THESIS

EFFECTS OF SOME PLANT EXTRACTS ON TOXICITY AND ACTIVITIES OF ESTERASE AND GLUTATHIONE-S-TRANSFERASE IN THE GREEN LEAFHOPPER (*Nephotettix virescens* (Distant))

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THESIS

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Isara Srisaard 2007: Effects of Some Plant Extracts on Toxicity and Activities of Esterase and Glutathione-S-transferase in the Green Leafhopper (*Nephotettix virescens* (Distant)). Master of Science (Biology), Major Field: Biology, Department of Zoology. Thesis Advisor: Associate Professor Suraphon Visetson, Ph.D. 80 pages.

The Green Leafhopper, *Nephotettix virescens* (Distant), is one of the major insect pests of rice (*Oryza sativa* L.) in Asia. It is a vector of viruses such as tungro, yellow dwarf, yellow-orange leaf, transitory yellowing and dwarf viruses. It also causes indirect damage to the crop because of the virus diseases that it transmits. This research was done to evaluate the effects in terms of detoxification mechanisms of some plant extracts against the Green Leafhopper. All extracts were done by Soxhlet's Extraction method using 95% ethanol as the solvent. Various concentrations of these extracts were trialed on adult Green Leafhoppers by a topical sprayer method using a completely randomized experimental design with 3 replicates to obtain their LC50 values. Mangosteen Pericarp (*Garcina mangostana* L.) extracts showed LC50 values of 8.86%w/v (Y =-11.62 + 6.95 X, r^2 = 0.95) and 5.27%w/v (Y =15.55 + 6.52 X, r^2 = 0.99) at 24 hours and 48 hours, respectively. Yam Bean Seed (*Pachyrrhizus erosus* L.) extracts showed LC50 values *ca.* 0.62%w/v (Y =19.42 + 49.13 X, r^2 = 0.98) and 0.30%w/v (Y= 37.34 + 42.13 X, r^2 = 0.95) at 24 and 48 hours, respectively. Moreover, Sugar Apple Seed (*Annona squamosa* Linneaus) extracts exhibited 0.26%w/v (Y = 42.09 + 30.44 X, r^2 = 0.98) and 0.05%w/v (Y = 48.44 + 31.57 X, r^2 = 0.98) LC50 values at 24 and 48 hours after exposure, respectively. Furthermore, the toxicity to Guppies (*Poecilia reticulata*) were determined at 24 hours after exposure. LC50 values were 3.27, 0.11 and 0.16%w/v for Mangosteen Pericarp Extracts, Yam Bean Seed extracts and Sugar Apple Seed extracts, respectively (r^2 =0.99 for all 3 extracts). *In vitro* studies of activities of the detoxification enzymes esterase and Glutathione-S-transferase (GST) in surviving Green Leafhoppers after 24 hours exposure were analyzed. Only Mangosteen Pericarp extracts was characterized as an inhibitor of esterase (*ca.* 0.5 fold inhibition). For GST, enzyme activity increased with increasing concentration for every extracts (ca. 1.25 fold induction).

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Student's signature Thesis Advisor's signature

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EFFECTS OF SOME PLANT EXTRACTS ON TOXICITY AND ACTIVITIES OF ESTERASE AND GLUTATHIONE-S-TRANSFERASE IN THE GREEN LEAFHOPPER (*Nephotettix virescens* (Distant))

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop. It is a principal food in diet of millions of Asian, Africans and Latin Americans. Rice belongs to the grass family of Gramineae or Poaceae and is classified as *Oryza* genus. Rice grows well from the hot through the warm climate. Rice is grown throughout the year if an adequate supply of water is available (Office of Agricultural Economics, 2004).

Although, there are at least 23 species of rice grown in the world, only 2 species of rice, Asian rice (*Oryza sativum* Linn.) and African rice (*Oryza glaberrima* Steud.) are commonly cultivated for local consumption and exportation in the world. Of these two species, *Oryza sativum* Linn.is the best known and commercially grown in Thailand (Office of Agricultural Economics, 2004).

Thai rice has faced many problems such as low quality soils, bad climate, out break of disease and most of all are the insects. There are Green Leafhopper (GLH) *Nephotettix virescens* (Distant) (Homoptera: Cicadellidae), brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), whitebacked planthopper (WBPH) *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae), Asian rice gall midge *Orseolia oryzae* (Wood-Mason) (Diptara: Cecidiomyiidae), and yellow stem borer *Scirpophaga incertulas* (Lepidoptera: Pyralidae) (Vungsilabutr *et al*., 1995).

All of the insect pests mentioned above decrease both quantity and quality of rice product.The Green Leafhopper (GLH) is considered one of the most destructive insect pests of rice and damages rice by sucking the plant sap and transmitting virus diseases.Generally, the Green Leafhopper feed on the leaves and upper parts of the plant. Infestation during the early stages of plant growth reduces tiller number, plant height and the general vigor of the crop. Heavy infestation may cause complete drying of the crop (IRRI, 2003).

The Green Leafhopper is commonly controlled by insecticides. The use of insecticide has several drawbacks, as it requires economic inputs insects often develop resistance to the pesticides and accumulation of undesirable residue causes environmental pollution. Thai farmer have been using too many synthetic pesticides, so a lot problems occurred. There are cereal contamination, soil, water and air pollution and most of all pest resistant.

In addition to the troublesome mentioned above, health problems and non target organisms as well as the competitiveness in the world rice markets are the most public concerns in Thailand. The government is trying to minimize the use of such the dangerous synthetic pesticides and introduce other alternatives such as bio-botanical

pesticide as the rice pest control.The crude extracts of insecticidal plants are the main targets.Unlike synthetic pesticides, plant extracts do not possess these problems because they are biodegradable (Visetson *et. al*, 2002).

Researches on plant extracts against agricultural pests have been developing by many scientists. A lot of insecticidal plants have revealed good tendency for insect control namely, neem (*Azadirachta indica* L.) (Siripassarapon, 1995), Chili (*Capsicum frutescans* L.) for the control of *Sitophilus zeamais* Motschulsky (Bullangpoti *et al.*, 2002), derris (*Derris elliptica* L.) for the control of *Plutella xylostella* L. (Visetson and Milne, 2001), lemon grass (*Cymbopogon winterinus*, Jewitti) for the control of *Culex pipien quinquefasciatus* (Thummasarangkon, 2000), nudsage(*Cyperus rotandus* L.) for the control of *Pomacea canaliculata* L.(Ruamthum, 2002). Most of plant extracts have been proven to be effective in the control of many pests in Thailand.

The aims of this research are studying toxicity of some plant extracts (aqueous from Soxhlet's extraction), Mangosteen Pericarp, the Yam Bean Seed and the Sugar Apple Seed against the Green Leafhoppers (*Nephotettix virescens*). Moreover, the mechanisms of detoxification enzyme activities namely, Esterase and Glutathione-S-transferase enzyme activities are trialed using enzyme-substrate assays. Also study the toxicity against fishes are trialed.The results benefits control efficiency of Green Leafhopper in the future which can decrease many problems created by synthesis insecticides.

OBJECTIVES

1. To determine toxicity of the plant extracts, Mangosteen Pericarp, the Yam Bean Seed and the Sugar Apple Seed to the Green Leafhopper.

2. To determine effects on Esterase and Glutathione–S–transferase activity in the Green Leafhopper before and after treatment of some efficient plant extracts; Mangosteen Pericarp, the Yam Bean Seed and the Sugar Apple Seed.

3. To determine the relationship between LC50 and enzyme activity for each plant extracts to reveal proper mechanisms.

4. To determine toxicity of each crude extract from Mangosteen Pericarp, the Yam Bean Seed and the Sugar Apple Seed on non-target organisms such as fishes.

LITERATURE REVIEW

Green Leafhopper

Phylum	:	Arthopoda
Class	:	Insecta
Order	:	Homoptera
Family	:	Cicadellidae
Scientific name	:	*Nephotettix virescens* (Distant)
Common name	:	Green Leafhopper (GLH)

Figure 1 Adult Green Leafhopper (*Nephotettix virescens* (Distant))
Source: IRRI (2003)

1. Characteristics

Green Leafhoppers are common in rain fed and irrigated wetland environment. They are not prevalent in upland rice. Both the nymphs and adults feed on the dorsal surface of the leaf blades rather than the ventral surface. They prefer to feed on lateral leaves rather than the leaf sheaths and the middle leaves. They also prefer rice plants that have been fertilized with large amount of nitrogen (IRRI, 2003).

The adult Green Leafhopper is slender and green. Its head is rounded. Its vertex is with or without an anterior black band and a sub marginal black band extending beyond the ocelli to the inner margins of the eyes. The face is green. Its pronotum is smooth with or without a black anterior margin. A pair of black spots is either present on the forewings. The insect is 4.2 – 4.3 mm. Neonate nymph measures 0.9 mm. It is transparent, white and shiny (IRRI, 2003).

As it matures, it turns yellowish to green with black markings on the head, thorax, and abdomen. A mature nymph is 3mm long. The shape of the nymph green leafhopper is similar to that of the adult except that the nymph is smaller and

wingless. As the insect matures, blackish markings on the abdomen become more prominent as well as the blackish band on the last abdominal segment (IRRI, 2003).

2. The Life cycle of Green Leafhopper

Green Leafhoppers possess five instars.The nymphal duration of the Green Leafhopper ranged from 16 – 18 days at temperatures of 25 – 30 ° C. The life cycle of the Green Leafhopper is about 23 – 30 days (IRRI, 2003).

The GLH begins laying eggs 6 – 9 days after adult emergence. The female GLH can lay up to 400 eggs during its life time. The average nymphal stage takes 14 days. Upon hatching, the lengths of the nymphs were about 0.6mm. The nymphs molt 5 times before becoming adult and usually stay on the lower parts of the rice plants. Most adults emerged early in the morning. The adult stage can survive up to 20 days. The GLH multiplied more rapidly on older plant than on young seedlings (IRRI, 2003).

Eggs of Green Leafhopper are white and elongate or cigar-shaped. Individual eggs are arranged neatly and lie parallel to each other in each egg batch. A single egg measures 0.9 – 0.8mm. Upon maturation, the egg turns brownish and develops red eyes. The major host of the Green Leafhopper is the rice plant. It also feeds on a number of grasses (IRRI, 2003).

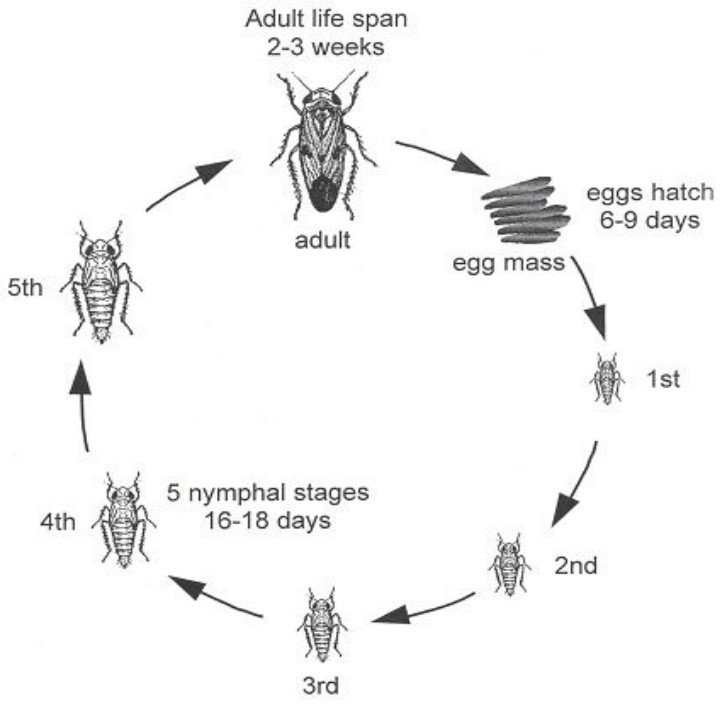


Figure 2 Life cycle of Green Leafhopper *(Nephotettix virescens* (Distant))
Source: Department of Agriculture Extension (2004)

3. Mechanism of damage

Green Leafhopper *(Nephotettix virescens* (Distant)) causes damage to the rice plant. Both nymphs and adults of the Green Leafhopper feed on rice by sucking the plant sap and plugging the vascular bundles, xylem and phloem tissues with its stylet sheaths during feeding. By direct feeding the GLH can cause yellowing and drying of the susceptible rice plants. After heavy feeding the plant becomes stunted and produces the poor yield. The Green Leafhoppers are most numerous during the tilling and panicle initiation stages of the crop. Seedling and booting stages are also susceptible. They migrate to the field soon after seedlings have emerged. Moreover they can cause indirect damage through transmission of virus diseases to the crop because of the virus diseases that they transmit (IRRI, 2003).

Green Leafhoppers are important pests because they are the vectors of virus such as tungro, yellow dwarf, yellow-orange leaf, transitory yellowing and dwarf. Tungro virus disease is transmitted by leafhoppers wherein the most efficient vector is the Green Leafhopper, *Nephotettix virescens* (Distant). The disease complex is associated with rice tungro baciliform virus (RTBV) and rice tungro spherical virus (RTSV). RTBV cannot be transmitted by leafhoppers unless RTSV is present. Insects could acquire the virus from any part of the infected plant. After acquiring the virus, the vector can immediately transmit to the plants. RTBV particles are rod shaped and 100 – 300 nm. in length and 30 – 35 nm. in width. The insect acquires the virus by feeding on the plant for a short time in an 8 hour acquisition access period (minimum of 30 minutes). It can transmit the virus immediately after feeding. Either or both viruses can be transmitted during a 1 hour inoculation access period (minimum of 7 minutes). The virus does not remain in the vector's body. After feeding on a diseased plant, the insect can transmit the virus for about 5 days and the longest is about a week. The insect becomes re – infective after re-acquisition feeding (IRRI, 2003).

Figure 3 Rice tungro baciliform virus (RTBV)
Source: IRRI (2003)

Tungro is one of the most damaging and destructive diseases of rice in countries in Southeast Asia. Outbreaks of the disease can affect thousands of hectares in many countries. Plant infected with the virus at the early crop growth stage could have as high as 100% yield loss in severe cases. The damage caused by the disease is depends on the variety used the plant stage when infection occurs, the virus particles,

and the environmental conditions. Tungro disease has signs and symptoms partially filled grains such as discoloration begins from leaf tip and extends down to the blade or the lower leaf portion. The infected leaves may also show mottled or striped appearance stunting and reduced tilling. It cause be delayed flowering, which may delay maturity panicles small and not completely exerted, most panicles sterile or partially filled grains and covered with dark brown blotches. Tungro incidence depends on the availability of the virus sources, population and composition of the vector, age and susceptibility of host plants and synchronization of the three factors mentioned (IRRI, 2003).

Figure 4 Leaf symptoms of Tungro caused by Green Leafhopper
Source: Entomological and Zoological Division (2002)

4. Management principles

There are three limitations of effective tungro management 1) the absence of symptoms at early growth stage of the disease development 2) lack of resistant varieties to the tungro viruses and 3) vector adaptation on GLH-resistant variety. Planting of resistant varieties against tungro virus disease is the most economical means of managing the disease. Among the cultural management practices, adjusting the date of planting is recommended. Likewise, observing a fallow period of at least a month to eliminate hosts and viruses and vectors of the disease and plowing and harrowing the field to destroy stubbles right after harvest in order to eradicate other tungro hosts are also advisable (IRRI, 2003).

Currently, there are many methods which developed by scientists for control Green Leafhopper such as rice varieties and synthetic insecticides.Another method by using control agents, which are available for the insect. For example, small wasps parasitize the eggs. Mire bugs also feed on them. Strepsipterans, small wasps, parasitize the eggs, pipunculid flies, and nematodes parasitize both the nymphs and adults. They are also attacked by aquatic veliid bugs, nabid bugs, emphid flies, damselflies, dragonflies, and spiders. A fungal pathogen infects both the nymphs and adults of the Green Leafhopper. In areas without tungro source, insecticides are not needed. Spraying of insecticide should be avoided because it is often unable to prevent or reduce tungro infections (IRRI, 2003).

Mangosteen

Family	:	GUTTIFERAE.
Genus	:	Garcina.
Scientific name	:	*Garcina mangostana* L.
Common name	:	Mang kut (Thailand), Mangosteen.

A B

Figure 5 A: Fruits of Mangosteen B: Mangosteen tree
Source: Wannasiri (1998)

1. Description

Mangosteen (*Garcina mangostana* L.) is classified in family GUTTIFERAE, genus Garcina and Thai people called mangosteen as Mang kut (Smitinand, 2001). Mangosteen is one of praised tropical fruits and certainly esteemed fruit in family GUTTIFERAE. It is perhaps most delicate of all Thai fruits. Thai reveres mangosteen as the queen of fruits. This kind of fruit is usually eaten as dessert; furthermore, they can be made processed products such as juice, jam, jelly and sugar. The seed are sometimes eaten alone after boiling or roasting (Anonymous, 2003a).

Mangosteen takes the form of dark – purple sphere with thick, waxy sepals surrounding a short length of stem on top (Figure 5). The bottom of these bears a flat, raised, asterisk-like ornament. The number of the points on this little star indicates the number of the sections in the fruit inside (Anonymous, 2003a).

Mangosteen is a densely foliaged broad-leaved evergreen with a dense pyramidal crown and mature trees reach up to 10 – 25 meters. It is slow growing tree with shiny dark green leaves (Partricia, 1998). The glabrous leaves up to15 – 25 cm. long (Boonyapapat and Choakchaijalernporn, 2000). Mangosteen tree has dark – brown or nearly black, flaked bark, the inner bark containing much yellow, gummy, bitter latex and new leaves are rosy.

The flowers are borne singly or in pairs at the end or the terminal raceme on the outer canopy. Although perfects are borne, they are effectively unisexual because pollen is not produced at any stage. The flowers are large (5 – 6 cm.diameters) and

creamy yellow in color. The flowers generally open in the afternoon and remain open for up to two days. Staminode or pollen is not produced at any stage; moreover, seed development is described as apodictic (without fertilization) and the fruits develop pathenocarpically (effectively vegetative). There are four sepals and four ovate, thick, fleshy petals, green with red spots on the outside and yellowish-red inside (Noichinda, 1992).

Mangosteen is a kind of berries that have diameter 4-5cm.The fruits pericarp change from a green to red, then a purple color at maturity; however, if fruit development occurs during the dry season and the trees suffer from water stress the fruits may change the brown color instead (Patricia, 1998).

Fruit development and enlargement proceed at a constant rate, taking between 100 – 160 days from flowering to fruit maturity is depend on the temperature. Fruit development is obviously slower during the cooler months of the year during the November to January period. In generally, Thai Mangosteen will be harvested during on May to July (Eastern) or August to October (Southern) (Wanichkul and Kosiyajinda, 1984).

Compared with other tropical fruits, mangosteen has a comparative small edible portion. Surrounding the edible portion or aril is the pericarp, which is thick and tough, about 5 – 7 mm. thick. If damaged, this smooth and hard pericarp exudes latex. The aril or flesh, which makes up 30% of the total fruit weight is a pearly white color, slightly translucent and consists of 4 to 8 segments. One or two of these segments usually contain seed. The seed are brown in color and almond shaped (20 to25mm long). The flesh is sweet, delicate and mellow (Patricia, 1998).

2. Soil and Climate

Mangosteen grows well on wide range of soils provided it is well drained. It prefers clay or sandy loam soil with high organic matter content and pH of 5.0 – 6.5. Mangosteen thrives well in the tropical rainforests, which receives 200 to 250cm of rainfall. It grows best in warm, humid and non-seasonal rainfall areas.

3. Propagation

Mangosteen has propagated by seed but the present has success in Graft method.

4. Pest and Diseases

Few pests have been reported. A small ant, Myrnelachista ramulorum, in Puerto Rico, colonizes the tree, tunnels into the trunk and branches and damages the new growth. Mites sometimes deface the fruits with small bites and scratches. In Asia reported that the fully ripe fruits are attacked by monkeys, bats and rat.

Pellicularia Koleroga, the fungus that found in Puerto Rico is often seen on branch, foliage and fruits of trees in the shaded, humid areas. In Malaya, the fungus, *Zingnoella garcineae* causes canker tuberous growths on the branches that make a dying-back of foliage, branches and eventually the entire tree. Breakdown in storage is caused from the fungi *Diplodia gossypina, Pestalotia* sp., *Phumopsis* sp., *Gloeosporium* sp. and *Rhizopus nigricans* (Morton, 1999).

5. Food Uses

Mangosteens are usually eaten as dessert; furthermore, they can be made processed products such as juice, jam, jelly and sugar. The seed are sometimes eaten alone after boiling or roasting. The rind is rich in pectin. After treatment with 6% sodium chloride to eliminate astringency, the rind is made into a purplish jelly.

6. Medical Uses

The sliced and dried rind is powdered and administered to overcome dysentery. Made into an ointment, it is applied on eczema and other skin disorders.The rind decoction is taken to relief diarrhea and cystitis, gonorrhea and gleets and is applied externally as an astringent lotion. The rind steeped in water overnight given as a remedy for chronic diarrhea in adults and children. In Philippines, decoct the leaves and bark as a febrifuge and to treat thrush, diarrhea, dysentery and urinary disorder. In Malaya, a fusion of the leaves, combined with unripe banana and a little benzoin is applied to the wound of circumcision. A root decoction is taken to regulate menstruation. Amibiasine, a bark extracts has been marketed for the treatment of amoebic mystery.

Chairungsrilerd et al. (1996) described the fruit hull (pericarp) of mangosteen is used as an anti – inflammatory agent, astringent and in the treatment of diarrhea. They reported the yellowish excretion of the fruit hull, alpha mangostin, gamma mangostin and mangostanol showed an inhibitory effect on phosphodiesterase.

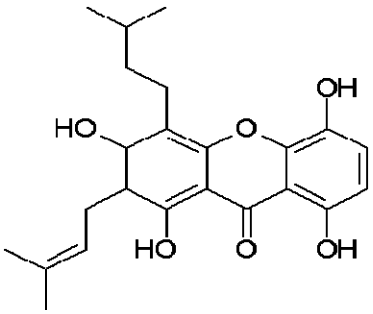
Moreover, extracts of the pericarp of the ripe fruits have been show to have immunomodulating (Chanarat et al., 1997), anti – bacteria (Inuma et al., 1996) and anti – cancer (Liou et al., 1993).

7. Other Uses

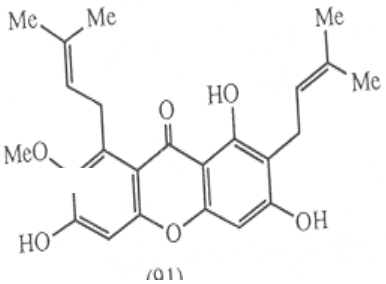
Twigs of mangosteen are used as chew sticks in Ghana. The fruit contains 7 to 14% catechin tannin and rosin, and is used for tanning leather in China. It also yields a black dye.

8. Toxicity

The rind of partially ripe fruits yields a polyhydroxy – xanthone derivative termed mangostin, also β – mangostin. The fully fruits contains xanthones, gartanin, 8 – dioxygartanin and normangostin. Mangostin – 6 – di – O – glucoside, a derivative of mangostin (Figure 6), is a central nervous system depressant and causes a rise in blood pressure (Morton, 1999).



A: Gartanin (class xanthone)



B: Mangostin

Figure 6 Some active principles in mangosteen pericarp
Source: National Cancer Institute (2003)

Asai et al. (1995) described chemical constituents in pericarp of mangosteen consist xanthone derivative as alpha mangostin, beta mangostin, gamma mangostin, gartanin, garcinone E, 1,5-dihydrozy-2-(3-methylbut-2-enyl)-3-methoxy-,1-7-di hydroxy -2-(3-methylbut-2-enyl)-3-methoxyxanthone and mangostinone.

Yam bean

Family	:	FABACEAE (Leguminosae)
Genus	:	*Pachyrrhizus*
Scientific name	:	*Pachyrrhizus erosus* (L) Urban.
Common name	:	Yam Bean, Jicama

Figure 7 *Pachyrhizus erosus*
Source: Jarinee (2004)

1. Description

The yam bean is native to the American tropics, but it was carried by man years ago to Asia and the Pacific islands. Now it is commonly cultivated in these places, and is also found growing wild in forested areas. This plant grows in wet areas of tropical regions (Sorensen, 1996).

Commercially, it is produced to some extent in such tropical regions as Puerto Rico, Hawaii, and Mexico, and in warm regions of the south-western United States. Centuries ago, it was carried to Asia with the Spanish galleons that annually voyaged from Mexico to the Philippines and it became a favorite crop of Chinese market gardeners throughout Asia (Sorensen, 1996).

The yam bean is a climbing plant of the bean family, with alternate, three-parted leaves and a turnip like root. The bluish or purplish flowers are pea like in shape. The plants are often so rampant that they cover the vegetation upon which they are growing. Yam bean is a coarse, hairy, herbaceous, legume that grows rapidly and can reach 5 – 6 m in length. The large tubers 600 – 2000 g, are edible and also use as livestock feed. The young pods are consumed and the hay used as feed. The plant contains rotenone that has insecticidal properties (Sorensen, 1996).

Roots: The brownish tubers are either sugar – beet shaped or long and slender, weighing up to 2kg. They have a thick, tough skin that pericarp off easily, exposing the white flesh beneath, which is crisp and succulent like that of an apple, with a sweet, pleasant flavor.The rhizobia in their root nodules make nitrogenous compounds available to the plants and even enrich the soil in which they are grown.

Leaves: It has compound leaves with pointed edges. It is great variation in the outline of the leaflets from dentate to palmate.

Flowers: It has many beautiful white or violet flowers. It does not have hairs on the petals. It has 4 – 11 bisexual flowers per lateral inflorescence axis i.e. by complex racemes and an inflorescence length of 8 – 45 cm.

Fruits: It has green lima bean-shaped pods. The pods are borne in clusters of around six pods per cluster, and may reach 6 – 13 cm. in length and 8 – 17 mm. in width. Color from pale is brown to olive-green – brown.

Seed: Seed ranges from olive-green to brown or reddish brown, the shape is flat, and square to round (Sorensen, 1996).

A

B

C

Figure 8 A: Leaf of Yam bean B: Flower of Yam Bean C: Seed of yam bean
Source: Srikong (2006)

2. Soil and Climate

In areas with an annual dry season, the habitat is along deciduous forest edges and in scrub vegetation. In general the plant is adapted to subtropical to tropical climates.

As with other root crops, the soil should be light and well drained so as not to restrict tuber growth or encourage fungal rot. Sandy loams or alluvial soils are best, especially when the crop is irrigated, but also deep clay soils are reported suitable for cultivation. It thrives in fertile soils (Sorensen, 1996).

3. Propagation

It is among the most vigorous-growing legumes.When the plants are propagated from seed, only 5-9 warm months are needed to produce large tubers. In warmer parts of Mexico where the plant is propagated by small tubers on light, rich soil, mature tubers are commonly harvested after only 3 months. Within its natural distribution area flowering plants have been seen in all months except January, with the majority (90%) from July to October.The latest flowering occurs in the southern parts of the distribution area, at the end of the rainy season. Mature legumes have been recorded from August to February.

The flowering in the three cultivars examined commenced 58 – 68 days after sowing, and lasted 92 – 103 days. The stigma was found to become receptive at 12 hours before and to last for 18 hours after opening of the flower. The anthers generally dehisced 8 – 12 hours before opening of the flower. When the pollen grains were stored at room temperature, their germination percentage is decreased after 4 hours (Sorensen, 1996).

4. Food Uses

The tubers are about the size of a turnip and they are crisp, sweet, and juicy and have a nutty flavor. They are nourishing and at the same time quench the thirst. Eat them raw or boiled. To make flour, slice the raw tubers, let them dry in the sun, and grind into flour that is high in starch and may be used to thicken soup. (Sorensen, 1996)

5. Other Uses

The beneficial effects on the environment, mainly as a result of biological nitrogen fixation, must be considered to outweigh the harmful ones. The positive environmental effects include highly efficient nitrogen fixation and increased vigor among the neighboring plants (especially on poorer soils); good drought tolerance and provision of high protein forage for livestock and other herbivores; effective control of soil erosion. (Sorensen, 1996)

Negative effects include: seed propagation, the crop may easily be spread into the wild vegetation bordering fields cultivated for seed production; rotenone content the poisonous seed may cause problems in livestock foraging on escaped plants

among the wild vegetations; vigorous growth with strong vines that may upset fragile indigenous flora. (Sorensen, 1996)

6. Toxicity

Rotenone is the most potent the chemicals in Yam Bean seed. It is unstable in light and heat and almost all toxicity can be lost after three days during the summer. It is very toxic to fish, one of its main uses by native people over the centuries being to paralyze fish for capture and consumption. Crystalline rotenone has an acute oral LD_{50} of 60, 132 and 3000mg/kg for guinea pigs, rats and rabbits (Matsumura, 1985). Because the toxicity of Yam Bean Seed content of rotenone, it is obvious that the other esters in crude preparations have significant biologic activity.

Acute poisoning in animals is characterized by an initial respiratory stimulation followed by respiratory depression, ataxia, convulsions, and death by respiratory arrest. The anesthetic-like action on nerves appears to be related to the ability of rotenone to block electron transport in mitochondria by inhibiting oxidation linked to NADH $_2$, this resulting in nerve conduction blockade (Corbett, 1974). The estimated fatal oral dose for a 70kg man is of the order of 10 to 100g.

Rotenone has been used topically for treatment of head lice and other ectoparasites but the dust is highly irritating to the eyes (conjunctivitis), the skin (dermatitis) and to the upper respiratory tract (rhinitis) and throat (pharyngitis).

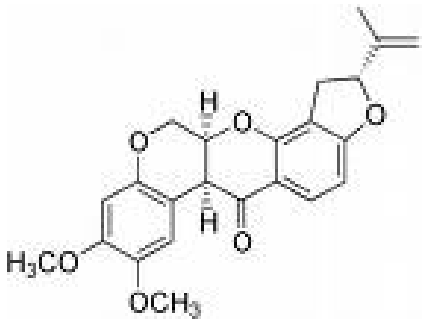


Figure 9 Structure of Rotenone
Source: Corbett (1974)

Sugar apple

Family	:	ANNONACEAE
Genus	:	Annona
Scientific name	:	*Annona squamosa* Linneaus.
Common name	:	Sugar apple, custard apple

Figure 10 Sugar apple *(Annona squamosa* Linneaus.)

1. Description

The original home of the sugar apple is unknown. It is commonly cultivated in tropical South America, not often in Central America, very frequently in southern Mexico, the West Indies, Bahamas and Bermuda, and occasionally in southern Florida. In Jamaica, Puerto Rico, Barbados and in dry regions of North Queensland, Australia it has escaped from cultivation and is found wild in pastures, forests and along roadsides(Morton, 1987).

The Spaniards probably carried seed from the New World to the Philippines and the Portuguese are assumed to have introduced the sugar apple to southern India before 1590. It was growing in Indonesia early in the 17th century and has been widely adopted in southern China, Queensland, Australia, Polynesia, Hawaii, tropical Africa, Egypt and the lowlands of Palestine. Cultivation is most extensive in India where the tree is also very common as an escape and the fruit exceedingly popular and abundant in markets. The sugar apple is one of the most important fruits in the interior of Brazil (Morton, 1987).

The sugar apple tree ranges from 3 – 6 m. in height with open crown of irregular branches and somewhat zigzag twigs. Deciduous leaves, alternately arranged on short hairy petioles are lance late or oblong; blunt tipped 5 – 15 cm. long and 2 – 5 cm. wide dull green on the upper side, pale with a bloom below and aromatic when crushed. Along the branch tips opposite the leaves, the fragrant flowers are borne singly or in groups. Drooping stalks and 3 fleshy outer petals is yellow-green on the outside and pale-yellow inside with a purple or dark red spot at the base.The compound fruit is nearly round, ovoid its thick rind composed of knobby segments, pale green, gray-green in one form, dull, deep – pink externally (nearly always with a bloom) separating when the fruit is ripe and revealing the mass of conically segmented, creamy white, glistening, delightfully fragrant, juicy, sweet and delicious

flesh. Many of the segments enclose a single oblong-cylindrical, black or dark brown seed about 1/2 in long. There may be a total of 20 to 38 or perhaps more, seed in the average fruit, however, bear seedless fruits (Morton, 1987).

Figure 11 The Sugar Apple Seed *(Annona.squamosa* Linneaus.)

2. Soil and Climate

The sugar apple tree requires a tropical climate.Generally, it does best in dry areas and it has high drought tolerance. During the blooming season, drought interferes with pollination and it is, therefore, concluded that the sugar apple should have high atmospheric humidity but no rain when flowering. In severe droughts, the tree sheds its leaves and the fruit rind hardens and will split with the advent of rain. The sugar apple is not particular as to soil and has performed well on sand, limestone and heavy loam with good drainage.Water logging is intolerable. The tree is shallow. Rooted and doesn't need deep soil (Morton, 1987).

3. Propagation

Sugar Apple Seed have a relatively long life, having kept well for 3 – 4 years. They germinate better a week after removal from the fruit than when perfectly fresh. Germination may take 30 days or more but can be hastened by soaking for 3 days or by scarifying. The percentage of germination is better in un-soaked seed. While the tree is generally grown from seed, vegetative propagation is practiced where the crop is important and early fruiting is a distinct advantage. Seedlings may be budded or grafted when one year old (Morton, 1987).

4. Pests and Diseases

In Florida and the Caribbean, a seed borer (chalcid fly), Bephratelloides cubensis, infests the seed and an associated fungus mummifies the partly grown fruits on the tree. This has discouraged many from growing the sugar apple, though in the past it was a fairly common dooryard fruit tree. Similar damage is caused by B. maculicollis in Colombia, Venezuela and Surinam, by B. ruficollis in Panama, and B. paraguayensis in Paraguay. The soft scale, Philephedra sp., attacks leaves and twigs and deposits honeydew on which sooty mold develops. Ambrosia beetles lay eggs on young stems and the larvae induce dieback during the winter. The mealy bug is the

main pest in Queensland, Australia, but is easily controlled. The green tree ant is a nuisance because of the nests it makes in the tree. Bird and animal predators force Indian growers to cover the tree with netting or pick the fruits prematurely and ripen them in straw. Serious leaf blight in India is caused by the fungus Colletotrichum annonicola. In1978 a new fruit rot of sugar apple was observed in India, beginning with discoloration at one end which turns brown or black in 4 or 5 days, and 2 or 3 days later the entire fruit starts to rot. Later, the fruit is covered with gray – black mycelium and spherical bodies (Morton, 1987).

5. Food Uses

The ripe sugar apple is usually broken open and the flesh segments enjoyed while the hard seed are separated in the mouth and spat out. It is so luscious that it is well worth the trouble.

6. Medical Uses

In India the crushed leaves are sniffed to overcome hysteria and fainting spells, they are also applied on ulcers and wounds. A leaf decoction is taken in cases of dysentery. Throughout tropical America, a decoction of the leaves alone or with those of other plants is imbibed either as an emmenagogue, febrifuge, tonic, cold remedy, digestive, or to clarify the urine. The leaf decoction is also employed in baths to alleviate rheumatic pain. The green fruit is very astringent, is employed against diarrhea in El Salvador. The ripe fruits of this plant are applied to malignant tumors to hasten suppuration. The dried unripe fruit powder is used to destroy vermin. In India, the crushed ripe fruit mixed with salt, is applied on tumors. The bark and roots are both highly astringent. The bark decoction is given as a tonic and to halt diarrhea. The root, because of its strong purgative action is administered as a drastic treatment for dysentery and other ailments (Kirtikar and Basu, 1993).

7. Other Uses

The seed kernels contain 14 – 49% of whitish or yellowish, non-drying oil with saponification. It has been proposed as a substitute for peanut oil in the manufacture of soap and can be detoxified by an alkali treatment. The leaves yield an excellent oil rich in terpenes and sesquiterpenes, mainly B-caryophyllene, which finds limited use in perfumes, giving a woody spicy accent (Morton, 1987).

8. Toxicity

The seed are acrid and poisonous. Bark, leaves and seed contain the alkaloid, anonaine. Six other aporphine alkaloids have been isolated from the leaves and stems: corydine, roemerine, norcorydine, norisocarydine, isocorydine and glaucine. Aporphine, norlaureline and dienone may be present also. Powdered seed, also pounded dried fruits serve as fish poison and insecticides. A paste of the seed powder

has been applied to the head to kill lice but must be kept away from the eyes as it is highly irritated and can cause blindness. If applied to the uterus, it induces abortion. Heat – extractsed oil from the seed has been employed against agricultural pests. It is also used for destroying worms in the wounds of cattle (Kirtikar and Basu, 1993). The compound annotemoyin-1 has been isolated from the chloroform extracts of the seed of *Annona squamosa* and shows significant cytotoxic anti-tumor, anti-bacterial and anti-fungal activity (Parvin, 2002).

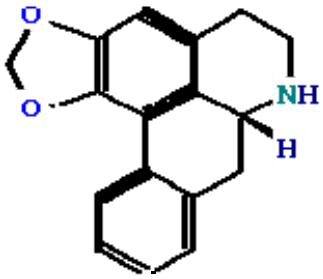


Figure 12 Structure of anonaine
Source: IUPAC (2002)

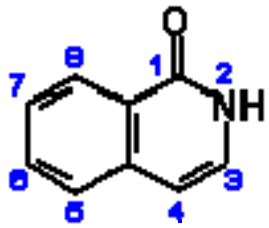


Figure 13 Isoquinolone alkaloid
Source: IUPAC (2002)

Detoxification Mechanisms

Xenobiotics are metabolized by a variety of reactions intended to speed up their excretion and reduce their activity. However, detoxification does not always occur and some chemicals will actually show increase physiological activity or toxicity as a result of metabolic change undergone after absorption (Pascoe, 1983). The metabolic transformation which is divided by toxicologist has two types of reactions known as metabolic transformation (Phase I) and conjugation (Phase II reaction).

Many researchers reported that the major sites of detoxification enzymes in insects these enzymes are accumulated in adipose cells.The detoxification enzymes work on various substrates to reduce poisons.Furthermore, the detoxification enzymes can regulate and induce when xenobiotics are coming.When insects are exposed to chemicals, they may change behavior known as behavior avoidance to decrease exposure to chemicals. If insects do not die, they collect substance in adipose tissue and any tissue. Insects have various behavior avoidance and various detoxification

systems.Insects use detoxification enzymes to decrease poisons because it is very fast mechanism (Visetson, 2001).

1. Metabolic Transformation (Phase I Reactions)

This reaction includes oxidation, reduction and hydrolysis whereby insoluble foreign compounds, which have passed through SER membrane, are transformed into more soluble compounds by the introduction of new functional groups. Metabolic transformation may result in the formation of compounds with variety of properties depending upon whether activity has been decreased or increased. Although the activation of accidentally absorbed compounds may present a hazard, the process also has useful applications such as in the development of pesticides and of pro-drugs, which may be converted into active pharmacological products by several metabolic transformation mechanisms. Depending on their water solubility, the products of metabolic transformation may excrete directly or may undergo conjugation.

2. Conjugation (Phase II Reactions)

During conjugation, functional groups on the compound such as carboxyl (COOH), amino (NH2), hydroxyl (OH) and sulphydryl (SH) react with compounds such as glucuronic acid, amino acids, sugars etc. to decrease toxic, more polar soluble derivatives which can be rapidly excreted. Their reduced lipid solubility decreases the capacity to diffuse across membranes and consequently their activity within the body is also reduced. Xenobiotics may undergo metabolic transformation and conjugation, or only one of these reactions. For example, metabolic transformation leads to a product sufficiently water soluble to be excreted then conjugation may not be necessary. On the other hand, if xenobiotics compound entering the body already possesses group that engage in conjugation, then metabolic transformation may not be needed.

3. Other Detoxification Mechanisms

Some poisons appear to be detoxicated by linking to a larger molecule, typically a protein, to produce a less toxic complex which can be stored in various body organs. For example metalothionein, a low molecule weight, cysteine-rich metal-binding protein which is widely produced in the animal in response to heavy metals such as cadmium, zinc, chromium and copper and is found in high concentration in the liver and kidney. In some case, xenobiotics not happened these metabolic reactions, particularly those of high lipid solubility, could remain within the body for extended periods after absorption, a lifetime (Pascoe, 1983).

4. Detoxification Enzymes

4.1 Esterase

Esterase is one of detoxification enzymes in phase I. There are two types for classified Esterase. A-type Esterase is not inhibited by organophosphates insecticides

and includes arylesterases. B-type Esterase is inhibited by several organophosphate insecticides due to irreversible phosphorylation of the active serine site (Dauterman, 1985). The B-type Esterase includes carboxylesterases and Cholinesterases.

A-type can be classified into 2 groups depend on activated by Ca^{2+} or Mn^{2+} and CO^{2+}. A-type esterase hydrolyzed paranitrophenylphosphate faster than paranitrophenylbutylate (Visetson, 1991).

B-type such as carboxylesterase and acetylcholinesterase are most studied from many scientists. B-type hydrolyzed paranitrophentlphosphate slower than paranitrophenylbutylate.Organophosphates usually process high levels of these enzymes (Mackness, 1983). Cholinesterases are capable of splitting acetylcholine to yield acetate and choline. The properties of cholinesterase in insects are similar to mammals. It is therefore called either acetylcholinesterase or cholinesterase. These enzymes are very sensitive to organophosphate inhibition (Visetson, 1991).

In insect, Esterase is found in cytosol, microsome as well as mitochondria and nuclei. There are many report shows esterase play a significant role in the metabolism in organophosphate, pyrethroid and chitin inhibitor.

Zhu and Brindley (1990) showed the highest specific activity was found in the mitochondrial fraction followed by the microsomal fraction; the activities in nuclei, cell debris and in the soluble fraction were relative low. Visetson (1991) said multiple forms of esterase are common among insect species.

The most common method for study multiple forms of insect esterase are by electrophoretic separation and determination of enzyme activity with color forming product. By using ion exchange chromatography, (Kao et al., 1985), found multiple forms of four types of esterases. They presented the molecular weights of being 220 kDa. Because all forms of esterase catalyzed hydrolysis reaction, therefore the uses of simple substrates are not likely to reveal differences in the level of each enzyme activity. Organophosphorus, carbamates, chelators and sulhydryl reagents have been reported to inhibit the activity of B type esterase. The mechanism of esterase of transforming the ester bond of xenobiotics to acid and alcohol is shown in Figure 14.

This research uses pNPA (paranitrophenyl acetate) assay that be modified from Mackness et al. (1983), Visetson (1991, 2001) and Visetson et al. (2001, 2002). These reactions show yellow color of Paranitrophenol generated that was detected by spectrophotometer at 400 nanometers.

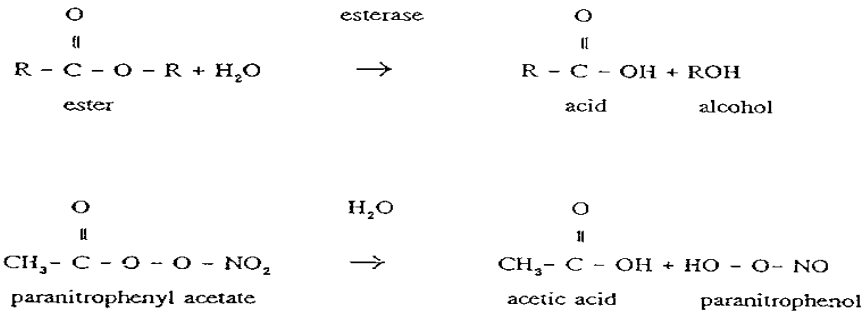


Figure 14 Mechanism of esterase
Source: Visetson (1991).

4.2 Glutathione–S–transferase (GST)

This enzyme system is an important detoxification enzyme in phase II conjugation of relative metabolite formed by microsomal oxidation, as phase I metabolic transformation with compounds. It is soluble, which catalyze the conjugation of reduced Glutathione with various compounds possessing an electrophilic center, include insecticides. Glutathione – S – transferase (GST) is found in mammals, insects, protozoa, algae, fungus and bacteria (Jakoby, 1978). Structure of GST consists of three amino acid, glutamic acid, cysteine and glycine. (Figure15). They metabolized by several steps and/or excreted to form mercapturic acids for increase solubility in water. At the results, compounds may be dealkylated, dehalogenated or cyanide may be released from organic thiocyanates. Glutathione – S – transferase is an important detoxification enzyme in phase II conjugation of relative metabolite formed by microsomal oxidation, as phase I metabolic transformation with compounds. The high levels of Glutathione – S – transferase activities are important in resistance to organophosphate and organochlorine (Motoyama and Dauterman, 1980). Since, it is one of important phase II detoxification enzymes, it is found in cytosol, membrane and liver microsomes. Visetson (1991) reported GST can be inhibited by DEM and Saligenin cyclic phosphate.

This research detects Glutathione – S – transferase levels by CDNB assay, which is modified from method of Visetson (1991, 2001) and Visetson et al. (2001, 2002). These data is recorded by spectrophotometer at 340 nanometers.

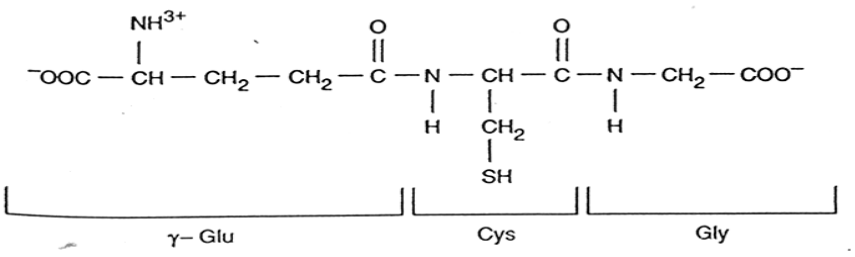


Figure 15 Structure of Glutathione – S – transferase
Source: Visetson (1991)

MATERIALS AND METHODS

Materials

1. The adult of Green Leafhopper *(Nephotettix virescens* (Distant)) was received from the Pathumthani Rice Research Center, Ministry of Agriculture and Cooperative, Pathumthani, Thailand.

2. Seed of rice (susceptible resistance rice), RD7 was gifted from the Pathumthani Rice Research Center, Ministry of Agriculture and Cooperative, Pathumthani, Thailand.

3. Sources of plant materials

Mangosteen Pericarp (*Garcina mangostana* L.) and seed of sugar apple *(Annona squamosa* Linneaus.) were collected from single source during the month of May, 2005 from Chantaburi, ca. 300 kms east of Bangkok, Thailand. Seed of Yam bean (*Pachyrrhizus erosus* (L.)) were collected from single source during the month of April, 2005 from Nakhonsawan, ca.200 kms north of Bangkok, Thailand.

4. Guppies (*Poecilia reticulata*) were purchased from Somboon Farm, Nakhonpratom, 100 kms west of Bangkok, Thailand.

5. Some potential equipment

- Beaker
- Cuvette
- Cylinder
- Dropper
- Flask
- Foam box
- Freeze dry (Heto-FD3)
- Gauze
- Glass rod
- Hot Air Oven (Memmert-600)
- Label paper
- Measurement engine (Sartorious-BP3105)
- Micropipette (NICHIRYO Model 5000)
- Mixer (Buchi Model 5000)
- 1.5 ml Microtube
- Plastic boxes
- pH meter (SartoriousPP-25)
- 1,5 ml Pipette
- Pipette tip
- Plate
- Refrigerator (4°C)

- 1.5 ml polystylene cuvette (Bio-Rad)
- Refrigerate high speed centrifuge (Hettich- Universal 16R)
- Rotary Evaporator (Buchi B-850)
- Round flask
- Spectrophotometer with Kinlab program
- Soxhlet extractsor
- Small Funnel
- Thimble
- 500 ml vacuum flask
- Stereomicroscopy (Olympus)
- Tank for fish experiment (30 X 30 X 30 cm)
- Cage for Green Leafhopper experiment, 25°, 70%RH day: night (16:8)

6. Reagents for *in vitro* enzyme assays

- Absolute Ethanol
- 1- Chloro -2, 4- dinitrobenzene (CDNB) (Sigma)
- Coomassie Brilliant Blue R (Sigma)
- Ethylene diamine tetra acetic acid (EDTA) (Fluka)
- Glutathione reduced form (Sigma)
- Paranitrophenyl acetate (pNPA) (Sigma)
- Polyvinylpolypyrolidone (PVPP) (Sigma)
- distilled water
- Sodium hydroxide (NaOH)
- Hydrogen chloride (HCl)

Methods

1. The Experimental Design

This research uses Completely Randomized Design with 3 replicates and 60 individual adults *Nephotettix virescens* (Distant) per replicate. In this experiment, 5 concentrations from Mangosteen pericarp extracts, the Yam Bean Seed extracts, the Sugar Apple Seed extract and distilled water was served as control. All concentrations were set as followed.

The Mangosteen Pericarp Extracts (Extracts A)

Treatment 1A	4% w/v Extracts A in distilled water
Treatment 2A	6% w/v Extracts A in distilled water
Treatment 3A	8% w/v Extracts A in distilled water
Treatment 4A	10% w/v Extracts A in distilled water
Treatment 5A	Distilled water (Control A)

The Yam Bean Seed Extracts (Extracts B)

Treatment 1B	0.1% w/v Extracts B in distilled water
Treatment 2B	0.5% w/v Extracts B in distilled water
Treatment 3B	1.0% w/v Extracts B in distilled water
Treatment 4B	1.5% w/v Extracts B in distilled water
Treatment 5B	Distilled water (Control B)

The Sugar Apple Seed Extracts (Extracts C)

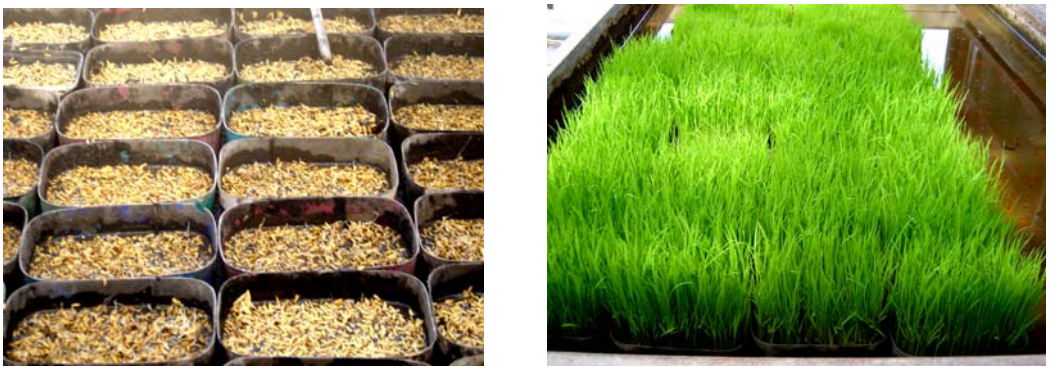
Treatment 1C	0.1% w/v Extracts C in distilled water
Treatment 2C	0.5% w/v Extracts C in distilled water
Treatment 3C	1.0% w/v Extracts C in distilled water
Treatment 4C	1.5% w/v Extracts C in distilled water
Treatment 5C	Distilled water (Control C)

The differences of mean mortalities and detoxification enzyme from each treatment were compared. Data was determined to be normally distributed and variances were homogeneous. So analysis of variance was used with SPSS. (Statistical Package for the Social Sciences) and IRRISTAT (2001 version).The LC50 and enzyme activity analysis were determined using computer program namely Sigma plot 2000 program and probit analysis.

2. Mass rearing of *Nephotettix virescens* (Distant)

The adults *Nephotettix virescens* (Distant) were received from the Pathum Thani Rice Research Center, Ministry of Agriculture and Cooperative, Pathumthani province, Thailand. Two hundred adults of Green Leafhopper in the specific cage were reared under controlled temperature at 30 ± 3°C. They were kept in 5 cages (24 x 24 x 24 inch) in Department of Zoology, Faculty of Science Kasetsart University

(Bang Khen) and day/night period was adjusted about 16:8 hours (Figure 18). The rice for *Nephotettix virescens* was received from Pathum Thani Rice Research Center.The RD7, susceptible rice varieties to *Nephotettix virescens* (Distant) was planted in the plastic boxes with the size10 x 15x 6 and 4 cm thick of soil. When the rice seedling was about 7-10 days old (Figure 16) were placed in the cage. Adults *Nephotettix virescens* were released in the cage with rice seedling boxes and then they lay eggs for 3 days. The rice seedling was changed when the leaf turn yellow and dry.



A B

Figure16 RD7 Rice for massing *Nephotettix virescens* (Distant)

Figure17 *Nephotettix virescens* (Distant) on Rice Plant

Figure18 Cage for mass *Nephotettix virescens* (Distant) at Department of Zoology, Faculty of Science, Kasetsart University.

3. Plant Extraction method

The Mangosteen Pericarp (*Garcina mangostana* L.) (5kg) and the Sugar Apple Seeds (5kg) were collected from single source during the month of May, 2005 from Chantaburi, ca. 300 kms east of Bangkok, Thailand. The Yam Bean Seeds (5kg) (*Pachyrrhizus erosus* (L.)) were collected from single source during the month of April, 2005 from Nakhonsawan, ca.200 kms north of Bangkok, Thailand. All of these plants were extractsed by using a method modified from Visetson et al. (2004). The Mangosteen Pericarp, the Yam Bean Seeds and the Sugar Apple Seeds were dried by Hot Air Oven (Memmert-600) at 65°C about 30 minutes and ground by mixer (BUCHI Mixer Model 5000) about 15 minutes. Then bring these powders in Soxhlet extractsor (Figure19). This research uses 95% Ethanol solution as solvent for extract at 75°C for about 8 hours. Then the extracts were evaporated to removed ethanol by the rotary evaporator (BUCHI B-850) (Figure20) and freeze dryer (Heto-FD3) (Figure21) then they were kept at 4°C until experiment commenced.

Figure 19 Soxhlet's Extraction, using 95% ethanol calibrated at 75ºC for 8 hours.

Figure 20 Rotary Evaporator

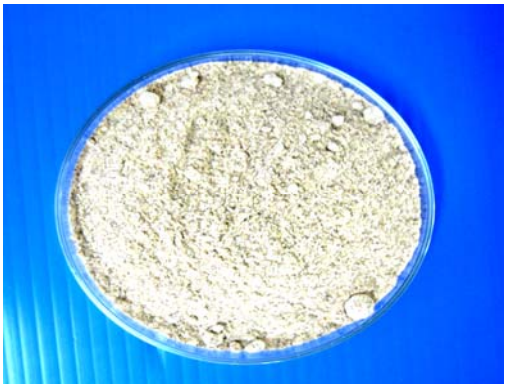
Figure 21 Freeze dryer for final products evaporated at 0°C for 8 hours.

(A)

(B)

Figure22 (A) Dried Mangosteen Pericarp (B) Ground Dried Mangosteen Pericarp

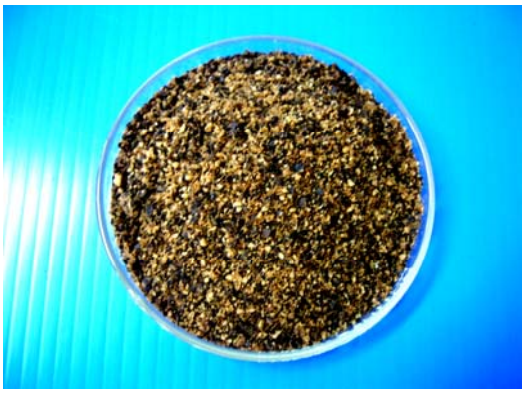
(A)



(B)

Figure23 (A) Dried Yam Bean Seed (B) Ground Dried Yam Bean Seed

(A)



(B)

Figure24 (A) Dried Sugar Apple Seed (B) Ground Dried Sugar Apple Seed

Figure 25 Crude Mangosteen Pericarp Extracts which extracted with 95% ethanol.

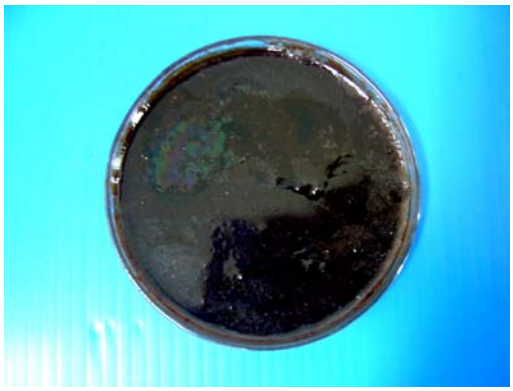


Figure 26 Crude Yam Bean Seed Extracts which extracted with 95% ethanol.

Figure27 Crude Sugar Apple Seed Extracts which extracted with 95% ethanol.

4. Toxicity analysis method

Analysis Method of Mortality

Every treatments, 60 adults Green Leafhopper with 3 replicated by using Completely Randomized Design were used with all extracts.The topical spray method modified from Visetson et al. (1991) was used to evaluate their efficacy in terms of LC50 with each extracts. All extracts were diluted into 4 concentrations with distilled water which contained 100 ml of the sprayer. The adults Green Leafhopper (60 samplings each replicate) were released from the cage, knocked by refrigerator (10 sec.) and put on the glass plate then treated with each concentration by sprayer (5 seconds). After that the Green Leafhopper were transfer to the Rice plant (RD7, 10 days old) and were kept at 28 – 33°C and day/night period was adjusted about 16:8 hours. For the control treatment the Green Leafhopper (60 samplings in each replicate) were sprayed with distilled water. Mortality was recorded for each experiment in 3 replicates at 24 and 48 hours after treatment. The differences of mean mortality and detoxification enzyme from each treatment were analyzed by Analysis of Variance with CRD. Bioassay data was analyzed using regression equation to obtain LC50 derived from simple linear regressions by Sigma plot program. The $LC50_{,}$ statistic analysis and enzyme activity analysis were determined using the SPSS (Statistical Package for the Social Sciences) for windows version 11.0 and Sigma plot 2000 program.

5. Method to determine detoxification mechanism of treated Green Leafhopper

The 60 adults *Nephotettix virescens* (Distant) were treated with the Mangosteen Pericarp extracts, the Yam Bean Seed extracts and the Sugar Apple Seed extracts at concentration at LC50 which was obtained from toxicity analysis. The Green Leafhopper which move lay down in the rice box is inferred as this insect is died. The Mortality was recorded for each experiment in 3 replicates 24 and 48 hours after treatment. If the mortality data in the control treatment appeared, the data would be corrected by Abbott's formula (Abbott, 1925) as shown below. Raw bioassay data was analyzed using regression equation to obtain LC50 by Sigma plot 2000 program.
Abbott's formula:

$$\% \text{ corrected mortality} = \frac{[(\% \text{ treatment mortality} - \% \text{ control mortality}) \times 100]}{(100 - \% \text{ control mortality})}$$

Detoxification Enzyme Activity

Extraction of detoxification enzymes method were modified from Yang et al. (2004) and Visetson and Milne (2001) that uses survival *Nephotettix virescens* (Distant) after 24 hours exposure. The 15 insects was placed on ice and were homogenized on cool mortal in homogenization buffer (0.1 M potassium phosphate

pH 7.5 and 8.0 then adds 1 mM Ethylene diamine tetra acetic acid (EDTA) and 10mM Glutathione reduced form grind the insects with 50 % w/w material Polyvinylpolypyrolidone (PVPP). The homogenate were centrifuged at 4° C, 18,000 rpm for 5 minutes. The supernatant were kept into a clean 1.5 ml microtube, placed on ice and use immediately for carboxylesterase and Glutathione-S-transferase (GST) assays with spectrophotometer as shown in Figure 28-30

Enzyme activity analysis

Enzyme assays for esterase activity was measured using the PNPA assay which modified from Visetson (2001) and Visetson et al. (2003 and 2004). The 3 ml incubation mixture contained 0.1 M potassium phosphate buffer and enzyme. The reaction was initiated by addition of substrate, pNPA (paranitrophenylacetate). The esterase activity is measured by hydrolysis of pNPA shows the yellow color solution of paranitrophenol generated and was detected by the change in absorbance at 400 nm using a spectrophotometer and Printer (Perkin Elmer- Lamda 25) with Winlab program.

For assays quantifying Glutathione-S-transferase (GST) activity, the CDNB assay is modified the method of Visetson et al. (2002, 2003) and Bullangpoti (2002). These assay uses 0.152g Dichloronitrobenzene in 5ml 100% Ethanol as substrate that conjugated with Glutathione as Glutathione-S-transferase as catalyst. The reaction mixture consists of 150 mM CDNB and enzyme solution, 130 µl of 10 mM GSH reduced form in 0.1 M potassium phosphate buffer to give a final GSH concentration of 1 mM, and 0.1 M potassium phosphate buffer (containing no GSH) to give a final volume of 1.31 ml. Reaction is initiated by addition of 150 mM CDNB to both the sample and reference (no enzyme) cuvettes. For analyzing the absorbability of monochloro-dinitrobenzene Glutathione which change in absorbance at 340 nm is recorded using a spectrophotometer and Printer (Perkin Elmer- Lamda 25) with Winlab program.

The pH optimum of all enzymes activity detections were preliminary test which was elucidated with various pH of potassium phosphate buffer (pH 4-13). The activities were test with enzymes of adult GLH with paranitrophenylacetate as substrate for carboxylesterase and 1- chloro-2, 4-dinitrobenzene (CDNB) for Glutathione-S-transferase. The pH which shows the highest activity level of substrate detected of each enzyme was selected to apply in this study.

Carboxylesterase activity was measured using the PNPA assay which modified from Visetson (2001) and Visetson *et al*. (2003, 2004) as shown in appendix A 3. The 3 ml incubation mixture contained 2900 µl, 0.1 M potassium phosphate buffer pH 8.0 and 50 µl enzyme. The reaction was initiated by addition of 50 µl substrate, pNPA (paranitrophenylacetate) to the mixture at 28-30°C of both the sample and reference (no enzyme) cuvettes. The hydrolysis of paranitrophenylacetate shows the yellow color of paranitrophenol generated and was detected by the change in absorbance at 400 nm using a spectrophotometer and Printer (Perkin Elmer- Lamda 25) with Winlab program.

Glutathione-S-transferase (GST) activity, the CDNB assay is modified the method of Visetson *et al.* (2002, 2003) and Bullangpoti (2002) as show in appendix A4 and A5. The reaction mixture consists of 10 µl, 150 mM - chloro-2, 4-dinitrobenzene (CDNB) and enzyme solution, 130 µl of 10 mM Glutathione reduced form in 0.1 M potassium phosphate buffer pH 8.0 to give a final glutathione concentration of 1 mM, and 1150 µl, 0.1 M potassium phosphate buffer pH 8.0 (containing no Glutathione) to give a final volume of 1.31 ml. Reaction is initiated by addition of 150 mM - chloro-2, 4-dinitrobenzene (CDNB) to both the sample and reference (no enzyme) cuvettes. Change in absorbance at 340 nm is recorded using a spectrophotometer and Printer (Perkin Elmer- Lamda 25) with Winlab program.

Moreover, Total protein content was determined by the Bradford method using bovine serum albumin (sigma) as a standard followed the method in appendix B.The 5.5 ml incubated mixture contained 0.5 ml sample and 5 ml Bradford solution (100mg coomassie brilliant blue R250 in 50ml 95% ethanol then add 100 ml 85% Phosphoric acid and make final volume to 1 L by distilled water). Incubated the mixture at room temperature for 5 minutes then the reaction was detected by the change in absorbance at 595 nm using a spectrophotometer and Printer (Perkin Elmer-Lamda 25) with Winlab program.

Figure 28 Refrigerated centrifuge at 18,000 rpm 4ºC for 5 minutes was used for separation of enzyme.

Figure 29 Computerized spectrophotometer methods modified from Visetson *et al.* (2003) with UV-visible light at 340 and 400 nm were used for esterase, Glutathione-S-transferase and protein concentrations in the enzyme homogenates.

Figure 30 Doing enzyme activity analysis experiment.

The chemical and protocol for analyze activity of esterase and glutathione-s-transferase and protein concentration were described in appendix A and B.

6. Evaluation effect to non-target organisms method

Guppies, one of bio-indicator, were treated with Mangosteen Pericarp Extracts, the Yam Bean Seed Extracts and the Sugar Apple Seed Extracts for determine toxicity to aquatic animal (Figure31). The guppies experiment uses Completely Randomized Design with 3 replicates and 30 sampling 3 months old of guppies in each replicate were treated with 3 concentrations of all extracts and control treatments (distilled water) which were doing in the tank (30 x 30x 30 cm) including 3000 ml water. No operculum moving of guppies was assumed as mortality to guppies. The differences of mean mortality were analyzed by Analysis of Variance with CRD, 3 replicates. The LC_{50} statistic analysis was determined using the SPSS (Statistical Package for the Social Sciences) for windows version 11.0 and Sigma plot 2000 program.

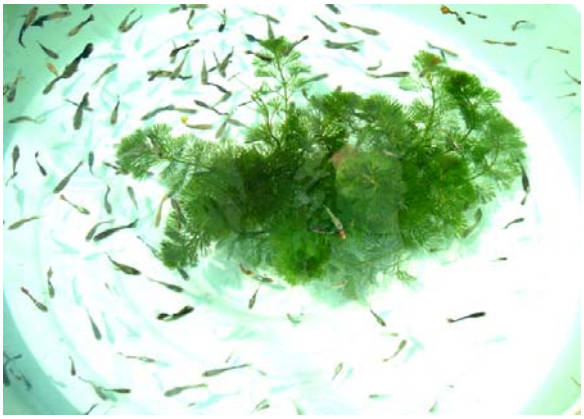


Figure 31 Guppy for testing

Figure 32 Toxicity test to Guppy

7. Place and duration

Place : Department of Zoology, Faculty of Science Kasetsart University (Bang Khen)
Duration : July 2005 to January 2007.

RESULTS AND DISCUSSION

Results

1. The toxicity of Mangosteen Pericarp Extracts on Green Leafhopper.

The Mangosteen Pericarp were extracted by Soxhlet's Extraction method with 95% ethanol as solvent and were evaporated by rotary evaporator and freeze dry. The yield of brown crude extract is 34.07% w/w. This crude extract was tested against adults of Green Leafhopper at 5 concentrations, 0 (distilled water: Control), 4, 6, 8 and 10% w/v. The actual death percentage and LC50 values at 24 and 48 hours of treated adults of Green Leafhopper (GLH) were measured against all of these extracts. It showed that the mortality percentage at 24 hours of treated adults of Green Leafhopper against various concentrations of these crude extracts are 0, 20.33 ± 2.58, 27.22 ± 2.50, 37.22 ± 3.90 and 63.33 ± 2.10, respectively (Table 1, Figure 33).The simple linear regression of this result is Y = -11.62 + 6.95 X (X means percent concentrations of Mangosteen Pericarp Extracts and Y means mortality percentage of treated adults of Green Leafhopper) thus, LC50 values is 8.86% w/v with correlation coefficient is 0.95 (Table2). The mortality percentage values differ among groups and increase with significantly different at 5% level of Duncan's Multiple Rang Test.

The mortality percentage at 48 hours of treated adults of Green Leafhopper against various concentrations of these crude extracts are 0, 41.66 ± 3.49, 53.89 ± 3.27, 69.44 ± 3.89 and 80.00 ± 2.97, respectively (Table 1, Figure 33). The simple linear regression of this result is Y = 15.55 + 6.52 X (X means percent concentrations of Mangosteen Pericarp Extracts and Y means mortality percentage of treated adults of Green Leafhopper) thus, LC50 values is 5.27% w/v with correlation coefficient is 0.99 (Table 2). The mortality percentage values differ among groups and increase with significantly different at 5% level of Duncan's Multiple Rang Test.

Table 1 Mortality percentage of adults Green Leafhopper against crude extracts of Mangosteen Pericarp after 24 and 48 hour under the laboratory condition.

Dose (% w/v)	Total Treated	No. Repeat	% Average Mortality adult/ treatment [(2)]	
			24 hr.	48 hr.
0 [(1)]	60	3	0.00[a]	0.00[a]
4	60	3	20.33 ± 2.58[b]	41.66 ± 3.49[b]
6	60	3	27.22 ± 2.50[c]	53.89 ± 3.27[c]
8	60	3	37.22 ± 3.90[d]	69.44 ± 3.89[d]
10	60	3	63.33 ± 2.10[e]	80.00 ± 2.97[e]

[(1)] Control A = Distilled water
[(2)] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.

Table 2 Regression equation, LC50, Correction coefficient (r) and coefficient determination (r^2) between concentrations of Mangosteen Pericarp Extracts.

Time (Hours)	Regression Equation [1]	LC50 [2]	r [3]	r^2 [4]
24	Y = -11.62 + 6.95 X	8.86	0.95	0.90
48	Y = 15.55 + 6.52 X	5.27	0.99	0.99

[1] Regression equations of mortality percentage of Green Leafhopper after exposure to various concentrations of Mangosteen Pericarp Extracts;
X = Percent concentration of Mangosteen Pericarp
Y = Mortality percentage of treated Green Leafhopper
[2] LC50 = The lethal concentration at which 50% died.
[3] r = correlation coefficient for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.
[4] r^2 = coefficient determination for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.

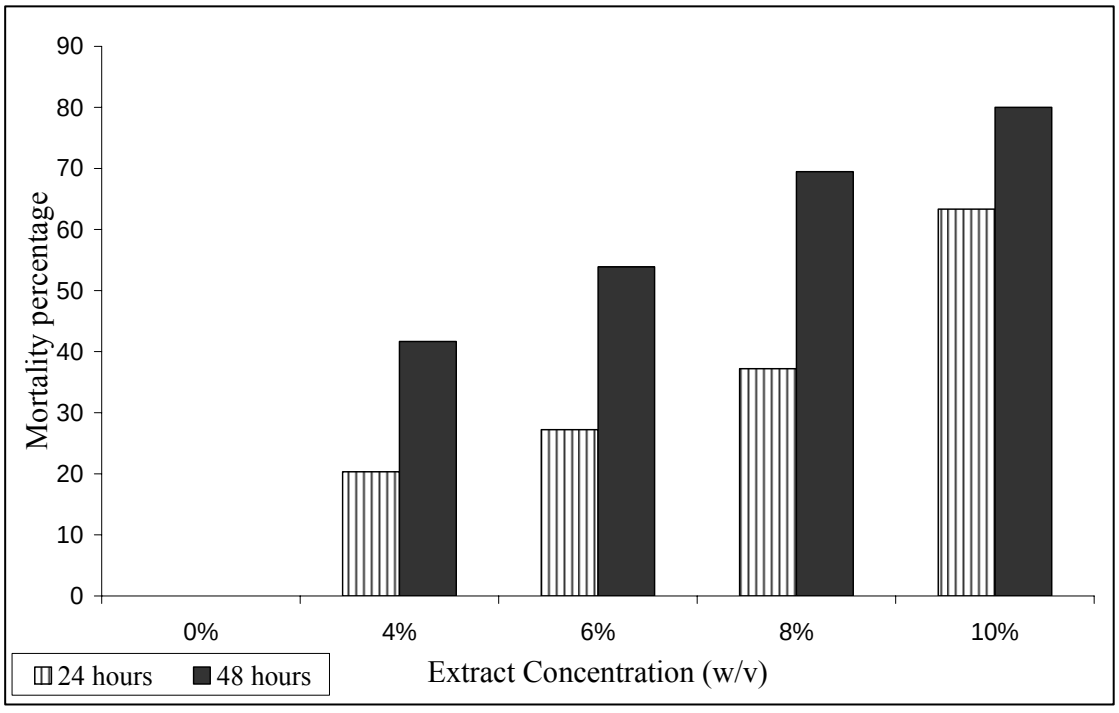


Figure 33 Mortality percentage of Green Leafhopper after exposure to various concentrations of Mangosteen Pericarp Extracts at 24 and 48 hours.

2. The toxicity of Yam Bean Seed Extracts on Green Leafhopper

The seed of yam bean were extracted by Soxhlet's Extraction method with 95% ethanol as solvent and were evaporated by rotary evaporator and freeze dry. The yield of yellow crude extract is 34.12% w/w. This crude extract was tested against adults of Green Leafhopper at 5 concentration as 0 (distilled water: Control), 0.1, 0.5, 1.0 and 1.5 % w/v and was collected real mortality percentage and LC50 values at 24 and 48 hours of treated adults of Green Leafhopper against these extracts. It showed the mortality percentage at 24 hours of treated adults of Green Leafhopper against various concentrations of these crude extracts are 0, 21.11 ± 1.92, 46.67 ± 3.33, 72.22 ± 1.92 and 90.00 ± 3.33, respectively (Table 3 and Figure 34).The simple linear regression showed Y = 19.42 + 49.13 X (X means percent concentrations of Yam Bean Seed extracts and Y means mortality percentage of treated adults Green Leafhopper thus, LC50 values is 0.62% w/v with correlation coefficient is 0.99 (Table 4). The mortality percentage values differ and increase with significantly different at 5% level of DMRT (Table 3 and Figure 34).

The mortality percentage at 48 hours of treated adults Green Leafhopper against various concentrations of these crude extracts are 0, 36.67 ± 3.33, 62.22 ± 1.92, 85.55 ± 1.92 and 95.55 ± 1.93, respectively (Table 3 and Figure 34). The simple linear regression showed Y = 37.34 + 42.13 X (X means percent concentrations of Yam Bean Seed extracts and Y means mortality percentage of treated adults Green Leafhopper thus, LC50 values is 0.30% w/v with correlation coefficient is 0.97 (Table 4). The mortality percentage values differ and increase with significantly different at 5% level of DMRT (Table 3 and Figure 34).

Table 3 Mortality percentage of adults Green Leafhopper against crude extracts of Yam Bean Seed after 24 and 48 hour under laboratory condition.

Dose (% w/v)	Total treated	No. Repeat	% Average Mortality adult/ treatment [(2)]	
			24 hr.	48 hr.
0 [(1)]	60	3	0.00 [a]	0.00 [a]
0.1	60	3	21.11 ± 1.92 [b]	36.67 ± 3.33 [b]
0.5	60	3	46.67 ± 3.33 [c]	62.22 ± 1.92 [c]
1.0	60	3	72.22 ± 1.92 [d]	85.55 ± 1.92 [d]
1.5	60	3	90.00 ± 3.33 [e]	95.55 ± 1.93 [e]

[(1)] Control A = Distilled water
[(2)] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.

Table 4 Regression equations, LC50, correlation coefficient (r) and coefficient determination (r^2) between concentrations of Yam Bean Seed Extracts.

Time (Hours)	Regression Equation [1]	LC50 [2]	r [3]	r^2 [4]
24	Y = 19.42 + 49.13 X	0.62	0.99	0.98
48	Y = 37.34 + 42.13 X	0.30	0.97	0.95

[1] Regression equations of mortality percentage of Green Leafhopper after exposure to various concentrations of Yam Bean seed extracts;
X = Percent concentration of Yam Bean Seed extracts.
Y = Mortality percentage of treated Green Leafhopper
[2] LC50 = The lethal concentration at which 50% died.
[3] r = correlation coefficient for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.
[4] r^2 = coefficient determination for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.

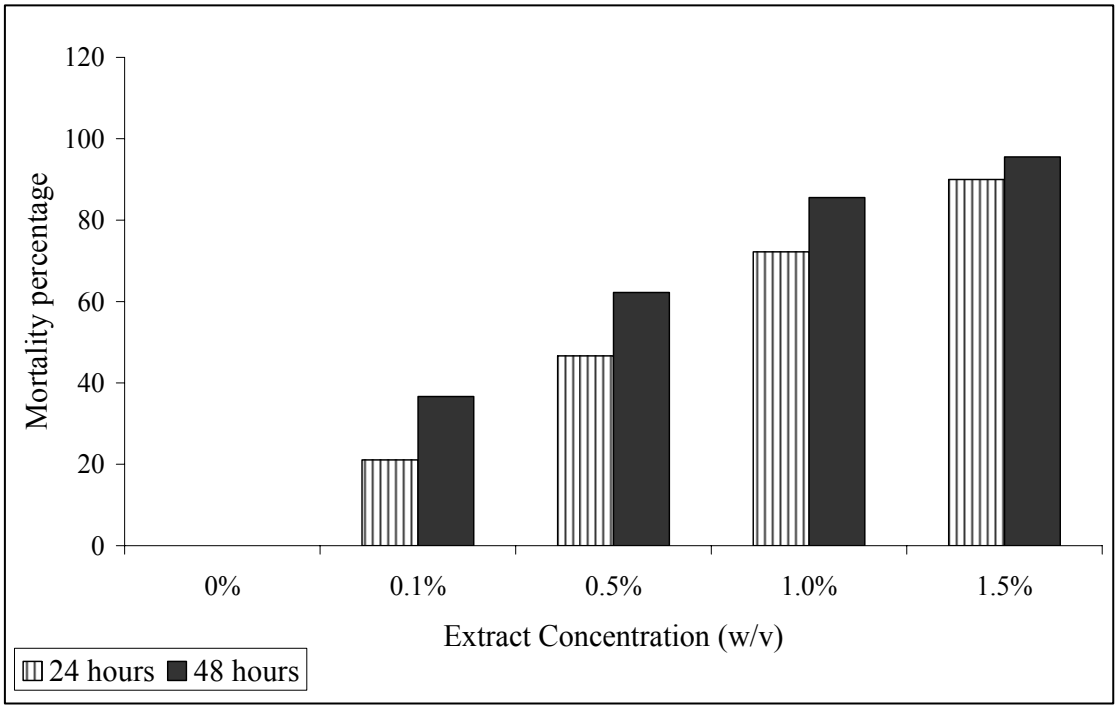


Figure 34 Mortality percentage of Green Leafhopper after exposure to various concentrations of Yam Bean Seed Extracts at 24 and 48 hours.

3. The toxicity of Sugar Apple seed extracts to Green Leafhopper

The seed of sugar apple were extracted by Soxhlet's Extraction method with 95% ethanol as solvent and were evaporated by rotary evaporator and freeze dry. The yield of yellow crude extract is 35.43% w/w. This crude extract was tested against adults of Green Leafhopper at 5 concentration as 0 (distilled water : Control), 0.1, 0.5, 1.0 and 1.5 % w/v and was collected real mortality percentage and LC50 values at 24 and 48 hours of treated adults of Green Leafhopper against these extracts. It showed the mortality percentage at 24 hours of treated adults of Green Leafhopper against various concentrations of these crude extracts are 0, 46.10 ± 0.22, 54.97 ± 0.75, 74.53 ± 1.66 and 87.15 ± 3.37, respectively (Table 5 and Figure 35). The simple linear regression showed Y = 42.09 + 30.44 X (X means percent concentrations of Sugar Apple Seed extracts and Y means mortality percentage of treated adult Green Leafhopper) thus, LC50 values is 0.26% w/v with correlation coefficient is 0.99 (Table 6). The mortality percentage values differ and trend to be increase with significantly different at 5% level of DMRT (Table 5 and Figure 35).

The mortality percentage at 48 hours exposed adults Green Leafhopper against various concentrations of these crude extracts are 0, 48.87 ± 0.97, 67.78 ± 1.92, 80.55 ± 2.54 and 94.44 ± 6.93, respectively (Table 5 and Figure 35). The simple linear regression showed Y = 48.44 + 31.57 X (X means percent concentrations of Sugar Apple Seed extracts and Y means mortality percentage of treated adults of Green Leafhopper) thus, LC50 values is 0.05% w/v with correlation coefficient is 0.99 (Table 6). The mortality percentage values trend to significantly increase at 5% level of DMRT (Table 5 and Figure 35).

Table 5 Mortality percentage of adults Green Leafhopper against crude extracts of Sugar Apple Seed after 24 and 48 hours under the laboratory condition.

Dose (% w/v)	Total Treat	No. Repeat	% Average Mortality adult/ treatment [(2)]	
			24 hr.	48 hr.
0 [(1)]	60	3	0.00[a]	0.00[a]
0.1	60	3	46.10 ± 0.22[b]	48.87 ± 0.97[b]
0.5	60	3	54.97 ± 0.75[c]	67.78 ± 1.92[c]
1.0	60	3	74.53 ± 1.66[d]	80.55 ± 2.54[d]
1.5	60	3	87.15 ± 3.37[e]	94.44 ± 6.93[e]

[(1)] Control A = Distilled water
[(2)] Means ± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.

Table 6 Regression equation, LC50, Correlation coefficient (r) and coefficient determination (r^2) between concentrations of Sugar Apple Seed Extracts.

Time (Hours)	Regression Equation [1]	LC50 [2]	r [3]	r^2 [4]
24	Y = 42.09 + 30.44 X	0.26	0.99	0.98
48	Y = 48.44 + 31.57 X	0.05	0.99	0.98

[1] Regression equations of mortality percentage of Green Leafhopper after exposure to various concentrations of Sugar Apple seed extracts;
X = Percent concentration of Sugar Apple seed extracts.
Y = Mortality percentage of treated Green Leafhopper
[2] LC50 = The lethal concentration at which 50% died.
[3] r = correlation coefficient for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.
[4] r^2 = coefficient determination for correlation of mortality percentage against extracts concentration of treated Green Leafhopper at 24 and 48 hours.

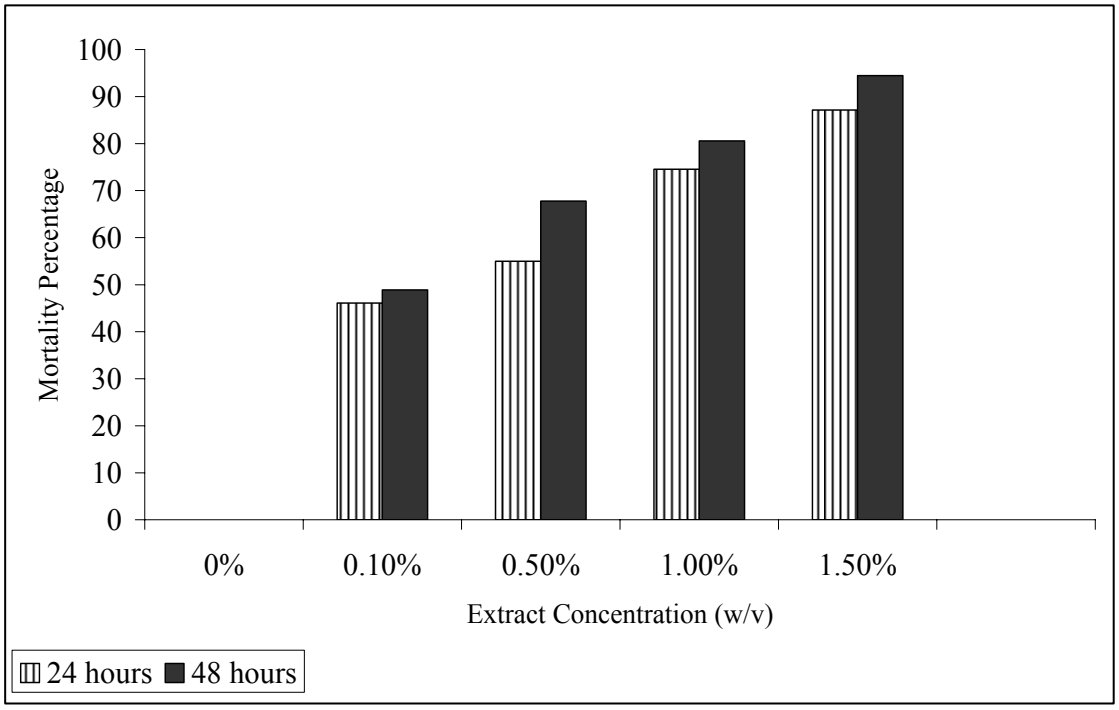


Figure 35 Mortality percentage of Green Leafhopper after exposure to various concentrations of Sugar Apple extracts at 24 and 48 hours.

Detoxification Enzyme Activity of the Green Leafhopper against Plant Extracts

1. pH optimum for esterase activity in Green Leafhopper

The method for detect activity of esterase was done by using UV – visible spectrophotometer with have paranitrophenylacetate as substrate. When vary pH of buffer, phosphate buffer, the data show pH 8.0 is the best pH for detect activity of esterase of Green Leafhopper as show in Figure 36.

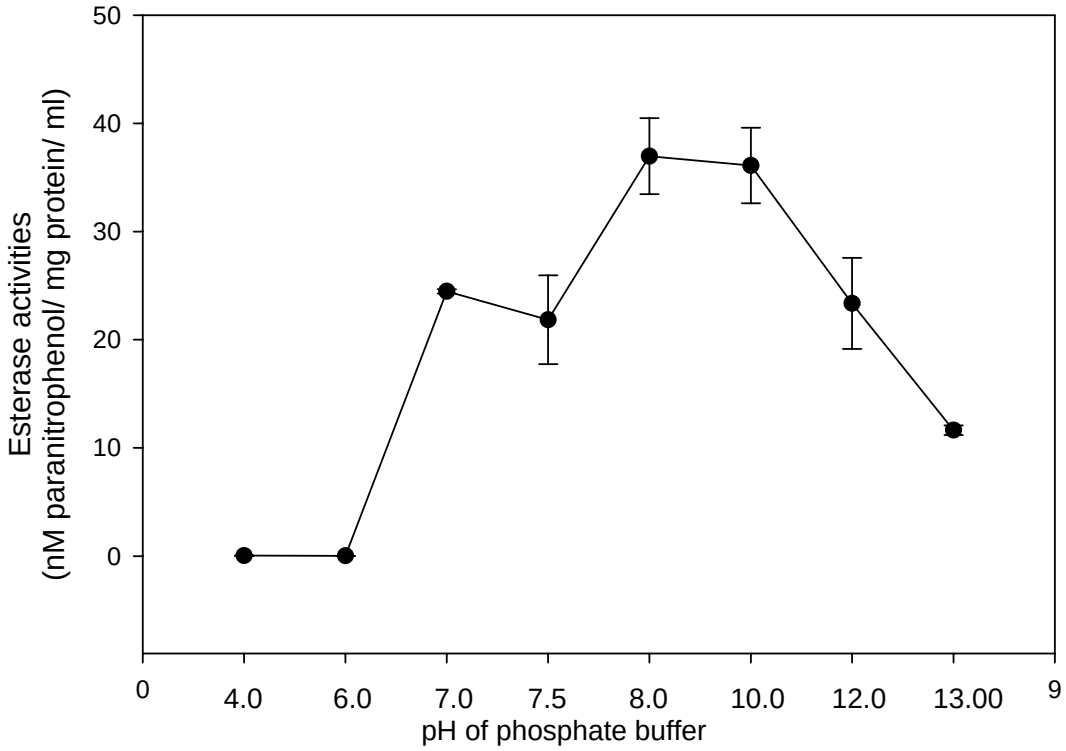


Figure 36 Esterase Activity in Green Leafhopper in various pH of phosphate buffer.

2. pH optimum for Glutathione-S-transferase activity in Green Leafhopper

The method for detect activity of Glutathione-S-transferase was done by using UV- visible spectrophotometer.The first substrate is chlorodinitrobenzene (CDNB) which analyze at 340 nm. When vary pH of buffer, phosphate buffer, the data show pH 7.5 is the best pH for detect activity of Glutathione-S-transferase of Green Leafhopper for CDNB as substrate as show in Figure 37.

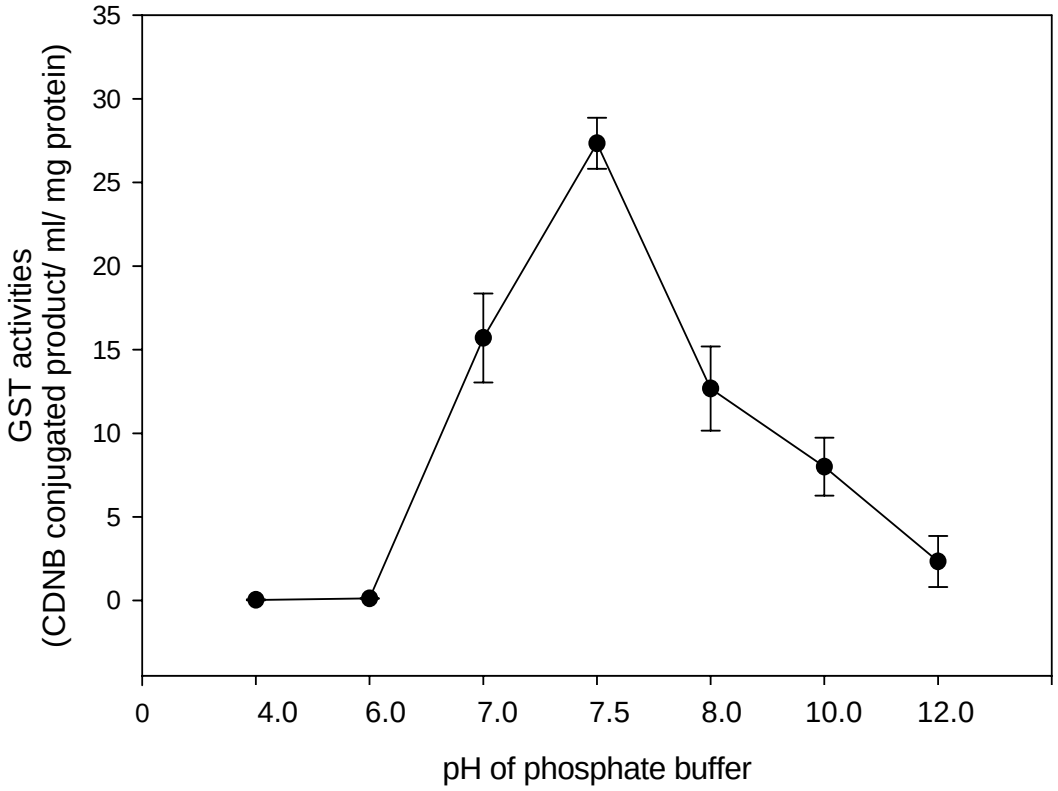


Figure 37 Glutathione-S-transferase activity in Green Leafhopper in various pH of phosphate buffer.

3. The Effects of Mangosteen Pericarp Extracts on Detoxification Enzymes of the Green Leafhopper

3.1 Esterase Activity Level.

The Mangosteen Pericarp extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water:Control), 4, 6, 8 and 10% w/v.The esterase activities from adults of Green Leafhopper after exposure for 24 and 48 hours are in vitro observed by using pNPA as a substrate. The spectrophotometer calibrated at 400 nm was used to identify the product, paranitrophenol, which produced yellowish colors.

The in vitro esterase activities against various concentrations at 24 hours are 26.32 ± 2.21, 24.53 ± 1.70, 19.44 ± 1.14, 17.12 ± 2.00 and 15.24 ± 0.95 nmole paranitrophenol/mg protein/ml, respectively (Table7, Figure38). Esterase activities values between control and 4% w/v of pericarp of mangosteen extracts did not differ among groups with significantly different at 5% level of DMRT. And the esterase activities values at 6%w/v, 8%w/v and 10%w/v of pericarp of mangosteen extracts did not differ with significantly different at 5% level of DMRT. After exposure 48 hours esterase activities against various concentrations are 28.67 ± 1.35, 19.34 ± 0.94, 16.86± 0.46, 15.92 ± 0.38 and 13.32 ± 0.93 nmole paranitrophenol/mg protein/ml,

respectively (Table8, Figure38).The esterase activities values of control differ among groups with significantly different at 5% level of DMRT. As this result, the esterase activity values is depend on concentrations of the extracts which showed the higher concentrations reduce the esterase activities more than the lower one. Furthermore, the total protein values did not differ among groups with significantly different at 5% level of DMRT. (Table 7 and Table 8)

Table 7 Esterase activities of Green Leafhopper against crude extracts of Mangosteen Pericarp at 24 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeated	% Average Esterase Activities (nM paranitrophenol / mg protein/ ml) [2]	CF [3]
0 [1]	2.010 ± 0.102[a]	3	26.32 ± 2.21[a]	-
4	2.097 ± 0.015[a]	3	24.53 ± 1.70[a]	1.07
6	1.996 ± 0.139[a]	3	19.44 ± 1.14[b]	1.35
8	2.082 ± 0.058[a]	3	17.12 ± 2.00[b]	1.54
10	1.986 ± 0.104[a]	3	15.24 ± 0.95[b]	1.73

[1] Control A = Distilled water
[2] Means ± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

Table 8 Esterase activities of Green Leafhopper against crude extract of Mangosteen Pericarp at 48 hours under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeated	%Average Esterase Activities (nM paranitrophenol / mg protein/ ml) [2]	CF [3]
0 [1]	2.010 ± 0.102[a]	3	28.67 ± 1.35[a]	-
4	2.097 ± 0.015[a]	3	19.34 ± 0.94[b]	1.48
6	1.996 ± 0.139[a]	3	16.86 ± 0.46[b]	1.70
8	2.082 ± 0.058[a]	3	15.92 ± 0.38[b]	1.80
10	1.986 ± 0.104[a]	3	13.32 ± 0.93[b]	2.15

[1] Control A = Distilled water
[2] Means ± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3]CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

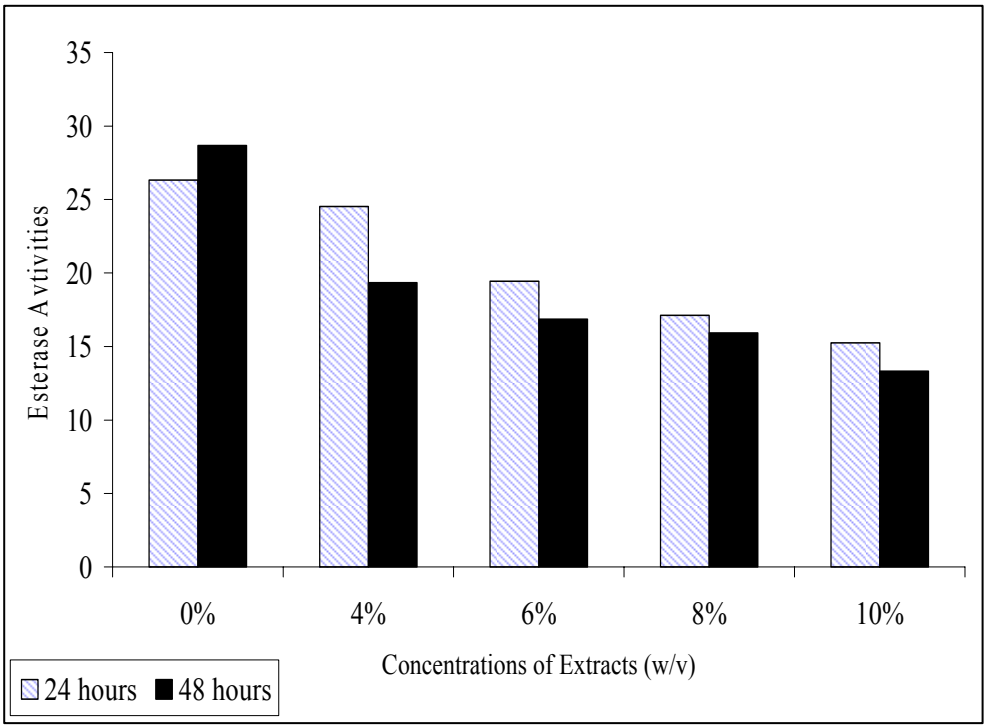


Figure 38 Comparison of Esterase activities from Green Leafhopper after tested with various concentrations of Mangosteens Pericarp Extracts at 24 and 48 hours under laboratory condition.

3.2 Glutathione-S-transferase (GST) Activity Level.

The Mangosteen Pericarp extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water: Control), 4, 6, 8, and 10% w/v. The GST activities from life treated adults Green Leafhopper after exposure for 24 and 48 hours are *in vitro* observed by using CDNB as a substrate.The spectrophotometer calibrated at 340 nm was used to identify the CDNB conjugated product, which produced no colors.

The *in vitro* GST activities against various concentrations at 24 hours are 1.23 ± 0.68, 3.73 ± 2.19, 8.40 ± 4.15, 18.33 ± 1.15 and 23.33 ± 1.52 (every means multiply with 10^{-7}) nM conjugated product/ mg protein/ ml, respectively (Table 9, Figure 39). After exposure 48 hours GST activities against various concentrations are1.46 ± 0.30, 3.00 ± 1.11, 3.76± 2.35, 10.11 ± 1.01 and 20.33 ± 1.52 (every means multiply with 10^{-7}) nM conjugated product/ mg protein/ ml, respectively (Table 10, Figure 39). The GST activity values differ with significantly at 5% level of DMRT. Moreover, GST activity values is depend on concentrations of these extracts which show the higher concentrations increased GST activities more than the lower one. Anyhow, the total protein values did not differ among groups with significantly different at 5% level of DMRT (Table 9 and Table 10).

Table 9 Glutathione–S–transferase activities of Green Leafhopper as affected by crude extracts of Mangosteen Pericarp at 24 hours under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Activities (nM conjugated product x10^{-7}/mg protein/ ml) [2]	CF [3]
0 [1]	2.01 ± 0.11[a]	3	1.23 ± 0.68[a]	-
4	1.98 ± 0.15[a]	3	3.73 ± 2.19[b]	0.33
6	1.99 ± 0.19[a]	3	8.40 ± 4.15[c]	0.15
8	2.02 ± 0.05[a]	3	18.33 ± 1.15[d]	0.07
10	1.96 ± 0.05[a]	3	23.33 ± 1.52[e]	0.05

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)

Table 10 Glutathione–S–transferase activities of Green Leafhopper as affected by crude extracts of Mangosteen Pericarp at 48 hours under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Activities (nM conjugated productx10^{-7}/mg protein / ml) [2]	CF [3]
0 [1]	2.01 ± 0.11[a]	3	1.46 ± 0.30[a]	-
4	2.02 ± 0.05[a]	3	3.00 ± 1.11[b]	0.49
6	1.99 ± 0.19[a]	3	3.76 ± 2.35[c]	0.39
8	2.00 ± 0.03[a]	3	10.11 ± 1.01[d]	0.14
10	2.06 ± 0.14[a]	3	14.33 ± 1.52[e]	0.10

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor =(enzyme activity of none)/ (enzyme activity of treatment)

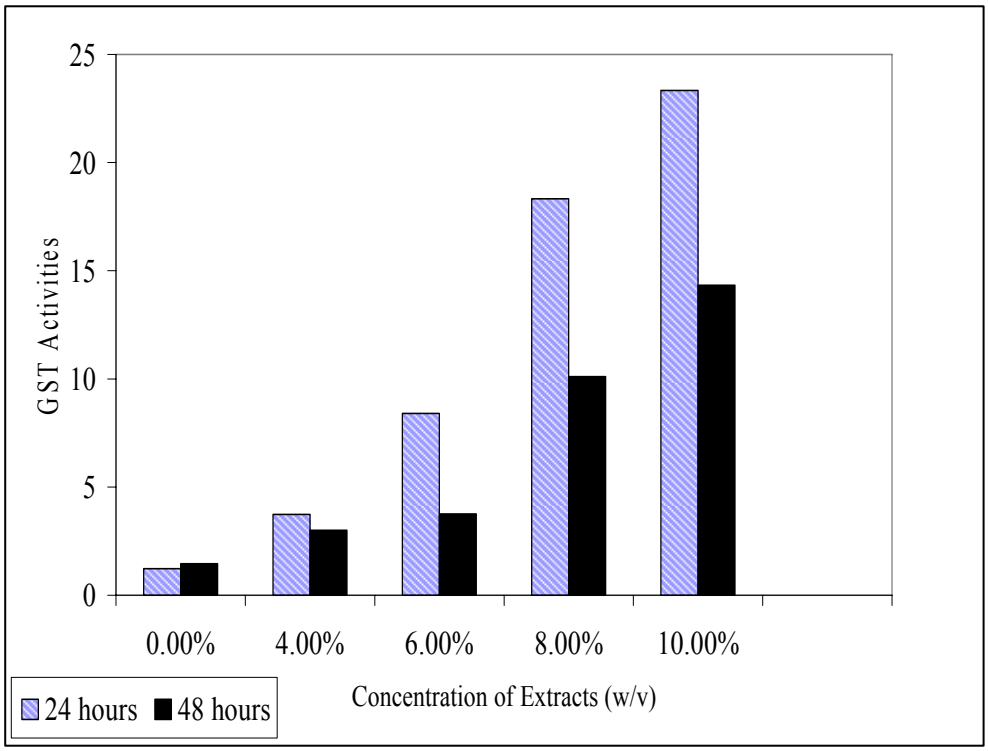


Figure 39 Comparison of Glutathione-S-transferase activities of Green Leafhopper after are tested with various concentrations of mangosteens pericarp extracts at 24 and 48 hours under laboratory condition.

4. The Effects of Yam Bean Seed Extracts to Detoxification Enzymes of the Green Leafhopper

4.1 Esterase Activity Level

The Yam Bean Seed Extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water: Control), 0.1, 0.5, 1.0, 1.5 % w/v. The esterase activities from adults of Green Leafhopper after exposure for 24 and 48 hours were *in vitro* observed by using pNPA as a substrate. The spectrophotometer calibrated at 400 nm was used to identify the product, paranitrophenol, which produced yellowish colors.

The *in vitro* esterase activities against various concentrations at 24 hours are 21.33 ± 1.06, 26.80 ± 4.22, 30.24 ± 1.53, 35.98 ± 1.73 and 46.19 ± 0.93 nmole paranitrophenol / mg protein / ml, respectively. Esterase activities of concentration at 0%, 0.1%, 0.5%, 1.0% and 1.5% w/v are different among group.But esterase activities concentration between 0.5% and 1.0% w/v did not differ with significantly different at 5% level of DMRT (Table 11, Figure 40). The esterase activities at 48 hours are 22.96 ± 1.91, 25.18 ± 1.38, 28.41 ± 0.95, 29.64 ± 0.65 and 36.81 ± 2.84 nmole paranitrophenol/mg protein/ ml, respectively. Esterase activities of Green Leafhopper against crude extracts concentration at 0%, 0.1%, 0.5% and 1.0% w/v of yam bean seed at 48 hours did not differ among group.But esterase activities of Green Leafhopper against crude extracts concentration at 1.5%w/v differ from group(Table 12, Figure 40). The esterase activity values were dependent on concentrations of these extracts showing higher concentrations increased esterase activities. Furthermore, the total protein values did not differ among groups with significantly different at 5% level of DMRT. (Table 11 and Table 12)

Table 11 Esterase activities of Green Leafhopper against crude extracts of Yam Bean Seed at 24 hours under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeat	% Average Esterase Activities (nM paranitrophenol / mg protein / ml) [2]	CF [3]
0 [1]	2.02 ± 0.12[a]	3	21.33 ± 1.06[a]	-
0.1	2.07 ± 0.01[a]	3	26.80 ± 4.22[a]	0.80
0.5	1.89 ± 0.29[a]	3	30.24 ± 1.53[b]	0.71
1.0	2.10 ± 0.08[a]	3	35.98 ± 1.73[b]	0.59
1.5	1.99 ± 0.04[a]	3	46.19 ± 0.93[c]	0.46

[1] Control = Distilled water
[2] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)

Table 12 Esterase activities of Green Leafhopper against crude extracts of the Yam Bean Seed at 48 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeated	% Average Esterase Activities (nM paranitrophenol / mg protein / ml) [2]	CF [3]
0 [1]	1.98 ± 0.22[a]	3	22.96 ± 1.91[a]	-
0.1	2.01 ± 0.01[a]	3	25.18 ± 1.38[a]	0.91
0.5	1.93 ± 0.13[a]	3	28.41 ± 0.95[a]	0.81
1.0	2.07 ± 0.12[a]	3	29.64 ± 0.65[a]	0.77
1.5	1.96 ± 0.10[a]	3	36.81 ± 2.84[b]	0.62

[1] Control = Distilled water
[2] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)

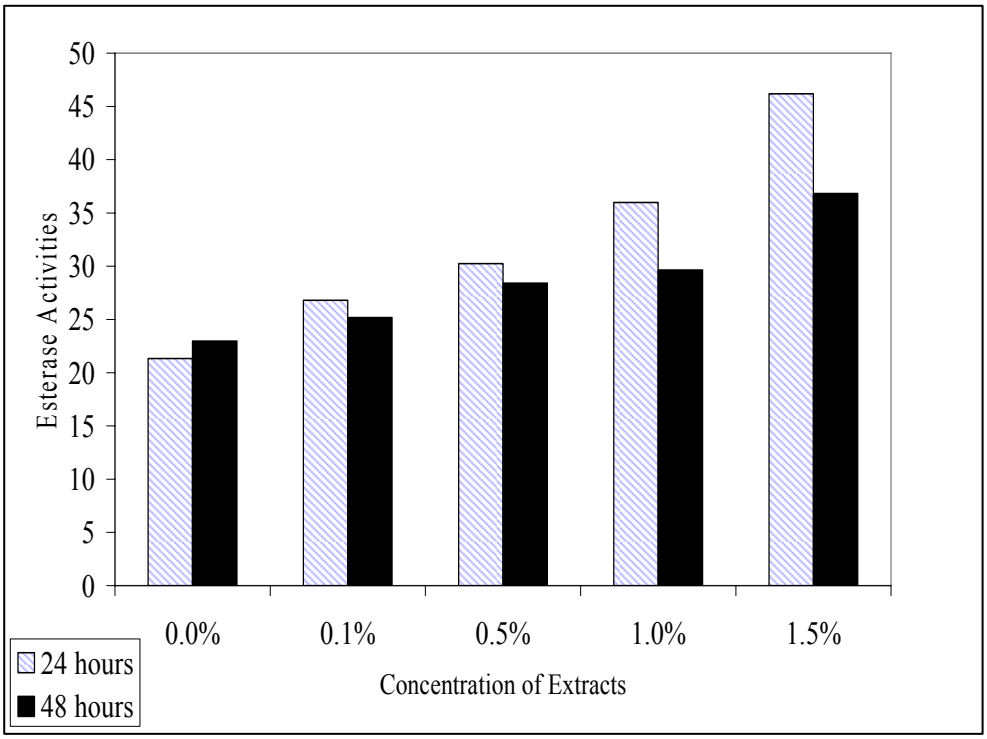


Figure 40 Comparison of Esterase activities of Green Leafhopper after are tested with various concentrations of Yam Bean Seed Extracts at 24 and 48 hours under laboratory condition.

4.2 Glutathione-S-transferase (GST) Activity Level

The Yam Bean seed Extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water: Control), 0.1, 0.5, 1.0, 1.5% w/v.The GST activities from treated adults Green Leafhopper after exposure at 24 and 48 hours are *in vitro* observed by using CDNB as a substrate. The spectrophotometer calibrated at 340 nm was used to identify the CDNB conjugated product, which produced no colors.

The *in vitro* GST activities against various concentrations at 24 hours are2.63 ± 0.40, 2.54 ± 0.15, 2.23 ± 0.04, 1.93 ± 0.66 and 1.84 ± 0.65 (every means multiply with10^{-6}) nM conjugated product / mg protein / ml, respectively. GST activities values did not differ with significantly at 5% level of DMRT (Table 13, Figure 41). After exposure 48 hours GST activities against various concentrations are 2.06 ± 0.11, 2.03 ± 0.27, 2.02 ± 0.14, 1.93 ± 0.11and 1.73 ± 0.15 (every means multiply with 10^{-6}) nM conjugated product/mg protein / ml, respectively (Table 14, Figure 41). The GST activity values did not differ with significantly at 5% level of DMRT. However, GST activity values depend on concentrations of these extracts showing higher concentrations decreased GST activities than the lower one. Anyhow, the total protein values did not differ among groups with significantly different at 5% level of DMRT (Table 13 and Table 14).

Table 13 Glutathione–S–transferase activities of Green Leafhopper as affected by crude extracts of yam bean seed at 24 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Acvities (nM conjugated product / mg protein / ml) [2]	CF [3]
0 [1]	2.00 ± 0.07[a]	3	2.63 ± 0.40[a]	-
0.1	2.07 ± 0.03[a]	3	2.54 ± 0.15[a]	1.03
0.5	1.95 ± 0.11[a]	3	2.23 ± 0.04[a]	1.09
1.0	2.01 ± 0.08[a]	3	1.93 ± 0.66[a]	1.18
1.5	1.92 ± 0.24[a]	3	1.84 ± 0.65[a]	1.43

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

Table 14 Glutathione–S–transferase activities of Green Leafhopper against crude extracts of Yam Bean Seed at 48 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Activities (nM conjugated productx10^{-6} / mg protein / ml) [2]	CF [3]
0 [1]	1.95 ± 0.14[a]	3	2.06 ± 0.11[a]	-
0.1	2.09 ± 0.04[a]	3	2.03 ± 0.27[a]	1.01
0.5	1.99 ± 0.19[a]	3	2.02 ± 0.14[a]	1.02
1.0	2.08 ± 0.08[a]	3	1.93 ± 0.11[a]	1.06
1.5	1.98 ± 0.04[a]	3	1.73 ± 0.15[a]	1.19

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column is not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

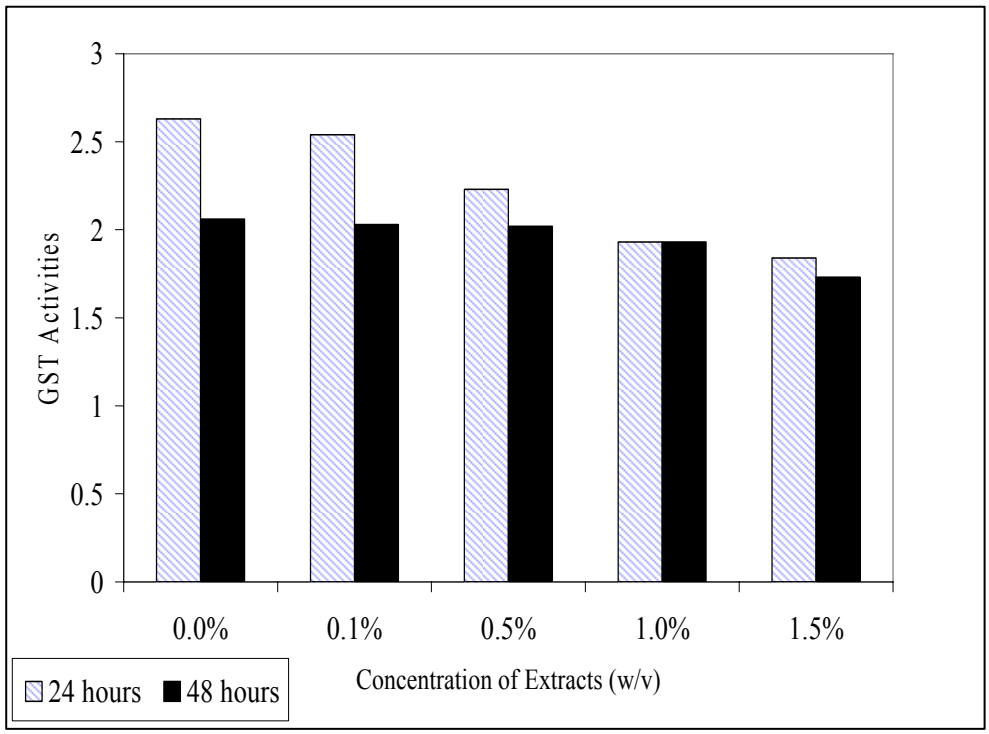


Figure 41 Comparison of Glutathione-S-transferase activities of Green Leafhopper after tested with various concentrations Yam Bean Seed extracts at 24 and 48 hours under laboratory condition.

5. The Effects of Sugar Apple Seed Extracts to Detoxification Enzymes of the Green Leafhopper

5.1 Esterase Activity Level.

The Sugar Apple Seed Extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water: Control), 0.1, 0.5, 1.0, 1.5 % w/v. The esterase activities from adults of Green Leafhopper after exposure for 24 and 48 hours are *in vitro* observed by using pNPA as a substrate. The spectrophotometer calibrated at 400 nm was used to identify the product, paranitrophenol, which produced yellowish colors.

The *in vitro* esterase activities against various concentrations at 24 hours are 27.41 ± 0.74, 14.62 ± 1.12, 18.35 ± 0.11, 22.23 ± 0.61 and 25.12 ± 0.90 nmole paranitrophenol/mg protein/ml, respectively. Esterase activity values are different at 5% level of DMRT (Table 15, Figure 42). The esterase activities at 48 hours are 25.49 ± 3.01, 13.74 ± 1.24, 17.32 ± 0.68, 20.67 ± 1.31and 23.95 ± 0.29 nmole paranitrophenol/mg protein/ml, respectively. Esterase activity values differ among groups. But concentration at 0% w/v and 1.5% w/v did not differ with significantly different at 5% level of DMRT (Table 16, Figure 42).The esterase activity values were dependent on concentrations of these extracts showing higher concentrations increased esterase activities. Furthermore, the total protein values did not significant different (Table 15 and Table 16).

Table 15 Esterase activities of Green Leafhopper against crude extracts of Sugar Apple seed at 24 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeated	% Average Esterase Activities (nM paranitrophenol / mg protein / ml) [2]	CF [3]
0 [1]	1.91 ± 0.21^{a}	3	27.41 ± 0.74^{a}	-
0.1	2.45 ± 0.03^{a}	3	14.62 ± 1.12^{b}	1.87
0.5	1.98 ± 0.19^{a}	3	18.35 ± 0.11^{c}	1.49
1.0	2.05 ± 0.08^{a}	3	22.23 ± 0.61^{d}	1.23
1.5	1.98 ± 0.12^{a}	3	25.12 ± 0.90^{e}	1.09

[1] Control = Distilled water
[2] Means ± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor=(enzyme activity of none) / (enzyme activity of treatment)

Table 16 Esterase activities of Green Leafhopper against crude extracts of Sugar Apple seed at 48 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [2]	No. Repeated	% Average Esterase Activities (nM paranitrophenol / mg protein / ml) [2]	CF [3]
0 [1]	1.92 ± 0.02[a]	3	25.49 ± 3.01[a]	-
0.1	2.03 ± 0.05[a]	3	13.74 ± 1.24[b]	1.85
0.5	1.96 ± 0.16[a]	3	17.32 ± 0.68[c]	1.47
1.0	2.02 ± 0.07[a]	3	20.67 ± 1.31[d]	1.23
1.5	1.94 ± 0.14[a]	3	23.95 ± 0.29[a]	1.06

[1] Control = Distilled water
[2] Means ± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

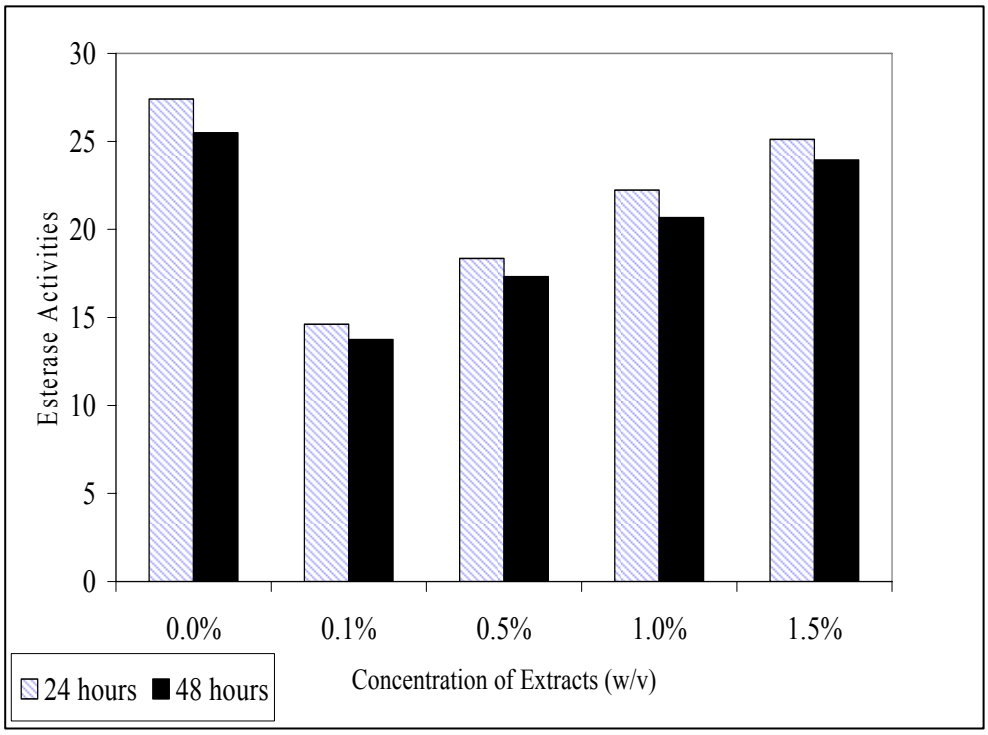


Figure42 Comparison of esterase activities of Green Leafhopper after are tested with various concentrations of Sugar Apple seed extracts at 24 and 48 hours under laboratory condition.

5.2 Glutathione-S-transferase (GST) Activity Level.

The Sugar Apple seed extracts were trialed against Green Leafhopper at 5 concentrations, 0 (distilled water:Control), 0.1, 0.5, 1.0 and 1.5% w/v. The GST activities from treated adults Green Leafhopper after exposure for 24 and 48 hours are *in vitro* observed by using CDNB as a substrate. The spectrophotometer calibrated at 340 nm was used to identify the CDNB conjugated product, which produced no colors.

The *in vitro* GST activities against various concentrations at 24 hours are17.00 ± 1.04, 5.46 ± 0.61, 7.53 ± 1.33, 14.00 ± 0.01 and 16.00 ± 0.01 (every means multiply with10^{-6}) nM conjugated product / mg protein / ml, respectively. GST activity values concentration at 0%, 0.1%, 0.5% and 1.0% w/v differ among group at 5% level of DMRT but 1.5% w/v did not differ between 1.0%w/v and control group at 5% level of DMRT (Table 17, Figure 43). After exposure 48 hours GST activities against various concentrations are15.06 ± 1.46, 5.30 ± 1.41, 7.05± 0.63, 10.23 ± 1.40 and 13.76 ± 0.70 (every means multiply with 10^{-6}) nM conjugated product/ mg protein/ ml, respectively (Table 18, Figure 43). The GST activity values differ among group with significantly at 5% level of DMRT. However, GST activity values depend on concentrations of these extracts showing higher concentrations increased GST activities than the lower one but still less than in control treatment. Anyhow, the total protein values did not differ among groups with significantly different at 5% level of DMRT (Table 17 and Table 18).

Table 17 Glutathione–S–transferase activities of Green Leafhopper as affected by crude extracts of Sugar Apple seed at 24 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Activities (nM conjugated product x10^{-6}/ mg protein / ml) [2]	CF [3]
0 [1]	2.04 ± 0.21^{a}	3	17.00 ± 1.04^{d}	-
0.1	2.07 ± 0.05^{a}	3	5.46 ± 0.61^{a}	3.11
0.5	1.98 ± 0.14^{a}	3	7.53 ± 1.33^{b}	2.25
1.0	2.00 ± 0.05^{a}	3	14.00 ± 0.01^{c}	1.21
1.5	1.97 ± 0.12^{a}	3	16.00 ± 0.01^{cd}	1.06

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column is not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

Table 18 Glutathione–S–transferase activities of Green Leafhopper as affected by crude extracts of Sugar Apple seed at 48 hr. under the laboratory condition.

Dose (% w/v)	Total protein (mg) [3]	No. Repeated	% Average GST Activities (nM conjugated productx10^{-6} / mg protein / ml) [2]	CF [3]
0 [1]	1.98 ±0.10[a]	3	15.06 ± 1.46[a]	-
0.1	1.99 ± 0.01[a]	3	5.30 ± 1.41[b]	2.84
0.5	2.06 ± 0.13[a]	3	7.05 ± 0.63[c]	2.13
1.0	2.18 ± 0.04[a]	3	10.23 ± 1.40[d]	1.47
1.5	1.96 ± 0.14[a]	3	13.76 ± 0.70[e]	1.09

[1] Control = Distilled water
[2] Means± SD followed by a common letter within the same column are not significantly different at 5% level of DMRT.
[3] CF is a correction factor = (enzyme activity of none) / (enzyme activity of treatment)

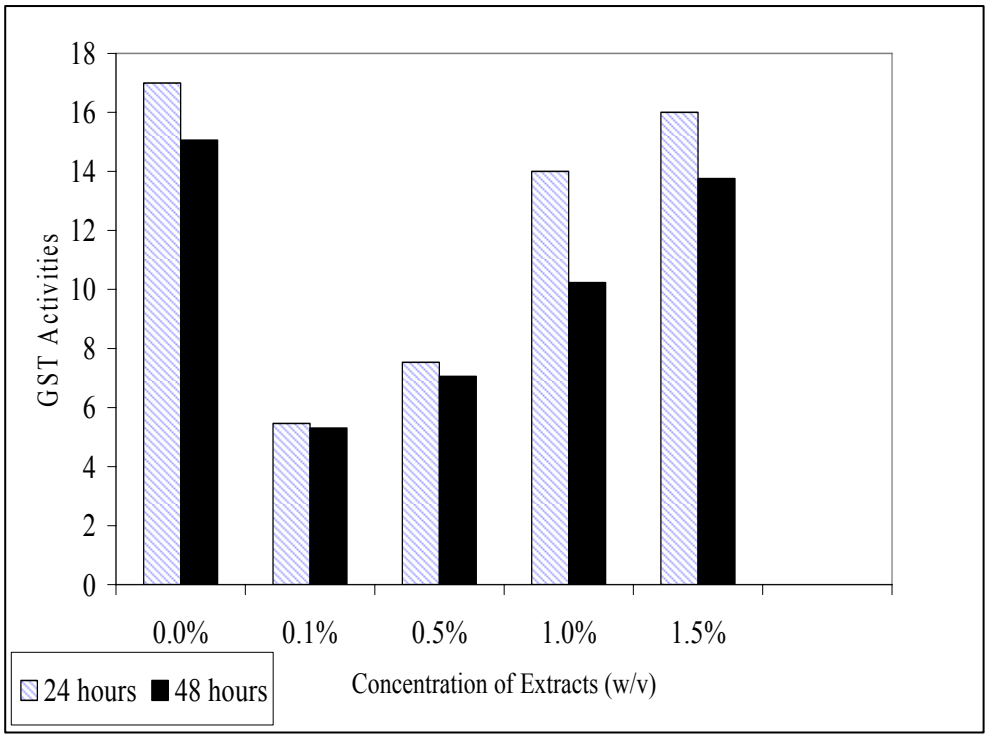


Figure 43 Comparison of Glutathione-S-transferase activities of Green Leafhopper after tested with various concentrations of Sugar Apple seed extracts at 24 and 48 hours under laboratory condition.

6. The Correction Factor of Each Plant Extracts to Detoxification Enzymes of Green Leafhopper.

The Mangosteen Pericarp Extracts inhibited of esterase activity as shown in correction factor (CF) ca. 1.07-1.73 fold comparing to the untreated control (Table 19). However, Glutathione–S–transferase activities showing dramatically increase after treated this extracts as of correction factor (CF) ca 0.05-0.33 fold.

The Yam Bean seed extracts gave increased esterase as ca 0.46-0.80 fold but Glutathione–S–transferase activities show decrease that showing of correction factor (CF) ca. 1.03-1.43 fold, comparing to untreated control (Table 20).

The Sugar Apple seed extracts inhibited esterase at the lowest concentration and seemed to be induce at the highest concentration; however it still less than the activity of control treatment as showing CF 1.09-1.87 fold. The Glutathione–S–transferase activities showed the same activity as esterase showing correction factor (CF) ca. 1.06-3.11 fold, respectively, comparing to untreated control (Table 21).

Table19 Esterase and Glutathione–S-transferase (GST) activities [(1)] of adults Green Leafhopper against of Mangosteen Pericarp extracts at 24 hours exposure.

Type of Extracts	Esterase	[CF] [(2)]	GST [(3)]	[CF] [(2)]
Control (Distilled water)	26.32 ± 2.21[a]	-	1.23 ± 0.68	-
Mangosteen Pericarp 4%	24.53 ± 1.70[a]	1.07	3.73 ± 2.19	0.33
Mangosteen Pericarp 6%	19.44 ± 1.14[b]	1.35	8.40 ± 4.15	0.15
Mangosteen Pericarp 8%	17.12 ± 2.00[c]	1.54	18.33 ± 1.15	0.07
Mangosteen Pericarp 10%	15.24 ± 0.95[d]	1.73	23.33 ± 1.52	0.05

[(1)] Means ± SD followed by different letters within the same column are significantly different at $P<0.05$, DMRT
[(2)] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)
[(3)] Every means multiply with 10^{-6}

Table 20 Esterase and Glutathione–S–transferase (GST) activities [1] of adult Green Leafhopper is against of Yam Bean seed extracts at 24 hours exposure.

Type of Extracts	Esterase	[CF] [2]	GST [3]	[CF] [2]
Control (Distilled water)	21.33 ± 1.06[a]	-	2.63 ± 0.40[a]	-
Yam Bean Seed 0.1%	26.80 ± 4.22[b]	0.80	2.54 ± 0.15[a]	1.03
Yam Bean Seed 0.5%	30.24 ± 1.53[c]	0.71	2.40 ± 0.66[a]	1.09
Yam Bean Seed 1.0 %	35.98 ± 1.73[d]	0.59	2.23 ± 0.04[a]	1.18
Yam Bean Seed 1.5 %	46.19 ± 0.93[e]	0.46	1.84 ± 0.65[a]	1.43

[1] Means ± SD followed by different letters within the same column are significantly different at P<0.05, DMRT
[2] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)
[3] Every means multiply with 10^{-6}

Table 21 Esterase and Glutathione–S–transferase (GST) activities [1] of adult Green Leafhopper is against of Sugar Apple seed extracts at 24 hours exposure.

Type of Extracts	Esterase	[CF] [2]	GST [3]	[CF] [2]
Control (Distilled water)	27.41± 0.75[a]	-	17.00 ± 1.04[a]	-
Sugar Apple Seed 0.1%	14.62 ± 1.12[b]	1.87	5.46 ± 0.61[a]	3.11
Sugar Apple Seed 0.5%	18.35 ± 0.11[c]	1.49	7.53 ± 1.33[a]	2.25
Sugar Apple Seed 1.0 %	22.23± 0.62[d]	1.23	14.00 ± 0.01[a]	1.21
Sugar Apple Seed 1.5 %	25.12± 0.90[e]	1.09	16.00 ± 0.01[a]	1.06

[1] Means ± SD followed by different letters within the same column are significantly different at P<0.05, DMRT
[2] CF is a correction factor = (enzyme activity of none)/(enzyme activity of treatment)
[3] Every means multiply with 10^{-6}

6. Toxicity of Some Plant Extracts to non – target organisms.

6.1 Toxicity of Mangosteen Pericarp Extracts to.Guppies.

The toxicity in term of LC50 *ca*. 3.27%w/v and 2.78 % w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 22, Figure 44). The correlation between concentration and mortality in most experiments indicated of $r^2 = 0.99$ and 0.96 for 24 and 48 hours, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated (Table25). The longer exposure time to 48 hours give significantly different at $P<0.5$ and the high concentration gave high toxicity than less extracts concentration.

Table22 Mortality percentage [(2)] of Guppies against crude extracts of Mangosteen Pericarp after 24 and 48 hr. under the laboratory condition.

Concentration	24 hours	48 hours
0 [(1)]	0 ± 0 [a]	0 ± 0 [a]
2	37.99 ± 1.92 [b]	49.44 ± 5.71 [b]
4	57.44 ± 2.77 [c]	69.33 ± 6.55 [c]
6	89.00 ± 3.77 [d]	94.78 ± 2.92 [d]
Regression equation	Y= 3.14+ 14.32X	Y= 7.75+15.21X
LC50	3.27	2.78
R^2	0.99	0.96

[(1)] Control A = Distilled water.
[(2)] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.

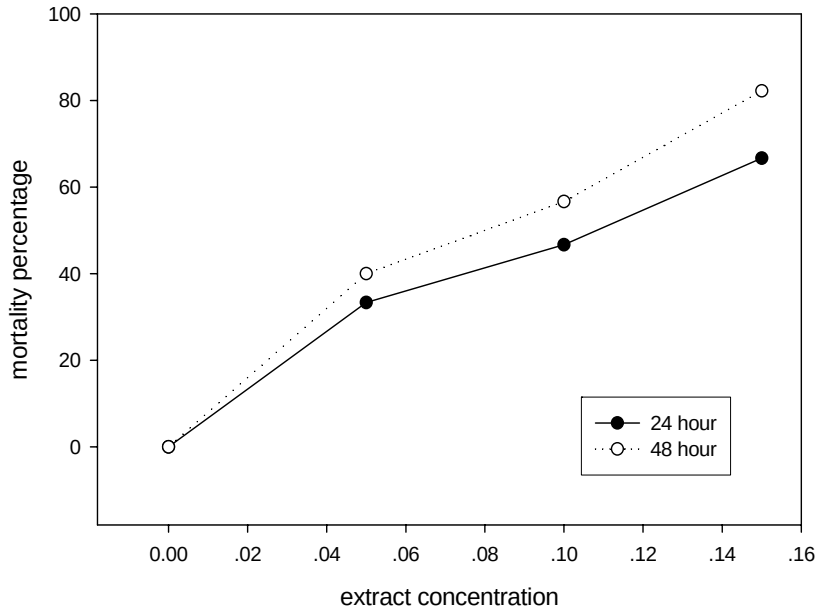


Figure 44 Mortality percentages of Guppies against crude extracts of Mangosteen Pericerp after 24 and 48 hr. under the laboratory condition.

6.2 Toxicity of crude of Yam Bean seed Extracts to.Guppies.

The toxicity in term of LC50 *ca*. 0.11%w/v and 0.09 % w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 23, Figure 45). The correlation between concentration and mortality in most experiments indicated of $r^2 = 0.97$ of both 24 and 48 hours, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated (Table23). The longer exposure time to 48 hours give significantly different at $P<0.5$ and the high concentration gave higher toxicity than less extracts concentration.

Table 23 Mortality percentage [2] of Guppies is against crude extracts of Yam Bean seed after 24 and 48 hr. under the laboratory condition.

Concentration	24 hours	48 hours
0[1]	0 ± 0 [a]	0 ± 0 [a]
0.05	33.33 ± 3.34 [b]	40.0 ± 3.33 [b]
0.10	46.67 ± 3.34 [c]	56.67 ± 3.34 [c]
0.15	66.67 ± 3.33 [d]	82.22 ± 3.85 [d]
Regression equation	Y= 4.67+ 426.7X	Y= 5.22+526.66X
LC50	0.11	0.09
R^2	0.97	0.97

[1] Control A = Distilled water
[2] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.

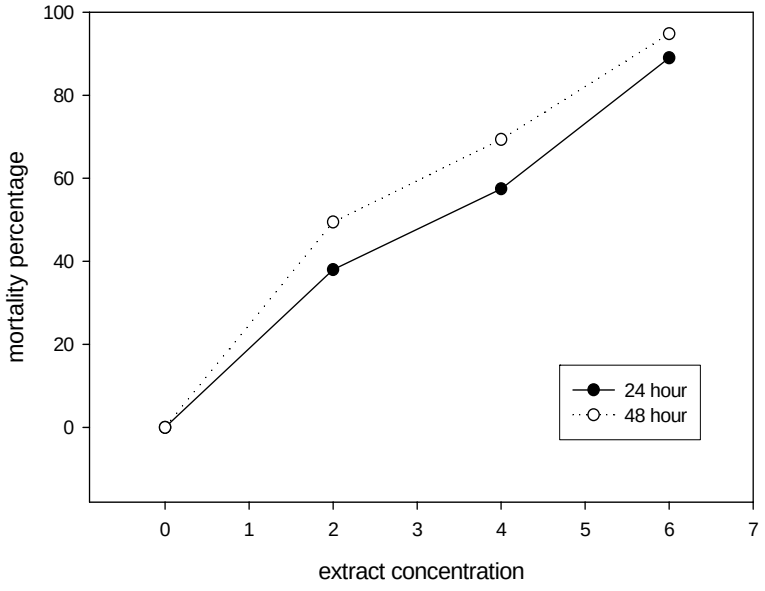


Figure 45 Mortality percentages of Guppies against crude extracts of Yam Bean seed after 24 and 48 hr. under the laboratory condition.

6.3 Toxicity of crude of Sugar Apple seed Extracts to Guppies.

The toxicity in term of LC50 *ca*. 0.16%w/v and 0.12 % w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 24, Figure 46). The correlation between concentration and mortality in most experiments indicated of $r^2 = 0.99$ of both 24 and 48 hours, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated (Table24). The longer exposure time to 48 hours give significantly different at $P<0.5$ and the high concentration gave high toxicity than less extracts concentration.

Table 24 Mortality percentage [(2)] of Guppies is against crude extracts of Sugar Apple seed after 24 and 48 hr. under the laboratory condition.

Concentration	24 hour	48 hour
0[(1)]	0 ± 0 [a]	0 ± 0 [a]
0.10	28.89 ± 5.10 [b]	45.00 ± 2.36 [b]
0.15	48.88 ± 5.09 [c]	68.33 ± 2.36 [c]
0.20	64.44 ± 5.09 [d]	80.00 ± 4.70 [d]
Regression equation	Y= 325.05X- 1.01	Y= 2.05+411.42X
LC50	0.16	0.12
R^2	0.99	0.99

[(1)] Control A = Distilled water
[(2)] Means ± SD followed by a common letter in the same column are not significantly different at 5% level of DMRT.

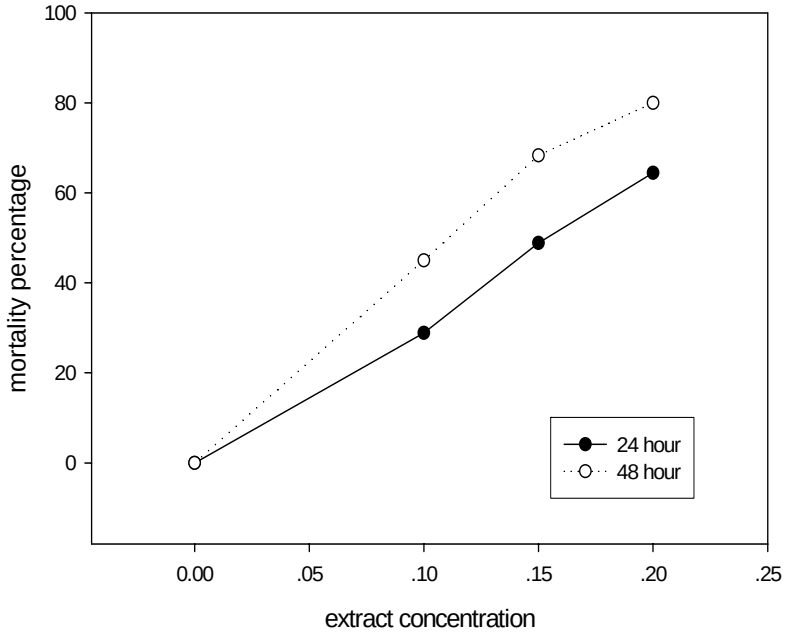


Figure 46 Mortality percentages of Guppies against crude extracts of Sugar Apple seed after 24 and 48 hr. under the laboratory condition.

Discussion

The successful researches on plant extracts against some key agricultural pests have been developing by many scientists. A lot of insecticidal plants have revealed good tendency for insect control namely, Chili (*Capsicum frutescans* L.) for the control of *Sitophilus zeamais* Motschulsky (Bullangpoti *et al.,* 2002), sugar apple (*Annona squamosa* L.) for control *Nephotettix virecens* (Distant) (Srisaard, 2005), nudsage (*Cyperus rotandus* L.) for the control of *Pomacea canaliculata* L. (Ruamthum, 2002) and *Aedes aegypti* (Srikong, 2006), The fruit rind of mangosteen (*Garcina mangostana* L.), rambutan's seed (*Nephilium lappaceum* L.) and kaffir lime's leave (*Citrus hystix* DC.) for the control of *Sitophylus oryzae* L. (Bullangpoti, 2004a), seed jam bean (*Pachyrhizus erosua* (Urb) and Ya-Knong-Chang (*Heliotropium indicum* L.) for control *Aedes aegypti* (Srikong, 2006).

The Mangosteen Pericarp, Yam Bean Seed and Sugar Apple Seed were extractsed by Soxhlet's Extraction method with 95% ethanol as solvent. The yields of crude extracts showed 34.07% w/w, 34.12% w/w and 35.43 % w/w for Mangosteen Pericarp, the Yam Bean Seed and the Sugar Apple Seed, respectively. The amount of all extracts are higher than yield from nutgrass extracts (12.81%w/w) (Ruamthum, 2002), lemon grass extracts which showed ca. 5.00 % w/w (Thummasarangkoon, 2000) and seed kernel extracts (20.12%w/w) (Visetson, 2001). In this research, Yam Bean Seed Extracts gave yield less than result of Srikong (2005) who show yield of seed Yam Bean Extracts ca. 34.32%w/w. The different yield can be happened. It is

depend on kind of plant type, growing sources, plant composition, plant part, solvent, temperatures and extracts method.

Although all of plant materials extractsed from the same method, solvent and temperature, the yields of extracts are usually different. Visetson *et al*. (2003) mentioned this situation that the yield of compounds extractsed may vary dramatically from one batch to the other owing to other components other that the active principle itself.

The Mangosteen Pericarp (*Garcina mangostana* L.) extracts, the Yam Bean Seed extracts and the Sugar Apple extracts Seed have been revealed promising efficiency on insect control under laboratory condition. From these extracts for controlling Green Leafhopper in terms of LC50 and the mechanisms of detoxification enzyme activities namely, esterase and Glutathione-S-transferase were trialed by using enzyme substrate assays with spectrophotometer. Inaddition, I studied the toxicity of these extracts to non-target organisms fishes in order to determine the safety commercial standard for the product in the future.

To clarify the objectives of this thesis, I would like to discuss my results as followed.

1. Toxicity of Plant Extracts Against Green Leafhopper (*Nephotettix virescens*)

Using the topical spray method with varying extracts concentrations against Green Leafhopper, the toxicity in term of LC50 values was determined for each plant extracts (Mangosteen Pericarp, Yam Bean Seed and the Sugar Apple Seed) against the adult of Green Leafhopper. The LC50 values were 8.86 % w/v,0.62% w/v and 0.26 % w/v at 24 hours after exposure, respectively and 5.27% w/v ,0.30% w/v and 0.05% w/v, respectively at 48 hours after exposure (Table 2,4 and 6) From the result, the Sugar Apple Seed Extracts show more toxicity than neem seed kernel extracts against *Callosobruchus maculatus* (Visetson, 2001) but the Yam Bean Seed Extracts dramatically less toxic when compared with derris extracts which showed LC50 about 0.5%w/w against *Plutella xylostella* L. (Visetson and Milne, 2001).The Mangosteen Pericarp Extracts with adult green leafhopper, LC50 values at 24 hour after exposure were 8.86%w/v and 5.27%w/v for 24 and 48 hour after exposure, respectively (Table 2). The toxicity data is different compare with toxicity data of Bullangpoti (2004) who tested this extracts on *Sitophilus oryzae* that showed LC50 value ca. 5.25%w/v.

Furthermore, the effectiveness of Yam Bean Seed Extracts was the same effect to Sugar Apple Seed Extracts that higher concentrations showed effectiveness more than lower ones.

As table1-6, the different time exposure (24 and 48 hour) can give different mortality data, thus we can conclude that time exposure is affected to toxicity to organisms. The different to control insect pest can be occurred because different organism have different detoxification mechanism and efficiency to release or avoid

toxic substance out from their body. The toxicity to control insect pest is different from another report such as Saisongkhroh (2005) who used Sugar Apple Seed or chili extracts to control *Spodoptera litura* L. which showed LC50 1.642 %w/v and 4.880 %w/v, respectively.

2. Detoxification Enzymes Activity of the Green Leafhopper (*Nephotettix virescens*) Against Plant Extracts.

The optimum pH of every detoxification enzymes which was studied in this research are esterase and Glutathione-S-transferase are 8.0 and 7.5, respectively as shown in Figure 36 and 37. This pH is different in other insect which pH is 7.5 such as *Stiophilus oryzae* (Bullangpoti, 2004), *Aedes aegypti* (Srikong, 2006) and *Spodoptera litura* (F.) (Saisongkhroh, 2006). This result shown that types of organisms can be affect to pH optimum of enzyme thus, before study about enzyme we should investigated the pH optimum first.

The detoxification enzymes which were investigated in this research were esterase and Glutathione-S-transferase. The detoxification enzyme mechanism in each organism is different. It is depends on type of organisms or toxic substance. This result is same with another paper such as Srikhong (2006) and Saisongkhroh (2006) who study carboxylesterase and Glutathione-S-transferase in *Aedes agypti* L. and *Spodoptera litura* L., respectively after treated insect with some botanical insecticides, the carboxylesterase have important role to be resistant as shows higher activity than Glutathione-S-transferase.

Comparison of detoxification enzyme mechanism of the Green Leafhopper (*Nephotettix virescens*) after treated Mangosteen Pericarp Extracts, Yam Bean Seed extracts and Sugar Apple Seed extracts. After treated Green Leafhopper with various concentration at 24 hour and 48 hour after exposure, the survival insects were used to investigate the detoxification enzyme mechanism for observe trend to be resistant in the future. Some insect or animal use this mechanism for protect themselves from toxic substance. The mechanism is various which depend on age, sex, toxic substance, organism type and dose.

In this research, the *in vitro* studies indicated that esterase activities against various concentrations of Mangosteen Pericarp Extracts were decreased by upto 1.07-1.73 fold. This evident was showing that the Mangosteen Pericarp Extracts acted as inhibitors for those enzyme systems and the Green Leafhopper may not recognize these compounds and sensitive to them. Both of the seed of Yam Bean Extracts and the seed of Sugar Apple Extracts were increased esterase activity was also depend on concentrations of extracts meaning the higher concentrations increased esterase activities more than the lower one.

Glutathione-S-transferase activities of Green Leafhopper against various concentrations all extracts were increased upto 0.05-3.11 fold but still less than the control treatment. Thus, if use the Mangosteen Pericarp Extracts, Seed of Yam Bean Extracts and Sugar Apple Seed Extracts for control Green Leafhopper (*Nephotettix*

virescens) at a long time, this insect can adapt itself to be resistant to these extracts via this enzyme mechanism.

3. Toxicity of some plant extracts against guppies.

This research used Guppy, *Poecillia reticulate* as the representative for observed the toxicity in aquatic. This kind of fish can be potential bioindicator for urban pollution, especially their spatial distribution over sites of all pollution region and variation in metal accumulation levels deflecting the degree of pollution (Widianarko et al., 2000).

After treated Mangosteen Pericarp Extracts with Guppies, the toxicity in term of LC50 *ca* 3.27%w/v and 2.78% w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 22, Figure 44). The correlation between concentration and mortality in most experiments indicated of r^2 of 0.99 and 0.96 for 24 and 48 hours, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated. The longer exposure time to 48 hours give significantly different at $P<0.5$ and the high concentration gave high toxicity than lesser extracts concentration.

The toxicity of Yam Bean Seed Extracts in term of LC50 *ca*. 0.11%w/v and 0.09 % w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 23, Figure 45). The correlation between concentration and mortality in most experiments indicated of r^2 of 0.97 of both 24 and 48 hour, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated. The longer exposure time to 48 hours give significantly different at $P<0.5$ and the high concentration gave higher toxicity than lesser extracts concentration.

The toxicity of the Sugar Apple Seed Extracts in term LC50 *ca*. 0.16%w/v and 0.12 % w/v in Guppies at 24 hours and 48 hours exposure, respectively (Table 24, Figure 46). The correlation between concentration and mortality in most experiments indicated of r^2 of 0.99 of both 24 and 48 hours, respectively showing that the effects of the extracts on the mortality of Guppies were highly correlated.The longer exposure time to 48 hours give significantly different at $P<0.5$ and the higher concentration gave higher toxicity than lesser extracts concentration.

The toxicity from all extracts is lower than another insecticide such as imidacloprid which have acutely toxic to adult fish at relative concentration (over 80 ppm) with juvenile fish being considerably more susceptible. However, extremely toxic at low concentration of imidacloprid to some species of aquatic animals including freashwater crustacean *Hyalella aztecaia* and the estuary crustacean *Mysidopsis behia* (Buffin, 2003), the result is different from result of Saisongkhroh (2005) who test toxicity of Yam Bean Extracts, chili extracts and seed of sweet apple extracts to *Poecilia latipiana*, the LC50 is 0.047% w/v, 3.00 %w/v and 0.34 %w/v, respectively. Thus, the extracts seems to be friendly to fish more then other botanical insecticides. However, Visetson *et al.* (2005) found that water was important role in hydrolysis of salinadiene from nutgrass tuber more than 80% after went through river.

CONCLUSION AND RECOMMENDATION

Conclusion

Green Leafhopper, *Nephotettix virescens* (Distant) is one of the major insect pests of rice (*Oryza sativa* L.) in the temperate rice growing region of Asia. They are important pests because they are vectors of viruses such as tungro, yellow dwarf, yellow-orange leaf, transitory yellowing, and dwarf. They migrate to the field soon after seedlings have emerged. They can cause indirect damage to the crop because of the virus diseases that they transmit.This research was done to evaluate the effects in terms of detoxification mechanisms of some plant extracts to against the Green Leafhopper, *Nephotettix virescens* (Distant).

The Mangosteen Pericarp (*Garcina mangostana* L.), Yam Bean Seed (*Pachyrrhizus erosus* L.) and Sugar Apple Seed (*Annona.squamosa* Linneaus) extractsed by Soxhlets Extraction using 95% ethanol as solvent. The yields of crude extracts showed 34.07% w/w, 34.12% w/w and 35.43 % w/w for Mangosteen Pericarps, the Yam Bean Seed and the Sugar Apple Seed, respectively. The different yield can be happened. It is depends on kind of plant type, growing sources, plant composition, plant part, solvent, temperatures and extracts method. Various of concentrations of all extracts were trialed with adults Green Leafhopper by topical sprayer method. In order to use as the alternative for control of Green Leafhopper for obtain toxicity data, the Mangosteen Pericarp Extracts showed toxicity value in term of LC50 values ca. 8.86 %w/v (Y = -11.62+6.95X, r^2 = 0.95) and 5.27%w/v (Y = 15.55 + 6.52X, r^2 = 0.99) after exposure at 24 hours and 48 hours, respectively. The Yam Bean Seed Extracts showed toxicity in term of LC50 values ca. 0.62%w/v (Y =19.42+49.13X, r^2 = 0.98) and 0.30%w/v (Y = 37.34+42.13X, r^2 = 0.95) after exposure at 24 and 48 hours, respectively. Moreover, the Sugar Apple Seed Extracts showed LC50 values ca. 0.26 %w/v (Y = 42.09 + 30.44X, r^2 = 0.98) and 0.05%w/v (Y = 48.44 + 31.57X, r^2 = 0.98) at 24 and 48 hours after exposure, respectively. Furthermore, the toxicity to non-target organism as Guppies (*Poecilia reticulata*) which is the representative of aquatic organism were determined at 24 hours after exposure. The LC50 value ca. 3.27, 0.11 and 0.16%w/v for Mangosteen Pericarp Extracts,the Yam Bean Seed Extracts and the Sugar Apple Seed Extracts, respectively (r^2 =0.99). As this result, the Yam Bean Seed Extracts showed the highest toxicity to Guppies compare with other extracts.*In vitro* studies, detoxification enzyme activity such as esterase and Glutathione-S-transferase (GST) from surviving of the Green Leafhopper after 24 hours exposure were analyzed. Only Mangosteen Pericarp extracts showed characterization as inhibitor to esterase. For GST, this enzyme trend to be induced for every extracts. The result can suggest that this insect can develop itself to be resistant to every extracts in the future and also suggested that the Mangosteen Pericarp Extracts, the Yam Bean Seed Extracts and the Sugar Apple Seed Extracts will give alternative choice for control of Green Leafhopper.

Recommendation

1. The investigate toxicity in environmental fate should be done in the future research and should be developed into a commercial product.

2. Trails for new method to extracts which can give higher yield.

3. Purify plant extracts by other means and isolation of the active compoundsshould be done to maximize the best formulation.

LITERATURE CITED

Agro-economic. 2004. **Statistic Agriculture in Thailand 2003** (in Thai). Department of Agriculture BKK, Thailand.

Anonymous. 2003a. Mangosteen. **Fruits Collection**.

Available source: http:// www. Holliday.org/fruits/mangoist.html, January 30, 2003.

Asai F., H. Tosa, T. Tanaka and M. Iinuma. 1995. A Xanthone from Pericarps of Garcina mangostana. **Phytochemistry**. 39(4): 943-944.

Boonyapapat, N. and A. Choakchaijalernporn. 2000. **Herbs** (in Thai). Faculty of Pharmacy, Mahidol University, Bangkok Thailand. pp. 740-742.

Buffin David. 2003. Imidacloprid. Pesticide News. Available sources: http://www.pan-uk.org/pestnews/Actives/imidaclo.htm, January 5, 2006.

Bullangpoti, V. 2004. **Effects of Some Plant Extracts on Toxicity and Activities on Rice Weeil (*Sitpphilus oryzae* L.)**. Master Degree Thesis, Kasetsart University, Bangkok Thailand.

_____, J. Penssok, P. Wisarntanon, P. Kannasutra and S. Visetson. 2002. Chili Extracts (*Capsicum frutescens* L.) for control of corn weevil (*Sitophilus zaemai* Motschulsky).**Agricultural Sci. J**. 33: 6 (Suppl.): 300-4.

Chairungsrilerd N.,K.Takeuchi, S. Nozoe and T. Ohta. 1996.Mangostanol, A Phenyl Xanthone From *Garcina mangostana*. **Phytochemistry**. 43(5):1-99-1103

Chanarat P., N. Chanarat, M. Fujihara and T. Nagumo. 1997. Immunopharmacological activity of Polysaccharide from the pericarp of Mangosteen Garcinia: Phagocytic Intracellular Killing Activities. **J. Med. Asso Thail**. 80:S1149-54.

Corbett, J. R. The Biochemical Mode of Action of Pesticides. Academic Press, New York. 1974.

Dauterman, W.C. 1985. Insect Metabolism: Extramicrosomal, pp. 713-730. In Kerkut, G.A. and Gilbert L.I., eds. **Comprehensive Insect Physiology, Biochemistry and Pharmacology**. Vol. 12. Pergamon Press, Oxford.

Department of Agriculture Extension. 2004. Rice Pest Spread Calendar. **Plant Pest Warning Group**. Available source:http://www.doae.go.th/pest/rice/ribph.htm, December 14, 2004.

Entomological and Zoological Division. 2002. **Method to Control Insect Pest and other Pest** 2002 (in Thai). Department of Agriclture Ministry of Agriculture and Cooperatives, Bangkok Thailand.

International Rice Research Institute.2003.**Green Leafhopper .**Available source: http://www.knowledgebank.irri.org/RiceDoctor_MX/Fact_Sheets/Pests/Green _leafhopper.htm ,December 7, 2003.

_____**Tungro.** Available source: http://www.knowledgebank.irri.org/RiceDoctor_MX/Fact_Sheets//Diseases/Tungro.htm ,December 7, 2003.

Inuma M., H. Tosa, T. Tanaka, F. Asai, Y. Kobsyashi, R. Shimano and KI. Miyauchi. 1996. Antibacterial Activity of Xanthones from Guttiferaeous Plants Against Methicilin -Resistant *Staphylococcus aureus.* **J. Pharm Pharmacol**. 48:861-865.

IUPAC.2002,Commision on Nomenclature of organic Chemistry.Blackwell scientific publication, copyright.

Jakoby, W.B. 1978. TheGlutathione-s-transferase: A Group of Multifunctional Detoxification Proteins. **Adv.Enzymol.Relat. Areas Mol.Biol**. 46:383-414.

Jarinee, 2006 **Yam Bean** Available source: http://kanchanapisek.or.th/kp6/BOOK5/chapter5/t5-5-l3.htm ,December 14, 2006

Kao, L.R., N. Motoyama and W.C. Dauterman. 1985. The Purification and Characterization of Esterase From Insecticide- Resistant and Susceptible Housefiles. **Pest. Biochem. Physiol.** 23:228-239.

Kirtikar and Basu, 1993. Indian Medicinal Plants, India, 2nd Ed.,pp:60-73.

Liou SS., WL. Sheih, TJ Cheng, SJ Won and CN. Lin. 1993. gamma-Pyrone Compounds as Potential Anti-cancer Drugs. **J. Pharm Pharmaco**. 45:791-794.

Mackness, ML. *et al.* 1983. Esterase Activity in Homogenous of Three Strains of the Rust Red Flour Beetle (*Tribolium castaneum* Herbst.) **Comp.Biochem.Physiol.** 74(C):65-68

Matsumura, F. Toxicity of Insecticides. Plenum Press, New York. 1985.

Morton, J. 1999. Mangosteen *Garcina mangostana* L. **Fruits of Warm Climates.** Available source: http://www.hort.pordue.edu/newcrop/morton/mangosteen.html. January 30,2005

Morton, J. 1987. Sugar Apple. p. 69–72. In: Fruits of warm climates. Julia F. Morton, Miami, FL.

Motoyama N., A.P. Kulkani, E. Hodgson and W.C. Dauterman. 1978. Endogenous Inhibitors of Glutathione-S-transferase in House Flies. **pestic. Biochem. Physiol**. 9:255-262.

Motoyama, N. and W.C. Dauterman. 1980. Glutathione – S- transferase: Their Role in the Metabolism of Organophosphorus insecticides. **Rev. Biochem. Toxicol**. 2:49-69.

National cancer institute. 2003. Structure and Data for Gatanin. **Molecular Targets Development Program**. Available Source:http//home.ncifcrf.gov /mtddp /compounds /692946.html, May 10, 2003.

Noichinda, S. 1992. Effect of Modified Atmosphere Condition on Quantity and Storage Life of Mangosteen (Garcina *mangostana* L.) fruit (in Thai). M.S. Thesis, Kasetsart University, BKK Thailand.

Parvin, M.S. 2002. Phytochemical and Biological Studies on *Annona squamosa* Linn. M. Pharm Thesis, Rajshahi University, bangladesh.

Patricia, C.P. 1998. Mangosteen: General Crop Management. **Horticulture and Fresh Produce**. Available Source: http://www.Dpi.gld.gov.au/horticulture/5447.html, January 30, 2003.

Ruamthum, W. 2002. **Effect of Nutgrass (*Cyperus rotundus* L.) Corn Extracts on Esterase and Sugar Apple Seed Level in Digestive System of the Golden Apple Snails (*Pomacea canaliculata* Lamarck)**(in Thai)**.** M.S. thesis, Kasetsart University, Bangkok Thailand.

Saisongkhroh, B. 2006. **Esterase and Glutathione-s-transferase Activities in *Spodopter litura* L. After Exposure to Some Plants Extracts**(in Thai)**.** M.S. thesis, Kasetsart University, Bangkok Thailand.

Siripassaraporn, A. 1995. **The Application of Neem Extracts in the Controlling of Insect pests of Okra (*Abelmoschus esculentus* L.) on Farmer's Field** (in Thai). M.S. thesis, Kasetsart University, BKK Thailand.

Smitinand, T. 2001. **Thai Plant Names** (in Thai). 2nd ed. (Revised edition). The Forest Herbarium, Department of Forest, Bangkok.

Sorensen, Marten. 1996. Yam bean (Pachyrhizus DC.). Promoting the conservation and use of underutilized and neglected crops. 2. Institute of Plant Genetics and Crop Plant Research, Gatersleben / International Plant Genetic Resources Institute, Rome.

Srikhong, P. 2006. **Effects of Some Plant Extracts on Esterase and Glutathione-S-transferase Activity in *Aedes aegypti* L.**(in Thai)**.** M.S. thesis, Kasetsart University, Bangkok Thailand.

Srisaard I *et al.* 2005. Effect of sweet apple seed on mortality and some detoxification enzyme in green leafhopper (*Nephotettix virescens*). **The Seventh Nation Plant Protection Conference.** 2-4 November 2005. Lotus Pangsuankaew, Chiengmai, Thailand.

Thummasarangkoon, W. 2000. **Efficiency of Lemon Grass (*Cymbopogon winterianus* Jewitti) Extracts on Mortality and The Levels of Detoxification Enzymes in Larva of *Culex pipien quinquefasciatus*** (in Thai)**.** M.S. thesis, Kasetsart University, BKK Thailand.

Visetson, S. 1991. **Insecticide Resistance Mechanism in the Red Rust Flour Beetle (Tribolium *castaneum* Burst).** PhD. Thesis. The University of Sydney, Australia.

_____. 2001. Effect of Azadirachtin from Various Thai Neem Extracts on Some Detoxification Enzyme Activity in Callosobruchus *maculates* F., pp. 38-46. **20th ASEAN/ 2nd APRC Seminar on Postharvest Technology, September 11-14, 2001**. Lotus Hotel Pang Suan Kaew, Chingmai Thailand.

_____ and M. Milne. 2001. Effect of Root Extracts From Derris (Derris eliptica Benth) On Mortality and Detoxification Enzyme Levels in the Diamondback Moth Larvae (Plutella xylostella L.). **Kasetsart J. (Nat.Sci.).**35:157-163.

_____, _____ and J. Milne. 2001. Toxicity of 4,11 – Selinnadien – 3 –one from Nut sedge (Cyperus *rotundus* L.) Tuber Extracts to Diamond Black Moth Larvae (*Plutella Xylostella* L.), Detoxification Mechanism and Toxicity to Non Target Species. **Kasetsart J. Nat. Sci.** 35: 284-292.

_____, W. Purivirojkul, P. Kannasutra and H. Rose. 2002. Insecticide Resistance Mechanisms in Various Strains of the Rust-red Flour Beetle (Tribolium *castaneum* Herbst). **Agr. Sci. J.** 33(6 Suppl.): 318-324.

_____ , J. Milne, M. Milne and P. Kannasutra. 2003. Synergistic Effects of sesame Oil With Cypermethrin on the Survival and Detoxification Enzyme Activity of

Plutella xylostella L. Larvae. In **The 6th International Conference on Plant Protection in the Tropics " Globalization and Plant Protection in Developing Economics" 11-14 August 2003**. Pan Pacific Hotel.

_____, S. Khanananukhulchai and J. Milne. 2004. Detoxification Mechanism of Larvae of Diamondback Moth (*Plutella xylostella* L.) Against Various Allelochemicals in Thailand. **The 2nd Asian International Conference on Ecotoxicology and Environmental Safety,** September 26-29, 2004, BP Samila Beach Hotel, Songkla Thailand.

Vungsilabutr, P. Suwat, R., Rewat, P., Chalermwon, T. and Wanich , Y. 1995. **Friend and enemy of farmer** (in thai). Division of Entomology and Zoology, Department of Agriculture, Bangkok.

Wanichkul, K. and S. Kosiyajinda. 1984. Postharvest Handing of Mangosteen in Thailand, pp. 188- 190. In Azucena, C.F., Thomas, D.K. and Fernandenz, J., eds. **Seminar paper, ASEAN-Postharvest Horticulture Training & Research Center.** Department of Horticulture, College of Agriculture University of the Philippines.

Wannasiri, S. 1998. Mangosteen 4th (in Thai). Thankasetkaum Printing, Nonthaburi Thailand.

Widianarko B. *et al.* 2000. Associations between Trace Metals in Sediment, water, and guppy, *Poecilia reticulate* (Peters) from urban streams of Semarang, Indonesia. **Ecotoxicology and Environmental safty.** 46: 101-107.

Zhu, K.Y. and W.A. Brindley. 1990. Acetyl- Cholinesterase and its Reduced Sensitivity to Inhibition by Paraoxon in Organophosphate-Resistant Lygus *hesperus* Knight (Hemiptera: Miridae). **Pestic. Biochem. Physiol.** 36: 22-28.

APPENDICES

APPENDIX A

ENZYME ACTIVITY ANALYZED METHOD

APPENDIX A

Enzyme Activity Analyzed Method

This research using modified method of Yang et al. (2004) and Visetson and Milne (2001) that's described below;

Apendix A1

The reagent for analyzed

1. Buffer : Potassium phosphate buffer (KH_2PO_4)
   Potassium dihydrogenthiophosphate (M.W = 136.09)

   0.1 M = M.W / L
   = 13.609 g / ddw 1 L

   Adjust pH

2. 1mM EDTA
   0.45224 g/ ddw 1 L

3. 1L 0.1 M Phosphate buffer + 1 ml 1mM EDTA

4. 10 mM GSH
   0.15g GSH reduced form in 50 ml 0.1 M Phosphate buffer

7. PVPP: 50%w/w material

Appendix A2

Enzyme Isolation Method

Homogenized material with 2000 µl 0.1 M PPB + EDTA

1000 µl 10 mM GSH

50% w/w PVPP

Centrifuged at 18,000 rpm, 4ºC 5 min in refrigerated centrifuge

Appendix A3

pNPA assay (Modified from Visetson (2001) and Visetson et al. (2003, 2004).

This protocol is for analyzed carboxylesterase activity using spectrophotometer at absorbance 400 nm (3 min)

Stock solution:

- 1 ml 1mM EDTA + 0.1 M Phosphate buffer
- 10 mM Glutathione reduced form (0.15 g GSH / 50 ml buffer)
- Substrate: 0.12 M paranitrophenylacetate(pNPA) (0.1 g / 5 ml ethanol)

Appendix Table A1 PNPA assay protocol

Solution reference	Blank	Sample
0.1 M Phosphate buffer	2900 µl	2900 µl
Substrate	50 µl	50 µl
0.1 M Phosphate buffer + EDTA (Homogenized buffer)	50 µl	-
Supernatant (enzyme)	-	50 µl

Enzyme activity (nM paranitrophenol product/ mg protein/ml)
= O.D x 58.8235 x (total volume)

Appendix A5

CDNB assay protocol (Modified from Visetson et al. (2002, 2003) and Bullangpoti (2002). This protocol is for analyzed Sugar Apple Seed activity using spectrophotometer at absorbance 340 nm (3 min)

Stock solution:

- 1 ml 1mM EDTA + 0.1 M Phosphate buffer
- 10 mM Glutathione reduced form (0.15 g GSH / 50 ml buffer)
- Substrate: 150 mM CDNB (0.152 g / 5 ml ethanol)

Appendix Table A2 CDNB assay protocol

Solution reference	Blank	Sample
0.1 M Phosphate buffer	1150 µl	1150 µl
0.1 M Phosphate buffer + GSH	20 µl	-
Supernatant (enzyme)	-	20 µl
Equilibrate 35 °C for ca. 3 min		
Substrate	10 µl	10 µl

Enzyme activity (CDNB conjugated product/ mg protein/ ml)
= (O.D x 1.316) / (9.6 x 1000)

APPENDIX B

BRADFORD METHOD

APPENDIX B

Bradford Method

This research use bovine serum albumin (BSA) as standard

Appendix B1

Stock solution:

- 100 mg Coomassie Brilliant Blue R250 in 50 ml 95% ethanol then add 100 ml 85% H_3PO_4 and make final volume to 1L by distilled water.

Appendix Table B1 Bradford assay protocol

Solution reference	Volume
Sample	0.5 ml
Bradford solution	5 ml
Incubate at room temp. for 5 minutes	
Determine by spectrophotometer at 595 nm	

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