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Effects of an organophosphate insecticide, chlorpyrifos on the survival of *Macrobrachium rosenbergii* at various life stages

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Abstract

The present study investigated the acute toxicity of an organophosphate insecticide, chlorpyrifos used in the paddy fields of Kuttanad, a part of the Vembanad wetland system which is a Ramsar site of international importance in the state of Kerala, India, to various life stages of *Macrobrachium rosenbergii*, a commercially important and indigenous prawn of Kuttanad. LC_{50} values were determined using the observations made on the mortality of prawns exposed to 5 different concentrations between the No Observable Effect Concentration (NOEC) and Lowest Observable Effect Concentration (LOEC) of chlorpyrifos. The LC_{50} values for 24, 48, 72 and 96 h was determined by probit analysis using SPSS, based on which safe concentrations were calculated using Hart's formula. The study revealed the 96 h LC_{50} concentrations as 0.00041, 0.022 and 0.038 ppm and safe concentrations as 0.0001, 0.004 and 0.008 ppm for post-larvae, juvenile and adult, respectively of *M. rosenbergii*. The results revealed that the application of chlorpyrifos in the paddy fields of Kuttanad poses a threat to the existence of the species in its homeland, as the field application concentration (0.0025 ppm) of chlorpyrifos is higher than the safe concentration determined for post-larvae.

Keywords: chlorpyrifos, acute toxicity, *Macrobrachium rosenbergii*, postlarvae, juvenile, adult

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, has received remarkable attention as an important commercial freshwater species because of its large size, rapid growth and high nutritive value [1]. It is an indigenous species of Kuttanad, a part of the Vembanad wetland ecosystem which is one of the internationally recognized Ramsar sites (location no. 1214) in Kerala. It is one of the most fertile lands of the world, having 55,000 ha of paddy fields, vast stretches of backwaters and bordering mangrove formations. The water bodies of Kuttanad serve as nursery grounds for postlarvae and juveniles of *M. rosenbergii*, where they feed upon rice grains and small worms [2, 3].

The Kuttanad Below Sea-level Farming System (KBSFS) is unique, as it is the only system in India that practices rice cultivation below sea level since the past 2 centuries. Moreover, the area is prone to pests such as Plant Hoppers, stem borers, leaf rollers, etc. There is no systematic pest control in the region and therefore, farmers indiscriminately apply pesticides in their farms. This unscientific and non-judicious application of pesticides was reported to be ineffective as well as unwanted as they caused severe damage to the Kuttanad ecosystem **[4]**.

Chlorpyrifos (O, O-diethyl 0-3, 5,6-trichloro-2-pyridyl phosphorothioate) is a broad-spectrum, organophosphate (OP) pesticide commercially used for more than a decade to control foliar insects that affect agricultural crops [5,6] in the paddy fields of Kuttanad. It is a non-systemic insecticide with contact stomach, respiratory action and a quick knockdown effect, that has been extensively used for controlling stem borers and leaf-eating caterpillars and several species of Coleoptera, Diptera, Homoptera, and Lepidoptera in soil or on foliage [7] in over a large number of crops including rice, cotton, oilseeds, pulses, vegetables and plantation. It is the second largest selling OP and found to be more toxic to fish than organochlorine compounds [8]. This OP insecticide is known to inhibit acetylcholinesterase, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate [9]. The inhibitory effects of chlorpyrifos is dependent on its binding capacity to the enzyme active site and by its rate of phosphorylation in relation to the behaviour and age [10, 11]. It is reported to be activated by contact, ingestion and vapour action, causing convulsions and paralysis. Chlorpyrifos can enter the body either by inhalation of air containing chlorpyrifos, ingestion of contaminated food or by dermal contact with chlorpyrifos. It can cause acute poisoning and well known symptoms include myosis, increased urination, diarrhoea, diaphoresis, lacrimation and salivation [12]. It is also reported to be involved in multiple mechanisms like causing hepatic dysfunction [13], Genotoxicity [14], Neurobehavioral and neurochemical changes [15]. Chlorpyrifos intoxication is shown to cause a significant decrease in the reduced glutathione (GSH), catalase (CAT) and glutathione Stransferase (GST) activities [16]. Symptoms of high level of exposure to OPs include accumulation of ACh in the synaptic cleft Causes overstimulation of the neuronal cells, which leads to hyperactivity caused by neurotoxicity, paralysis and eventually death [17].

The universal nature of chlorpyrifos in terms of accessibility and extensive usage has led to increasing concern about its toxicity. Chlorpyrifos passes via air drift or surface runoff into surrounding waters and gets accumulated in different aquatic organisms, particularly fish, thus making it vulnerable to several discernible effects [18, 19, 20, 21]. The resultant consequence of extensive use of chlorpyrifos is that more nontarget organisms, especially fish and aquatic organisms will become exposed to it and would thus increase the frequency of toxic sequelae attributed to this compound [22, 23, 24].

Several toxicity studies of chlorpyrifos in crustaceans [25 -35] and *Machrobrachium* sp., *viz. Macrobrachium lanchesteri* [36, 37] and *Macrobrachium nipponense* [38, 39] has been done. In view of these data, the present study was focused to determine the LC_{50} concentration of chlorpyrifos in different life stages of *M. rosenbergii viz.* postlarvae, juvenile and adult, since the effect of chlorpyrifos in *M. rosenbergii* at these life stages remains unexplored except postlarvae [40].

2. Materials and Methods

2.1. Animal rearing

The toxicity assessment was carried out with postlarvae, juvenile and adult M. rosenbergii procured from Sea View Prawn Hatchery, Thriprayar, Kerala, India. They were brought to the aquatic animal rearing facility of the Department of Marine Biology, Microbiology and Biochemistry located at the School of Marine Sciences, Cochin University of Science and Technology, Kerala, India, wherein the study has been conducted. Prior to the experiment, prawns were held in experimental glass tanks for a period of 15 days and fed with commercial pellet feed to get acclimatized to the ambient condition. Acclimatized prawns were starved for 24 h and used for experimentation.

2.2. Analysis of water quality

The water quality parameters such as temperature (Amber mercury thermometer, Amber hydrometers, Ahmedabad, India.), pH (Cyberscan pH 510 Meter, Aarkey Labtronix India.) and dissolved oxygen (Winkler's method) were evaluated daily. Alkalinity (titration method), total ammonia (Phenate method) and hardness (EDTA titrimetric method) were determined at the beginning and the end of the experiment [41]. Water quality variables were determined independently for each treatment to establish whether the prawns were maintained at appropriate favorable conditions [42].

2.3. Experimental setup

Assessment of the median lethal concentration (LC_{50}-96 h) was done based on the manual of the Environmental Protection Agency USA [43]. Experiments were carried out using 540 prawns (180 postlarvae / 18 experimental units, 180 juveniles / 18 experimental units and 180 adults / 18 experimental units). The units consisted of 60 l glass tanks with 10 postlarvae (0.041 ± 0.02 g; 1.45 ± 0.25 cm) in 20 l of test solution, 10 juveniles (3.5 ± 1.5 g; 7.26 ± 0.51 cm) in 30 l of test solution and 10 adult (17.5 ± 2.5 g; 12.63 ± 0.42 cm) in 40 l of test solution. Experimental units were equipped with aeration systems and a natural photoperiod of 12 h / 12 h (light / dark).

2.3.1. Range finding test

A preliminary range-finding test was conducted for a period of 96 h with a wide range of concentrations *viz.*, 0.001, 0.01, 0.1, 1.0, 10 and 100 ppm. The concentrations between the No Observable Effect Concentration (NOEC) and Lowest Observable Effect Concentration (LOEC) of chlorpyrifos were determined by observing the mortality after 96 h and used for the acute toxicity experiments.

2.3.2. Acute toxicity test

Acute toxicity tests were employed with six different concentrations *viz.* 0, 0.0002, 0.0004, 0.0006, 0.0008 and 0.0010 ppm; 0, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm; and 0, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm Commercial-grade chlorpyrifos (Cheminova India – Classic 20 having active ingredient of 20% EC), respectively for postlarvae, juvenile and adult *M. rosenbergii* each in triplicates. Test solutions were renewed every 24 h to maintain even concentration of toxicants, to remove organic wastes and to avoid oxygen depletion.

The criterion used to affirm the lethality was the total absence of any kind of movement or reaction to mechanical stimuli using a glass rod. Prawns were observed every 1 h for the first 8 h and every 12 h between 8 h and 96 h [44].

2.3.3. Determination of LC_{50} concentration

Mortality of postlarvae, juvenile and adult stages of *M. rosenbergii* exposed to each concentration of chlorpyrifos after 24, 48, 72 and 96 h were recorded and used for estimation of the LC_{50} values. The LC_{50} values were determined by looking up the concentration corresponding with probit in the probit list generated by estimating linear regression using SPSS version 16.0. [45].

2.3.4. Determination of Safe concentration

LC_{50} values of postlarvae, juvenile and adult stages of *M. rosenbergii* exposed to chlorpyrifos after 24 and 48 h estimated by probit analysis [45] using SPSS version 16.0 were used for the estimation of safe concentration (concentration of pollutants that supposedly has no adverse effect on the organism). Safe concentration of chlorpyrifos to postlarvae, juvenile and adult *M. rosenbergii* was calculated by the method described by Hart *et al.* (1945) [46] using the formula.

$$\text{Safe concentration} = \frac{48\text{ h } LC_{50} \times 0.2}{S^2}$$

$$\text{Where } S = \frac{24\text{ h } LC_{50}}{48\text{ h } LC_{50}}$$

2.4. Statistical analysis

The median lethal concentration of chlorpyrifos was calculated by the probit method [45]. Briefly, probits (short for probability unit) for a 50% response (LC_{50}) were determined as 5.00 by looking up those corresponding to the % responded in Finney's table [45]. The LC_{50} values for 96 h with 95% confidence intervals were determined by looking up the concentration corresponding with probit of 5.00 in the probit list generated by estimating linear regression with observed cumulative mortality and the logarithm of the concentrations of chlorpyrifos using SPSS version 16.0.

3. Results and Discussion

The acute toxicity study results of the giant freshwater prawn *M. rosenbergii* at different life-history stages, *viz.* postlarvae, juvenile and adult to chlorpyrifos are as follows:

3.1. Water quality parameters

During the experimental period, water temperature ranged from 27 to 28 °C, pH from 7.2 to 7.7, dissolved oxygen from 5.6 to 7.4 mg.l^{-1}, hardness from 27.2 to 30.8 mg.l^{-1}, alkalinity from 20.2 to 23.2 mg.l^{-1} CaCO$_3$ and Total ammonia from 0.203 to 0.93 mg.l^{-1} (Table 1).

Table 1: Water quality parameters of acute toxicity tests of chlorpyrifos in different life-history stages (postlarvae, juvenile and adult) of M. rosenbergii.

Life-history stages	**Variables**	**Concentrations (ppm)**					
		0.000	**0.0002**	**0.0004**	**0.0006**	**0.0008**	**0.0010**
Postlarvae	Temperature (°C)	27.2±0.5	27.2±0.5	27.2±0.5	27.2±0.5	27.2±0.5	27.2±0.5
	pH	7.3±0.1	7.3±0.1	7.3±0.1	7.3±0.1	7.3±0.1	7.3±0.1
	Dissolved oxygen (mg·l^{-1})	6.6±0.4	6.6±0.8	6.7±0.7	6.7±0.6	6.8±0.4	6.8±0.2
	Hardness (mg·l^{-1} CaCO$_3$)	28.4±0.8	28.4±1.2	29.0±1.4	29.4±1.2	29.4±1.0	30.2±0.4
	Alkalinity (mg·l^{-1})	22.6±0.1	22.4±0.2	21.8±0.2	21.5±0.3	21.1±0.5	20.8±0.6
	Total ammonia (mg·l^{-1})	0.205±0.3	0.44±0.2	0.48±0.44	0.47±0.40	0.48±0.32	0.46±0.70
		0.00	0.02	0.04	0.06	0.08	0.10
Juvenile	Temperature (°C)	27.2±0.5	27.5±.5	27.3±0.2	27.4 ±0.5	27.6±0.4	27.1±0.3
	pH	7.5±0.1	7.4±0.2	7.3±0.1	7.4±0.1	7.5±0.1	7.5±0.1
	Dissolved oxygen (mg·l^{-1})	6.6±0.2	6.2±0.8	6.6±0.3	6.4±0.7	6.6±0.1	6.5±0.2
	Hardness (mg·l^{-1} CaCO$_3$)	28.2±0.7	28.2±1.2	29.2±1.2	29.4±1.4	29.4±1.2	29.1±0.4
	Alkalinity (mg·l^{-1})	22.5±0.1	22.3±0.3	21.7±0.2	21.6±0.2	21.1±0.5	20.9±0.5
	Total ammonia (mg·l^{-1})	0.207±0.2	0.44±0.3	0.47±0.47	0.47±0.34	0.48±0.20	0.47±0.92
		0.00	0.02	0.04	0.06	0.08	0.10
Adult	Temperature (°C)	27.4±0.6	27.4±0.4	27.4±0.5	27.7±0.2	27.6±0.3	27.6±0.4
	pH	7.3±0.1	7.3±0.1	7.4±0.2	7.4±0.1	7.4±0.2	7.5±0.1
	Dissolved oxygen (mg·l^{-1})	6.5±0.4	6.6±0.2	6.3±0.5	6.4±0.3	6.3±0.8	6.4±0.8
	Hardness (mg·l^{-1} CaCO$_3$)	28.5±0.8	28.6±1.1	29.1±0.4	29.2±1.2	29.8±0.1	30.2±0.4
	Alkalinity (mg·l^{-1})	23.3±0.1	22.4±0.2	21.6±0.2	21.5±0.8	21.4±0.2	20.8±0.3
	Total ammonia (mg·l^{-1})	0.204±0.3	0.36±0.2	0.45±0.34	0.47±0.52	0.47±0.16	0.47±0.72

Water quality parameters remained within the optimal range reported for the growth and survival of *M. rosenbergii*. Temperature ranges from 28–31 °C, dissolved oxygen ranges from 3 to 7 mg.l^{-1}, pH ranges from 7–8.5, hardness ranges from 20–150 mg.l^{-1} Total ammonia 0.1-0.5 mg.l^{-1} and alkalinity ranges from 20–60 mg.l^{-1} of CaCO$_3$ [47]. As none of the water quality Parameters measured in this study was stressful for the prawns, the lethal effects observed could be due to the toxicity of chlorpyrifos.

3.2 Acute toxicity of chlorpyrifos to *M. rosenbergii*

During the acute toxicity study of chlorpyrifos to postlarvae of *M. rosenbergii* exhibited escape tactics like sticking to the walls of the glass tanks above water level and clinging on to the nets covering the experimental tanks. Juveniles and adults of *M. rosenbergii* exposed to high concentrations of chlorpyrifos exhibited hyperactivity with a series of abnormal behavioral patterns such as erratic swimming, flashing, restlessness, etc. Later they became paralyzed and eventually fell dead. In all life stages examined, the mortality of prawns exposed to chlorpyrifos increased with that of exposure period and concentration. Mortality observed for control *M. rosenbergii* (not exposed to chlorpyrifos) during the trial period was negligible and may be related to the agonistic behavior of conspecifics attacking/eating the other leading to injury or death known as cannibalism.

The behavioral changes noticed in the postlarvae, juveniles and adult *M. rosenbergii* during bioassay test indicate escape tactics similar to those described for *M. malcolmsonii* on exposure to monocrotophos [48], *M. rosenbergii* to endosulfan [49] and *M. lamarrei lamarrei* to dichlorvos [50]. Negligible rate of mortality of control prawns is expected in experimental conditions [44, 51].

3.2.1. Acute toxicity of chlorpyrifos in postlarvae of *M. rosenbergii*

Median lethal concentration of post-larvae of *M. rosenbergii* exposed to different concentrations of chlorpyrifos, *viz.* 0, 0.0002, 0.0004, 0.0006, 0.0008 and 0.0010 ppm for 24, 48, 72, and 96 h are given in Fig. 1. Postlarvae of *M. rosenbergii* exposed to chlorpyrifos for 24 h showed a median lethal concentration of 0.00145 ppm. Whereas, after 48 h and 72 h median lethal concentration decreased to 0.00105 ppm and 0.00062 ppm, respectively. However, the lowest value of median lethal concentration were obtained after 96 h exposure (0.00041 ppm). Thus, a safe level of exposure for the postlarvae of *M. rosenbergii* was calculated to be 0.0001 ppm.

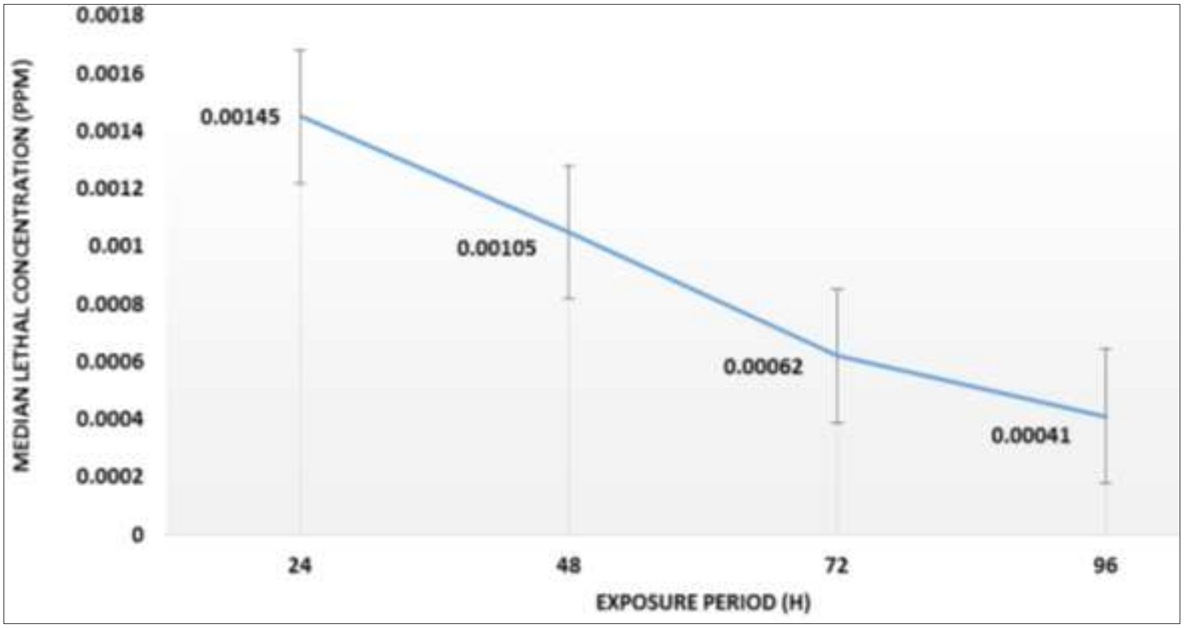

Fig 1: Median lethal concentration of post-larvae of M. rosenbergii exposed to different concentrations of chlorpyrifos for 24, 48, 72, and 96 h.

3.2.2. Acute toxicity of chlorpyrifos in juvenile *M. rosenbergii*

Median lethal concentration of juvenile *M. rosenbergii* exposed to different concentrations of chlorpyrifos, *viz*. 0, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm for 24, 48, 72, and 96 h are given in Fig. 2. Median lethal concentration of juveniles exposed to chlorpyrifos showed a declining trend with increase in exposure period. The median lethal concentration determined by probit analysis were 0.044, 0.035, 0.028 and 0.022 ppm for 24, 48, 72, and 96 h, respectively. Thus, a safe level of exposure for the juveniles of *M. rosenbergii* was calculated as 0.004 ppm.

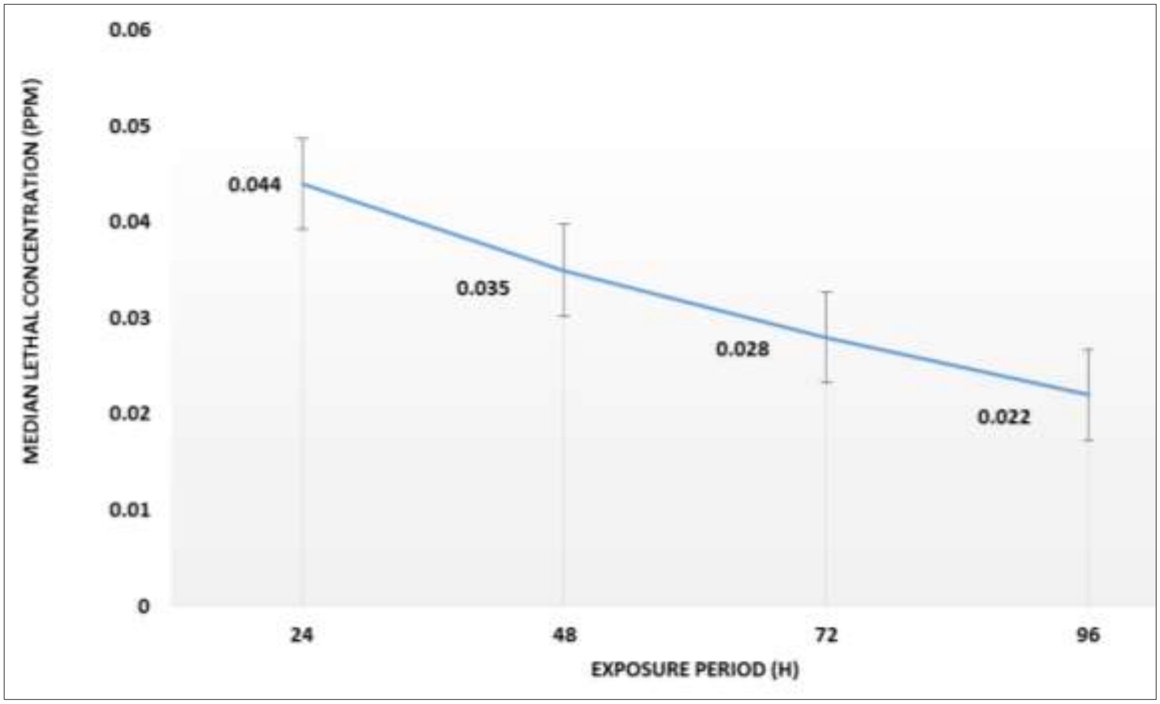

Fig 2: Median lethal concentration of juvenile M. rosenbergii exposed to different concentrations of chlorpyrifos for 24, 48, 72, and 96 h.

3.2.3. Acute toxicity of chlorpyrifos in adult *M. rosenbergii*

Median lethal concentration of adult stage of *M. rosenbergii* exposed to different concentrations of chlorpyrifos, *viz*. 0, 0.02, 0.04, 0.06, 0.08 and 0.10 ppm for 24, 48, 72, and 96 h are given in Fig. 3. Adult *M. rosenbergii* exposed to chlorpyrifos showed highest value of median lethal concentration after 24 h (0.059 ppm) and lowest value after 96 h (0.038 ppm). Whereas, after 48 h and 72 h median lethal concentration determined by probit analysis were 0.052 ppm and 0.043 ppm, respectively. Thus, a safe level of exposure for the adults of *M. rosenbergii* is 0.008 ppm.

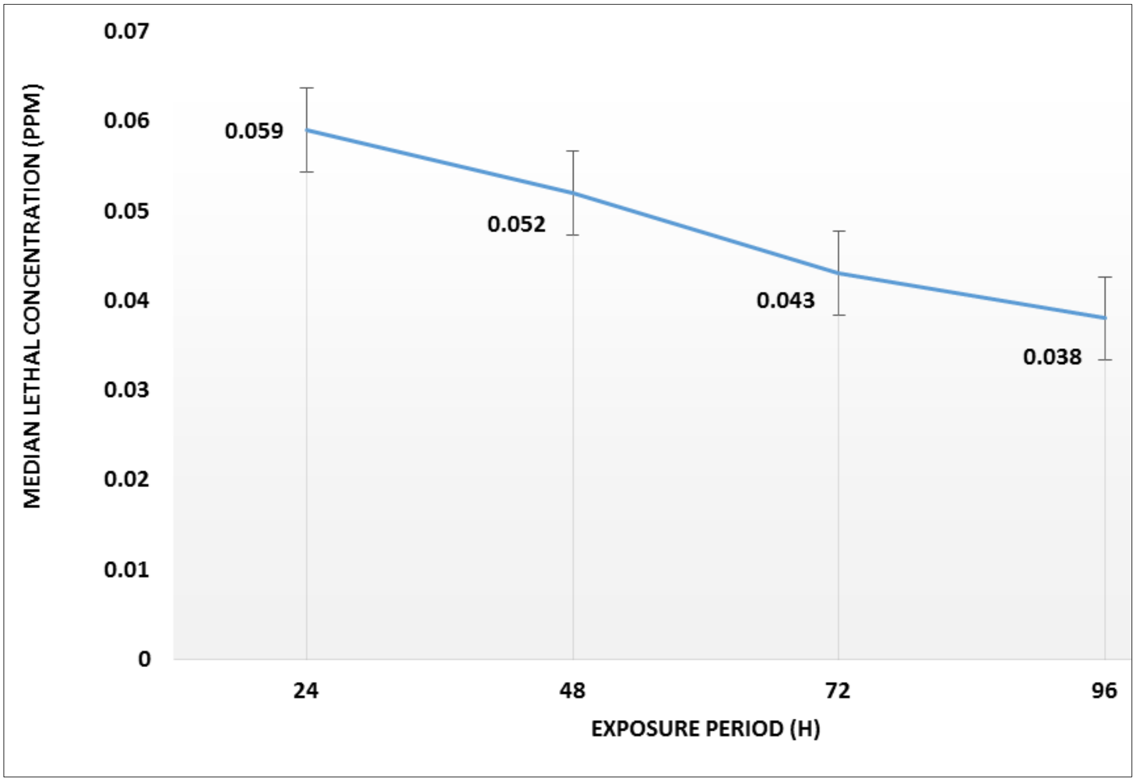

Fig 3: Median lethal concentration of adult M. rosenbergii exposed to different concentrations of chlorpyrifos for 24, 48, 72, and 96 h.

The toxicological impact of chlorpyrifos exposure poses a critical threat to *M. rosenbergii*, known to support a very lucrative fishery in Vembanad Lake adjacent to Kuttanad wetland areas. Since most pesticides are lipophilic in nature, they easily diffuse into cells, thereby causing alterations in the pH of the medium, physico-chemical properties of the cytoplasm, destruction of the membranes of the organelles, disruption of the normal functioning of the cell proteins and inhibition of the actions of the enzymes [52, 53, 54]. At sublethal levels, toxicants can severely impair the physiological functions of organisms, which in turn will terminate the productivity [55]. Responses to organophosphate insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and species [56, 57, 58]. Crabs and shrimps are relatively more sensitive to pesticides than higher aquatic organisms [59]. Acute toxicity is an important parameter for toxicity test, which throw light on whether the changes resulting from exposure is detrimental to aquatic organisms [60]. The consequent estimation of the lethal concentrations (LC_{50}) is a standard practice in aquatic toxicology studies [61, 62].

Comparing toxicity of chlorpyrifos in the different life stages of *M. rosenbergii*, it was found that the sensitivity to chlorpyrifos is related to the developmental stage of the organism. In the present study, it has been found that the postlarvae of *M. rosenbergii* more sensitive to chlorpyrifos than juvenile and adult, as the field application concentration (0.0025 ppm) is higher than the safe concentration determined for post larvae but lower than those of juvenile and adult. The 96 h LC_{50} of the chlorpyrifos for post larvae has been calculated as 0.00041 ppm. This is in agreement with the previous research that the larvae of *M. rosenbergii* are more sensitive than later life stages of the same species or other species as later stages showed higher resistance [63-66]. The sensitivity of an organism to a toxicant can vary depending on its size, age and stage of development [67], because several enzymes may have differential activities along with development or aging [68].

In the early stages of development, the organisms are generally more sensitive due to increased mitotic activity [69], and also possibly their higher surface area to volume ratios, being smaller in size. This variation in sensitivity of different ontogenetic stages has been observed previously in *Macrobrachium* sps., in studies with other pesticides [70], heavy metals [71] and nitrogen compounds such as ammonia and nitrite [64, 72, 73]. Chlorpyrifos is found to induce endocrine disruption, mitochondria dysfunction, oxidative stress and immunodeficiency in various invertebrates [74]. Prawns are highly sensitive to chlorpyrifos when compared with results reported on *Oreochromis mossambicus* [75], *Channa punctatus* [76, 77] and *Cyprinus carpio* [78, 79]. Once the accumulation of the insecticide in hepatopancreas exceeds its capacity to metabolize it the ill effects of insecticide begin to set in. Inhibition of AChE is reported to be accompanied by an increase in acetylcholine (ACh) levels that can be dangerous since it will impact feeding capability, swimming activity, identification and spatial orientation of the organism [80]. Indeed, immobility of the organism exposed to chlorpyrifos makes them easy prey vulnerable to attacks by numerous predators inhabiting the rice-agro ecosystems. In this regard, it must be pointed out that, given the short life cycles of these organisms, the affected populations will certainly vanish in a very short time. Nonetheless, it is hard to reconcile mortality endpoints (LC_{50}) as a reliable predictor of environmental effects of organophosphates, because a paralysis effect takes place at much lower concentrations than those required to cause death. As a consequence, immobilization tests and EC_{50} values are recommended for this class of compounds, while caution should be excised in environmental risk assessment of this and possibly other related organophosphate insecticides with similar activity.

3.2.4. Safe concentration of chlorpyrifos for different life-history stages of *M. rosenbergii*

Fig. 4. Presents the safe level of chlorpyrifos exposure for different life-history stages of *M. rosenbergii*. Safe concentration of chlorpyrifos is related to the developmental stage of the *M. rosenbergii*. The safe level determined by Hart's formula based on the 96 h LC_{50} values were 0.0001 ppm, 0.004 ppm and 0.008 ppm for postlarvae, juvenile and adult, respectively. These results indicate that postlarvae are the most vulnerable to chlorpyrifos toxicity being having the lowest concentration when compared to later life stages. Whereas, adults are the most resistant to chlorpyrifos toxicity being having the highest safe concentration when compared to early life stages.

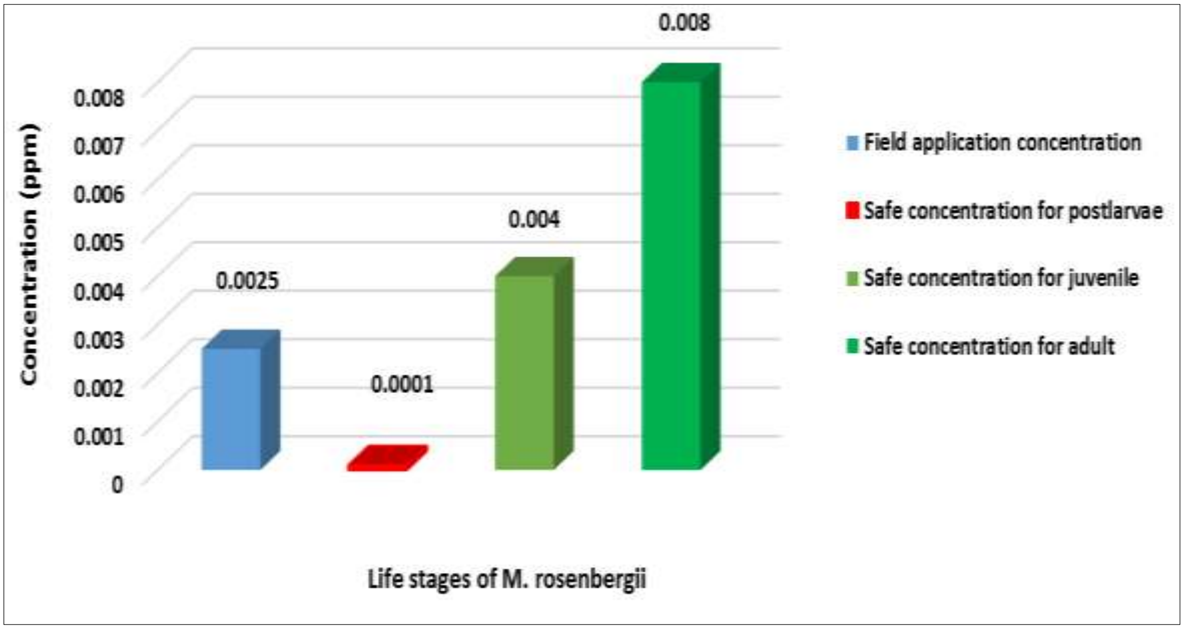

Fig 4: Safe concentration of chlorpyrifos in different life-history stages (postlarvae, juvenile and adult) of M. rosenbergii.

4. Conclusion

Effects of an organophosphate insecticide, chlorpyrifos on the survival of *M. rosenbergii* at various life stages clearly illustrated that the postlarvae of *M. rosenbergii* are more vulnerable to chlorpyrifos than juveniles and adults, as they have the lowest value of 96 h LC_{50} (0.00041 ppm). Indeed, field application concentration (0.0025 ppm) of chlorpyrifos is higher than the safe concentration (0.0001 ppm) estimated for postlarvae. The consequence of lethality caused by chlorpyrifos to postlarvae of *M. rosenbergii* in its natural habitat may end up in the complete devastation of the species in its homeland within a short span. Hence, it is exhorted that chlorpyrifos should be subdued below this level in surface water systems near the agricultural area to restrain the potential menace of chlorpyrifos. As paralysis occurred at concentrations much lower than lethal dose, perhaps a more useful indicator of toxic sequel for chlorpyrifos is the EC_{50} derived from immobilization bioassays. Thus, a safe value determined based on the EC_{50}, used as a toxicity threshold, may be warranted as a promising tool in ecotoxicology, to assess the maximum permissible levels of chlorpyrifos in water bodies and establish better environmental policies and regulations. Further studies on the sublethal effects of chlorpyrifos on *M. rosenbergii* need to be performed to elucidate toxicity pathways that result in the death of the organism.

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