# CHAPTER I

# INTRODUCTION

Rice is a staple food of the world, especially in Asian countries like Thailand. More than 3 billion people in Asia consumed rice and about 75% of people in Thailand work in agricultural lines. Thailand is one of the world's biggest rice producers, with paddy output of 27 million tons in 2003. Thailand is also the world's biggest rice exporter: annual shipments are worth more than $2,000 million and reached 7.5 million tons in 2003. Its main export markets are Indonesia, Nigeria, Iran, the United States and Singapore. In addition, the productivity of rice is brought up as the majority of the export agricultural product of the country. Referring to the Thailand export data, for a few decades, it was found that the majority of the world's market share of rice was performed from Thailand. However, the productivity of rice in Thailand could not significantly increase whereas the world-demand was highly increased. These problems may arise from many reasons such as weather, the quality of the plant field, rainfall, weed and rice pest.

Rice pests were directly affected to the productivity of rice and one of the most rice pests that could vastly damaged to the productivity of rice in very short time and became seriously plaque was "brown planthopper". Brown planthopper is the sucking insect, was the most significant insect pest of the tropical as well as temperature region, throughout rice growing countries including Thailand. It could attack the basal portion (stem), in every its life-cycle and removed plant sap from xylem and phloem tissue of rice. Severely damaged of rice obviously gave the brownish appearance of plants like fire damage, hence, hopper virus disease, such as grassy stunt virus. In 1973, the brown planthoppers was firstly reported that its outbreak was occurred in central region and some of the northern region of Thailand that could vastly damage the productivity of rice. Furthermore, it was known that brown planthopper could develop its resistant population to the synthetic insecticides.

### 1.1 Taxonomy of *Nilaparvata lugens* (Stal)

Superfamily Flugoroidae is reported to contain more than 20 insect families. (Todatora, 1977) However, three species: *Nilaparvata* lugens (Stal), *Sogatella furcifera* and *Laodelphax striatellus* are in both tropical and temperature areas. In this region, the family is a particular group of Fulgoroidae, and identification of Delphacidae, structure of the following sclerotized characters of adults is usually used: vertex, frons, clypeus, gene, antenna, pronotum, mesonotum, scutellum, carinae, wing, legs, and abdomen. The brown planthopper is classified in Kingdom: Animilar, Class: Insecta, Order: Homoptera, Series: Auchenorhyncha, Superfamily: Fulgoridae, Family: Delphacidae, Genus: *Nilaparvata*, Scientific name: *Nilaparvata lugens*(Stal), Common name: Brown planthopper.

### 1.2 Life cycle of *N. lugens* (Stal)

The life cycle of brown planthopper can be divided into 3 cycles (Figure 1.1), (Preecha, 2545). The eggs of brown planthopper are laid as the single or aggregated unit. In facts, laid eggs normally are on center of the rice's leaf sheath. An adult is open its ovipositor and takes it eggs inside to the leaf sheath. The eggs are crescent-shaped and 0.99 mm long. Newly laid eggs are whitish. They turn darker when about to hatch. Before egg hatching, two distinct spots appear. representing the eyes of the developing nymph. Some eggs are united near the base of the flat egg cap and others remain free. The nymph was divided into 5 stages: the nymph first stage is white and 1 mm long. After 1-2 days it was molting to second stage, its thorax turned to be grey. Just for a few days after it was laid, the grayish body was appeared and developed its state following 5 states. An adult could be divided into 2 forms are yellowish brown or dark brown. Macropterous form male length 2.3-2.4 nm female length 2.8-3.2 nm Brachypterous form male length 2.0-3.1 nm female 2.7-3.5 nm. Carinae of vertex was relatively less obvious, faintly prominent. Frons normal, not excavated centrally, with a distinct median carina which is not cut short. Aplical cells of the anterior half of macropterous forewing not marked distinctly with dark brown. Post-tibia can be spur with 30-36 teeth.

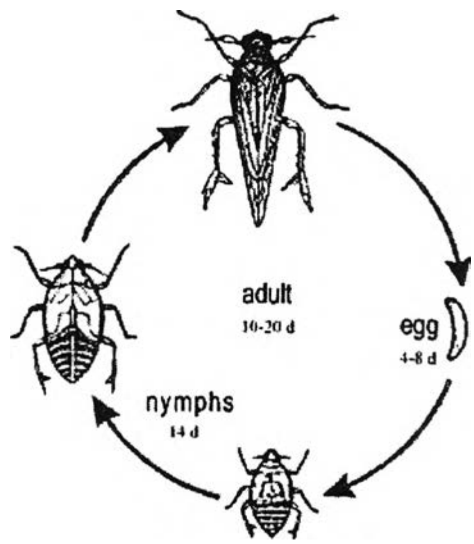



**Figure 1.1** The life cycle of brown planthopper

## 1.3 *N. lugens* (Stal) biotype

It is important to interpret the brown planthopper in Asia. Pathak and Heinrichs in 1982 reported that many researchers have investigated the genetics of resistance to brown planthopper. It is well-recognized fact that genetic diversity is existing. These physiological or genetic strains in the BPH population are commonly referred to as biotypes, although the adoption of term biotype may be control versial from different conceptual viewpoints. The International Rice Research Institution (IRRI) classified into 3 biotypes of brown planthopper by gene in rice. The population that was unable to infest varieties that carried *Bph 1* was unable to infest varieties that carried *Bph 1* gene was designated as biotype 1, while those population capable of infesting resistant varieties carrying *Bph 1* and *Bph 2* gene referred to as biotypes 2 and 3 respectively. However, in Thailand brown planthoppers could not be interpreted their biotype.

## 1.4 Feeding of *N. lugens* (Stal)

The brown planthopper causing direct damage rice by sucking assimilates from the phloem (Kasushi, 1973) which can result in the complete drying of the plants, a condition known as "hopper burn" is the causing action between insect physiology and rice complex. The mouth parts are specialized for intake of liquid diet. The most conspicuous element of the mouth parts are the stylets, are about 650-700 μm long which serve as a piercing and sucking organ. It consists of outer pair of mandibular and inner pair of maxillary stylets. The maxillary stylets are interlocked ferming two canals. The dorsal one functions as the sucking canal and communication with the sucking *via* the pharyngeal duct. A hypothesized process of feeding in the plant of brown planthooper is displayed in Figure 1.2.

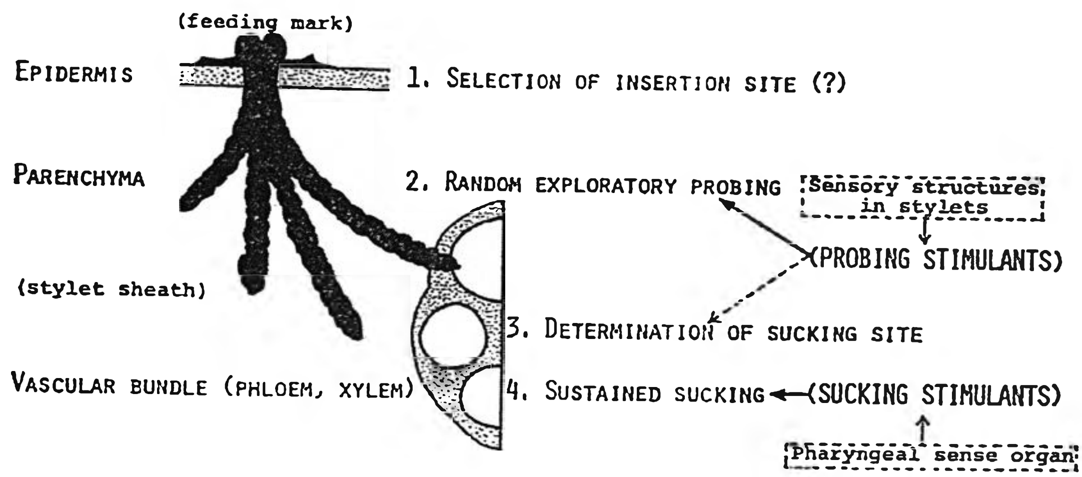
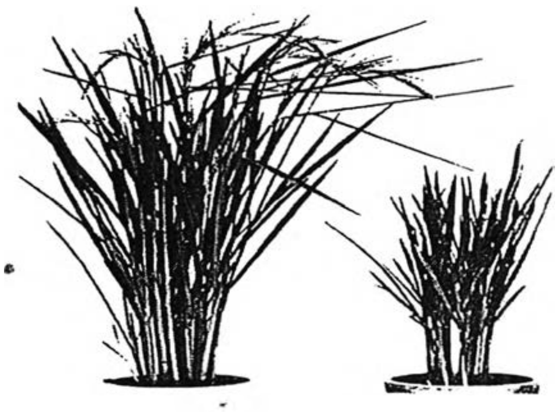



**Figure 1.2** A hypothesized process of the feeding in the plant of brown planthopper

In addition the brown planthopper causing sucking damage and transmitting plant viruses, such as ragged stunt virus and rice grassy stunt virus were directly damaging the rice crop. This disease has severely damaged rice crops. The brown planthoppers could be transmitted glass stunt disease, which had become one of the major diseases of the rice plant. Ling *et al.*, in 1978 reported this as a new virus disease, occurred sporadically in the Philippines in 1977. The symptom of grassy stunt disease displayed at leaves are short, narrow, usually pale green or pale yellow and often have numerous small dark-brown dots or sports of various shapes which may coalesce to from blotches. Young leaves of some varieties may be mottled or striped. The leaves may remain green if supplied with adequate nitrogenous fertilizer and the yield loss depends on the susceptibility of rice variety. The symptom of grass stunt disease are shown in Figure 1.3.



Healthy Grassy stunt

**Figure 1.3** The symptom of rice ragged stunt disease

**1.5 Distribution in Asia**

The distribution of brown planthopper could be found in tropical and temperate Asia. For example, Australia, Bangla Desh, China, Cambodia, Fiji, India, Indonesia, Japan, Korea, Malaysia, New caledonia New Guinea, Philippines, Sarawak, Solomon Is., Sri Lanka, Taiwan, Thailand and Vietnam.

**1.6 Distribution in Thailand**

The distribution of brown planthopper in Thailand came from two mainly reasons, the first one is the weather. As the matter of fact, the weather in Thailand was generally suitable for planting rice twice a year, especially in central region. Therefore, the brown planthopper could completely be survived without the lack of food over the year giving it outbreak completely in short time. The other factor stems from the migration of brown planthopper from the colder area as in China to the warner area as in Thailand.

**1.7 Methods of controlling of *N. lugens* (Stal)**

Controlling methods for the brown planthopper could be divided into 5 methods.

### 1.7.1 Physical control

Physical control could be divided into 2 submethods depending on the state of brown planthopper. As the adult state, female brown planthopper could be trapped *via* a light trap source, so called as "Light Trap Method". The second one was specifically suitable for controlling brown phanthopper's eggs. This could be done by giving the water over to the height area of brown planthopper's egg that could interrut the respiratory system of the eggs and made them died.

### 1.7.2 Biological control (Jirapong, 2543)

The biological control of brown planthopper could be divided into 2 methods 1) parasite and 2) predator. Eggs of brown planthopper theparasite *Oligosita yasumatsui*, *Anagrus optabilis* and *Tetrastichus formosanus* preadator *Cyrtorhinus lividipennis* Reuter nymps were used. While for the adult stage, the parasite *Elenchus yasumatsui*, *Haplogonatopus orientalis*, *Echthrodelphax fairchildii*, predator *Lycosa pseudoannulata* was generally employed. (Preecha, 2545)

### 1.7.3 Host – plant resistance control

The resistant rice to brown planthopper, such as Patumtanee 1, Chinat 60 and Suphanburi 60 must be performed in the controlling of brown planthoppers.

### 1.7.4 Intergrated pest management (IPM) (Suwat, 2544)

When the brown planthopper outbreak was more complex, the use of method over than a method must be performed, this called as "host-plant control." For instance, the combination use of physical control and chemical control.

### 1.7.5 Chemical control

There are several insecticides available for control brown planthopper. Chemical substances which widely used can be classified two types (Ministry of Agriculture and Cooperative, 2545).

1.7.5.1 Inorganic insecticides

Inorganic insecticides could be classified into 5 groups: (Cremlyn, 1978).

1.7.5.1.1 Carbamate group

The successful development of organophosphorus insecticides stimulated the examination of other compounds known to possess anticholinesterase activity *i.e.* carbaryl **(1)** carbofuran **(2)** and carbosulfan **(3)**.

1.7.5.1.2 Organochlorine group

Organochlorine group is the most important of this insecticide. It is referred as second-generation insecticides They are characterized by being long-lived (persistence), with a broad spectrum of action, as both contact and stomach poison *i.e.* DDT **(4)** methoxychlor **(5)**.

1.7.5.1.3 Organophosphate group

The organic chemistry of phosphorus goes back to 1820. Serious investigation on toxic organophosphorus compounds as potential nerve gases began during the second world war. They are however very effective insecticides, *i.e.* methyl parathion **(6)** and ethyl parathion **(7)**. The organophosphorus insecticides apparently inhibit the action of several enzymes.

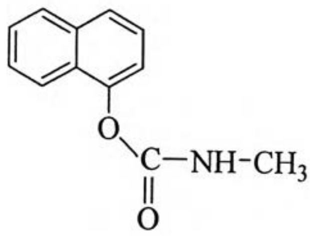
1.7.5.1.4 Synthetic pyrethroid group

This is an alternative to the naturally occurring pyrethrums whose main drawback is its short life as an effective chemical. The modern chemical analogues are however, more removed from the original pyrethrins, *i.e.* etofenprox **(8).**

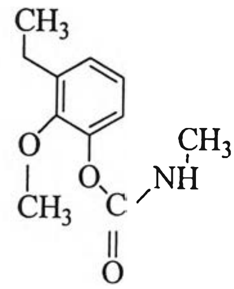
1.7.5.1.5 Chloronicotinyl group

Chloronicotinyl is a relatively new chemical class containing a number of systemic insecticides, *i.e.* imidarcloprid **(9)**.

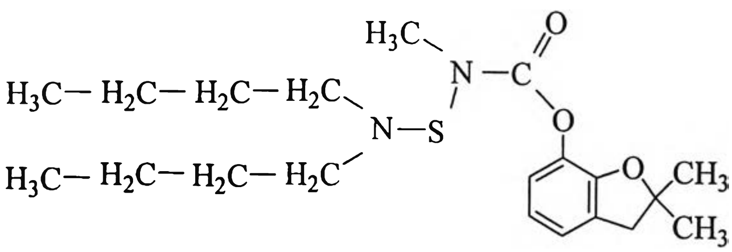
The effects of carbamate and organophosphate insecticides could be inhibited acetylcholinesterase enzyme.



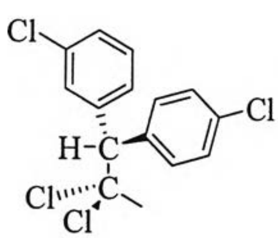
carbaryl **(1)**



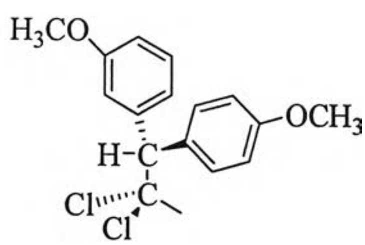
carbofuran **(2)**



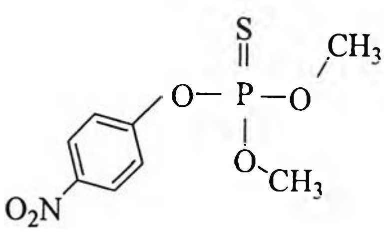
carbosulfan **(3)**



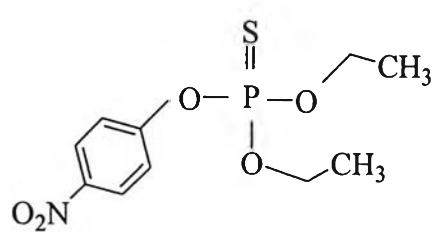
DDT **(4)**



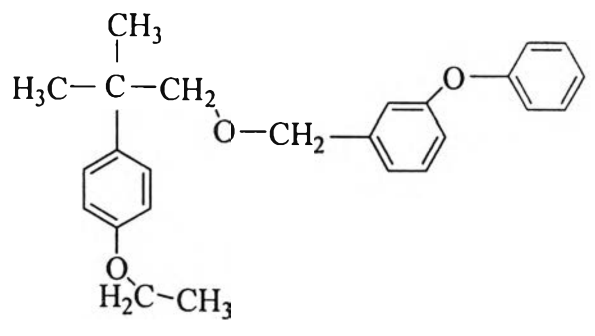
methoxychlor **(5)**



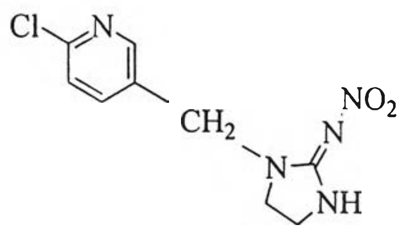
methyl parathion **(6)**



ethyl parathion **(7)**



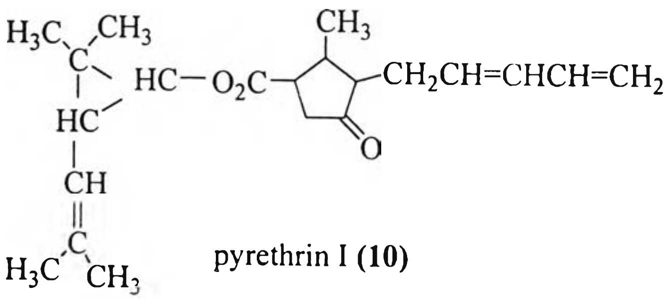
etofenprox **(8)**



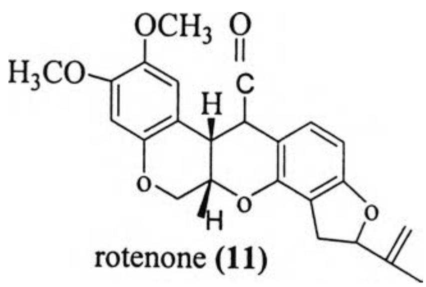
imidarcloprid **(9)**

1.7.5.2. Organic insecticides

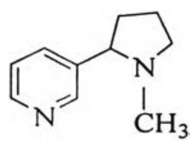
Plants have evolved over some 400 million years (Cremlyn, 1978). To combat insect attack they have developed a number of protective mechanisms, such as repellency, and insecticidal action. Thus, a large number of different plant species contain natural insecticidal materials. Many isolated compounds were identified and proved to progress agrochemical activity for example pyrethrin I **(10)** a complex of esters extract from the flowers of *Chrysanthenum cinerariefolium* (Compositae). Rotenone **(11)** is a flavonoid extracted from the roots of two plants: *Derris spp.* (Fabaceae) and *Lonchocarpus spp.* (Fabaceae). The first one gives up to 13% of rotenone while the second only about 5%. *Derris spp.* is a native to eastern tropics, while *Lonchocarpus spp.* is native to western hemisphere. Rotenone is a contact and ingestion compound. Nicotine **(12)**, a piperidine-pyridine alkaloids was isolated from *Nicotiana tabacum*. Anabasine **(13)** was found in plants in the *Nicotiana spp.* Sabadilla **(14)** is derived from the seeds of the plant *Schoenocaulon officinale*. The active component, ryania **(15)** is derived from the roots and woody stems of *Ryania speciosa*. Ryania is an alkaloid from *Anabasis aphylla.* Neem seeds yield azadiractin **(16)** from the fruits of *Azadirachta indica*.



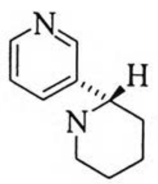
pyrethrin I **(10)**



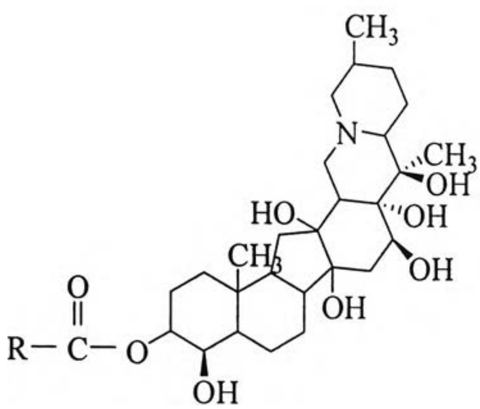
rotenone **(11)**



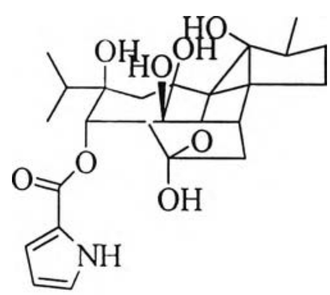
nicotine **(12)**



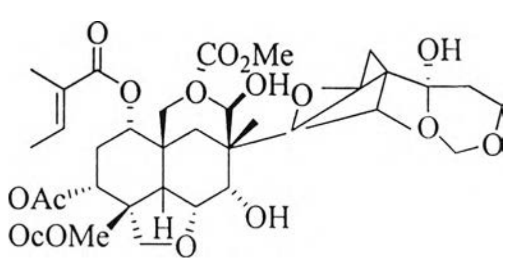
anabasine **(13)**



sabadilla **(14)**



ryania **(15)**



azadiractin **(16)**

## 1.8 Study on *Piper spp.*

From the literature review, there are a few reports on the chemical constituents of *Piper spp.* *Piper* is an economically and ecologically important genus of the family Piperaceae that includes a fascinating array of species for studying natural history, natural products chemistry, community ecology, and evolutionary biology. It contains 1,500 – 2,000 species of shrubs, herbs, and lianas, many of which are keystone species in their native habitat, while others are a major invasive species in areas where they are introduced.

### 1.8.1 Characteristics of *Piper spp.*

The general characteristics of leaves are simple, alternate broadly ovate or rounded, 5-18 by 2-10 cm, having apex acute or a acuminate, unequally rounded at the base or broadly heart-shaped. Flowers are very minute, in cylindrical male of female. *Piper* species have a pan tropical distribution, and are most commonly found in the understudy of lowland rainforests, but can also occur in clearings and in higher elevation life zones such as cloud forests; they are typically a dominant plant wherever they are found. Obligate and facultative ant mutuality found in some *Piper* species have a strong influence on their biology, making them ideal systems for research on the evolution of symbioses and the effect of mutualisms on biotic communities. The diversity and ecological relevance of this genus makes it an obvious candidate for ecological and evolutionary studies, but surprisingly most research on Piper species has focused on the economically important plants, such as *P. nigrum* (black pepper), *P. methysticum* (kava), and *P. betle* (betel leaf).

### 1.8.2 Literature survey of chemical constituents of *Piper spp.*

In 1979, Miyakada *et al.* reported that pipericides **(17)**, dihydropipericides **(18)** and guineensine **(19)** from *Piper nigrum* L. showed insecticidal activity against the *Callosobruchus chinesis*.

In 1981, Helen *et al.* addressed that (*E*,*E*)-*N*-(2-methylpropyl)-2,4-decadienamide, (*E*,*E*,*E*)-13-(1,3-benzodioxol-5-yl)-*N*-(2-methyl-propyl)-2,4,12-tridecatrienamide and (*E*,*E*,*E*)-11-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,10-undecatrienamide could be extracted from *Piper nigrum* and showed insecticidal activity against *Calloosobruchus maculates*.

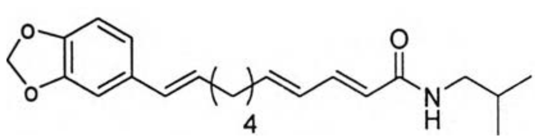
In 1993, Parmar *et al.* isolated (2*E*, 4*E*)-*N*-Isobutyl-7-(3,4-methylenedioxyphenyl)-hepta-2,4-dienamide **(20)** was isolated from *Piper falconeri*. This compound exhibited insecticidal and showed insecticide activity against *Musca domestica* and *Aedes aegyptii*.

In 1994, Lehamberg *et al.* reported that ryanodine **(21)** was an active ingradient of ryania which presented insecticidal activity against *Musca domestica.*

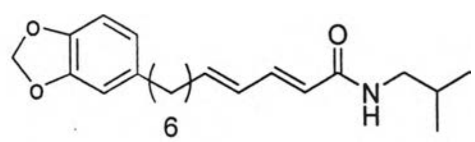
In 2004, Siddiqui *et al.* separated and characterized two new amide insecticides from *Piper nigrum* L. as pipnoohine **(22)** and pipyahyine **(23)**. These compounds were exhibited toxicity at $LC_{50}$ 35 and 30 ppm against fourth instar larvae of *Aedes aegypti* L. by WHO method.

In 2005, Park *et al.* addressed the isolation of retrofractamide A **(24)** guineensine **(19)**, pellitorine **(25)**, piperine **(26)** from *Piper nigrum* L. as new insecticidal principals against third instar larvae *Aedes aegypti.*

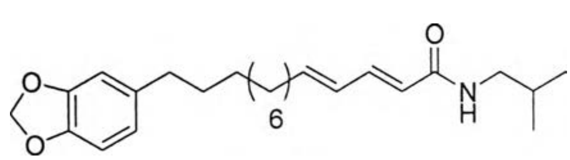
In 2005, Christodoulopoulou *et al.* disclosed two new piperidine amides: *N*-[2*E*,4*E*,8*Z*)-tetradecatrienoyl]piperidine **(27)** and *N*-[2*E*,4*E*,8*Z*,11*Z*)-tetradecatetrenoyl]piperidine **(28)** from *Otanthus marittimus*.



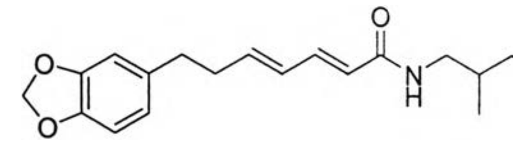
pipericides **(17)**



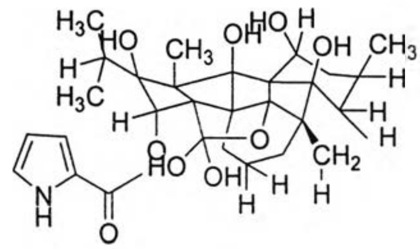
dihydropipericides **(18)**



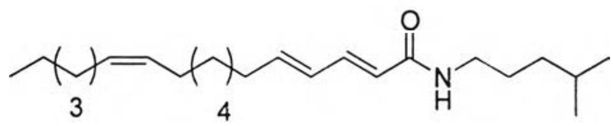
guineensine **(19)**



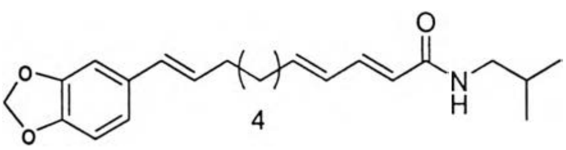
(2*E*,4*E*)-*N*-Isobutyl-7-(3,4- methylenedioxyphenyl)-hepta-2,4-dienamide **(20)**



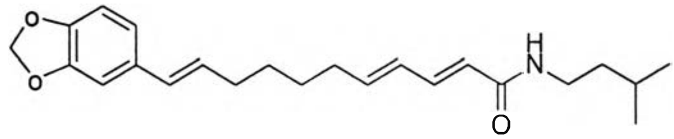
ryanodine **(21)**



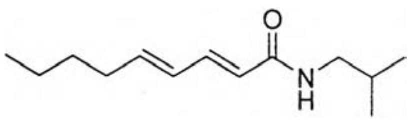
pipnoohine **(22)**



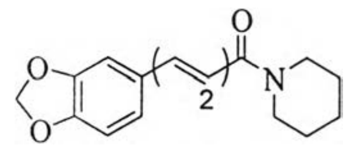
pipyahyine **(23)**



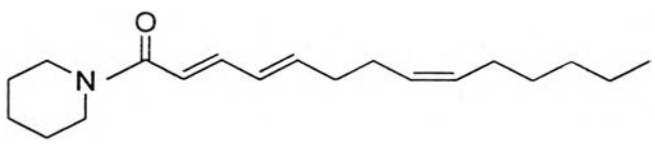
retrofractamide A **(24)**



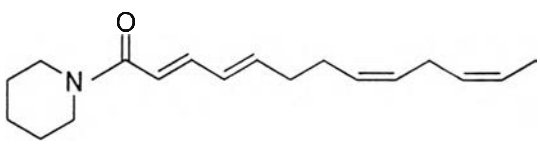
pellitorine **(25)**



piperine **(26)**



*N*-[2*E*,4*E*,8*Z*)-tetradecatrienoyl]piperidine **(27)**



*N*-[2*E*,4*E*,8*Z*,11*Z*) - tetradecatetrenoy]piperidine **(28)**

### 1.8.3 Characteristics of *Piper sarmentosum*

Other local names are Cha phlu (central); Nom wa (peninsular), Phak pu na, Phak phlu nok, Phlu ling (northern), Ye-thoei (karan-mae hong son). (Tem, 2544). *P. sarmentosum* is a medicinal plant belonging to the Piperaceae family, Piper genus. The taxonomy description is as follows: Glabrous, creeping, terrestrial herbs with procambant branches. Leaves 6-16 cm × 5-9 cm, thin lower leaves. Usually ovate-cordate, upper leaves rather oblong or ovate-oblong, ovate to obliquely or rounded at base, shortly acuminate at apex 5-7 radiatiny nerves from base; petiole 2.5-50 cm long spikes short, dense blunt cylindric white stamens short, stigma 3-4; fruit obovaid 1.5 × 1 cm.

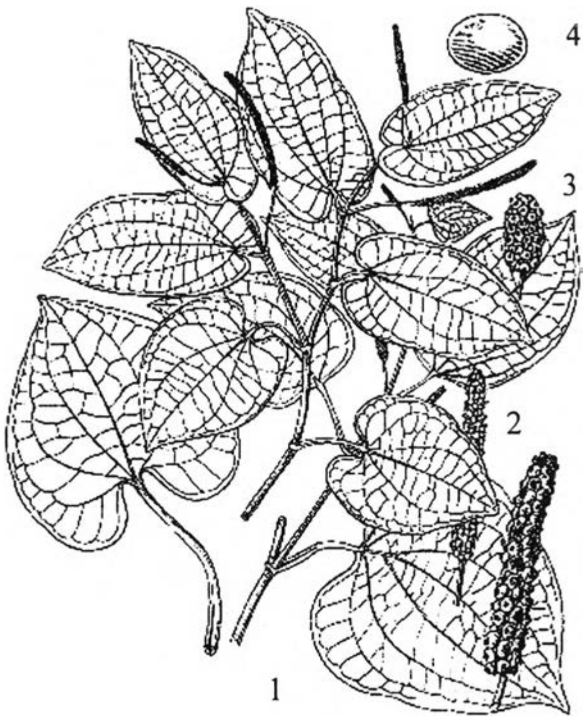


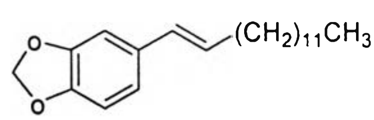

**Figure 1.7** (1) male flowering branchlet (2) male inflorescense (3) female inflorescense (4) bract from female flower

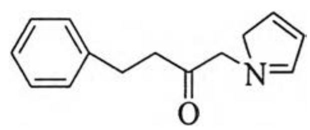
### 1.8.4 Literrature reviews of the chemical constituents of *P. sarmentosum*

In 1987, Likhitwitayawuid and Ruangrungsi reported the isolation of six compounds from the fruits of *P. sarmentosum* as 1-(3,4 methylene dioxyphenyl 1*E*-tetradecane **(29)**, *N*-(3-phenylpropanoyl) pyrrole **(30)**, *β*-sitosterol **(31)**, pellitorine **(25)**, sarmentine **(32)**, sarmentosine **(33)**.

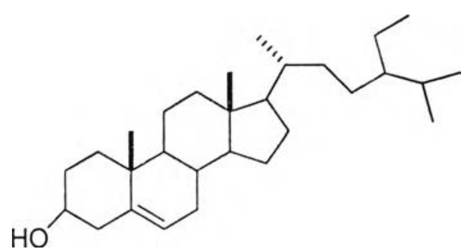
In 2004, Rukachaisirikul *et al.* isolated fourteen compounds from the fruits of *P. sarmentosum* and characterized as pellitorine **(25)**, guineensine **(34)**, brachystamide B **(35)**, sarmentine **(32)**, brachyamide B **(36)**, 1-piperetthyl Pyrrolidine **(37)**, 3′,4′,5′-trimethoxycinnamoyl pyrrdidine **(38)**, sarmentosine **(33)**, (+)asarinin **(39)**, sesamine **(40)**, 1-(3,4 methylene dioxyphenyl 1*E*- tetradecane **(29)**, methyl piperate **(41)**, *β*-sitosterol **(31)** and stigmasterol **(42)**. Sarmentine **(32)** and 1-piperetthyl pyrrolidine **(38)** showed antituberculosis activity at MIC 100, 50 μg/mL and showed antiplasmodial activity $IC_{50}$ 18.9 and 6.5 μg/mL, respectively.

In 1991, Masuda *et al.* separated 4 compounds as from the leaves of *P. sarmentosum* 3 phenylpropanoids and a novel compound. The compounds are characterized as 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene **(43)**, 1-allyl-2,4,5-trimethoxybenzene **(44)** 1-(1-*E*-propenyl)-2,4,5-trimethoxybenzene **(45)** and 1-allyl-2-methoxy-4,5-methylenedioxybenzene **(46)** as a new compound which showed antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* at 100 ppm (MIC 100 μg/mL).

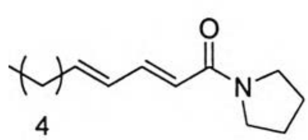


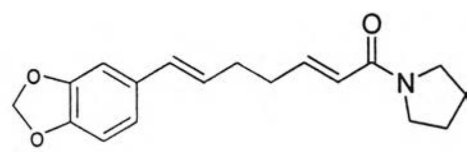

1-(3,4 methylene dioxyphenyl)-1*E*- tetradecane **(29)** *N*-(3-phenylpropanoyl) pyrrole **(30)**



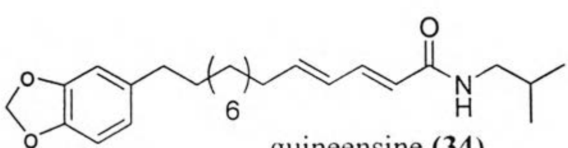

*β*-sitosterol **(31)**



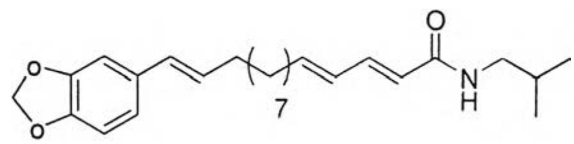

sarmentine **(32)**



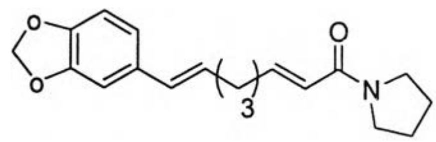

sarmentosine **(33)**



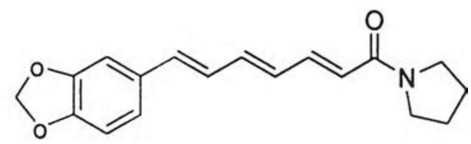

guineensine **(34)**



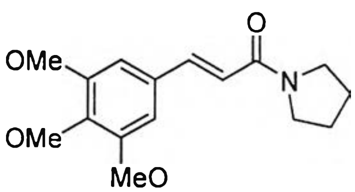
brachystamide B **(35)**



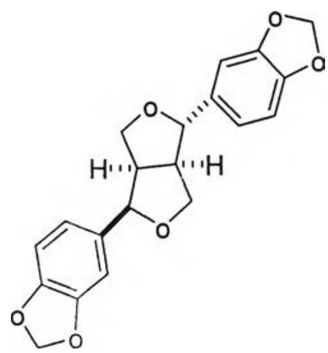
brachyamide B **(36)**



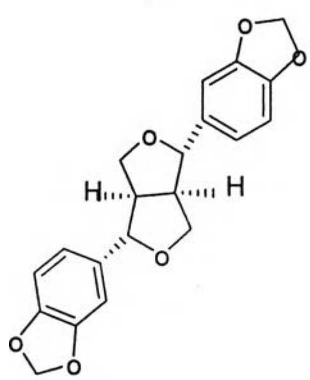
1-piperettyl pyrrolidine **(37)**



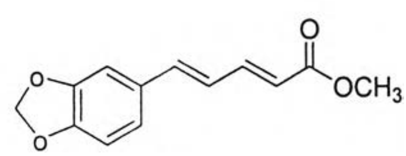
3′,4′,5′-trimethoxycinnamoyl pyrrdidine **(38)**



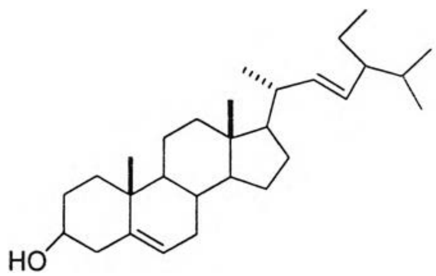
(+)-asarinin **(39)**



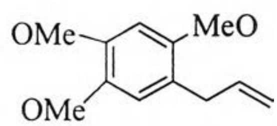
sesamine **(40)**



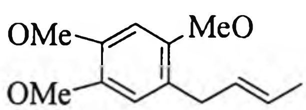
methyl piperate **(41)**



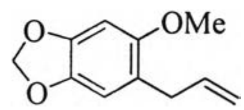
stigmasterol **(42)**



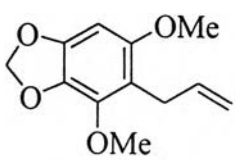
γ-asarone **(43)**



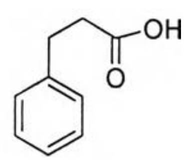
α-asarone **(44)**



asaricin **(45)**



1-allyl-2-methoxy-4,5-methylenedioxybenzene **(46)**



hydrocinnamic acid **(47)**

### 1.9 The objective of this research

The goal of this research could be summarized as follows:

1 Preliminary screening on insecticidal activity test against adult brown planthoppers by Topical application method from the ethanolic extracts of Thai plants belonging to family Amaranthaceae, Cleomaceae, Euphorbiaceae, Leguminosae, Piperaceae, Rubiaceae and Zingiberaceae.

2 To extract and separate pure compound from the most active crude extract.

3 To elucidate the structure of the isolated compounds

4 To search for the bioactive ingredients that can be used in agriculture by using bioassay results as a guide.