

## CHAPTER VI

### CONCLUSION

1. Chlorpyrifos decreased from initial concentration (6.81, 13.62, 27.24, 54.48, and 68.10  $\mu\text{g/l}$ ) to almost 0 within 48h.
2. The calculated 24, 48, 72, and 96 h  $\text{LC}_{50}$  value of chlorpyrifos for juvenile *P. monodon* were 52.43, 28.21, 23.64, and 20.74  $\mu\text{g/l}$ , respectively
3. At the lethal concentration, AChE activities observed in shrimp exposed to 68.1 and 681  $\mu\text{g/l}$  of chlorpyrifos were 1.7 and 3.3 times lower than that of control shrimp after 30 min of exposure ( $P < 0.05$ ). For sub-lethal exposure, the AChE activity in shrimp exposed to 0.681  $\mu\text{g/l}$  chlorpyrifos was 1.9 times lower than that of control shrimp after 72 h of exposure. The sensitive reduction of AChE activity at the sub-lethal concentration (0.681  $\mu\text{g/l}$ ) which was 30 times lower than 96 h  $\text{LC}_{50}$  found in this study indicates the potential use as biomarker of chlorpyrifos exposure.
4. Chlorpyrifos could cause cytotoxicity and genotoxicity on *P. monodon* haemocyte. Significant increases of the DNA tail moments were detected from the haemocytes exposed to 0.034 and 0.170  $\mu\text{g/l}$  of chlorpyrifos when compared to that of control group within 1 h ( $P < 0.05$ ) and the evidence was still detected after 6 h of exposure to 0.170  $\mu\text{g/l}$  of chlorpyrifos.
5. Full length cDNA of carboxylesterase, cytochrome P450, and glutathione-S-transferase were obtained by using RACE-PCR.
6. Carboxylesterase was composed of 1,746 bp ORF encoding a putative polypeptide of 582 amino acids. Deduced amino acid sequence shared significant identities (36%) with from many animals.
7. Cytochrome P450 was composed of 1,530 bp ORF encoding a putative polypeptide of 510 amino acids. Deduced amino acid sequence shared significant identities (59%) with from many animals.

8. Glutathione-s-transferase was composed of 654 bp ORF encoding a putative polypeptide of 218 amino acids. Deduced amino acid sequence shared significant identities (59%) with from many animals.

9. Forty four differential displayed transcripts, including 20 up-regulated and 24 down-regulated PCR products were obtained from mRNA DDRT-PCR. Result from BLASTx (NCBI) search identified 22 transcripts (16 up-regulated and 6 down-regulated) were known genes. Twenty two transcripts were unknown genes and hypothetical proteins found in other species (8 up-regulated and 14 down-regulated).

10. Expression analysis of gene including cytochrome P450 (*CYP4C39*), beta glucuronidase, heat shock protein 70, heat shock protein 90, vitellogenin, OPA07G350-27-1 (LDL receptor member LR3), UBC101C-1,000-D-3 (esterase), UBC119A-650-F-5 (*CYP330A1*), glutathione-s-transferase, OPA18G-600-4-1 (ubiquitin-like-7), OPA01G-415-1 (leucine zipper protein 5), and OPA02G-450-2 (sequence of unknown gene) using semi-quantitative RT-PCR did not show difference in expression level among group of shrimp exposed to 0-27.24  $\mu\text{g/l}$  chlorpyrifos within 96 h.