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Assessment of Larval Health and Metamorphosis of the Giant Freshwater Prawn *Macrobrachium rosenbergii* Larvae during Acute Exposure to Imidacloprid

Stephy Rose K. V.[1,2], Sruthy C. Sunil[2], Niladri Sekhar Chatterjee[1] and Anandan Rangasamy[1]

[1]ICAR-Central Institute of Fisheries Technology, Matsyapuri, Cochin, Kerala (682 029), India
[2]Faculty of Marine Sciences, Cochin University of Science and Technology, Cochin, Kerala (682 016), India

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Corresponding kranandan@rediffmail.com

0000-0003-0759-2064

ABSTRACT

An acute toxicity study of imidacloprid on the larval health and metamorphosis of the giant freshwater prawn *Macrobrachium rosenbergii* larvae were carried out during the months of January–August, 2021 at the aquatic animal raring and experimenting facility in the Cochin University of Science and Technology, Kerala, India. Imidacloprid is a broad-spectrum neonicotinoid insecticide used against sucking pests infecting agricultural crops as both contact poison and stomach poison. The present study investigated the adverse effects of imidacloprid on the survival rate, feeding behaviour, larval quality and incidence of metamorphosis in *Macrobrachium rosenbergii* larvae. The 48 h median lethal concentrations (LC_{50} values) and safe concentration of imidacloprid determined for different larval stages (Zoea I–XI) of *M. rosenbergii* ranged between 0.000635–0.001388 mg l^{-1} and 0.000055–0.000191 mg l^{-1}, respectively. Larvae exposed to imidacloprid for 48 h showed decrease in survival rate with increase in concentration of imidacloprid. The results of larval condition index (LCI) that indicates the larval quality showed that imidacloprid reduced the larval health significantly (p<0.05) at high concentrations and is potent to delay the incidence of metamorphosis. The feeding rate of larvae exposed to different concentrations of imidacloprid were found to be significantly (p<0.05) reduced due to paralytic effect of imidacloprid. The present work provides relevant data beneficial for the risk assessment of imidacloprid in *M. rosenbergii*.

KEYWORDS: Acute toxicity, feeding, imidacloprid, *M. rosenbergii*, metamorphosis, survival

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1. INTRODUCTION

Macrobrachium rosenbergii, is an economically important, extensively farmed crustacean with excellent demand in markets (Rose et al., 2022). It emerged as the most valuable species in the inland waters of Kerala by virtue of being an important earner of foreign exchange (Kurup and Harikrishnan, 2000). It is an indigenous species of Kuttanad (Rose and Joseph, 2020), a part of the Vembanad wetland ecosystem that is considered as an important Ramsar site in Kerala. Kuttanad is traditionally known as the 'rice bowl of Kerala' with an estimated total area of 1,10,000 ha. 55,000 ha of the land in Kuttanad is used for paddy cultivation where pesticides are used indiscriminately, resulting in serious ecological imbalance (Kumar et al., 2013).

Pesticides protect crops from pest attack and improves agricultural productivity (Mahmood et al., 2016). 99.9% of the pesticides used in the field seeps into different components of the environment (Jin et al., 2016). Imidacloprid is a potent, broad-spectrum, nitromethylene insecticide of the neonicotinoid family (Jeschke et al., 2010) widely used against several species of Coleoptera, Diptera and Lepidoptera (Bass et al., 2015) infecting chilly, cotton, grapes, groundnut, okra, paddy, sugarcane, sunflower and tomato (Anonymous, 2020). Imidacloprid contaminate surface and ground waters and gets easily transported into estuaries (Van Dijk et al., 2013, Butcherine et al., 2019) where *M. rosenbergii* spawns and completes the larval phase of its life cycle (Ling, 1969). It is generally persistent in water (Rose and Joseph, 2019) and not easily degradable (Sharma and Singh, 2014). Imidacloprid derive their toxicity by acting agonistically on post-synaptic nicotinic acetylcholine receptors (nAChRs) (Tomizawa and Yamamoto, 1992, Buckingham et al., 1997, Matsuda et al., 2001) interfering feeding behavior leading to paralysis (Schmuck et al., 2003), starvation and death (Alexander et al., 2007). Within this context, non-target aquatic organisms including the larvae of *M. rosenbergii* are at higher risk of imidacloprid exposure via water, sediments (Cox et al., 1998) and food sources (Anonymous, 1995). Thus, imidacloprid gets biomagnified within different trophic levels of the aquatic niche (Amiard et al., 1980).

Drobne et al., 2008 pointed out the differential response to imidacloprid by different species. Therefore, the toxic effects caused by imidacloprid may not be generalized. There are several reports showing toxic effect of imidacloprid in crustaceans (Blazic et al., 2005, Sánchez-Bayo and Goka, 2006, Drobne et al., 2008, Chen et al., 2010, Lukancic et al., 2010). Toxicity of several pesticides also have been studied in *Machrobrachium* sp., viz. *Macrobrachium amazonicum* (Dutra et al., 2017), *Macrobrachium nipponense* (Yuan et al., 2004, Qiu et al., 2013, Hong et al., 2018), *Macrobrachium olfersii* (Barbieri et al., 2016), and *M. rosenbergii* (Chang et al., 2013, Gaume et al., 2015, Lafontaine et al., 2016). Acute toxicity assessment of imidacloprid to various life stages viz. postlarvae, juvenile and adult stages of *M. rosenbergii* also have been done by Rose and Joseph (2020).

Although there are toxicity studies of several pollutants in larval stages of *Machrobrachium* sp., viz. *Macrobrachium amazonicum* (Hayd et al., 2014, Dutra et al., 2016), *Macrobrachium carcinus* (Gomez et al., 2016), *Macrobrachium malcolmsonii* (Arun and Subramanian, 1998), and *M. rosenbergii* (Piyan et al., 1985, Cavalli, 2000, Mallasen and Valenti, 2005 and 2006, Shanker, 2014, Tavabe et al., 2015, Rafiee et al., 2015), a knowledge gap still exists about the toxic sequel attributed to imidacloprid in the larval stages of *M. rosenbergii*. Hence, this study aimed to elucidate the vulnerability of *M. rosenbergii* larvae, and determine the LC_{50} concentration, safe concentration, feeding inhibition, survival rate, metamorphosis and larval condition index (LCI) to each larval stage of *M. rosenbergii*.

2. MATERIALS AND METHODS

The experimental work was conducted during January-August 2021 at aquatic animal rearing and experimentation facility at Cochin University of Science and Technology, Kerala, India, with larvae of *M. rosenbergii* procured from Regional shrimp hatchery, Kodungallur, Kerala, India. Experiments were done using 540 larvae of each stage (zoea I–XI), randomly divided into six groups (control and 5 test concentrations) of 10 larvae, each exposed in four replicates in 24 experimental units (10 larvae experimental unit^{-1}). The units consisted of 500 ml glass beakers with 100 ml of test solution prepared in brackish water (14 ppt), according to Anonymous (2005). Experimental units were equipped with gentle aeration systems and a natural photoperiod of 12 h 12 h^{-1} (light dark^{-1}).

2.1. Analysis of water quality

The water quality parameters such as temperature and pH were measured twice a day. Dissolved oxygen, alkalinity, total ammonia and hardness were determined at the beginning and the end of the experiment (Anonymous, 1975).

2.2. Determination of LC_{50} concentration

Assessment of the median lethal concentration (LC_{50}) were done based on the methodology adapted from Mallasen and Valenti (2006) with six different concentrations of Commercial-grade imidacloprid (Bayer CropScience Pty Ltd - Confidor having active ingredient of 17.8% SL), viz. 0, 0.0002, 0.0004, 0.0006, 0.0008, 0.001 mg l^{-1}. Test solutions were renewed every 24 h to maintain even concentration

of toxicants and to avoid oxygen depletion. Larvae were observed every 1 h for the first 8 h and every 6 h between 8 h and 48 h (Armstrong et al., 1976). Mortality of larvae at different stages viz., zoea I-XI exposed to each concentration of imidacloprid after 24 and 48 h were recorded and used for estimation of the LC_{50} values using SPSS version 16.0.

2.3. Determination of safe concentration

24 and 48h LC_{50} values of *M. rosenbergii* larvae at different stages viz., zoea I–XI exposed to imidacloprid were used for the estimation of safe concentration. Safe concentration of imidacloprid to *M. rosenbergii* larvae at different stages viz., zoea I–XI were calculated by the method described by Hart et al. (1945).

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

Where, $$S=\frac{24\text{h } LC_{50}}{48\text{h}LC_{50}} \quad \text{.....(1)}$$

2.4. Estimation of survival rate

The survival rate was estimated by the method adapted from Krebs (1999). Briefly, the number of dead larvae at different stages viz., zoea I-XI exposed to different concentrations of imidacloprid during acute toxicity test was recorded after 48h for the estimation of survival rate (S) using equations 2.

$$S=N_t.N_0^{-1} \quad \text{..... (2)}$$

Where, N_t=Number of live larvae at time t, N_0=Number of live larvae in the beginning of the experiment, *ln*=Natural logarithm, *T*=Exposure time in days.

2.5. Feeding inhibition tests

Feeding inhibition tests at 6, 12, 18 and 24h were executed based on the methodology adapted from Allen et al. (1995). Experiments were carried out simultaneously with 600 larvae, viz. zoea I–XI exposed to 1000 ml of 5 different conc. of imidacloprid and control in 1000 ml beaker for 24 h. After every 6 h, 10 larvae from each concentration were randomly transferred to 10 units of 50 ml test tubes, each containing 10 ml 14 ppt saline water and 20 Artemia nauplii (instar-I). The experimental set up were left covered with a black plastic sheet to prevent disturbance from external stimuli. Larvae were allowed to feed on artemia nauplii for a period of 4h. At the end of the feeding period, Artemia remained unfed in tubes were preserved with Lugol's solution and left to settle down for at least half an hour. After this, Artemia were transferred to the sedgewick rafter counting chamber using a glass dropper, counted using a microscope (Labomed LX-400 Binocular Microscope, Los Angeles, U.S.A), at low power (10X) and feeding rate of a single animal (F) were calculated using simplified Gauld's equation (Allen et al., 1995) (Eq.3.).

$$F=\frac{C_0-C_t}{t} \quad \text{..... (3)}$$

Where, C_0=initial amount of food (20 Artemia), C_t=final amount of food (remaining Artemia),

T=Time animals were allowed to feed (4 h).

2.6. Assessment of larval health

Larval condition index (LCI) were estimated by the method described by Tayamen and Brown (1999) and Maciel and Valenti (2014) for each larval stage, viz. zoea I–XI after 48 h acute toxicity test. Larvae survived after 48h acute exposure to different concentrations of imidacloprid were observed in cavity slides with water. Fullness of gut, lipid content of hepatopancreas, setation, distribution of chromatophores and abdominal colouration, width of gut and muscle, transparency of abdominal muscles, presence of necrosis, severity of infection by fouling organisms and swimming behaviour were scored as poor (p=0), satisfactory (p=1) or excellent (p=2) and larval condition index (LCI) were calculated using equation 4.

$$LCI=(\sum P)\ 10\ N\text{-}1 \quad \text{.....(4)}$$

Where, P=the score recorded for each larvae, N=number of larvae examined.

2.7. Assessment of metamorphosis

Metamorphosis of the larvae exposed to different concentrations of imidacloprid for 48 h were studied by checking the larval stage. Briefly, larvae survived after 48h acute exposure to different concentrations of imidacloprid were observed in glass slides under a compound microscope (Leica DM500) by the method described by Uno & Kwon (1969) and the result was expressed as percentage of larvae metamorphosed.

2.8. Statistical analysis

The median lethal concentrations of imidacloprid were calculated by the probit method (Finney, 1952) using SPSS version 16.0. LCI, feeding rate, survival rate and coefficient of mortality were analysed by analysis of variance (one-way ANOVA) and significant differences among the means were found by Duncan's test in SPSS version 16.0.

3. RESULTS AND DISCUSSION

3.1. Effect of imidacloprid on water quality

During the experiment, water temperature ranged from 27 to 28 °C, pH from 7.2 to 8.1, dissolved oxygen from 5.8 to 7.4 mg l^{-1}, hardness from 27.3 to 30.6 mg l^{-1}, alkalinity from 20.4 to 23 mg l^{-1} $CaCO_3$ and Total ammonia from 0.23 to 0.48 mg l^{-1}. As the water quality parameters remained within the optimal range for the growth and survival of *M. rosenbergii* larvae in comparison with the reports of Boyd and Zimmermann (2000), the lethality observed could be attributed to that of imidacloprid toxicity.

3.2. Determination of LC_{50} concentration

Acute toxicity study of imidacloprid to *M. rosenbergii* larvae at different stages viz., zoea I–XI revealed the 24 h and 48 h LC_{50} values (Figure 1). Mortality occurred predominantly after 18 h of exposure to imidacloprid. Larvae exposed to high concentrations of imidacloprid exhibited pale body coloration and reduced motility. LC_{50} values ranged between 0.000635–0.001388 mg l^{-1} and showed a decline pattern with an increase in exposure time in all larval stages. Results revealed that the susceptibility to imidacloprid varies with the larval stage of the organism. This is in agreement with the previous studies in *Macrobrachium* sp., with other pesticides (Dai et al., 2014), heavy metals (Asih et al., 2013) and nitrogenous compounds (Lin et al., 1993, Mallasen and Valenti, 2005, Mallasen and Valenti, 2006). In this study, early zoeal stages were found more vulnerable to imidacloprid than the later stages. This has been observed previously in *Macrobrachium* sp. by Armstrong et al. (1976), Mallasen and Valenti (2005), Figueroa-Lucero et al. (2012), Dutra et al. (2016) and Rose and Joseph (2020). Acute toxicity is considered as a vital parameter in aquatic toxicology studies, which throw light on the detrimentality of a toxicant to aquatic organisms (Ruparelia et al., 1984, Anonymous, 1985, Greenberg et al., 1992).

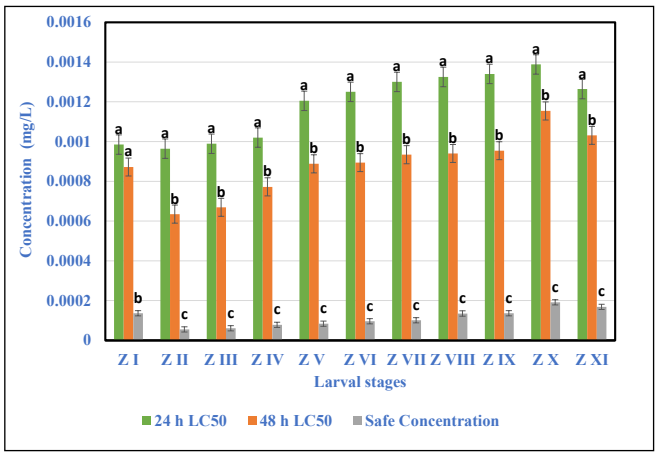

Figure 1: LC_{50} values and Safe concentration of imidcloprid to different larval stages (zoea I–XI) of *M. rosenbergii*. Bars with different superscripts in each larval stage showed significant difference in values (p<0.05).

3.3. Determination of safe concentration

The safe concentration of imidcloprid to the larvae of *M. rosenbergii* at different stages viz., zoea I–XI are given in Figure 1.

The safe concentrations determined from 24 h and 48 h LC_{50} values ranged between 0.000055–0.000191 mg l^{-1} with the lowest value at zoeal stage II and highest value at zoeal stage X. The field application concentration (0.003 mg L^{-1}) of imidacloprid is higher than the safe concentration estimated for the larvae of *M. rosenbergii*. Thus, application of imidacloprid in paddy fields of Kuttanad contributes to the factors diminishing the existence of the species in its homeland.

3.4. Estimation of survival rate

Exposure to imidacloprid had a significant effect on the survival rate of *M. rosenbergii* larvae (p<0.05). A negative correlation was observed between survival rate and the concentration of imidacloprid. Either, survival rate decreased with the increase in concentration of imidacloprid in all larval stages. Earlier larval stages showed lower survival rate when compared to that of later larval stages. Larvae of all zoeal stages in control group (not exposed to imidacloprid) exhibited the highest value of survival rate (1) whereas the survival rate were less than or equal to 0.4 at the highest exposure concentration (0.001 mg l^{-1}) in most larval stages. The lowest survival rate observed were 0.1 in the third larval stage at imidacloprid concentration 0.001 mg l^{-1}. Significant difference in survival rates (p<0.05) observed between imidacloprid exposure concentrations at each larval stage is denoted with different superscripts in figure 2.

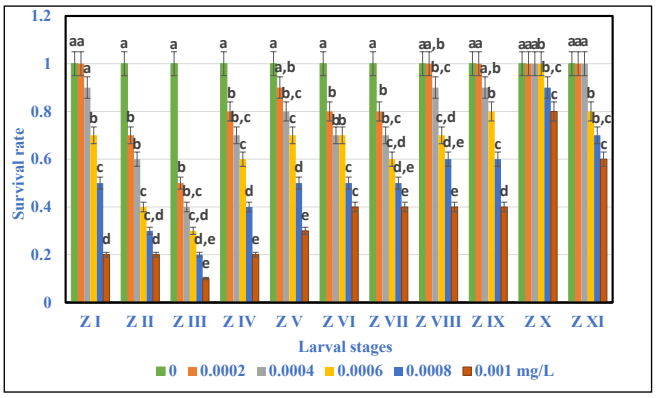

Figure 2: Survival rate of different larval stages of *M. rosenbergii* after 48 h exposure to different concentrations of imidacloprid. Bars with different superscripts in each larval stage showed significant difference in values (p<0.05).

These results are in agreement with previous studies in *Macrobrachium* sp. by Mallasen and Valenti (2005), Gomes et al. (2016) and Rose and Joseph (2020). The greater sensitivity to toxicants during early larval stages has been attributed to the differential enzyme activities during the development of the larvae which in turn influence the uptake and metabolism of imidacloprid (Barbieri et al., 2002).

3.5. Assessment of larval health

Results of Larval Condition Index (LCI) of *M. rosenbergii* larvae (zoea I–XI) exposed to different concentrations of imidacloprid for 48 h indicated that imidacloprid is potent to reduce the larval quality within a short period of time (Figure 3).

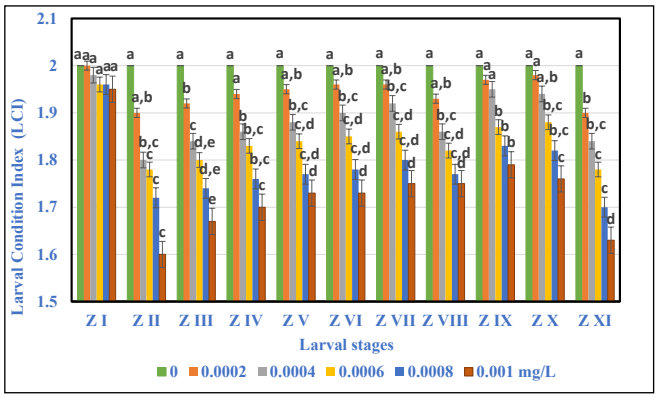

Figure 3: Larval Condition Index of *M. rosenbergii* larvae (zoea I–XI) exposed to different concentrations of imidacloprid. Bars with different superscripts in each larval stage showed significant difference in values (p<0.05)

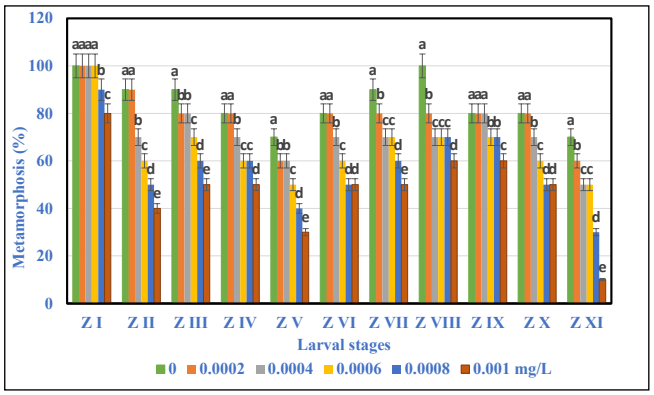

Figure 4: Metamorphosis of *M. rosenbergii* larvae (zoea I–XI) exposed to different concentrations of imidacloprid. Bars with different superscripts in each larval stage showed significant difference in values (p<0.05)

All larval stages except the first zoeal stage showed drastic reduction in larval quality with a minimum LCI value of 1.6 at the highest concentration of 0.001 mg l^{-1} for Z II larval stage. There were no significant reduction in larval quality at first zoeal stage even at the highest concentration of imidacloprid. This inconspicuous reduction may be due to the minimum interaction of larvae (Z I) with the external environment as they completely depend on the food stored in their yolk sac for energy. Larvae of *M. rosenbergii* starts feeding form the environment only from Z II stage. Z II stage was found most vulnerable to imidacloprid in this study being the earliest life stage to interact with the surroundings (Mallasen and Valenti, 2005). The potency of imidacloprid to decrease the LCI values were diminishing as the larval stage progresses. Similar results were also reported by Rafiee et al. (2015) and Tavabe et al. (2015) in *M. rosenbergii* during stress.

3.6. Assessment of metamorphosis

Study on the metamorphosis of the *M. rosenbergii* larvae (zoea I–XI) exposed to different concentrations of imidacloprid for 48 h revealed that imidacloprid is potent to delay the metamorphosis (Figure 4).

Influence of imidacloprid in the incidence of metamorphosis varied with the concentration of imidacloprid and larval stage of the organism. Percentage of larvae metamorphosed was found to significantly (p<0.05) decrease with the increase in concentration. Eleventh zoeal stage showed the highest sensitivity to imidacloprid with the lowest percentage of metamorphosis. Only 10% of the Z XI larvae exposed to 0.001 mg l^{-1} of imidacloprid metamorphosed to postlarvae after 48h. Lowest sensitivity to imidacloprid was observed in first larval stage with no significant change in metamorphosis percentage at concentrations 0.0002, 0.0004 and 0.0006 mg l^{-1}, and only 5–10% decrease even at higher concentrations. This insensitiveness is associated with the limited interactions with the habitat (Mallasen and Valenti, 2005). Environmental conditions play a major role in channeling energy gained through food for growth and metabolism of the larvae (Dawirs, 1983). Previous studies (Sastry, 1983, Mallasen and Valenti, 2006) have reported the delay in metamorphosis as an indicator of pollutants in the aquatic system.

The delay in larval development, as observed in the present work for *M. rosenbergii*, is a generalized response of decapod larvae exposed to some type of pollutant (Sastry, 1983). Probably, *M. rosenbergii* larvae kept in high nitrite concentration (≥4 mg l^{-1} NO2-N) allocated energy to adjust physiological mechanisms against the toxic effect, thus reducing weight gain and delaying the metamorphosis.

3.7. Feeding inhibition tests

The feeding rate of *M. rosenbergii* larvae (zoea I–XI) exposed to different concentrations of imidacloprid varied over the different concentrations of imidacloprid and larval stages (Figure 5). In the control group, the feeding rate increased gradually from 0.5 to 4 as the larval stage progressed. However, the consumption of artemia nauplii was reduced significantly in all larval stages after exposure to imidacloprid (p=0.001). The first larval stage were excluded from this study as the larvae of *M. rosenbergii* starts feeding only from Z II stage. The Z II stage showed the highest sensitivity with the lowest feeding rate after imidacloprid exposure. The feeding was completely inhibited for Z II even at concentrations 0.0006 mg l^{-1} and above. Although the feeding was not completely inhibited in later stages, there were significant reduction in the feeding rate with the increase of imidacloprid concentration. This study corroborate the results of Satapornvanit et al. (2009). The decline in feeding is due to the inability of the organism to locate food and lack of muscular movement control to conquer the prey. This situation is because of the action

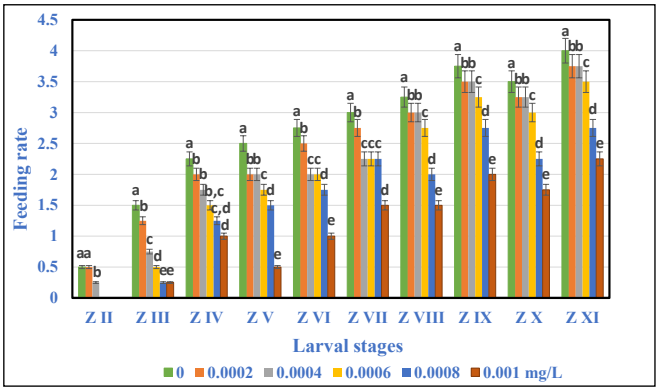

Figure 5: Feeding rate (F) of different larval stages of *M. rosenbergii* after 24 h exposure to different concentrations of imidacloprid. Bars with different superscripts in each larval stage showed significant difference in values (p<0.05)

of imidacloprid on the nervous system that impaired the neuronal coordination of the larvae (Schmuck et al., 2003).

4. CONCLUSION

The results of the present study have shown that imidacloprid is potent to cause significant reduction in the survival rates, feeding rates and larval condition index with substantiate delay in the metamorphosis of *M. rosenbergii* larvae. The present observation reflects the potent threat caused by imidacloprid to the species. Hence, the current study suggests that the concentration of imidacloprid must be kept below safe level in the farm lands to reduce the imidacloprid induced toxicity in *M. rosenbergii* larvae.

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