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Effect of Insecticide Containing Deltamethrin on Immune Response of the Giant Freshwater Prawn, *Macrobrachium rosenbergii* (De Man 1879)

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Abstract

Study on natural immune responses of freshwater prawn, *Macrobrachium rosenbergii* (De Man 1879) exposed to deltamethrin in laboratory condition was performed. Prawns (17±2.3 g) were maintained in water containing concentrations of deltamethrin at 0.0025; 0.025 and 0.125 $mg{\cdot}L^{-1}$. Prawns in control group were maintained in water without deltamethrin. Haemolymph samples from prawns in all groups were collected at 0, 24, 48, 72 and 96 h after exposure to deltamethrin. Haemolymph analyses carried out include total haemocyte count and identification, phenoloxidase, respiratory burst and superoxide dismutase activities. Results of immunological analyses revealed that total haemocyte count, granular cells, hyaline cells and phenoloxydase activity decreased significantly ($P<0.05$) in treatment groups compared to the control group at 24 ,48, 72 and 96 h after exposure. Respiratory burst activity in the treatment groups increased significantly ($P<0.05$). However, in the group exposed to 0.125 $mg{\cdot}L^{-1}$ deltamethrin, superoxide dismutase activity decreased at 24, 72 and 96 h exposure to deltamethrin compared to the control group, but no difference was observed at 48 h. These results suggest that exposure to deltamethrin suppressed the immune responses of *M. rosenbergii*.

Introduction

Decapod crustaceans, such as prawns lack the components of the vertebrate adaptive response (e.g., immunoglobulins, major histocompatibility complex antigens, T cell receptors) that provide protective immunity and allow vaccination against viruses (Arala-Chaves and Sequeira 2000). Therefore, these animals have to rely on innate immune mechanisms.

Prawns have a capacity for innate, non-adaptive immunity which involves the detection of foreign ligands (e.g., lipopolysaccharides, mucopeptides) by pattern recognition molecules, providing a useful defense against bacteria and parasites (Bachere et al. 1995). The innate immune

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response in prawn consists of both cellular and humoral responses. Haemocytes are responsible for most cellular responses (Lee and Soderhall 2002). Both semi-granular and granular cells carry out functions of the prophenoloxidase (proPO) system, which has a role in recognition and defense (Johansson and Soderhall 1989). Phenoloxidase (PO) is the terminal enzyme in the proPO system and is activated by several microbial polysaccharides, including b-1,3-glucan or lipopolysaccharide from fungal cell walls (Smith et al. 1984). The humoral factors originate from granulocytes (Destoumieux et al. 2000; Kimbrell and Beutler 2001). Cellular responses include encapsulation, phagocytosis, melanisation, cytotoxicity, cell-to-cell communication, and the ProPo activating system (Johansson et al. 2000). In prawns, phagocytosis generally occurs in the lymphoid organ, where the hyaline and semigranular haemocytes engulf and degrade foreign particles (Van de Braak et al. 2002). Humoral factors include lectins, defensive enzymes, reactive oxygen intermediates and a wide array of antimicrobial peptides (Kimbrell and Beutler 2001; Lee and Soderhall 2002; Soderhall 1999).

Several reactive oxygen species are produced during phagocytosis in the immunity response of crustaceans. In the beginning of this process, the membrane-bound enzyme complex, Nicotinimide adenine dinucleotide phosphate (NADPH) oxidase, assembles after binding of the cell to a foreign particle and reduces molecular oxygen to the superoxide anion (O_2^-), subsequently leading to the production of hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radicals (OH^-), and numerous other reactive compounds (Munoz et al. 2000). This phenomenon, known as respiratory burst, plays an important role in microbicidal activity (Bell and Smith 1993), and can be considered as functional tests for the evaluation of immune potential at a cellular and organismal level. The superoxide anion is the first product released from the respiratory burst, and can be scavenged by superoxide dismutase (SOD).

Environmental contaminants have been reported to cause a reduction in the haemocyte count and PO activity of *Crangon crangon* (Linnaeus 1758) and *Carcinus maenas* (Linnaeus 1758) (Truscott and White 1990; Smith and Johnston 1992; Le Moullac and Haffner 2000). In *M. rosenbergii*, variations of the haemocyte count, PO activity, respiratory burst, superoxide dismutase and generation of superoxide anion in haemocytes have been investigated under stress from copper sulphate, benzalkonium chloride, potassium permanganate and organophosphorus insecticide such as Trichlorfon (Cheng and Wang 2001; Cheng et al. 2003a; Cheng et al. 2003b).

The giant freshwater prawn is widely cultured in freshwater areas in Vietnam. Pyrethroids as insecticidal and antiparasitic formulations have widely been used as agriculture pesticide, to eliminate wild crustaceans in prawn farming, to treat water and to reduce disease problems (Tu et al. 2006). Among pyrethroid, deltamethrin has a potent insecticidal activity with an appreciable safety margin (Mestres and Mestres 1992).

It kills insects by contact and works by paralysing their nervous system and therefore gives a quick knockdown effect (Velisek et al. 2007). The rapid disappearance of deltamethrin from the

water and its low bioconcentration capacities indicate that this molecule will not accumulate through the food chain. Nevertheless, its high toxicity and rapidity of action could cause significant harm to aquatic animals after direct treatment. L'Hotellier and Vincent (1986) showed that the 96 h LC_{50} value of deltamethrin (25% purity) in pink shrimp (*Penaeus duorarum* Burkenroad 1939) was 0.35 $mg{\cdot}L^{-1}$. Besides, 96 h LC_{50} value of deltamethrin in black tiger shrimp (*Penaeus monodon* Fabricius 1798) was 1.05 $mg{\cdot}L^{-1}$ (Nguyen et al. 2010). Our previous studies have indicated that the 96 h LC_{50} values of deltamethrin for *M. rosenbergii* were 0.25 $mg{\cdot}L^{-1}$ (Oanh et al. unpublished data). When black tiger shrimp were exposed to deltamethrin for 4 days, acetylcholinesterase (AChE) activity in muscle was significantly inhibited (49% at a concentration of 0.07 $mg{\cdot}L^{-1}$ deltamethrin). Therefore, the activity of AChE in muscle is considered as biomarkers when shrimp are exposed to deltamethrin (Tu et al. 2009). However, the effect on the immune response in prawns under deltamethrin stress remains to be investigated. Thus, the objective of this study was to determine the effect of insecticide containing deltamethrin on immune response of *M. rosenbergii* at 24, 48, 72 and 96 h sub-lethal stress of deltamethrin concentrations. Haemocyte counts, PO activity, respiratory bursts (production of superoxide anions) and superoxide dismutase were used as indicators.

Materials and Methods

Experimental prawns

Apparently healthy giant freshwater prawns *M. rosenbergii* (17±2.3 g) were obtained from a prawn farm in Cantho city, and kept in freshwater in $1m^3$ tank, with continuous aeration, and maintained at 28-30 °C. Prawns were fed commercial pellet feed *ad libitum* twice per day. Prior to the start of the experiment, these prawns were randomly collected from the stock to examine for fouling parasites, *Vibrio* bacteria and white muscle viruses to make sure prawns were free from these pathogens. Only prawns in the intermolt stage (stage C) were used for the study. Commercial insecticide containing 2.5% of deltamethrin (Toxcis 2.5 EC) was purchased from An Nong Company Limited.

Effect of insecticide containing deltamethrin on immune response of giant freshwater prawns

At the start of experiment, prawns were transferred to an experimental facility and stocked in cylindrical 200 L experimental tanks (58 cm in diameter, 35 cm water depth) filled with 70% volume of freshwater, each fitted with continuous aeration, and maintained at 28-30 °C. All treatments were performed in triplicate. Fifteen prawns per tank were stocked in twelve experimental tanks. Three tanks served as control treatment. Nine other tanks were exposed to three sub lethal concentrations of deltamethrin (0.0025; 0.025 or 0.125 $mg{\cdot}L^{-1}$). These concentrations were selected as 1%, 10% and 50% of 96 h LC_{50} values (0.25 $mg{\cdot}L^{-1}$) of deltamethrin for *M. rosenbergii*, respectively (Oanh et al. unpublished data).

There was no water exchange during the 4 days exposure and shrimp were feed *ad libitum* twice per day at 7 am and 5 pm. Haemolymph samples were collected from three prawns·tank^{-1} samples in all groups at 0, 24, 48, 72 and 96 h after exposure to deltamethrin to analyse total haemocyte count, haemocyte identification, PO, respiratory burst and SOD activities.

Total haemocyte count

Total haemocyte count (THC) (cells·mL^{-1}) was performed as described by (Le Moullac et al. 1997). Haemolymph (100 µL) was collected using a 1 mL syringe and 26G needle containing 900 µL anticoagulant solution (trisodium citrate 0.114 M, sodium chloride 0.1M, pH 7.45, osmolality 490 mOsm·kg^{-1}). The haemocytes on the hemocytometer were observed under light microscope and counted manually in all 25 squares (0.1 mm^3).

Differential haemocyte count

Differential haemocyte count (DHC) was performed by the method of Cornick and Stewart (1978) with some modifications. Briefly, 200 µL haemolymph was collected using a 1 mL syringe and 26G needle containing 200 µL formaline anticoagulant solution (pH 4.6) and kept in an eppendorf tube, mixed well and centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellet was rinsed once with 200 µL formaline anticoagulant solution (pH 4.6) and resuspended gently in50 µL of the same solution. A drop of the haemocyte suspension was spread on glass slides, air-dried, fixed for 5 min in ethanol, washed in distilled water and stained with Giemsa stain for 30 min. Stained slides were rinsed in acetone and xylene and observed under light microscope (100X).

Phenoloxidase activity

Phenoloxidase (PO) activity was measured by the method or Herández-López et al. (1996). The optical density of PO activity was measured by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA). Two hundred microlitre of diluted haemolymph was centrifuged at 5000 rpm at 4 °C for 10 min. The supernatant fluid was removed and the pellet was suspended gently in 1 mL cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride and 0.10 M trisodium citrate, pH 7.0), then centrifuged at 2000 rpm at 4 °C for 10 min. The pellet was suspended in 200 µL cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.01 M calcium chloride and 0.26 M magnesium chloride, pH 7.0). One hundred microlitre of resuspended pellet was incubated with 50 µL trypsin (1 mg·mL^{-1}) for 10 min at 25-26 °C, then 50 µL L-DOPA (3 mg·mL^{-1}) was added and left for 5 min at room temperature followed by adding 800 µL cacodylate buffer. The optical density at 490 nm was measured using a microplate reader. The control solution, which consisted of 100 µL cell suspension, 50 µL cacodylate buffer (to replace the trypsin) and 50 µL L-DOPA, was used to measure the background PO activity in all test conditions.

The background PO optical density values were in the range of 0.02-0.05. The PO activity optical density values of prawns for all test conditions were expressed as dopachrome formation per 50 mL of haemolymph.

Respiratory burst assay

Respiratory burst activity (RES) of haemocytes was quantified by the method of Song and Hsieh (1994). Haemolymph (100 µL) in anticoagulant solution was deposited in the microplate coated with 100 µL solution of poly-L-lysine (0.2%) to increase adhesion of cells. Microplate was centrifuged at 5000 rpm for 15 min and plasma was removed. A volume of 100 µL zymosan (0.1% in Hanks' solution minus phenol red) was added and allowed to react for 30 min at room temperature. Zymosan was removed and haemocytes were washed 3 times with 100 µL Hanks' solution, stained with 100 µL nitroblue tetrazolium (NBT) solution (0.3%) for 30 min at room temperature. The NBT solution was discarded and cells were washed 3 times with 100 µL of 70% methanol and air-dried for 30 min. One hundred and twenty microlitre of 2 M KOH and 140 µL of dimethyl sulfoxide (DMSO) were added to dissolve the insoluble formazan crystals formed by the reduction of NBT. The optical density at 630 nm was measured in triplicate using a microplate reader. Respiratory burst activity is expressed as NBT-reduction in 10 µL of haemolymph.

Superoxide dismutase activity

Superoxide dismutase activity (SOD) was measured by the method described by Sarathi et al. (2008). Haemolymph (50 µL) in 50 µL anticoagulant solution (pH 7.0) was added to a cold eppendorf tube containing 500 µL phosphate buffer (50 mM, pH 7.8), mixed well and centrifuged at 5000 rpm at 4 °C for 5 min. Five hundred microlitre of the supernatant was transferred to a new eppendorf tube, incubated for 5 min at 65 °C, then centrifuged at 5000 rpm at 4 °C for 5 min. New supernatant (crude extract) was transferred to a new eppendorf tube and stored at -20 °C until use. Samples were always kept on ice to avoid protein denaturation. A mixture of NBT, 20 mM of reaction mixture (0.1 mM EDTA, 13 µM methionine, 0.75 mM NBT and 20 µM riboflavin in 50 mM phosphate buffer, pH 7.8) and 0-100 µL of the crude extract were placed under fluorescent light for 2 min or until OD A560 in the control tubes reached 0.2-0.25. The results were expressed as relative enzyme activity.

Statistical analysis

The obtained data were analysed using the software SPSS 16.0. One way ANOVA and Tukey's Multiple Comparison Test were used to test differences in the same group and between different groups at significance level $P < 0.05$.

Asian Fisheries Science **27** (2014): 90-103

Results

Experimental water parameters

Daily temperature, pH, dissolved oxygen, ammonium, nitrite and nitrate in experimental tanks were not significantly different among treatments. Water temperature ranged from 28±1 to 30±0.5 ºC, pH from 7.1–7.5 and dissolved oxygen from 5.8-6 $mg \cdot L^{-1}$. Ammonium, nitrite and nitrate were below 0.2 $mg \cdot L^{-1}$, 0.03 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$, respectively. In general, water parameters in experimental tanks were suitable for *M. rosenbergii*.

Total haemocyte count

Total haemocyte count (THC) of prawns that were exposed to insecticide containing deltamethrin at all tested concentrations was significantly lower than prawns in the control at different sampling times (Table 1).

Table 1. Mean (±SD) total haemocytes, granular cells and hyaline cells in *M. rosenbergii* exposure to different concentrations of insecticide containing deltamethrin at different time intervals.

Deltamethrin concentration ($mg \cdot L^{-1}$)	0 h	24 h	48 h	72 h	96 h
			Total haemocyte count		
0	71.6±0.1^{Aa}	70.2±4.7^{Aa}	69.0±6.5^{Aa}	74.7±2.1^{Aa}	75.6±1.8^{Aa}
0.0025	71.6±0.1^{Aa}	57.0±4.4^{Bb}	57.7±1.9^{Bb}	58.9±1.6^{Bbc}	62.7±1.8^{Bc}
0.025	71.6±0.2^{Aa}	49.7±5.2^{Bb}	47.1±4.3^{Cb}	46.5±3.8^{Cb}	45.2±2.7^{Cb}
0.125	71.6±0.3^{Aa}	34.8±4.8^{Cb}	32.8±5.3^{Db}	36.4±1.6^{Db}	37.0±0.2^{Db}
			Granular cells		
0	63.5±1.3^{Aa}	62.1±4.7^{Aa}	52.9±13.9^{Aa}	66.5±2.5^{Aa}	67.5±1.7^{Aa}
0.0025	65.8±1.2^{Aa}	49.6±4.7^{ABb}	48.5±1.9^{Ab}	49.5±1.4^{Bb}	52.5±1.8^{Bb}
0.025	63.3±1.0^{Aa}	39.0±13.5^{BCb}	40.0±4.5^{ABb}	39.6±3.9^{Cb}	34.1±6.4^{Cb}
0.125	64.7±0.7^{Aa}	25.8±5.7^{Cb}	27.7±5.2^{Bbc}	32.3±1.3^{Dbc}	33.2±0.3^{Cc}
			Hyaline cells		
0	8.4±0.225^{Aa}	8.2±0.147^{Aa}	8.3±0.267^{Aa}	8.2±0.165^{Aa}	8.1±0.137^{Aa}
0.0025	8.6±0.059^{Aa}	8.3±0.318^{Aa}	9.3±0.085^{Bb}	9.4±0.280^{Bb}	10.2±0.231^{Bc}
0.025	8.7±0.023^{Aa}	5.8±0.228^{Bc}	7.1±0.298^{Cb}	6.9±0.460^{Cb}	5.7±0.254^{Cc}
0.125	8.7±0.031^{Aa}	4.7±0.288^{Cb}	5.1±0.231^{Dc}	4.1±0.274^{Dd}	3.8±0.230^{Dd}

Capital letter (A, B, C and D): denotes a significant difference between treatments and control prawns at different time intervals using one-way ANOVA ($P < 0.05$). Small letter (a,b,c and d) denotes a significant difference different time intervals using one-way ANOVA ($P < 0.05$).

After 24 h, no significant difference in THC was observed among prawns exposed to 0.0025 and 0.025 $mg{\cdot}L^{-1}$ deltamethrin. However, THC in prawn exposed to 0.125 $mg{\cdot}L^{-1}$ was significantly lower to those at lower concentrations and control group. After 48, 72 and 96 h, THC was significantly reduced and was inversely proportional to the concentration of deltamethrin.

There was no significant difference in THC of prawns in the control group at different sampling times. However, THC in prawns that were exposed to 0.0025 $mg{\cdot}L^{-1}$ deltamethrin decreased significantly at 48 h and continued to decrease significantly at 96 h. Total haemocyte count in prawns that were exposed to 0.025 and 0.125 $mg{\cdot}L^{-1}$ reduced significantly after 24 h but no significant difference was observed from 48, 72 and 96 h (Table 1).

Granular cells

After 24 h, granular cells in prawns exposed to 0.0025 $mg{\cdot}L^{-1}$ deltamethrin did not decrease significantly compared to prawns in the control group, whereas, significant decrease was observed in those exposed to 0.025 and 0.125 $mg{\cdot}L^{-1}$ of deltamethrin (Table 1). After 48 h, GC decreased significantly in prawns exposed to 0.125 $mg{\cdot}L^{-1}$ compared to prawns in the control and 0.025 $mg{\cdot}L^{-1}$. After 72 and 96 h, GC was significantly reduced and the reduction was inversely proportional to the concentration of deltamethrin.

There was no significant difference in GC of prawns in the control group at different sampling times. There was a significant difference in GC in prawns exposed to 0.125 $mg{\cdot}L^{-1}$ after 24 h but no difference was found at 48, 72 and 96 h. Granular cells in prawns that were exposed to 0.025 and 0.125 $mg{\cdot}L^{-1}$ reduced significantly after 24 and 96 h (Table 1).

Hyaline cells

Hyaline cells in prawns exposed to all concentrations of deltamethrin decreased significantly ($P < 0.05$) compared to control group over the sampling times, except for concentration of 0.0025 $mg{\cdot}L^{-1}$ at 24 h. After 24, 48, 72 and 96 h, HC decreased significantly and the decrease was inversely proportional to the concentration of deltamethrin (Table 1). No significant difference was found across different sampling times (0, 24, 48, 72 and 96 h) of prawns against the control. However, at the same concentrations of deltamethrin, HC was significantly different over the sampling times (Table 1).

Asian Fisheries Science **27** (2014): 90-103

Phenoloxidase activity

Phenoloxidase activity in prawns exposed to deltamethrin was significantly less than prawns in the control group. However, PO activity at 72 h was not significantly different especially in prawns exposed to deltamethrin at 0.125 mg·L^{-1} compared to other concentrations and the control prawns (Fig. 1).

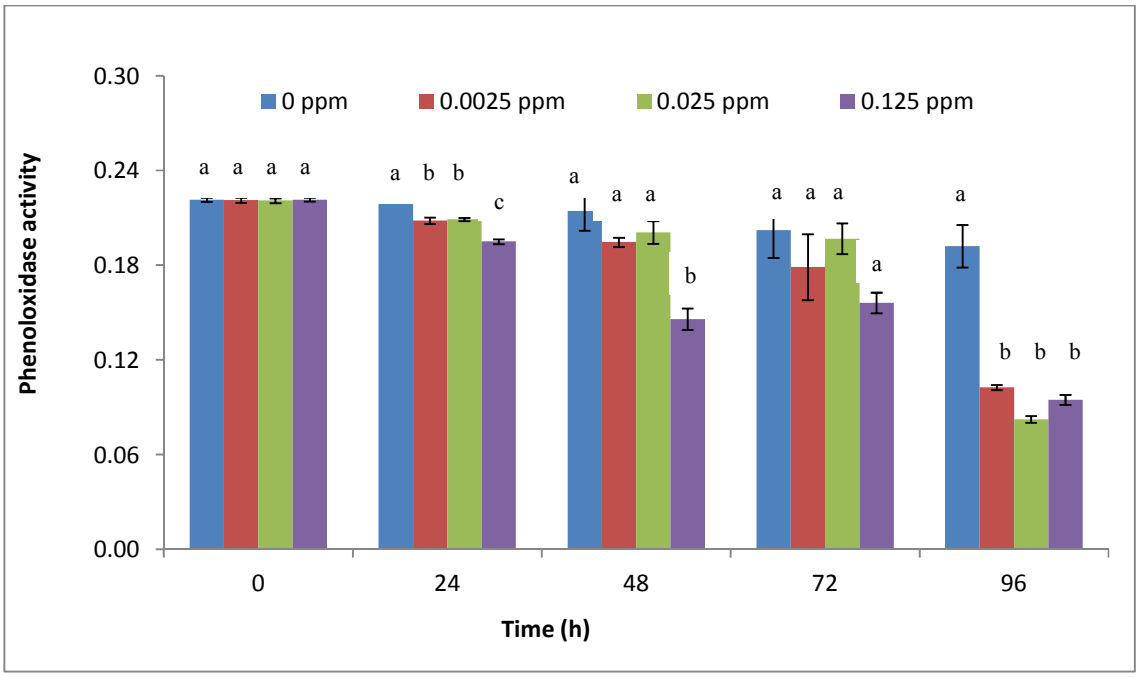

Fig. 1. Phenoloxidase activity in the haemocytes of *M. rosenbergii* at 0, 24, 48 and 96 h exposure to insecticide containing deltamethrin at different concentrations. Bars with different letters are significantly different ($P<0.05$). The bars indicate standard error mean between samples from the triplicate groups.

At different times, PO activity reduced significantly after 72 h exposure to deltamethrin at 0.0025 mg·L^{-1} and after 48 h exposure to deltamethrin at 0.025 mg·L^{-1}. At 24 h exposure to deltamethrin at 0.125 mg·L^{-1} PO activity was significantly reduced and then increased slightly at 72 h but this difference was not significant compared to 48 h. In general, PO activity decreased more quickly at higher concentrations of deltamethrin (Fig. 1).

Respiratory burst

No significant differences in RES were observed for prawns in control group, 0.0025 mg·L^{-1} and 0.025 mg·L^{-1} groups at 0, 48, 72 and 96 h. However, RES increased significantly with increasing exposure time in groups exposed to 0.125 mg·L^{-1} deltamethrin (Fig.2).

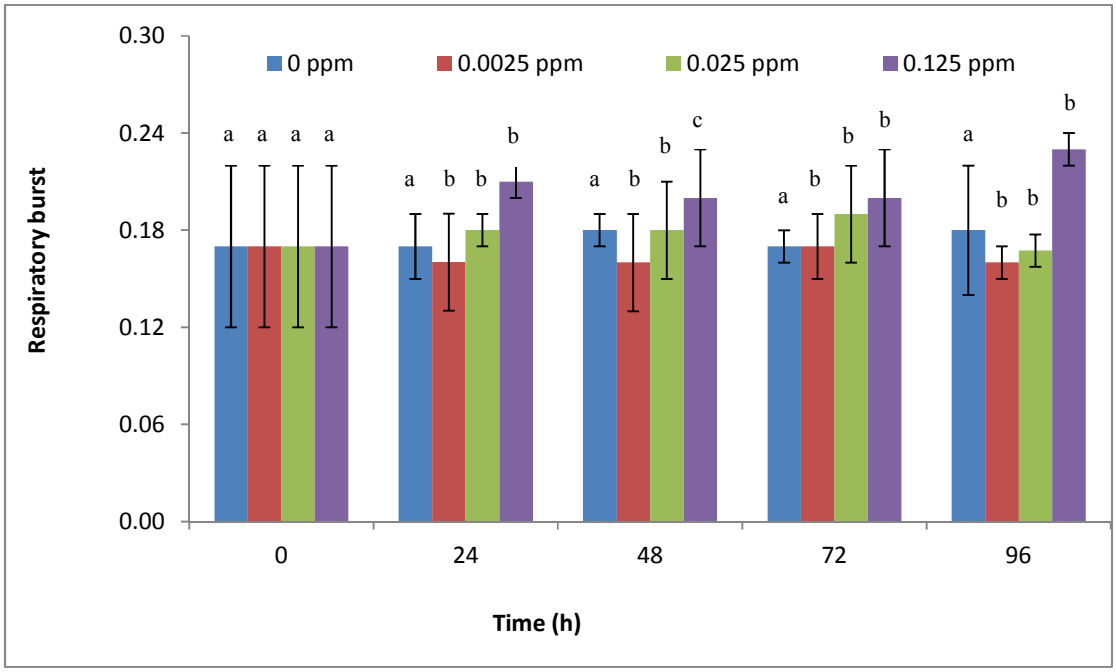

Fig. 2. Respiratory burst in the haemocytes of *M. rosenbergii* at 0, 24, 48 and 96 h exposure to insecticide containing deltamethrin at different concentrations. Bars with different letters are significantly different ($P<0.05$). The bars indicate standard error mean between samples from the triplicate groups.

Superoxide dismutase activity

Superoxide dismutase activity in prawns exposed to deltamethrin at all concentrations was significantly reduced in comparison to the control ($P<0.05$) at 24, 72 and 96 h but there was no significant difference of SOD activity from prawns at different concentrations of deltamethrin (Fig.3). The SOD activity in samples collected at different times showed an increased activity at 24 h and reached the highest at 48 h, decreased at 72 h and increased again at 96 h. However, the SOD activity in prawns exposed to deltamethrin was always lower than those in the control group (Fig.3).

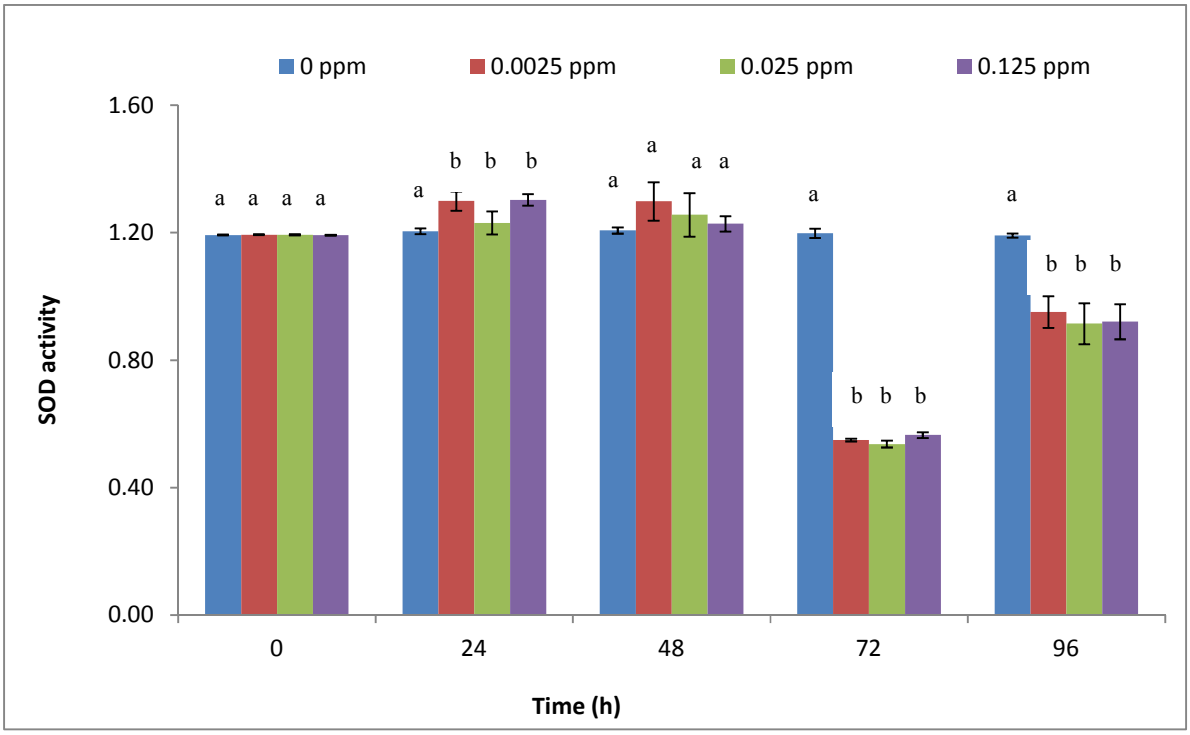

Fig. 3. Superoxide dismutase activity in the haemocytes of *M. rosenbergii* at 0, 24, 48 and 96 h exposure to insecticide containing deltamethrin at different concentrations. Bars with different letters are significantly different ($P<0.05$). The bars indicate standard error mean between samples from the triplicate groups.

Discussion

Physico-chemical parameters such as temperature, salinity, dissolved oxygen and nitrite have been reported to affect THC in several shrimp species. In addition, environmental pollutants have also been reported to affect THC in several species of invertebrates. Le Moullac et al. (1998) observed decreased THC in blue shrimp *Penaeus stylirostris* (Stimpson 1874) after 24 h exposure to dissolved oxygen as low as 1 $mg{\cdot}L^{-1}$. Le Moullac and Haffner (2000) observed a significant decrease in THC in blue shrimp *P. stylirostris* exposed to low temperature (18 °C) compared to prawns at 27 °C. They also observed a significantly higher THC in Brazilian shrimp (*Penaeus paulensis* Pérez-Farfante 1967) that were reared at 34 ppt compared to shrimp reared at 22 and 13 ppt. Tseng and Chen (2004) observed significant differences in haemocyte counts in white shrimp, *Litopenaeus vannamei* (Boone 1931) exposed to 4.94, 9.87 and 19.99 $mg{\cdot}L^{-1}$ nitrite-N for 96 h suggesting that nitrite in water caused a depression in the immune response of *L. vannamei* and an increased susceptibility to *Vibrio alginolyticus* infection. Victor et al. (1990) reported that freshwater prawn *Macrobrachium idea* (Heller 1862) exhibited hyperplastic gill lamella engorged with haemocytes after 30 days exposure to 1 $mg{\cdot}L^{-1}$ mercuric chloride. Le Moullac and Haffner (2000) found that THC of *P. stylirostris* decreased by 15 and 50% following exposure to ammonia at 1.5 and 3.0 $mg{\cdot}L^{-1}$. In this study, physico-chemical parameters of the environment were suitable for experimental prawns which help to exclude the effects of these parameters on THC. However, THC of prawns exposed to insecticide containing deltamethrin was significantly reduced inversely

proportional to the concentration of deltamethrin and at different sampling times. These observations indicate the risks for *M. rosenbergii* associated with different levels of tested deltamethrin.

The present study demonstrated that exposure to insecticide containing deltamethrin significantly reduced PO activity in *M. rosenbergii*. Moreover, PO activity was significantly reduced more quickly at higher concentrations of deltamethrin. This was similar to the effects that were previously shown with ammonia, copper sulfate and benzalkonium chloride (Cheng and Wang 2001; Cheng and Chen 2002; Cheng et al. 2003a). Environmental factors such as hypoxia (Le Moullac et al. 1998), temperature or pH (Cheng and Chen 2000; Le Moullac and Haffner 2000) and chemicals such as polychlorinated biphenyls 15 (Smith and Johnston 1992) were shown to affect PO. Reduction of PO activity resulted in the susceptibility of prawns to infectious pathogens such as *Lactococcus garvieae* (Cheng et al. 2003a).

Production of superoxide anion increased in *M. rosenbergii* following exposure to deltamethrin in this study. This result was similar to the effect of exposure to ammonia, nitrite, copper sulfate and benzalkonium chloride (Cheng and Wang 2001; Cheng et al. 2002; Cheng et al. 2003a). Exposure to ammonia-N at 0.55 $mg{\cdot}L^{-1}$, nitrite-N at 1.15 $mg{\cdot}L^{-1}$, copper sulfate at 0.3 and 0.4 $mg{\cdot}L^{-1}$, and benzalkonium chloride at 0.3 $mg{\cdot}L^{-1}$ or greater in *M. rosenbergii* results in increased immunity or cytotoxicity (Cheng et al. 2003a). In this study following 24 h exposure to 0.125 $mg{\cdot}L^{-1}$ deltamethrin, the superoxide anion increased significantly but PO activity decreased significantly suggesting that deltamethrin promoted cytoxicity and acted as an immunodepressor in *M. rosenbergii*.

Conclusion

The present study demonstrated that *M. rosenbergii* exposed to deltamethrin showed reduced non-specific immunity as evidence by the decrease in total haemocyte count, granular cells, hyaline cells, PO and SOD activities but an increase in respiratory burst activity. These results suggest that when *M. rosenbergii* is exposed to deltamethrin it supresses the immune responses which may result in the susceptibility of prawn to infectious pathogens. Our work suggests caution in the use of deltamethrin for wild crustaceans elimination, treatment of water quality and reduction of disease problems in grow out ponds of *M. rosenbergii*.

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