Chapter 2

Literature Review

2.1. Aphid in Thailand

Aphids are one of the most injurious insect groups to agricultural economic crops. They colonize underneath the leaves, at the terminal leaves or young shoot, flower, fruits even though on the stem of host plants. The economic importance of aphids is outlined in three ways.

(1) Robbing of the plant sap.

(2) Toxic action of their salivary secretions, injected during feeding, thus causing stunting of growth, deformation of leaves and fruits, and producing galls on leaves, stem and root

(3) By acting as vectors of viral diseases to various host plants

Dispersion of plant viral diseases occurred mainly by winged aphids (alatae) since they can fly for several meters and can be blown up to ten kilometers by wind. Within-field spread of viruses, wingless aphids (apterae) may also be important due to their crawling ability from plant to plant (Bänziger, 1976)

Classification of Aphids is controversial, once belongs to order Homoptera, but currently being classified under the order Hemiptera (Triplehorn and Johnson, 2005). They are very small insects about 1-5 mm, soft bodied and relatively slow moving. It is quite difficult to distinguish aphids from each other due to their similarity in morphological appearance, hence, the detailed studies on taxonomy, biology and ecology are highly prerequisite. Some 60 species of aphids have been recorded in Thailand of which 11 species appear to be of major economic importance, including, *Toxoptera odinae* (Van der Goot); *T. aurantii* (Boyer de Fonscolombe); *T. citricitus* (Kirkaloy); *Myzus persicae* (Sulzer); *Lipaphis erysimi* (Kaltenbach); *Aphis gossypii* Glover; *A. craccivora* Koch; *A. citricola* Van der Goot; *Hysteronevra setariae* (Thomas); *Melanaphis sacchari* (Zehntner); and *Rhopalosiphum maidis* (Fitch) (Bänziger, 1976.).

Malanont (1981) reported 20 species of aphids occurred in Bangkhen area, he had also provided details on the species descriptions and illustrations.

2.2. *Lipahis erysimi* (Kaltenbach)

Synonymy:

L. contermin Walker

L. papaveri Takahashi

Apterous viviparous females.

Color when alive is yellowish green or pale green to green. Color when macerated, head and antennae (except antennal segments 1, 2 and base of 3 are pale) are brown. Pale abdomen with cornicles, cauda, genital and anal plates are light brown.

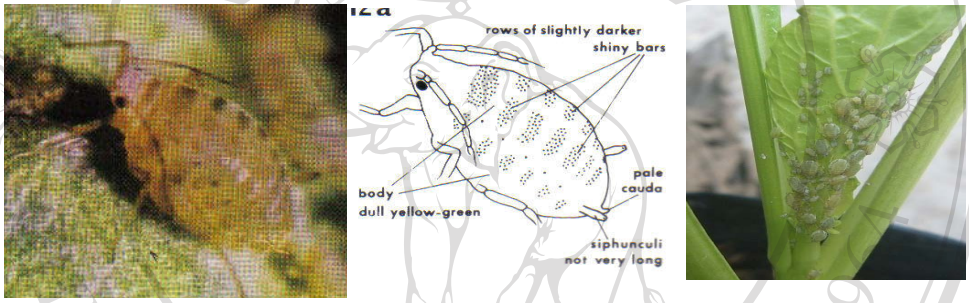


Figure 2.1 *Lipaphis erysimi* Kaltenbach with row of slightly darker shiny bars on body (Bänziger,1977)

Morphology

Body length is approximately 1.55-1.97 mm. Antennae are six-segmented, imbricated with 1.02-1.19 mm long. Rostrum reaching to middle coxae: rostrol IV+V is 0.09-0.1 mm long, Cornicles imbricated with 0.18-0.21 mm long; triangular cauda of 0.15-0.18 mm long with 5 setae (Bänziger,1977).

2.3. Plants with botanical insecticide property for controlling aphids

Areekul (1991) compiled a list of 18 plant species with toxicants against most aphids as followings:

Plant		Plant part	Efficacy
Acorus	*calamus*	Rhizome	high
Alpinia	*conchigera*	Rhizome	high
Calotropis	*gigantean*	Leave, flower, fruit	high
Cassia	*gorrettiana*	Leave, flower, fruit	high
Croton	*tiglium*	Fruit	high
Datura	*alba*	Leave, seed	high
Derris	*scandens*	Root	high
Dioscorea	*hispida*	Rhizome	high
Erythropholocum succirubrum		Seed, seedling	high
Euphorbia	*erigona*	Stem	high
Gloriosa	*superba*	Seed, Rhizome	high
Helianthus	*annuus*	Flower	high
Jantropha	*gossypifolia*	Seed	high
Lantana	*camara*	Leave	high
Melia	*azedarach*	Leave	high
Pachyrhizus	*angulatus*	Seed	high
Sophora	*tomentosa*	Stem	high
Strychnos	*nux-vomica*	Fruit	high

Sudto (1992) reported that 0.5 kilogram broken seed of *Anona squnmosa* L. maculated for 1-2 days in 20 liters of water; and another 2 kilograms of tobacco dry leaves maculated for 24 hours in 100 liters of water. Both of these plant solutions provided effective control of the aphids under field conditions.

Other plant species that contain secondary metabolites with potential use as commercial insecticides including, *Acorus calamus*, *Artemisia tridentata*, *Heliopsis longipes*, *Mammea americana*, and *Tagetes minuta* (Balandrin *et al*., 1985), and also *Stemona* spp. (Pimsamarn *et al.*, 2003).

2.4. Pesticidal properties of derris plant.

Derris elliptica Bentham (local variety) and *Derris malaccensis* Prain (cultivar variety) were another pesticide alternative. Rotenone substance in derris root is a widely used insecticide of botanical origin and also known by Thai people as “Lotin”. It is a selective non-systemic insecticide with some acaricidal properties. Rotenone is used alone or in combination with pyrethrins, pyrethrum and piperonyl butoxide to control a wide variety of insect pests of food crops including, flea beetle: *Phyllotreta sinuata* Stephens (Sottikul, 2001); leaf cutter: *Deporaus marginatus* Pascoe; cucurbit leaf beetle: *Aulacophora similis* Olivier; cotton leafhopper: *Amrasca biguttula* Ishida; mango leafhopper: *Idioscopus niveospasus* Lethierry; cabbage aphid: *Lipaphis erysimi* Kaltenbach; broad mite: *Polyphagotarsoremus latus* (Bank) (Sottikul, 2000; Worawong and Pimsamarn, 2003). In veterinary medicine the compound has been applied directly to treat dog flea: *Ctenocephalides canis* (Curtis); dog tick: *Rhipicephalus* sp.; southern cattle tick: *Boophilus microplus* (Canestrini); and chicken mite: *Megninia* sp. (Sangmanidet *et al.*, 2005). The effectiveness of methanolic extract of *D. elliptica* on broad mite using two bioassay techniques, direct spray with Potter’s spray tower and leaf dip bioassay were conducted. The extract exhibited both contact and stomach poisons against broad mite, with an LC_{50} of 0.0037% (direct spray) and 0.035% (leaf dip bioassay) at one hour after treatment. This study demonstrated the possibility of using *D. elliptica* extract in management of field populations of broad mite (Worawaong and Pimsamarn, 2003). Rotenone solution (root extract of *D. elliptica*; 10% or 20% a.i.) was tested as a bait with 2% hydrolysate protein in field trials to determine its efficiency against olive fly (*Bactrocera oleae* Gmelin). Rotenone toxicity was tested against both adult and immature stages of olive fly in laboratory trials. A notable repellent action of the high concentration of rotenone solution was found while no selective activity of rotenone was observed between the two olive fly sexes (Stavroulakis *et al.*, 2001).

For centuries, South and Central American people have used the Jewel vine to stun fish. When the vine is crushed up and thrown into the water, the fish cannot inhale oxygen through their gills and come to the surface, making it possible for them to kill the fish with their bows and arrows. Also, in World War Two, rotenone was used to kill lice in the trenches. Derris has not appeared on the insecticide market for

two decades since more highly effect synthetic pesticides have been introduced. However these pesticides cause obvious problems including, development of pesticide resistant insects, the threat of contaminate in human food chains, and highly increasing of the production cost, thus awakening derris to return to the market in 1995. These are the two main commercial uses of rotenone today, as a piscicide and as an insecticide (Visetson and Milne, 2001). Rotenone obtained from roots of several tropical and subtropical plant species belonging to genus *Lonchocarpus* or *Derris*. Both exhibite contact and stomach poisons on insects although kill them very slowly, never the less, activate them to stop their feeding almost immediately, exerting the toxic action by acting as a general inhibitor of cellular respiration. Rotenone is sold in dispersible powder, emulsifiable concentrate, and wettable powder formulations. In the U.K., two professional products are registered: Devcol Liquid Derris and Liquid Derris, Another product marketed by pbi contains a mixture of rotenone and sulfur, both a fungicide and insecticide. Products containing rotenone are registered in Denmark, Ireland, United Kingdom, France, Spain, Italy. In the U.K., rotenone products are approved for use against aphids on flowers, ornamentals, protected crops, soft fruit, top fruit and vegetables, and against in pears and rose. Other target organisms of rotenone include maggots, bagworms, coding moths, Japanese beetles, leafhoppers, Mexican bean beetles, cabbage worms, thrips, stinkbugs, flea beetles, and vegetable weevils. Sangmaneedet *et al.* (2005) report that fresh *Derris elliptica* powder (FDP) and dried *D. elliptica* powder (DDP) were effective on killing fly larvae when compared to insecticide powder, Negasunt® DDP (containing 6.69% rotenone) was the most effective treatment which yielded cumulative death percentages of larvae at 38.0%, 70.0%, 80.0% and 88.0% after an exposure for 3, 6, 9 and 12 hours, respectively. FDP (containing 1.94% rotenone) yielded lower cumulative death percentages of 22.0%, 48.0%, 64.0% and 86% with the same exposure periods. Negasunt® was also gradually effective in killing fly larvae and reached the same efficacy as DDP and FDP after the exposure for 12 hours. In vivo, DDP was effective in a treatment of cutaneous myiasis in pigs. All experiment pigs had normal appetite and condition throughout the experiment without any signs of side effect from DDP. Larvae exposed to DDP died within 20 hours and an inflammation caused by larval migration was gradually recovered within 7 days.

The leaves of *Derris malaccensis* were extracted with hexane, ethyl acetate and methanol, respectively. The ethyl acetate extract was subjected to preliminary fractionation using quick column chromatographic technique and was further purified by using column chromatography. The isolated compounds were finally purified by recrystallization. The structures of the purified compounds were determined by using spectroscopic techniques. The structure of the major components in ethyl acetate extract was found to be β-sitosterol (1) and a new prenylated chalcone (2). Study on biological activity of compound (2) showed cytotoxicity to KB cells (LD_{50}: 15 g/ml) and HuCCA-1 cells (LD_{50}: 15 g/ml). Different concentrations of *D. urucu* root extract were tested against fourth instar larvae of *Aedes aegypti*. One hundred percent mortality was observed at application rate of 150 µg/ml (LC_{50}: 17.6 µg/ml) 24 h following treatment (Gusmão, 2002).

Cytological studies of nine species of *Lonchocarpus* and four species of *Derris* reveal only one variant from 2n = 22, *L. utilis* with 2n = 44. Sterility in *L. utilis*, *L. cyanescens*, and *D. elliptica* is probably in each case a clonal rather than species mutation and has been preserved by cultivation. Rotenone production in *Lonchocarpus* apparently increases with polyploidy. No correlation has yet been discovered in *Derris*. The value of further genetic and cytological exploration to the development of commercial rotenone is pointed out (Atchiso,1949).

Some of the plants containing rotenone [Order *Fabales*; Family *Fabaceae* (or *Leguminosae*) – Pea family]: Hoary Pea or Goat's Rue (*Tephrosia virginiana*) - North America, Cubé Plant or Lancepod (*Lonchocarpus utilis*) - South America , Barbasco (*Lonchocarpus urucu*) - South America, Tuba Plant (*Derris elliptica* and *Derris malaccensis*) - southeast Asia & southwest Pacific islands, Jewel Vine (*Derris involuta*) - southeast Asia & southwest Pacific (Ridley, 1922).

2.5. Derris propagation and root production

Sottikul (1999) obtained appropriate stem cutting method with following steps:

1. Selection of the plant stem with approximately 0.80-1.00 cm in diameter
2. Shortening of the selected stem down to approximately 20-25 cm long
3. Defoliation of the selected stem
4. Dipping the stem in 0.3% of IBA

5. Watering 0.1% (w/v) urea at 10 day intervals for 3 times after stem cutting

Tongma *et al*. (2004) founded that derris plants grown at 1.0x0.5 cm spacing provided more root yield than the plants grown at 1.0x1.0, 1.0x1.5 cm, and 1.0x2.0 m spacing. The application of 15-15-15 (N-P_2O_2-K_3O) fertilization at the rate of 25, 50, 75 and 100 kg/rai increased the root yield but no effect on the concentration of rotenone in roots. The comparative studies on growth and yield production of derris plants grown in cement pots filling soil and those of the plants grow in soil at 1.0x1.0 m spacing in the field, the result showed that root of derris cut in slices and dried in shade for 1-2 days, then grinded to power and extracted with alcohol in stirring machine for 20 hr. The extract was filtered and evaporated to almost dryness. Then took one to bioassay with the second instar of diamondback moth larvae using no choice dipping method of Chinese kale leaves. The other one was brought to analyze chemical composition by TLC and HPLC/MS. The results showed that the extract at level of 25 ppm could kill diamondback moth at 50% within 2 day and the chromatographic result showed mainly containing rotenone 12% and rotenone derivatives such as sumatrol, deguelin, toxicarol, elliptone, malaccol, munduserone, pachyrrhizone dolinoone and erosone (Pitiyont and Sangwanich, 1997).

Tissues and cells obtained by in vitro culture of *Derris elliptica* and *Tephrosia vogelii* were extracted with 9:1 CHC_{l3}/MeOH, purified by gradient filtration of the C-18 reversed-phase column and analyzed by HPLC with 66:34 MeOH/H_2O or 42:58 CH_3CN/H_2O as mobile phase. The results showed that rotenone was biologically synthesized by the callus cultures induced from both species though the content of rotenone were low, 17.4 µg/g and 1.8 µg/g dry weights, respectively. The rotenone content of root-differentiated callus culture of *Derris* was dramatically increased to 136.2 µg/g dry weight, being 7.8 folds of that of the callus culture. The cell suspension culture of *Tephrosia* showed higher increase in rotenone content than the tissue culture, but the rotenone content was still lower than that of callus culture of *Derris*. These results might imply that genotype played an important role in the secondary metabolism and that the differentiation of tissues or cells favored in vitro biosynthesis of rotenone (Xinnian *et al*., 2000).

2.6. Physical and chemical properties of rotenone

Rotenone is a naturally occurring rotenoid plant extract from South America, Australia and many countries in Southern Asia. It is found in the roots and stems of several tropical plants, jewel vine (*Derris* spp.), lancepod (*Lonchocarpus* spp.) and hoary pea (*Tephrosia* spp.) being the most common. It has been used for centuries as a selective fish poison and more recently as a comment insecticide, it is a selective, non-specific botanical insecticide with some acaricidal properties. Rotenone is used in home gardens for insect control for lice and tick control on pets and fish eradications as part of water body management. Rotenone containing extracts are taken from the roots, seeds and leaves of the various plants. Recent research in rotenone stems mainly of biochemical interest focusing on its highly specific action in selectively inhibiting mitochondrial activity and its possible anticancer properties (Fang and Casida, 1998).

Physical Properties.

Rotenone is a colorless, crystalline solid with a melting point of 165-166ºC and has a very low solubility in water at ambient temperatures. It is soluble in acetone, carbon disulfide, ethyl acetate, and chloroform. It is less soluble in ether, carbon tetrachloride, and petroleum solvents. Solutions are readily oxidized, in the presence of light and alkali, to products with weaker insecticidal properties. Rotenone is an unstable compound that breaks down when exposed to the environment. It is ultimately converted to carbon dioxide and water. The breakdown process is rapid and is affected by temperature, light, oxygen and alkalinity (Brunson, 1954).

Rotenone

Chemical Name: (2*R*,6a*S*,12a*S*)-1,2,6,6a,12,12a–hexahydro-2-isopropenyl-8,9-dimethoxychromeno [3, 4-*b*] furo [2,3-h]chromen-6-one

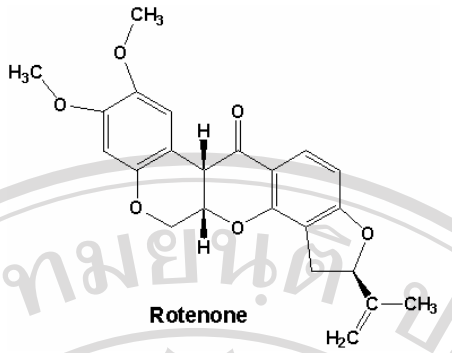
Empirical formula: $C_{23}H_{22}O_6$

CAS Number: 83-79-4

Molecular weight: 394.41

Water solubility: 15 mg/l 100ºC, slightly soluble in water

Melting point: 165-166ºC



Chemical structure of rotenone

2.7 The rotenone extraction

The extraction of active compounds from plants is one of the most critical steps in the commercial development of natural products for medicinal, herbicidal or pesticidal use. Extraction methods, such as Soxhlet extraction, stirring soaking, and solvent extraction, are commonly used to extract rotenone from derris plants (Visetson and Milne, 2001). However, these methods are time consuming, involve high solvent consumption and may have lower extraction efficiencies. In order to reduce the use of organic solvents and improve the extraction processes, pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) has been used since 1995 as an alternate technique. The pressurized liquid extraction (PLE) technique was applied for the efficient extraction of rotenone from the stem and root of *Derris elliptica* Bentham and *Derris malaccensis* Prain. The effects of experimental variables, such as solvent, temperature and pressure, on PLE efficiency had been carried out. Chloroform was determined as a superior extraction solvent (rotenone content 40.6%, w/w) comparative with commonly used solvent, 95% ethanol (rotenone content 15.0%, w/w). The optimal condition for PLE application was 50ºC with 2000 psi. PLE showed better extraction efficiency (rotenone content 46.1%, w/w) as compared with conventional maceration method (rotenone content 40.6%, w/w). The rotenone content sequence from the highest to the lowest were detected from root (46.1%, w/w) and stem (9.4%, w/w) of *D. elliptica* and stem of *D. malaccensis* (5.2%, w/w), respectively. In addition, PLE application was convincingly consumed less time and solvent (30 min, 3 ml/g of dried sample) than the conventional maceration techniques (72 h, 10 ml/g of dried sample) (Sae-Yun *et al*., 2006)**.**

The chemical components of derris root extract were rotenoids such as deguelin, β-dihydrorotenone, dehydrorotenone, rotenone, 6alpha beta, 12alpha beta-rotenolone, and tephrosin. They were chromatographed on 8-12 mum silica. A mobile phase of chloroform-isooctane (35+65) pumped at a flow rate of 1 ml/min through a 30 cm column was used and the absorbance of the eluate was monitored at 294 nm. Rotenone, β-dihydrorotenone, deguelin, and dehydrorotenone are completely resolved while 6alpha beta, 12alpha beta-rotenolone and tephrosin chromatograph as one peak. This method has potential as a preparative separation technique for rotenoids. Also described is a procedure to quantitatively measure rotenone in pesticide formulations. Samples were extracted with chloroform and chromatographed at a flow of 2.5 ml/min. The method is rapid (rotenone is eluted in 12 min) and reproducible (Bushway *et al*., 1975).

Rotenone and rotenoids (deguelin, rotenolone (12a-hydroxyrotenone), tephrosin (12a-hydroxydeguelin), 12aR-hydroxyrotenone, and dehydrorotenone) were determined in cubé resins and formulations. Cubé resins from Lonchocarpus contain large quantities of deguelin (ca. 21.2%) and smaller quantities of tephrosin (ca. 3.5%) and rotenolone (ca. 3.0%). The composition of commercial formulations may present very different rotenoid contents depending on the extracts used to prepare them. Because these rotenoids also present insecticide activity, the efficacy of these formulations may be very different. The storage stability and photodegradation of some rotenone formulations were studied. Rotenone and rotenoids are very sensitive to solar radiation, which degrades them rapidly, with half-lives in the order of a few tens of minutes. Some formulations show greater disappearance rates than that of cubé resin, indicating that not much attention has been paid to protecting the active ingredients from photodegradation in the formulation. A study on the residues on olives was also carried out to assess not only the rotenone content, but also that of the main rotenoids. At harvest, the residues of deguelin, tephrosin, and rotenolone were 0.10, 0.06, and 0.10 mg/kg, respectively, very similar to rotenone (0.08 mg/kg), and though a few data indicate similar acute toxicity values for deguelin, only rotenone is taken into consideration in the legal determination of the residue (Cabizza *et al*., 2004).

Cubé resin, the root extract from *Lonchocarpus utilis* and *L. urucu*, is an important insecticide, acaricide, and piscicide. The four major active ingredients are

rotenone, deguelin, rotenolone, and tephrosin, totaling 77 % wt. As a commercial pesticide, the minor constituents are also of chemical interest and toxicological relevance. This study identifies 25 minor rotenoids in cube resin "brittle" of which 12 are new compounds, the most unusual being 7'-chloro-5'-hydroxy-4',5'-dihydrodeguelin (the first chlororotenoid from a plant extract) and four isomers of 4',5'-dihydro-4', 5'-dihydroxytephrosin. Several of the minor rotenoids may be decomposition products from free radical processes during sample preparation, extraction with trichloroethylene, and processing the resin. Assays of the 29 rotenoids as inhibitors of NADH: ubiquinone oxidoreductase activity (primary target for toxicity) and phorbol ester-induced ornithine decarboxylase activity (indicator of cancer chemopreventive action) and for cytotoxicity establish similar structure-activity relationships in each system and the importance of the overall molecular conformation and the E-ring substituents (Fang and Casida, 1999).

2.8 Toxicological effects of rotenone

Acute toxicity: Local effects on the body include conjunctivitis, dermatitis, sore throat, and congestion. Ingestion produces effects ranging from mild irritation to vomiting. Inhalation of high doses can cause increased respiration followed by depression and convulsions. The compound can cause a mild rash in humans and is a strong eye irritant to rabbits (Ray, 1991). The oral LD_{50} of rotenone ranges from 132 to 1500 mg/kg in rats. The reported oral LD_{50} of rotenone in white mice is 350 mg/kg (Kidd and James, 1991). The oral LD_{50} of rotenone ranges from 300 to 500 mg/kg in humans (Ray, 1991).

Chronic toxicity: Growth retardation and vomiting resulted from chronic exposures of rate and dogs. Rats fed diets containing rotenone at doses up to 2.5 mg/kg for 2 years developed no pathological changes that could be attribute test to rotenone. Doge fed doses of rotenone up to 50 mg/kg day for 28 days experienced vomiting and excessive salivation, but no decreased weight gain. Dogs fed rotenone for six months at dose up to 10 mg/kg/day had reduced food consumption and therefore reduced weight gain (National Research Council, 1983).

Reproductive effects: Pregnant rats fed 10 mg/kg/day on day through 15 of gestation experiences decreased fecundity, increased fetal resumption, and lower birth weight (U.S. National Library Databank, 1995).

Mutagenic effects: The compound was determined to be non mutagenic to bacteria and yeast ant in treated mice and rats. However, it was shown to cause mutations in some cultured mouse cells (Ray, 1991; U.S. National Library Databank,, 1995).

Carcinogenic effects: Studies in rats and hamsters have provided limited evidence for carcinogenic activity of rotenone. No evidence of carcinogenic activity was seen in hamsters at oral dose as high as 120 mg/kg/day for a period of 18 months. Studies of two species of rats evidenced no statistically significant cancerous changes in any organ site, including mammary glands, at oral dose of up to 75 mg/kg/day for 18 months. Significant increase in mammary tumors have been reported in albino rats with intraperitoneal doses of 1.7 mg/kg/day for 45 days, and in wistar rats at approximately 1.5 mg/kg/day orally for 8 to 12 months. In the later study, however, higher dose rates (3.75 and 7.5 mg/kg/day) over the same period did not produce increased tumor. Thus, the evidence for carcinogenicity is inconclusive (National Institute of Health, 1984).

Fate in humans and animals: Absorption in the stomach and intestines is relatively slow and incomplete, although fats and oils promote its uptake. The liver breaks down the compound fairly effectively. Animal studies indicate that possible metabolites are carbon dioxide and a more water-soluble compound that can be excreted in the urine. Studies incited that approximately 20% of the applied oral dose may be eliminated from animal systems within 24 hours (Ray, 1991).

Effects on birds: Rotenone is slightly toxic to wildfowl. The LD_{50} values for rotenone in mallards and pheasants are 1 (greater than) 2000 mg/mg and 1680 mg/kg respectively. A dietary LC_{50} of 4500 to 7000 ppm is reported in Japanese quail (Hill, *et al.,* 1975).

Effects on aquatic organisms: Since rotenone is use as a fish toxin, it follows that it is very highly toxic to fish. Reported 96 hour LC_{50} were

0.031 mg/l in rainbow trout

0.0026 mg/l in channel catfish

0.023 mg/l in bluegill for the 44% pure formulation (Rejesus *et al.,* 1995).

Acute toxicity test of derris extract (*D. elliptica*) (0.17%) and derris formulation (0.24%) on *Tilapia nilotica* L. (2.75+0.28 cm and 7.25+0.89 cm) were investigated. Both derris solutions were made in various dilutions and dissolved into water. The results were determined after 96 hours. LC_{50} of rotenone from derris extract was 0.0007 mg/l and 0.0008 mg/l in formulation for the small tilapia. On the other hand, LC_{50} of rotenone from derris extract for the large tilapia was 0.008 mg/l and 0.08 mg/l in the formulation. The study on cholinesterase activity in the survived tilapia after experiments revealed that Rotenone could inhibit the cholinesterase activity in fish brain (Unjitwatana *et al*., 2006).

Environmental fate: Breakdown in soil and ground water; Rotenone is rapidly broken down in soil and in water. The half-life in both of these environments is between 1 and 3 days. It does not readily leach from soil and it is not expected to be a groundwater pollutant. Rotenone breaks down readily by exposure to sunlight. Nearly all of the toxicity of the compound is lost in 5 to 6 days of spring sunlight or 2 to 3 days of summer sunlight (Kidd and James, 1991).

2.9 Bioassay

Broad mite: *Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae) is an important pest of chili because of its short life cycle. Chemicals were used regularly for broad mite control. The aim of this study is to evaluate for the effectiveness of methanolic extract of *D. elliptica* on broad mite using two bioassay techniques, direct spray with Potter's spray tower and leaf dip bioassay. The extract exhibited both contact and stomach poisons against broad mite, with an LC_{50} of 0.0037% (direct spray) and 0.035% (leaf dip bioassay) at one hour after treatment. This study demonstrated the possibility of using *D. elliptica* extract in management of field populations of broad mite (Worawong and Pimsamarn, 2003).

Two types of ethanol extraction methods. Soxhlet and stirring soaking, were carried out. The rotenone content determined by the HPLC was 8.6% w/w for the former method compared to 5.2% w/w for the latter one. Third instar larvae of the diamondback moth gave LD_{50} of 24.25 ppm and 89.07 ppm for the Soxhlet and stirring methods, respectively (Visetson and Milne, 2001).

2.10. Mode of action of rotenone

The mechanism of action in higher organisms is on interference with the electron transport chain at the inner mitochondrial membrane**.** It has low toxicity to mammal (Matsumura,1985). It including shutdown of the electron transfer through respiratory chain complex I, decreasing cellular ATP level, increasing mitochondrial reactive oxygen species (ROS) production, and decreasing mitochondrial membrane potential (Li *et al*., 2003).

2.11 Formulation

Rotenone toxicity is highly species specific with exceptional toxicity in gill breathing organisms. Rotenoids are detoxiced by cytochrome P-450 mixed function oxidases, enzymes inhibited by the aromatic aliphatic polyether piperonyl butoxide (PBO) as such. PBO is a rotenone synergist and commercial piscicide formulations containing both materials have increased potency and residual activity (William *et al.*, 1999). Insecticidal formulations in the form of dusts or aerosols containing rotenone from the root of *Derris elliptica* and pyrethrum from the flower of *Chrysanthemum cinerariaefolium* are commonly used in homes and gardens. Pyrethrin is a natural product extracted from white Chrysanthemum flowers, that have been used as insecticides for over a century. It works against a very wide range of insect pests. The main active principles of Pyrethrum flowers are known as Pyrethrins. Pyrethrin is a fast-acting poison which disrupts the nervous system and causes paralysis of the insect, while at the same time being non-toxic to warm-blooded animals. Pyrethrins are also biodegradable and they break down very quickly in sunlight, moisture and oxygen. Pyrethrins are usually combined with other insecticides like rotenone to ensure their effectiveness. Ronnel (fenchlorphos), a systemic insecticide is used to control house flies and upon oral treatment ectoparasites of cattle. These insecticides are extensively used and their teratology potential has not been fully investigated. Technical grades of rotenone at 0, 2.5, 5, or 10 mg/kg, pyrethrum at 0, 50, 100, or 150 mg/kg (rotenone and pyrethrum were of natural origin) and ronnel at 0, 400, 600 or 800 mg/kg were tested. Each of these was suspended in corn oil and administered orally in single daily doses on d 6-15 of pregnancy to Wistar rats. The dams were killed on the last day of pregnancy, and all fetuses were evaluated following routine teratologic

methods. Rotenone was associated with an increased number of nonpregnant rats and resorptions, at a dose of 10 mg/kg; reductions in maternal body weight gain, fetal weight, and skeletal ossification, together with an increased incidence of extra rib, were found at 5 and 10 mg/kg; but no significant effects were found at 2.5 mg/kg. Increases in the incidence of resorptions in pyrethrum-treated groups and of extra rib in runnel-treated groups were also observed (Khera *et al.,* 1982). Chili or chili pepper, the ripe fruits and seed contain insecticidal compounds and they can control aphids and caterpillars. Highly concentrated chili solution has been known to produce similar results to synthetic insecticides. Nicotine, derived from tobacco, is extremely toxic and fast acting on most animals. The nicotine of half a cigarette if injected into the blood is enough to kill a full-grown man. An additional danger of using tobacco leaf extract is that this extract may contain a virus disease called Tobacco Mosaic Virus or TMV. This virus disease affects a wide range of plants, for example tomato. Nicotine kills insects by contact and if inhaled or eaten. The most common use is to control soft-bodied insects such as aphids, mites, and caterpillars. Thungrabeab and Tongma (2007) reported that the derris extract was use at 1% and 0.1% incorporated to a culture medium (malt extract peptone agar) and distributed into petri-dishes for their effects on vegetative growth and conidia viability. The vegetative growth of all fungi test was inhibited by 1% and 0.15 of derris extract but they did not affect the viability of conidia.