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Effect of a commercial rice pesticide on embryonic and larval development of climbing perch (*Anabas testudineus*)

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Abstract

Pesticides are chemicals that are used to kill pests, including insects, rodents and fungi. Pesticides are very toxic to other organisms, including humans, animals and environments. This research was aimed to examine the effect of a commercial brand rice pesticide on embryonic and larval development of *Anabas testudineus*. The treatment used in this experiments were divided into two; embryonic and larval development. For embryonic development, treatments were prepared in different concentrations of a commercial brand rice pesticide, *Brand X*, as follows: control, 50 ppm, 100 ppm, 150 ppm and 200 ppm. Embryonic development was observed from 0 hour until hatched, approximately 24 hours. Meanwhile, for larval development, treatments were prepared in different concentration of *Brand X* as follows: control, 10 ppm, 30 ppm, 50 ppm, 70 ppm, 100 ppm, 150 ppm and 200 ppm. The results showed deformities found in embryonic development in more than or at 100 ppm of the pesticide. Larval development results showed the behavioural changes in the larval and all the larval died after 5 days of pesticide treatments. Gills histology of the fish larvae treated with the pesticide showed degradation, necrosis, adjacent of secondary lamellae and blood congestion. Furthermore, treatment at 10 ppm of the pesticide in larvae also showed bone deformity. This study showed the harmful effects of rice pesticides to the aquatic organisms and the pesticides is not suitable to be used in a high concentration (>50 ppm) as it will disrupt biodiversity for a sustainable agriculture.

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Introduction

Aquaculture industries become one of the contributors of global seafood production by 44.1% in 2014 (FAO, 2016). Fisheries industries was no longer as one of the important source of seafood for human consumption but have been placed by aquaculture sector. It is estimated the fish consumption in Asia, Africa, American and European countries will increase during 2010 to 2030 (Kobayashi *et al.*, 2015). The significant trend of aquaculture industry in Malaysia was too intense to achieve higher production. However, at the same time, pollutions from nearer industries that contained chemicals such as pesticides or herbicides could harm the aquaculture system (Mohamed *et al.*, 2000). Pesticides are used for insect, and animal control. Negative impacts of some pesticides could lead to low survival rate and female reproduction effects on fish. Pesticides cause toxicity through several different methods especially when they go directly in the water. The exposure to pesticides resulting in decreased fertility, developmental abnormalities and endocrine disruption in most amphibian, fish and reptiles (Khan *et al.*, 2005). Substantial development of the land use and the significant changes effect on different aquatic habitats that lead to immerse the pesticides in water (Rabalais *et al.*, 2009). Pesticides carried by run-off into aquatic habitats where it affected the physiological traits and ecological consequences (Méjanelle and Laureillard, 2008). The exert considerable effects on the physiology, metabolism, reproduction, behaviour and survival of the aquatic organisms. The Mekong Delta is one of the examples of cultivated area that used a massive amount of pesticides (Toan *et al.*, 2013), where this area in Vietnam is one of the largest production of rice. The massive use of chemical products including pesticides increased the contamination of water (Stampini and Davies, 2009). Previous study was carried out to observe the effect of pesticides in catfish (*Clarias batrachus*) and zebrafish (*Danio rerio*). The pesticide concentration affected the growth and survival performance of *C. batrachus*. The results showed reduction in liver mass of *C. batrachus* (Narra *et al.*, 2015) and behavioural changes such as slow swimming and loss appetite when *C. batrachus* exposed to pesticides (Jordaan *et al.*, 2013). Pesticide also exerts more toxic effect in zebrafish. The physiological, morphological, behavioural and biochemical characteristics of the embryonic stage was not fully developed (Li *et al.*, 2014).

In Malaysia, few studies have been conducted on the effect of pesticides towards fish or any aquatic organisms. There is no available data on the effect of pesticide on survival and growth in one of native species in Malaysia, *Anabas testudineus* (Bloch, 1792), also known as climbing perch. Climbing perch is a tropical climate species that live between 22°C to 30°C in freshwater and brackish environment. This species can be found in cannels, lakes, ponds, estuaries as well as rice paddy fields (Vidthayanon, 2002). In this study, the effect of pesticide towards hatching rates, survival and growth of the species were observed in details. This study would provide information on the effect of a local brand of rice pesticide towards development of embryonic and larval of climbing perch.

Materials and methods

Induced breeding of Climbing Perch

Broodstock of female and male of climbing perch were injected with Ovatide® hormones, which help the induction of oocytes ovulation and stimulate pituitary gland to produce testosterone hormone for matured male sperms. The male and female broodstock were weighed to measure the correct dosage of the hormone, 0.5 ml/kg for female fish and 0.25 ml/kg for male fish, respectively.

The broodstocks were put into water containing clove oil to calm the fish. The injections were performed through intramascular muscle (IM) and the needle position must be at 45° from the fish body. The fish were put back into an 80 L aquarium tank with aeration to gain conscious and the top of the tank was covered with a net to avoid from jump out. After eighteen hours, the male and female of the climbing perch were already spawned and laid eggs inside the tank.

Preparation of Different Treatments for Embryonic Development of Climbing Perch

A commercial rice pesticide was obtained from Dr. Izera Ismail, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. The stock concentration of the rice pesticide, *Brand X,* was 10 g/L = 1 ppt (10 g = 1 ppt in 1 L of water). Different concentrations were prepared as follow: Control, 0 ppm (250 mL of water + 0 mg of *Brand X*), 50 ppm (250 mL of water + 12.5 mg of *Brand X*), 100 ppm (250 mL of water + 25.0 mg of *Brand X*), 150 ppm (250 mL of water + 37.5 mg of *Brand X*), and 200 ppm (250 mL of water + 50.0 mg of *Brand X).*

Treatment of Pesticides in Embryonic Development of Climbing Perch

Ten fish eggs of the climbing perch were put into each well of a tissue culture plate (6x4 wells). Five different treatment of a rice pesticide *Brand X*: Control (0 ppm), 50 ppm, 100 ppm, 150 ppm and 200 ppm), each in triplicate, were applied on the fish eggs.

Embryonic developments of the eggs were observed under a compound microscope in every 3 hours for 24 hours, until hatched. The water temperature was maintained at 25-32°C. The hatching rate was observed and recorded.

Preparation of Different Treatments for Larval Development of Climbing Perch

From previous experiment, similar commercial rice pesticide, *Brand X*, was applied for the treatment (Stock concentration: 10 g = 1 ppt in 1 L of water). Different concentrations were prepared as follow: Control, 0 ppm (1L of water + 0 mg of *Brand X*), 10 ppm (1L of water + 10 mg of *Brand X*), 30 ppm (1L of water + 30 mg of *Brand X),* 50 ppm (1L of water + 50 mg of *Brand X),* 70 ppm (1L of water + 70 mg of *Brand X),* 100 ppm (1L of water + 100 mg of *Brand X),* 150 ppm(1L of water + 150 mg of *Brand X),* and 200 ppm (1L of water + 200 mg of *Brand X).*

Treatment of Pesticides in Embryonic Development of Climbing Perch

After two weeks of larval rearing, climbing perch larvae were treated with the rice pesticide (*Brand X*) with different treatments: Control (0 ppm), 10 ppm, 30 ppm, 50 ppm, 70 ppm, 100 ppm, 150 ppm and 200 ppm. All treatments were run in triplicate in 2 L beakers. All the treatments were aerated and the temperature maintained at 25-32°C.

The larvae were fed twice daily with *Artemia* sp. The experiments continued for 2 weeks, and the behavioural changes were observed including swimming behaviour and feeding appetite and mortality.

Fish histology

After two weeks, samples of climbing perch larvae for from every treatment were sacrificed following fixation process with 70% ethanol for 24 hours.

The samples were then preserved with 10% formalin. For histological process, the larvae were embedded in paraffin, followed by sectioned at thickness of 5-7 µm and stained with haematoxylin and eosin solution. The slides were examined under a light microscope (Leica DFC 295, Leica Microsystems, and Queenstown, Singapore).

Results

Effect of a Rice Pesticide Treatment in Embryonic Development of Climbing Perch

Results of embryonic development of climbing perch after treatment with a rice pesticide, *Brand X,* for every 3 hours were showed in Fig. 1. The development were observed and determined under a compound microscope.

At 0 hour of *Brand X* treatment (Fig. 1A), all embryo were observed at mid gastrula stage. After 3 hours of treatment (Fig. 1B), for Control (0 ppm) treatment, the embryonic development reached at 6 somites, where the brain and optic vesicle were formed.

For 50 ppm treatment, an early neurula stage, where the head was formed. For 100 ppm, 150 ppm and 200 ppm treatments, the embryo showed late gastrula stage.

Table 1. The behaviourial changes of climbing perch (*A. testudineus*) recorded and observed during the larval development after treatment with pesticide. The behaviourial changes recorded are mortality, swimming behaviour and feeding appetite.

Observations	Treatments							
	Control 0 ppm pesticide *Brand X*	10 ppm pesticide *Brand X*	30 ppm pesticide *Brand X*	50 ppm pesticide *Brand X*	70 ppm pesticide *Brand X*	100 ppm pesticide *Brand X*	150 ppm pesticide *Brand X*	200 ppm pesticide *Brand X*
Mortality	No mortality	Died after 5 days of treatment	Died after 2 days of treatment	Died 24 hours after treatment	Died after 5 hours of the treatment	Died after 11 minutes of treatment	Died after 6 minutes of treatment	Died immediately after 2-3 minutes of the treatment
Swimming behaviour	Swimming normally	Stayed at the bottom of the beaker and less swimming activity, gasped for the air at the surface of the water	Stayed at the bottom and no swimming behaviour showed, gasped for the air after 24 hours of the treatment	Gasped for the air after the treatment and swam vigorously to the surface of the wate	Stayed at the surface of the water for air throughout the 5 hours treatment	Swam towards the surface of the water and died	Swam towards the surface of the water and died	Swam towards the surface of the water and died
Feeding appetite	Normal feeding activity	Normal swimming appetite for the first 3 days of the treatment	Low feeding appetite	No feeding appetite	No feeding appetite	No feeding activity	No feeding activity	No feeding activity

After 6 hours of treatment (Fig. 1C), the embryonic development for Control (0 ppm) treatment reached at the stage of 16 somites, where the heart has started to beat, while treatments at 50 ppm and 100 ppm, the embryonic development reached at the stage of 12 somites, where the heart was formed.

Treatments of 150 ppm and 200 ppm showed the embryonic development at the stage of 9 somites, where there was an appearance of heart anlage. After 9 hours treatment (Fig. 1D), newly hatched larvae were observed in Control (0 ppm) and 50 ppm treatments, while treatment at 100 ppm was at the stage of 18-19 somites, where there was onset of blood circulation.

For 150 ppm treatment, there were at 16 somites stage, where the heart start to beat, while at 200 ppm treatment, the heart started to formed at stage of 12 somites. After 20 hours (Fig. 1E), the Control (0 ppm), 50 ppm and 100 ppm treatments, there were one day old of larvae, however for 100 ppm, deformaties were detected on the larvae. In 150 ppm treatment, the embryo were still at 16 somite stage, where the heart beat was started, while at 200 ppm treatment, the heart just formed at 12 somite stage.

Effect of a Rice Pesticide Treatment in Larval Development of Climbing Perch

Data for the effect of a rice pesticide in larval development could only be reported for Control (0 ppm), 10 ppm and 30 ppm of treatments. For 50 ppm, 70 ppm, 100 ppm, 150 ppm and 200 ppm treatments, all the larvae died within 2 minutes to 24 hours after treated with the pesticides.

Table 1 below showed the behaviourial changes including mortality, swimming behaviour and feeding appetite that were observed and recorded during the larval development after treatment with the pesticide.

After five days of treatment, the Control (0 ppm) treatment grew normally, with a good appetite towards live feed and the size are larger compared to 10 and 30 ppm of pesticide treatment.

There was no mortality recorded and all the larvae swam normally. For 10 ppm pesticide treatment, majority of climbing perch larvae died after five days. After 24 hours of treatment, most of the larvae stayed at the bottom of the beaker and there were less swimming activity was observed. The larvae gasped for the air at the surface of the water.

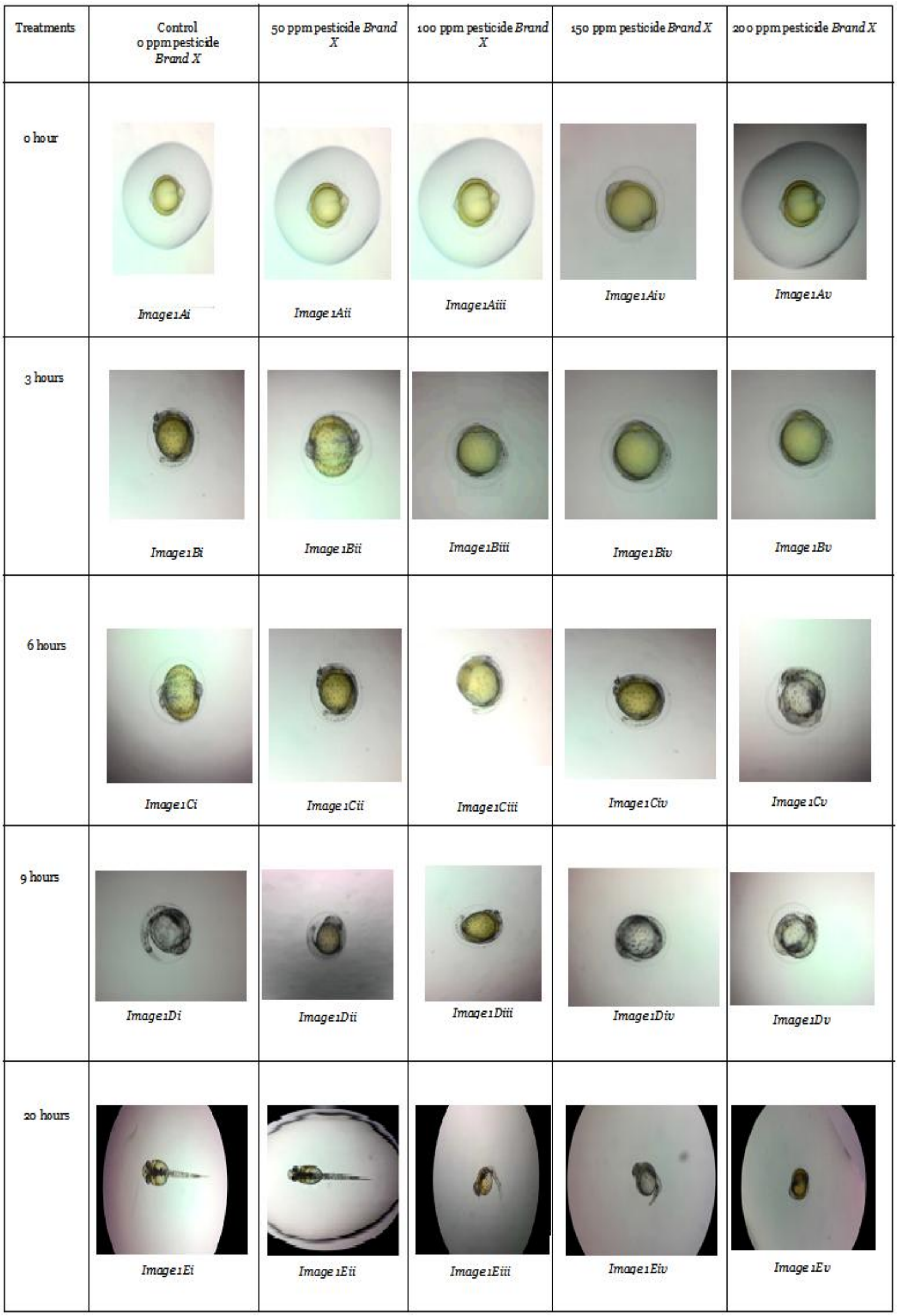

Fig. 1. Embryonic development of climbing perch (*A. testudineus*) after treatment with a commercial rice pesticide, *Brand X*.

The feeding appetite was normal for three days after treatment, but the appetite was reduced after five days of treatment. For 30 ppm pesticide treatment, majority of larvae died after 48 hours of post-treatment. During the treatment, the larvae had less feeding appetite and stayed at the bottom of the beaker and no swimming behavior was observed. The larvae gasped for the air at the surface of the water.

Fig. 2. Gills histology of climbing perch (*A. testudineus*) larvae in Control treatment; (bv) blood vessel, (s) secondary lamellae.

Histology

The histology of the gills from the climbing perch were obtained and compared between the Control (0 ppm) and pesticide treated groups. The gills in Control (0 ppm) treatment had normal blood vessel and secondary lamellae (Fig. 2). For the pesticide treated larvae, the gills revealed degradation, necrosis, adjacent second lamellae and blood congestion (Fig. 3 and Fig. 4). Most of the pesticide treatments led to gills degradation.

Discussion

In this study, a commercial rice pesticide, *Brand X*, was found to be toxic to the embryo of climbing perch at high concentration, respectively. The chemical from the pesticide directly affected the fertilized eggs and developing embryo. The study showed that after 20 hours, the fertilized eggs did not hatch when treated with the pesticide at the concentrations up to 150 ppm and 200 ppm, while at 100 ppm the fish larvae was hatched but deformity. Previous study revealed that the complete cycle embryonic development in climbing perch from the fertilized eggs until the newly hatched larvae was around 20 hours, respectively (Zalina *et al.*, 2012).

The behavioural changes of climbing perch larvae development were detected after the pesticide treatments, while higher concentration of *Brand X* killed the fish in less than 5 hours. Initially, they loss their feeding appetite as they were gasping for oxygen at the surface of water. Study from Renick *et al.* (2016), the estuarine fish showed negative effects on their swimming behaviour after treated with pesticides. The swimming speed of the fish were lower.

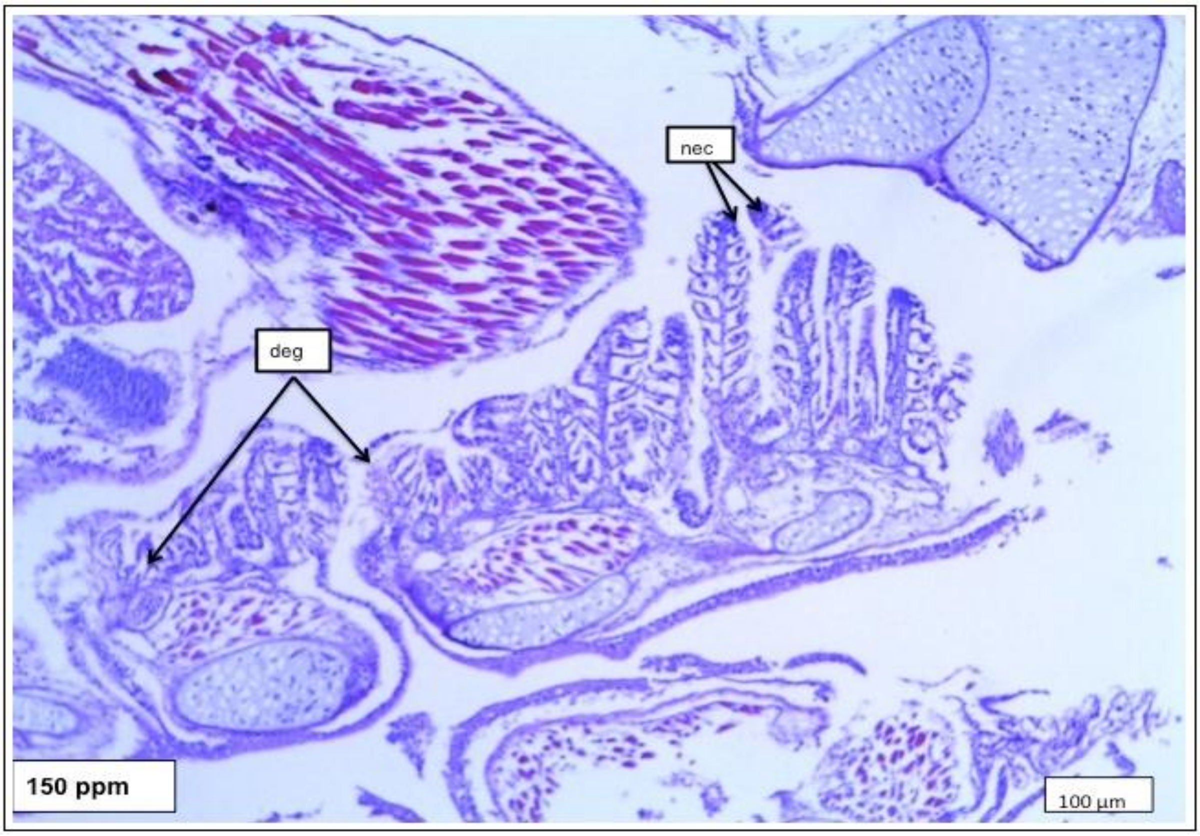

Fig. 3. Gills histology of climbing perch (*A. testudineus*) larvae in 150 ppm treatment; (deg) degradation, (nec) necrosis.

The estuarine fish exposed to sublethal concentrations of organophosphate pesticide that exhibits changes in their neurotransmitter activity and hormone release rates.

Previus study showed tilapia fish that survived at highly polluted area could accumulate heavy metal rather than less poluted area (Bahnasawy *et al.*, 2009). From the study, the heavy metals detected at high concentration were recorded in liver and gills (Lasheen *et al.*, 2008).

The heavy metal detected in gills may cause due to lower binding affinity of metals on the surface of the gills. Low accumulation also could happen due to the development of some defensive mechanism, for example excessive mucos secretion that can clog the gills (Osman and Kloas, 2010).

Study on estuarine fish (California killifish) reported the effect from pesticide residue showed the brain and lateral muscle tissue contain higher pesticide residue when treated with pesticide (Sandahl and Jenkins, 2002). In addition, study of bioaccumulation and biochemical on Nile tilapia showed heavy metal residues in the tissues of Nile tilapia exhibited different patterns of accumulation and distribution among the selected tissues and localities. Effects of high concentrations of a heavy metal on fish are threatening and combinations with other heavy metals produce a toxic effect to the fish (Yacoub, 2017).

Another previous study on a catfish (*Clarias batrachus*) showed sub-lethal of pesticide were negatively effected the growth and survival performances. Continuous application of pesticides resulting in deppression in feeding or energy, and reduction of liver mass which related with protein degradation genitival alteration (Narra *et al.*, 2015). From this study, the results showed similar pattern on the infected of gills, where fish treated with commercially *Brand X* pesticide had been highly effected in their gills.

Fig. 3. Gills histology of climbing perch (*A. testudineus*) larvae in 150 ppm treatment; (deg) degradation, (nec) necrosis.

Treatment of a commercial rice pesticide, *Brand X*, to embryo and larvae of climbing perch (*Anabas testudineus*) showed changes on the growth and development. Based on the results, there were clear differences between treatments of the pesticide in embryonic development (>100 ppm) and larval development (>30 ppm). High concentration of the pesticide led to deformities and unhatched climbing perch embryo.

This study indicated high concentration of pesticides, may not suitable to be used in paddy field area, as it could effect the aquatic organisms including fishes, mollusc and gastropods. In future, farmers who are using pesticides should concern and use the pesticide at low concentration (<10 ppm) in order to maintain the aquatic ecosystem for a sustainable agriculture.

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