Chapter 8

General discussion and conclusion

8.1 General discussion

The identification of the two varieties of derris, *Derris malaccensis* Prain (cultivar variety) and *Derris elliptica* Bentham (local variety) were investigated by the difference pattern appearances for the basic enzymes of young and mature derris leaves analyzed by peroxidase (POX), esterase (EST), and acid phosphatase (ACP) method. Obviously, in determining the derris plants varietal species the POX isozyme pattern is being one of the methods in identifying the derris mature leaves, while the EST isozyme pattern is better for the young leaf identification. Certainly, the ACP pattern is the best method for mature and young leaf identifications of both derris varietal species. Besides, the distincquishable morphological characteristics of *D. elliptica* and *D. malaccensis* were also observed.

Rotenone contents from various parts of derris cultivar and local varieties, including, mature leaf, young tip, stem, and various root sizes of 0.4, 0.9, and 1.5 cm in diameters were investigated. The superior quantity of the rotenone content existed in the cultivar variety (*D. malaccensis*) in almost all plant parts with the highest content of 4.105% prevailed in 0.9 cm diameter root. Accordingly, in pest management practice the mass cultivation of the cultivar variety for better rotenone quantity is highly recommended. Hence, *D. malaccensis* with the optimum root size range of 0.8-1.0 cm in diameter should provide the highest level of rotenone content.

Trials on maximizing of the derris root system and rotenone content with appropriate plant ages cultivated in various container types and sizes as compare to the field condition were conducted. Overall, the plant biomass in terms of total fresh and dry weights of each plant (gm/pt); the root biomass in terms of root fresh and dry weights (gm/pt); and rotenone quantity in terms of percentages of rotenone content in fibrous and branch roots are normally growth with ages, hence, the twelve-month derris plants exhibited significantly highest levels of all mentioned variables than other plant ages in the trials. Types of containers either plastic bags or plastic pots showed no consequence on those variables, although the biggest values of tested variables were

recorded in the plant cultivated in the bigger size container (13"x 26"), they demonstrated non-significant different to certain variables of plants developed in smaller size containers. When considering root harvesting advantage, the bigger size, especially 13"x 26" container, provided easily harvested with less time and labor consuming in addition with less root damage than obtaining from the field derris plants. Thus, the twelve-month derris plant developed in 13"x 26" container is highly recommended for better root biomass and higher rotenone quantity percentage.

Experiments on modifying conventional maceration extraction technique by combining with certain volumes of ethanol to maximize obtainable rotenone and its derivatives for effectiveness of the economic insect pest management were conducted. Maceration the samples in 10 ml of 95% ethanol certainly ample dissolvability of the rotenone substances in the solutions, the means of rotenone content of the samples were enormously increased. Hence, the modified conventional water extraction method by adding 10 ml of 95% ethanol in the treatment combination could furnish better rotenone quantity. The Rotenone concentration (y) can be calculated from the value of light absorbance (X) using the linear equation of $y = 814.20(X) - 79.128$ and the R^2 was 0.9893. This equation was applied to estimate rotenone content in water extraction of derris fresh root by mean of the Spectrophotometer instruments, which demonstrated less time-consuming and less expensive than application of the HPLC instruments. There were 14 chemical substances occurred in derris root precipitate, however; only 7 compounds were verified. The rotenone quantities extracted from 1 gm of derris root powder in non evaporate state with varying volumes of 5, 10, 15, and 20 ml of 95 % ethanol were 7.74, 6.89, 4.80, and 3.87 %, respectively. The rotenone contents detected from all treatments in evaporate state were not significantly different, the average rotenone content of 10% concentration (w/v) of crude extract was approximately 12 %. The extraction of 1 gm derris root with 5 ml of 95 % ethanol (200 gm /1 liter of 95 % ethanol) was demonstrated to be the appropriate volume for ethanol supplement in modified water extraction of derris root powder.

Trials on determination of rotenone degradation for sprayed residues in selected environment were investigated. For indoor condition depends on methods of extractions, 30 hours after treatment applications the percentages of rotenone degradations were between 75.15–90.12% by water extractions and rotenone residue concentrations was

within a range of 17.44-57.80 ppm, while ethanol extractions provided the percentages of rotenone degradations was within a range of 59.6–62.34% and rotenone residue concentrations were between 20.46 – 46.23 ppm. For outdoor condition at 30 hours after treatment applications the percentages of rotenone degradations was within a range of 91.72-96.05% and rotenone residue concentrations were between 4.50 – 9.19 ppm. In the cabbage field condition, 12 hours after treatment applications rotenone residue concentrations were less than 1 ppm and percentages of rotenone degradations was within a range of 78.40–90.72%, the amount of rotenone toxicants were very low and safe. The rotenone residue concentrations were rapidly decreased to below the detection limit within 24 hours. Hence, the appropriate harvest time for field cabbage was 24 hours after assigned treatment applications in order to prevent possible contamination of rotenone toxicants in the human food chains to ensure the food safety practice and environment protection.

Experiments on determination of the appropriate chemicals for shelf-life extension and efficacy enhancement of the rotenone formulated product for controlling the cabbage aphid, *Lipaphis erysimi* (Kaltenbach), were conducted. Overall, after 4 months of storage at room temperature, derris extract mixed with 1% ascorbic acid exhibited the highest percentages of rotenone residue (84.39%), while derris extract added with 1% propylene glycol provided the second highest percentages of rotenone residue (78.48%). Thus, this experiment confirmed that ascorbic acid and propylene glycol should be appropriated chemical substances to extend shelf life and prolong activity of the derris formulated product. Under laboratory condition, the extract of derris root mixed with Tween 80 furnished the highest insect mortality within 24 hrs with the value of LC_{50} was 4.33 ± 0.23, while the non-mixed derris root extract contributed less efficacy with the value of LC_{50} was 34.12 $\pm$ 3.52 ppm. At the field condition, 24 hrs after the treatments application the aphids numbers in the check treatment (299.98 insects/plant) was significantly higher than the obtained aphid numbers from the mixed derris root extract with Tween 80 treatment (44.90 insects/plant). The similar consequences also achieved through the second and third treatment applications. Hence, Tween 80 demonstrated strongly synergistic effect on rotenone toxicants, adding 1% of Tween 80 to the derris root extract was firmly enhanced the efficacy of the derris formulated product for the cabbage aphid control. However, Tween 80 played no

important role on the fresh weight, including width and length, and leaf chlorophyll activity of the field cabbages.

8.2 Conclusion

In identification of the two varieties of derris, *Derris malaccensis* Prain (cultivar variety) and *Derris elliptica* Bentham (local variety), the ACP (acid phosphatase) pattern is the best method for mature and young leaf identifications of both derris varietal species. The distincquishable morphological characteristics of both varieties were also observed.

D. malaccensis with the optimum root size range of 0.8-1.0 cm in diameter should provide the highest level of rotenone content. The twelve-month derris plants exhibited significantly highest levels of the plant biomass, the root biomass, and rotenone quantity than other plant ages in the trials. Types of containers either plastic bags or plastic pots showed no consequence on those variables. When considering root harvesting advantage, the bigger size, especially 13"x 26" container, provided easily harvested with less time and labor consuming in adjunct with less root damage than obtaining from the field derris plants. Hence, the twelve-month derris plant developed in 13"x 26" container is highly recommended for better root biomass and higher rotenone quantity percentage.

Modification of conventional water extraction method by adding 10 ml of 95% ethanol in the treatment combination could furnish better rotenone quantity. The rotenone contents detected from all treatments in evaporate state were not significantly different, the average rotenone content of 10% concentration (w/v) of crude extract was approximately 12 %. The extraction of 1 gm derris root with 5 ml of 95 % ethanol (200 gm /1 liter of 95 % ethanol) was demonstrated to be the appropriate volume for ethanol supplement in modified water extraction of derris root powder. There were 14 chemical substances occurred in derris root precipitate, however; only 7 compounds were verified.

Under selected environmental conditions: indoor; outdoor; and in the cabbage field; the rotenone residue concentrations on crop plants were rapidly decreased to below the detection limit within 24 hours. Hence, the appropriate harvest time for field cabbage was 24 hours after assigned treatment applications in order to prevent possible contamination of rotenone toxicants in the human food chains to ensure the food safety practice and environment protection.

Tween 80 demonstrated strongly synergistic effect on rotenone toxicants, adding 1% of Tween 80 to the derris root extract was firmly enhanced the efficacy of the derris formulated product for controlling the cabbage aphid, *Lipaphis erysimi* (Kaltenbach).

8.3 Recommendations for field application and storage

Rotenone is rapidly broken down by sunlight; hence, the critical spray application times could be applied either in the evening or very early in the morning to achieve the best pest protection results. Since rotenone is a broad-spectrum botanical insecticide effective against several economic insect pests include true bugs, caterpillars, beetles, aphids, flies, whiteflies, thrips, leafhoppers, and others. The appropriate time for treatment application could enhance rotenone efficacy for controlling several caterpillars of noctuid moths and other nocturnal pests who active at night and also other slow-moving pests aggregated on crop leaves in the morning. The farmers could provide the extensive delicate spray coverage to allow the bioactive compound to contact the pests directly. Derris formulated product can have an extended shelf life if the storage area is cool, dry, and out of direct sunlight. Protection from temperature exthemes is important because heat or cold can shorten its self life. After treatment application, do not enter the treated area for 12 hours and the crops may be harvested 24 hours later to allow no residue problem along with assurance of the food safety practice and environment protection.

8.4 Suggestion for future experiment

Rotenone is an environmentally friendly biopesticide with limited duration of efficacy in the field. The short residual activity of this pesticide due to sunlight induced degradation process, thus, decreases the usefulness of this pesticide and requires extensive amount of toxicant to achieve the desired effect. Hence, there a need for product enrichment with alternative additive materials to improve the degradation resistance and enhanced rotenone activity. Intensive investigations on the following additive materials include the UV protectants, such as Benzotriazole Anti UV 328, Benzotriazole Anti UV 5411 (Octrizole), sulfite lignin, sulfonated lignin, sulfonated lignite, sulfonated tannins, naphthalene sulfonates, and other related compounds; activity enhancers, such as astaxanthin, α-tocopherols (vitamin E), beta-carotene, diazabicyclooctane (DABCO),

hexamethylenetetramine (HMTA), tetraehtylenepantamine (TEPA), petaethylenehexamine (PEHA), egg albumin, bovine serum albumin (BSA), and other related compounds; synergistic additives, such as Tween 20, Tween 40, Tween 60, piperonyl butoxide (PBO), bis(2,3,3,3-tetrachloropropyl) ether (S-421), N-Octyl bicycleheptene dicarboximide (MGK-264), and other related compounds; stickers or spreaders, such as APSA-80, Fiercer, Sunlight, and other related compounds; to acquire satisfactory additive composition amount to provide enhanced pesticidal protection by extending half life of the rotenone toxicant activity.

Although the current study highly recommended the twelve-month derris plant developed in 13"x 26" container for better root biomass and higher rotenone quantity percentage, recently, some investigators observed bigger root biomass and larger rotenone content colleted from eighteen-month derris plant cultivated in 40 x 80 cm circular cement container. Thus, the appropriate container type and size in addition with harvesting time available for maximizing rotenone content level of the derris root need to be further investigated.

Since derris plants are scarcely existed in the natural habitats, a massive scale on derris plant cultivation is needed to obtain sufficient raw material supply for the future commercially derris root product purpose. Applications of appropriated technologies on plant propagation and cultivation, root extraction, product formulation, handling, packaging, and storage, could be attentively administered. Deviation from pesticidal property, rotenone bioactive compounds are also effective in controlling pests of veterinary and medical importance along with certain chemical properties for specific human ailment remedy, thus, integrated research project by scientists from all related fields should be established for compiling research documents and thus strengthening the National Database on beneficial and proper application aspect of derris plants of Thailand.