Evaluation of abamectin effect on some biochemical constituents and histological alterations in Asian sea bass (*Lates calcarifer*)

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Abstract

The aim of this study was to evaluate the effect of abamectin at the concentrations of 5, 10 and 15 µg/L for 24, 48 72 and 96 h on Asian sea bass (*Lates calcarifer*, Bloch). The results indicated that plasma electrolyte (Na^+ and Cl^-) in the exposed Asian sea bass significantly increased with an increasing of time after exposed to 5 µg/L of abamectin, at the same time, protein content in gill and liver significantly decreased. For histological alteration, we found that the alteration levels in gill, kidney and liver were increased with increasing exposure time. The histological alteration in gills was epithelial lifting and partial fusion of lamellae. It was found that the degradation of tubular, melanomacrophage aggregate, glomerulus deformed and necrosis of tubular cell in the kidney of exposed fish. Moreover, the results revealed vacuolation, blood congestion, and enlargement of sinusoid and necrosis of hepatocyte in the liver of the exposed fish.

Keywords: Abamectin, Asian sea bass, Electrolyte, Histological alteration

Introduction

Pharmaceuticals, drugs and cosmetics, have been continuously dispersed into the environment resulting in health risk concerns. Abamectin, which generally known in many trademark names such as A.G.BA, Dimatin, Agrotin, Abama and Jacket, is wildly used as insecticide. In the family of abamectin, avermectin B1a is generally used in both as a pesticide and an anthelminthic drug in animals. It naturally occurs in the fermentation process of *Streptomyces avermitilis*. Abamectin is a mixture of homologues B1a and B1b containing at least 80% B1a and the rest is B1b.

In 2009, Thailand imported abacmectin to eliminate several kinds of insects such as Cotton Leafhopper (Seeduangkaew, Kulsarin, Buranapanichpan, & Kumpiro, 2015). The farmers and veterinarians tend to use this pesticide because of their spectrum of activity, convenience and safety to the non-target animals. After getting in the insect body, abamectin has the effect on nervous system including neuron and muscle and especially the synapse in the brain resulting in blocking blood flow and eventually causing death.

In general, most pesticides are not only toxic on the target organism but also on non-target organisms such as fish (Al-Kahtani, 2011). Hedayati Vajargah, Yalsuyi, Abarghoei, and Hajaihmadyan. (2014) suggested that fish and aquatic organisms are prone to be sensitive to the toxicant, thus it has a potential to be used as bioindicator. Abamectin is high toxic substance to fish. Its LC_{50} (water exposure) at 96 h in rainbow trout are 3.2 ppb, in bluegill sunfish 9.6 ppb (Jenčič, Cerne, Erzen, Kobal, & Cerkvenik-Flajs, 2006). In fish, the abamectin or avermectins can be assimilated through blood/brain barrier and cause toxic effects (El-Said, 2007). Their primary target of is the nervous system. They interact with the glutamate-gated

chloride channels and GABA (c-amino butyric acid)-gated chloride channels resulting in strong chloride influx and disrupting neural signal transmission. Their mode of action is not specific to parasitic arthropods and nematodes, thus, they may affect to other useful organisms.

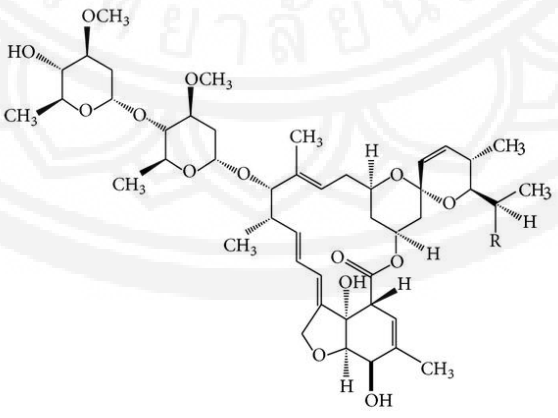
The nutritional value of fish to the consumer depends on their biochemical composition, which is mainly affected by surrounding environments it lives (Prado, Rioboo, Herrero, & Cid, 2009). The biochemical composition or its alterations are influenced from their environmental stress and being reported by many authors (Al-Kahtani, 2011). In addition, we can apply histological alterations as biomarker for stressors exposure. This kind of biomarkers allows the environmentalist to study the effect of toxicant on specific target organs and cells under in vivo condition. Moreover, it is quite rapid method to investigate both in acute and chronic effects in other tissues (Hinton & Laurén, 1993).

In Thailand, the study of the effect of abamectin in the aquatic organisms is few though the amount of insecticide has been being increased every year. The excessive of insecticide reaches to the aquatic environment by drift or runoff. The environmentalist tries to test the toxicity of each insecticide in laboratory and apply the results to predict its effects in the field. Thus, the aim of this study was to evaluate the effect of abamectin on Asian sea bass (*Lates calcarifer*, Bloch) which was cultured countrywide in both pond and cage. The Asian sea bass may expose to abamectin from surrounding agricultural area and carry that toxicant to human being which is the top consumer. The study on the effect of abamectin was performed by evaluating the amount of electrolyte (Na^+, Cl^- ions), protein content and histological alteration in gill, liver and kidney.

Materials and methods

1. Chemicals

Abamectin in technical standard grade (purity: 98.7%) was purchased from Sigma Aldrich (Thailand) (Figure 1). For other analyses; protein assays, analytical of electrolyte ions and histological alteration, the chemicals used were analytical grades and purchased from Amersham Pharmacia Biotech Inc and GE healthcare life science (Thailand).



(i) R = $-CH_2CH_3$ (avermectin B_{1a})
(ii) R = $-CH_3$ (avermectin B_{1b})

Figure 1 Abamectin structure

2. Animals husbandry and Abamectin treatment

Asian sea bass (*Lates calcarifer*, Bloch) used in this study was collected from Ang Sila, Chonburi province. Sampled fishes were filled in 2000 L tanks with aerated freshwater and feeding twice a day. They were acclimated for 14 days and water condition was kept constantly with temperature at 25.0±1.4°C, pH at 6.84± 0.02, dissolved oxygen at 3.6±0.20 mg/L and conductivity at 115.1±1.02 µmhos/cm. After that, juvenile fishes were selected, weighed and measured their size. The experiment was performed repeated three times (n=5). Abamectin (purity: 98.7%) was serially diluted with distill water until concentration at 5, 10, and 15 ug/L in the experimental tank of 500 L.

At the end of every exposure time (24, 48, 72, and 96 h), fishes from each experimental tank were collected. Their blood was drawn from the heart region by cardiac puncture which filled with heparin as an anticoagulant and then was centrifuged at 5,000 rpm for 10 mins. Then, the clear plasma was taken for further evaluation of sodium and chloride content by using colorimetric method. Finally, fish samples were sacrificed and their tissues such as gill, liver and kidney were collected (Figure 2).

Figure 2 Internal organs and general appearance of Asian sea bass (*Lates calcarifer*, Bloch)

For gill and liver, two grams of each tissue were separately collected and homogenized in phosphate buffer solution, then centrifuged at 5,000 rpm for 15 min. The supernatant was taken for further analysis of total protein estimation by Bradford protein assay.

Other tissues, liver, gill and kidney, were cut to be small pieces and then immediately fixed in 10% phosphate buffer formalin solution for 24 h. Next, tissue was dehydrated in a series of ethanol (50%, 70%, 80%, 90%, and absolute ethanol), and then cleared in xylene and infiltrated in paraffin and finally embedded in paraffin. The blocks of specimens were sectioned at 6 µm thickness on a microtome. The sections were stained with hematoxylin and eosin (H & E), and then studied the changes under light microscope.

Statistical analysis of sodium, chloride ions and protein content was performed using one way analysis of variance (ANOVA). The significant difference was considered at $p<0.05$.

Results

In this study, the effect of abamectin on Asian sea bass was evaluated by comparing the alteration observed in 2 groups of experiment: (1) fish exposed to abamectin to the concentrations of 5, 10 and 15 µg/L and (2) non-exposure fish. From the

results, we found 100% mortality in the group exposed to abamectin at the concentrations of 10 and 15 µg/L for 24 h. Thus, we performed in only one group of experiment remained; the group which exposed to the concentrations of 5 µg/L. There were 2 types of the effect of abamectin in Asian sea bass studied: (1) the changes of plasma electrolyte (Na^+ and Cl^-) and protein content in liver and gills, and (2) the histological alteration in gill, kidney and liver, after exposed to abamectin for 24, 48, 72 and 96 h.

1. Electrolyte in plasma

After the changes of plasma electrolyte (Na^+ and Cl^-) were studied, we found that it increased with an increasing in exposure time compared to the control group. In the Asian sea bass exposed to abamectin, Na^+ increased from 158±1.6 mmol/L (after 24 h of exposure) to 235.3±18 mmol/L after 96 h while it increased from 113.4± 2.3 mmol/L to 133±2.4 mmol/L for Cl^- as shown in Figure 3.

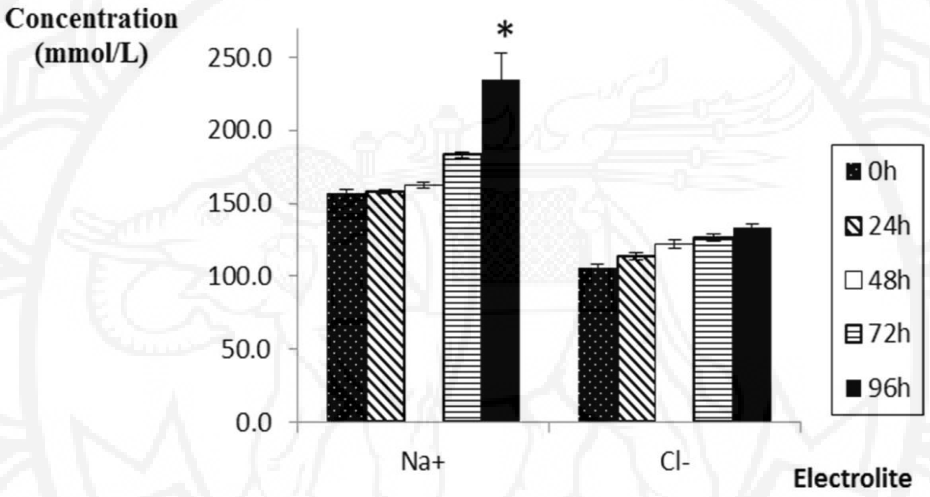


Figure 3 Plasma electrolyte (Na^+ and Cl^-) in Asian sea bass after exposed to abamectin at the concentrations of 5 µg/L for 24, 48, 72, and 96 h, respectively

Remark: * Significantly different from control group ($p < 0.05$)

2. Protein content

For protein content, the effect of abamectin was performed in liver and gill and the results indicated that protein content of exposed fish significantly deceased with an increasing in exposure time compared to the control group ($p < 0.05$). In the liver, protein content of non-exposed fish was 5.34±0.31 mg/g while it was 1.12±0.6 mg/g in the fish exposed to abamectin for 96 h. In addition, it remained only 1.75±0.23 mg/g in gill of fish which exposed for 96 h compared to 1.75±0.23 mg/g in the non-exposed as illustrated in Figure 4.

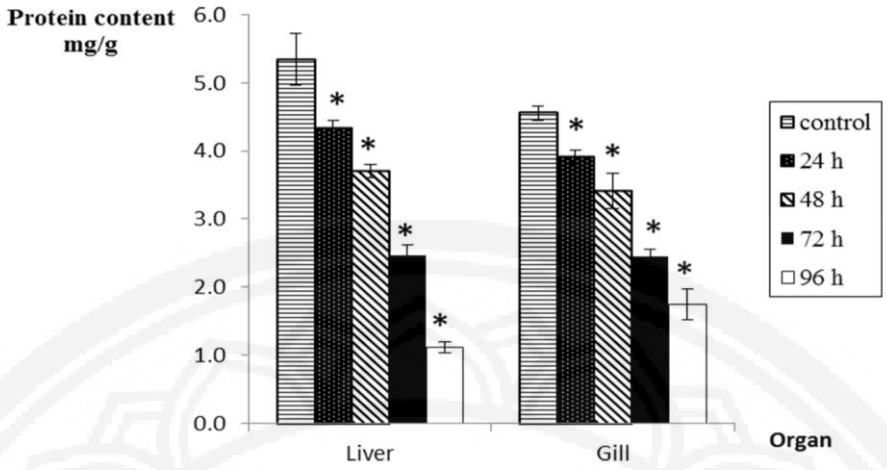


Figure 4 Protein content in liver and gill of Asian sea bass from non-exposed and treated group for 24, 48, 72 and 96 h, respectively

Remark: * Significantly different from control group ($p < 0.05$)

3. Histological alterations

After histological alterations were studied, the results indicated that the alterations were found in the exposed fish compared to the non-exposed. Figure 5A shows the normal gills of Asian sea bass. The alterations increased with an increasing in exposure time. After exposed to abamectin for 72 and 96 h, it was observed epithelial lifting and partial fusion of gill lamellae (Figure 5B-5C).

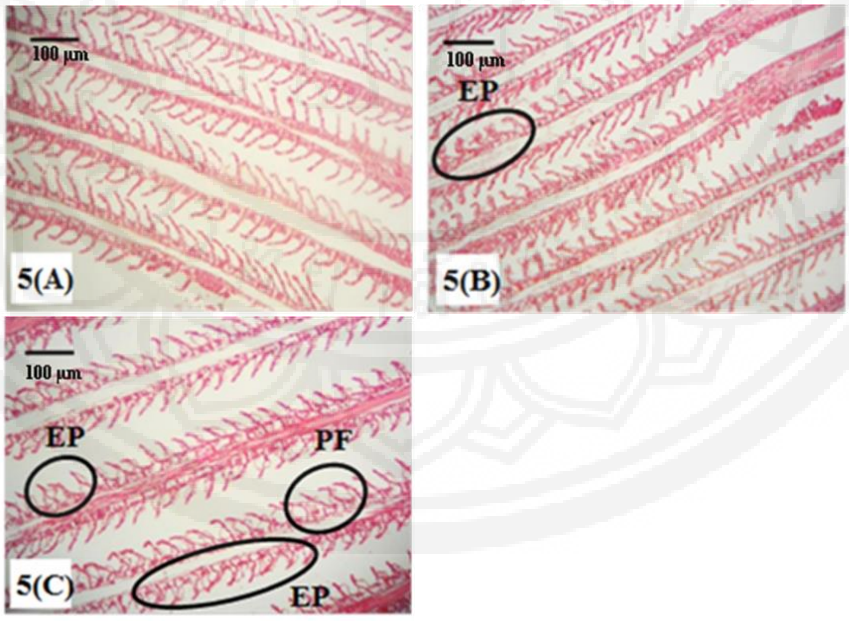


Figure 5 Five pairs of gill in the non-exposed fish (5A) and exposed fish for 72 h (5B), and 96 h (5C); where, EP: epithelial lifting, PF: Partial fusion of gill lamellae (H & E, 10X)

After studied the alteration in kidney tissue, the alteration was observed after 48 h of exposure (data not shown). We found the transformation of tubular epithelium. When exposure time increased (72 and 96 h), melanomacrophage aggregated, glomerulus deformed and necrosis of tubular epithelium were found (Figure 6B and 6C).

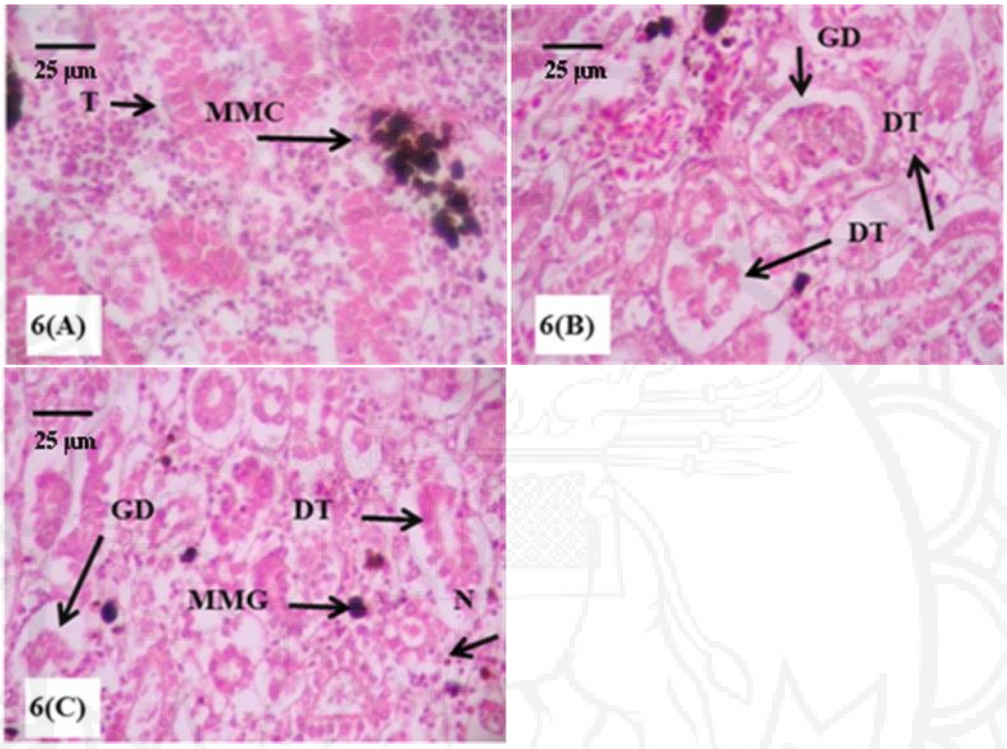


Figure 6 Kidney tissues of non-exposed fish (6A) and exposed fish for 72 h (6B) and 96 h (6C); where, T: Tubular epithelium, DT: Degeneration of tubular, MMC: Melanomacrophage center, MMG: Melanomacrophage aggregate, N:Necrosis, GD: Glomerulus deformed (H & E, 40X)

Figure 7(A) shows the normal hepatocyte cells and sinusoid in the liver. After exposed to abamectin for 72 and 96 h, it was found the alteration as shown in Figure 7B and 7C. The alteration observed was vacuolation, blood congestion, enlargement of sinusoid and necrosis of hepatocyte.

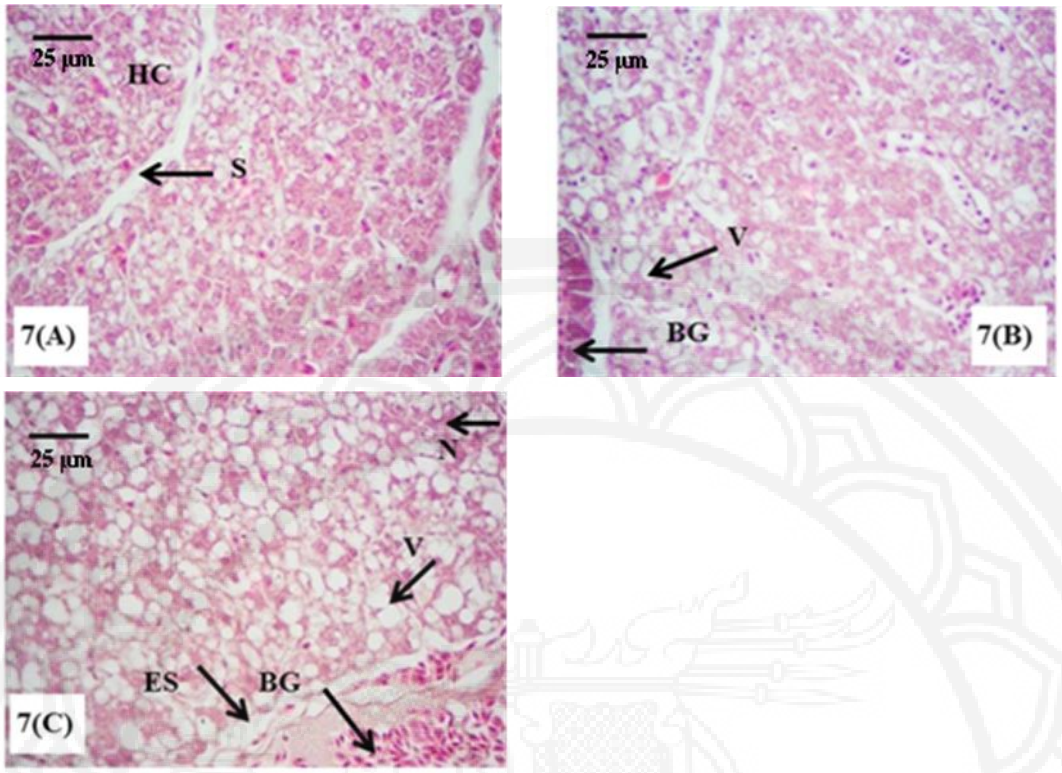


Figure 7 Liver tissues of non-exposed fish (7A) and exposed fish for 72 h (7B) and 96 h (7C); where, HC: Hepatocyte, S: Sinusoid, V: Vacuolation, BG: Blood congestion, ES: Enlargement of sinusoid, N: Necrosis of hepatocytes (H & E, 40X)

Discussions

The amount of abamectin is found in the ecosystems and its mortality effect depends on the degradation processes in any conditions. Although applied in a few amounts, it can cause harmful effect on bee, birds, fish, and other aquatic organisms (Hedayati et al., 2014).

In the case of toxicant exposure, the regulations of cations such as Na^+, K^+, Ca^{2+} and Mg^{2+} in animal body are disturbed. The level of any ions in blood or haemolymphs, which can be measured by osmolarity pressure alteration, may be used as potential biomarkers for water pollution. The reduction in plasma electrolyte may be caused by two important factors; (1) increased passive efflux of ions across the gill because of disturbing occurred in branchial permeability leading to heamodilution by enhanced osmotic uptake of water across the gills and (2) the process of ions uptake is inhibited by the chloride cells of the gills contributing to the negative ions balance of the blood (Freda, Cavdek, & McDonald, 1990). El-Said (2007) found significant increase in the levels of the Na^+ and Cl^- ions in the fish (*Orechromis niloticus*) after exposed to abamectin for 7 days which is in agreement with the alteration observed in this study. This alteration might be caused by the stimulation of Na^+- K^+ ATPase activity which led to an increasing in influx of Na^+ and Cl^- ions.

Protein is an important organic substance which organisms use in tissue building and energy metabolism (Remia, Logaswamy, Logankumar, & Rajmohan, 2008). The results of our study showed that protein content in the liver and gill significantly decreased. This phenomenon may be caused by

proteolysis and an increasing in metabolism under toxicant stress (Remia et al., 2008). Many studies showed a reduction of protein levels in many organs and tissues after exposed to toxicants. Al-Kahtani (2011) evaluated protein content in Tilapia fish (*Oreochromis niloticus*) after exposed to abamectin and found that protein in studied tissues significantly decreased. And, Kumar & Saradhamani (2004) also found a significant reduction of protein content in all studied tissues of the fish *Cirrihinus mrigala* after exposed to the pesticide.

For histopathological changes, it has been widely used as biomarker for fish exposure to contaminants in both laboratory (Thophon et al., 2003) and field studies (Camargo & Martinez, 2007). The most important advantages of using this biomarker is that it allows examination of specific target organs including gills, kidney, and liver which play the important role in controlling response processes to vital infections such as respiration, excretion, accumulation and biotransformation of xenobiotics (Gernhofer, Pawet, Schramm, Müller, & Triebskorn, 2001). For this study, histological alteration in three important organs was studied; gills, livers and in the respiration having an opportunity to expose to toxins, in metabolism of toxicant, and kidneys involve in elimination process, respectively. After alteration observed, lamella in gills of exposed fish was changed. Genten Terwinghe, and Danguy (2009) described the important role of gills that it brings the blood haemoglobin to absorb oxygen from the water and release carbon dioxide released. El-Said (2007) studied histological alteration in fish exposed to abamectin and found necrosis of lamella and infiltration of acidophils leukocyte in gills. Moreover, Hasan, Ghayyur, Hassan, and Rafique (2015) reported that they observed histological changes in the gills of grass carp (*Ctenopharyngodon idella*) at secondary gills lamellae, congestion, accumulation inflammatory cells, and disorganization after fishes exposed to endosulfan which is pesticide wildly used. Kidney is an organ which composed of numerous renal corpuscles connecting with well-developed glomeruli and a system of renal tubules. In this study, we found the degeneration of tubular, melanomacrophage aggregate, deformed glomerulus and necrosis of tubular epithelium in the kidney of exposed fish. There are many reports about the histological alterations in the kidney of fish exposed to toxicant or insecticide such as the study on effect of abamectin on Rainbow trout (*Oncorhynchus mykiss*) (Jenčič et al., 2006) and *O. niloticus* (El-Said, 2007). The liver plays an important role in degradation process of toxic compounds, but its regulating mechanisms may be affected by these toxicants, and ultimately result in inhibition degradation process by damaging its liver tissue structure (Bruslé & Anadon, 1996). As found in this study, vacuolation and dead cells were found in liver tissue which was also observed in the study of Jenčič et al. (2006) which indicated that abamectin could affected on hepatic in fish (*O. mykiss*). Additionally, cypermethrin had damaging effect on the hepatocytes that were altered with pycnotic nucleus. The inhibition of hepatocytes may affect to the distribution of nutrients to the other vital tissues of the fish (Karthigayani, Denis, Rexlin, Remy, & Shettu, 2014).

Conclusions

Based in the results achieved, it could be concluded that abamectin had toxic affect in the Asian sea bass (*Lates calrifer*, Bloch) at the concentration of 5 μg/L. The plasma electrolyte level (Na^+ and Cl^-) in exposed fish was increased, and protein contents and histological characteristics were altered. Thus, the alteration induced by

abamectin in Asian sea bass can be applied as fundamental data in aquatic environmental management and assessment.

References

Al-Kahtani, M. A. (2011). Effect of an Insecticide Abamectin on Some Biochemical Characteristics of Tilapia Fish (*Oreochromis Niloticus*). *American Journal of Agricultural and Biological Sciences, 6*(1), 62-68.

Bruslé, J., & Anadon, G. G. (1996). The structure and function of fish liver. In J. S. D. Munshi & H. M. Dutta (Eds.), *Fish Morphology* (pp. 77-93). North-Holland: Science Publishers.

Camargo, M. M. P., & Martinez, C. B. R. (2007). Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. *Neotropical Ichthyology, 5*(3), 327-336.

El-Said, M. M. (2007). Evaluation of Abamectin toxicity on some biochemical constituents and osmoregulation in freshwater fish *Oreochromis niloticus* (*Tilapia niloticus*). *Journal of Egypt society of Toxicology, 37*, 1-10.

Freda, J., Cavdek, V., & McDonald, D. G. (1990). Role of organic complexation in the toxicity of aluminum to *Rana pipiens* embryos and *Bufo americanus* tadpoles. *Canadian Journal of Fisheries and Aqauculture, 47*, 217-224.

Genten, F., Terwinghe, E., & Danguy, A. (2009). *Atlas of Fish Histology*. New Hampshire: Science Publisher.

Gernhofer, M., Pawet, M., Schramm, M., Müller, E. & Triebskorn, R. (2001). Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *J. Aquatic Ecosystem, Stress and Recovery, 8*, 241-260.

Hinton, D. E., & Laurén, D. J. (1993). Liver structural alterations accompanying chronic toxicity in fishes: potentioal biomarkers of exposure. In J. F. McCarthy & L. R. Shugart (Eds.), *Biomarkers of Environmental Contamination* (pp. 51-65) Boca Raton: Lewis Publishers.

Hasan, Z., Ghayyur, S., Hassan, Z. U., & Rafique, S. (2015). Histomorphometric and hematological profile of grass carp (*Ctenopharyngodon Idella*) during acute endosulfan toxicity. *Pakistan Veterinary Journal, 35*(1), 23-27.

Hedayati, A., Vajargah, M. F., Yalsuyi, A. M., Abarghoei, S., & Hajaihmadyan, M. (2014). Acte toxicity test of pesticide abamectin on common carp (*Cyprinus carpio*). *Journal of Coastal Life medicine, 2*(11), 841-844.

Jenčič, V., Cerne, M., Erzen, N. K., Kobal, S, & Cerkvenik-Flajs, V. (2006). Abamectin effects on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology, 15*(3), 249-257.

Kumar, K., & Saradhamani, N. (2004). Effect of insecticide, AVAUNT, on glycogen content of the fresh water fish *Cirrhinus mrigala*. *Nature Environment and Pollution Technology, 3*(4), 515-518.

Karthigayani, T., Denis, M., Rexlin, A., Remy, A., & Shettu, N. (2014). Histological study of the intestine and liver tissues in the fish *Oreochromis mossambicus* exposed to cypermethrin. *Journal of modern biotechnology*, *3*(4), 48-54.

Prado, R., Rioboo, C., Herrero, C., & Cid, A. (2009). The herbicide paraquat induces alterations in the elemental and biochemical composition of nontarget microalgal species. *Chemosphere, 76*, 1440-1444.

Remia, K. M., Logaswamy, S., Logankumar, K., & Rajmohan, D. (2008). Effect of an insecticides (Monocrotophos) on some biochemical constituents of the fish *Tilapia mossambica*. *Pollution Research Journal, 27*, 523-526.

Seeduangkaew, J., Kulsarin, J., Buranapanichpan, S., & Kumpiro, S. (2015). Biology of Cotton Leafhopper and Efficacy of Insecticides for Controlling in Purple Eggplant. *Journal of Agriculture, 31(2)*, 193 - 201.

Thophon, S., Krutrachue, M., Upatham, S., Pokethitiyook, P., Sahaphong, S., & Jaritkhuan, S. (2003). Histopathological alteration of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. *Environmental pollution, 121(3)*, 307-320.