Microscopic Anatomy of Gill and Kidney of Nile Tilapia (*Oreochromis niloticus* L.) Treated with Bioinsecticide from *Stemona curtisii* Hook.F. and *Mammea siamensis* Miq. T. in Comparison to Lannate

Panase P*., Saenphet K. Jatisatienr A. and Saenphet S.
Department of Biology, Faculty of Science, Chiang Mai University, Thailand

บทคัดย่อ

หนอนตายหยาก (*Stemona curtisii* Hook.F.) และสารภี (*Mammea siamensis* Miq. T.) เป็นพืชสมุนไพรที่ใช้ในการกำจัดแมลง แต่อย่างไรก็ตามสารสกัดจากพืชดังกล่าวอาจจะส่งผลกระทบต่อสิ่งมีชีวิตที่ไม่ใช่เป้าหมายในการกำจัด งานวิจัยนี้จึงสนใจศึกษาการเปลี่ยนแปลงของเนื้อเยื่อเหงือกและไตปลานิล (*Oreochromis niloticus* L.) ที่ได้รับสารกำจัดแมลงชีวภาพที่ผลิตจากหนอนตายหยากและสารภี (0.019 mg/l และ 0.009 mg/l) เปรียบเทียบกับสารฆ่าแมลงยี่ห้อแลนเนท (0.972 mg/l and 0.486 mg/l) เป็นเวลา 75 วัน พบว่าเนื้อเยื่อเหงือกของปลาที่ได้รับสารชีวภาพความเข้มข้น 0.019 mg/l และ lannate ทั้ง 2 ความเข้มข้น มีการเรียงตัวของ 2^0 lamellae (SL) อย่างไม่เป็นระเบียบ SL หักงอลู่เข้าหากันและเชื่อมติดกัน และมีการโป่งพองที่บริเวณส่วนปลาย กลุ่มที่ได้รับ lannate ความเข้มข้น 0.972 mg/l มีการฝ่อตัวของ SL อย่างเห็นได้ชัด ส่วน 1^0 lamellae มีเลือดคั่งตรงบริเวณแกนกลาง การสลายตัวของ SL นั้นไม่พบในปลาที่ได้รับสารสกัดชีวภาพ สำหรับเนื้อเยื่อไตพบว่าปลาที่ได้รับ lannate และสารสกัดชีวภาพมีพยาธิสภาพที่คล้ายคลึงกัน คือ เซลล์ของท่อไตมีช่องว่างแทรกอยู่ มีเลือดคั่งในเนื้อไต เซลล์ท่อไตหลุดแยกออกจากฐาน พบการตายของเซลล์ทั้งแบบ karyolysis และ necrosis เกิดการหดตัวของ glomerulus ในปลากลุ่มที่ได้รับ lannate ที่ความเข้มข้นทั้ง 2 ระดับยังพบการเพิ่มขนาดของเซลล์ Bowman's capsule ด้วย ผลที่ได้จากการศึกษาครั้งนี้ชี้ให้เห็นว่าสารกำจัดแมลงชีวภาพที่มีส่วนผสมของหนอนตายหยากและสารภีและ lannate อาจมีผลรบกวนการทำงานของเหงือกและไตของปลาได้ ดังจะเห็นได้จากพยาธิสภาพของเนื้อเยื่ออวัยวะดังกล่าว

Abstract

Stemona curtissi and *Mammea siamensis* are the medicinal plants used for insecticidal purpose. However, the extract of these plants may also affect non-target organisms. Thus, the histopathological changes of gills and kidneys in nile tilapia, *Oreochromis niloticus*, exposed to bioinsecticide produced from *S. curtisii* and *M. siamensis* (0.019 mg/l and 0.009 mg/l) as compared to lannate, a commercial insecticide, (0.972 mg/l and 0.486 mg/l) for 75 days were investigated. The disorganization, bending and fusing including the aneurisms at tip area of 2^0 gill lamellae (SL) were found in fish exposed to bioinsecticide at concentration of 0.019 mg/l and all doses of

lannate. SL dysplasia was obviously noticed in lannate group. Blood congestion in central area of 1^0 gill lamellae was also observed. Nevertheless, SL dysplasia was not evident in fish exposed to bioinsecticide. Both bioinsecticide and lannate caused similar histopathological changes in kidney. These included large extracellular space, blood congestion, detachment of tubular cells from basement membrane, karyolysis, necrosis and shrinkage of the glomerulus. The enlargement of cells in Bowman's capsule was also observed in lannate group. The results obtained from this study indicated that the bioinsecticide produced from *S. curtisii* and *M. siamensis* as well as lannate at concentrations used may alter the gill's and kidney's functions evidenced by the morphological damages.

Key words: *Stemona curtisii*; *Mammea siamensis*; Gill; Kidney; Nile Tilapia; *Oreochromis niloticus* ; bioinsecticide ; lannate

Introduction

Organisms living in aquatic habitats near agricultural fields are of high risk to be affected by several agricultural substances including insecticides. They may be directly exposed to those substances via spray drift or indirectly throughout runoff inputs. Toxic effects of synthetic insecticides on fish have well documented by several workers (Barat *et al.*, 1998; Tripathi and Singh, 2003). As a result of these disadvantages, the less hazardous alternatives to synthetic pesticides are needed and plant-derived pesticides is one of the interesting alternatives. In Thailand, the insecticidal properties of *Stemona curtissi* and *Mammea siamensis* were known for centuries. The research on biopesticide development using these 2 plants as active ingredient have been reported previously (Plank, 1994; Jacobson and Crosby, 1971; Stoll, 1986). The safety of *S. curtisii* extract on laboratory mice has been evaluated (Pandee *et al.*, 2003). Nevertheless, toxicity test of both *S. curtisii* and *M. siamensis* in fish have not been reported. The present study, thus, aimed to assess the effect of bioinsecticide produced from these 2 plants on gill and kidney of nile tilapia (*Oreochlomis niloticus*) while lannate, a commercial insecticide, was used as positive control.

Materials and Methods

Plant extracts

Roots of the *S. curtisii* and seeds of the *M. siamensis* were washed, died, homogenized , extracted with 95 % ethanol and evaporated by rotary evaporator, respectively. The crude extracts were mixed to ratio 1: 1 and dissolved in distilled water for using as bioinsecticide.

Experimental design

Nile tilapia, *Oreochromis niloticus*, with an average body weigh of 3±1.4 g and 3-5 cm total length was used in this study. The fish were acclimated to the laboratory conditions for least 5 days prior to the experiment in a glass aquarium (29x59x87 cm). During the acclimatization and experimental period, the fish were fed twice daily with commercially fish feed.

The fish were divided into five groups (10 each) and placed in separate glass aquarias Group 1 was maintained in pesticide-free water to serve as control. Groups 2-3 were exposed to bioinsecticide at concentrations of 0.019 and 0.009 mg/l, respectively. Groups 4-5 were exposed to lannate at concentrations of 0.972 and 0.486 mg/l, respectively. The concentrations used were based on the 5 and 10 times of concentrations lower than their LC_{50} for *O. niloticus* (Jatisatienr, 2005). Exposure time was 75 days and the experiment was conducted in three replicas. The substance and water in each aquaria were changed in five-day-intervals. At the end of the treatment period, fish were anesthetized with MS222 (tricaine methansulphonate) for histological investigation.

Histology

Immediately after anesthesia, the gill and kidney tissues were removed and fixed in Bouin's solution for histopathological examination using a routine histology technique . 5 µm sections were made and stained with hematoxylin and eosin (H&E) for light microscopic examination.

Results

Gill

No histopathological changes were observed in the gill of control fish. The structural details of the gill of *O. niloticus* are shown in Fig. 1 A. The gill is made up of primary lamellae. Secondary lamellae were found on the lateral sides of primary lamellae. The surface of the gill lamellae is covered by epithelium.

The gill damages were obviously seen in fish treated with lannate at 0.468 mg/l. Irregular appearance of gill lamellae, blood congestion, aneurisms at tip area, bending and fusing of SL were observed (Fig. 1 D and 2 B). Moreover, desquamation of epithelial SL and PL, dysplasia and necroses of SL and PL were pronounced in fish treated with lannate at 0.972 mg/l (Fig. 1 E and 2 E).

For bioinsecticide treatment, only some aneurisms at tip area of SL were seen in the group of 0.009 mg/l (Fig. 2 D). Most of the gill structure were found normal (Fig. 1 B.). In 0.019 mg/l group, however, structural alterations of the gill were similar to those of lannate (Fig. 1C and 2 E).

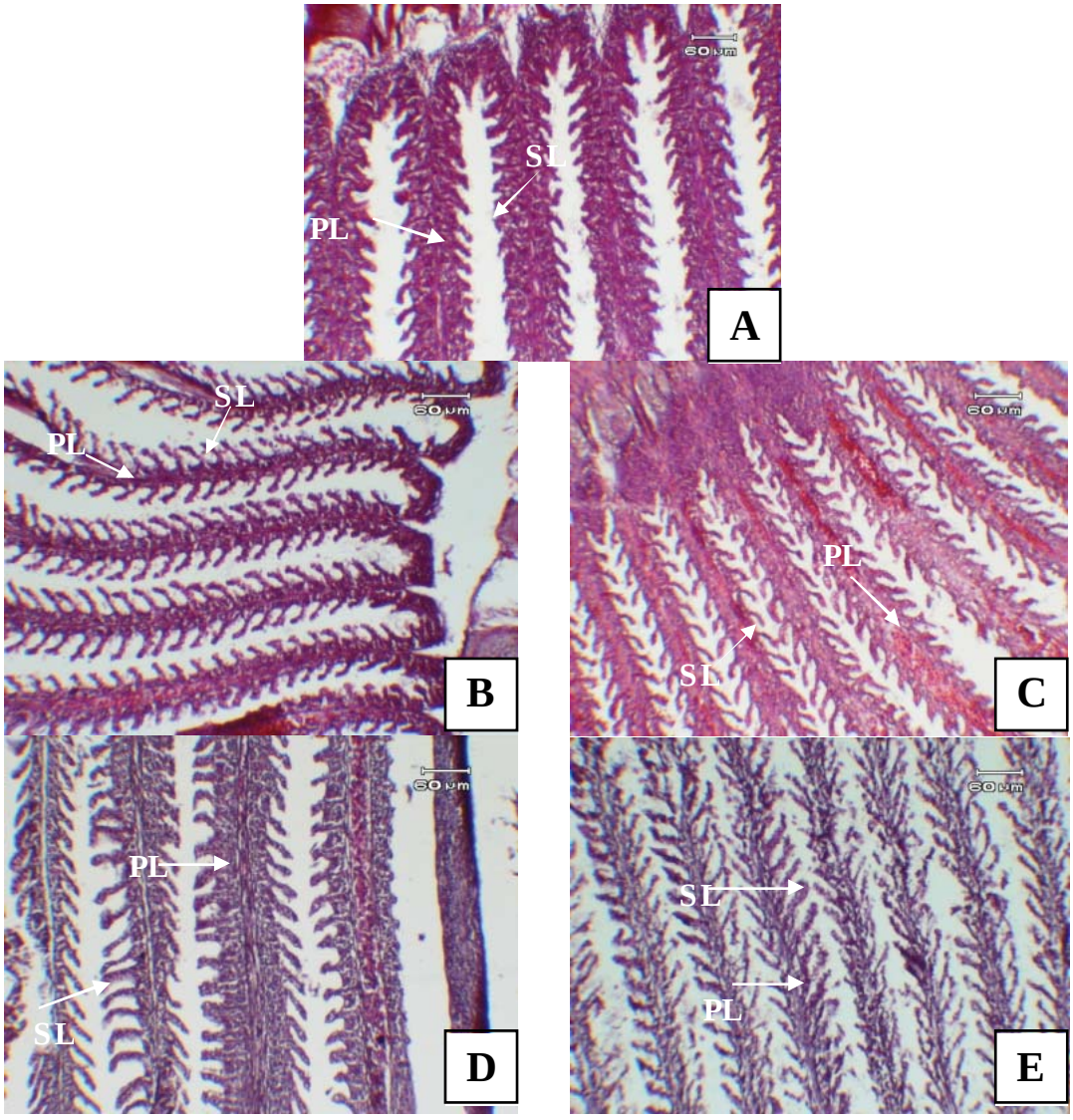

Fig. 1. Gill structure of control *O. niloticus* (A) as compared to those of fish exposed to 0.009 mg/l (B) and 0.019 mg/l of bioinsecticide (C), 0.486 mg/l (D) and 0.972 mg/l of lannate (E) for 75 days; primary lamellae (PL) and secondary lamellae (SL) (H&E, 100x)

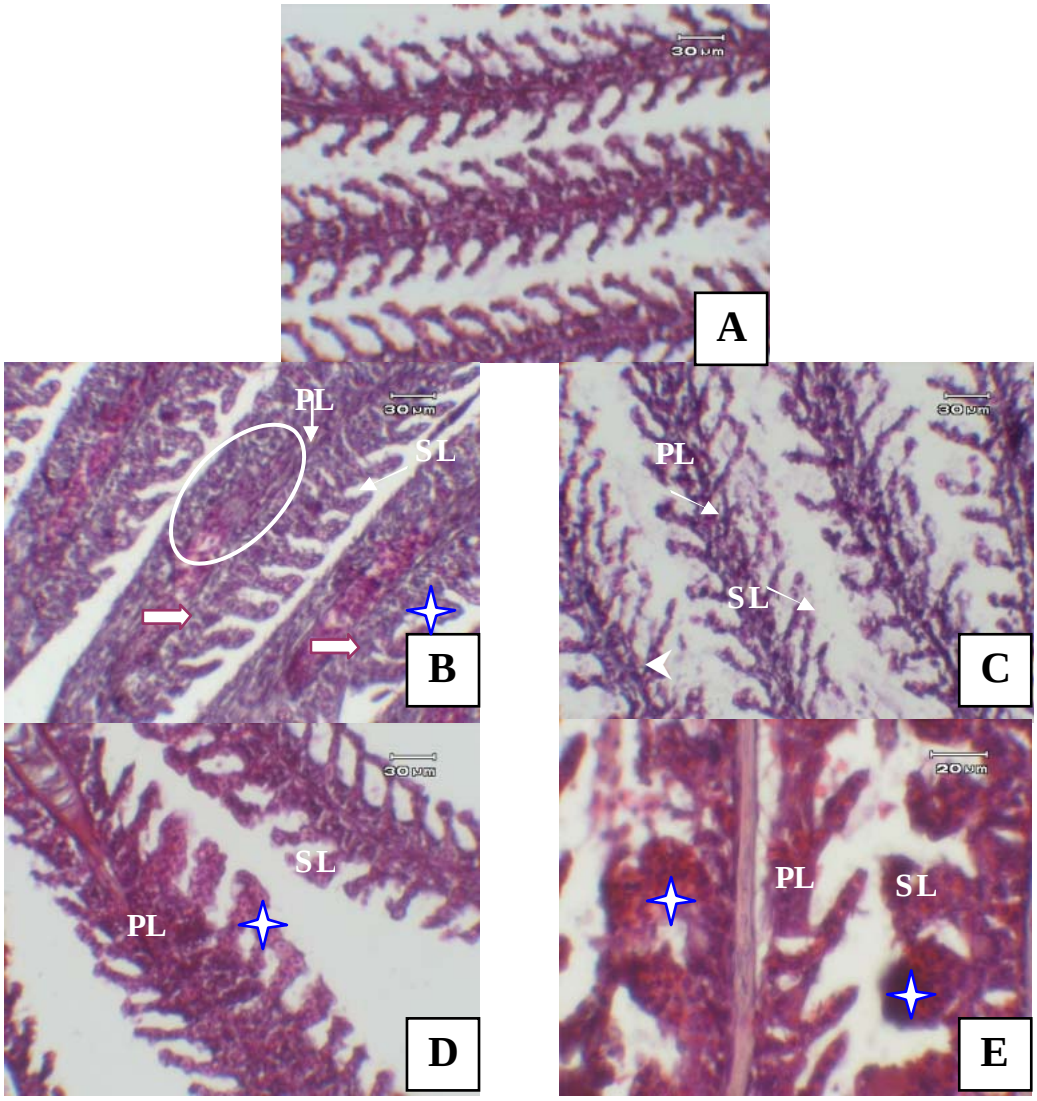

Fig. 2. Histopathological changes of gill of *O. niloticus* exposed to 0.009 mg/l (D) and 0.019 mg/l of bioinsecticide (E) and exposed to 0.486 mg/l (B) and 0.972 mg/l of lannate (C) for 75 days as compared to controls (A); bending and fusing of SL (circle), aneurisms (✦), blood congestion on the PL (➡), and desquamation of SL (➤) ; PL= primary lamellae, SL=secondary lamellae ; (Fig.2. A, B, C, and D H&E 200x; E H&E 400x)

Kidney

Fig. 3 A and B showed no histological changes in the kidney of control fish. In contrast, fish treated with bioinsecticide (Fig. 3 C-D) and lannate (Fig. 3 E-F)showed severe kidney damages and the degree of tissue damages was in concentration-dependence manner. The damages included large extracellular space, blood congestion, detachment of tubular cells from

basement membrane, cell necrosis, shrinkage of the glomerulus and enlargement of cells in Bowman's capsule.

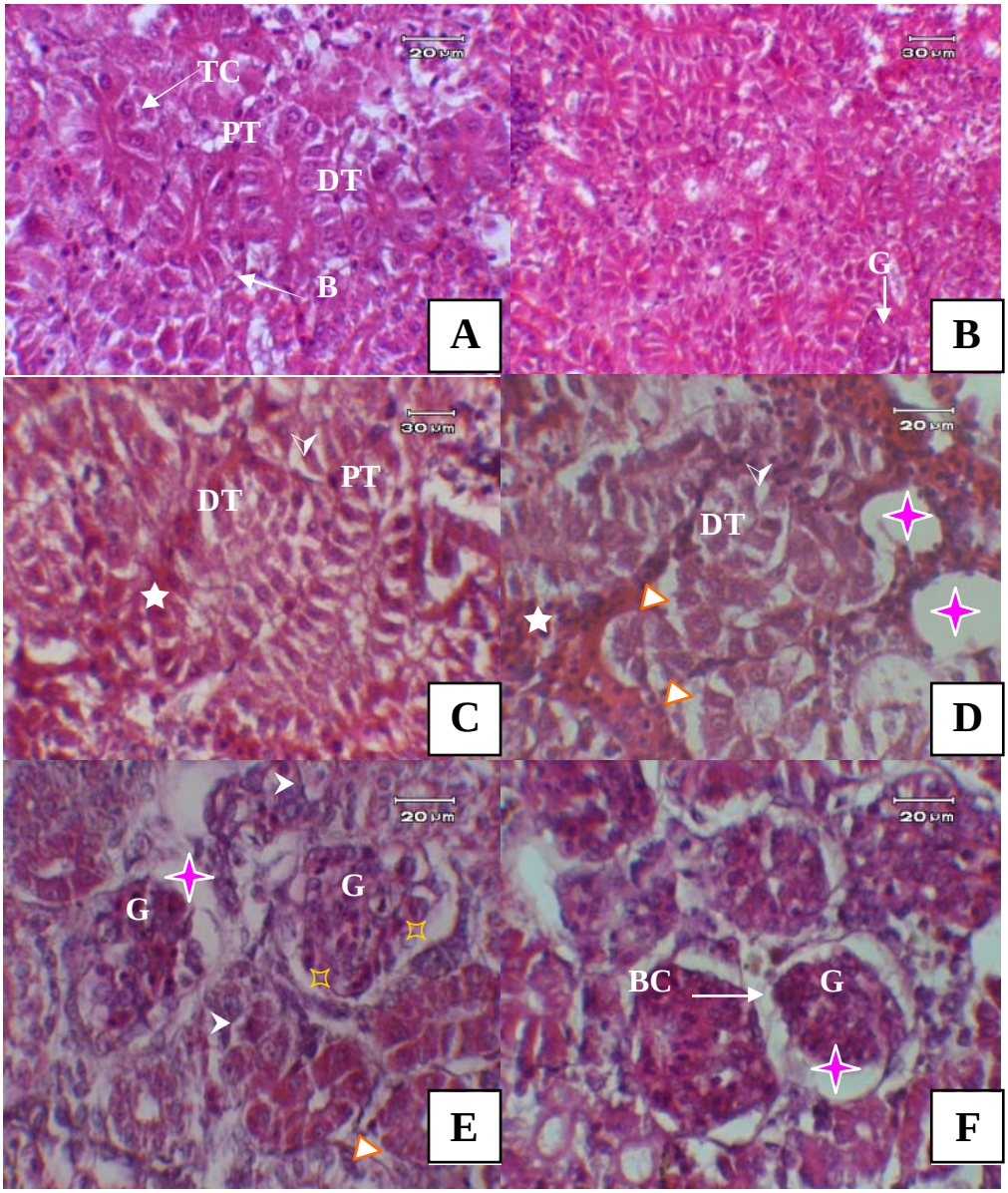

Fig. 3. Histopathological changes of kidney of *O. niloticus* exposed to 0.009 mg/l (C) and 0.009 mg/l of bioinsecticide (D: 400x), 0.486 mg/l (E) and 0.972 mg/l of lannate (F: 400x) for 75 days as compared to controls (A: 400x and B: 200x). Large extracellular space of tubular cells ( ), detachment of tubular cells from basement membrane ( ), cells necrosis ( ),The enlargement of cells in Bowman's capsule ( ), blood congestion ( ), expansion of space inside the Bowman's capsule due to shrinkage of the glomerulus ( ), PT :proximal convoluted tubule, DT: distal convoluted tubule, B: basement membrane, BC: Bowman's capsule, TC: tubular cell

Discussion

Respiratory distress is one of the early symptom of pesticide poisoning (McDonald, 1983). In this study, gill of *O. niloticus* exposed to 0.972 and 0.486 mg/l of lannate showed the fusion and aneurism at tip area of gills lamella. This damage caused a drastic reduction in the respiratory surface area (Leino *et al.*, 1987; Dutta *et al.*, 1996). In addition, blood congestion in central area of primary lamellae (PL) indicated that the blood vessels were break down. Moreover, the complete destruction of PL and SL in 0.972 mg/l group may lead to the loss of respiratory function. Fish treated with bioinsecticide showed the similar histopathological changes of gill structure to the lannate groups, but less toxicity showing by no destruction of PL and SL. The present investigation indicates that due to continuation or long time of the toxic impact of bioinsecticide produced from *S. curtisii* and *M. siamensis* and lannate, the protective role of the thin layer of the gill collapses and fails to prevent the penetration of treated substance. These caused various degrees of the damage to the delicate protective device of the gill epithelium and may lead to efficiency-decrement respiratory, finally, to death.

Fish, as in higher vertebrates, the kidney performs an important function relate to electrolyte and water balance and maintenance of a stable internal environment. Following exposure of fish to toxic agents, histological alterations have been found at the level of tubular epithelium and glomerulus (Teh *et al.*, 1997). Ortiz *et al.* (2003) indicated that the kidney of fish receives much the largest proportion of post-branchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution. Elsan treatment in *Channa puntatus* resulted in a significant decrease in the dimension of Bowman's capsule and glomerulus, and the tubules had irregular shape due to precipitation of cytoplasm and karyolysis (Banerjee and Bhattacharya, 1994). In this study, *O. niloticus* exposed to all doses of bioinsecticide and lannate revealed similar histopathological changes of their kidney to the several other studies such as large extracellular space of tubular cells, detachment of tubular cells from basement membrane, cells necrosis, enlargement of cells in Bowman's capsule, blood congestion, and expansion of space inside the Bowman's capsule due to shrinkage of the glomerulus. Since, structural deformities of the kidney tissues could be detected at low levels of bioinsecticide and lannate, the alteration of kidney's functions could be expected. Further investigation concerning the evaluation of blood biochemistry is necessary. In conclusion, bioinsecticide produced from *S. curtisii* and *M. siamensis* in concentrations used in this study caused the histopathological changes in gill and kidney tissue of

O. niloticus. Although this bioinsecticide showed milder toxicity than lannate, the direct use in freshwater without a detailed study should be aware.

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