Chapter 7

The Longevity Extension and Efficacy of the Derris Formulated Product for Controlling the Cabbage Aphid

7.1 Introduction

Rotenone is a natural product and chemically unstable. It is rapidly broken down in soil, water, groundwater, and in vegetation. The half-life in soil and water is between 1-3 days. It degraded readily by exposure to sunlight, nearly all of the compound toxicity is lost 2-6 days in sunlight. Rotenone is a highly active but short-lived photosensitizer and also sensitive to heat, it quickly lost at high temperature (Extoxnet, 1996). Rotenone has been used for years against fleas and lice, but is also effective against aphids, beetles, caterpillars, maggots, bagworms, cabbage worms, thrips, leafhopper, Japanese beetles, vegetable weevils, codling moths, sawflies and slug sawflies (Ray, 1991). 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).

The cabbage aphid, *Lipaphis erysimi* Kaltenbach (Aphididae) is an economically importance pest of Cruciferous in northern vegetable production area of Thailand, leading to significant reduction in quality and quantity of the yield product (Kameya and Ratanabhumma, 1998). Their outbreaks were occurred during November to February of the vegetable growing season (Sottikul, 2000). They caused damage directly through feeding and indirectly through the transmission of more than 10 viral plant diseases (Kennedy *et al*., 1962).

The objectives of the current experiments are to determine the appropriate chemicals for longevity extension and efficacy enhancement of the rotenone formulated product for controlling the cabbage aphid.

7.2 Materials and Methods

7.2.1 The shelf-life extension of the derris formulated product

Two-hundred gram of derris root powder was macerated in 1,000 ml of 95% ethanol for 5 days then filtered. The obtained solution samples were measured for the

rotenone content by HPLC method. The solution samples were separately mixed to obtain 1% of either glycerin or ascorbic acid or EDTA (Ethylene diamine tetracecetic acid) or propylene glycol or sorbates or benzoate. The untreated solution was assigned as the check treatment. The mixed and unmixed solutions were stored at room temperature for duration of 4 months; the rotenone contents were measured for a total of 4 times at monthly interval.

7.2.2 Efficacy enhancement of derris product to control the aphid (*L. erysimi*)

Tween 80 (polysorbate 80) is a nonionic surfactant and emulsifier derived from sorbital which is obtained from various types of fruit. Tween 80 is a water soluble liquid used as a dispersing agent to mix oil and water and to solubilize fragrances and essential oils (Wikipedia, 2006). Tween 80 was assigned to the current experiment to determine its synergistic effect on rotenone toxicants. Derris root powder was extracted by macerated in 200gm/L of 95% ethanol for 3 days, and filtered. The obtained solutions were measured for rotenone contents by HPLC method as described in Chapter 3. The extracted solution was divided into 2 parts: one part was added with nothing, then diluted with water to obtain the rotenone contents of 125, 100, 50, and 25 ppm; the other part was added with 1% Tween 80, then diluted with water and adjusted the rotenone content to be 9.6, 7.2, 4.8, and 2.4 ppm. Tween 80 was also individually applied as the checked treatment of the experiment. The third nymphal stage of the cabbage aphids were selected and treated with the assigned treatments by topical tower sprayer. The total numbers of the death cabbage aphids were counted at 24 hrs after sprayed and calculated for percent correction mortality by Abbott's formula (Abbott,.. 1925). The 50 % insect mortality was calculated with M stat C program based on Probit analysis.

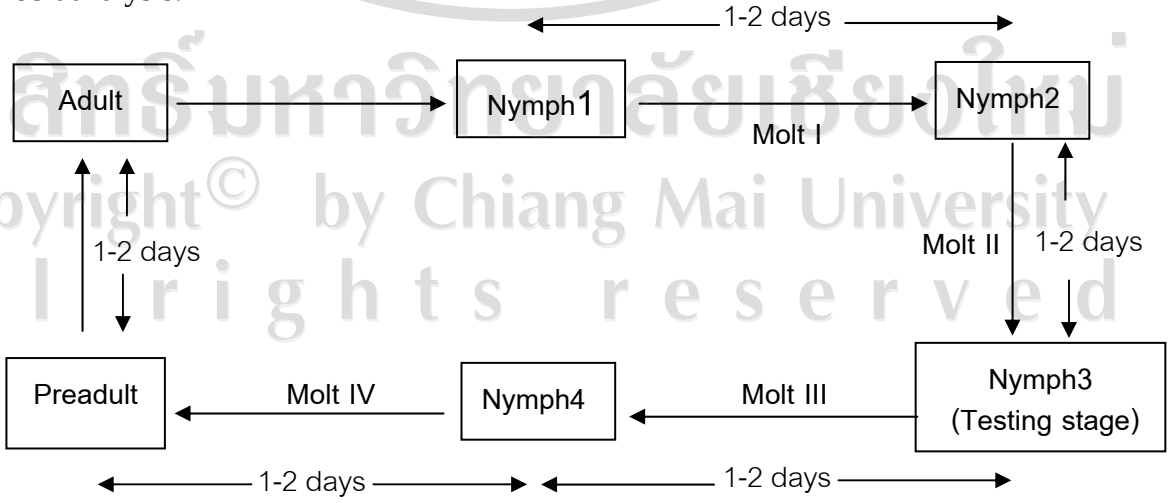

Figure 7.1 Schematic view of the life cycle of *Lipaphis erysimi* Kaltenbach

7.2.3 Field evaluation of the efficacy of the derris formulated product for controlling the cabbage aphid

Under laboratory condition the derris formulated product demonstrated very high promising for controlling the cabbage aphid. However, the field efficacies of rotenone are strongly influenced by various environmental factors including, UV light, heat, soil, water, and method of applications. This experiment was conducted to evaluate the efficacy of the derris formulated product to control *L. erysimi* in the cabbage field.

1. Seedling preparation

Cabbage seed of the OK 23 variety, elephant brand were applied in 20 x 30 cm plastic basket filled with soil and rice husk charcoal (1:1), the seedlings were germinated on November 15, 2006. After 14 days, the seedlings were transplanted to 3"x 5" cultivated plastic bags, and transplanted to experiment field at 7 days later.

2. Experimental field

Total of 8 plots were assigned as experimental units. Each plot consisted of a 1x5 m^2/planting bed was planted to cabbages at 50 cm row spacing for a total of 10 plants per plot. The soil planting bed was prepared with planting materials of 200 gm of farm manure and 25 gm of 15-15-15 fertilizer mixture. 15 days after transplant all plants were provided with additional 25 gm/plant of 13-13-21 fertilizer mixture.

Figure 7.2 Soil planting bed preparations

Figure 7.3 Ten-day old cabbage seedlings

3. Preparation of derris formulated product

Two-hundred gram of derris root powder was extracted by macerate in 1,000 ml of 95% ethanol for 3 days, and then filtered. The obtained clear solution was analyzed for rotenone content by HPLC method. The extracted solution was adjusted to 5% rotenone content by Pearson square (Wagner and Stanton, 2006) as shown in figure 7.4.

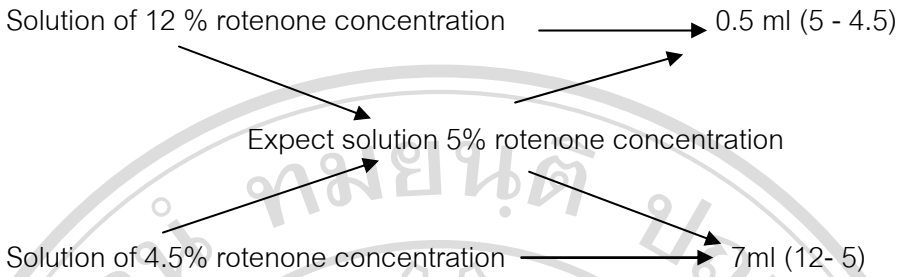

Figure 7.4 Pearson square for adjusted rotenone concentration (Wagner and Stanton, 2006)

The prepared solution was added of 1% Tween 80. One percent concentration of this solution was applied in the cabbage fields for a total of 4 plots (5 liters/40 plants) to determine the treatment efficacy compare with the check treatment.

Data collection

1. The first treatment applications were conducted as soon as the field aphid population abundances reached the predetermined tolerance limit. Numbers of aphids at pre- and post- treatment applications were recorded at followed

(1) The first treatment applications were conducted on December 24, 2006 (18 days after transplanting)

(2) The second treatment applications were conducted on January 3, 2007 (27 days after transplanting)

(3) The third treatment applications were conducted on January 9, 2007 (33 days after transplanting)

2. The cabbages were harvested three times, for weighting and head size measuring on 12, 16, and January 19, 2007

3. Chlorophyll activity of cabbage leaves were measured at thirty minute after the third treatment application (January 9, 2007) by fluorescent chlorophyll apparatus (chlorophyll efficacy meter).

7.3 Result and discussion

7.3.1 The shelf-life extension of the derris formulated product

Percentages of rotenone residues after 4 months of storage at room temperature for assigned treatments: derris + ascorbic acid, derris + propylene glycol, derris alone, derris + EDTA, derris + sorbate, derris + glycerin, and derris + benzoate were 84.39, 78.48, 78.47, 74.39, 67.24, and 59.86%, respectively (Table 7.1 and Figure 7.1). The derris extract mixed with 1% ascorbic acid exhibited the highest percentages of rotenone residue (84.39%). This was probably the ascorbic acid reduce oxidation and concurrent by

decrease the rotenone degradation due to a higher affinity of ascorbic acid to be oxidized than rotenone (Mottram *et al*., 1975). Derris extract added with 1% propylene glycol provided the second highest percentages of rotenone residue (78.48%). Propylene glycol had a better property to adsorb humidity from air and from preservative product (Pornpisit, 1997). Thus, this experiment confirmed that ascorbic acid and propylene glycol should be appropriated chemical substances to extend shelf life and prolong the activity of the derris formulated product.

Table 7.1 Rotenone contents and percentages of rotenone residues by treatments at monthly intervals stored at room temperature

Treatments	Duration of product storage (month)					% rotenone residues
	0	1	2	3	4	
1. Derris + 1% glycerine	4.10	4.04	3.17	2.99	2.75	67.07
2. Derris + 1% ascorbic acid	4.08	4.01	3.77	3.73	3.28	84.39
3. Derris + 1% EDTA	4.10	4.08	3.89	3.44	3.05	74.39
4. Derris + 1% Propylene glycol	4.09	3.79	3.43	3.27	3.21	78.48
5. Derris + 1% sorbates	4.06	3.68	3.64	3.29	2.73	67.24
6. Derris + 1% benzoate	4.16	4.12	3.64	3.17	2.49	59.86
7. Derris	4.18	3.80	3.53	3.46	3.28	78.47

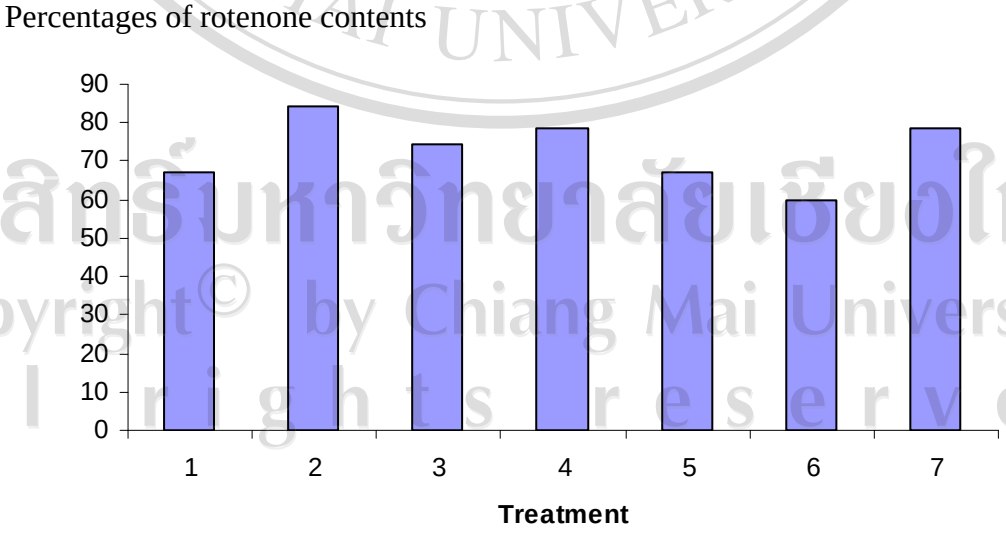

Figure 7.5 Percentages of rotenone contents by treatments after 4 months storage at room temperature

7.3.2 Efficacy enhancement of derris product to control the aphid (*L. erysimi*)

Although the obtained results of the previous studies showed a promising efficacy of certain derris formulated product for controlling the cabbage aphid, further investigation on efficacy enhancement of the product is needed. This study revealed the bioassay of the third nymphal instars of the cabbage aphids with the mixed and unmixed derris root extracts. 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 ± 3.52 ppm (Table7.2). The LC_{50} of Tween 80 alone was 15,900 ± 500 ppm indicated that polysorbate 80 had no effect on the cabbage aphid mortality. Hence, Tween 80 has 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.

Table 7.2 Rotenone contents and LC_{50} values by treatments

Treatments	Rotenone content in solution (ppm)	LC_{50} (ppm)
Derris root powder + 95%ethanol+1%Tween 80	50,000	4.33 ± 0.23
Derris root powder + 95%ethanol	50,000	34.12 ± 3.52
Tween 80	-	15,900 ± 500

Figure 7.6 Cabbage aphid clusters on the leaf of Chinese cabbage

Figure 7.7 Bioassay of Cabbage aphids on Chinese cabbage leaves

Figure 7.8 Cabbage aphids on Chinese cabbage leaves prepared for topical tower sprayer

Figure 7.9 Topical tower sprayer

7.3.3 Field evaluation of the efficacy of the derris formulated product for controlling the cabbage aphid

At pre-treatment applications, numbers of aphids for derris extracts mixed 1% Tween 80 and check treatment were 59.45 and 63.10 insect/plant, respectively (Table 7.3 and Figure 7.6). At 24 hours after the first treatment applications the numbers of aphids were 44.90, and 299.98 insects/plant, respectively. The aphids numbers in the check treatment was significantly higher than the obtained aphid numbers from the derris extracts mixed 1% Tween 80 treatment. By the second treatment applications, the numbers of aphids for derris extracts mixed 1% Tween 80 and check treatment were 34.18 and 232.41 insect/plant, respectively. The similar consequences also achieved by the third treatment applications, the numbers of aphids were 22.69 and 207.98 insect/plant, respectively. The average percentage of aphid decreased numbers from the first treatment applications through the third treatment applications for the derris extracts mixed 1% Tween 80 were 28.84, 28.61, and 43.02 insect/plant, respectively. The check treatments number aphid increase at the first and the second treatment applications were 404.59 and 11.44% but at the third applications aphid decreased 10.50 %, respectively

The fresh weight, including width and length of cabbage for mixed and check treatments with the values of 1.37 and 1.39 kg/pt; 13.19 and 12.88 cm; and 15.63 and 15.66 cm., respectively, all variables were not significant difference at $T_{.05}$ level (Table 7.4), indicated Tween 80 played no important role on the fresh weight, including width and length of the field cabbages.

The Fv/Fm value measured by chlorophyll efficacy meter after treatment applications were also not significantly affected by mixed Tween 80 to the derris root extract (Table 7.4) .

Table 7.3 Average numbers of aphids for pre- and post-treatment applications and percentages of aphid decrease conducted at 18, 27, and 33 days after transplanted

Treatments	1% Derris extract	check	**t-test**
18 days after transplanted (1st treatment applications)			
Pre-	63.10 [b]	59.45[b]	ns
Post-	44.90[b]	299.98[a]	*
% Decrease	28.84	+404.59	
27 days after transplanted (2nd treatment applications)			
Pre-	47.88[b]	208.56[a]	*
Post-	34.18[b]	232.41[a]	*
% Decrease	28.61	+11.44	
33 days after transplanted (3rd treatment applications)			
Pre-	39.82[b]	232.48[a]	*
Post-	22.69[b]	207.98[a]	*
% Decrease	43.02	10.50	

Note: Pre- = shortly before treatment applications
Post- = 24 hours after treatment applications

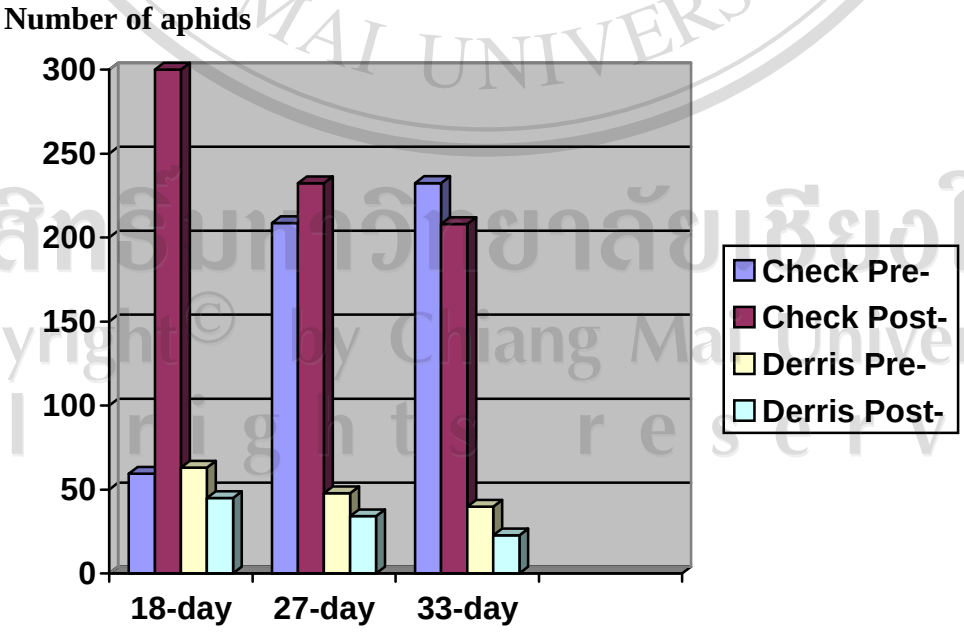

Figure 7.10 Numbers of aphids on cabbage for pre- and post-treatment applications at 18, 27, 33 days after transplanted

Table 7.4 Fresh weight, width and length of cabbages at harvest period and Fv/Fm values of cabbage leaf chlorophyll after treatment applications

Treatments	Weight (kg)	Width (cm)	Length (cm)	Fv/Fm value
derris root extract	1.37	13.19	15.63	0.78
check	1.39	12.88	15.66	0.81
t-test	ns	ns	ns	ns

7.4 Conclusion

The derris extract mixed with 1% ascorbic acid the highest percentages of rotenone residue (84.39%). 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 longevity 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 ± 3.52 ppm. At the field condition, 24 hrs after the first treatment applications the aphids numbers in the check treatment (299.98 insects/plant) was significantly higher than the obtained aphid numbers from the derris extract mixed 1% Tween 80 treatment (44.90 insects/plant). The similar consequences also achieved through the second and third treatment applications, Hence, Tween 80 has 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. Although, Tween 80 played no important role on the fresh weight, including width and length, and leaf chlorophyll activity of the field cabbages.

OK 23 cabbage seed variety, elephant brand

Seed germinated (15/11/2006)

21-day old seedlings transplanted in 3"x 5" cultivated plastic bag

14-day old seedlings transplanted in plastic basket filled with soil and charcoal husk (1:1)

Figure 7.11 Preparation of cabbage seedling

Figure 7.12 Thirty three day old cabbages

Figure 7.13 Forty day old cabbages

Figure 7.14 Aphid clusters on cabbage leaves

Figure 7.15 Stunt caused by aphid

Figure 7.16 Ready-to-harvest cabbage (40 days old) of harvesting ready and harvesting

Figure 7.17 Cabbage fresh weighting

Figure 7.18 Width and length of cabbage

Figure 7.19 Fluorescent chlorophyll apparatus and sensor on cabbage leaf