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EFFECTIVENESS OF NORN-TAI-YARK, *STEMONA* SPP. (STEMONACEAE) PRODUCTS FOR CONTROLLING SOME INSECT PESTS OF CAULIFLOWER

CHANTHA KIM

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By

CHANTHA KIM

APPROVED BY

Advisory Committee Chairperson ..... D. Pongphan .....

(Assistant Professor Damkoeng Pongphan)

..... 6 / Oct / 2008 .....

Advisory Committee Member ..... Charnnarong Duangsa-ard .....

(Associate Professor Dr. Charnnarong Duangsa-ard)

..... 6 / Oct. / 2008 .....

Advisory Committee Member ..... Chantana Wicharatana .....

(Assistant Professor Chantana Wicharatana)

..... 6 / Oct / 2008 .....

Chairperson, Committee on Master of Science Program in Horticulture ..... Prasit Noree .....

(Assistant Professor Dr. Prasit Noree)

..... 7 / Oct. / 08 .....

CERTIFIED BY

GRADUATE SCHOOL .....

(Associate Professor Dr. Thep Phongparnich)

..... 7 / Oct. / 2008 .....

Title Effectiveness of Norn-Tai-Yark, *Stemona* spp. (Stemonaceae) Products for Controlling Some Insect Pests of Cauliflower

Author Mr. Chantha Kim

Degree Master of Science in Horticulture

Advisory Committee Chairperson Assistant Professor Damkoeng Pongphan

ABSTRACT

Norn-Tai-Yark (*Stemona* spp. (Stemonaceae) is distributed mainly in tropical Asia. *Stemona* plants have long been known for their medicinal use in traditional medicine. Currently, interest has become highly focused on using the plant as pest control agent. The effectiveness of *Stemona tuberosa* Lour. fermented (FS) and *Stemona curtisii* Hook f. ethanol extracts (Biopes) on Diamondback moth, *Plutella xylostella* (L) and Common cutworm, *Spodoptera litura* (F), the insect pests of cauliflower, *Brassica oleracea* L. var. *botrytis* L., were investigated under laboratory and field conditions. The best contact activities against third-instar larvae of *P. xylostella* were observed from FS with the LC_{50} (72 hr) of 25, 000 ppm and from Biopes with the LC_{50} (72 hr) of 25, 000 and 3, 000 ppm. Similarly, the best antifeedant activities against third-instar larvae of *P. xylostella* was also observed from FS with LC_{50} (72 hr) of 20, 000 and 25, 000 ppm within 0.40 to 0.60 mm^2 leaf damage area per larva and from Biopes with the LC_{50} (48 hr) of 2, 500 and 3,000 ppm within 2.60 mm^2 and 0.60 mm^2 leaf damage area per larva. For the *S. litura* as observed from FS and Biopes, the results could not increase up to 50% accumulated mortality for all treatments and various times of application.

Under field conditions, highest yield of 7,466.70 and 8,160.00 kg/ha of cauliflower, the lowest population larvae of *P. xylostella* and percentage leaf damage by *P. xylostella* larvae were detected when using *S. tuberosa* fermented and *S. curtisii* extracts at the rate of 25 ml and 3 ml per liter of water, respectively.

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Abbreviations

AMLD	Accumulation mortality by leaf dipping method
AMTA	Accumulation mortality by topical application method
ANOVA	Analysis of Variance
Biopes	*Stemona curtisii* ethanol extract (trade name)
DAT	Day after transplanting
DBM	Diamondback Moth
dpi	Dots per Inch
DT	Data transformed
FAO	Food and Agriculture Organization
FS	Fermented *Stemona tuberosa*
GIF	Graphics Interchange Format
JPEG	Joint Photographic Exerts Group
LC_{50}	Lethal concentration, that kills 50% of the test animals in a given time
LD	Leaf dipping method
LZW	Lempel-Ziv-Welch
LDA	Leaf damaged area
LSD	Least Significant Difference
PLDA	Percentage leaf damage area
ppm	Part per Million
ppb	Part per Billion
TA	Topical application method
TLD	Total leaf area (4 cm x 4 cm)
TIFF	Tagged Image File Format
WHO	World Health Organization

Chapter 1

Introduction

Cauliflower, *Brassica oleracea* L. var. *botrytis* L., is a cole crop that belongs to the family Cruciferae. It is one of the important vegetable crops in the world. It contains most of the minerals and vitamins necessary for human diet (Holland *et al.*; 1991). It is cultivated in areas where the optimum temperature is 27°C, usually ranging from 7-29°C. Some cultivars can also be grown over 30°C although most varieties are very sensitive to climatic changes, especially temperatures. It grows best on soils which are slightly acidic to neutral (pH 5.7 to 6.6).

The problems which usually arise during the growing season of cauliflower consist of severe damages to the plant caused by some insect pests, which can be defined as follow: (1) The insect pests may damage the economic product for which the crop is grown with primary effect on the reduction in product quality (direct damage). (2) The insect pests may cause indirect damage to crops by attacking their non-economic vegetative or reproductive parts with primary effect on the overall yield loss. (3) The insect pests may not attack the plant parts but they may decrease the crop value by contaminating the harvested product with their presence as living organisms, as dead bodies or parts or as by-products of their metabolism. These foreign materials in economic products diminish the product quality and consequently the economic value. (4) Some insects may not attack the economic plants, however, they can serve as vectors, acquiring a pathogen from an infected host and transmitting it to another host (Acquaah, 2005). Major insect pests commonly found attacking cauliflower in almost all growing areas and in all seasons are 'diamondback moth' (DBM) and 'common cutworm' (Siemonnsma and Piluck, 1994).

To control these insect pests, most of the growers relied only on the use of insecticides which are applied on scheduled or calendar spraying without any consideration whether the pests are present in the field or damage occurs or not. Such practices can create the following problems (Luckmann and Metcalf, 1994): (1) selection of resistance in population, (2) destruction of beneficial species, (3) resurgence of treated populations, (4) outbreak of secondary pests, (5) residues in foods and environment, and (6) hazard to human and environment.

Sole reliance on insecticides has created problems for controlling the insects and maintaining environment. Thus intensified search for alternative strategies of pest control is

necessary. Among the strategies available, botanical insecticide is one of the promising tactics to control the vegetable pests. Examples include extracts or products derived from Norn-Tai-Yark (*Stemona* spp).

To obtain basic information on the effects of *Stemona* products on controlling some insect pests of cauliflower, this study was conducted with the following objectives:

1. To evaluate the effectiveness of *Stemona* products to control the diamondback moth *Plutella xylostella* (L) and common cutworm *Spodoptera litura* (F), both under laboratory and field conditions, and

2. To evaluate the effects of *Stemona* products on growth and development of cauliflower.

Chapter 2

Literature Review

Cauliflower

Cauliflower (*Brassica oleracea* var *botrytis*) is a member of cole crop group and is native to the Mediterranean region. Cole crop falls under mustard family, *Cruciferae*. Cauliflower originated from the word caulis, meaning stem or stalk of a plant. It is a biennial crop, but is generally grown as an annual crop. It has diploid chromosome number (2n = 18) and seed weight of 1,000 seeds is around 3 grams (Tindall, 1993). It contains most of the minerals and vitamins necessary for human diet.

Cauliflower is classified as a "cool season" crop that can be resistant to frost and light freezes. Throughout the U.S. it is grown in the spring or fall; so that normal growth takes place when the temperatures are cool (Martin *et al.*, 2006). Cold period is necessary for curd formation, the minimum and maximum growing temperatures for cauliflower are 0°C and 30°C, with the optimum growing temperature between 15 to 22°C. The minimum, optimum and maximum germination temperatures for cauliflower seed are 7, 27, and 29°C, respectively. High temperatures during cauliflower production delay maturity and increase vegetative growth while cool temperatures hasten maturity and may induce bolting. Fluctuating temperatures may induce heading in some cauliflower cultivars to revert back to vegetative phase resulting in poor quality curds. Optimum soil pH is in the region of 6.0-7.0 with acid soils are sometimes treated with lime or limestone if the pH value is too low (Viliam *et al.*, 2007).

Insects of economic importance of cauliflower

Diamondback Moth, *Plutella xylostella* (L)

Description of stages of development and damage. The adult moth of the Diamondback Moth is small and slender with long antennae. It is grayish-brown with a broad cream or light brown band along its back. The band can have constrictions, which give it a diamond-like pattern. When viewed from the side, the wing tips appear to turn up slightly. Eggs are oval and flattened, yellow to pale green, and approximately 0.02 inches long and 0.01 inches wide. There are four larval instars. Even the oldest is quite small and very active. Larvae will wriggle violently if disturbed and will drop on the leaf suspended by a stand of silk. The body tapers at both ends and the fifth pair of prolegs protrudes from the posterior. After the first instars, which are colorless, the larvae become green. Larvae pupate in loose cocoon on lower or outer leaves or in florets of cauliflower. The female mostly attaches her eggs to the lower leaf surface, either singly or in groups of two or three. The eggs hatch, and the larvae begin to feed on the underside of the leaf. Newly hatched larvae are pale white with a pale brown head while the fully grown caterpillars are light green, measuring 10 mm in length. The population growth is most rapid at high temperature. Pupation takes place in a loose mesh of silken cocoon spun by the caterpillar. The mature pupae are 6 mm long and of light brown color, which are usually attached to the underside of leaves. Plant at all stages of growth may be attacked. Larvae chew small holes in leaves, with larger larvae making larger holes. Often young larvae feed on one surface of the leaf, leaving a thin layer or "window" of leaf epidermis. The resulting damage deforms the head and leaves entry points for decay pathogens (Webb, 2002).

Duration of developmental stages. The incubation period of eggs ranges from 3 to 6 days (Abraham and Padmanabhan, 1968). Patil and Pokharkar (1971) reported an incubation period of 4 to 6 days under laboratory conditions while Jayarathnam (1977) observed an incubation period of 3 to 4 days under both laboratory and field conditions. Total larval period extends from 14 to 21 days (Abraham and Padmanabhan, 1968). Patil and Pokharkar (1971) observed five larval instars and a larval period of up to 11 days; however, Jayarathnam (1977) reported only four instars. The first instar was occupied 3 days in the hot season, 3 to 4 days in the rainy season and

4 to 5 days in the cold season. The second instar stage extends for 2 days in the hot and rainy seasons and 2 to 3 days in the cold season. The third instar larvae generally feed on mature leaves for 2 days in the hot and rainy seasons and for 2 to 3 days in the cold season. The fourth instar larvae, excluding the prepupal period, consume the largest quantity of leaf tissue and last for 2 days in the hot season, 2 to 3 days in the rainy, and 3 to 4 days in cold season. The pre-pupal period lasts for 1 day in all 3 seasons. The total larval and prepupal periods are estimated as 10 days in the hot and rainy seasons and 12 to 15 days in the cold season. The pupal stage ranges from 7 to 11 days (Abraham and Padmanabhan, 1968). However, Patil and Pokharkar (1971) reported the pupal period to vary from 3 to 7 days, with an average of 5 days. Jayarathnam (1977) observed that the pupal period may last up to 4 days in the hot and rainy seasons and 4 to 5 days in the cold season. These results show significant variations in pupal period in different parts of India possibly due to climatic variations. Adults are grey moths with a wing expanse of 14 mm. Their longevity ranges from 3 to 11 days (Abraham and Padmanabhan, 1968). Patil and Pokharkar (1971) reported the lifespan of male and female to be 10.4 and 12.1 days, respectively. Jayarathnam (1977) found adults to survive for 3 to 6 days without food and for 11 to 16 days when provided with food.

Common Cutworm, *Spodoptera litura* (F)

Description of stages of development and damage. Between 2 and 5 days after emergence, females lay 1,000-2,000 eggs in egg masses of 100-300 on the lower leaf surface of the host plant (Miyahara *et al.*, 1971). Waterhouse and Norris (1987) reported egg masses (4-7 mm diameter) were laid at nigh in clusters of 200 to 300. The masses are covered by hair-like scales from the end of the insect's abdomen. Fecundity is adversely affected by high temperature and low humidity (about 960 eggs laid at 30°C and 90% RH and 145 eggs at 35°C and 30% RH) (Miyahara *et al.*, 1971). The larvae feed on the surface of the leaf in cluster. If the leaf is unpalatable, they drop on silken threads and are carried elsewhere on the wind. Young larvae are pale green and later instars are dark green to brown. Although coloration is variable, the bright yellow stripe along the dorsal surface is characteristic. The older larvae are night feeders but usually remain on the plant during the daytime (Waterhouse and Norris, 1987). The young larvae (first to third instar) feed in groups, leaving the opposite epidermis of the leaf intact. Later, the

(fourth to sixth instar) larvae disperse and spend the day in the ground under the host plant, feeding at night and early in the morning (Miyahara *et al.*, 1971). Schreiner (2000) reported larvae feeding on both foliage and curd of host plant. Common cutworm larvae consume greater amounts of leaf tissue than the diamondback moth. Since adults can readily invade a field from nearby crops or weeds, monitoring the crop twice a week for common cutworm presence and damage is recommended. The pupation takes place in the soil close to the plants. The adults are grayish-brown, 15-20 mm long with a wingspan of 30-38 mm (Schreiner, 2000). The forewings are grey to reddish-brown with a strongly variegated pattern and paler lines along the veins; the hindwings are grayish-white with grey margin. Females mate three or four times during their lifetime and lay up to 2500 eggs (Waterhouse and Norris, 1987; Hill, 1975).

Duration of developmental stages. The eggs hatch in about 4 days in warm conditions, or up to 11-12 days in winter (Miyahara *et al.*, 1971). Waterhouse and Norris (1987) reported that the eggs take 2-3 days to hatch. The larvae pass through six instars in 15-23 days at 25-26°C (Miyahara *et al.*, 1971). Waterhouse and Norris (1987) observed that the larvae go through six instars lasting 13-30 days, depending on temperature. The pupal period is spent in earthen cells in the soil and lasts about 11-13 days at 25°C. Longevity of adults is about 4-10 days, being reduced by high temperature and low humidity. Thus, the life cycle can be completed in about 5 weeks (Miyahara *et al.*, 1971). Waterhouse and Norris (1987) reported that pupation takes place in the soil close to the plants, lasts a period of 7-10 days. In Japan (Nakasuji, 1976), four generations develop between May and October, while in the humid tropics there may be eight annual generations. In the seasonal tropics, several generations develop during the rainy season, while the pupal stage survives the dry season.

Insect Pests Control

Insect pests can damage vegetables in several different ways. Pests like diamond back moths, common cutworms, flea beetles and imported cabbage worms, feed primarily on the leaves, whereas aphids suck sap from the plant and cause indirect damage. The ability of plants to produce curd or flower stalk can be reduced if they lose enough leaf area or sap. An estimated 50% of crops are lost to pests in the field, making crop protection a necessity. Chemical insecticides are the most powerful tool available for use in pest management. They are highly

effective, rapid in curative action, adaptable to most situations, flexible in meeting changing agronomic and ecological conditions, and relatively economical. Insecticides are the only one tool for pest management that is reliable for emergency action when insect pest populations approach or exceed the economic threshold. Despite these impressive credentials, much use of insecticides has been ecologically unsound, leading to such disadvantages as insect pest resistance, outbreaks of secondary pests, adverse effects on non-target organisms, objectionable pesticide residues, and direct hazards to the user (Smith, 1970). There are many problems arising from pesticide use and misuse that result to undesirable impacts of various chemicals used as insecticides, such as:

1. Effects on non-target pests: Pesticides may harm useful organisms, such as honey bee or beneficial insects that are critical in the biological control of pest populations. Some insecticides are toxic to wildlife (within the agroecosystem). The repeated applications of pesticides to crops obviously have a destructive effect on beneficial insect populations. As a result, fields and orchards under heavy insecticide treatment schedules may become veritable "biological deserts" (Van den Bosch and Stern, 1962). Many wildly used insecticides are highly toxic to vertebrates as well as to invertebrates and act as general biocide (Cope, 1971; Newsom, 1967). The honeybee is important for producing honey and beeswax and more importantly, the honeybee is estimated to pollinate 80% of deciduous fruits, vegetables, legumes and oil crops. This is a major problem of beekeepers especially where applications are made to crops during the bloom period.

2. Cost of pesticides. Although low cost maybe a reason for the use of the pesticides but in other situations, pesticides are an expensive choice. This is especially true for management of certain insects where biological control is a feasible alternative. In many developing countries, the cost of pesticides is prohibitive. Often cheap labor offers alternatives to pesticides as, for example, in hand weeding or in hand picking worms from crop plants.

3. Residues and drift. Residues remain in the soil, water or in harvested produce after application of a pesticide. Residues may be a particular concern if pesticides are applied incorrectly. Drift occurs when pesticides are applied during unfavorable weather conditions. Wind may carry pesticides to areas adjacent to the crop fields, resulting in damage to neighboring plants and animals. Contamination of soil and water in the highlands of northern Thailand by organochlorine pesticides has been monitored since 1974. Several pesticides and their metabolites

namely DDT, heptachlor, dicofol, lindane, endosulfan, BHC, aldrin and dieldrin have been found. However, none of these were over maximum residue limits. Applications of pesticides often drift long distances from the target site, and indeed it appears that less than half the total amount of insecticides applied as sprays to crops is deposited on plant surfaces for effective control and less than 1% is applied to insects themselves. Insecticides containing organochlorines, pyrethoids, endrin, endosulfan, and toxaphene, have caused massive fish kills when present in the water in the ppb range. Insecticide residues are nearly everywhere and highly persistent non-degradable compounds such as DDT, DDE, diedrim, heptachlor epoxide, lindane, endrin, toxaphene, and dicofol are detectable in the soil, water and human adipose tissue as well as in wildlife (Metcalf, 1986).

4. Food contamination. The possibility exists that pesticide residues are lead to long-term, adverse health consequences for consumers. Residues are of particular concern on food, for instant, as tolerance may quite be different from those for adults. When tests have been done, pesticide residues are typically either not detectable or are within established tolerances. Hayes (1969) estimated that an average of 100 non-fatal poisoning occur for each fatality and enumerates 85 insecticides known to have caused poisoning in humans.

5. Hazard to farm workers. Due to toxicity, pesticides have the potential to cause illness in farm workers, especially for those working in hand-harvested fresh market crops. Because these must be free of cosmetic damage, they require pesticide application close to harvest. Grandstaff (1992) reported that farmers harvested their crops according to market prices, which occurred frequently before the end of the recommended waiting period. Farmers' behavior in spray mixing and handling of pesticides indicated an unawareness of the actual danger. Pesticide use is the greatest occupational health hazard to agriculture in Thailand based on the consensus of officials interviewed in Occupational Health Division, Ministry of Public Health and Occupational Health Faculties at two major Thai Universities (Chase, 1990).

6. Create pest problems. Several potential problems can develop with repeated use of pesticides:

a. Pesticide resistance. The repeated use of a single pesticide may lead to the selection of pests that are resistant to the pesticide. This is an enormous problem for the use of all types of pesticides but has been particularly important with pesticides, herbicides, fungicides and

bactericides (antibiotics). Insecticide resistance is believed to develop largely, if not entirely, as a result of the natural selection of pre-adaptive mutants that process genetically controlled mechanisms for detoxification, target-site insensitively or other means of survival in the presence of pesticide, for example, the enzyme DDT-ase that converts DDT to nontoxic DDE or altered acetyl clolinesterase that is not readily inactivated by organophosphorus insecticides. These genetic factors may be present in a very low frequency in the population before insecticidal treatment. For example, *Anopheles gambiae* Giles in northern Nigeria was found to contain heterozygotes for the single gene of diedrin resistance in 0.4-6% of the population. Intensive selection by dieldrin or BHC residues, house spraying for malaria control, increased the gene frequency for resistance to 90% after 1-3 years (Brown and Pal, 1971). Georghiou (1990) reported that more than 500 species of insects had developed resistance to one or more classes of insecticide.

b. Pest resurgence. When the pesticide (usually an insecticide) kills the target pest and also kills beneficial insects, the pest population often will increase to a level higher than the level preceding the application. The phenomenon is called pest resurgence. When the survivors of the target population begin to produce, their numbers grow exponentially because the beneficial pests that once limited population growth are no longer present. Pest resurgence often leads to repeated pesticide applications. Resurgences following the disruption of natural enemies are much more prevalent than generally realized. About 24 out of the 25 most destructive insect pests of California agriculture are resurgences that lead to increased use of insecticides (Luck *et al.*, 1977).

c. Secondary pest outbreaks. When the pesticide kills the key pest but not a minor (secondary) pest, the minor pest population may increase and become important. Kenmore (1984) reported that the population of the brown planthopper in the pesticide treated field was much higher than in the untreated field because of disruption of population regulation factors by insecticides. Both parasites and predators were killed and their population could not build up fast enough because the pest population was temporarily too low to provide adequate food.

7. Pesticide treadmill. Misuse of pesticide may lead to more frequent applications and require higher rates of the product needed to control the same pest. This phenomenon has been

called the pesticide treadmill and it is the result of severe ecosystem disruption. The pesticide treadmill has been of particular concern in the excessive use of pesticides.

Alternatives for Insect Pests Control

There are many control methods besides chemical insecticides used on field vegetable crops that can reduce insect pest population, such as agricultural method, mechanical control, biological control and especially, plant derivation. Robert *et al.* (2003) reported that botanical insecticides derived from plants were some of the first known pesticides. As plants evolved, the selection pressures exerted by animal pests and by pathogens resulted in the evolution of plant chemical defenses that act to inhibit pest attack. Defensive compounds that plants produce at all times regardless of whether the pest is present, are termed constitutive defenses. In some plants, defensive chemicals are produced only after pest attack, and those chemicals are referred to as inducible defenses. Plants that produce active constitutive chemical defenses may be grown so that the chemicals can be extracted and applied as pesticides. Hellebore, a plant in the buttercup family, was used by Romans to control rats, mice and insects. Pyrethrum, derived from plants in the genus Pyrethrum and rotenone from the Derris plant were used in widespread manner in the mid-nineteenth century. But the use of nicotine for insect control was well established by the mid-eighteenth century. The chemical azadirachtin obtained from the neem tree, *Azadirachta indica*, is an effective insecticide. Many plants produce various alkaloids especially *Stemona* spp; Botanical insecticides can be degraded rapidly under environmental conditions such as sunlight, humidity, and rainfall. This means that they are less persistent thus reducing their impact on benefit and non-target organisms. Botanical pesticides kill insects quickly or stop insects from feeding almost immediately after application. Most of botanical insecticides have a low mammalian toxicity (based on oral LD_{50}) and are generally non-toxic to humans, animals, and honeybees. Similarly, they are not harmful to plants (less phytotoxic) when applied according to the label directions (Dorner, 2004). Thus it is considered to be more suitable for use to control the insect pests of vegetables.

Norn-Tai-Yark (*Stemona* spp) as Botanical Insecticides

'Norn-Tai-Yark' is a vernacular name of *Stemona* spp in Thai. *Stemona* belongs to the family Stemonaceaea and consist of about 27 species such as *Stemona sessilifolia* Miq; *Stemona japonica* (Bl.) Miq; *Stemona tuberosa* Lour; *Stemona burkilii* Prain; *Stemona collinsae* Craib; *Stemona curtisii* Hook.f.; *Stemona phyllantha* Gangeb; *Stemona aphylla* Craip; *Stemona beerii* Craib and *Stemona hutunguriana* Nov. It is a perennial climbing shrub, reaching a length of 5-6 m with tuberous roots and fascicled. Leaves are opposite or alternate, oblong and heart-shaped; main nerves are curved with secondary nerves very close. Flowers which are in the axil of leaves, are greenish-yellow externally or reddish-purple within fetid (Figure1). The plant grows in the wild mainly in the hills and mountains, and is considered native to eastern and southeastern Asia including northern Australasia (Anthony, no date; Pimsa-man, no date). According to WHO (1990), the tuberous roots of *Stemona* contain alkaloids, such as stemonine, tuberostemonine, isotuberostemonine, stemonidine, sinostemonine, and 2.3% glucides, 0.83% lipids, 9% proteins and organic acids (citric, formic, malic, succinic etc.).

Figure 1 *Stemona* morphology

Source: Johann *et al.* (2007)

The decoction or extract of the plant is applied externally against impetigo and scabies. It can also be used as an insecticide for mosquito larvae, fleas and bugs (Brem *et al.*, 2002). The insecticidal effects have been confirmed recently.

Structural *Stemona*

Johann *et al.* (2007) revealed three different types of alkaloid derived from *Stemona*: Stichoneurine type alkaloids such as (1) tuberostemonine, (2) tuberostemonine A, (3) tuberostemonine N, (4) neotuberostemonine and (7) parvistemonine (Figure 2); Croomine type alkaloids, for instance (5) croomine and (6) 6-hydroxycroomine (Figure 3); and, Protostemonine-type alkaloids such as (8) Protostemonine, (9) Stemofoline, (10) 2'-Hydroxystemofoline, (11) Didehydrostemofoline (Asparagamine A), (12) Oxystemokerrine, (13) Oxystemokerrine N-oxide, (14) Stemocurtisinol, (15) Stemocurtisine (=Pyridostemine), and (16) Stemokerrine (Figure 4).

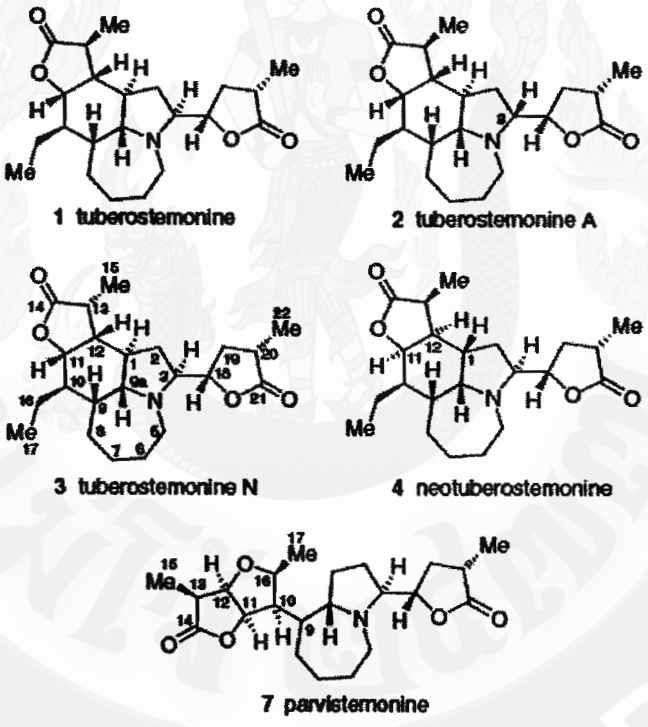

Figure 2 Stichoneurine type alkaloids

Source: Johann *et al.* (2007)

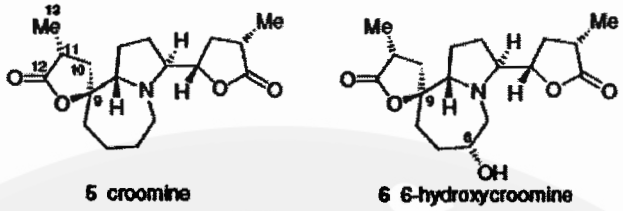

Figure 3 Croomine type alkaloids

Source: Johann *et al.* (2007)

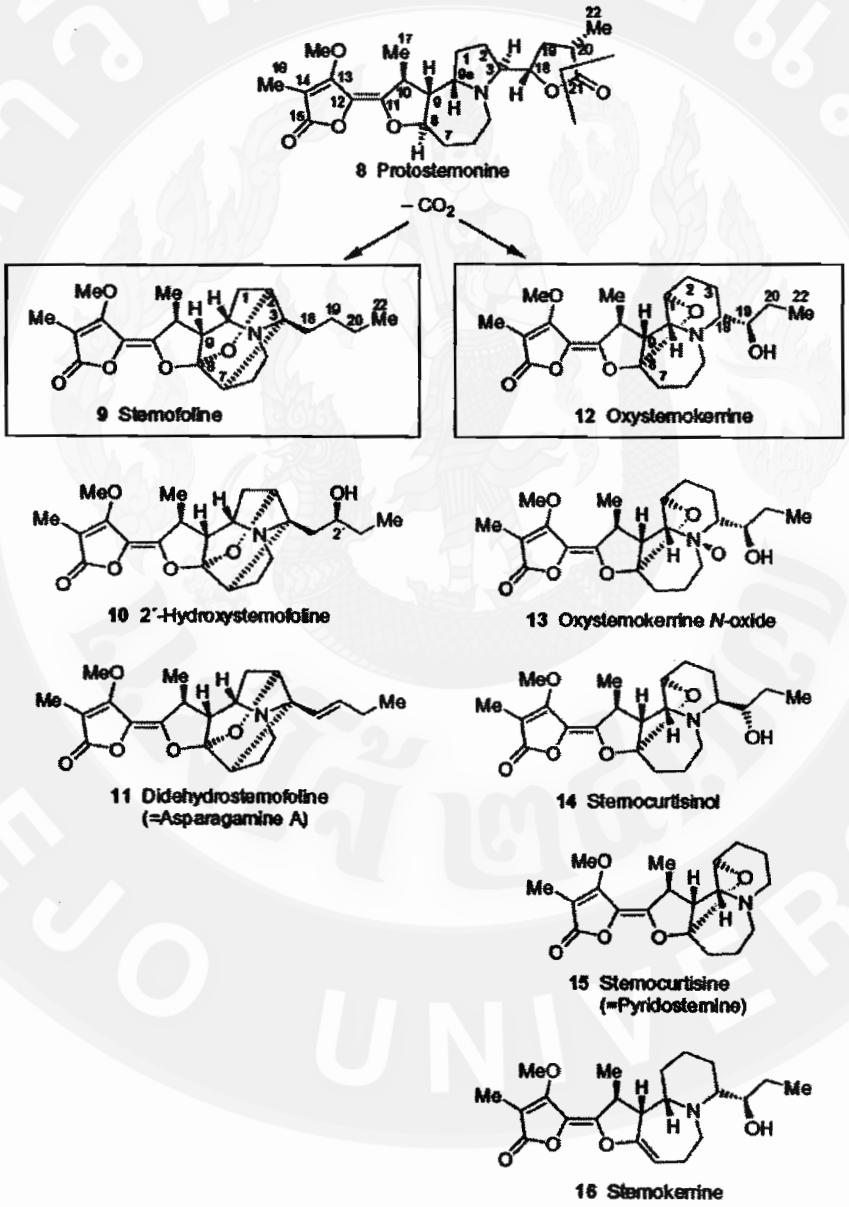

Figure 4 Protostemonine-type alkaloids

Source: Johann *et al.* (2007)

Effect of *Stemona* against pests

The roots of *Stemona* (Stemonaceae) have long been prescribed in traditional Chinese medicine as insecticidal and antitussive agents (Jiangsu New Medical College, 1986; Tsi and Duyfjes, 2000). Up to now, extracts from roots of these plants are used for respiratory disorders, including pulmonary tuberculosis and bronchitis, and externally used against different insect pests (Philli and Ferreira de Oliveira, 2000; Xu, 2000). Reid (1993) reported that stemonine has properties of being antitussive, demulcent to lungs, anthelmintic, kills lice and the herb decoction could inhibit the growth of multiple types of bacteria and skin fungi. It was found lethal to maggots, mosquitoes, mandarin aphids, cutworms, etc. Stemonine calms the respiratory centre and is strongly effective against *Pediculus capitus, P. corporis and Phthirus pubis* without irritation or toxicity (Keys, 1976). According to WHO (1990), the tuberous roots are well known for their antibacterial, antiparasitic and expectorant properties. They are prescribed in the therapy of cough, ascariasis and oxyuriasis in a dose of 4 to 12 g per day in the form of a decoction, extract, powder or pills, for 4 to 6 days. The decoction or extract is applied externally against impetigo and scabies, can also be used as an insecticide against mosquito larvae, fleas and bugs. The insecticidal effect was confirmed and the aqueous and the 70% alcoholic extract of the herb were found lethal to *Pediculus capitis* and *P. vestimenti*. It was also able to kill lice ova. The alcoholic extract killed *P. pubis* in a few minutes after contact. Tuberostemonine produced an inhibitory effect on the motility of *Angiostronglyus cantonensis, Dipylidium caninum* and *Fasciola hepatica* at 6.7 x 10-6 – 6.7 x 10-5 M in vitro. It is used for killing insects and worms and applied externally in *Pediculosis capitis*, *Pediculosis corporis*, oxyuriasis (infestation with pinworms) and pudendal itching (Zhu, 1998).

Christroph *et al.* (no date) found that the biological tests performed on larvae of *Spodoptora littoralis* (Noctuidae), a polyphagous pest insect:

Anti-feeding test (leaf disk choice test) on the crude extracts (leaves and roots) of the tested *S. collinsae* and *S. tuberosa* showed completely different results. *S. tuberosa* extracts showed very strong repellent activities, no feeding occurred at all, whereas *S. collinsae* extracts unfolded anti-feedant activity which may be associated with the high toxicity of the stemofolines present in this plant.

Insecticidal/growth inhibition test (artificial diet test), in this test system *didehydro-stemofoline* showed the highest activities (LD_{50} = 0.84 ppm), modifications in the side chain lowered the activities dramatically (*Stemofoline* LD_{50} = 2.04 ppm and isolate from *Stemona collinsae* LD_{50} = 30.33 ppm). The most prominent alkaloid from *S. tuberosa,* tuberostemonine, had only marginal activity (LD_{50} > 500 ppm).

Jiyavorranant *et al.* (2003) reported the investigation on the effect of *Stemona tuberosa* Lour. ethanol extracts on the diamondback moth (DBM), *Plutella xylostella* (Linnaeus). It was found that a 0.5% *Stemona tuberosa* extract had the best activity against third-instar larvae of *Plutella xylostella* by leaf dipping method. At this concentration, accumulated mortality was significantly 53.3% with 3.67 mm^2 in feeding sites per larva within 72 hours.

Plant extracts from *Stemona* spp for insect control were conducted to determine the efficacies of *Stemona* against the diamondback moth, *Plutella xytostella* and the common cutworm *Spodoptera litura* under laboratory conditions and found that the highest contact activity was observed from metabolic extract with the EC_{50}(48 hr) of 535; 2,313 and 2,108 ppm, respectively. Highest antifeedant activity against the diamond back moth and common cutworm, was also observed from methanolic extract with the EC_{50}(48 hr) of 129.22 and 701.11 ppm, respectively (Pimsa-man, no date).

Sanguanpong (no date) reported the efficacy of different crude extracts from *S. tuberosa* against *Spodoptera exigua,* with results suggesting that water extract with high concentration could promote higher mortality. On the other hand, hexane extract caused approximately 30% mortality with highest concentration at 15%. Besides, dichloromethane and acetone extract could be considered as having similar efficacy.

Chapter 3

Materials and Methods

Laboratory Experiments

Stock culture

Field collected larvae of *P. xytostella* were kept in a plastic box measuring 9 x 17 x 24 cm, provided with cauliflower leaves as food for the larvae which were raised until they reached the pupal stage. The pupae were then transferred to the cage provided with young cauliflower plant in pot and 10% solution of honey as food for adults (Figure 5). The eggs laid on the leaves were collected and the larvae hatched were raised as the same method as the beginning. By this method, it was possible to maintain a stock culture of *P. xytostella* for various experimental purposes. The stock culture of *S. litura* was also done by the same method as *P. xytostella.*

Figure 5 Cage for raising adults of *P. xytostella* and *S. litura*

***Stemona* products**

Samples of fermented *S. tuberosa* (F.S) were obtained from the Department of Land Development, Chiang Mai province while another bio-insecticide, *S. curtisii* (Biopes) was obtained from the Biological Laboratory, Faculty of Science, Chiang Mai University, Thailand (Figure 6).

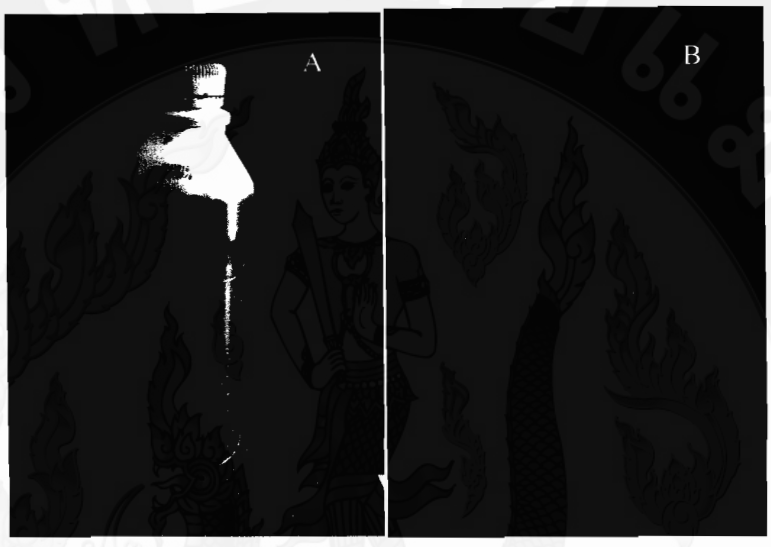

Figure 6 (A) Fermented *S. tuberosa*, (B) Bio-insecticide of *S. curtisii* (Biopes)

Contact activity test

The contact activity of active substance was investigated by using a topical application method (TA or contact poison). It was applied on the body surface of the third-instar larvae of both diamondback moth and common cutworm with a micropipette. The concentrations of FS were 5,000; 10,000; 15,000; 20,000 and 25,000 ppm while the concentrations of Biopes were 1,000; 1,500; 2,000; 2,500 and 3,000 ppm, respectively. Each treatment was applied at 3 µl per larva. For control treatment, only 3 µl of tap water was used. The cauliflower leaves were wrapped with wet tissue paper to maintain the fresh leaves and placed in plastic boxes covered with net for air circulation inside the box (Figure 7). Each box contained 5 larvae and the number of dead larvae was recorded 24, 48, 72 and 94 hours after application. The experiments were carried out using a Completely Randomized Design comprised of 6 treatments with 3 replications.

A total of 4 experiments were conducted. Active contact toxicity (LC_{50}) was evaluated when accumulated mortality was observed at more than 50%.

Figure 7 Box for contact activity test

Anti-feedant activity test

The anti-feedant activity of active substance was carried out by leaf dipping method (LD). Pieces of 4 x 4 mm cauliflower leaves were dipped into tap water (control) together with different concentrations (5,000; 10,000; 15,000; 20,000 and 25,000 ppm) of FS and (1,000; 1,500; 2,000; 2,500 and 3,000 ppm) of Biopes, for 30 seconds. The dipped leaves were kept at room temperature to evaporate the solvents. After drying, they were transferred into the Petri-dishes with 5 larvae of the third instar larvae placed in each-petri dish (Figure 8). The feeding areas were recorded after 24, 48 and 72 hours and the number of dead larvae from both FS and Biopes treatments were counted. The cauliflower leaves were changed every 24 hours after recording. Data were calculated in terms of percentage accumulated mortality (Janprasert, 1992) while feeding areas were computed by using Scion Image Software (O'Neal *et al.*, 2002). Active anti-feedant toxicity (LC_{50}) was evaluated when accumulated mortality was observed at more than 50%.

Figure 8 Petri-dish for anti-feedant activity test

Detecting the damaged leaf areas

Individual leaves were scanned into a digital format using a Compaq Notebook Computer with HP DeskJet F 4185 scanner (images were scanned with resolution at 200 dpi). The preliminary image was converted from color to grayscale. The highlight and shadow levels within the exposure adjustment (selected from the tool menu) were manipulated to create a black image on the white background. The final version was saved as a TIFF file without LZW compression. Formats that involve compression (GIF, JPEG and TIFF with LZW compression) were not compatible with the image analysis software. The leaves were calculated by using Scion Image software and set scale option (cm) (O'Neal *et al.*, 2002).

The Percentage Leaf Damage Area (PLDA) was calculated using the following formula:

$$PLDA = \frac{LDA \times 100}{TLA}$$

where:

LDA = leaf damage area per 5 larvae

TLA = total leaf area (4 cm x 4 cm)

Field Experiment

Location

The experiment was carried out from October 2007 until January 2008 at the experimental field of the Organic Section, Division of Vegetable Technology, Department of Horticulture, Faculty of Agricultural Production, Maejo University, which is located 10 km north of Chiang Mai. The area is situated in the Chiang Mai valley, 700 km north of Bangkok at an altitude of 300 m. The lowest average temperature was 21°C and the highest was 26°C (Appendix1).

Effects of *Stemona* products on the population of *P. xytostella, S. litura* and growth and development of cauliflower

The seeds of cauliflower (White Gold) were sowed on 28 October 2007 in the plastic trays at 2-3 seeds per pit for a total of 12 trays with 104 pits and then placed on the shelf in the greenhouse (Figure 9). The seedlings were thinned at 7-10 days after sowing. Field was prepared by dividing it into 5 m long and 2.4 m wide plots for a total of 21 plots. After 26 days of sowing, the seedlings were transplanted in 40 x 40 cm spacing between rows and plants (2 rows per bed and 2 beds per plot), 48 plants per plot. The total area and plants were 252 m^2 and 1,008 respectively (Figure 10). The experiment was layout using the Randomized Complete Block Design (RCBD) with 7 treatments and 3 replications (Appendix 2).

Treatments comprised of:

T_1 = Control (applied water)

T_2 = 5 ml of FS per liter of water (5ml/L)

T_3 = 15 ml of FS per liter of water (15ml/L)

T_4 = 25 ml of FS per liter of water (25ml/L)

T_5 = 1 ml of Biopes per liter of water (1ml/L)

T_6 = 2 ml of Biopes per liter of water (2ml/L)

T_7 = 3 ml of Biopes per liter of water (3ml/L).

All plots except the control plots were sprayed with *Stemona* products using knapsack sprayer 20 days after transplanting at 5 days interval for 4 times continuously (Figure 11).

Sampling

The plant samples were determined randomly at 5 plants per row thus 10 plants per plot were represented for each treatment. The number of larvae diamondback moth (DBM) *P. xytostella* and common cutworm (*S. litura*) larvae on each plant sampled were recorded by counting (Kirby and Slosser, 1981) 4 days after spraying. Assessment of leaf damage and plant growth (number of leaves, plant height and plant canopy) were done from 10 days after transplanting and continued every 5 days interval until 40 days of transplanting.

The plant weight, curd diameter, leaf length, leaf width and curd yield per plot were measured at harvesting time on 6th January 2008.

Figure 9 Cauliflower seedlings on the greenhouse shelf

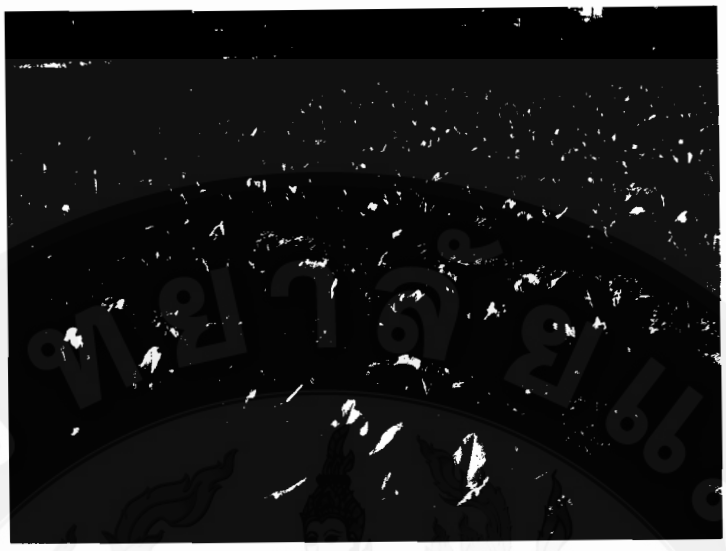

Figure 10 Cauliflower experimental field

Figure 11 *Stemona* products applied on field experiment of cauliflower

Data analysis

Analysis of variance (ANOVA) was used to determine significant differences between treatments in each experiment. Data related to the number of insects and leaf damaged were transformed to logarithms $Z = \ln(x+1)$ by using SAS (Statistical Analysis System) program. When the treatment effects had significant difference, the least significant difference (LSD) was used for mean comparison.

Chapter 4

Results

Laboratory Experiments

Toxicity of FS on the third-instar larvae of *Plutella xylostella*:

1. Contact activity. Five concentrations of FS at 5,000; 10,000; 15,000; 20, 000 and 25, 000 ppm were applied to the third-instar larvae of *P. xylostella* to determine the contact activity by using topical application method (TA). Percentage accumulated mortality of the tested larvae were used as a criteria to justify the "effective threshold" (i.e. > 50 % accumulated mortality) of FS. The effectiveness of FS at various concentrations was recorded at 24, 48, 72 and 96 hours after treatment, respectively. The results showed that there were no significant differences in percentage accumulated mortality among the treatments within 24 hrs. At 48 hrs, significant difference was found among treatments but percentage accumulated mortality was not high enough to reach an "effective threshold". Concentrations at 20,000 and 25,000 ppm provided 46.67 and 53.33 percent accumulated mortality, which were highly significantly different from those other treatments within 72 hrs. The percentage accumulated mortality at various concentrations within 96 hrs was also significantly different, which increased from 60.00 to 73.33 percent as the concentration increased from 20,000 to 25,000 ppm. These data are summarized in Table1.

Table 1 Percentage of accumulated mortality at different concentrations of FS on *Plutella xylostella* by topical application method.

Concentration (ppm)	Percentage of accumulated mortality							
	24 Hours		48 Hours		72 Hours		96 Hours	
	%	DT	%	DT	%	DT	%	DT
5,000	0.00	0.10	13.33	0.52^{ab}	26.67	0.87^{ab}	33.33	1.00^{c}
10,000	6.67	0.31	6.67	0.31^{b}	13.33	0.52^{bc}	33.33	1.00^{c}
15,000	6.67	0.31	26.67	0.87^{a}	33.33	1.00^{ab}	40.00	1.13^{bc}
20,000	6.67	0.31	33.33	1.00^{a}	46.67	1.22^{a}	60.00	1.41^{ab}
25,000	13.33	0.44	33.33	1.00^{a}	53.33	1.29^{a}	73.33	1.55^{a}
Control	0.00	0.10	6.67	0.31^{b}	6.67	0.31^{c}	33.33	0.96^{c}
F-test		ns		*		**		*
$LSD_{0.05}$				0.54		0.50		0.37

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

^{ns} = not significant; * = significantly different at $P \leq 0.05$;

** = Highly significant difference at $P \leq 0.01$

DT = data transformed.

2. Anti-feedant activity. Leaf dipping method (LD) was used to evaluate anti-feedant activity of FS on the third-instar larvae of *P. xylostella*. Data collected in this experiment used the same method as in contact activity test. The leaf damage area and percentage accumulated mortality were recorded at 24, 48 and 72 hrs, respectively.

The results of anti-feedant activities were shown in Table 2. It was found that leaf damage areas were increased as the concentration of FS decreased. At the concentrations of 20,000 and 25,000 ppm, the lowest leaf damage areas of 0.40 and 0.60 mm^2 per larva and the highest accumulated mortality of 60 and 80 percent were detected within 72 hrs after treatment.

At 24 hrs after treatment, leaf damage areas were relatively high, ranging from 23.60 to 68.40 mm^2 per larva in all treatments. All concentrations did not provide percentage accumulated mortality.

At 48 hrs after treatment, leaf damage areas also increased, ranging from 15.80 to 51.60 mm^2 per larva at all concentrations and untreated (control), but concentrations at 20,000 and 25,000 ppm provided only 40 percent accumulated mortality which were not enough to achieve an "effective threshold".

Table 2 Percentage of accumulated mortality and leaf damage area per larva at different concentrations of FS on *Plutella xylostella* by leaf dipping method

Concentration (ppm)	24 hours		48 hours		72 hours	
	LDA per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality
5,000	54.00	0	38.00	20	49.00	40.00
10,000	53.20	0	28.20	0	26.40	20.00
15,000	48.80	0	19.00	20	6.20	40.00
20,000	39.00	0	21.80	40	0.40	60.00
25,000	23.60	0	15.80	40	0.60	80.00
Control	68.40	0	51.60	0	53.40	20.00

LDA = Leaf damage area.

3. Percentage comparison of leaf damage area and accumulated mortality by both methods against *P. xylostella* larvae treaded with different concentrations of FS. The percentage leaf damage areas in untreated and treated with concentrations at 5,000; 10,000; 15,000; 20,000 and 25,000 ppm within 72 hours, were observed in decreasing trend of 16.69, 15.31, 8.25, 1.94, 0.13 and 0.19%, respectively (Figure 12 C). In contrast, they showed increasing trend in percentage accumulated mortality from 6.67 to 53.33% by TA and 20 to 80% by LD. In other hand, at highest concentration (25,000 ppm) the percentage damaged leaf area was also seen to have decreased at 7.37, 4.93 and 0.19% within 24, 48 and 72 hrs, respectively (Figure 12 A, B and C) and (Table 3).

At 24 hours, the LD method did not show percentage accumulated mortality but it provided even higher percentage accumulated mortality than TA method after 48 and 72 hours of application, especially concentrations at 20,000 and 25,000 ppm (Figure 12 A, B and C).

Table 3 Percentage leaf damage area and accumulated mortality by topical and leaf dipping methods of FS on *Plutella xylostella* larvae.

Concentration (ppm)	Time after application (%)								
	24 hrs			48 hrs			72 hrs		
	LDA	AMTA	AMLD	LDA	AMTA	AMLD	LDA	AMTA	AMLD
5,000	16.87	0	0	11.87	13.33	20	15.31	26.67	40.00
10,000	16.62	6.67	0	8.812	6.67	0	8.25	13.33	20.00
15,000	15.25	6.67	0	5.937	26.67	20	1.94	33.33	40.00
20,000	12.18	6.67	0	6.812	33.33	40	0.13	46.67	60.00
25.000	7.37	13.33	0	4.937	33.33	40	0.19	53.33	80.00
Control	21.37	0	0	16.12	6.67	0	16.69	6.67	20.00

LDA = Percentage leaf damage areas

AMTA = Percentage accumulated mortality by topical application

AMLD = Percentage accumulated mortality by leaf dipping.

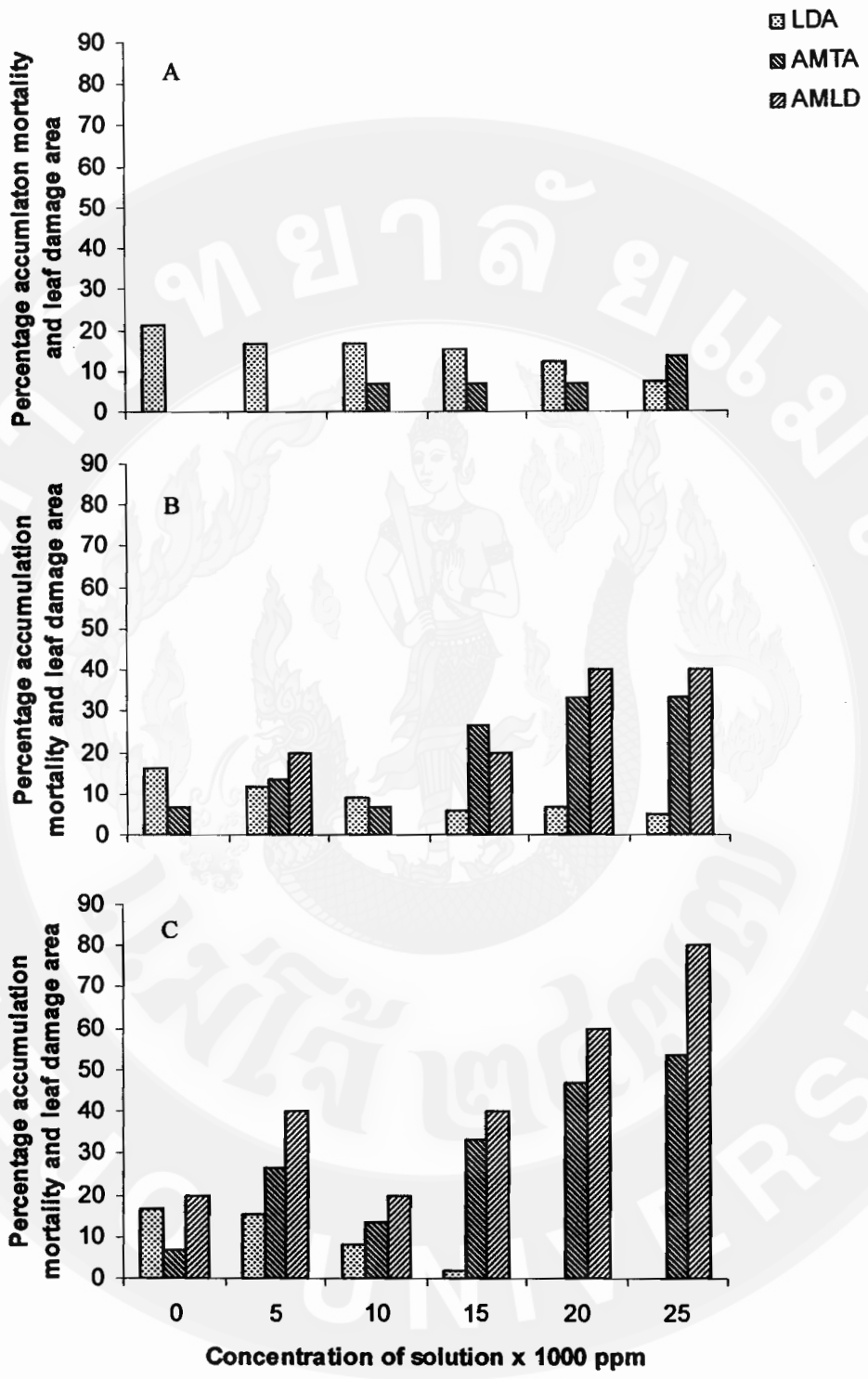

Figure 12 Comparison of percentage leaf damage area (LDA), accumulated mortality by topical applications (AMTA) and accumulated mortality by leaf dipping (AMLD) methods against *P. xylostella* larvae treated with different concentrations of FS within A = 24, B = 48 and C = 72 hours, respectively.

Toxicity of Biopes on the third-instar larvae of *Plutella xylostella*:

1. Contact activity. Five concentrations of Biopes at 500; 1,500; 2,000; 2,500 and 3,000 ppm were applied to the third-instar larvae of *P. xylostella* to determine the contact activity by using topical application method (TA). Percentage accumulated mortality of the tested larvae were used as a criteria for judging the "effective threshold" (i.e. > 50 % accumulated mortality) of Biopes. Data collected and method in this experiment were used the same as in contact activity test of FS.

Results revealed no significant differences in percentage accumulation mortality among treatments within 24 hrs. At 48 hrs, highly significant difference was found among the treatments but percentage accumulation mortality was not high enough to reach an "effective threshold". Concentrations at 2,000; 2,500 and 3,000 ppm provided 53.33, 60.00 and 73.33 percent accumulation mortality, respectively, which were highly significantly different from those of other treatments within 72 hrs. Percentage accumulation mortality at various concentrations within 96 hrs, also differed significantly, providing 93.33 percent accumulation mortality at concentration 3,000 ppm but not significantly different from 66.67 and 60.00 percent accumulation mortality at concentrations 2,500 and 2,000 ppm, respectively. This was followed by 46.67 percent accumulation mortality at concentration 1,500 ppm while lowest values were 26.67 and 33.33 percent accumulation mortalities at concentration 1,000 ppm and untreated, respectively. These data as indicated are shown in Table 4.

Table 4 Percentage of accumulated mortality at different concentrations of Biopes on *Plutella xylostella* by topical application.

Concentration (ppm)	Percentage of accumulated mortality							
	24 Hours		48 Hours		72 Hours		96 Hours	
	%	DT	%	DT	%	DT	%	DT
1,000	6.67	0.31	26.67	0.87^{a}	26.67	0.87^{b}	26.67	0.87^{c}
1,500	20.00	0.65	26.67	0.87^{a}	26.67	0.87^{b}	46.67	1.22^{bc}
2,000	6.67	0.31	40.00	1.13^{a}	53.33	1.31^{a}	60.00	1.41^{ab}
2,500	13.33	0.44	46.67	1.22^{a}	60.00	1.41^{a}	66.67	1.48^{ab}
3,000	13.33	0.52	46.67	1.22^{a}	73.33	1.55^{a}	93.33	1.75^{a}
Control	0.00	0.10	6.67	0.31^{b}	6.67	0.31^{c}	33.33	1.09^{c}
F-test		ns		**		**		**
$LSD_{0.05}$				0.39		0.38		0.36

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

^{ns} = not significant; ** = Highly significant difference at $P \leq 0.01$

DT = data transformed.

2. Anti-feedant activity. Leaf dipping method (LD) was used to evaluate the anti-feedant of Biopes on third-instar larvae of *P. xylostella*. Data collected and method in this experiment were the same as using in anti-feedant activity test of FS.

Results of anti-feedant activity test were presented in Table 5. It was found that leaf damage areas were decreased as the concentration of Biopes increased. The lowest leaf damage areas of 2.60 and 0.60 mm^2 per larva and the highest accumulation mortality of 60 percent were found at concentrations 2,500 and 3,000 ppm, respectively, within 48 hrs after treatment. At 72 hrs, lowest leaf damage areas of 1.60 and 0.20 mm^2 per larva were observed at 2,500 and 3,000 ppm, providing highest accumulated mortality of 60 and 80 percent. At 24 hrs, showed percentage leaf damage areas decrease from 68.40 to 13.40%. However, percentage leaf damage areas still high compare to 48 and 72 hrs after treated and all concentrations did not provide any percentage accumulation mortality.

Table 5 Percentage of accumulated mortality and leaf damage area per larva at different concentrations of Biopes against *Plutella xylostella* by leaf dipping method.

Concentration (ppm)	24 hours		48 hours		72 hours	
	LDA Per larva (mm^2)	% accumulated mortality	LDA Per larva (mm^2)	% accumulated mortality	LDA Per larva (mm^2)	% accumulated mortality
1,000	47.80	0	19.60	20	14.40	40.00
1,500	40.40	0	6.20	20	9.40	40.00
2,000	40.60	0	5.80	40	13.40	60.00
2,500	19.80	0	2.60	60	1.60	60.00
3,000	13.40	0	0.60	60	0.20	80.00
Control	68.40	0	51.60	0	53.40	20.00

LDA = Leaf damage area.

3. Percentage comparison of leaf damage area and accumulated mortality by both methods against *P. xylostella* larvae treated with different concentrations of Biopes. Concentrations at 1,000; 1,500; 2,000; 2,500 and 3,000 ppm provided the percentage leaf damage areas in decreasing trend (4.50, 2.93, 4.18, 0.50 and 0.06%) as compared to the untreated control (16.69%) as indicated in Table 6 and Figure 13 C. In opposite, the same concentrations gave percentage accumulated mortality in increasing rate (26.66, 26.66, 53.33, 60.00 and 73.33%) by TA and (40, 40, 60, 60 and 80%) by LD. High concentration (3,000 ppm) also showed decreased percentage of leaf damage area (4.18, 0.18 and 0.06%) within 24, 48 and 72 hours, respectively (Figure 13 A, B and C). At 24 hours, the LD method did not show percentage accumulated mortality, but it provided percentage accumulated mortality higher than TA method after 48 and 72 hours of application, especially concentrations at 2,000; 2.500 and 3,000 ppm.

Table 6 Percentage leaf damage area and accumulated mortality by topical and leaf dipping methods of Biopes on *Plutella xylostella* larvae.

Concentration (ppm)	Time after application (%)								
	24 hrs			48 hrs			72 hrs		
	LDA	AMTA	AMLD	LDA	AMTA	AMLD	LDA	AMTA	AMLD
1,000	14.94	6.67	0	6.13	26.67	20.00	4.50	26.67	40.00
1,500	12.63	20.00	0	1.94	26.67	20.00	2.94	26.67	40.00
2,000	12.69	6.67	0	1.81	40.00	40.00	4.19	53.33	60.00
2,500	6.19	13.33	0	0.81	46.67	60.00	0.50	60.00	60.00
3.000	4.19	13.33	0	0.19	46.67	60.00	0.06	73.33	80.00
Control	21.38	0	0	16.13	6.67	0	16.69	6.67	20.00

LDA = Percentage leaf damage areas

AMTA = Percentage accumulated mortality by topical application

AMLD = Percentage accumulated mortality by leaf dipping.

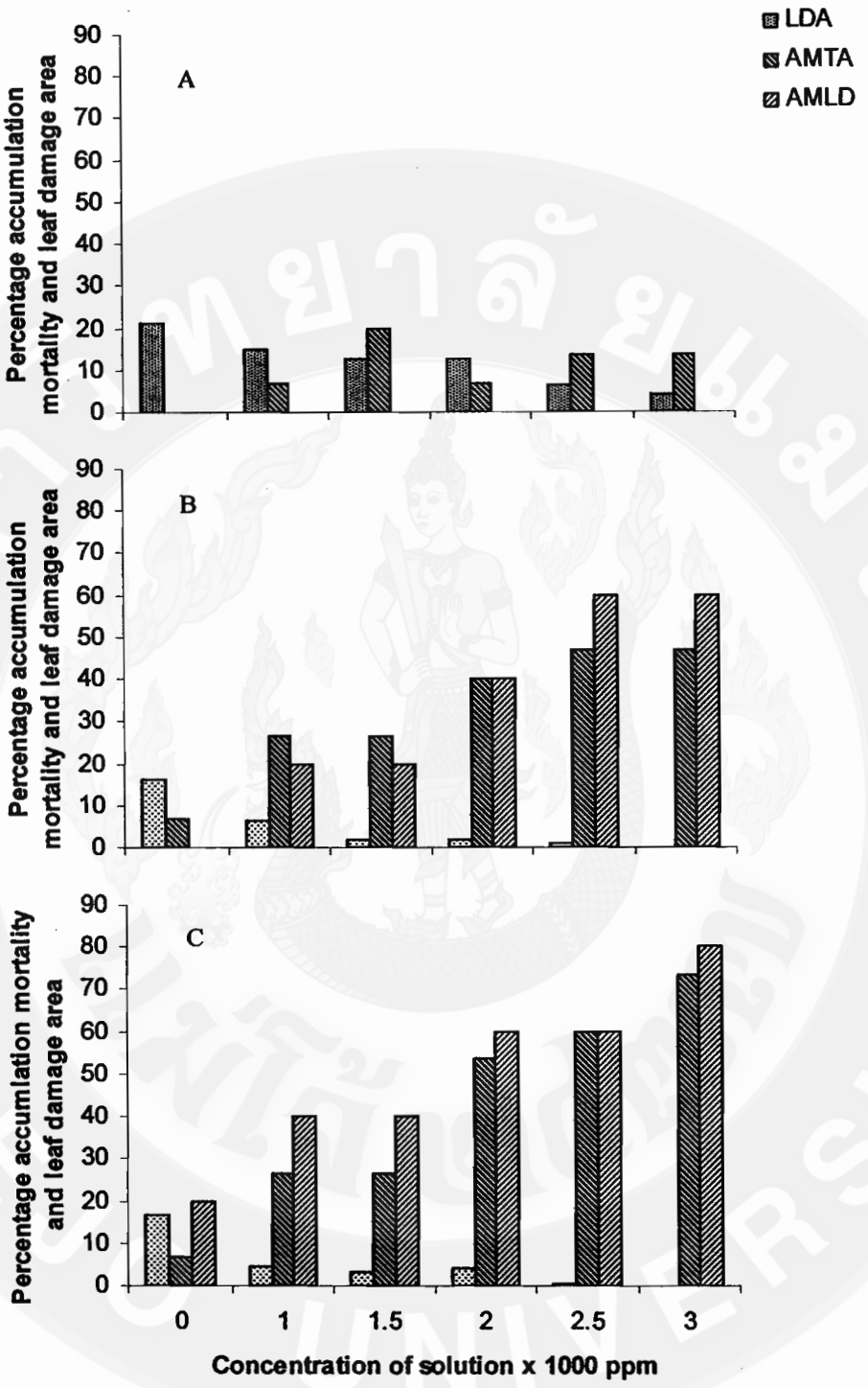

Figure 13 Comparison of percentage leaf damage area (LDA), accumulated mortality by topical applications (AMTA) and accumulated mortality by leaf dipping (AMLD) methods against *P. xylostella* larvae treaded with different concentrations of Biopes within A = 24, B = 48 and C = 72 hrs, respectively.

Toxicity of FS on the third-instar larvae of *Spodoptera litura*

1. Contact activity. Five concentrations of FS were applied to the third-instar larvae of *S. litura* by topical application (TA), which were used the same method as experiment of FS against third-instar larvae of *P. xylostella*. The results showed that there were no significant differences in percentage accumulated mortality among the treatments within 24, 48, 72 and 96 hrs. These data were presented in Table 7.

Table 7 Percentage of accumulated mortality at different concentrations of FS on *Spodoptera litura* by topical application.

Concentration (ppm)	Percentage of accumulated mortality							
	24 Hours		48 Hours		72 Hours		96 Hours	
	%	DT	%	DT	%	DT	%	DT
5,000	0	0.10	7	0.31	7	0.31	13	0.52
10,000	0	0.10	0	0.10	7	0.31	27	0.87
15,000	0	0.10	20	0.74	20	0.74	33	1.00
20,000	0	0.10	13	0.52	20	0.74	33	1.00
25,000	0	0.10	20	0.74	27	0.87	33	1.00
Control	0	0.10	0	0.10	7	0.31	13	0.52
$LSD_{0.05}$		ns		ns		ns		ns

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

^{ns} = not significant

DT = data transformed.

2. Anti-feedant activity. Leaf dipping method (LD) was used to evaluate anti-feedant activity of FS on the third-instar larvae of *S. litera*. Data collected in this experiment were used the same method as in anti-feedant activity test of FS against the third-instar larvae of *P. xylostella*. The results of anti-feedant activity test were shown in Table 8. It was found that leaf damage areas at all concentrations were increased as activity time increased from 24 hrs to 72 hrs.

Accumulated mortality provided only 20 percent at concentrations of 15,000; 20,000 and 25,000 ppm within 48 and 72 hrs.

Table 8 Percentage of accumulated mortality and leaf damage area per larva at different concentrations of FS on *Spodopera litura* by leaf dipping method.

Concentration (ppm)	24 hours		48 hours		72 hours	
	LDA Per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality
5,000	20.00	0	34.40	0	62.20	0
10,000	26.00	0	23.80	0	86.60	0
15,000	21.80	0	56.60	20	55.60	20
20,000	19.60	0	29.20	20	42.60	20
25,000	25.60	0	36.20	20	84.60	20
Control	23.80	0	35.80	0	124.40	0

LDA = Leaf damage area.

3. Comparison of percentage leaf damage area and accumulated mortality by both methods against *S. litera* larvae treated with different concentrations of FS. All concentrations of 5,000; 10,000; 15,000; 20,000, 25,000 ppm of FS and untreated treatments did not show any decrease in percentage damaged leaf area, and both TA and LD method did not give percentage accumulated mortality (Figure 14 A). Highest concentration (25,000 ppm) provided only 27% accumulated mortality by TA method and 20% accumulated mortality by LD method within 72 hours (Figure 14 C). In all concentrations and study periods (24, 48 and 72 hours), percentage leaf damage areas were increased (Figure 14 A, B and C) and Table 9.

Table 9 Percentage leaf damage area and accumulated mortality by topical and leaf dipping methods of FS on *Spodoptera litura* larvae.

Concentration (ppm)	Time after application (%)								
	24 hrs			48 hrs			72 hrs		
	LDA	AMTA	AMLD	LDA	AMTA	AMLD	LDA	AMTA	AMLD
5,000	6.25	0	0	10.75	7	0	19.44	7	0
10,000	8.13	0	0	7.44	0	0	27.06	7	0
15,000	6.81	0	0	17.69	20	20	17.38	20	20
20,000	6.13	0	0	9.13	13	20	13.31	20	20
25,000	8.00	0	0	11.31	20	20	26.44	27	20
Control	7.44	0	0	11.19	0	0	33.88	7	0

LDA = Percentage leaf damage areas

AMTA = Percentage accumulated mortality by topical application

AMLD = Percentage accumulated mortality by leaf dipping.

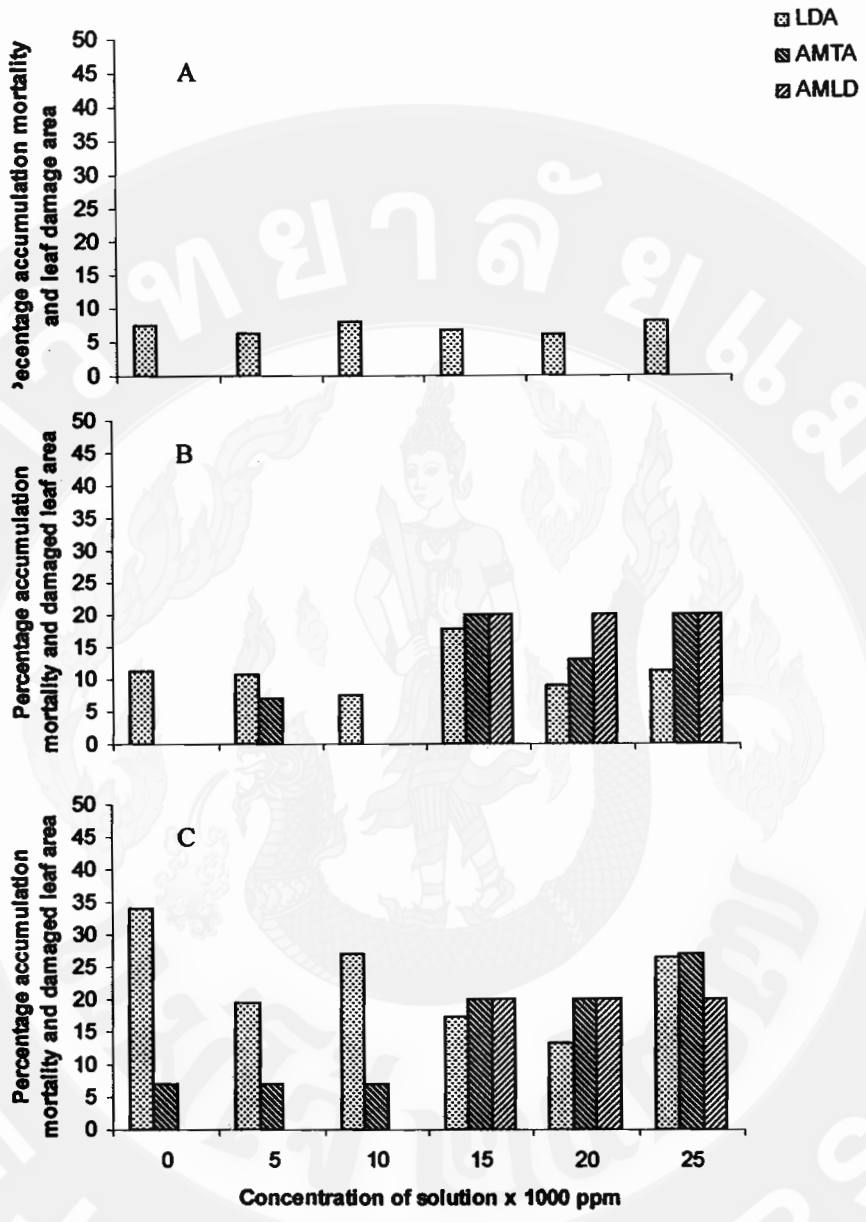

Figure 14 Comparison of percentage leaf damage area (LDA), accumulated mortality by topical applications (AMTA) and accumulated mortality by leaf dipping (AMLD) methods against *S. litura* larvae treated with different concentrations of FS within A = 24, B = 48 and C = 72 hrs, respectively.

Toxicity of Biopes on the third-instar larvae of *Spodoptera litura*

1. Contact activity. Five concentrations of Biopes (500; 1,500; 2,000; 2,500 and 3,000 ppm) were applied to the third-instar larvae of *S. litura*, which were used in the same previous method (contact activity test of FS on the third-instar larvae of *P. xylostella*). Results showed no significant differences in percentage accumulated mortality among treatments within 24, 48 and 96 hrs. At 72 hrs, significant difference was found among the treatments but percentage accumulated mortality were not high enough to reach an "effective threshold". These data were indicated as in Table 10.

Table 10 Percentage accumulated mortality of different concentrations of Biopes on *Spodoptera litura* by topical application.

Concentration (ppm)	Percentage of accumulated mortality							
	24 Hours		48 Hours		72 Hours		96 Hours	
	%	DT	%	DT	%	DT	%	DT
1,000	0	0.10	7	0.31	7	0.31[b]	27	0.87
1,500	0	0.10	7	0.31	13	0.52[ab]	20	0.65
2,000	7	0.31	20	0.74	27	0.87[ab]	27	0.87
2,500	0	0.10	20	0.74	33	1.00[a]	47	1.22
3,000	7	0.31	27	0.87	40	1.09[a]	60	1.39
Control	0	0.10	0	0.10	7	0.31[b]	13	0.52
F-test		ns		ns		*		ns
$LSD_{0.05}$						0.57		

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

ns = not significant; * = Significantly different at $P \leq 0.05$

DT = data transformed.

2. Anti-feedant activity. Leaf dipping method (LD) was used to evaluate anti-feedant activity of Biopes on the third-instar larvae of *S. litura*. This experiment was conducted using the same method as in anti-feedant test of FS. Results as indicated in Table11 showed that leaf damage areas were increased as time increased from 24 hrs to 72 hrs. Highest accumulated mortality provided only 40 percent at concentration 3,000 ppm within 48 hrs and; 2,500 and 3,000 ppm within 72 hrs.

Table 11 Percentage of accumulated mortality and leaf damage area per larva at different concentrations of Biopes on *Spodoptera litura* by leaf dipping method.

Concentration (ppm)	24 hours		48 hours		72 hours	
	LDA per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality	LDA per larva (mm^2)	% accumulated mortality
1,000	42.00	0	29.80	0	61.00	0
1,500	23.80	0	51.00	0	28.80	0
2,000	13.40	20	11.00	20	23.00	20
2,500	12.80	0	22.60	20	34.60	40
3,000	10.80	20	20.40	40	35.60	40
Control	23.80	0	35.80	0	124.40	0

LDA = Leaf damage area.

3. Comparison of percentage leaf damage area and accumulated mortality by both methods against *S. litura* larvae treatsed with different concentrations of Biopes. At all various concentrations of Biopes, the percentage leaf damage areas were increased due to expanded the time after treated as indicated in Table 12 and Figure 15 A, B and C. At the highest concentration (3,000 ppm), there was only 40% accumulated mortality by both TA and LD methods within 72 hours and percentage leaf damage area reached to 11.13 % (Figure 15 C).

Table 12 Percentage leaf damage area and accumulated mortality by topical and leaf dipping methods of Biopes on *Spodoptera litura* larvae.

Concentration (ppm)	Time after application (%)								
	24 hrs			48 hrs			72 hrs		
	LDA	AMTA	AMLD	LDA	AMTA	AMLD	LDA	AMTA	AMLD
1,000	13.13	0	0	9.31	7	0	19.06	7	0
1,500	7.44	0	0	15.94	7	0	9.00	13	0
2,000	4.19	7	20	3.44	20	20	7.19	27	20
2,500	4.00	0	0	7.06	20	20	10.81	33	40
3.000	3.38	7	20	6.38	27	40	11.13	40	40
Control	7.44	0	0	11.19	0	0	38.88	7	0

LDA = Percentage leaf damage areas

AMTA = Percentage accumulated mortality by topical application

AMLD = Percentage accumulated mortality by leaf dipping.

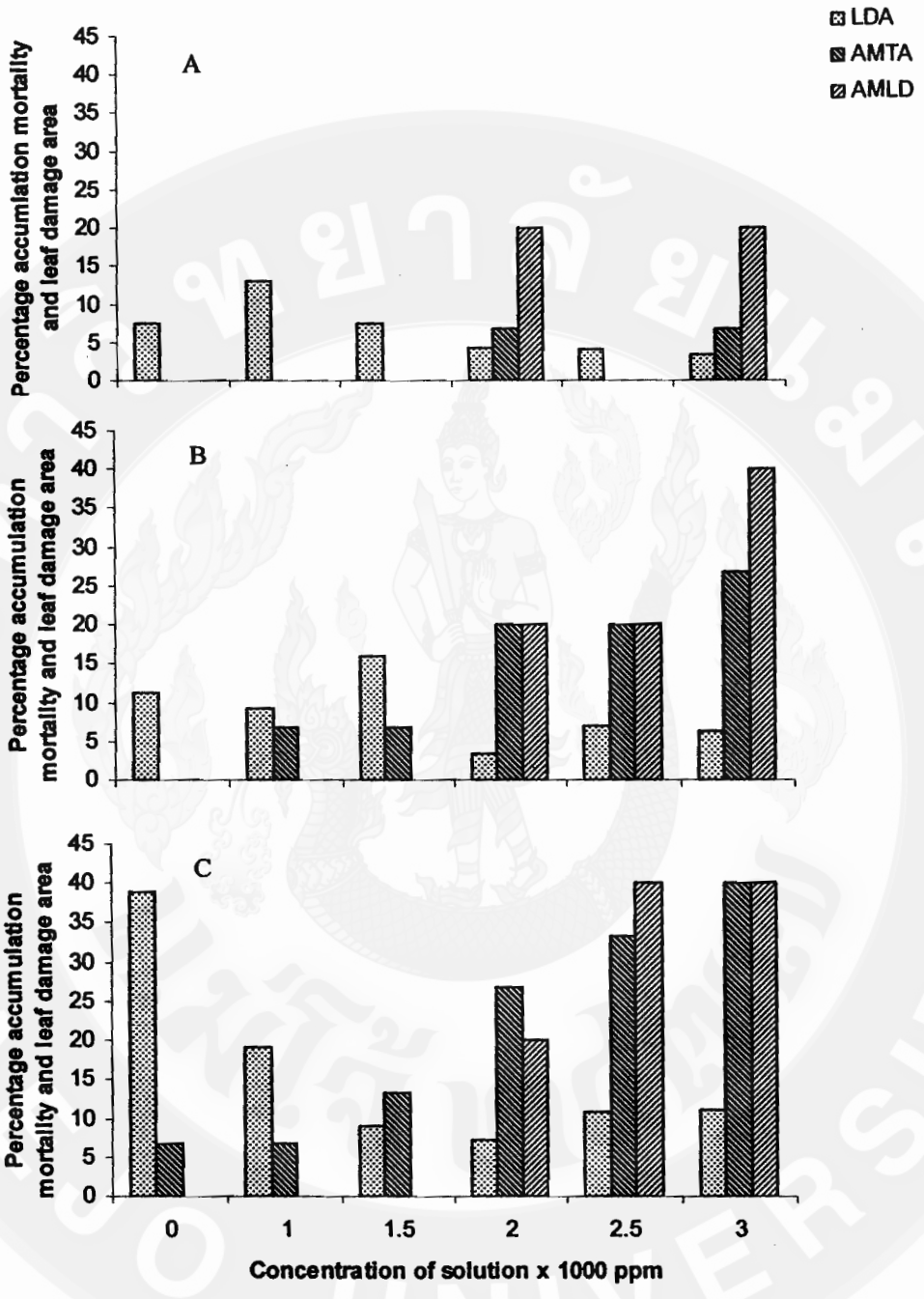

Figure 15 Comparison of percentage leaf damage area (LDA), accumulated mortality by topical applications (AMTA) and accumulated mortality by leaf dipping (AMLD) methods against *S. litura* larvae treated with different concentrations of Biopes within A = 24, B = 48 and C = 72 hrs, respectively.

Field Experiment

Effect of *Stemona* products for controlling *Plutella xylostella*

Table 13 showed the effectiveness of FS and Biopes at various concentrations on the larvae population of *P. xylostella*. FS at the concentration of 25 ml/L and Biopes at the concentration 3 ml/L provided good control as indicated by the lowest number of *P. xylostella* larvae throughout the sampling period.

Table 13 Populations of *Plutella xylostella* (L) larvae on cauliflower after treated with different concentrations of *Stemona* products (no. larvae/plant) from December 17, 2007 to January 1, 2008.

Stemona products and concentration	Days after transplanting no. larvae/plant			
	25	30	35	40
Control	4.63 (1.72) ^{a}	7.47 (2.13) ^{a}	9.33 (2.33) ^{a}	9.80 (2.37) ^{a}
FS 5 ml /L	4.43 (1.69) ^{ab}	6.73 (2.04) ^{ab}	8.27 (2.22) ^{ab}	7.40 (2.12) ^{b}
FS 15 ml /L	4.70 (1.74) ^{a}	6.50 (2.01) ^{ab}	7.17 (2.09) ^{bc}	6.90 (2.06) ^{bc}
FS 25 ml /L	3.27 (1.45) ^{c}	4.23 (1.65) ^{e}	4.97 (1.78) ^{d}	5.23 (1.80) ^{de}
Biopes 1 ml /L	4.80 (1.75) ^{a}	7.60 (2.15) ^{a}	9.10 (2.31) ^{a}	8.33 (2.23) ^{ab}
Biopes 2 ml /L	4.20 (1.64) ^{ab}	5.87 (1.92) ^{c}	6.30 (1.98) ^{c}	5.83 (1.92) ^{cd}
Biopes 3 ml /L	3.73 (1.55) ^{bc}	4.93 (1.78) ^{d}	4.70 (1.73) ^{d}	4.37 (1.67) ^{e}
F-test	*	**	**	**
$LSD_{0.05}$	0.16	0.11	0.14	0.18

Numbers of larvae per plant were averaged from 3 replications

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

^{ns} = not significant; * = significantly different at $P \leq 0.05$;

** = Highly significant difference at $P \leq 0.01$

Data in the parenthesis were transformed using ln(x+1).

Table 14 showed the effectiveness of FS and Biopes at various concentrations on the percentage leaf damage by *P. xylostella* larvae. The percentage leaf damage treated with various concentrations of FS and Biopes was significantly decreased as the concentrations increased with lowest percentage leaf damage found at the concentration of 25 ml/L of FS and 3 ml/L of Biopes throughout the sampling period.

Table 14 Percentage leaf damage under field condition after treated with different concentrations of *Stemona* products.

Stemona products and concentration	Day after transplanting PLDA/plant			
	25	30	35	40
Control	3.70 ^{a}	5.97 ^{ab}	7.46 ^{a}	7.52 ^{a}
FS 5 ml /L	3.54 ^{ab}	5.38 ^{bc}	6.61 ^{ab}	6.80 ^{ab}
FS 15 ml /L	3.76 ^{a}	5.20 ^{cd}	5.73 ^{bc}	5.97 ^{b}
FS 25 ml /L	2.61 ^{c}	3.38 ^{e}	3.97 ^{d}	4.08 ^{d}
Biopes 1 ml /L	3.84 ^{a}	6.08 ^{a}	7.28 ^{a}	7.46 ^{a}
Biopes 2 ml /L	3.36 ^{ab}	4.69 ^{d}	5.04 ^{c}	5.09 ^{c}
Biopes 3 ml /L	2.98 ^{bc}	3.94 ^{e}	3.76 ^{d}	4.05 ^{d}
F-test	*	**	**	**
$LSD_{0.05}$	0.68	0.64	0.88	0.84

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

* = significantly different at $P \leq 0.05$; ** = highly significant difference at $P \leq 0.01$

PLDA = Percentage leaf damage area

Effect of *Stemona* products on growth and development of cauliflower

The number of leaves, size of plant canopy, plant height, leaf width, leaf length, plant weight, curd diameter and curd yield were used as parameters to determine the effects of *Stemona* products on cauliflower crop. It was found that almost all of these parameters in all treatments exhibited significant difference except for the leaf length, number of leaves, plant canopy size and plant height which were not significantly different among treatments.

1. Marketable curd yield. The curd yield of cauliflower treated with dose 3 ml/L of Biopes provided highest curd yield (8,160.00 kg/ha), which was highly significantly different among all entries but this treatment was not significantly different from treatment of 25 ml/L of FS. Dose treatment of 5 ml/L of FS and control gave the lowest marketable curd yield at 5,593.30 and 5,000.00 kg/ha, respectively, as presented in Table 15.

2. Curd diameter of cauliflower treated with dose of 3 ml/L of Biopes was significantly different among all entries, providing the highest size of 10.40 cm. Dose treatments of 25 ml/L of FS and 2 ml/L of Biopes gave similar sized curds as provided by 3 ml/L of Biopes (Table 15). The treatments of dose 15 ml/L and control provided the lowest curd diameter 8.26 and 8.25 cm, respectively (Table 15).

3. For plant weight, dose treatment of 3 ml/L of Biopes gave the highest of 470.50 g/plant which was significantly different with all other entries. However, this treatment was not significantly different from treatments with dose of 2 ml/L of Biopes, 25 ml/L of FS, 1 ml/L of Biopes and 5 ml/L of FS. The control treatment provided the lowest plant weight of 328.25 g/plant (Table 15).

4. The widest leaf was observed in dose treatments of 3 ml/L of Biopes and 25 ml/L of FS 13.47 and 13.24 cm, respectively. However, they were not significantly different from treatment doses of 2 ml/L of Biopes and 5 ml/L of FS. The treatment dose of 15 ml/L and control provided the narrowest leaf of 11.86 and 11.59 cm, respectively (Table 15).

Table 15 Plant weight, curd diameter, leaf length, leaf width per plant and marketable curd yield of cauliflower harvested with different concentrations of *Stemona* products application.

Stemona products and concentration	Plant weight (g)	Curd diameter (cm)	Leaf length (cm)	Leaf width (cm)	Curd yield (kg/ ha)
Control	328.25 ^{c}	8.25 ^{c}	20.55	11.59 ^{c}	5000.00 ^{d}
FS 5 ml /L	390.00 ^{abc}	8.60 ^{bc}	22.13	12.30 ^{abc}	5593.30 ^{d}
FS 15 ml /L	369.67 ^{bc}	8.26 ^{c}	20.48	11.86 ^{c}	5966.70 ^{cd}
FS 25 ml /L	451.33 ^{ab}	9.91 ^{ab}	23.24	13.24 ^{a}	7466.70 ^{ab}
Biopes 1 ml /L	383.33 ^{abc}	8.81 ^{bc}	21.29	11.90 ^{bc}	5960.00 ^{cd}
Biopes 2 ml /L	456.04 ^{ab}	9.60 ^{abc}	23.45	13.20 ^{ab}	6852.60 ^{bc}
Biopes 3 ml /L	470.50 ^{a}	10.40 ^{a}	23.60	13.47 ^{a}	8160.00 ^{a}
F-test	*	*	ns	*	**
$LSD_{0.05}$	87.17	1.40	2.50	1.31	1203.10
C.V (%)	12.04	8.65	6.37	5.90	10.51

Different letters indicated significant difference of means by LSD ($P \leq 0.05$)

* = significantly different at $P \leq 0.05$; ** = highly significant difference at $P \leq 0.01$.

For growth and development of all samples, there were no significant differences in number of leaf, plant canopy and plant height from 15 days to 40 days after transplanting (Table 16).

Table 16 Growth and development in terms of the number of leaves, plant canopy and plant height due to different concentrations of *Stemona* products application.

Horticulture characters	Day after transplanting					
	15	20	25	30	35	40
No. of leaf (Unit)	4.42 ^{ns}	6.13 ^{ns}	8.04 ^{ns}	10.07 ^{ns}	14.07 ^{ns}	17.97 ^{ns}
Plant canopy (Cm)	10.14 ^{ns}	14.44 ^{ns}	21.55 ^{ns}	29.25 ^{ns}	35.08 ^{ns}	39.63 ^{ns}
Plant height (Cm)	10.69 ^{ns}	14.07 ^{ns}	19.09 ^{ns}	24.75 ^{ns}	31.20 ^{ns}	35.64 ^{ns}

^{ns} = not significant.

All numbers are grand means.

Chapter 5

Discussion

Insecticidal activity of FS against third instar larvae of *Plutella xylostella* and *Spodoptera litura* larvae

The result from contact activity test of FS against third instar larvae of *P. xylostella* provided 53.33% accumulated mortality at concentration of 25,000 ppm (2.5%) within 72 hours. Meanwhile at the same concentration, anti-feedant activity test of FS against third instar of *P. xylostella* larvae gave higher percentage accumulated mortality (80%) than contact activity test that provided 53.33% accumulated mortality. In this study, however, using higher concentration of FS was found more effective in comparison to the *S. tuberosa* ethanol extracts. Jiyavorranant *et al.* (2003) reported that the effect of *S. tuberosa* ethanol extracts at only 0.5% concentration was the best activity against third-instar larvae of *P. xylostella*, which provided 53.30% accumulated mortality with 3.67 mm^2 leaf damage area per larva within 72 hours by the leaf dipping method. These different results indicated that different products made by different extraction methods could be affected differently. In most cases, immersion in parallel extraction technique showed a better performance than in serial extraction technique (Sanguanpong, no date). Sanguanpong (no date) also mentioned that the highest extraction yield was dependent on the solvent used, which was found by using acetone, followed by water, ethanol, methanol, dichloromethane and hexane respectively. Whereas, solid residue or marc was found highest by using hexane extraction followed by dichloromethane, water, ethanol, methanol and acetone respectively (Sanguanpong, no date). Moreover, the same extraction method but from different places, also offered accumulated mortality at different concentrations. Greger *et al*, (2003) presented that crude extracts of *S. kerrii* showed less insecticidal activity with LC_{50} values of 89 ppm from Khao Chomphu sample than 48 ppm from Doi Sutep sample. But interestingly, the contact and anti-feedant activity tests (TA and LD method) of FS did not affect the third instar larvae of *S. litura*. Berm *et al.* (2002) reported that Tuberostemonine, a dominating alkaloid in the roots of *S. tuberosa*, showed outstanding repellency but no toxic effects.

Similarly, Christroph *et al.* (no date) mentioned the most prominent alkaloid from *S. tuberosa*, tuberostemonine, had only marginal activity (LD_{50} > 500 ppm) against *S. littoralis* (N). In

contrast, Pimsa-man, (2002) revealed that *S. tuberosa* root methanolic extract indicated higher insecticidal activity against second instar *S. litura* larvae than neem seed extract using the same bioassay technique LD_{50} 0.0054 mg/insect, LC_{50} 2,866 ppm.

Insecticidal activity of Biopes against third instar larvae of *Plutella xylostella* and *Spodoptera litura* larvae

The result from this study showed that the same concentrations at 2,500 and 3,000 ppm provided faster anti-feedant activity than contact activity against third-instar larvae of *P. xylostella*. The anti-feedant activity gave 60% accumulated mortality in both concentrations within 48 hours, while contact activity gave 60% and 73.33% accumulated mortality at 2,500 and 3,000 ppm within 72 hours, respectively. This indicated that Biopes performed more on anti-feedant activity than contact activity.

The concentrations at 2.000; 2,500 and 3,000 ppm gave the highest contact activity against third-instar larvae of *P. xylostella* up to 53.33; 60.00 and 73.33% accumulated mortality respectively, within 72 hours. This result conformed with the report of Pimsa-man, (no date). He stated that *Stemona* spp. extract against the diamondback moth showed the highest contact activity with the LC_{50} (48hr) of 2, 313 and 2, 108 ppm, although it was found that the contact activity of Biopes was 24 hours late to achieve the effective threshold compared to the report of (Pimsa-man, no date).

Concentrations at 2,500 to 3,000 ppm used by LD method of Biopes gave 60% accumulated mortality in leaf damage area from 2.60 to 0.60 mm^2 within 48 hours. These concentrations were still of high concentrations than reported by Pimsa-man (no date). He said that the highest anti-feedant activity against the diamondback moth was observed from *Stemona* spp; as extracted with the LC_{50} (48hr) at 129.22 and 701.11 ppm. On the other hand, all concentrations of Biopes did not affect the third instar larvae of *S. litura* in the study. Greger *et al.* (2203) reported that pyrido[1,2- a]azepine *Stemona* alkaloids 1-5* only led to paralysis and

* The structures of stemocurtisine1, stemokerrin 2, oxystemokerrin 3, oxystemokerrin N-oxide 4 and methoxystemokerrin N-oxide 5

softening of larval bodies. These alkaloids were isolated from the root extracts of *S. kerri*, *S. curtisii* and an unknown species. Moreover, the majority of the *S. litura* strains collected in South India exhibited high resistance levels, 61 to 148 fold to organic pesticides (Kranthi *et al.*, 2002).

Effects of FS and Biopes against *Plutella xylostella* larvae

The effectiveness of FS by contact poison at concentration of 25,000 ppm provided 53.33% accumulated mortality within 72 hrs, similarly with Biopes using the same method that provided 53.33% accumulated mortality at concentrations of 2,000 ppm within 72 hrs. However, effectiveness of FS by anti-feedant poison provided 60% accumulated mortality at concentration of 20,000 ppm and caused leaf damage areas of 0.40 mm^2 per larva within 72 hrs after treatment. The same effect of Biopes anti-feedant poison at concentrations of 2,500 and 3,000 ppm caused 60% accumulated mortality while leaf damage areas were 2.60 and 0.60 mm^2 per larva but Biopes took time for only 48 hrs after treatment.

So these results conformed that Biopes were more poisonous than FS against third-instar larvae of *P. xylostella*. Moreover, anti-feedant activity of Biopes gave faster effect than anti-feedant activity of FS.

Effects of *Stemona* products on *P. xylostella* larvae in the cauliflower field

The population levels of *P. xylostella* larvae and leaf damage were significantly lower in cauliflower plants treated with high concentrations of FS and Biopes than another treatment and control plots. This conformed to the results of the laboratory test on *Stemona* products against third instar larvae of *P. xylostella* which provided a high percentage of accumulated mortality and reduced leaf damage area. The treated plots at high concentrations of FS and Biopes also gave a high curd yield of cauliflower. Acquaah (2005) stated that insects indirectly affect photosynthesis by reducing leaf area through defoliation. When photosynthates are reduced, subsequent growth and yield were decreased. On the other hand, insects may bore through curd in the field, reducing harvestable yield. This result from field experiment was also similar with the result of previous laboratory trials done by Charleston *et al.* (2007) in which they showed that feeding damage by *P. xylostella* larvae was significantly lower on cabbage plants treated by botanical extracts derived

from *Melia azedarach* and *Azadirachta indica*. Other field experiments conducted in crucifer systems using neem products showed significantly lower damage to cabbage heads, with significantly higher marketable yield than control plots (Saucke *et al.*, 2000; Verkerk and Wright, 1994).

Results from field plots of cauliflower plants treated with *Stemona* products also showed effects on extended leaf width or leaf area indices, which provided increasing curd yield of cauliflower because leaf area indices could improve photosynthesis rates (crop growth rate). In order to get maximum crop yield, leaf area should expand to reach its optimum rapidly. By these results, both types of *Stemona* products were nearly the same, such as 25 ml/L of FS producing curd yield of 7,466.70 kg/ha, cased leaf damage 4.08% per plant at 40 days after transplanting and 3 ml/L of Biopes providing curd yield of 8,160.00 kg/ha with leaf damage 4.05% per plant at the same time. However, the high dose differences were due to different extract method, *Stemona* species and location of growth.

Effects of *Stemona* products on *S. litera* larvae could not be observed due to its absence during the field experiment.

Chapter 6

Conclusion and Recommendations

Conclusion

The present study was to find out the effectiveness of *Stemona* products to control *P. xylostella* and *S. litura* larvae both under laboratory and field conditions. In the laboratory test for toxicity of *Stemona* products test, it was found that 25,000 ppm of FS had the best activity against third-instar larvae of *P. xylostella* by the TA method. This concentration gave 53.33% accumulation mortality within 72 hours. LD method of FS at 20,000 and 25,000 ppm concentrations also provided the best activity against third-instar larvae of *P. xylostella*, which decreased leaf damage area to the lowest levels (0.40 and 0.60 mm^2/larva) and increased accumulated mortality (60 and 80%).

Biopes at concentrations of 2.000; 2,500 and 3,000 ppm had the best activity against third-instar larvae of *P. xylostella* by TA method, resulting to 53.33, 60.00 and 73.33% accumulated mortality, respectively, within 72 hours. Concentrations at 2,500 and 3,000 ppm of Biopes also provided the best activity against third-instar larvae of *P. xylostella* by LD method, thus decreasing the leaf damage area (2.60 and 0.60 mm^2/larva) but increased at 60% accumulated mortality within 48 hours. However, both methods and both *Stemona* products did not affect the third-instar larvae of *S. litura*, providing only 50% accumulated mortality. They only provided abnormal growth of *S. litura* larvae. Moreover, in the field condition, *S. litura* larvae were absent, whereas, larval population of *P. xylostella* was found lowest when treated with 3 ml/L of Biopes and 25 ml/L of FS. These doses had reduced percentage leaf damage of cauliflower crops. On the other hand, they also gave horticultural characteristics of the plant such as plant weight, curd diameter and expanded leaf width, especially curd yield of cauliflower crops.

Recommendations

From such findings in these experiments it could be recommended for the further studies as follows:

1. Effects of *Stemona* products against *P. xylostella* and *S. litura* larvae could be studied in other cole crops like cabbage and broccoli, etc.

2. *Stemona* products should be reevaluated for *S. litura* control to find out their effects.

3. *Stemona* products should be applied on the cauliflower crop at the minimum rate of 3 ml/L of Biopes and 25 ml/L of FS at 3-5 days interval depend on population density and the growth stages of *P. xylostella* larvae.

4. Analysis of cost and benefit ratio of *Stemona* products application against insects control in cole crops should be considered for future study.

5. It will also be important to study the effects of *Stemona* products on natural enemies of the insect pests of cole crops.

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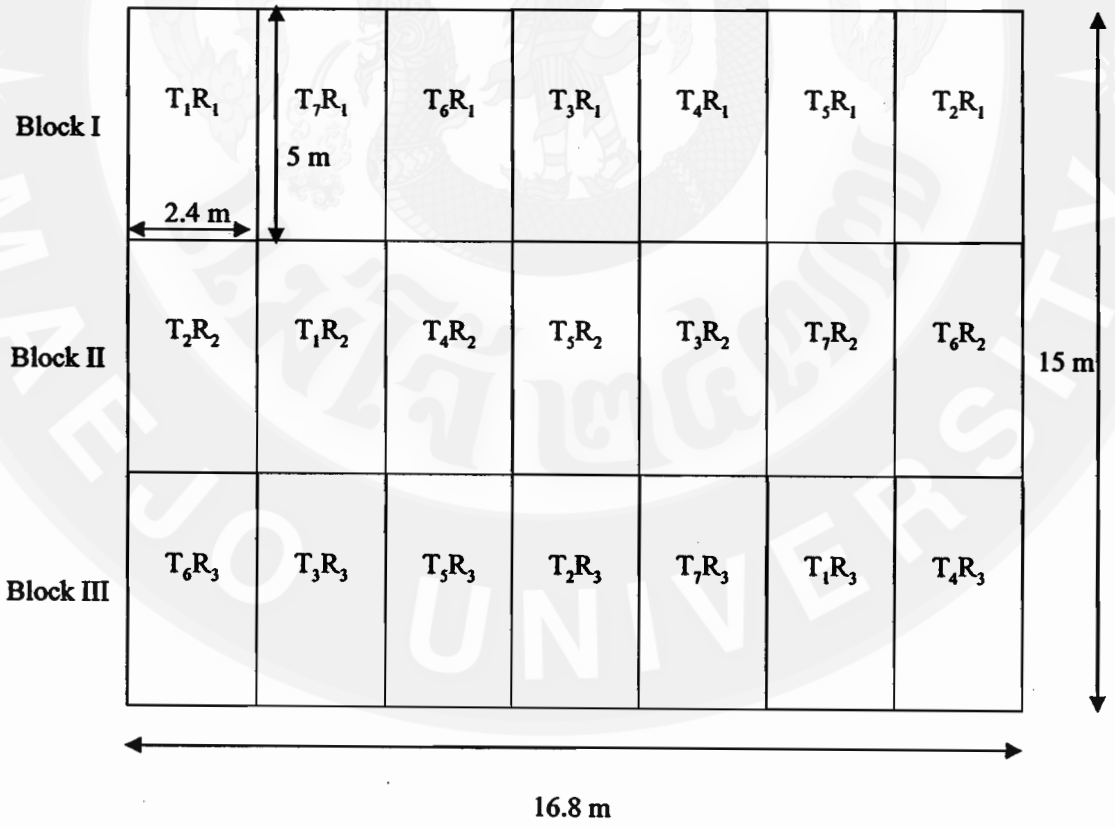
Appendices

Appendix1: Temperature during Oct 2007 and Dec 2008

Temperature	2007			2008
	Oct	Nov	Dec	Jan
Maximum (°C)	31	30	28	29
Minimum (°C)	21	19	15	13
Average (°C)	26	24.5	21.5	21

Source: http://www.chiangmai.net/weather/.

Appendix2: Layout at the experimental plot in (RCBD)



The main plot experimental area 15 m x 16.8 m (252 m^2)

Curriculum Vitae

Name:	Chantha Kim
Date of Birth:	January 04, 1968
Educational Background:	
1987-1991	B.Sc. Agriculture Royal University of Agriculture, Phnom Penh, Cambodia
2006-2008	M.Sc. Horticulture Maejo University, Chiang Mai, Thailand
Scholarship:	Thailand International Development Cooperation Agency (TICA)
Work Experience:	
1992-1997	Technical Staff Department of Agriculture Forestry and Fisheries, Kompot Province.
1997-2003	Extension Staff Department of Agriculture, Kompong Cham Province
2003-2006	Chief office of Agricultural Stong Trang District, Kompong Cham Province, Cambodia
Publication:	Paper presented in 7th National Horticultural Congress held on 27-29 May, 2008 at Naresuan University; Phitsanulok.