

CHAPTER 9

CONCLUSION

A total of six traditional entomopathogenic fungal isolates, including two isolates of *Beauveria bassiana* and two species of *Metarhizium* were observed from dead arthropods at different locations of Chiang Mai province. These different isolates showed variation in growth, sporulation, germination and virulence in this study. From two isolates of *B. bassiana*, isolate MF03 produced red pigment in culture broth. When the collected isolates were tested for the pathogenic activity, all evaluated entomopathogenic fungal strains were capable of infecting *Bactrocera* spp. pupae. However, *P. lilacinus*, *B. bassiana* (MF03) and *M. flavoviride* are the most virulent strains based on the LD₅₀ and percent mortality. Moreover, the virulence strains were able to cause disease in soil dwelling *Bactrocera* pupae. The results from this study could give an idea of how to control fruit fly pupae in the fallen fruits on the soil. Furthermore, this three virulence strains and isolate MG03 showed high efficacy in infection against two-spotted spider mite (*T. urticae* (Koch)) in both laboratory and pot experiment using soybean as host-plant. The results of our finding pointed out that this four isolates are capable of killing two different host ranges. Finally, we would recommend these virulence isolates as promising agents for effective control of *Bactrocera* spp. and *T. urticae* (Koch) on the soil and plant.

The clear outcomes from the conidia production of collected insect fungi demonstrated that *P. lilacinus*, *M. flavoviride* and *B. bassiana* (MF03) grew well in solid grains and produced inocula significantly better than other strains. Though

fungal growth was highest in corn, sorghum yielded the maximum spore production for the isolates *P. lilacinus* and *M. flavoviride*, while rice had the highest for *B. bassiana* (MF03). Furthermore, the virulence of the harvested dried spore was found in *P. lilacinus* followed by *B. bassiana* (MF03) when conidial suspension was treated against *Bactrocera* pupae. High spore germination and the absence of contaminated microorganisms were observed when conidia were checked for viability and purity after 60 days incubation. Therefore, high spore germination with high purity of tested EPFs would be practical for the field works and ability to maintain longer shelf-life at room temperature. The method used in this experiment is a promising strategy for the large scale production of native fungal conidia of Thailand strains as mycoinsecticide at low cost.

Certain entomopathogenic fungal isolates evaluated for antimicrobial activity produced some antibacterial and antifungal compounds without the addition of any insect-derived materials. Our result pointed out the red pigment producing isolate MF03 has antibacterial activity towards Gram-negative bacterium, *Proteus mirabilis*. In addition, the water extract of native *P. lilacinus* have both anti-*Bacillus* (Gram-positive) and anti-*Proteus* (Gram-negative) activities. To our knowledge, this is the new report of anti-bacterial activity for above two strains against Gram-negative bacteria.

The extracellular cuticle-degrading enzymes and toxins bring as infective activity from tested fungal pathogens were detected. From our findings, the tested fungal isolates produced chitinase and protease which are seem to be participated in the mode of action during infection mechanism. Moreover, the production of secondary metabolites, DPA, was determined from *M. flavoviride* which have

marginal insecticidal activity. The concentration of DPA from this fungal strain was 43.72 mg/L. Auspiciously, this is the first examination of dipicolinic acid from the fungal crude extract of *M. flavoviride*.

From this study, two species of *Cordyceps* showed low infectivity against two different hosts and low activity in producing secondary metabolites. This may be due to the different in the nature of fungi (telomorph and anamorph) and longer storage time, and application method in controlling insects.

The result obtained by the study indicates an absence of toxicity of crude extracts from all selected virulence native insect pathogens to normal cell line (Vero cell) although not identified their specific metabolites. This is the very first finding of the safety of pathogenic crude extract from native *P. lilacinus* and *B. bassiana* in monkey kidney cell line (Vero cell).

Lastly, the potential of the native fungal isolates *M. flavoviride*, *P. lilacinus* and *B. bassiana* (MF03 and MG03) from Chiang Mai province, were firstly discovered in this study as microbial control agents in alternate approach of IPM strategy.