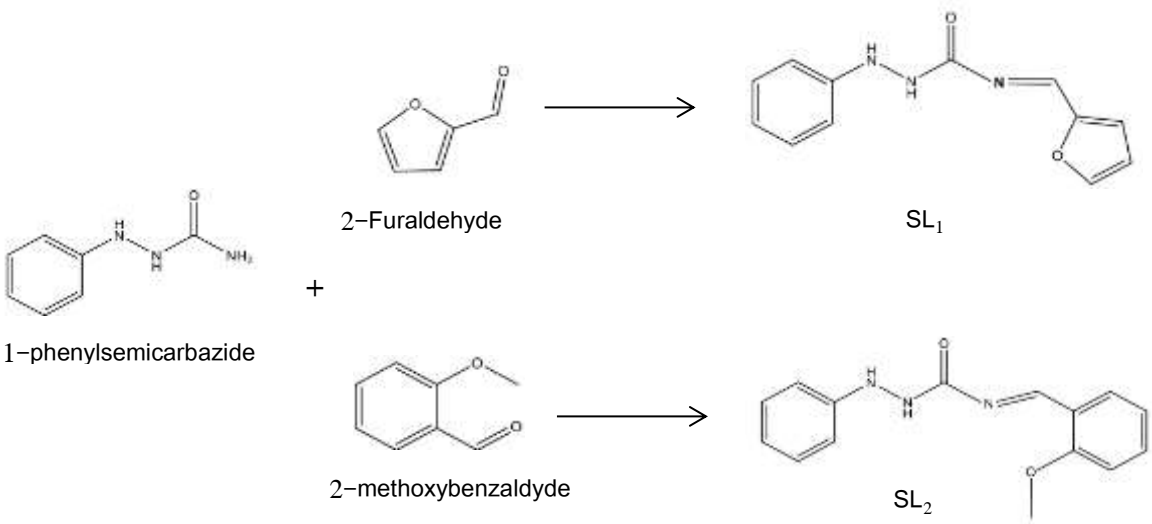
*Ae. aegypti* (Diptera; Culicidae) larvae were obtained from the mosquito laboratory at the Medical Entomology Laboratory, Biological Science Department, Faculty of Science, Sana'a University. The *Ae. aegypti* larvae were reared in a laboratory environment using diet media fish food for more than 4 generations. The various treatments reported in this study involved larvae from the culture. It was intended to keep the stock colony at lab temperature.

### 2.2 Organophosphorus Schiff base compounds

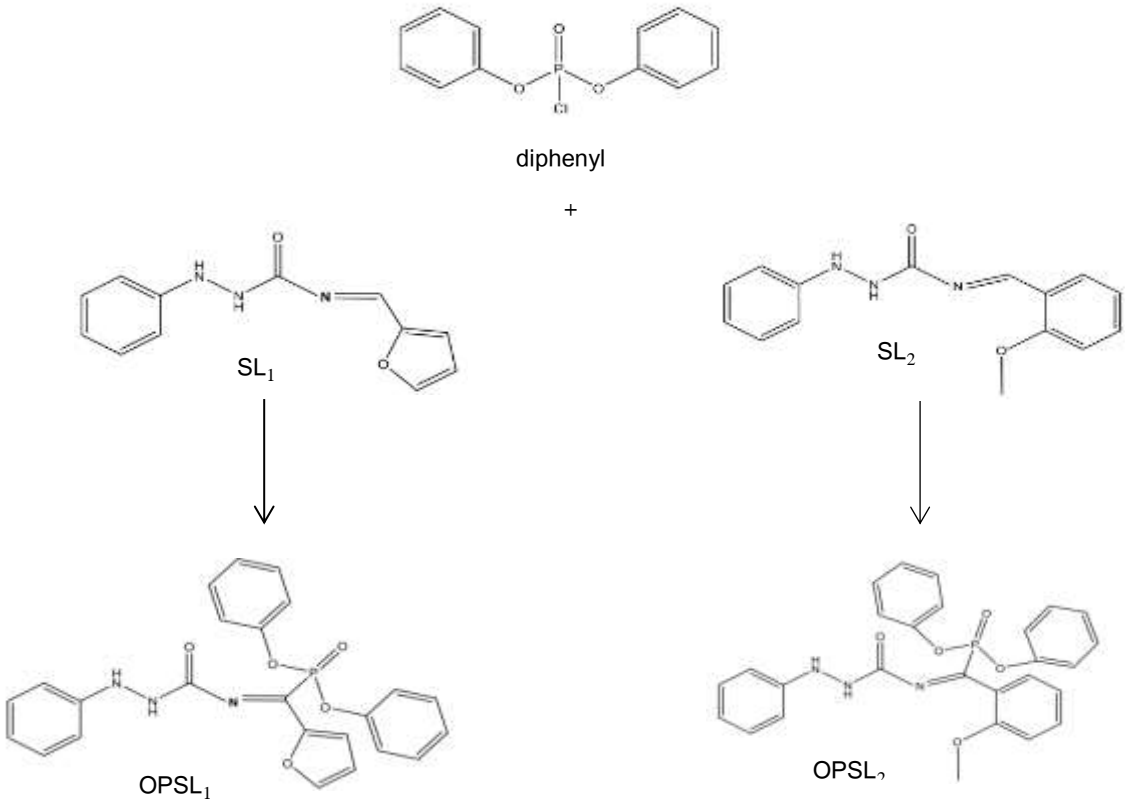
The new Organophosphorus Schiff base compounds used for larval bioassay were (2-Furalidenediphenylphosphate-1-phenylsemicarbazide **OPSL$_1$**) and 2-methoxybenzalidenediphenylphosphate-1-phenylsemicarbazide **OPSL$_2$**).

In the beginning, the Schiff bases (**SL$_1$** and **SL$_2$**) were prepared by condensation between an ethanolic solution of 1-phenylsemicarbazide (7.55g, 0.05mol) with an ethanolic solution of 2-Furaldehyde (4.80g, 0.05 mol) and 2-methoxybenzaldyde (6.80 g, 0.05 mol) respectively (Scheme 1). After cooling, the product separated. It was filtered, washed with ethanol and ether multiple times, and then recrystallized with ethanol to produce the corresponding Schiff base. Secondly, the Organophosphorus Schiff bases (**OPSL$_1$** and **OPSL$_2$**) were prepared by condensation between a solution of Schiff bases **SL$_1$** (3.43g, 0.015mol) and SL$_2$ (4.03g, 0.015mol) respectively in dry benzene (50 ml) with a diphenyl chlorophosphate (0.015 mol) solution in dry benzene (50 ml) in 1:1 molar ratio in presence of triethyl amine (Scheme 2). The reaction mixture was heated under reflux for two hours after complete addition. After evaporation in a water bath, the formed solid (triethyl

amine hydrochloride) was filtered and the product was obtained, the prepared compounds were characterized and screened for their antioxidant, antibacterial and antifungal activity [29].




**Scheme 2: Preparation of Schiff bases OPSL1 and OPSL2**




**Scheme 2: Preparation of Schiff bases $OPSL_1$ and $OPSL_2$**

### 2.3 Bioassay of compounds against *Ae. Aegypti* larvae.

The WHO standard protocols were followed for the larvicidal bioassay [30]. The concentrations (150, 300, 600, 800, 1000, and 1200 ppm) were prepared in 2% DMSO for each compound. Larval treatments were carried out by continuously exposing late third or early fourth instar larvae to various concentrations of the compounds**.** During these experiments, the larvae were fed their normal larval food**.**

The percentage of mortality was calculated after 24 hours of exposure.

### 2.4 Statistical Analysis

This study used a completely randomized design (CRD) in a factorial experiment. Analysis of variance (ANOVA) software was used to statistically examine the collected data, then the Minitap software program was used to compare the means using LSD at $p \leq 0.05$. $LC_{50}$ (Concentration which kills 50% of mosquito larvae) and according to the Probit analysis application, $LC_{95}$ (Concentration which kills 95% of mosquito larvae) was estimated [30,31]. The 95% confidence intervals by a computerized log-probit analysis.

## 3. RESULTS AND DISCUSSION

In the present study, the **$OPSL_1$**, and **$OPSL_2$** compounds showed larvicidal activity against *Aedes aegypti* when exposed to concentrations ranging from 150 to 1200 ppm for 24 hours.

The results of the larvicidal activity of tested compounds against, *Ae. aegypti* larvae are presented in (Table 1) and (Figures 1 and 2). Both **$OPSL_1$** and **$OPSL_2$** compounds revealed 0% larval mortality at 150ppm, however they showed 100% larval mortality at 1200ppm. The **$OPSL_2$** compound showed higher larvicidal activity against *Ae. aegypti* compared with the **$OPSL_1$** compound at 1000, 800, and 600ppm respectively. The $LC_{50}$/ $LC_{95}$ values were 642.9/1188.4, 600.6/1074.6 ppm for $OPSL_1$ and $OPSL_2$ compounds respectively (Table 2).

**Table 1: Toxicity effect of $OPSL_1$ and $OPSL_2$ against *Ae.aegypti* larvae**

| Compound | Conc. (ppm) | Larval Mortality (%) |
|---|---|---|
| **$OPSL_1$** | 150 | 0 |
| | 300 | 25 |
| | 600 | 33 |
| | 800 | 60 |
| | 1000 | 88 |
| | 1200 | 100 |
| **Control** | 2% DMSO | 0 |
| **$OPSL_2$** | 150 | 0 |
| | 300 | 23 |
| | 600 | 43 |
| | 800 | 68 |
| | 1000 | 95 |
| | 1200 | 100 |
| **Control** | 2% DMSO | 0 |

**Table 2: Statistical parameters of $OPSL_1$ and $OPSL_2$ against *Ae. aegypti* larvae**

| Statistical parameters | $OPSL_1$ | $OPSL_2$ |
|---|---|---|
| **$LC_{50}$ (ppm)** | 642.9 | 600.6 |
| **95% (*F. L)** | 621.5-663.4 | 580.3-619.8 |
| **$LC_{95}$ (ppm)** | 1188.4 | 1074.6 |
| **95% (*F. L)** | 1142.8-1241.7 | 1037.4-1117.4 |
| **Slope** | 2.38 | 2.52 |
| **$R^2$** | 0.94 | 0.96 |

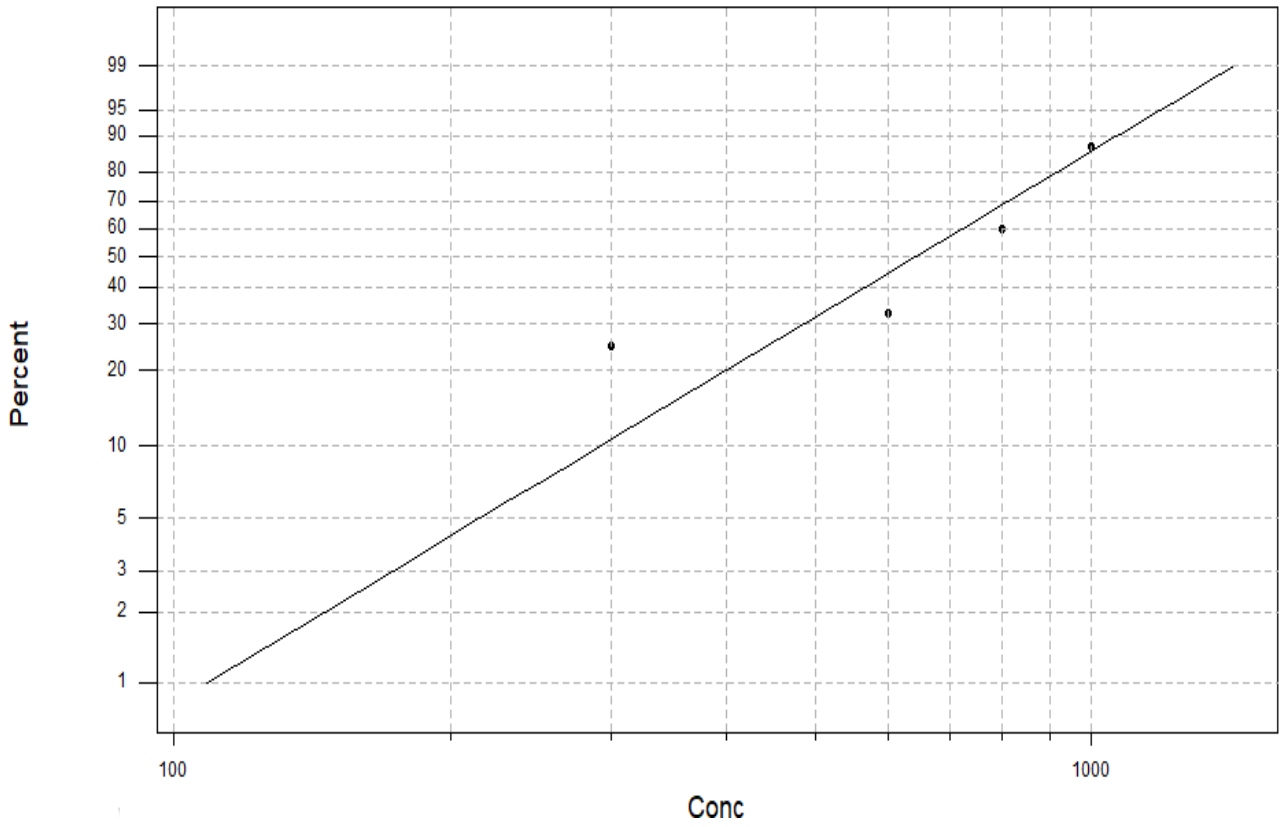



**Figure 1: The relationship between concentration of OPSL$_1$ and mortality percentage of *Ae. aegypti* larvae**

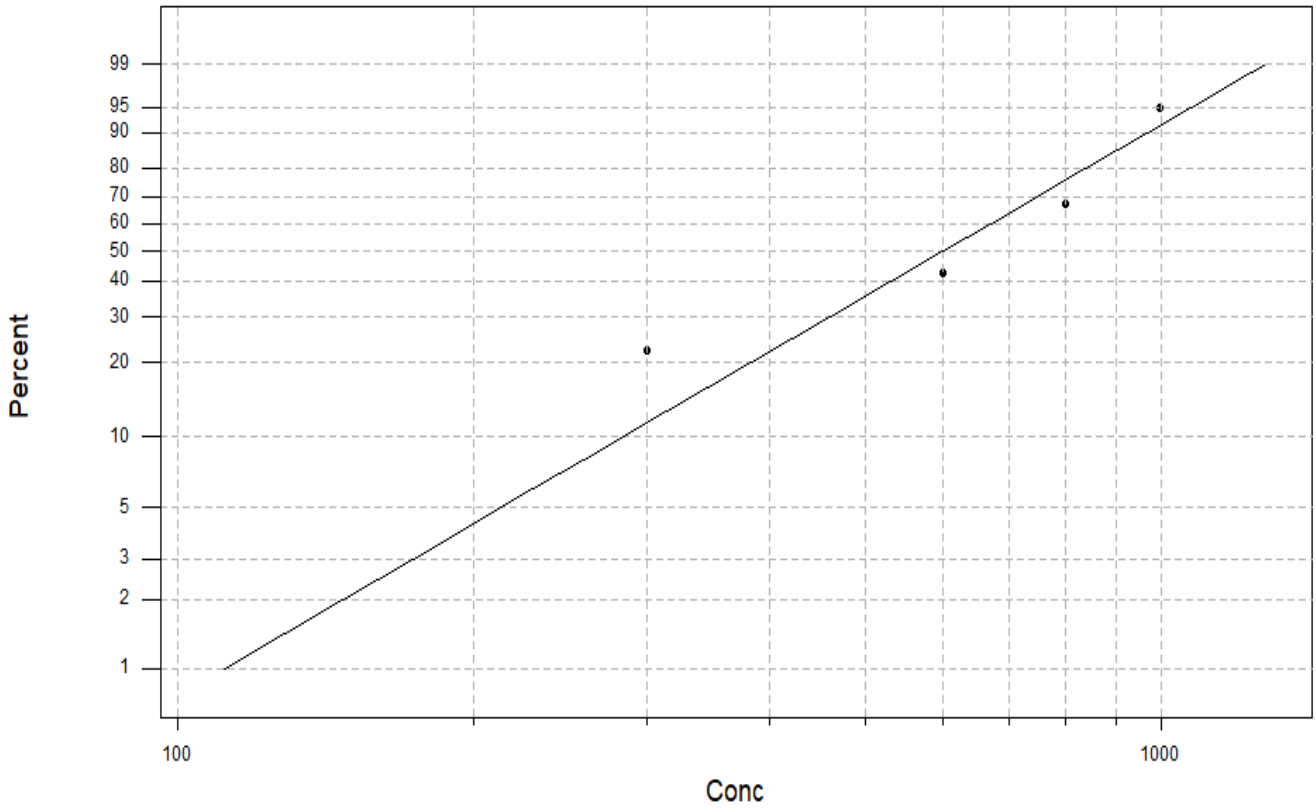
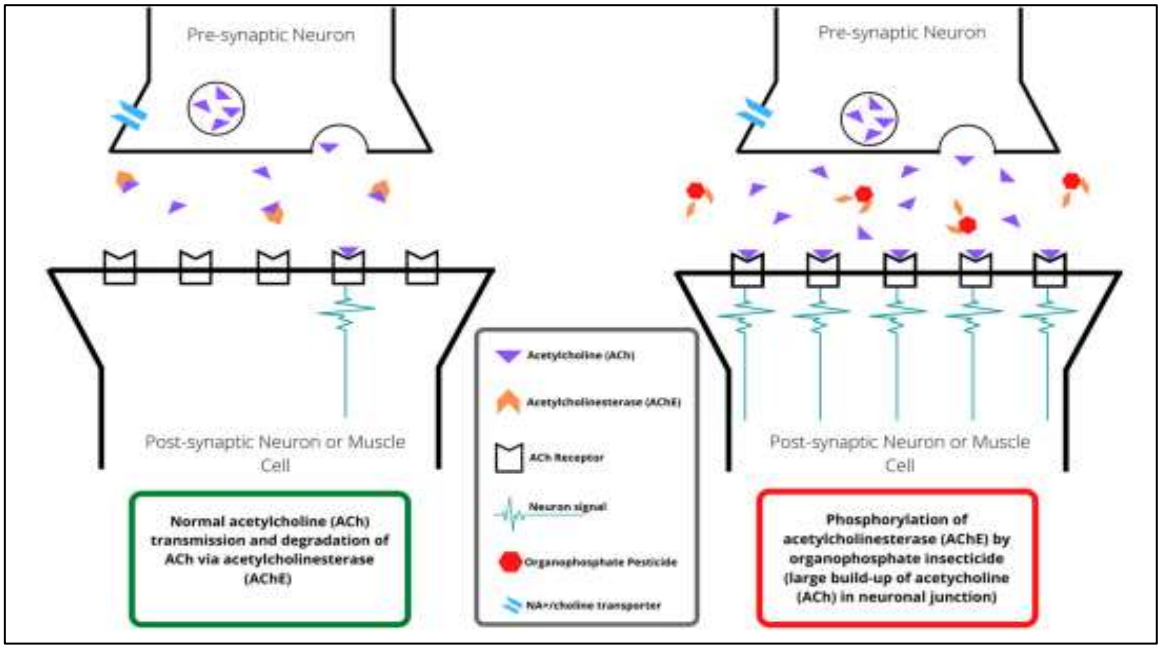



**Figure 2: The relationship between concentration of OPSL$_2$ and mortality percentage of *Ae. aegypti* larvae**

Studies using organophosphorus insecticides have demonstrated that a cholinergic system regulates an insect's muscular motions when it emerges from the egg [32]. As a larva develops from the egg stage, AChE activity multiplies five-fold [33]. Between the first and last larval instars, cholinergic component levels also rise**.** The cholinergic system does not considerably alter during the larva-pupa molt, although it does so significantly during the development of pharate adults**.** Just before adult emergence, the cholinergic components are at their highest levels [34].

The main neurotoxic effect of exposure to organophosphate (**OP**) insecticides is the irreversible inhibition of acetyl cholinesterase (AChE) in the synaptic junction of neurons (Figure 3) [35], which causes the hyperstimulation of post-synaptic cells [36,37]. To stop AChE from hydrolyzing ACh, OPs bind to the hydroxyl group of the enzyme by phosphorylation [38]. By causing an unusual buildup of ACh at synaptic connections as a result of decreased AChE activity, muscarinic and nicotinic receptors that are implicated in cholinergic pathways are hyper stimulated [39]. As a result, increased OP exposure is anticipated to significantly affect neural function, and AChE levels and activity in blood as well as pesticide metabolites can be used to quantify the risk of OP-dependent toxicity [40]. OPs can seriously harm cells as a result of acute and long-term exposure, including cytotoxicity, apoptosis, genotoxicity, and ensuing DNA alterations [41,42,43]. In earlier research, it was shown that pesticides create covalent adducts with DNA, which lead to the formation of interstrand cross-links that prevent cellular reproduction and transcription [44]. Despite the fact that DNA adducts are harmful substances that harm cells, they are nonetheless helpful biomarkers for detecting oxidative stress and genotoxicity [45].




**(Figure 3: The physiological action of acetylcholine (ACh; purple triangles) at the neuronal cell synapse, the breakdown of ACh through acetyl cholinesterase (AChE; orange diamonds), and the phosphorylation of AChE through organophosphate insecticide (OP; red hexagons) exposure).**

## 4. Conclusion

The results lead to the conclusion that the two Organophosphorus Schiff base compounds, OPSL1 and OPSL2 showed high efficacy against *Ae. aegypti* larvae under lab. conditions. The results from this study indicate that OPSL1 and OPSL2 compounds are more advisable to use for the control of mosquito- borne diseases. These results might motivate future research into additional active organophosphorus compounds.

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