CHAPTER 1

INRODUCTION

In natural ecosystems, phytophagous insects coexist in a complex relationship with plant communities. Some insects may be harmful to the plant. The degree of yield loss depends upon the plant organs attacked by the insects. To overcome pest problems in modern crop production systems, agrochemicals- generally synthetic pesticides, are used (Panda and Khush, 1995). Through over-use of pesticides and poor spraying techniques, pests developed resistance to most of the available insecticides, hazard to the environment, non-target organisms and human being. Biological control using entomopathogens (bacteria, fungi, virus, nematodes or protozoa) is one of the alternatives in reversing agriculture's hazardous dependence on synthetic pesticides and establishing a more environmental friendly paradigm. One of the biological control options is the use of arthropod pathogens (Maniania and Takasu, 2006). Entomopathogens are widespread in nature and contribute to the natural regulation of insects and mites. They can be exploited for pest management in a number of ways. These natural enemies are being used as microbial control agents (MCAs) of a range of insect and mite pests of horticultural and agricultural crops (Chandler, 2009).

Fruit flies are serious pests that cause enormous losses for farmers in many countries. Fruit flies belonging to the family Tephritidae (Order: Diptera) are considered a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers

(Diamantidis, 2008). In Southeast Asia, fruit fly is economically the most important insect pest of fruit, and can cause losses as high as 90-100%. Fruit flies damage fruits by puncturing and laying eggs under the soft skin in both mature and green fruits (Hollingsworth and Allwood, 2000). The eggs hatch and feed inside the fruit causing the fruits to rot (Dhillon *et al*., 2005) resulting in unmarketable fruits. The use of insecticides as the only way to control pests in fruit and vegetables causes environmental pollution and hygienic problems that represent a risk for people and animals (Gallo, 2007). The alternative to the use of a chemical stressor is to combine entomopathogenic fungi (EPFs) with other microbial pathogens (Zimmermann, 1994).

The biological control of fruit flies can be partially replace other control methods in integrated management programs of fruit flies, especially the use of agrochemicals, presenting economic and environmental advantages for tropical fruit (de Oliveira, 2010). Controls of fruit fly have motivated the search for biological control alternatives, including enotmopathogenic bacteria, nematodes and fungi (Toledo, 2002).

Particularly, in Thailand, two species of fruit fly namely Melon fly (*Bactrocera cucurbitae* (Coquillet)) and Oriental fruit fly (*B. dorsalis* (Hendel)) (Ramadan and Messing, 2003). Sarango, 2009 stated that the amount of insecticides used in the fields is great and the flora of the compounds is much diversified in controlling fruit flies according to the survey done in Kamphaeng saen, Thailand. Bait sprays made up of a mixture of protein hydrolysate and insecticides have been used to attract and kill fruit flies (Sookar *et al.*, 2008).

The biological control of fruit flies is given by parasitoids, predators and pathogens (Salles, 1991). Major hymenopteran parasitoids of flies belong to the families Braconidae, Pteromalidae and Figitidae (Salles, 1995; Malavasi and Zucchi, 2000). Generally, predators have little effect on fruit fly populations in an orchard or vegetable production situation. Invertebrate predators may include spiders, ants, carabid beetles, assassin bugs, staphylinid beetles and probably others (http://www.spc.int/Pacifly/Control/Biocontrol.htm). Tephritid fruit fly biological control relies heavily on larval/egg prepupal braconid parasitoids (Purcell, 1998; Ovruski *et al*., 2000). The microbial control of fruit flies can partially replace other methods of control in an integrated management program for these insects. Aemprapa, 2007 found that *Beauveria bassiana* 6241 was the most suitable strain for controlling *B. dorsalis* (Hendel) in Lampang Province, Thailand.

The two-spotted spider mite (TSSM) *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests of many crops in temperate and tropical climates. Chemical control is the main method of combating this mite (Oliveira *et al*., 2007). As the adverse effect of extensive using of chemicals, biological control of spider mites has been tried as an alternative method to chemical control. Several biological control agents are commercially available for the control of TSSM. Several species of naturally occurring insects and mite preys on spider mite (Opit *et al*., 2005), a lady beetle the spider mite destroyer (*Stethorus* sp.); predaceous thrips, six spotted thrips (*Scolothrips sexmaculatus* (Pergande)) and minute pirate bugs (*Orious* sp.) are important (Parvin *et al*., 2010). Predatory phytoseiid mites are the most reliable biological agent (McMurtry and Croft, 1997), and the adult female *Phytoseiulus persimilis* (Athias-Henriot) preys on all stages of mites in the U.S (Osborne *et al*., 1999).

The most promising microbial control agents of *T. urticae* (Koch) are EPFs, which invade their hosts by growing through the cuticle. Pathogenicity of TSSM using EPFs have been reported by many studies (Tamai *et al.*, 2002; Chandler *et al.*, 2005; Alves *et al.*, 2002, Irigaray *et al*., 2003; Bugeme *et al.*, 2009). *Neozygites floridana* (Weiser & Muma) (Zygomycetes: Entomophthorales) has been reported infecting naturally at least 18 species of tetranychids worldwide. Furtado *et al*., 2007 and Wekesa *et al*., 2007 demonstrated that a Brazilian strain of the predatory mite *Phytoseiulus longipes,* Evans and the pathogenic fungus *Neozygites floridana,* Weiser and Muma have shown promising results in laboratory experiments.

Fungi invade insects by penetrating their cuticle or "skin." Death is caused by tissue destruction and, occasionally, by toxins produced by the fungus (http:// www.nysaes.cornell.edu/ent/biocontrol/pathogens/fungi.htm). Potentially, fungi have great biotechnological importance as a source of new pharmaceutical compounds, secondary metabolites, and other useful 49 compounds and as agents of biological control (Wildman, 1997; Azevedo *et al.*, 2000). During the fungal penetration through the host cuticle, hydrolytic enzymes such as proteases, chitinases and lipases are produced and secreted and are important for the initiation of the infection process (St. Leger *et al*., 1986, 1996). Fungal proteases are believed to play an important role in cuticle penetration (St. Leger, 1995). As with all biological pesticides, safety is a major factor in its public acceptance and use and is very important when it comes to registration (Genthner *et al*., 1998).

It is sometimes assumed a prior that microbial pesticides must be considerably safer to humans and their environment than synthetic chemical insecticides (Hajek *et al.*, 2007a). Among the pathogens, traditional fungal entomopathogens were the only types of pathogens introduced worldwide until about the 1950s. The use of entomopathogenic microbial agents of pest control is very important and often necessary to control some pests that have a high reproductive capacity and short life cycle.

Therefore, this research was conducted to investigate whether the naturally occurring insect pathogenic fungi will infect on these two target pests, mentioned above, of different order even from primary host species under laboratory and pot experiments. To elucidate the role of secondary metabolites in the infection process, it is essential to characterize these fungal metabolites. The results from this research will be used as the guide line for integrated control of insects for growers supporting as organic farming system.

Research Objectives

The main objectives of this study are listed below:

1. To isolate and identify the entomopathogenic fungi from natural habitat.
2. To detect the virulence strains of collected entomopathogenic fungi.
3. To investigate the pathogenicity of entomopathogenic fungi on agricultural pests.
4. To examine the growth conditions of insect pathogenic fungi on artificial culture.
5. To partially characterize the secondary metabolites from entomopathogenic fungi.

Usefulness of the Research

1. Discovery of insect pathogenic fungi from natural habitat.
2. Occurrence of pathogenic ability of entomopathogenic fungi against tested agricultural pests in laboratory bioassay and pot experiments.
3. Point out the possible bioactive compounds and fungal metabolites from insect pathogenic fungi