Effect of 17 Beta-Estradiol Hormone and Cypermethrin Insecticide on Nile Tilapia

Chutima Thanomsit[1]*, Samnao Saowakoon[1], Amnuay Wattanakornsiri[2], Jakkaphun Nanuam[3], Witchuda Prasatkaew[4] and Phochit Nanthanawat[5]

[1]Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin 32000 Thailand

[2]Program of Environmental Science, Faculty of Science and Technology, Surindra Rajabhat University, Surin 32000 Thailand

[3]Program of Natural Resources and Environment, Faculty of Science and Social Sciences, Burapha University, Sakaeo 27160 Thailand

[4]Department of Environmental Science, Faculty of Science and Technology, Dhonburi Rajabhat University, Samutprakan 10540 Thailand

[5]Department of Biotechnology, Faculty of Science, Burapha University, Chonburi 20131 Thailand

* Corresponding author. E-mail address: chutima.tn@rmuti.ac.th

Abstract

This research aimed to investigate the effect of 17-beta estradiol hormone and cypermethrin onto Nile tilapia in both male and female. After exposure to 17-beta estradiol and cypermethrin, they were assessed for the changes in behaviors, external physiological expressions, internal organ characteristics and protein forms. We found that the fish in the control and 17-beta estradiol induction group swam normally, which contrasted with those fish that exposed to cypermethrin in sub lethal level with and without inducing vitellogenin production. Their swimming was very fast, abnormal and directionless. Additionally, the operculum were always moving and there was lesion around their mouth. The scales were loosened and bleeding as well as the internal organs was decayed. After studying the vitellogenin induction using Western blotting technique, we found the protein band of vitellogenin (210 kDa) in only the female fish induced by 17-beta estradiol (6 mg/kg of body weight) and soaked in 0.25 ppm of cypermethrin. Moreover, their plasma gave a positive result (dark brown) in Dot blot testing. Based on our results, we concluded that cypermethrin and 17-beta estradiol hormone resulted in vitellogenin expression in the female of Nile tilapia. Besides, cypermethrin affected the behaviors, physiological expressions and internal organs. This research is concluded that vitellogenin could be used to assess the exposure of 17-beta estradiol mixed with cypermethrin affecting on endocrine gland functions. It would be beneficial to the investigation of environmental quality and fish population in aquatic environment.

Keywords: Cypermethrin, Nile tilapia, Vitellogenin, Insecticide, Endocrine disrupting chemicals

Introduction

Thailand is classified as agricultural country. There are many agricultural areas that their products are locally consumed, internationally traded and even exported. Thus, farmers continuously look for the best procedure to achieve high quantity and quality of products to meet market needs. As a result, they normally apply pesticide as an important alternative way to increase their yields. However, pesticide application causes an adverse effect in the long term such as contamination in vegetables (Petchoy & Pung, 2017) and in the environment. A report indicated that only 0.01% of applied pesticide affected target organisms (Pimentel, 1995). In addition, contaminated pesticide may cause health effect in farmer and consumer. Prasopsuk, Saisuphan, and Srisawangwong (2014) studied chemical accumulation in vegetables and fruits from farms, applying certification in good agricultural practice in Eastern Thailand in between 2011 - 2013. They found contamination of chlorpyrifos, cypermethrin, methomyl and carbaryl, respectively sorted by percentage of samples found. Moreover, previous studies report that some pesticides have been found the estrogenic effect on aquatic animals and leading to the disruption of reproductive endocrine control and androgen and estrogen balance (Tian, Ru,

Bing, & Wang, 2010; Wang et al., 2015). Therefore, these chemicals are defined as Endocrine Disrupting Chemicals (EDCs).

EDCs can imitate or induce the response of estrogen in the organism (Campbell et al., 2006). For examples, chemicals having these capability consist of household chemical, insecticide, ago-chemical, drug or other pharmaceuticals. However, there are differences of toxic level in each chemical (Mills & Chichester, 2005). The sources of these chemicals are domestic sewage, effluent of treatment plant and run-off from agricultural area. After contaminating into the organism, EDCs can cause various adverse effects (Nanthanawat, 2015). After fishes are exposed to EDCs, their reproductive cycle including ovulation and spawning is affected in case of ending their reproductive cycle. Moreover, reproductive capability of male fish is also affected (Nicolas, 1999). Exposure to EDCs in male or juvenile fish has been reported to induce vitellogenin (VTG) induction. Normally, vitellogenin or Female-Specific Serum Protein (FSSP) is a protein that can be found in blood of oviparous vertebrate in sex matured stage (Nicolas, 1999). This estrogenicity of EDCs is due to permanently altered sexual differentiation and impairment of fertility in different species (Sumpter & Jobling, 1995).

An increasing in vitellogenin level in plasma of male fish may be caused by EDCs in either single or combined species. In addition, it can cause by chronic exposure in low concentration or acute exposure in high concentration (Marx, Sherry, Hansen, & Hock, 2001). Moreover, the level of vitellogenin synthesis can be affected by exposure time of fish to estrogen. Based on this relationship, vitellogenin level in male fish can be used as indicator for exposure or contamination. Thus, the induction of vitellogenin production in male or juvenile fish has been applied as bio-indicator for EDCs exposure in the environment (Watts, Pankhurst, Pryce, & Sun, 2003).

In this study, we aimed to investigate the effect of cypermethrin which is an insecticide in pyrethroid group widely applied in Thailand and cause severe effects to aquatic organisms and water quality. For the fish sample, we used Nile Tilapia because of being found nationwide and being a popular economic fish. After consuming contaminated fish, the contaminants may cause health risk, sex maturation or abnormal functions of endocrine system. In this study, male and female fish were injected by 17-beta estradiol hormone and cypermethrin with single treatment and both hormones and then exposed to cypermethrin. After that, they were assessed for behaviors, external physiological appearances and internal organs compared to the control group and the group having only vitellogenin induction by 17-beta estradiol hormone. Moreover, their protein form and cross-reaction of vitellogenin in plasma and monoclonal antibody being specific to vitellogenin from Asian sea bass (MAb 1:10,000) were also studied using immunological technique.

Methods and Materials

Chemicals

17-beta estradiol was purchased from Sigma-Aldrich (Thailand). Cypermethrin was obtained from the local company with a purity of 25% w/v. Other reagents for SDS-PAGE and Western Blot including molecular weight standard, nitrocellulose membrane, and Coomassie brilliant blue R-250 (CBR-250) were obtained from Bio-Rad (Thailand). Monoclonal antibody being specific to vitellogenin from Asian sea bass (MAb-sea bass VTG 23) was obtained in our lab, as previously described by Prasatkaew, Thanomsit, and Nanthanawat (2016).

Fish sample

Nile Tilapia used in this study was the sex mature having their weight in between 40.0±42.2 g and average length was 22±2.8 cm. Each sex was separately raised in cage in earthen pond of Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus. Then, Nile Tilapia were acclimated for 7 days and this study was done three replicates.

Laboratory testing of 17-beta estradiol and cypermethrin

This study was designed as Randomized Complete Block Design (RCBD) and divided into 8 groups as the follows;

Group 1: three male fishes were raised without any exposure and then their blood sample was taken (control group of male).

Group 2: three female fishes were raised without any exposure and then their blood sample was taken (control group of female).

Group 3: three male fishes were raised and injected by 17-beta estradiol in their muscle at the ratio of 4 mg hormone/kg fish weight. Then, their blood sample was taken for further study on vitellogenin induction.

Group 4: three female fishes were raised and injected by 17-beta estradiol in their muscle at the ratio of 4 mg hormone/kg fish weight. Then, their blood sample was taken for further study on vitellogenin induction.

Group 5: three male fishes were raised and injected by 17-beta estradiol in their muscle at the ratio of 6 mg hormone/kg fish weight. Next, they were placed in a 250 L of plastic tank filled with 0.25 ppm of cypermethrin for 3 days. After that, blood sample was taken to study vitellogenin induction and effect of cypermethrin.

Group 6: three female fishes were raised and injected by 17-beta estradiol in their muscle at the ratio of 6 mg hormone/kg fish weight. Next, they were placed in a 250 L of plastic tank filled with 0.25 ppm of cypermethrin for 3 days. After that, blood sample was taken to study vitellogenin induction and effect of cypermethrin.

Group 7: three male fishes were raised in a 250 L of plastic tank filled with 0.25 ppm of cypermethrin for 3 days. Then, blood sample was taken for further study on effect of cypermethrin.

Group 8: three female fishes were raised in a 250 L of plastic tank filled with 0.25 ppm of cypermethrin for 3 days. Then, blood sample was taken for further study on effect of cypermethrin.

For blood sample collection, a 1-2 mL of fish blood was taken from blood vessel in tail base and placed in a microcentrifuge tube coated with anticoagulants. Then, blood sample was centrifuged at 5,000 rpm in 4oC. The plasma or supernatant was then taken and placed in a microcentrifuge tube coated with phenylmethylsulfonyl fluoride (PMSF). Next, it was kept at -80^{o}C for further analysis.

The assessment of behavior, external physiological appearance and internal organ condition in fish sample after insecticide exposure

For closely monitor and assess their swimming behaviors and external physiological appearances of male and female fish after induced by 17-beta estradiol hormone, some fishes were transferred to white plastic tank for clearly seen any change. Their appearances were assessed every 24 h for 3 days before blood taken and then the fish were dissected for study internal organs compared to the condition in each experimental group.

Protein determination

Protein in plasma in both male and female fish was determined and protein concentration was used in calculating the amount for applying to Gel-electrophoresis and cross-reaction testing by Western blotting

technique. A 1.4 mg/mL of BSA standard protein solution (Bio-Rad, Thailand) was diluted as same as the sample reaching concentrations of 0.7, 0.35, 0.175 and 0.0875 mg/mL. Then, plasma was measured absorbance at wavelength of 280 nm. The absorbance level and BSA protein concentration was used in plotting standard graph in Linear Regression: X axis: protein concentration in plasma (mg/mL), and Y axis: absorbance level.

Study on protein form using 7.5 % Sodium Dodecyl Sulfate Gel Electrophoresis (7.5 % SDS-PAGE) technique

A 40 µg of fish plasma protein was mixed with 2x sample buffer in the ratio of 1:1. Then, it was boiled in hot water for 5 min and left in room temperature. The solution of 10% separating gel and 4% stacking gel were prepared for protein separation using electricity (120 Volt). Protein was moved from anode to cathode until tracking dye reached to separating gel. Then, electric potential was reduced to 110 V until tracking dye closed to end of gel plate then it was stopped. Gel plate was carefully removed and marked because it was generally thin (0.75 mm thickness). The part of stacking gel was cut off and then being stained by 0.1% Coomassie Brilliant Blue R-250 for 30 min. Non-attached dye was washed by dye remover Destaining solution I until blue band of protein was clearly seen. Then, it was washed by dye remover Destaining solution II for clearer seen. Next, it was washed by distilled water and further recording protein band. For the gel being not dyeing, its protein was transferred to nitrocellulose paper for Western blotting.

Western blot

For preparation, nitrocellulose paper, mini Trans-Blot Filter Paper, filter paper No.1 and gel plate were soaked in cold Towbin's Transfer buffer. Then, it was transferred to place sequentially (Mini Trans-blot Filter Paper, filter paper No.1, nitrocellulose paper, gel plate, filter paper No.1 and Mini Trans-Blot Filter Paper) on Bio-Rad Trans Blots SD Semi-Dry set. Electricity having electric potential of 15 V was applied for 15 min. Nitrocellulose paper on which protein placed was removed and then soaked in 5% Blotto solution for 1 h. Then, it was washed by 0.5% Blotto solution for 5 min three times. Next, nitrocellulose paper was removed to be soaked in 1st antibody solution (MAb-sea bass VTG 23) overnight. The excessive protein was washed out by 0.5% Blotto solution for 5 min three times. It was then soaked in 2nd antibody solution (GAM-HRP) in the dilution of 1:1,000 overnight at 4°C. The excessive protein was washed out by 0.5% Blotto solution for 5 min and then washed in PBS for 5 min two times. Finally, the color showing substrate reaction in solution was developed. The point at which reaction occurred was black brown after being incubated in substrate solution (DAB 0.03%, H_2O_2 0.03% and $CoCl_2$ 0.05% in PBS).

Dot blot

Protein determination was performed in each plasma sample from experimental conditions. Plasma concentration of 4 µg/µL were dropped onto nitrocellulose membrane and left for 10 min. Then, it was incubated in 5% skimmed milk solution in PBS for 1 h. Next, the membrane was washed by 0.1% Tween-20 solution in PBS for 5 min three times. Then, it was incubated in 1st antibody solution produced from Asian sea bass (MAb-sea bass VTG 23 in dilution of 1:10,000) overnight. Next, it was washed and incubated in 2nd antibody labeled GAM IgG-HRP enzyme in the dilution of 1:1,000 for 3 h in room temperature. It was then washed and assessed for occurred reaction showing by black brown after incubation in substrate solution.

Results

Fish behavior

After assessing swimming behaviors of male and female fish, we found that they swam normally as same as in the group of vitellogenin induction by injecting 17-beta estradiol hormone in the muscle. However, their behaviors altered after being transferred into plastic tank filled with cypermethrin solution. They swam fast but directionless. Their operculum were always moving. After 24 h, they swam slowly and sank to the bottom.

After assessing fish behaviors, their external physiological appearances were also studied comparing in control group and the group exposed to cypermethrin in concentration of 0.25 ppm (Figure 1). We found the noticeable alteration in exposed fish. There were lesion and colorless around the mouth after two days. Their scales were loosened and hemorrhagic patches were observed (Figure 2). Moreover, the internal organs in male fish were decayed while they were swollen abdomen and soft and friable tissues in the female. In female, we noticed the decaying in some parts (Figure 3). For internal organs of the control, we found they were in normal condition having bright color and normal shape.

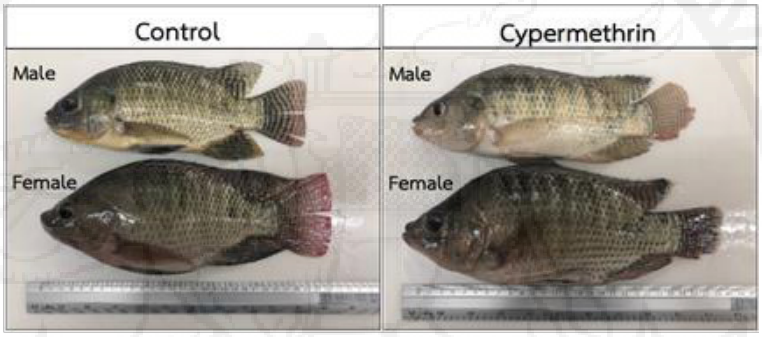

Figure 1 Nile Tilapia in control and cypermethrin exposure groups

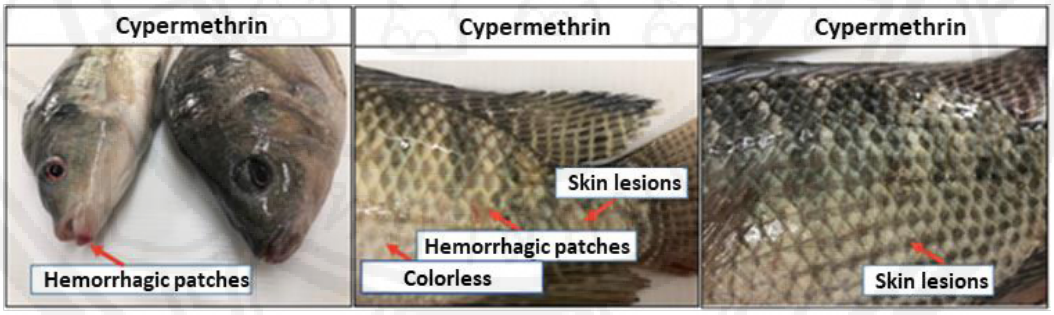

Figure 2 Alteration of external physiological appearances after cypermethrin exposure

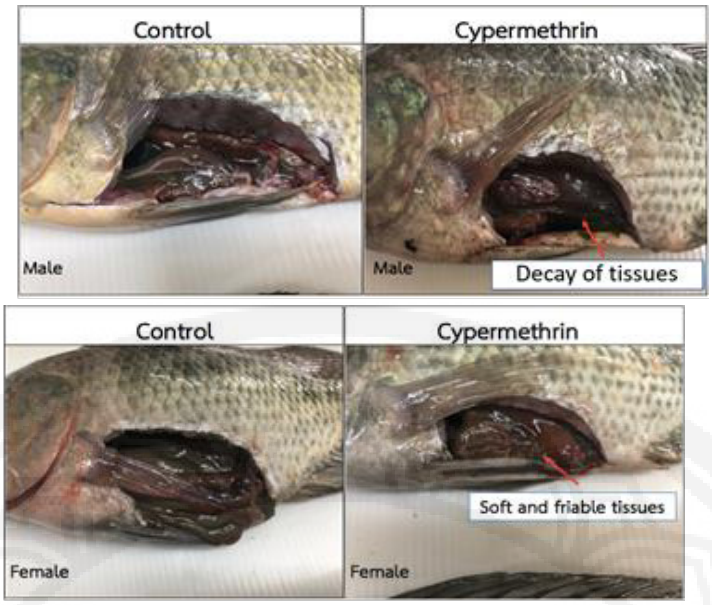

Figure 3 Internal organs in male and female fish in control and cypermethrin exposure groups

Study on form of plasma protein by using SDS-PAGE

The pattern of protein in plasma of Nile Tilapia in both male and female from different experimental conditions were shown in Figure 4. The expression of different proteins was compared to standard protein (Lane 1 in Figure 4). Proteins tested were extracted from plasma of male fish, plasma of female fish, plasma of male fish induced by 17-beta estradiol hormone injection (4 mg/kg fish), plasma of female fish induced by 17-beta estradiol hormone injection (4 mg/kg fish), plasma of male fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin, plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin, plasma of male fish and being soaked in 0.25 ppm of cypermethrin, and plasma of female fish and being soaked in 0.25 ppm of cypermethrin (Figure 4). It was found that vitellogenin (210 kDa) being dominant protein in only female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin (Lane 7 in Figure 4).

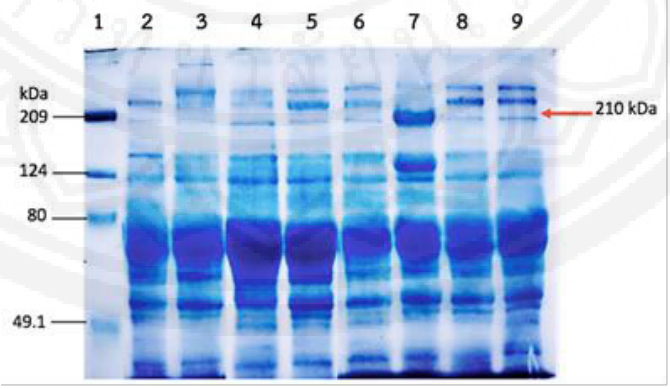

Figure 4 Soduim dodecyl sulphate Gel Electrophoresis (SDS-PAGE 10%) showing protein form:

Lane 1 Standard protein consisting of Myosin (209 kDa), B-galactosidase (124 kDa), BSA (80 kDa), Ovabumin (49.1 kDa), Carbonyc anhydrase (38.1 kDa), Soybean trypsin inhibitor (28.9), Lysozyme (20.1 kDa) and Apotinin (7.1 kDa)

Lane 2 Plasma of male fish

Lane 3 Plasma of female fish

Lane 4 Plasma of male fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 5 Plasma of female fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 6 Plasma of male fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 7 Plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 8 Plasma of male fish and being soaked in 0.25 ppm of cypermethrin

Lane 9 Plasma of female fish and being soaked in 0.25 ppm of cypermethrin

Western blot

After the form of plasma protein in each experimental condition was assessed by using Gel Electrophoresis, protein samples were then tested for cross-reaction of vitellogenin of Nile Tilapia and monoclonal antibody being specific to vitellogenin of Asian sea bass. We found a positive result (black brown of protein band having molecular weight of 210 kDa) in only plasma of female fish induced by 17-beta estradiol hormone injection (4 mg/kg fish) (Lane 5 in Figure 5) and plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin (Lane 7 in Figure 5).

Figure 5 Cross-reactivity in plasma of Nile tilapia by using Western blot technique and incubation in monoclonal antibody being specific to vitellogenin in the concentration of 1:10,000. Protein weight was 40 µg/lane

Lane 1 Standard protein

Lane 2 Plasma of male fish

Lane 3 Plasma of female fish

Lane 4 Plasma of male fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 5 Plasma of female fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 6 Plasma of male fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 7 Plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 8 Plasma of male fish and being soaked in 0.25 ppm of cypermethrin

Lane 9 Plasma of female fish and being soaked in 0.25 ppm of cypermethrin

Dot blot

Dot blot is a technique applied to study cross-reaction of monoclonal antibody being specific to vitellogenin of Asian sea bass and plasma of male and female fish. We found positive result only in plasma of female fish induced with 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

for 3 days. And, the results achieved from Dot blot technique were similar with those from Western blot technique.

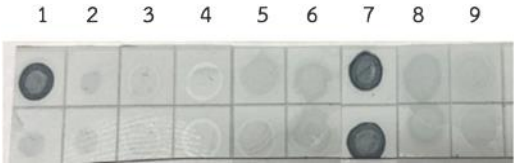

Figure 6 Cross-reactivity testing in plasma (1 μL) of Nile tilapia incubated in monoclonal antibody being specific to vitellogenin (1:10,000) by using Dot blot technique.

Lane 1 Positive expression (upper line) plasma from female of Asian sea bass induced by 17-beta estradiol hormone injection (4 mg/kg fish) for three times (boosting). And, negative expression was in lower line (plasma from male of Asian sea bass)

Lane 2 Plasma of male fish

Lane 3 Plasma of female fish

Lane 4 Plasma of male fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 5 Plasma of female fish induced by 17-beta estradiol hormone injection (4 mg/kg fish)

Lane 6 Plasma of male fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 7 Plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin

Lane 8 Plasma of male fish and being soaked in 0.25 ppm of cypermethrin

Lane 9 Plasma of female fish and being soaked in 0.25 ppm of cypermethrin

Discussion

In Thailand, agro-chemicals have been applied to increase crop production. In the case of excessive application, it can cause alteration or accumulation in the surrounding environment. Moreover, it can effect to aquatic environment such as freshwater fishes. Mill and Chichester (2005) reported that insecticide and agro-chemicals had an effect on functions of endocrine systems which further resulting in reproductive system. Thus, many researchers have tried to study and assess contamination of the chemicals in this group. They found that many techniques can be applied in evaluating the effects such as cell proliferation, ligand binding, attachment onto estrogenic binding site and vitellogenin induction in plasma (Sole, Porte, & Barcelo, 2001; Desforges, Peachey, Sanderson, White, & Blais, 2010; Nanthanawat, 2015).

Vitellogenin is a precursor protein of egg yolk which found in female of invertebrate having reproductive by spawning such as freshwater fish and amphibian. Vitellogenin in female was synthesized only after estrogen exposure while it was synthesized after endocrine disruption in male or juvenile. The organism can be exposed to disrupting agent in the environment. Thus, vitellogenin synthesis can be applied as indicator for endocrine disruption (Luo, Zhou, & Jiang, 2011). Vitellogenin can be assessed by many methods; however, immunoassay can give determination in both quantitative and qualitative. It can be performed rather fast, precise, and cheap (Prasatkaew et al., 2016).

Moreover, Le et al. (2010) suggested that vitellogenin expression could be applied in assessing agro-chemical contamination. They assessed the expression by using Reverse transcriptase- Polymerase chain reaction (RT - PCR) technique. It was found that glyphosate and methidathion affected to vitellogenin expression in Daphnia magna or red spider mites. These two chemicals resulted in lowering vitellogenin expression compared to control group (without exposure). In this study, we aimed to study the effect of cypermethrin insecticide on vitellogenin induced in plasma, behaviors, physiological appearances, internal organs, and protein form. In addition, we evaluated the specification of extracted vitellogenin using Western blot and Dot blot technique. In previous study, Prasatkaew et al. (2016) studied on vitellogenin induction as biomarker for endocrine disruption. And, they found that this biomarker could show effect in aquatic organism after exposed to chemicals. They tested cross-reaction of monoclonal antibody specific to vitellogenin from Asian Sea Bass with vitellogenin induced in other fish species for evaluating the efficiency of vitellogenin application to monitor contamination of endocrine disrupting agent in the environment. They found that produced antibody could specifically interact with vitellogenin in Asian sea bass and cross-react with vitellogenin in groupers and Nile tilapia. They concluded that these findings could be applied to assess contamination of endocrine disrupting agent in aquatic organism by using vitellogenin as biomarker and Asian sea bass or groupers as the sentinel.

Fish Behavior is an important factor used for assessment that this study emphasized on the effects of cypermethrin, being used worldwide, such as protein change and fish behavior. The study of fish behavior would illustrate its characteristics after cypermethrin exposure. When compared with the control group they could be used for its contamination. After Nile tilapia in both male and female exposed to cypermethrin, we noticed abnormal moving and swimming in the control group. In the fish having vitellogenin synthesis induction, their swimming was very fast, abnormal and directionless. The operculum were always moving more frequently than that in the control group. The alteration was noticed after 6 h of exposure. However, the concentration we used was in sub-lethal level causing all fish alive all experimental period (3 days). Changed behavior we found was similar to the study of Halappa and David (2008) that investigated effect of Chlorpyrifos insecticide on carp (*Cyprinus carpio*). They found that clearly seen alteration compared to the control. The fish abnormally moved and unbalanced swam. This findings caused by physiological alteration and surrounding environment (Little & Brewer, 2001) and abnormal behavior related to functions of nervous system (Keenleyside, 1979).

Nile tilapia in both male and female being exposed to cypermethrin showed clearly the alteration of external physiological appearances. Noticeable evidences were lesion and colorless around the mouth. The scales were loosened and the internal organs were decayed. There were swollen abdomen and colorless tissues. Some parts were misshaped. These alterations were caused by fish received chemicals in dissoluble form in both active and passive transportation (Sancho, Fernandez-Vega, Ferrenado, & Andreu-Moliner, 2003). A factor causing alteration of external physiological appearances and internal organs was route of exposure. Walker, Hopkin, and Peakall (2006) indicated that route of exposure was an important factor for alteration. Generally, the fish exposes to chemicals via feeding, breathing, and skin assimilation. In our experiment, Nile Tilapia exposed to cypermethrin by skin assimilation and breathing via gill and then dissipated in blood vessel to other organs.

It was surprise that vitellogenin induction was found in only female fish induced by 17-beta estradiol injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin. Previous studies have shown the effect of pesticide exposure in fish generally occurred in stage of juvenile and reproductive development (Ullah & Zorriehzahra, 2015). Thus, insecticide or herbicide exposure was then related to an expression of vitellogenin

that is induced by hormone or disruption of endocrine system in reproduction process. Wang et al. (2015) reported the vitellogenin expression in gold fish (*Carassius auratus*) after exposed to monocrotophos insecticide assessed using immunological technique. And, the results of SDS-PAGE showed that extracted vitellogenin molecular weights were 460, 130, 106 and 81 kDa, respectively. Vitellogenin expression in exposure fish was higher than that of the control. This finding might be caused by the structure of monocrotophos as like as of 17-beta estradiol hormone. Moreover, Nanthanawat (2015) suggested that, EDCs affected reproductive cycle of female fish. Besides, EDCs inhibited ovulation and spawning in female if they exposed to EDCs before spawning period. Furthermore, EDCs may cause abnormality of male sexual development, spermatogenesis and altered sexual differentiation and impairment of fertility.

The presence of vitellogenin can be assessed by using immunological technique based on antigen-antibody specification (Western blot and Dot blot technique). Molecular weight of extracted vitellogenin was 210 kDa. However, vitellogenin (MW 210 kDa) was not found in Nile Tilapia in both of male and female being induced by once 17-beta estradiol hormone injection (4 mg/kg fish). In this case, vitellogenin is non-detectable because the induction in matured fish might require higher hormone injection and boosting as suggested in the study of Paetrangsi, Prasatkaew, and Nanthanawat, 2017). They described the effect of nonylphenol as estrogen-like substance. This chemical is applied in textile and detergent industries. They induced vitellogenin synthesis in Asian Sea Bass by injecting nonylphenol in concentration of 2.5 mg/kg fish for three times. For the positive control group, fishes were injected by 17-beta estradiol (2.0 mg/kg fish) for three times. After injection, fish plasma was taken every three days for vitellogenin monitoring by Dot blot and Western blot technique. For Dot blot technique application, the results showed that vitellogenin was found at 3 days after exposure in both nonylphenol exposed fish and the control group (with 17-beta estradiol injection). However, it was negative in plasma of fish with nonylphenol injection when assessed by Western blot technique. Only positive control group gave a positive result in western blotting. In 2016, Daud, Jasmani, Sung, and Bolong (2016) studied on application of vitellogenin as biomarker for exposure of male grouper (*Epinephelus lanceolatus*). They found that vitellogenin synthesis can be induced in male fish by three times injection of 17-beta estradiol (2 mg/kg fish) using by SDS - PAGE and Western blot technique.

Hoeger, Kollner, Dietrich, and Hitzfeld (2005) reported that chemical exposure in each fish species can cause different effects on behavior, function of hormone, and reproductive system. In addition, chemical concentration and exposure time also influence on its effect (Walker et al., 2006). Moreover, difference of sex effects on toxicity. Gopi, Sathya, Goparaju, and Murthy (2012) studied vitellogenin synthesis in male and female of zebra fish by inducing with 2 substances Fenvalerate (Pyrethroid) and Mancozeb (Fungicide). It was found that Fenvalerate classified in pyrethroid group could induce vitellogenin synthesis in male of zebra fish after exposed to concentration of 10 µg/L while it was induced by concentrations of 1 and 3 µg/L. For Mancozob, the fish was induced in the concentrations of 0.08 and 0.2 mg/L. In the concentration of 0.8 mg/L, vitellogenin synthesis was induced in male while it was inhibited in female. That result was contrast with our results which found that cypermethrin in concentration of 0.25 ppm could not induce vitellogenin synthesis in both male and female of Nile Tilapia. These different results might be caused by structure of each chemical resulting in vitellogenin induction capability.

Finally, this research found that the main factors affecting vitellogenin expression were 17 beta-estradiol hormone and cypermethrin. Besides, the chemical concentration and exposure time were important.

Conclusion and Suggestions

The effect of insecticide (cypermethrin) on male and female of Nile Tilapia was assessed by monitoring on behaviors, external physiological appearances, internal organs, and forms of vitellogenin protein in plasma. After cross-reaction of vitellogenin in plasma of Nile Tilapia with monoclonal antibody being specific to vitellogenin produced in Asian Sea Bass was tested, we found a positive result (protein band with MW of 210 kDa) in plasma of female fish induced by 17-beta estradiol hormone injection (6 mg/kg fish) and being soaked in 0.25 ppm of cypermethrin. In contrast, vitellogenin synthesis was not found in the fish being soaked without injection. Based on our results, we concluded that cypermethrin and 17-beta estradiol influenced on vitellogenin expression in female of Nile Tilapia. In addition, cypermethrin also affected on behaviors, external physiological appearances and internal organs thus it is very important to further assess its contamination in aquatic organisms. Moreover, this data can be basically used to assess freshwater fish population in aquatics environment.

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