Acute Toxicity of Imidacloprid to Various Life Stages of the Giant Freshwater Prawn *Macrobrachium rosenbergii*, de Man, 1879

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Abstract

The present study was conducted invitro to evaluate the acute toxicity of a broad spectrum neonicotinoid, imidacloprid used by farmers contently to control piercing-sucking pests in the paddy fields of Kuttanad, a part of the Vembanad wetland ecosystem which is a Ramsar site of international importance in the state of Kerala, India, to various life stages of *Macrobrachium rosenbergii*, a commercially important indigenous prawn of Kuttanad. The median tolerance limit (LC$_{50}$) were calculated 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 biocides for a period of 96 h. LC$_{50}$ values for 24, 48, 72 and 96 h of imidacloprid was determined by probit analysis using SPSS version 16.0. The study revealed that the 96 h LC$_{50}$ concentrations for imidacloprid as 0.009, 5.0234 and 42.611 mg/l in post-larvae (0.041 ± 0.02 g), juvenile (3.5 ± 1.5 g) and adult (17.5 ± 2.5 g), respectively of *M. rosenbergii*. Based on the LC$_{50}$ values, safe concentrations found out for imidacloprid using Hart’s formula were 0.002, 1.000 and 15.138 mg/l, in post-larvae, juvenile and adult *M. rosenbergii*, respectively. The field application concentration of imidacloprid is 0.003 mg/l which is 3 times higher than the safe concentration of imidacloprid in post-larvae, whereas it is lower than the safe concentration in juvenile and adult *M. rosenbergii*. The results revealed that the application of imidacloprid in the paddy fields of Kuttanad has a lethal effect on post-larvae of *M. rosenbergii* causing a threat to the existence of species in its homeland.

Keywords: Imidacloprid, acute toxicity, *Macrobrachium rosenbergii*, postlarvae, juvenile, adult.

INTRODUCTION

*Macrobrachium rosenbergii*, the giant freshwater prawn of the Palaemonidae family is a prime species for aquaculture because of its large size, rapid growth and high nutritive value[1]. It is a true denizen of Kuttanad, a part of the Vembanad wetland ecosystem, which is one of the internationally recognized Ramsar sites (location no. 1214) in Kerala, southwest coast of India. The region extends from 9° 17’ to 9° 40’ N latitude and 76° 19’ to 76° 33’ E longitude, comprising an area of 1100 sq. km. 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[2]. A very abundant fishery of *M. rosenbergii* is reported in Vembanad Lake system, its confluent rivers and proximate inundated paddy fields[3]. The postlarvae and juveniles of *M. rosenbergii* have been reported to feed upon rice grains and small worms in the polders adjacent to Vembanad Lake[4, 5]. Its supreme role in international merchandise, earning overseas revenue for India, uplifted its concernment in the inland waters of the nation[3].

However, paddy cultivation has been facing severe agrarian distress for the last five decades owing to a variety of factors[6]. Pesticides act as a vital tool in promoting food productivity necessary to meet the increasing human demand. About 485 tonnes of pesticides are being applied in the paddy fields of Kuttanad every year[7]. Only 0.1 % of the pesticides applied to control the target pests pacify the purpose and the remaining is left to impact the surrounding biota and environment [8]. Imidacloprid (1- (6-chloro-3-pyridyl)methyl) -N-nitroimidazolidin-2-ylideneamime) is a nitroguanidine insecticide that belongs to the neonicotinoid family[9] commonly used in the paddy...
fields of Kuttanad, as a possible alternative for commonly used organophosphorus pesticides. It is a versatile, broad-spectrum insecticide which acts as a contact and stomach poison against sucking insects [10-12] including strains resistant to conventional pesticides. The application of imidacloprid is done as foliar sprays at the first sign of aphid infestation, with a maximum of two applications per season, as its insecticidal activity lasts for about 3 months [13]. As its application is relatively for a short period when compared to other insecticides, it is used in the largest volume world wide [14,15]. Due to its relatively high solubility (0.61 g/l) [16], imidacloprid moves easily from paddy fields to irrigation channels of the field [17] via input from spray drift, leaching, or runoff and eventually reaches the nearby aquatic ecosystem [18] inhabited by the postlarvae and juveniles of Macrobrachium rosenbergii [4], where it is reported to be persistent [19]. The molecule exhibits a novel mode of action as it is an agonist of the nicotinic acetylcholine receptor (nAChR) interfering with neural control and coordination of various physiological activities [20]. Imidacloprid causes muscle cramps, weakness and twitching resulting in impairment of invertebrate movements [21], which inturn can lead to starvation and death via dysfunctional feeding behavior [22] due to paralysis [23]. Within this context, non-target aquatic organisms potentially could be exposed to imidacloprid via water, sediments [24] and food sources [25]. Dissipation time 50% for the water phase (DT50water) values ranged 30 to 150 d and a DT50water value of 90 d could possibly lead to long-term exposure of aquatic ecosystems [26]. Significant effects have been recognized in different trophic levels of the aquatic niche after biomagnification [27].

Toxicity of several pesticides have been studied in Machrobrachium sp., viz. Macrobrachium amazonicum [28], Macrobrachium borellii [29], Macrobrachium lamarrei [30-32], Macrobrachium malcolmsonii [33-35], Macrobrachium naso and Macrobrachium dayanum [36], Macrobrachium nipponense [37-39], Macrobrachium olfersii [40] and M. rosenbergii [41-46]. The toxicity of imidacloprid has been studied in several crustaceans [47-51]. However, no serious attempt has been made on the toxic sequelae attributed to imidacloprid in M. rosenbergii. The present study was conducted to elucidate the susceptibility of the giant freshwater prawn M. rosenbergii at different life-history stages, viz. postlarvae, juvenile and adult to imidacloprid, and thereby determine the median lethal concentration (LC50) and safe concentration of imidacloprid to postlarvae, juvenile and adult stages of M. rosenbergii.

MATERIALS AND METHODS

Animal rearing

The toxicity assessment was carried out on different life stages of M. rosenbergii such as postlarvae, juvenile and adult, procured from Sea View Prawn Hatchery, Thrirprayar, 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. Prawns were acclimatized and starved for 24 h prior to the experimentation.

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 every day. Alkalinity, total ammonia and hardness were determined at the beginning and the end of the experiment [52]. Water quality variables were determined independently for each treatment to establish whether the prawns were maintained at appropriate favorable conditions [53].

Experimental setup

Assessment of the median lethal concentration (LC50) of imidcloprid to various lifestages of M. rosenbergii were done based on the manual of the Environmental Protection Agency USA [54]. 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).

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 of imidcloprid. The concentrations between the No Observable Effect Concentration (NOEC) and Lowest Observable Effect Concentration (LOEC) of imidcloprid were determined by observing the mortality after 96 h and used for the acute toxicity experiments.

Acute toxicity test

Acute toxicity tests were carried out with six different concentrations viz., 0, 0.002, 0.004, 0.006, 0.008 and 0.010 ppm; 0, 2, 4, 6, 8 and 10 ppm; and 0, 20, 40, 60, 80 and 100 ppm Commercial-grade imidacloprid (Bayer CropScience Pty Ltd - Confidor having active ingredient of 17.8% SL), 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 [55].

**Determination of LC$_{50}$ concentration**

Mortality of postlarvae, juvenile and adult stages of *M. rosenbergii* exposed to each concentration viz., 0, 0.002, 0.004, 0.006, 0.008 and 0.010 ppm; 0, 2, 4, 6, 8 and 10 ppm; and 0, 20, 40, 60, 80 and 100 ppm of imidacloprid after 24, 48, 72 and 96 h were recorded and used for estimation of the LC$_{50}$ values by the probit method, using SPSS version 16.0 [56].

**STATISTICAL ANALYSIS**

The median lethal concentration of imidacloprid was calculated by the probit method [56]. Briefly, using different concentrations viz., 0, 0.002, 0.004, 0.006, 0.008 and 0.010 ppm; 0, 2, 4, 6, 8 and 10 ppm; and 0, 20, 40, 60, 80 and 100 ppm of imidacloprid and corresponding mortality data collected after 24, 48, 72 and 96 h of exposure, linear regression were carried out in SPSS version 16.0 to generate a probit (short form of probability unit) list. The LC$_{50}$ values of imidacloprid to postlarvae, juvenile and adult *M. rosenbergii* after 24, 48, 72 and 96 h with 95% confidence intervals were determined by looking up the concentration corresponding with probit of 5.00 (probit for 50% mortality / LC$_{50}$) [56].

**RESULTS**

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

**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 imidacloprid in different life-history stages (postlarvae, juvenile and adult) of *M. rosenbergii***

| Life-history stages | Variables | Concentrations (mg/l) | 0.000 | 0.002 | 0.004 | 0.006 | 0.008 | 0.010 |
|---------------------|-----------|-----------------------|-------|-------|-------|-------|-------|-------|
| Postlarvae          | Temperature (°C) | 27.5±0.5 | 27.5±0.5 | 27.5±0.5 | 27.5±0.5 | 27.5±0.5 | 27.5±0.5 |
|                     | pH        | 7.4±0.1 | 7.4±0.1 | 7.4±0.1 | 7.5±0.1 | 7.5±0.1 | 7.5±0.1 |
|                     | Dissolved oxygen (mg·l$^{-1}$) | 6.8±0.4 | 6.6±0.8 | 6.7±0.7 | 6.7±0.9 | 6.8±0.8 | 6.8±0.6 |
|                     | Hardness (mg·l$^{-1}$ CaCO$_3$) | 28.6±0.8 | 28.4±1.1 | 29.2±1.4 | 29.6±1.2 | 29.4±1.1 | 30.2±0.6 |
|                     | Alkalinity (mg·l$^{-1}$) | 22.7±0.1 | 22.3±0.2 | 21.7±0.2 | 21.4±0.3 | 21.4±0.2 | 20.8±0.4 |
|                     | Total ammonia (mg·l$^{-1}$) | 0.206±0.3 | 0.41±0.2 | 0.49±0.44 | 0.47±0.42 | 0.48±0.36 | 0.46±0.72 |
| Juvenile            | Temperature (°C) | 27.4±0.5 | 27.3±0.5 | 27.5±0.2 | 27.2±0.5 | 27.6±0.4 | 27.1±0.5 |
|                     | pH        | 7.5±0.1 | 7.4±0.1 | 7.4±0.1 | 7.4±0.1 | 7.5±0.1 | 7.6±0.1 |
|                     | Dissolved oxygen (mg·l$^{-1}$) | 6.6±0.4 | 6.2±0.8 | 6.7±0.3 | 6.2±0.7 | 6.8±0.1 | 6.8±0.2 |
|                     | Hardness (mg·l$^{-1}$ CaCO$_3$) | 28.2±0.8 | 28.4±1.2 | 29.3±1.2 | 29.6±1.4 | 29.4±1.3 | 30.1±0.4 |
|                     | Alkalinity (mg·l$^{-1}$) | 22.6±0.1 | 22.4±0.3 | 21.8±0.2 | 21.6±0.2 | 21.6±0.8 | 20.7±0.5 |
|                     | Total ammonia (mg·l$^{-1}$) | 0.208±0.2 | 0.43±0.3 | 0.48±0.47 | 0.47±0.32 | 0.48±0.23 | 0.46±0.92 |
| Adult               | Temperature (°C) | 27.3±0.6 | 27.4±0.5 | 27.5±0.5 | 27.8±0.2 | 27.5±0.3 | 27.6±0.4 |
|                     | pH        | 7.4±0.1 | 7.3±0.1 | 7.4±0.1 | 7.4±0.1 | 7.4±0.1 | 7.5±0.1 |
|                     | Dissolved oxygen (mg·l$^{-1}$) | 6.4±0.4 | 6.6±0.4 | 6.3±0.7 | 6.7±0.3 | 6.2±0.8 | 6.4±0.6 |
|                     | Hardness (mg·l$^{-1}$ CaCO$_3$) | 28.5±0.8 | 28.6±1.2 | 29.1±0.8 | 29.7±1.5 | 29.9±0.1 | 30.2±0.6 |
|                     | Alkalinity (mg·l$^{-1}$) | 23.1±0.1 | 22.6±0.2 | 21.8±0.2 | 21.4±0.8 | 21.4±0.2 | 20.9±0.3 |
|                     | Total ammonia (mg·l$^{-1}$) | 0.207±0.3 | 0.38±0.2 | 0.47±0.34 | 0.47±0.72 | 0.48±0.16 | 0.47±0.82 |
Acute toxicity of imidacloprid to \textit{M. rosenbergii}

The 24, 48, 72 and 96 h LC$_{50}$ concentrations of imidacloprid in different life-history stages of \textit{M. rosenbergii} is shown in Table 2. With an increase in time period, there was a decline in the LC$_{50}$ value viz., 0.046 to 0.009, 7.362 to 5.031 and 88.104 to 42.611 mg/l, respectively for post larvae, juvenile and adult \textit{M. rosenbergii}. The 96 h LC$_{50}$ values indicate that postlarvae are the most vulnerable to imidacloprid toxicity being having the lowest concentration for lethality when compared to later life stages. Whereas, adults are the most resistant to imidacloprid toxicity being having the highest concentration for lethality when compared to early life stages. In all life stages examined, the mortality of prawns exposed to imidacloprid surged with that of exposure period and concentration. Mortality observed for control \textit{M. rosenbergii} (not exposed to imidacloprid) 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.

| Exposure period (h) | LC$_{50}$ values (mg/l) in different life-history stages of \textit{M. rosenbergii}. |
|---------------------|----------------------------------------------------------------------------------|
|                     | Postlarvae | Juvenile | Adult |
| 24                  | 0.046      | 7.362    | 88.104 |
| 48                  | 0.025      | 6.471    | 83.752 |
| 72                  | 0.015      | 5.445    | 63.679 |
| 96                  | 0.009      | 5.023    | 42.611 |

Acute toxicity of imidacloprid in postlarvae of \textit{M. rosenbergii}

Percentage mortality of post-larvae of \textit{M. rosenbergii} exposed to different concentrations of Imidacloprid for 24, 48, 72, and 96 h are given in Fig. 1. Postlarvae of \textit{M. rosenbergii} exposed to 0.002 mg/l imidacloprid showed no mortality throughout the experimental period. Whereas 10% and 40% mortality were observed, respectively at 0.004 mg/l and 0.008 mg/l of imidacloprid after 96 h. At a higher concentration, 0.010 mg/l of imidacloprid, mean mortality was 60% after 96 h. However, when comparing the relationship between mortality and imidacloprid concentration by the probit method and regression analysis, the lethal concentration for 50% of the prawns after 96 h was determined as 0.009 mg/l with 95% confidence intervals 0.005-0.017 mg/l. Thus, a safe level of exposure for the postlarvae of \textit{M. rosenbergii} was calculated to be 0.002 mg/l.

![Fig-1: Mean cumulative mortality (%) of post-larvae of \textit{M. rosenbergii} exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h.](image)

Acute toxicity of imidacloprid in juvenile \textit{M. rosenbergii}

Mean cumulative mortality (%) of juvenile \textit{M. rosenbergii} exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h are given in Fig. 2. Juveniles of \textit{M. rosenbergii} exposed to 2 mg/l imidacloprid showed no mortality up to 48 h but showed 10% and 20% mortality, respectively after 72 and 96 h. After 96 h, juveniles exposed to imidacloprid showed 40% mean mortality at concentration 4 mg/l.
and 60% mortality at concentration 6 mg/l. 100% of mortality was observed at 10 mg/l after 24 h. The median lethal concentration by the probit method and regression analysis, after 96 h was determined as 5.031 mg/l with 95% confidence intervals 3.488-6.945 mg/l. Thus, a safe level of exposure for the juveniles of *M. rosenbergii* was calculated as 1.000 mg/l.

**Acute toxicity of imidacloprid in adult *M. rosenbergii***

Mean cumulative mortality (%) of adult *M. rosenbergii* exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h are given in Fig. 3. Adult *M. rosenbergii* exposed to 20, 40 and 60 mg/l imidacloprid showed 20%, 45% and 60% mortality, respectively after 96 h. However, 80% and 100% mortality were observed, respectively at 80 mg/l and 100 mg/l of imidacloprid after 96 h. The concentration for 50% mortality of the prawns after 96 h was determined as 42.6579 mg/l with 95% confidence intervals 29.643-55.402 mg/l by probit analysis. Thus, a safe level of exposure for the adults of *M. rosenbergii* is 15.138 mg/l.

![Mean cumulative mortality (%) of juvenile *M. rosenbergii* exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h](image1)

**Fig-2: Mean cumulative mortality (%) of juvenile *M. rosenbergii* exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h.**

![Mean cumulative mortality (%) of adult *M. rosenbergii* exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h](image2)

**Fig-3: Mean cumulative mortality (%) of adult *M. rosenbergii* exposed to different concentrations of imidacloprid for 24, 48, 72, and 96 h.**
DISCUSSION

Water quality parameters
Water quality parameters were maintained within the optimal range reported for *M. rosenbergii* [58]. 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 imidacloprid.

Acute toxicity of imidacloprid to *M. rosenbergii*
Acute toxicity assessment of imidacloprid to various life stages of the giant freshwater prawn, *M. rosenbergii* revealed that the postlarvae of *M. rosenbergii* are susceptible to imidacloprid, which is said to be used to control aphids, whiteflies and leafhoppers affecting paddy, cotton, chilly, okra and ground nut [59]. Hence the widespread use of imidacloprid in the paddy fields of Kuttanad poses a critical threat to postlarvae of *M. rosenbergii*, a prime candidate for the freshwater aquaculture in Kuttanad. Acute toxicity is an important parameter for toxicity test, which throw light on whether the changes resulting from exposure is detrimental to aquatic organisms [57]. The consequent estimation of median lethal concentration (LC50) is a standard practice in aquatic toxicology studies [60, 61].

Comparing toxicity of imidacloprid in the different life stages of *M. rosenbergii*, it was found that the sensitivity to imidacloprid is related to the developmental stage of the organism. In the present study, it has been found that the postlarvae of *M. rosenbergii* are more sensitive to imidacloprid than juvenile and adult, as the field application concentration (0.003 mg/l) is higher than the safe concentration determined for postlarvae (0.002 mg/l), but lower than those of juvenile (1.000 mg/l) and adult (15.138 mg/l). The safe concentration found out for postlarvae of *M. rosenbergii* in this study corroborate the idea of EFSA, 2008, which suggested the maximum permissible concentration between 0.0002 and 0.019 mg/l. The 96 h LC50 of imidacloprid for the postlarvae has been calculated as 0.009 mg/l. This is in agreement with the previous research that the postlarvae are more sensitive than later life stages of *M. rosenbergii* [62, 63] or other *Macrobrachium* species [64, 65] as later stages developed resistance. The susceptibility of an organism to a toxicant can differ depending on its size, age and developmental stage [66], because several enzymes may have differential activities along with development or aging [67]. Lipophilic nature of imidacloprid helps them to easily diffuse into cells [68], thereby causing alterations in the physico-chemical properties and pH of the cytoplasm, destruction of the membranes of the organelles, disruption of the normal functioning of the cell proteins [69] and alteration in activity of enzymes viz. acetylcholinesterase, acid phosphatase, alkaline phosphatase, cytochrome oxidase, glutamic oxalic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, protease and succinic dehydrogenase [70]. Imidacloprid is found to reduce the food availability and quality for postlarvae of *M. rosenbergii* by accumulating in green algae: Desmodesmus subspicatus [71], Desmodesmus sp [72], diatoms: Gomphonema gracile, Planodithium lanceolatum [72]; chironomids: Chironomus riparius [73], 74], Chironomus tentans [11], Chironomus dilutes [75]; amphipods: Hyalella Azteca [11, 76, 77], Gammarus fossarum [72], Gammarus pulex [78], Gammarus roeseli [79] and other small crustaceans: Daphnia magna [80-82], Ceriodaphnia dubia [76]. Thus the bioaccumulative nature of imidacloprid in food webs may be exacerbated and lead to biomagnification between the lower and upper trophic levels [83]. Even at sublethal levels, toxicants are found to severely impair the reproductive capacity of *Macrobrachium* sp. by disrupting germinal epithelium and thereby spermatogenesis and oogenesis [36].

In the early stages of development, the organisms are generally more sensitive due to increased mitotic activity [84], and also possibly their higher ratios of surface area to volume, being small sized. This variation in susceptibility of various ontogenetic stages has been observed in *Macrobrachium* spp., exposed to other pesticides[85], heavy metals [86] and nitrogen compounds such as ammonia [87] and nitrite [88]. Imidacloprid is found to induce endocrine turmoil [89], impairment of mitochondria [90], oxidative desecration [91], and immunological disorders [92] in various invertebrates. 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 paralysis, as it leads to starvation due to feeding incapability, swimming disability, difficulties in identification and spatial orientation of the organism [93] and almost surely, will not be able to reproduce [94]. Indeed, insensitivity of the organism makes them defenceless to predator attacks in the rice-agro ecosystems.

Postlarvae of *M. rosenbergii* are highly sensitive to imidacloprid when compared with results reported on juvenile *Labeo rohita* [95] and common carp larvae [96]. The reason behind the higher toxicity of imidacloprid to invertebrates than those of vertebrates is due to the mode of action of imidacloprid. Imidacloprid functions by disrupting the nicotinic acetylcholine receptors (nACHR) in the central nervous system of insects. The vertebrates’ nACHRs differ from those in insects’, and hence exert less toxicity in vertebrates than invertebrates. In this regard, it must be pointed out that, given the short life cycles of *M. rosenbergii*, the affected populations will certainly diminish in a very short time [29].

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CONCLUSION

Acute toxicity assessment of imidacloprid to various life stages of the giant freshwater prawn, *M. rosenbergii* highlights that the postlarvae of *M. rosenbergii* have the lowest value of 96 h LC₅₀ (0.009 mg/l) when compared to that of juveniles and adults. Indeed, field application concentration (0.003 mg/l) of imidacloprid is higher than the safe concentration (0.002 mg/l) estimated for postlarvae. Thus postlarvae of *M. rosenbergii* are susceptible to imidacloprid in its habitat. Although, juveniles and adults are not susceptible to imidacloprid in its ecosystem, being having a safe concentration (1.000 mg/l and 15.138 mg/l for juveniles and adults, respectively) higher than the field application concentration, the lethal effect of imidacloprid to postlarvae of *M. rosenbergii* poses a threat to the existence of the species in its homeland. Hence, the present study reveals that imidacloprid should be maintained below safe concentration in the agricultural area to suppress the potential hazard caused by imidacloprid to the species in the natural ecosystem. Immediate attention of regulators, who currently assess the environmental impact of this toxicant is warranted. Further studies on the sublethal effects of imidacloprid on *M. rosenbergii* need to be performed to elucidate toxicity pathways that result in the death of the organism.

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