อัตราการตายและการเปลี่ยนแปลงเนื้อเยื่อของกุ้งก้ามกราม (*Macrobrachium rosenbergii*) ที่ได้รับสัมผัสกับอะบาเม็กติน

Mortality rate and histological alterations of prawn (*Macrobrachium rosenbergii*) exposed to abamectin

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บทคัดย่อ

กุ้งก้ามกรามถูกนำมาใช้ในการศึกษาถึงความเป็นพิษของอะบาเม็กตินเมื่อเกิดการปนเปื้อนในน้ำ ทั้งนี้เนื่องจากกุ้งก้ามกรามสามารถพบได้ทั่วไปตามแหล่งน้ำธรรมชาติและในฟาร์มที่มีการเพาะเลี้ยง ในการศึกษาครั้งนี้ได้ประเมินถึงความเป็นพิษของอะบาเม็กตินจากอัตราการตาย และการเปลี่ยนแปลงของเนื้อเยื่อที่ระดับความเข้มข้นของอะบาเม็กติน 2.5, 5.0, 7.5 และ 10.0 ไมโครกรัมต่อลิตร เมื่อได้รับสัมผัสที่ระยะเวลา 24, 48, 72 และ 96 ชั่วโมง อัตราการตาย 50 เปอร์เซ็นต์ของกุ้งก้ามกรามที่ได้รับสัมผัสกับอะบาเม็กตินที่ตรวจสอบได้จากการศึกษาครั้งนี้ คือ 16.18, 8.99, 5.79 และ 4.88 ไมโครกรัมต่อลิตรที่ระยะเวลา 24, 48, 72 และ 96 ชั่วโมง ตามลำดับ เมื่อทำการศึกษาการเปลี่ยนแปลงของเนื้อเยื่อกล้ามเนื้อและเหงือกพบว่ามีการเปลี่ยนแปลงเพิ่มขึ้นตามระดับความเข้มข้นและระยะเวลาที่ได้รับสัมผัส โดยการเปลี่ยนแปลงที่พบในเนื้อเยื่อกล้ามเนื้อ คือเกิดการบวมและมีการสะสมของเม็ดเลือดในเซลล์กล้ามเนื้อ ส่วนการเปลี่ยนแปลงที่พบในเนื้อเยื่อเหงือก คือพบการบวม การสะสมของเม็ดเลือดและเกิดการตายของเซลล์ในซี่เหงือก ดังนั้นจึงอาจจะสรุปได้ว่าการเปลี่ยนแปลงที่ตรวจสอบได้ในกุ้งก้ามกรามสามารถนำมาประเมินถึงการได้รับสัมผัสอะบาเม็กติน และกุ้งก้ามกรามสามารถนำมาประยุกต์ใช้เป็นตัวชี้วัดการปนเปื้อนของสารพิษในสิ่งแวดล้อมทางน้ำได้

คำสำคัญ : อะบาเม็กติน พฤติกรรม สารกำจัดแมลง การวิเคราะห์โพรบิท

Abstract

Prawn (*Macrobrachium rosenbergii*) was applied to study the toxicity of abamectin in the aquatic environment because it is the common species found in natural waters and cage. In this study, the toxicity of abamectin was assessed based on mortality rate and histological alterations in prawn after exposed to abamectin at the concentrations of 2.5, 5.0, 7.5 and 10.0 μg L^{-1} for 24, 48, 72 and 96 h. The median lethal concentration (LC50) values obtained were 16.18, 8.99, 5.79 and 4.88 μg L^{-1} at 24, 48, 72 and 96h, respectively. Moreover, the study on histological alterations in muscle

and gill tissues revealed that it increased with an increasing in abamectin concentration and exposure time. In the muscle tissue, the changes were swelling of muscular and infiltration of hemocyte. For the gills, they were swelling of gill lamellae, hemocytic infiltration, lifting of lamellae and necrosis of lamellae. Thus, it can be concluded that the alterations in exposed prawn can be used to assess abamectin exposure and further applied as bio-indicator monitoring chemical contamination in waters.

Keywords : Abamectin, Behavior, Insecticide, Probit analysis

Introduction

Industrialization and increasingly in natural resource consumption of human results in more environmental problems worldwide (Bela and Prasad, 2008). The major sources of water pollutant are industrial, domestic, and agricultural effluent (Maruthanayagam and Sharmila, 2004). Pesticides are extensively used to control or eliminate insects, pests or disease vectors. Ultimately, they reach to aquatic environment such as rivers, lakes and ocean. After that, they cause adverse effect on not only fish but also useful aquatic species. In addition, veterinary drugs; avermectins or other names (ivermectin, abamectin, and doramectin) are widely used as anthelmintics to control the parasites of both internal and external in cattle, pigs, horses, sheep, and goats (Campbell and Benz, 1984; Suarez, 2002).

Abamectin or avermectin B1a, widely used in both as pesticide and anthelminthic drug in animals, is a natural product made from *Streptomyces avermitilis* in fermentation process. It is a mixture of homologues of B1a and B1b containing a minimum of 80% B1a and a maximum of 20% B1b (Fisher and Mrozik, 1989). In 1993, Roth *et al*. (1993) revealed that abamectin is highly lipophilic. It tends to be dissolved in most organic solvents and then can be direct assimilated in animal body (Halley *et al*., 1993).

In many studies such as Martin (1997) and Martin *et al*. (2002) indicated that the target of abamectin was the nervous system of parasites. It tends to bind with the glutamate-gated chloride channels and GABA (c-amino butyric acid)-gated chloride channels in arthropods and nematodes and result in chloride influx and further disrupt neural signal transmission. However, abamectin not only effect on parasites in arthropods and nematodes but also on other organisms (Mc Kellar, 1997). Especially in fish, abamectin can pass through blood/brain barrier and cause adverse effects. Many researches indicated that abamectin have toxicity in many fishes such as rainbow trout (*Oncorhynchus mykiss*) and Tilapia (*Oreochromis niloticus*) (Jenčič *et al*., 2006; El-Said, 2007). The assessment of abamectin effect previously reported was to measure histological alterations,

acetylcholinesterase (AChE) level, biochemical constitute and oxygen consumption (Jenčič *et al*., 2006; El-Said, 2007; Al-Kahtani, 2011). In some studies, it also focused on behavior and physiological alterations. Some aquatic invertebrates or crustaceans involving in food chains may be used as bio-indicators of water contamination (Vijayraman and Geraldine, 1996). However, there were lack of knowledge about the effect of abamectin on each aquatic species such as shrimp, crab and mollusk. Thus, this study focused on the effect of abamectin on prawn (*M. rosenbergii*) by assessing mortality rate and behavior and histological alteration in muscle and gill tissue in different concentration and exposure time.

Materials and Methods

1. Animals and abamectin treatments

Prawn (*M. rosenbergii*) in post larvae state was collected from Roi-Et Inland Fisheries Research and Development Center and then transferred to Department of Fisheries in the Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus. Next, they were maintained until juvenile state by feeding twice a day. After that, the prawns (weight between 5.12±0.29 g and total body length of 6.9±1.7 cm as shown in Figure 1 were applied to study the effect of abamectin. They were placed in 50 L tank (n=20). The abamectin concentrations were varied as 2.5, 5.0, 7.5 and 10.0 μg L^{-1} and untreated condition used as the control. The experiment was triplicately performed. The mortality rate was calculated using the following equation 1. Besides, LC50 assessment was performed using probit analysis (Minitab software).

$$\text{Mortality rate (\%)} = \frac{\text{No. of prawn died}}{\text{Total No. of prawn stocked}} \times 100 \qquad (1)$$

In addition, water conditions; temperature, dissolved oxygen, pH and conductivity, were recorded every day. The treated prawns were collected at 24, 48, 72 and 96 h after exposure for further study of the alterations in muscle and gill tissues. They were knocked by freezing in ice box and immediately taken to avoid lysis and placed in 10% phosphate buffer formalin for fixation.

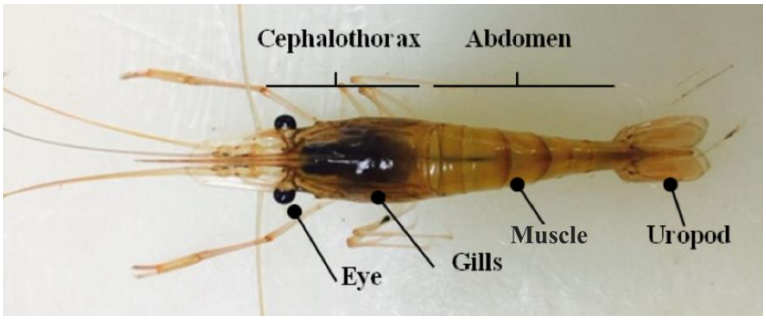

Figure 1 Morphological illustration of prawn (*M. rosenbergii*) used in this study

2. Histological alterations of prawn

Muscle and gill tissue of prawn were collected from treated and controlled conditions for further histological study. The tissue samples were dissected and fixed in 10% formalin for 24 h. After that, the fixed sample was cut in 3 cm cubes using microtome. After that, the sectioned organ was washed under running tap for 2 h and then dehydrated using 95% ethanol. Next, it was infiltrated and embedded in paraffin wax in an embedding chamber and then stained using hematoxylin and eosin. Smears and tissue imprint were made on glass slides for further analysis under light microscope (X40). Lesions and degenerations were used to assess the effect of the abamectin. The alteration was observed in both muscle and gill tissues, and then being classified into five severity factors, i.e., unchanged (-), mild occurrence (+), moderate occurrence (++), severe occurrence (+++) and most severe occurrence (++++)

Results

1. Behavior alterations and percentage of mortality of prawns exposed to abamectin

In experiment period, the water conditions: temperature, pH, dissolved oxygen values, turbidity and conductivity were kept at 27.2±0.4ºC, 6.98±0.03, 3.9±0.20 mg L^{-1}, 22.5±2.2 ppm and 113.1±1.01 µmhos/cm, respectively. For behavior changes, the movement and swimming of exposed prawn was faster than that of the control. And, tremor in the appendage was also found. Moreover, the bark of prawn after 96 h of exposure was paler than observed in non-exposed (data not shown). The mortality percentage of exposed prawn increased with an increasing in abamectin concentration and exposure time. The mortality rate (%) of the prawn exposed to abamectin in the concentrations of 2.5 µg L^{-1} for 48, 72 and 96 h were 10, 15 and 20%, respectively. At the concentration of 5.0 µg L^{-1} after 24, 48, 72 and 96 h of exposure, it was 25, 35, 40 and 50%. And, the mortality rate (%) sharply increased at the concentration of 7.5 µg L^{-1}. It was 30, 45, 60±5.0 and 70±5.0% after 24, 48, 72 and 96 h of exposure. The highest was 85±10.0% found in the concentration of 10.0 µg L^{1}after 96 h. Figure 2 shows mortality rate (%) of exposed prawn in the concentrations of 0, 2.5, 5.0, 7.5 and 10 µg L^{-1} for 24, 48, 72 and 96 h.

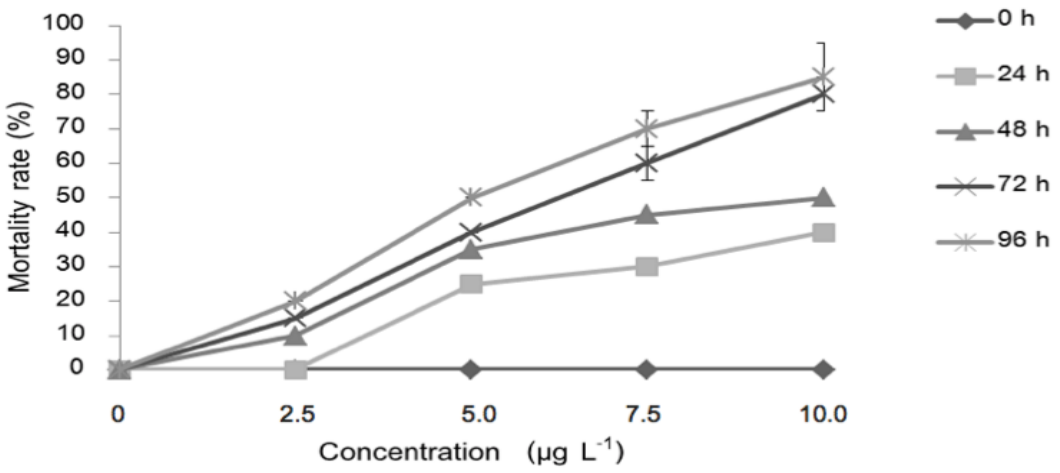

Figure 2 Mortality rate (%) in prawn after exposed to abamectin concentrations of 0, 2.5, 5.0, 7.5 and 10.0 µg L^{-1} for 24, 48, 72 and 96 h In addition, the determining of LC50 by probit analysis indicated that it was 16.18, 8.99, 5.79 and 4.88 µg L^{-1} at 24, 48, 72 and 96 h, respectively (Table 1 and Figure 3A-3D and Table 1).

Table 1 LC 50 values calculated by probit analysis

Time (h)	Results	
	LC50 (µg L^{-1})	95% confident
24	16.18	10.69-5932.55
48	8.99	7.64-11.4689
72	5.79	5.22-6.42
96	4.85	4.34-5.37

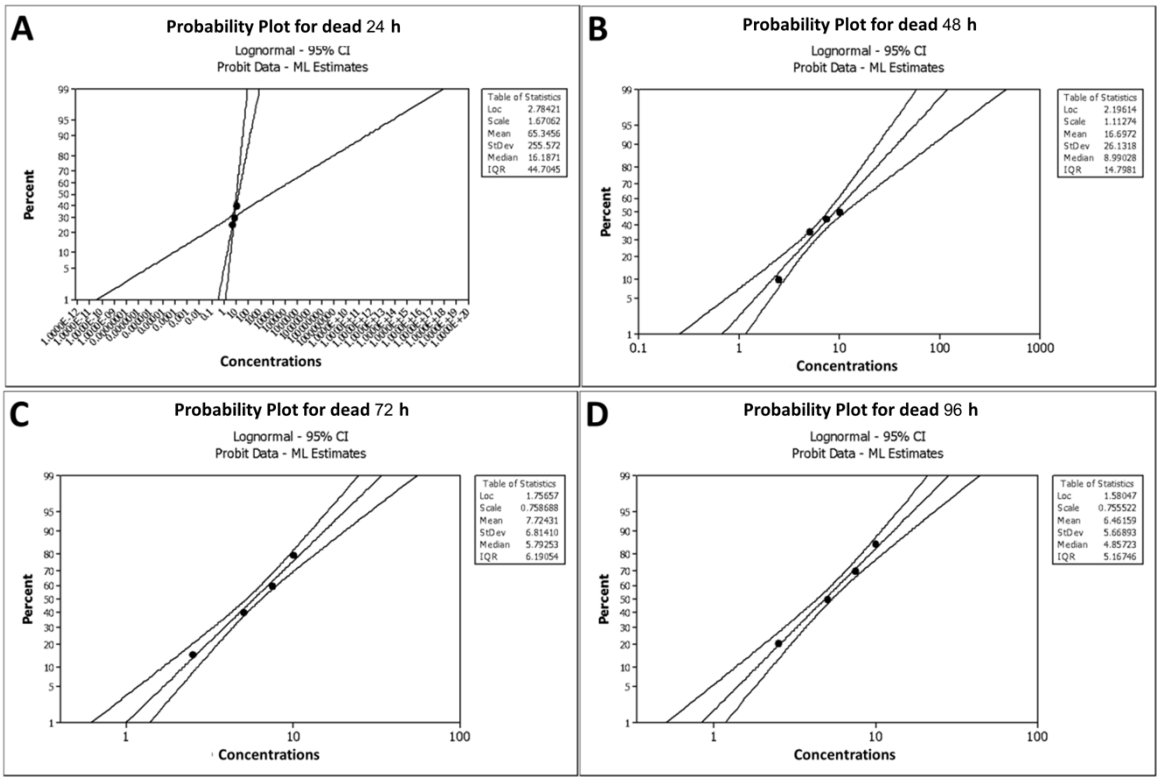

Figure 3 Probit plotted expressing mortality percentage in prawn after exposed to abamectin in the concentrations of 0, 2.5, 5.0, 7.5 and 10.0 μg L^{-1} for 24 (A), 48 (B), 72 (C) and 96 (D) h

2. Histological alterations in muscle and gill

In this study, histological alterations were assessed in two parts; (1) gill tissue and (2) muscle tissue. The abamectin concentrations tested were 2.5, 5.0, 7.5 and 10.0 μg L^{-1} and exposure time was 24, 48, 72 and 96 h. After histological alterations in prawn after exposed to abamectin being assessed, it found swelling of muscular and infiltration of hemocyte (Figure 4B-4E) compared to the normal condition (Figure 4A). These alterations increased with an increasing in concentration and exposure time as shown in Figure 4 and Table 2. At the lowest concentration observed a swelling of muscular was the concentration of 7.5 μg L^{-1} after 72 h of exposure. For infiltration of hemocyte, it could be found at 10.0 μg L^{-1} after 96 h.

Gill is an organ widely used in the study of toxicity. The level of alteration in gill depended on abamectin concentration and exposure time as same as found in the muscle but more severe. The condition of gill tissue in exposed prawn differed to the control (Figure 5A). In the prawn exposed to abamectin at the concentration of 10.0 μg L^{-1} for 48 h, gill alterations were swelling of lamellae, hemocytic infiltration and lifting of lamellae. In the higher concentration, it also found necrosis of gill lamellae as shown in Table 3 and Figure 5B-5E.

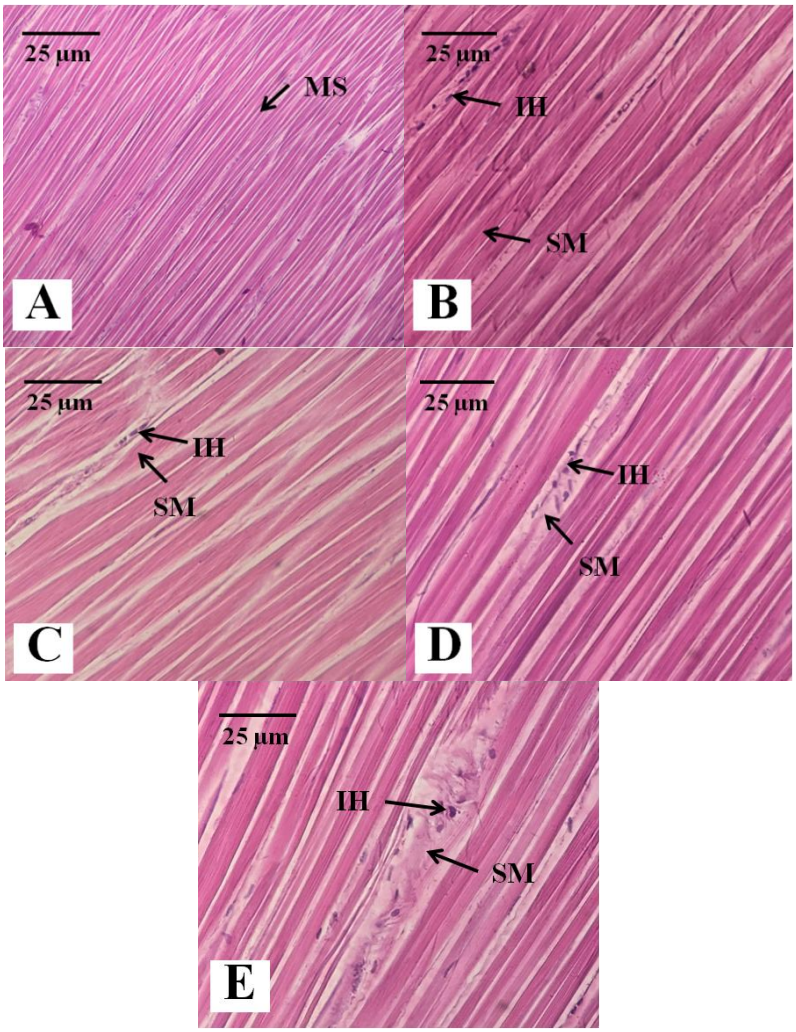

Figure 4 Histological alterations in muscle tissue of prawn in the control group (A), prawns exposed to abamectin at 2.5 (B), 5.0 (C), 7.5 (D) and 10.0 (E) µg L^{-1} for 96 h; where MS: Muscular, SM: Swelling of muscular, IH: Infiltration of hemocyte

Table 2 Histological alterations in muscle tissue in prawn after exposed to abamectin in the concentrations of 0, 2.5, 5.0, 7.5 and 10.0 μg L^{-1} for 24, 48, 72 and 96 h

Times	Concentrations	Histological alterations of muscle	
		Swelling of muscular	Infiltration of hemocyte
24 h	0 μg L^{-1}	-	-
	2.5 μg L^{-1}	-	-
	5.0 μg L^{-1}	-	-
	7.5 μg L^{-1}	-	-
	10.0 μg L^{-1}	-	-
48 h	0 μg L^{-1}	-	-
	2.5 μg L^{-1}	-	-
	5.0 μg L^{-1}	-	-
	7.5 μg L^{-1}	-	-
	10.0 μg L^{-1}	-	-
72 h	0 μg L^{-1}	-	-
	2.5 μg L^{-1}	-	-
	5.0 μg L^{-1}	-	-
	7.5 μg L^{-1}	+	-
	10.0 μg L^{-1}	++	+
96 h	0 μg L^{-1}	-	-
	2.5 μg L^{-1}	+	+
	5.0 μg L^{-1}	++	++
	7.5 μg L^{-1}	+++	+++
	10.0 μg L^{-1}	+++	++++

Remark: unchanged (-), mild occurrence (+), moderate occurrence (++), severe occurrence (+++) and most severe occurrence (++++)

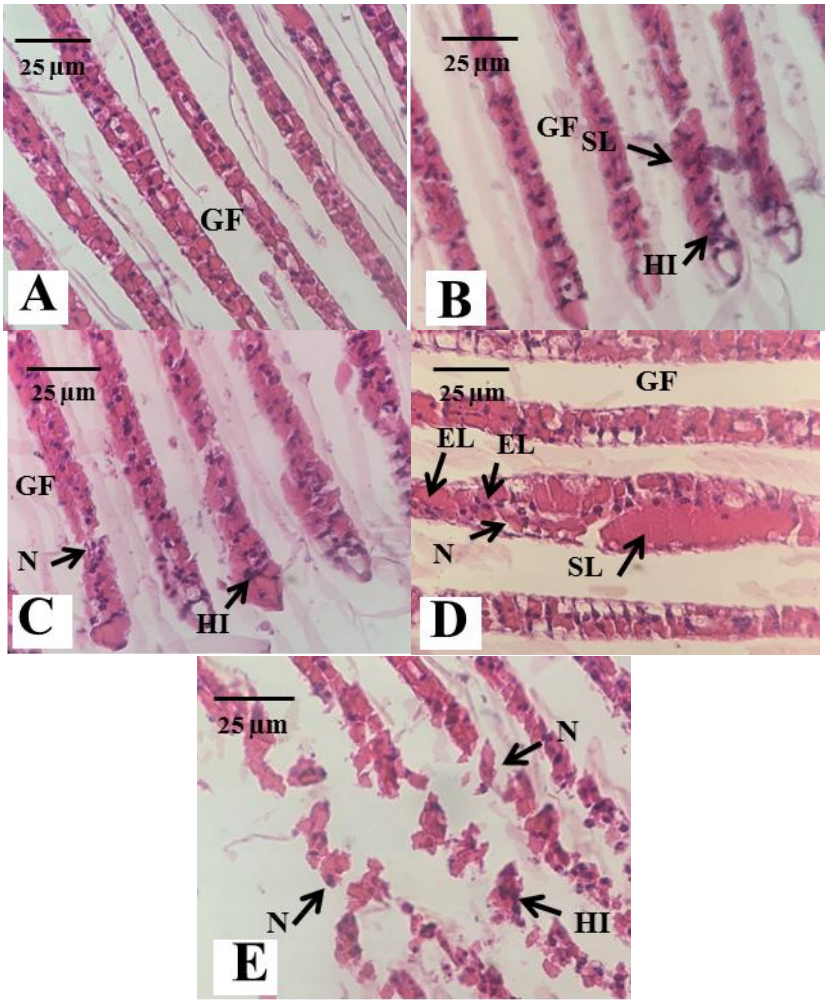

Figure 5 Histological alterations in gills tissue of prawn in the control group (A), prawns exposed to abamectin at 2.5 (B), 5.0 (C), 7.5 (D) and 10.0 (E) µg L^{-1} for 96 h; where GF: Gill filament, SL: selling of lamellae, HI: Hemocytic infiltration, EL: Epithelial lifting, N: Necrosis of lamellae

Table 3 Histological alterations in gill tissue in prawn after exposed to abamectin at the concentrations of 0, 2.5, 5.0, 7.5 and 10.0 μg L^{-1} for 24, 48, 72 and 96 h

Times	Concentrations	Histological alterations of gill			
		Swelling of lamellae	Hemocytic infiltration	Lifting of lamellae	Necrosis of lamellae
24 h	0 μg L^{-1}	-	-	-	-
	2.5 μg L^{-1}	-	-	-	-
	5.0 μg L^{-1}	-	-	-	-
	7.5 μg L^{-1}	-	-	-	-
	10.0 μg L^{-1}	-	-	-	-
48 h	0 μg L^{-1}	-	-	-	-
	2.5 μg L^{-1}	-	-	-	-
	5.0 μg L^{-1}	-	-	-	-
	7.5 μg L^{-1}	-	-	-	-
	10.0 μg L^{-1}	+	+	+	-
72 h	0 μg L^{-1}	-	-	-	-
	2.5 μg L^{-1}	-	-	-	-
	5.0 μg L^{-1}	-	-	-	-
	7.5 μg L^{-1}	+	+	+	-
	10.0 μg L^{-1}	++	++	++	+
96 h	0 μg L^{-1}	-	-	-	-
	2.5 μg L^{-1}	+	+	-	-
	5.0 μg L^{-1}	++	++	+	+
	7.5 μg L^{-1}	+++	+++	++	++
	10.0 μg L^{-1}	++++	++++	+++	+++

Remark: unchanged (-), mild occurrence (+), moderate occurrence (++), severe occurrence (+++) and most severe occurrence (++++)

Discussion

In present, insecticide and herbicide contamination is higher than that in the past because they have been used to produce more food to serve higher demand. However in the case of excessive or inappropriate use, it can cause environmental problem. In Thailand, abamectin, is used widely in many proposes such as a veterinary anthelmintic, medicine against a variety of animal parasites and insects. Abamectin can reach the aquatic environment by water runoff (Tišler and Eržen, 2006). Ultimately, abamectin causes toxic effect in both aquatic organism and human. Thus, the aim of this

study was to assess toxicity of abamectin on prawn which is an economic important animal. Its toxicity was tested based on mortality rate and histological alteration. The concentrations of abamectin applied as 0, 2.5, 5.0, 7.5 and 10.0 μg L^{-1} and exposure time as 24, 48, 72 and 96 h.

Many behavior alterations could be observed after pollutants exposure. The assessment of behavior changes provides the important tool to evaluate sublethal exposure effect. However, comprehensive knowledge about the relations of behavior alterations observed in the laboratory in the field is still required. After shrimps exposed to pesticides in sublethal level, they showed many behavioral alterations; restlessness and hyperexcitability, tremor in the appendages, uncoordinated swimming movement, spams and violent action of legs (Reddy and Rao, 1990; Roque *et al*., 2005; Garcĭa de la Parr *et al*., 2006). These symptoms were also observed in this study. The exposed prawn movement and swimming were faster than that of the non-exposed. Moreover, it was found tremor in the appendage. All alterations increased with an increasing in concentration. The toxicity of contaminant is influenced from many processes such as solubility, bioaccumulation, and biomagnification (Larter *et al*., 2010; Cui *et al*., 2011) which result in physiological, biochemical, cellular processes and nervous system in each aquatic organism (Tu *et al*., 2010). Devi and Fingerman (1995) found the alterations of acetylcholinesterase level in crustaceans. They hypothesized that it may result in improper impulse transmission which makes loss of coordination. Moreover, they suggested that mucous secretion in gills might be used as warning signal indicating prawn expose to toxicant. This phenomenon is a responding process to inhibit uptake of abamectin. This finding is in agreement with the study of Verma (2012) which reported that heavy metals the prawn exposed were trapped in the mucous layer. The excessive of mucous excretion may cause death because of coagulation precipitation with metals on gill surface.

Histological alterations are the potential indicator to monitor toxicant exposure (Cengiz and Ünlü, 2003). In aquatic organisms, fish is the popular species used in this propose. In prawn, there have been some researches focusing histological alterations after exposed to toxicants. The target organs studied in prawn were ovaries and muscle (MS and Ali, 2014), hepatopancreas and gill (Fŕas-Espericueta *et al*., 2008). However, the organ being selected to study was depended on the objective of the research. Moreover, Bernet *et al*. (1999) also reported that the alterations were influenced from species, sex, and surrounding conditions.

The primary site of exposure is muscle tissue and it tends to be affected the muscle epidermis abruptly. Pigment cells are dominant part of chronic inflammatory response (Ms and Ali, 2014). Many histological alterations observed in the muscle of exposed prawn such as swelling of muscular and infiltration of hemocyte. This finding is in agreement with the study of MS and Ali (2014) which studied

prawn after exposed to malathion and glyphosate. They found degeneration of muscle, necrosis of muscle fibers, hemorrhage and appearance of pigmented cells. However, it was different from the study of Stalin *et al*. (2013) which found shrinkage of muscular fiber and necrotic musculature in prawn irradiated with ^{60} Co gamma radiation.

Gills are the sensitive target in many crustaceans exposing to toxicants. The gill plays an important role in gas exchange, osmoregulation and excretion (Fŕas-Espericueta *et al*., 2008). Thus, the alteration of gill after exposed to abamectin was also studied. The alterations in gill of exposed prawn were swelling of gill lamellae, hemocytic infiltration, lifting of lamellae and necrosis of lamellae. Our results is in agreement with the study of Fŕas - Espericueta *et al*. (2008) which reported that they found necrosis, loss of regular structure and infiltration of hemocyte in gill of *Litopenaeus vannamei* after exposed to copper. In 2009, Bahavan and Geraldine found hemocoelic space of gill lamellae, fused gill lamellae and necrosis of lamellae in gill of prawn (*M. malacolmsonii*) after exposed to cabaryl. And Satalin *et al*. (2013) also observed freshwater prawn (*M. rosenbergii*) irrated with ^{60} Co gamma radiation having altered gill structure; swollen and fused lamellae and necrosis of lamellae.

Conclusion

Abamectin is an insecticide widely used thus it could contaminate aquatic environment and further cause adverse effect to human being. In the study, its effect on exposed prawn was both mortality rate and histological alterations. The severity depended on exposure time and concentration of abamectin prawn exposed. The highest alteration found in prawn exposed to highest concentration which was 10.0 µg L^{-1} at 96 h. Histological alterations in muscle which were found were swelling of muscular and infiltration of hemocyte. And, swelling of gill lamellae, hemocytic infiltration, lifting of lamellae and necrosis of lamellae were observed in altered gill. However, the alterations in both tissues also increased with an increasing in abamectin concentration and exposure time. Based on this study, prawn (*M. rosenbergii*) could be applied as bio-indicator to monitor abamectin in the aquatic environment.

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