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Acute Toxicity of Malathion, Permethrin, and Glyphosate on the Tropical Freshwater Shrimp *Xiphocaris elongata*

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Article

Acetylcholinesterase Enzyme Activity and Acute Toxicity of Malathion, Permethrin, and Glyphosate on the Tropical Freshwater Shrimp *Xiphocaris elongata*

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Abstract: Urban and agricultural runoffs can transport contaminants and pesticides into freshwater ecosystems, particularly in the developing tropics. For instance, organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin have been found in tropical streams. The uncontrolled application of these pesticides has become a growing concern due to their adverse effects on various non-targeted organisms. Unfortunately, most studies have focused on a few selected model species, ignoring the effects on other nontarget organisms, which may play an important role in tropical lotic ecosystems. In addition, the biological characteristics of aquatic crustaceans, including their morphology, physiology, and behavior, make them susceptible to toxic chemicals. For this reason, this study used the widely distributed freshwater shrimp *Xiphocaris elongata* as a model organism to determine the acute toxicity of permethrin, malathion, and glyphosate. Our results show that the proportion of mortality of *X. elongata* in each concentration group became progressively higher as the concentration of exposure increased We also found that the synthetic pyrethroid permethrin is the most toxic pesticide tested with a median lethal concentration (LC_{50}) values for 96h of 3.96 $x10^{-6}$ $\mu g.L^{-1}$. Followed by organophosphate malathion (13.44 $\mu g.L^{-1}$) and glyphosate (748.92 $\mu g.L^{-1}$). Experiments with this freshwater shrimp showed a good control performance and reproducibility for the tested pesticides. This study demonstrated that *X. elongata* is a suitable test organism that can be a representative bioindicator of pesticide toxicity in tropical streams.

Keywords: aquatic toxicology; organophosphate; pesticide; Puerto Rico; pyrethroids

1. Introduction

Pesticide contamination has become a growing concern in the tropics due to its adverse effects on a variety of non-targeted organisms. Faunal assemblages of lotic ecosystems are threatened by anthropogenic activities, including pesticide pollution [1,2]. Urban and agricultural runoff can transport contaminants and pesticides into streams [3,4]. Pesticides, however, are expected to have a much higher effect on aquatic environments because these are the ultimate recipients of contaminants [5,6]. The adverse effects of pesticides can vary depending on the organism and the chemical's mode of action. Most ecotoxicological studies have focused on a few selected model species, ignoring the effects on other nontarget organisms that may play important roles in tropical lotic ecosystems [7,8].

Organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin have been present in many tropical freshwater environments [9,10]. For years, glyphosate has been a common organophosphate pesticide that controls weeds in cultivated croplands. However, due to its toxicity to humans, it has been recently banned in several countries [11]. The primary mode of action is in the shikimate pathway that links primary and secondary metabolisms [12]. Specifically, glyphosate inhibits the activity of the enzyme 5-enolpyuvylshikimate-3-phosphate synthase (EPSPS) found in plants [13]. The exposure of glyphosate to nontarget organisms is of great concern to aquatic toxicologists because of their extensive use, high water solubility, and slow degradation rate in freshwater environments.

Permethrin is a synthetic pyrethroid used in agricultural and residential zones to control a wide range of insects. The application of permethrin is increasing because of its use in mosquito control programs [14]. Toxicological data for permethrin are available for aquatic organisms, but the information is needed to apply to tropical regions [7,14]. Synthetic pyrethroids induce a continuous series of nerve impulses that disrupts the normal functioning of the nervous system. The mode of action is related to the inhibition of sodium channels in nerve membranes and acetylcholinesterase (AChE) enzyme activity [15,16].

Malathion is a common organophosphate insecticide used to control mosquitoes and fruit flies. As an organophosphate insecticide, malathion inhibits acetylcholinesterase (AChE). Acetylcholinesterase is a critical enzyme for the normal function of the nervous system [17]. The inhibition of this enzyme can result in the accumulation of the neurotransmitter acetylcholine in the synaptic gap leading to disruption of the nervous system [16]. Previous studies found that malathion is highly toxic to many fish and aquatic invertebrates [18–22]. In addition, the morphology, physiology, and behavior of aquatic invertebrates, such as crustaceans, make them particularly susceptible to toxic chemicals [23]. Because of those biological characteristics, this study used the widely distributed tropical freshwater shrimp *Xiphocaris elongata* [24] as a model organism to determine the acute toxicity of permethrin, malathion, and glyphosate.

2. Materials and Methods

2.1. Collection and Acclimation of Freshwater Shrimp

Adults of *Xiphocaris elongata* (cephalothorax larger than 13.0 mm) were collected at Río Sabana near Sabana Field Research Station (18°19'29" N, 65°43'47" W) in Luquillo (elevation 113 above sea level), Puerto Rico. Freshwater shrimp were collected using an electrofishing backpack (Model 12-B, Smith-Root, Vancouver, Washington, USA)[25]. Collections consisted of five upstream electrofishing passes in each sampling reach (10 m). Hand nets were used to collect the organisms. The habitats sampled included riffles, runs, pools, and aquatic vegetation. The cephalothorax length (CL) of each shrimp was measured from the post-orbital region to the end of the carapace with a dial caliper (0.01 mm precision). The measurement from the tip of the rostrum was not used because the length of Xiphocaris elongata varies depending on the presence of fish predators. Acclimation was performed where the organisms were maintained for at least one week in aerated water, at 20°C, and with a photoperiod of 12h:12h (light: dark).

2.2. Acute Toxicity Tests

Acute toxicity tests were performed to determine the effect of pesticides on the survival of *X. elongata*. Toxicities were determined by applying a static procedure without renewal of test solutions. All experiments were conducted in glass aquaria with a 5L capacity to which the pesticides were added at different concentrations. Toxicities were expressed based on the concentration of the active ingredient rather than the formulation.

Ten test concentrations of pesticides (between 0.0 to 0.000040 μg/L) and controls were used for each bioassay. Adult freshwater shrimp were selected from the acclimation tanks and distributed randomly into exposure tanks. The exposure tanks (N= 25) (cubic dimensions: length- 15.4 cm, height 15.4- cm, depth- 15.4 cm) were filled with 2.0 L of dechlorinated water, pesticide, and the shrimp. Dead shrimp were counted and removed from the tanks every 24 hrs (24, 48, 72, 96 hrs); the alive organisms continued in the experiment until we reached 96 hrs. This procedure was repeated every 24 hrs. The control and the exposure tanks were kept under constant aeration during the experiment. If the control tanks' mortality rate reached 10% during the first 24 hrs, the experiments had to be reset.

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One hundred shrimp were used for each concentration group. The organisms were not fed during the test period. After pesticide exposure, mortality was recorded between 24h and 96h.

2.3. Statistical Analyses

Lethal concentration (LC_{50}) estimates were determined using probit analyses. Dose–response results were modeled in JMP Pro version 17 Software Software SAS Institute Inc., Cary, NC, USA [26]. Individual mortality per dose was used in the probit analyses to predict the median lethal concentrations. One-way variance analysis (ANOVA) was used to compare the mortality rates among different test groups. A post hoc Tukey's test was used to identify organophosphate and pyrethroids pesticides test with highly significant differences ($P<0.05$).

3. Results

The proportion of mortality of Xiphocaris elongata in each concentration group became progressively higher as the concentration of exposure increased. The mortality observed in acute toxicity tests for permethrin (ANOVA, $F_{(9,39)}=66.13$; $P < 0.001$), malathion (ANOVA, $F_{(9,39)}=84.35$; $P < 0.001$), and glyphosate (ANOVA, $F_{(9,39)}=107.64$; $P <0.001$) were statistically different (Table 1). Median lethal concentration (LC_{50}) values for 24h and 96h for permethrin were 6.91×10^{-6} $\mu g.L^{-1}$ and 3.96×10^{-6} $\mu g.L^{-1}$, respectively (Table 1; Figures 1 and 2). However, malathion median lethal concentration values for 24 h and 96 h were 13.44 $\mu g.L^{-1}$ and 8.87 $\mu g.L^{-1}$, respectively (Table 1; Figures 3 and 4). The least toxic pesticide was glyphosate with a LC_{50} value of 1156.4 $\mu g.L^{-1}$ and 748.92 $\mu g.L^{-1}$ for 24h and 96h, respectively (Table 1; Figures 5 and 6).

Table 1. Median lethal concentration (LC_{50}) estimates for permethrin, malathion, and glyphosate after 96h exposure. CI: confidence interval. Asterisks indicates significant difference *** P< 0.001.

Pesticide	LC_{50} $\mu g . L^{-1}$ (95% CI)		df	F	P
Permethrin	3.96×10^{-6}	($4.49 \times 10^{-6} – 4.52 \times 10^{-6}$)	9	66.13	<0.001 ***
Malathion	8.87	(8.31 – 9.49)	9	84.35	<0.001 ***
Glyphosate	748.92	(701.01 – 802.45)	9	107.64	<0.001 ***

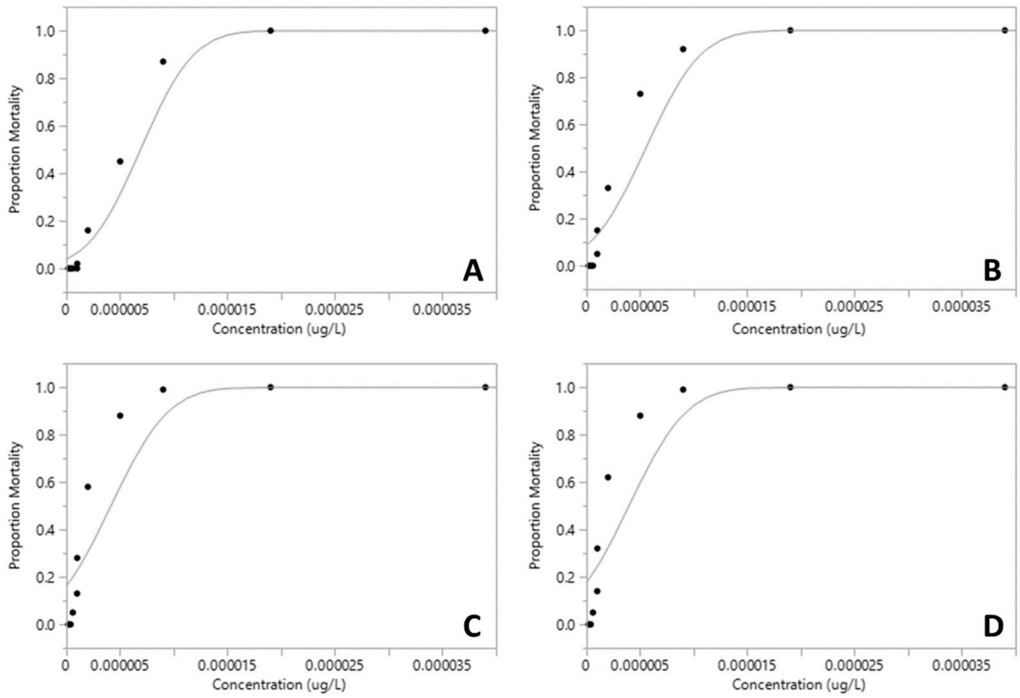

Figure 1. Dose-response curves of permethrin for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

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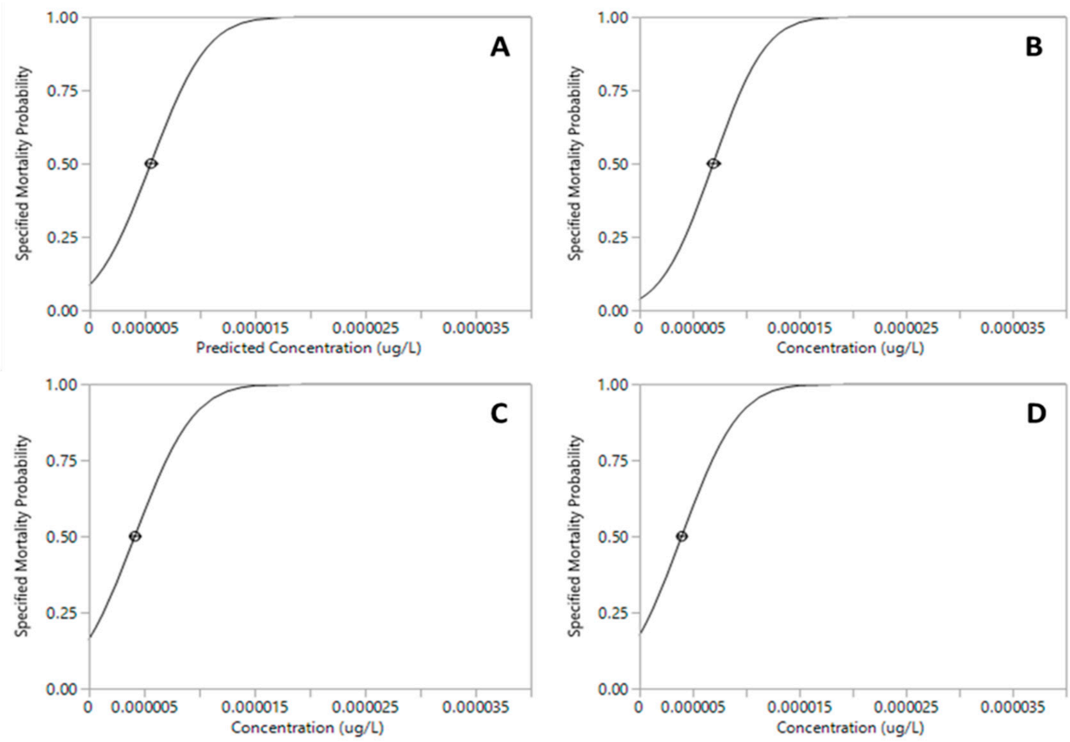

Figure 2. Median lethal concentration (LC_{50}) curves of permethrin for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

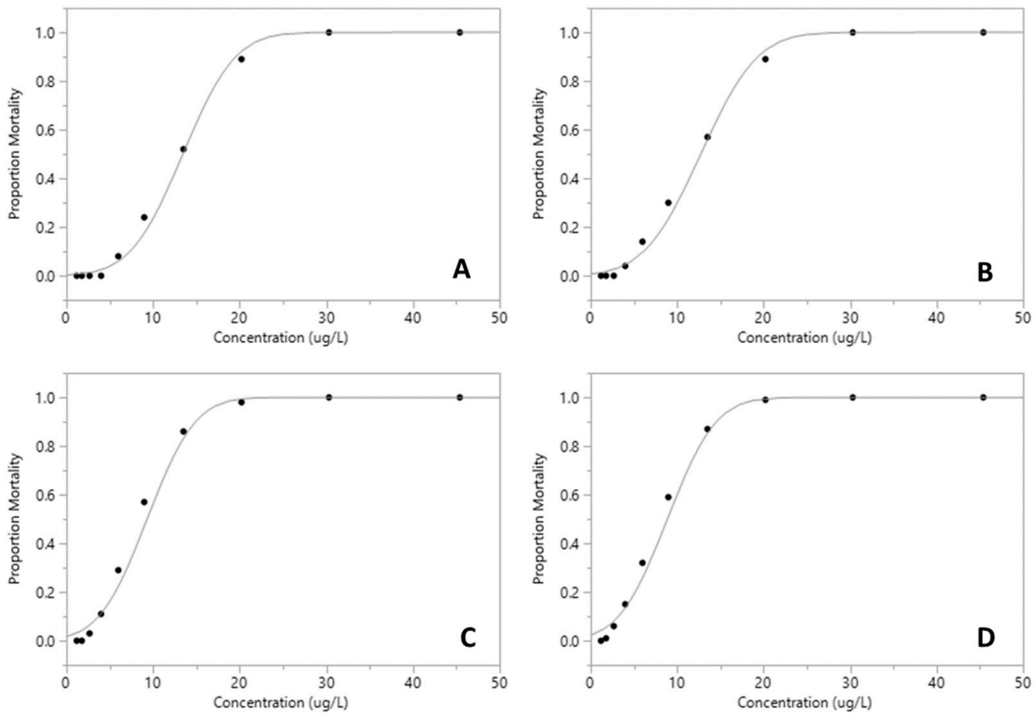

Figure 3. Dose-response curves of malathion for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

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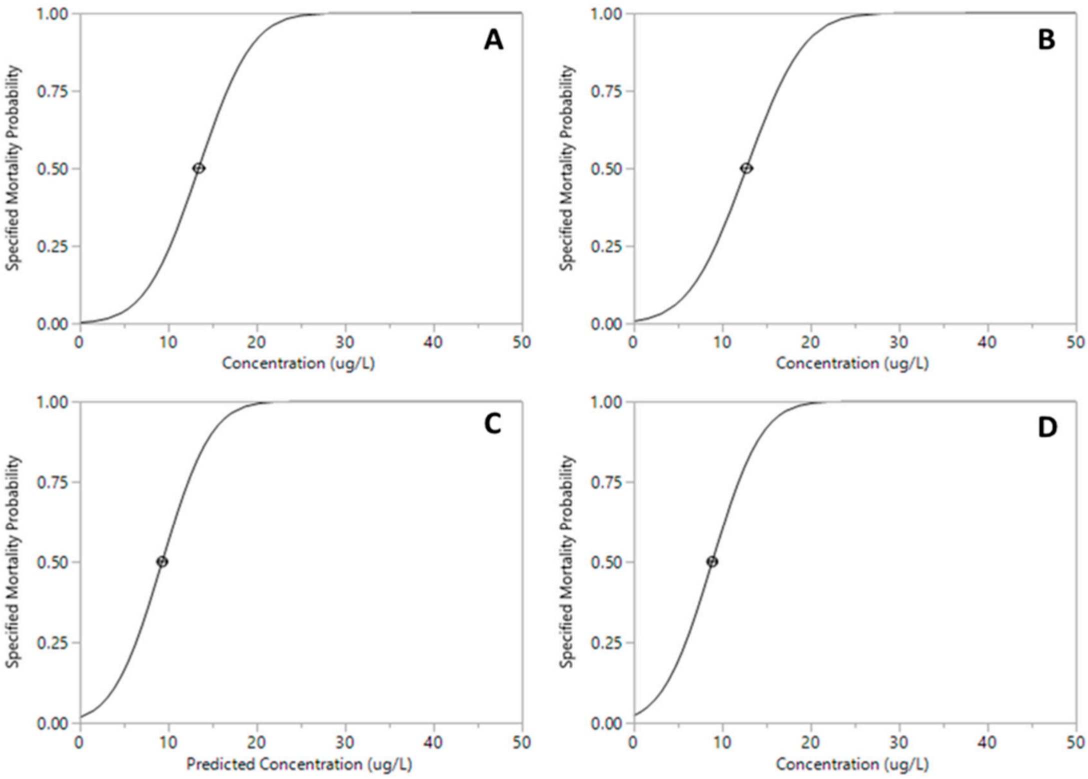

Figure 4. Median lethal concentration (LC_{50}) curves of malathion for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

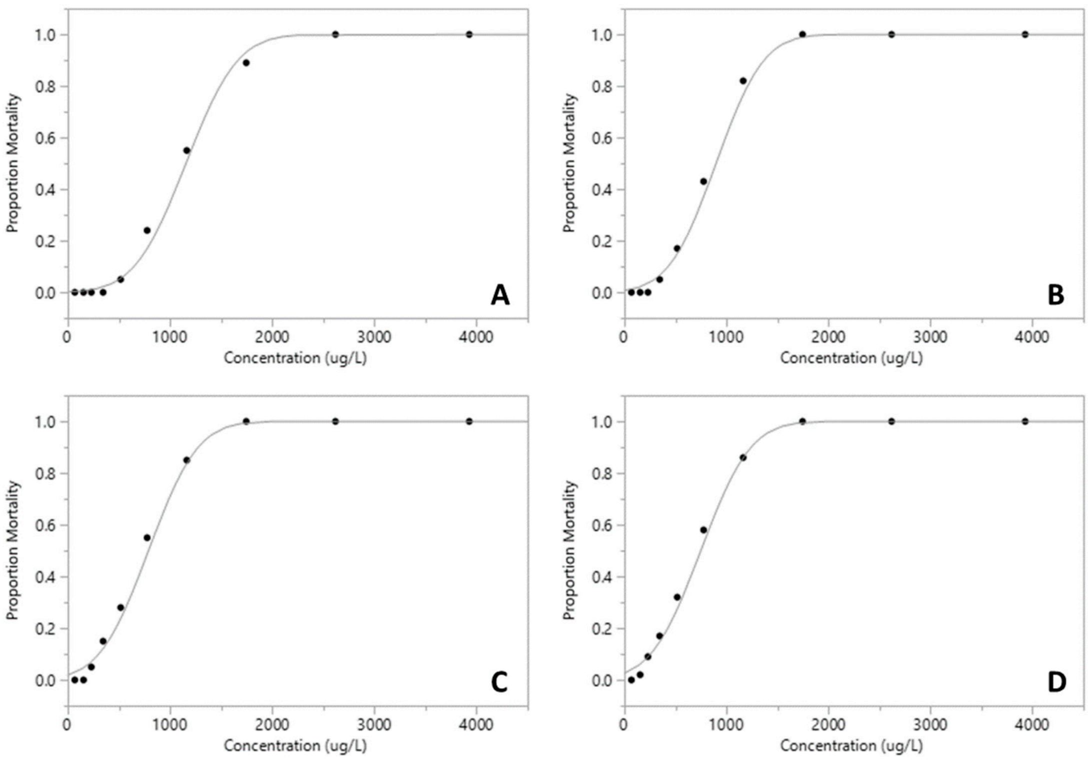

Figure 5. Dose-response curves of glyphosate for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

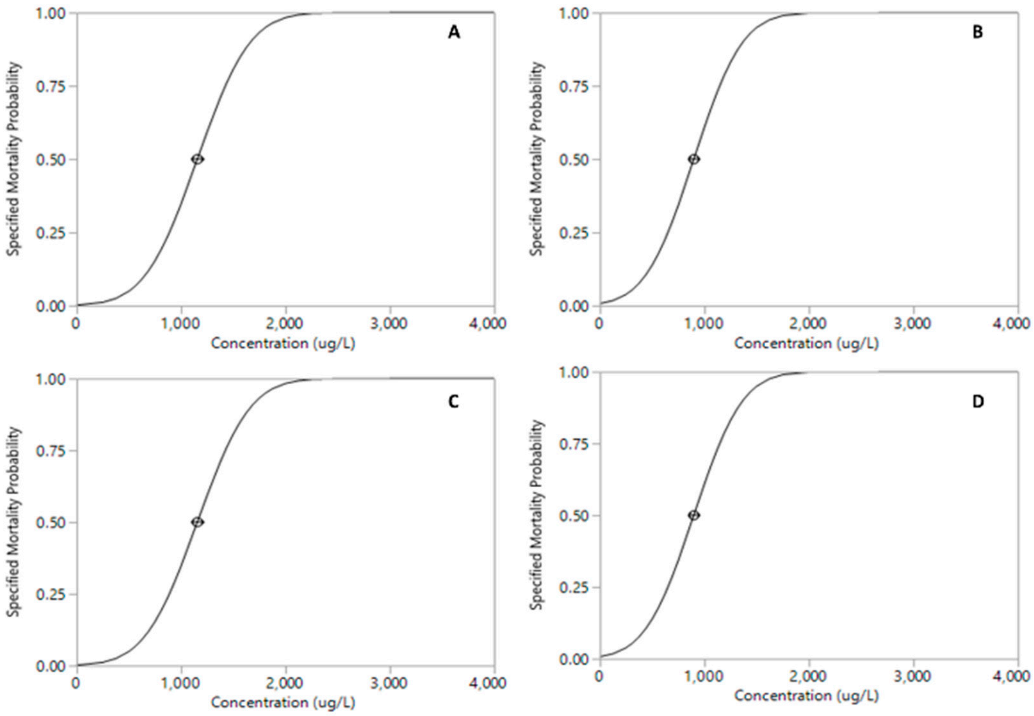

Figure 6. Median lethal concentration (LC_{50}) curves of glyphosate for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

4. Discussion

This study assessed the acute toxicity of permethrin, glyphosate, and malathion for the tropical freshwater shrimp *Xiphocaris elongata*. To evaluate the biological outcome of such exposure, it is necessary to determinate lethal and sublethal concentrations for organisms. Due to the high costs and long duration of chronic tests, there is a need to do more acute toxicity research. One advantage of using acute toxicity data is that it has been proven to be highly accurate in making long-term predictions [19]. Permethrin was the most toxic pesticide tested for our model organism, followed by malathion and glyphosate. Similarly, Sánchez-Fortun and Barahona [27] have found that the aquatic invertebrates most sensitive to pyrethroids pesticides are surface-dwelling insects, mayfly nymphs, and crustaceans. Among crustacean species, *Daphnia magna*, *Daphnia pulex*, *Palaemonetes pugio*, and *Macrobrachium rosenbergii*, have been used as model organisms in aquatic toxicology [28–30]. However, the disadvantage of using those species is that some are not present in tropical freshwater environments. In tropical regions, aquatic organisms are often exposed to pesticides and other contaminants, for this reason there is a need to develop model organisms for this geographic region such as we have done with the tropical freshwater shrimp *Xiphocaris elongata*.

Shrimp are highly susceptible to pyrethroids pesticides [31,32]. Even at sublethal concentrations, significant behavioral changes in shrimp feeding behavior and locomotion activities could affect their survival. In this study, permethrin median lethal concentration (LC_{50}) value for 96h exposure was 6.91x10-6 $\mu g.L^{-1}$. A study about the effect of permethrin on the grass shrimp *Palaemonetes pugio* proved to be acutely toxic at 0.21 $\mu g.L^{-1}$ [33]. Another study found that the freshwater prawn *Palaemonetes argentinus* are susceptible to pyrethroids with a median lethal concentration of 0.0031$\mu g.L^{-1}$ [34]. This study also determinated that malathion is high to moderate toxic with a 96h LC_{50} value of 13.44 $\mu g.L^{-1}$. Similar results were found in a study where malathion exhibited severe toxicity to the grass shrimp *Palaemonetes pugio* with a LC_{50} of 38.19 µg.L-1 [35].

Few studies have evaluated the toxicity of glyphosate on freshwater organisms [36–38]. In this study, glyphosate median lethal concentration value for 96 h was 748.92 $\mu g.L^{-1}$. Mensah among others [39] also investigated the acute toxicity of glyphosate on freshwater shrimp *Caridina nilotica*. Their results show a median lethal concentration of 25.3 $mg.L^{-1}$. The low toxicity of glyphosate exposure

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may be because the shikimic acid pathway is absent in animals. However, some studies demonstrated that high concentrations could alter animal mitochondrial activity by uncoupling oxidative phosphorylation during cellular respiration [38].

In summary, the tropical shrimp *Xiphocaris elongata* is an ecologically important organism in freshwater environments that can be exposed to synthetic pyrethroid and organophosphate pesticides. This study demonstrates that *X. elongata* is a suitable test or model organism that can be a representative bioindicator of pesticide toxicity in tropical freshwater environments. Similar results were observed in a study where AChE activity were used as biomarker to detect and assess organophosphate and pyrethroid pesticide exposure using *X. elongata* as model to assess the risk of pesticide contamination in tropical freshwater environment (Torres-Pérez & Pérez-Reyes, 2023). Experiments with this freshwater shrimp species showed a good control performance and reproducibility for the different pesticides tested. Future studies should evaluate sublethal effects using environmentally relevant concentrations to specific pesticides and mixtures.

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Conflicts of Interest: The authors declare no conflict of interest.

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