CHAPTER 8

GENERAL DISCUSSION

This study was conducted to investigate whether the pathogenic activity of collected insect pathogenic fungi from natural habitats and culture collected strains will affect on wide range of hosts, and evaluate their potential efficacy as microbial control agents. EPFs were collected from the naturally infected arthropods in some parts of Chiang Mai province, northern Thailand. The application efficient of different conidial concentrations of collected fungal isolates and two species of *Cordyceps* on two different insect orders was examined. In addition, the infectivity of tested isolates was checked for fruit fly pupae in soil and two-spotted spider mites in both laboratory and pot experiments.

Here we observed six isolates out of five species of EPFs were obtained by morphological and molecular identifications. We observed two *Beauveria* isolates and two species of *Metarhizium* from different infected arthropods at different locations.

The ability of insect pathogens to cause disease in fruit fly (*Bactrocera* spp.) and two-spotted spider mites (*T. urticae* (Koch)) are clearly observed in this study. The results of our findings pointed out that all fungal isolates are capable of infecting different host ranges. However, *P. lilacinus, M. flavoviride, B. bassiana* (MF03), and MG03 (in *T. urtice*) were more effective than others according to *in vitro* screenings at high conidial concentrations (10^7 and 10^8 conidia ml^{-1}). It was found that in cultured medium (agar plate) though the mycelia growth of fungi was not

significantly different; the virulence strains produced conidia faster than the less virulence strains. This feature brings these isolates to be virulence strains against tested hosts by rapid colonization of conidia in killing mechanism. Because of the application method of fungal suspension and lack of aerial sporulation on agar plate, two species of *Cordycceps* were found to be less virulence strains against two tested hosts. Another reason of less pathogenicity in *Cordyceps* sp. may be due to the longer storage in culture collection. As an agreement with the report of Claydon *et al.* (1979) that loss of pathogenicity by stored cultures is of common occurance.

In general, fruit flies pupate and hibernate in the soil to avoid adverse environmental condition. Regarding to this behavior we tried to use the fungal isolates in order to evaluate their infection ability to pupae by soil bioassay with two application methods. It was found that the virulence strains were high efficient in killing fruit fly pupae in soil bioassays too. Although most of the studies reported the control of fruit flies by using microbial control agents, it is very difficult to compare the results of our findings to others because of a lack of published study in controlling fruit fly (*Bactrocera* spp.) pupae in soil bioassay. However, similar study was carried out by Anand *et al.*, (2009) in controlling the pupae of *Spodoptera litura* (Fabricius) in the soil by *M. anisopliae* (ARSEF 7487) and *Lecanicillium muscarium* (Petch) (ARSEF 7037).

In TSSM control programs, two-time spraying intervals of selected virulence strains was the most effective in controlling mite population in pot trials on soybean to a leaser extends. Practically, EPFs are mostly used in control of insect pests of greenhouse crops because environmental factors: temperature and humidity are optimal for their development and efficacy (Fiedler and Sosnowska, 2007). It is

conquered with the result of Chandler *et al*. (2005) who found that the two-time interval sprays of *B. bassiana* 432.99, *H. thompsonii* 463.99, *M. anisopliae* 442.99, *V. lecanii* 450.99 and Naturalis- L reduced the population of *T. urticae* on tomato in greenhouse. Adult *T. urticae* were found to be more susceptible to fungal infection than the developmental stages. The differential susceptibility could be attributed to the interaction between the arthropod integument being penetrated by the fungus and ecdysis. Molting has been reported to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals (Vey and Fargues, 1977). Generally, our finding show that mortality increased with age as adults are more susceptible to fungal suspensions.

In this study, we reported the mortality of insects determined by the presence of external mycelia (mycosis) on the dead body of hosts, and the immotile body when touched with fine brush.

It was shown that the selected virulence strains of native insect pathogens are found to be safety for human and the environment when *in vitro* cytotoxicity was carried out against monkey kidney cell (Vero cell line).

The collected fungal strains were cultured in cereal grains to determine the suitable media for large scale production of aerial spores. A successful microbial insecticide should be able to produce high qualities inocula with long-term storage and viable on inexpensive substrates (Goettel and Robert, 1992; Miniania, 1991; McCoy, 1990; Soper and Ward, 1981). High spore production capability is a requirement for the successful development of microbial agents for pest management (Goettel and Robert, 1992). In this study we examined the suitable substrate for the mass production of traditional insect fungi by using rice, wheat, rye, sorghum and

corn as solid substrates. These substrates are available at a relatively lower price in market. Moreover, the experiment was done without adding any supplement in substrate so as to reduce the production cost. The virulence strains such as *P. lilacinus, M. flavoviride* and *B. bassiana* (MF03) produced large number of spores on their respective grain substrates. Though all fungal isolates grew well in all substrates, corn substrate gave the highest growth of mycelium. Among all tested fungal strains, *P. lilacinus* grew very well in all substrates. The maximum amount of conidia was produced in sorghum grain for *P.lilacinus* and *M. flavoviride*, and in rice grain for *B. bassiana* (MF03). The viability of conidia was over 80% for all isolates and no other contaminated microorganisms in harvested dry spores 60 days inoculation. When the virulence of aerial conidia was examined, *P. lilacinus* killed 100% to fruit fly pupa followed by *B. bassiana* (MF03).

Generally the fungus penetrates thinner, non-sclerotised areas of the cuticle, like joints, between segments or the mouthparts. Before penetration, germ tubes may produce so-called appressoria and infection pegs. The penetration process is by mechnical means and by the production of several enzymes, including proteases, chitinases and lipases (Zimmermann, 2007). EPFs secrete specific extracellular enzymes (invasions) during pre-penetration and penetration events (St. Ledger *et al.*,1998).The adhesion of entomopathogens to the insect cuticle is mediated by specific and non-specific factors including adhesins (e.g. hydrophobins), integrins (molecules containing the tripeptide sequence arginine-glycine-aspartic acid (RGD) recognizable by molecules on host cells, lipids, and polysaccharides (Tucker *et al.*,2001). Therefore, in this study an approach was conducted to detect the presence of metabolites in insect pathogens which are playing a part in infection mechanism to

the hosts. All tested insect fungi displayed the examined cuticle degrading enzymes, chitinase and protease, in this study. However, the most virulence four strains activated higher in chitinase activity when glucose was added in combination with 1.0% colloidal chitin. Similar result presented by Ali *et al.* (2010) that the higher rates of chitinase activity was observed when glucose was used in combination with higher colloidal chitin (1.5%). Though, all tested strains produced protease clear zone in screening, the highest proteolytic activity was detected in *P. lilacinus* among selected effective strains. The possible reason for the protease reduction in virulence strains was probably due to the composition of nutrient level of the medium affecting the enzyme synthesis. De Moraes *et al*. (2003) analyzed the secretion of proteases in single and combined carbon sources as compared to the complex substrates like chitin and *Boophilus microplus* cuticle.

Furthermore, in this study we worked out the presences of antimicrobial activity in insect pathogenic fungal isolates by using agar-well diffusion method. Fungal isolates were grown in five culture broths to evaluate the effect of media on antimicrobial properties against seven pathogenic bacteria and six pathogenic fungi. *C. militaris* and the virulence strains, *M. flavoviride* did not show antimicrobial activities except anti-yeast activity in *M. flavoviride* while strain, *P. lilacinus,* did not have antifungal activities. The tested insect pathogens have no antibacterial activity against three tested Gram- negative bacteria. Fortunately, red pigment producing *B. bassiana* (MF03) and water extract of *P. lilacinus* showed antibacterial activity against Gram- negative bacteria, *P. mirabilis* where the final pH of the culture broths was not acidic. When five fungal pathogens were used to test antifungal activity, none

of the insect pathogenic fungi showed clear zone against *Collectotrichum* sp. and *S. solani*.

The native strain, *M. flavoviride*, was observed the effective microbial control agent in causing disease against two different orders of arthropod host with the presence of pathogenic metabolites, dipicolinic acid. The toxicity of DPA to blowflies (Claydon and Grove, 1982) and third-instar nymphs of whitefly (Asaff *et al*., 2005) was studied. Moreover, the maximum concentration of DPA was examined in the fungal crude of *M. flavoviride* from this result.

FURTHER WORKS

The further investigation of the infectivity of the selected virulence strains to diverse number of arthropod species will be necessary in order to manufacture as commercial scale in crop management (IPM) strategy. Moreover, the particular metabolic toxins or enzymes from these strains that play an important role in invasion mechanism should be determined and purified in the future study. In addition, the specific antibacterial and antifungal compounds for respective strains should be investigated.