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Acute toxicity and histopathological effects of Malathion on shrimp *Macrobrachium nipponense* (De Haan, 1849) (Caridea: Palaemonidae)

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Abstract: In the south of Iraq, Malathion is frequently used in agricultural activities and overfishing, significantly harming the environment and animals that live in the region's waterways. The 96-h LC_{50} toxicity of malathion was determined in *Macrobrachium nipponense* and the value was 61.27 μg/L. Based on the 96-h LC_{50} value, two concentrations (1:10 and 1:5 of the 96-h LC_{50}) were selected as sublethal concentrations for 1 week exposure for histopathology analysis. The most histopathological alterations in hepatopancreas were abnormal lumen ALU after 1 week of exposure to Malathion with the sublethal concentration 6.12 μg/L. In the other sublethal concentration of Malathion 12.25 μg/L for 1 week exposure, abnormal lumen ALU; necrosis of epithelial cells NC; and necrosis of tubules were recorded.

Keywords: Malathion; 96-h LC_{50} toxicity; Hepatopancreas.

1 Introduction

Malathion [S-1,2 (bis-ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate], an organophosphate insecticide, is widely used in public health programs to protect grain in storage, control important arthropods, animal ectoparasites, human head and body lice, and home insects (Maroni et al., 2000). Malathion's bio-activated analog, malaoxon, is known to cause excitotoxicity (Berktas and Peker, 2021).

Malathion is widely used in agricultural activities and overfishing in the south of Iraq, wreaking havoc on the ecology and creatures that live in the region's waters (Yasser et al., 2008; Yasser et al., 2010; Yasser and Naser, 2011; Yasser, 2012; Yasser et al., 2021).

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In situ cellular, tissue, and organ system organization and spatial interactions are preserved in histological slices. As a result, visual localisation of substantial abnormalities in various tissues and organs of the organism can offer information on the combined impacts of molecular, biochemical, and physiological changes caused by pollutants (Hinton and Lauren, 1990; Myers and Fournie, 2002; Wester et al., 2002). Because cells and tissues are extremely sensitive to chemical stimuli, even low amounts of the pollutant can cause structural damage to tissues and organs. As a result, histopathology tests can operate as early warning indications of animal health harm (Hinton and Lauren, 1990) and as a useful screening approach for assessing potential ecological impacts.

The freshwater prawn shrimp *Macrobrachium nipponense* is an alien species widely distributed in Shatt Al-Arab and southern marshes of Iraq (Salman et al., 2006). The study being reported was an attempt to investigate the effects of malathion on the histoarchitecture of hepatopancreas of the prawn, *M. nipponense*.

2 Materials and Methods

Prawn shrimp *M. nipponense* were collected from a pool near the local fish breeder near Marine Science Center, University of Basrah, Iraq in September 2011. The average carapace length of the shrimp used in the experiment was 18.56 mm (± 1.70). Shrimp were maintained per beaker in 10 L beakers filled with dechlorinated tap water with continuous aeration (pH 7.8, dissolved oxygen 7.9 mg/l, salinity 0.9 practical salinity units, total hardness 137 mg/l as CaCO3) and a 12 L: 12 D photoperiod for 7 days prior to the start the experiment.

Static acute bioassays were conducted using commercial grade (50 % malathion, made in Iran). The nominal concentrations used were 20, 35, 50, 70 and 90 μg/L. Ten shrimp were tested at each concentration, with each shrimp kept in a separate 5 L beaker.

Every three hours for 96 hours, each shrimp was gently prodded under a dissecting microscope with a needle. Shrimp that did not respond within one minute were recorded as dead and were removed from the experiment.

Based on the 96-h LC_{50} value, two concentrations (1:10 and 1:5 of the 96-h LC_{50}) were chosen for sublethal shrimp exposures.

Histopathological examination:

M. nipponense specimens were immediately dissected in the laboratory. Hepatopancreas samples were removed using sterilized scissors. After 24 hours, the samples were kept in Davidson's solution in appropriately labeled and sealed vials (Arockia-Vasanthi et al., 2014). After 24 hours, the samples were washed and stored in 70 % ethanol. After dehydrating the gills in increasing concentrations of ethanol, diaphanizing them in xylol, and impregnating them with paraffin, Hematoxylin and eosin were employed to stain cross-sections with a thickness of approximately 5 μm. The changes were captured using a compound microscope connected with a digital camera.

Statistical Analysis:

LC_{50} values were determined using Finney's (1971) probit analysis LC_{50} determination method and version 1.00 of the software developed by EPA (1999).

3 Results and Discussions

Acute toxicity:

The present study discovered that the organophosphorus insecticide Malathion is harmful to *M. nipponense*. The toxicity of this chemical on the tested shrimp increased with increasing concentration and/ or exposure time in acute toxicity tests. Malathion's 96-h LC_{50} value was determined for *M. nipponense*. The concentration of was found to be 61.27 μg/L. (Table 1). The concentration-response curve is shown in Figure 1.

Table 1. The 95 % confidence intervals (Lower and Upper Confidence interval) for *M. nipponense* exposed to Malathion. (n=10 per replicate, No. of replicates per concentration= 3).

Concentration (μg/L)	Probability	95 % Lower Confidence interval	95 % Upper Confidence interval
-15.5799	0.01	-104.72638	11.64957
-6.5743	0.02	-84.93371	17.74005
-3.47601	0.025	-78.14312	19.85429
6.93401	0.05	-55.42718	27.05793
18.93609	0.1	-29.53953	35.66564
33.46967	0.2	0.87142	47.02587
38.99104	0.25	11.86778	51.89856
61.27355	0.5	47.8652	79.94363
83.55606	0.75	68.76706	123.08425
89.07743	0.8	72.98899	134.73137
103.61102	0.9	83.47606	166.01547
115.6131	0.95	91.76682	192.22007
126.02312	0.975	98.8149	215.09158
129.1214	0.98	100.89513	221.91618
138.12701	0.99	106.90778	241.78667

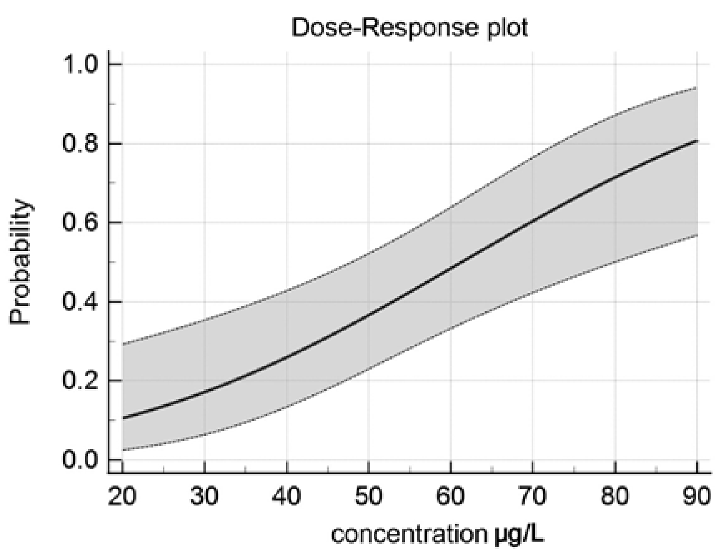

Figure 1: Concentration-response plot.

Hepatopancreas:

Pesticides and other water-borne chemicals can harm the hepatopancreas of crustaceans, which is analogous to the liver of higher animals (Baticados and Tendencia, 1991). Branched tubules bordered by various types of epithelial cells (E-cells, R-cells, F-cells, and B-cells) make up the majority of the hepatopancreas (Figure 2a). Freshwater prawns *Macrobrachium nipponense* were subjected to two sublethal concentrations of the organochlorine insecticide Malathion (6.12 and 12.25) μg/L for one week in the current investigation. The prawns' hepatopancreas were then dissected and processed for light microscopic examination. Malathion exposure was observed to cause various changes in the histoarchitecture of *M. nipponense*'s hepatopancreas. ALU (abnormal lumen), NC (necrosis of epithelial cells), and NC (necrosis of tubules) were among the changes (Figure 2b, c and d).

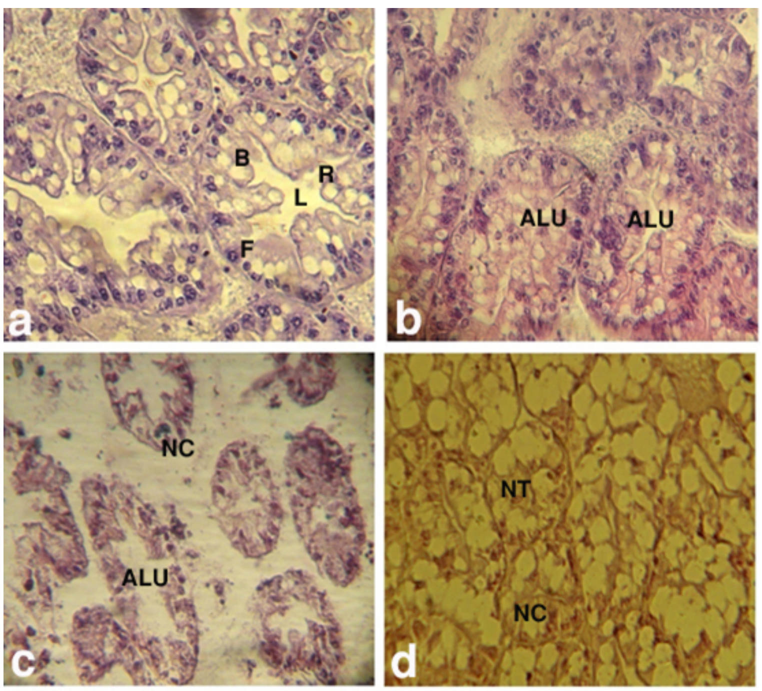

Figure 2: Hepatopancreas of a *Macrobrachium nipponense* control. (a) transverse section of tubules in the middle proximal region demonstrating that tubules are well organized and appear as a star shape in the lumen L., Different cell types are visible in transverse section, including secretory (blasenzellen) cells (B-cells), fibrous (fibrillenzellen) cells (F-cells), and absorptive, storage (restzellen) cells (R-cells) tenfold N, H & E stain, 20x. (b) After one week of exposure, shrimp were exposed to sublethal concentration of malathion 6.12 μg/L , resulting in abnormal lumen ALU. In 12.25 μg/L after 1 week of exposure, (c & d) exposed shrimp to sublethal concentration of malathion pesticides, abnormal lumen ALU; necrosis of epithelial cells NC; and necrosis of tubules N, H & E stain, 10x.

The acute toxicity of Malathion to aquatic crustacea species has been studied in a variety of ways. Eisler (1969) investigated the acute toxicities of insecticides to the marine decapods. He estimated the acute toxicity of Malathion to the grass shrimp *Palaemonetes vulgaris* (Say) and to the hermit crab *Pagurus longicarpus* (Say), and the 96-h LC_{50} were 82 μg/L and 83 μg/L, respectively. In comparison with Eisler's 96-h LC_{50}, *M. nipponense* showed more sensitive to Malathion than marine decapods *Palaemonetes vulgaris* and to the hermit crab *Pagurus longicarpus*. However, *M. nipponense* is more tolerant to Malathion when it compared with the grass shrimp, *Palaemonetes pugio* where the 96-h LC_{50} was 38.19 μg/L (Key et al., 1998). Rico et al. (2011) estimated the 96-h LC_{50} of Malathion and carbendazim on Amazonian freshwater organisms. According to their study, the freshwater shrimp *Macrobrachium ferreirai* is more than six fold tolerant to Malathion with 96-h LC_{50} 398 μg/L compared to *M. nipponense*.

It is well-known that the liver and hepatopancreas are extremely susceptible to many dietary and water-borne contaminants; as a result, these organs are frequently utilized to monitor the effects of various pollutants (Vogt, 1987; Bautista et al., 1994). The hepatopancreas is mostly made of branched tubules, with distinct types of epithelial cells lining the tubules and lining the lumen of the tubules. The presence of toxic substances or xenobiotics in the environment is therefore likely to result in changes to the tubule and epithelial cell architecture (Wu et al., 2008; Boudet et al., 2015). Researchers have looked at the effects of exposure to various toxicants on the histological and cellular changes that occur in the liver and hepatopancreas of a variety of aquatic organisms (Doughtie and Rao, 1984; Wu et al., 2008; Boudet et al., 2015).

This study tested the structural changes that occurred in the hepatopancreas of *M. nipponense*, when it was subjected to two sub-lethal concentrations of Malathion. According to our findings, structural alterations in the hepatopancreas occurred as a result of Malathion exposure. In this work, abnormal lumen ALU after 1 week of exposure to Malathion with the sublethal concentration 6.12 μg/L was recorded. In the other sublethal concentration of Malathion 12.25 μg/L for 1 week exposure, abnormal lumen ALU; necrosis of epithelial cells NC; and necrosis of tubules were observed. Because the same symptoms have been reported in other prawn species exposed to higher concentrations of Endosulfan, or a fungicide and heavy metals (Lightner et al., 1996; Bhavan and Geraldine, 2000; Wu et al., 2008; Boudet et al., 2015), these symptoms are likely common and typical responses when prawn are exposed to toxicants that cause lesions in the hepatopancreas.

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Conflict of interests

The authors have no conflict of interest to declare.

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