



## Behavior, survival rate and histological alterations in Nile tilapia (*Oreochromis niloticus-mossambicus*) after exposed to abamectin

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### Abstract

Nile tilapia (*Oreochromis niloticus-mossambicus*) was applied to assess the toxicity of abamectin contamination in aquatic environment because it is normally found. It is classified as important protein source in many areas. In the case of being contaminated, it causes adverse effects in both fish health and human which is the top consumer. In this study, its toxicity was assessed based on behavior, survival rate and histological alterations in gill, liver, and intestine. The abamectin concentrations were 2.5, 5.0, 7.5 and 10  $\mu\text{g L}^{-1}$  and exposure times at 24, 48, 72 and 96 h. After fish was exposed to abamectin, their behavior was changed that they swam without direction. Besides, their operculum frequently opened and closed. Then, they floated on surface of water and eventually died. In the concentration of 2.5  $\mu\text{g L}^{-1}$  survival rate remained 100% until the end of experiment. For other concentrations, the rate decreased with an increasing in abamectin concentration and exposure time. The alterations observed in gill were hyperplasia, partial fusion of gill lamellae, edema and epithelial lifting. In the liver, cell was deformed and blood congestion, vacuolation and necrosis of hepatocyte were observed. And, lesion and necrosis in intestine of exposed fish were observed. Thus, it concluded that all alteration observed could be used as warning signal in other fish and Nile tilapia has a potential to be alternative bio-indicator for toxicant contamination in the waters.

**Keywords:** Abamectin, Behavior, Histology, Fish, Survival

### Introduction

Abamectin or generally known in the trade name as A.G.BA, Dimectin, Agrotin, Abama, and Jacket is insecticide obtained from fermentation process by soil bacterium, *Streptomyces avermitilis*. The products extracted from this process are avermectin B1a and avermectin B1b having the same physical properties and toxicity; however, avermectin B1a showing prominent insecticidal properties. Thus, most products contain at least 1.44 % or 1.8 % W/V EC of avermectin B1a (Fisher & Mrozik, 1989).

For controlling insects, United States Environmental Protection Agency (USEPA, 2010) has recommended that abamectin can be used to

control mites, thrips, leafminers, leafhoppers, psyllids, potato beetles, skeletonizers, and pinworms. Seeduangkaew, Kulsarin, Buranapanichpan, and Kumpiro (2015) studied the efficiency of three insecticide; abamectin, cypermethrin and carbaryl, and found that they could eliminate 100% cotton aphid pests (state 3) on the eggplant in netted house. However in the excessive application, abamectin can cause adverse effect in both environment and human being.

Abamectin is high lipophilic insecticide, thus, it tends to accumulate in soil. After that, it may be assimilated into organism body especially invertebrates and fish (Nazifi, Firoozbakhsh, & Bolouki, 2000). These phenomenon cause high



concerns in human health issue, because in many parts of the world, fish is a very important protein source and it is high sensitive to contamination. However, the sensitivity to toxicant vary in each fish species, thus, toxicological assessment should be performed with concerning the difference of fish species and other surrounding conditions (Riehl & Baensch, 1996). In 1990, Høy, Horsberg and Nafstad reported that avermectin which is a constituent of abamectin can pass the blood / brain barrier of the organism. Abamectin can disturb osmoregulation, hormonal regulation, biochemical constitutes and oxygen consumption (El-Said, 2007; Al-kahtani, 2011). Moreover, it was reported that abamectin also inhibits the function of gamma amino acid butyric acid in the nervous system to cause death (Hedayati, Vajargah, Yalsuyi, Abarghoei, & Hajiahmadyan, 2014). Jenčič, Cerne, Kozuh Erz'en, Kobal, and Cerkvenik Flajs (2006) suggested that LC50 at 48 h of abamectin is 1.5 mg L<sup>-1</sup> based on the results of their study performed in rainbow trout, while it is 1.24 mg L<sup>-1</sup> in common carp (*Cyprinus carpio*) (Hedayati et al., 2014).

Recently, the assessment of abamectin toxicity in Thailand has not been so far studied; thus, this study was performed that gap. The aim of this study was to assess the toxic effect of abamectin on Nile tilapia which is a very important economic fish. The toxicity was assessed based on behavior, survival rate, and histological alterations.

## Material and Method

### Animal husbandry and abamectin treatment

Juvenile Nile tilapia were obtained from farms located in Surin Province, northeastern Thailand. They were placed in 2,000 L tanks of aerated freshwater and temperature kept at 25 °C for 7 days of acclimatization with no feeding. After that, fishes

(n=10) were immersed with abamectin to a final concentrations of 2.5, 5.0, 7.5 and 10 µg L<sup>-1</sup>. Tested fish was maintained in 50 L tank. Next, they were monitored for behavior alteration and survival rate that was also calculated by using following equation.

$$\text{Survival of fish (\%)} = \frac{\text{No. of fish survival}}{\text{Total No. of fish stocked}} \times 100$$

Then, the fish was sacrificed after 0, 24, 48, 72 and 96 h and immediately fixed in 10% phosphate buffer formalin.

### Histological alterations

The target tissues of fish i.e. gill, liver and intestine, were cut into small pieces and fixed in bouin's fixative solution for 24 h. Next, it was dehydrated in a graded series of ethanol i.e. 40%, 50%, 70%, 80%, 90%, and absolute ethanol. Then, it was immediately cleared with xylene, infiltrated, and embedded in paraffin. After that, the prepared tissue was cut into a 6 µm thickness and stained with hematoxylin and eosin (H & E). Then, the coverslips with permount were added. The alterations in tissue were studied under the light compound microscope (Primo Star, ZEISS) and photographed by digital camera (Nikon coolpix S 5100).

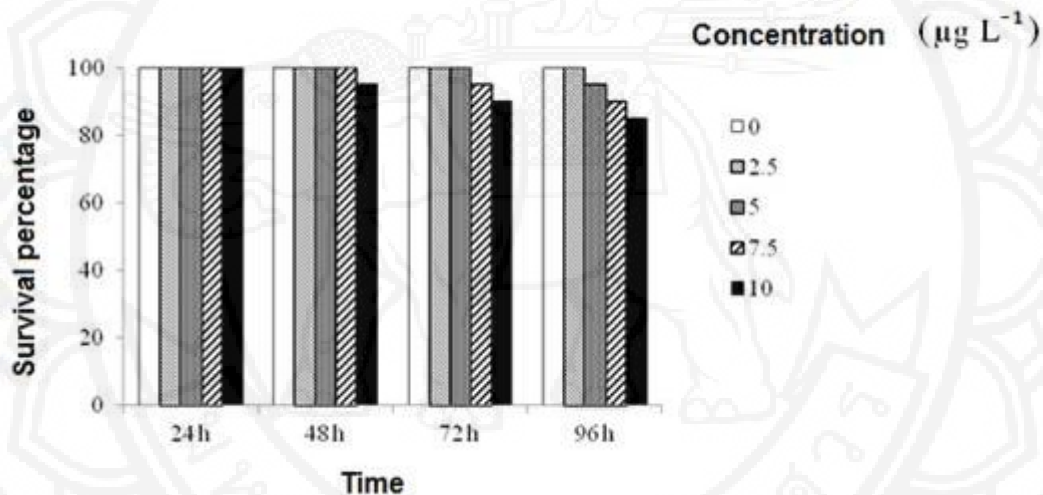
### Histological record

The alterations were classified based on the injury index described by Bernet et al. (1999) and Genten, Terwinghe, and Danguy (2009) with the slight modification. The morphological changes that observed in gill, liver, and intestine were classified in four severity factors, i.e., unchanged or changing less than 10% (-), mild occurrence with changing 10-30% (+), moderate occurrence with changing 31-70% (++) and severe occurrence with changing 71-100% (+++).

## Results

In this study, abamectin toxicity was tested in Nile tilapia because it is very important economic fish and can be found in all parts of Thailand. The toxicity was assessed based on behavior, survival rate and histological alterations in the fish after exposed to abamectin at the concentrations of 2.5, 5, 7.5 and 10  $\mu\text{g L}^{-1}$  for 24, 48, 72 and 96 h. Basically, exposed fishes swam unusually and hyperactivity while body pigmentation was greatly reduced. Operculum frequently opened and closed. Moreover, surfacing and gulping of air were observed. In high

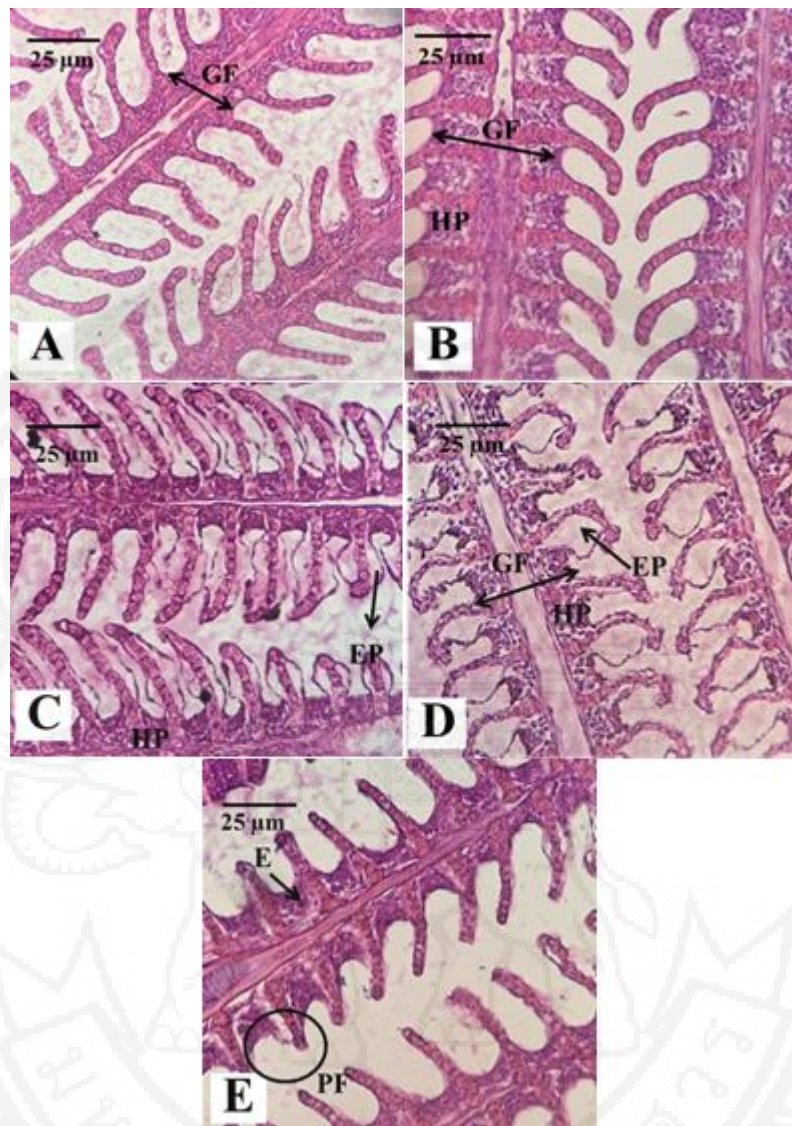
concentrations, 7.5 and 10  $\mu\text{g L}^{-1}$ , it was found that exposed fish floated on the water surface and died. However, the survival rate remained 100% until the end of experiment in lowest exposure concentration at 2.5  $\mu\text{g L}^{-1}$ . At the concentration of 5  $\mu\text{g L}^{-1}$ , survival rate decreased with an increasing in exposure time until it was 95% at 96 h. The survival rates in the concentration of 7.5  $\mu\text{g L}^{-1}$  were 100%, 100%, 95% and 90% at 24, 48, 72 and 96 h, respectively. For the highest concentration, it was found the lowest survival rate at 85% after 96 h of exposure as shown in Figure 1.



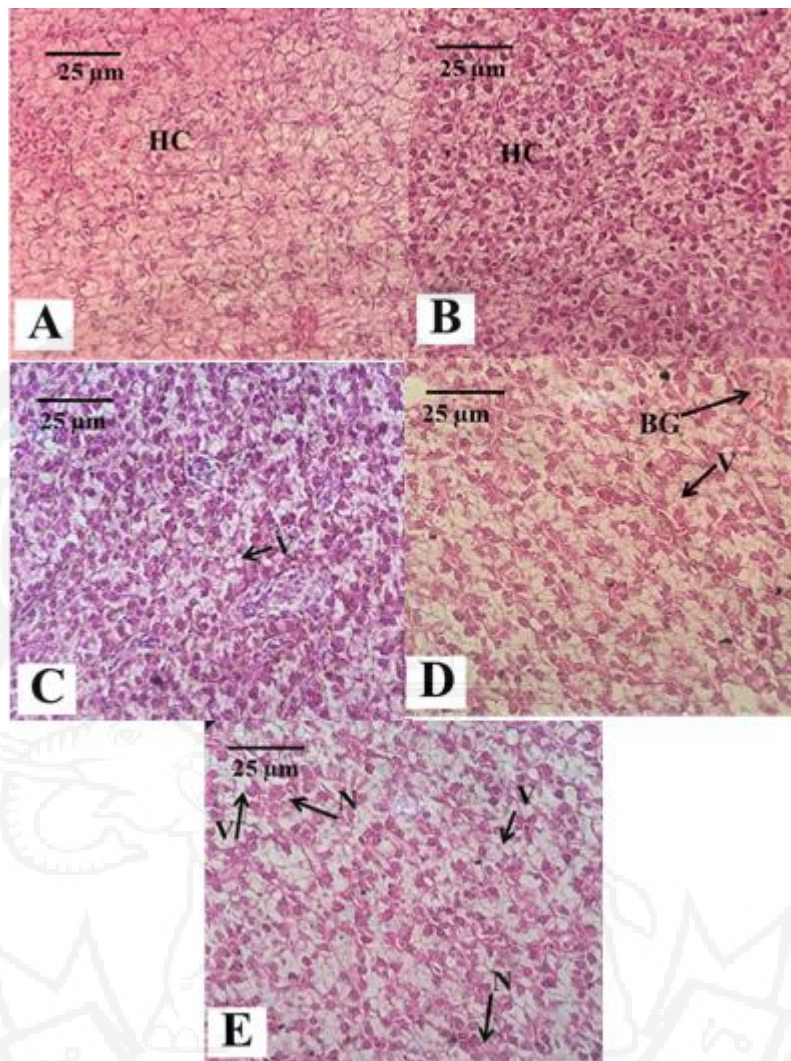
**Figure 1** Survival rate in Nile tilapia after exposed to abamectin at 0, 2.5, 5.0, 7.5 and 10  $\mu\text{g L}^{-1}$  for 24, 48, 72 and 96 h

In histological alteration, the assessment was performed in three organs: gill, liver and intestine. In the gill of exposed fish, it was found hyperplasia, epithelial lifting, edema, and partial fusion of the lamellae (Figure 2B-2E and Table 1). And, the most alterations were observed in the fish exposed to highest concentration of abamectin at 10  $\mu\text{g L}^{-1}$  for 96 h.

In the liver of exposed fish, it was observed vacuolation, blood congestion, necrosis of hepatocyte were observed (Figure 3B-D and Table 2) when compared to the control which had normal hepatic tissue, hepatocytes were observed with granular cytoplasm and central and round nucleus (Figure 3A). The alteration increased with an increasing in abamectin concentration and exposure time

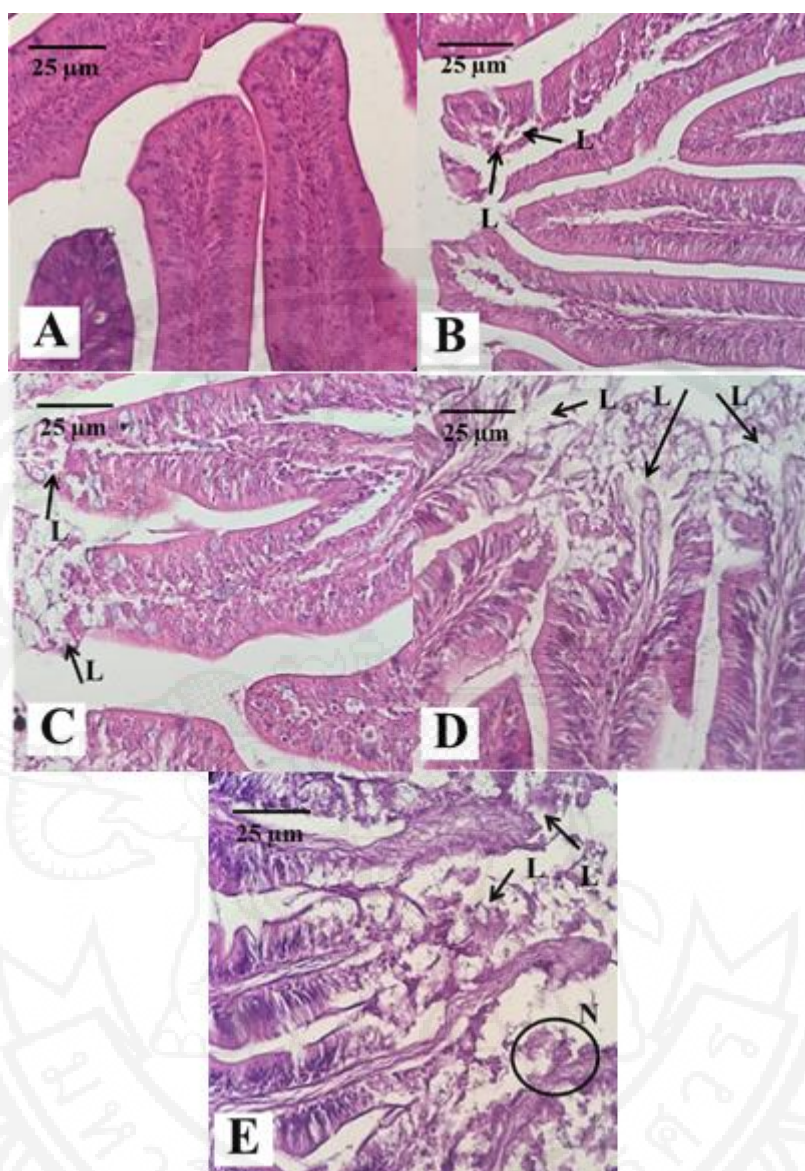


**Figure 2** Histological alterations in gill tissue of Nile tilapia in the control group (A), fishes exposed to abamectin at 2.5 (B), 5.0 (C), 7.5 (D) and 10 (E)  $\mu\text{g L}^{-1}$  for 96 h, where GF: Gill filament, HP: Hyperplasia, EP: Epithelial lifting, E: Edema, PF: Partial fusion the lamellae



**Figure 3** Histological alterations in liver tissue of Nile tilapia in the control group (A), fishes exposed to abamectin at 2.5 (B), 5.0 (C), 7.5 (D) and 10 (E)  $\mu\text{g L}^{-1}$  for 96 h, where HC: Hepatocyte, BG: Blood congestion, V: Vacuolation, N: Necrosis of hepatocyte

In the intestine of the control, it is composed of four tissue layers. Microvilli had a small protrusions on the surface and goblet cells in between the epithelium inside of the small intestine. In exposed fish, lesion and necrosis in microvilli were found (Figure 4B–4E) and the severity increased with increasing in concentration and exposure time as same as observed in gill and liver.



**Figure 4** Histological alterations in intestine tissue of Nile tilapia in the control group (A), fishes exposed to abamectin at 2.5 (B), 5.0 (C), 7.5 (D) and 10 (E)  $\mu\text{g L}^{-1}$  for 96 h, where: L: Lesion, N: Necrosis

**Table 2** Histological alterations in Nile tilapia exposed to abamectin at the concentrations of 2.5, 5.0, 7.5 and 10  $\mu\text{g L}^{-1}$  for 24, 48, 72 and 96 h

Organs	Histological alterations	2.5 $\mu\text{g L}^{-1}$				5.0 $\mu\text{g L}^{-1}$				7.5 $\mu\text{g L}^{-1}$				10 $\mu\text{g L}^{-1}$			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Gill	Hyperplasia	-	-	-	+	-	+	+	++	-	+	++	++	+	++	+++	+++
	Edema	-	-	-	+	-	-	+	++	-	+	++	++	+	++	++	+++
	Partial fusion	--	-	-	+	-	-	+	+	-	-	+	++	-	+	+	++
	Epithelial lifting	-	-	-	-	-	-	+	++	-	+	++	++	+	++	++	+++

**Table 2** (Cont.)

Organs	Histological alterations	2.5 µg L <sup>-1</sup>				5.0 µg L <sup>-1</sup>				7.5 µg L <sup>-1</sup>				10 µg L <sup>-1</sup>			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Liver	Vacuolation	-	-	-	-	-	-	-	+	-	-	+	++	-	+	++	+++
	Necrosis of hepatocyte	-	-	-	-	-	-	-	-	-	-	+	++	-	+	++	+++
Intestine	Lesion	-	-	-	+	-	-	+	++	-	+	+	++	+	++	++	+++
	Necrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++

**Remark:** Level of alteration: unchanged or changing less than 10% (-), mild occurrence with changing 10–30% (+), moderate occurrence with changing 31–70% (++) and severe occurrence with changing 71–100% (+++)

### Discussion

Generally known, fishes are very sensitive to the environmental changes and contamination. Many contaminants especially insecticides cause significant damage on physiological and biochemical processes resulting in serious impairment of fish health. In many areas around the world, aquaculture is fast growing sector in food industry to serve increased human need. However, it also makes the harmful effect on the environment by releasing the contaminants or toxicants into the aquatic environment. They can suppress immune system, lower metabolism, and damage gills and epithelia. The level of their effect (acute, sub-chronic and chronic) depends on the difference of concentrations. They can also make the alterations in behavior, genetic and immune system of fish (Sabra & Mehana, 2015). However, these changes can also be used as biomarker indicating toxicant exposure or the effects of environmental pollutants. For the advantage of biomarker, it can present toxicant effect in the term of biologically available; moreover, it can show the sum of each effects of multiple stressor and mechanisms of action.

Behavior alteration caused by toxicant exposure may affect to the fitness and survival in natural

ecosystems, and in some cases cause significant mortality. However, the effect of many toxicants in behavior alterations is not understood. Moreover, they can also disrupt sensory, hormonal, neurological, and metabolic systems which further effect fish behaviors. Unfortunately, the knowledge about integrative effect of behavior and physiology is still unclear. It requires multidisciplinary study to deal with possible mechanisms of behavioral alteration. Many researches focused on behavioral disruption such as cholinesterase (ChE) inhibition, the change in brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels (Scott & Sloman, 2004). Many behavior described in previous study was also found in this study, abamectin exposed fish showed swimming unusually, operculum frequently opened and closed. The muscle contracted and gill was inflamed and the some case the exposed fish died. As generally known, brain neurotransmitter levels and enzyme function strongly correlate and control fish behavior (Hofmann & Fernald, 2000; Höglund, Kolm, & Winberg, 2001). In 2014, Panigrahi Choudhury, and Tarafdar reported that fish exposed to the organophosphate pesticide for 96 h showing abnormal swimming, loss of equilibrium, fading of color, coughing and opercular



movements. Then, it was observed excited swimming and coughing in *Cyprinus carpio* because of disturbed ventilation in breathing resulting in operculum cavities quickly expansion and contract to clean debris accumulated in the gills.

Gill is an organ plays the important role in respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Heath, 1987). The fish gill can be damaged by the exposure of salts, heavy metals, pesticides, sewage and fertilizers (Temmink, Bouweister, De Jong, & Van Den Berg, 1983). Moreover, the direct exposure to xenosubstance can cause pathological alteration in fish (Mallatt, 1985). The gill is very vulnerable organ because it directly contacts to the surrounding environment; thus, it can be damaged by dissolved or suspended toxicant in the water (Roberts, 1978). In the gill of exposed fish, it was found hyperplasia, epithelial lifting, edema, and partial fusion of the lamellae compared to the normal state in the control. This finding is different from observed in *Oreochromis niloticus* which found necrosis in gills lamellae (El-Said, 2007). The contrast may be caused by the difference of fish species, toxicant concentration, and exposure time. In this study, the highest abamectin concentration was  $10 \mu\text{g L}^{-1}$  and exposure time was 96 h while it was  $103.68 \mu\text{g L}^{-1}$  and 14 days in the experiment of *Oreochromis niloticus*. And, Bernet et al. (1999) suggested that species and size had the influence on those alterations (Walker, Hopkin, Sibly, & Peakall, 2006).

The liver is an important organ playing a role in nutrient assimilation, production of bile, maintaining the body metabolic homeostasis and detoxification (Genten et al., 2009). After exposed to abamectin, Nile tilapia showed the alterations in liver tissue because this organ involving in metabolic homeostasis and detoxification. The alterations

observed were vacuolation, blood congestion, necrosis of hepatocyte. Our finding is in agreement with the study of Jenčić et al. (2006) which found vacuolar degeneration and single cell necrosis in the liver of rainbow trout (*Oncorhynchus mykiss*) after exposed to abamectin. Moreover, Olufayo and Alade (2012) also reported that catfish (*Heterobranchus bidorsalis*) having vacuolation and necrosis of hepatocyte in the liver after exposed to abamectin.

Intestine is the organ involving in food digestion and nutrient absorption (Genten et al., 2009). The alterations observed in fish after exposed to abamectin were lesion and necrosis of microvilli. The fish may expose to abamectin which dissolved in the water and then diffuse to all parts in fish body. In rainbow trout (*Oncorhynchus mykiss*) which exposed to abamectin, it was also found the alteration in intestine (Jenčić et al., 2006).

### Conclusion

Abamectin is the insecticide widely used thus it may reach to aquatic environment and cause the harmful effect on fishes. In the study, abamectin toxicity was assessed based on behavior, survival rate and histological alterations of exposed fish. After performed, these alterations were clearly observed; thus, it may be used as a potential bio-indicator for abamectin or other insecticide contamination in aquatic environment.

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