1. GENERAL INTRODUCTION

During the past few decades, our environmental quality, biological resources and biodiversities became in danger and some were damaged. El-Hage Scialabba (2003) described that the preferred use of uniform cultures have reduced dramatically the number of plants and animals produced in agriculture. Moreover, agricultural activities affect 70% of all threatened bird species and 49% of all plant species. FAO estimates that about three-quarters of the genetic diversity of agricultural crops have been lost over the last Century. Furthermore, a number of 1,350 from 6,300 animal breeds are endangered or already extinct (FAO, 2004). One of the many reasons is the use of chemical pesticides to improve agricultural productivity. While the study for alternative ways of pest control still continues, there is an increasing public concern on the health and environmental effects of highly residual synthetic pesticides. Therefore, during the last decade, especially in developed countries, more reliable synthetic pesticides with high efficiency were developed and introduced. Apart from that, particularly in developing countries an increasing interest in searching for new plant sources to find new secondary metabolites possessing toxicity to insects and plant pathogens can be observed (Sanches and Ohsawa, 1994; Manasikarn, 1996).

Several botanical insecticides such as pyrethrum, rotenone and neem are widely used, but some of them are partial very toxic against the environment to agricultural crops and non target organisms as well as human health. Also, many of the known botanical insecticides are not persistent Therefore, searching for new botanical insecticides is still essential.

Many insecticides cause an adverse effect on the plants in terms of phytotoxicity. Phytotoxicity or plant toxicity is an unwanted detrimental deviation from the normal patterns of appearance, growth, and function of plants in response to pesticides and other toxic chemicals. The phytotoxic response may occur during germination, growth, differentiation, and maturation of plants, and may be of a temporary or long-term nature. Phytotoxic responses include adverse effects on growth habit, yield, and quality of plants or their commodities (EPA, 1996).

The persistence of some chemical pesticides in the environment is relatively long, ranging from days to years and can thus affect ecosystems by adverse effects on non-target organisms. Similar to conventional pesticides, the botanical insecticides may cause sometimes negative effects on non-target organisms. For example, pyrethrum is easily destroyed by light and air but it is toxic to fish and honeybees under laboratory testing (Perry *et al.*, 1998). Neem oil is also toxic to several fish species. The seeds, whole fruits, or leaves of *Melia azedarach* contain compounds which are highly toxic to mammals (Schmutterer, 1995).

Therefore, it is very important to study the negative side effects of a new insecticide on agricultural products and also the ecosystem before marketing and use. The main objective of this research is to identify and to produce a new botanical insecticide from selected Thai local plants. The adverse effects of this new botanical insecticide on agricultural crops using the example of Chinese kale and the ecosystem are also studied.

Outline of the thesis

Chapter 2 contains the summary of the thesis. The selection of Thai native plants for their insecticidal activity is described in Chapter 3. The plant with highest insecticidal effective is chosen for the purification and identification of its active compound. The active compound is confirmed for its anti-insect properties in the laboratory. The crude extract of the selected plant is formulated for using as botanical insecticide and its fingerprint is also established for the quality control.

In Chapter 4, the produced insecticide is studied for its insecticidal efficiency in the experimental field. Moreover, its effects on physical properties and some quality parameters of Chinese kale are also determined. The residue of this insecticide in the environment and its adverse effects on non-target organisms are described in Chapter 5. Finally, general conclusion and some recommendation for future study are presented in Chapter 6.

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2. ABSTRACT

Botanical insecticides derived from plants may be an alternative to the use of chemical pesticides. In general, they may consider being beneficial, safe to human and the environment. However, some of botanical pesticides have adverse effects. In this study, nine species of Thai local plants were selected for their highest insecticidal activity. *Mammea siamensis* was selected for purification and chemical identification of the active compound. Surangin B was identified as the active compound which had a higher insecticidal activity on the larvae of diamond back moths than methomyl insecticide. Crude ethanolic extract of *M. siamensis* was formulated as emulsifiable concentrate (EC). The quality control of the extract from each batch was carried out with HPTLC.

In the field study with Chinese kale, the mammea insecticide was slightly less efficient in the insect control than the synthetic methomyl insecticide. However, it showed a better effect in controlling the population of diamondback moth larvae than the studied synthetic insecticide. Moreover, the environmental friendly effect was also demonstrated by giving a slightly higher number of beneficial insects (natural enemies) than the methomyl treated and the control groups. Nevertheless, the yield of Chinese kale sprayed with mammea insecticide was not significantly different from that of the control group.

In the net green house no insect pest interference was observed and mammea insecticide had a tendency to increase the yield of Chinese kale. Most of the physical plant properties i.e. height, fresh weight, dry weight, number of leaf and leaf length of Chinese kale were slightly higher than those after methomyl treatment and the control, respectively. Furthermore, all the analyzed quality parameters i.e. chlorophyll and carotenoid contents, reducing sugars, total dietary fiber, total ascorbic acid, total antioxidative activity, ascorbate peroxidase activity and free amino acid contents were not significant different. From these results, it could be generally concluded that mammea insecticide had no adverse effect on both physical and quality parameters of Chinese kale.

Furthermore, mammea insecticide showed no significant negative effect on the ecosystem. Its active ingredient had a shorter persistence in the environment than the insecticide methomyl. Its residue was found on Chinese kale only one day after spraying whereas in soil and water no residues were already detected at the day of harvest. The toxicity of mammea insecticide on non target organisms was also studied. The results showed that it had no toxic effect on both earthworm and honeybee. However, it was more toxic in *Tilapia* than methomyl insecticide. Nevertheless, because of its short-term persistence on environment, it could be assumed that under real field conditions the application of mammea insecticide followed the instruction (0.5 g L^{-1}) might not produce a strongly adverse effect on any aquatic organisms.

Therefore, from its insecticidal effectiveness especially on diamondback moth, with no significant adverse effects on vegetable quality and the ecosystem, the mammea plant extract could be one of the potentially natural resources for a botanical insecticide.

3. BOTANICAL INSECTICIDE IDENTIFICATION AND PRODUCTION

3.1 Introduction

Botanical insecticides are defined as phytochemicals having the capacity to kill or severely impair the function of insects. Naturally occurring plant toxins were first recognized as insect controlling agents by indigenous cultures long before they were exploited by modern societies. By the late 1800s, about half a dozen botanical insecticides were in common use in Europe and North America and this situation continued until the introduction of the first synthetic organic insecticide in the early 1940's (Duke, 1990).

Soejarto and Farnsworth (1989) estimated that out of the 250,000 species of flowering plants, only 5,000 species had been thoroughly investigated according to the Natural Product Alert (NAPRALERT) database, leaving 98% of these species with potential for phytochemical discovery. Heal *et al.* (1950) also reported that approximately 2,500 plants in 247 families had some toxic properties against insects. From an academic point of view, plants representing a vast storehouse of potentially useful natural products. Many laboratories worldwide have screened thousands of species of higher plants, not only searching for pharmaceuticals, but also for pest control products. These studies have indicated that numerous plant species possess potential pest-controlling properties under laboratory conditions, but the step from the laboratory to the field eliminates many contenders (Isman, 1997).

With the rising concern for environmental safety there has been a renewed interest in the use of naturally occurring substances as pesticides, including plant bioactive compounds. Many insecticides have active control agents for a variety of insect pests**.** Although a considerable number of botanical insecticides have been reported, some of them with outstanding biological activity, only four have been used for crop protection: nicotine from tobacco leaves, rotenone from Derris roots, pyrethrum from Chrysanthemum flowers, and azadirachtin from the neem tree. Today, pyrethrum and azadirachtin are the most important botanical insecticides, representing around 1% of the global insecticide market (Casanova *et al.,* 2002).

3.1.1 Plants used as insecticides in Thailand

***Acorus calamus* Linn.**

The common English name for *A. calamus* is 'sweet flag' or 'wann nam' in Thai. It is a perennial herb growing on the water edge and in wetlands (Saralamp *et al.,* 1997).

Part used: Rhizome (Tang and Eisenbrand, 1992).

Chemical investigation: The major components are sesquiterpenes and volatile oil (Yadava, 1971; Yamamura *et al.*, 1971; Tang and Eisenbrand, 1992).

Biological activities: The insecticidal and fungicidal properties of *A. calamus* have been investigated, for example, the spore growth of *Alternaria* sp., *Botrytis* sp. and *Fusarium* sp. could be inhibited by a dichloromethane extract of this plant. This extract also showed insecticidal activity against *Plutella xylostella* (Jiyavoranan, 2001; Mungkornasawakul, 2001).

***Eugenia caryophyllus* (Spreng.) Bullock et Harrison**

The common English name for *E. caryophyllus* is clove or 'kann plu' in Thai. It is a 5-10 m height. The stem contain simple leaves, opposite elliptic or lanceolate. The young leaf is red or brownish red; inflorescence in axillary corymbs. The fruit is a berry, ellipsoid-ovoboid and dark red (Saralamp, 1997).

Part used: Flower buds and oil from flower buds.

Chemical investigation: The major components are eugenol, β-caryophyllene, α-humulene and eugenyl acetate (Jirovetz *et al*., 2006)

Biological activities: The flower buds and oil are used as acaricides and insecticides especially in store product insect (Ho *et al*., 1994, Kim *et al*., 2003)

***Mammea siamensis* (Miq.) T. And.**

M. siamensis belongs to the Family Guttiferae, it is called "saraphee" in Thai. It is 10-15 m high and has white yellow fragrant flowers (Poobraseart *et al*., 1998; Mahidol *et al*., 2002)

Part used: Seeds, twigs and flowers (Poobraseart *et al*., 1998, Kaweetipob *et al.* 2000; Srioon, 2001).

Chemical investigation: The major components are coumarins and xanthones (Poobraseart *et al*., 1998; Prachyawarakorn *et al.,* 2000)
Biological activities: Seeds are used as an insecticide and flowers as a heart tonic in local medicine (Srioon, 2001; Mahidol *et al*., 2002).

***Stemona* spp.**

S. burkillii, S. curtisii, S. kerrii and *S. tuberosa* belong to the Family Stemonaceae. There are about 10 species of *Stemona* growing in an all parts of Thailand, in the deciduous and evergreen green forests. The characteristic of each species are different and have been described. (Maxwell, 1991; Duyfjes, 1993).
Part used: Rhizome (Suzuki, 1934; Kuo and Chu,1978; Zou *et al.* 2000)
Chemical investigation: The major components are *Stemona* alkaloids (Pilli and Ferreira de Oliveira, 2000).
Biological activities: The rhizome extracts of many species of *stemona* are used as insecticides and antibacterials (Pacher *et* al*,* 2002 .; Brem *et al*., 2002; Pham *et al*., 2002; Seger *et al.,* 2004).

3.1.2 Bioassay

The detection of the biological activity in natural product mixtures can be divided into two groups for screening purposes: general screening bioassays and specialized screening bioassays.

The two most popular general screening bioassays are the brine shrimp lethality test (BST) and the crown-gall tumor inhibition test (Ghisalberti, 1993; McLaughin, 1991). The first technique is an *in vivo* lethality test using a tiny crustacean, the brine shrimp (*Artemia salina*). Since its inception in 1982 (Mayer *et al*., 1982), this test has been used for the *in vivo* detection of active anti-tumor agents and pesticides produced by plants. It can also be used to evaluate plants for different pharmacological activities while the details of the crown-gall tumor inhibition test have been described (McLaughin, 1991).

The specialized screening bioassays are procedures which are used for detecting insecticidal activity. There are many well-known procedures for the insecticidal properties investigation e.g. topical application, feeding and drinking method, injection method, dipping method and residue method (Busvine, 1971, 1980; Perry, 1998).

3.1.3 Pesticide formulation

A pesticide product consists of two parts: the active and the inert ingredients. The active ingredient is any substance that will prevent, destroy, repel or mitigate any pest, or a substance that functions as a plant regulator, desiccant or defoliant. An inert ingredient refers to any substance other than the active ingredient which is intentionally included in a pesticide product. Some examples of inert ingredients include solvent, stabilizer, spreader or sticker, preservative, surfactant and defoamer, etc. (US Environmental Protection Agency, 2006a). The active ingredients in pesticide products come from many sources. Many such as nicotine, pyrethrum, and rotenone, are extracted from plants. Others are synthetic or inorganic origin, while a few are derived from microbes. Regardless of their sources, pesticide active ingredients have different solubility. Some are dissolved readily in water, others only in oils. Other active ingredients may be relatively insoluble in both water and oils. These different solubility characteristics, coupled with the intended use of the pesticide, define the types of formulations in which the active ingredient may be delivered (Blessing, 2001).

In the first research chapter of this project, Thai local plants which are known to have very active insecticidal properties were selected using brine shrimp lethality test and then purified and examined for active substances. The insecticidal efficiency of the active compound was determined in comparison with synthetic insecticides using diamondback moths. The ethanolic crude extract of the selected plant was further formulated for a botanical insecticide.