

การตอบสนองในระดับ โมเลกุลและความเป็นพิษของคลอโรไพริฟอสในกึ่งกลางดำ

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MOLECULAR RESPONSES AND THE TOXICITY OF CHLORPYRIFOS
IN GIANT TIGER SHRIMP

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ทัศนีย์ เอี่ยมกมล : การตอบสนองในระดับโมเลกุลและความเป็นพิษของคลอโรไพริฟอสในกุ้งกุลาดำ (MOLECULAR RESPONSES AND THE TOXICITY OF CHLORPYRIFOS IN GIANT TIGER SHRIMP) อ. ที่ปรึกษา: ศ. ดร. เปี่ยมศักดิ์ เมนะเสวต, อ.ที่ปรึกษาร่วม : ดร. ณรงค์ศักดิ์ พ่วงลาภ 210 หน้า.

การศึกษาถึงผลของสารกำจัดแมลงคลอโรไพริฟอสในกุ้งกุลาดำ *P. monodon* ในการศึกษาที่ประกอบด้วยความเป็นพิษเฉียบพลัน การยับยั้งระดับของเอนไซม์ acetylcholinesterase และ ความเป็นพิษต่อสารพันธุกรรม สำหรับการศึกษาถึงความเป็นพิษเฉียบพลันด้วยระบบปิดพบว่าค่า LC_{50} ที่คำนวณได้ที่เวลา 24 ถึง 96 ชั่วโมงคือระหว่าง 52.43 และ 20.74 $\mu\text{g/l}$ การยับยั้งระดับของเอนไซม์ acetylcholinesterase ในเหงือกของกุ้ง พบว่าที่ความเข้มข้นที่ทำให้เกิดพิษเฉียบพลัน ที่ 68.1 และ 681 $\mu\text{g/l}$ ระดับของเอนไซม์ลดลง 1.7 และ 3.3 เท่าอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุมภายใน 30 นาที ภายหลังการสัมผัสสาร ($N=5$, $P<0.05$) สำหรับความเข้มข้นต่ำ ที่ 0.681 $\mu\text{g/l}$ ระดับของเอนไซม์ลดลง 1.9 เท่าอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุมภายหลัง 72 ชั่วโมงที่ได้รับการสัมผัสสาร ($N=5$, $P<0.05$) การลดลงอย่างชัดเจนของระดับของเอนไซม์ acetylcholinesterase ที่ความเข้มข้นต่ำ 0.681 $\mu\text{g/l}$ ซึ่งต่ำกว่าค่า LC_{50} ที่เวลา 96 ชั่วโมง ถึง 30 เท่า แสดงให้เห็นถึงศักยภาพในการใช้เป็นเครื่องหมายทางชีวภาพต่อการได้รับคลอโรไพริฟอส การทดสอบความเป็นพิษต่อสารพันธุกรรมด้วยวิธี single-cell gel electrophoresis ซึ่งให้เห็นถึงการเพิ่มขึ้นของ DNA tail length และ tail moment เมื่อเซลล์เม็ดเลือดของกุ้งสัมผัสคลอโรไพริฟอสที่ 0.034 and 0.170 $\mu\text{g/l}$ เป็นเวลา 1 ชั่วโมง เมื่อเทียบกับกลุ่มควบคุม ($N = 3$, $P<0.05$) และผลยังปรากฏอยู่ 6 ชั่วโมงภายหลังที่สัมผัสสารที่ 0.170 $\mu\text{g/l}$ การค้นหาชิ้นที่มีศักยภาพในการใช้เป็นเครื่องหมายทางชีวภาพต่อการได้รับคลอโรไพริฟอสด้วยเทคนิค mRNA DDRT-PCR พบ 44 transcript ที่มีการแสดงออกที่แตกต่างระหว่างกลุ่มของกุ้งที่ได้รับคลอโรไพริฟอส ผลการเปรียบเทียบลำดับกรดอะมิโนด้วยวิธี BLASTx (NCBI) พบว่า 22 transcript เป็นชิ้นที่มีการศึกษาและระบุชนิดแล้ว (16 up-regulated และ 6 down-regulated) และ 22 transcript เป็นชิ้นที่ยังไม่ได้มีการศึกษาระบุชนิด (8 up-regulated และ 14 down-regulated) การศึกษาถึงระดับการแสดงออกของยีนประกอบด้วย cytochrome P450, beta glucuronidase, heat shock protein 70, heat shock protein 90, vitellogenin และ glutathione-s-transferase และ ยีนจาก mRNA DDRT-PCR ประกอบด้วย OPA07G350-27-1 (LDL receptor member LR3), UBC101C-1,000-D-3 (esterase), UBC119A-650-F-5 (*CYP330A1*), OPA18G-600-4-1 (ubiquitin-like-7), OPA01G-415-1 (leucine zipper protein 5), และ OPA02G-450-2 (sequence of unknown gene) ด้วยวิธี semi-quantitative RT-PCR พบว่าไม่มีความแตกต่างในระดับการแสดงออกของยีนเหล่านี้ในกลุ่มของกุ้งที่สัมผัสคลอโรไพริฟอสที่ความเข้มข้น 0-27.24 $\mu\text{g/l}$ ภายใน 96 ชั่วโมง การค้นหา full length cDNA ด้วยเทคนิค RACE-PCR ทำให้ได้ full length cDNA ของ 3 ยีน ประกอบด้วย carboxylesterase (1,746 bp ORF คิดเป็น 582 amino acid), cytochrome P450 (1,530 bp ORF คิดเป็น 510 amino acids), และ glutathione-s-transferase (654 bp ORF คิดเป็น 218 amino acids) ซึ่งเป็นการพบครั้งแรกในกุ้งกุลาดำ ผลการศึกษาในครั้งนี้แสดงข้อมูลพื้นฐานสำหรับความเป็นพิษของคลอโรไพริฟอสต่อกุ้งกุลาดำและศักยภาพของการใช้ระดับของเอนไซม์ acetylcholinesterase ในการใช้เป็นเครื่องหมายทางชีวภาพต่อการได้รับคลอโรไพริฟอส

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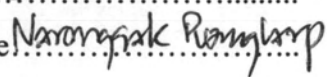
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TASSANEE EAMKAMON : MOLECULAR RESPONSES AND THE TOXICITY OF CHLORPYRIFOS IN GIANT TIGER SHRIMP. THESIS ADVISOR : PROFESSOR PIAMSAK MENASVETA, Ph.D., THESIS CO-ADVISOR : NARONGSAK PUANGLARP, Ph.D., 210 pp.

To examine the effects of chlorpyrifos to *P. monodon*, acute toxicity, inhibition of acetylcholinesterase activity, genotoxicity, and molecular responses of the exposed shrimps to chlorpyrifos were studied. Using static system, the LC₅₀ values after 24 to 96 h of exposure were between 52.43 and 20.74 µg/l. Acetylcholinesterase activities observed in shrimp exposed to the lethal concentration of 68.1 and 681 µg/l of chlorpyrifos were 1.7 and 3.3 times lower than that of control shrimp after 30 min exposure (N=5, P<0.05). The enzyme activity in shrimp exposed to the sub-lethal concentration at 0.681 µg/l of chlorpyrifos was 1.9 times lower than that of control shrimp after 72 h exposure (N=5, P<0.05). The sensitive reduction of AChE activity at the sub-lethal concentration was 30 times lower than 96 h LC₅₀ value, indicating the potential use as biomarker of chlorpyrifos exposure. The *in vitro* testing for genotoxicity using single-cell gel electrophoresis revealed significant increase of the DNA tail length and tail moments detected from the haemocytes exposed to 0.034 and 0.170 µg/l of chlorpyrifos in comparison with that of control group within 1 h (N = 3, P<0.05) and the evidences were still detected after 6 h exposure to 0.170 µg/l of chlorpyrifos. Screening for candidate genes of the shrimp induced by chlorpyrifos exposure using mRNA DDRT-PCR revealed forty-four differential displayed transcripts. BLAST result identified 22 transcripts (16 up-regulated and 6 down-regulated) as known genes and 22 transcripts as unknown genes and genes similar to hypothetical proteins found in other species (8 up-regulated and 14 down-regulated). Expression analysis of well-characterized genes including cytochrome P450, beta glucuronidase, heat shock protein 70, heat shock protein 90, vitellogenin, and glutathione-s-transferase as well as genes obtained from mRNA DDRT-PCR, including OPA07G350-27-1 (LDL receptor member LR3), UBC101C-1,000-D-3 (esterase), UBC119A-650-F-5 (*CYP330A1*), OPA18G-600-4-1 (ubiquitin-like-7), OPA01G-415-1 (leucine zipper protein 5), and OPA02G-450-2 (sequence of unknown gene) were carried out using semi-quantitative RT-PCR. The results showed no significant difference in expression level among group of shrimp exposed to 0-27.24 µg/l chlorpyrifos within 96 h. Using RACE-PCR, full length cDNA of carboxylesterase (1,746 bp ORF encoding 582 amino acids), cytochrome P450 (1,530 bp ORF encoding 510 amino acids), and glutathione-s-transferase (654 bp ORF encoding 218 amino acids) were firstly reported. The results from this study provided basic information for toxicity of chlorpyrifos to *P. monodon* and revealed the potential use of acetylcholinesterase activities as biomarker for detecting the pesticide exposure.

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Academic year2006..... Co-advisor's signature.....

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