Chapter 6

Determination of Rotenone Degradation for Sprayed Residues in Selected Environment

6.1 Introduction

Rotenone is classified by the World Health Organization (WHO) as moderately hazardous. It is mildly toxic to humans and other mammals, but extremely toxic to insects and aquatic life including fish (Wikipedia, 2007). Concentration of rotenone safe for human consumption has been estimated at 10 ppm (Lehman, 1950) and European food exposure limits are between 0.04 and 0.1 mg/kg (WHO, 1992). Rotenone is quickly degraded in sunlight and water. Soil application studies of rotenone demonstrated its very short persistency with a half-life range of only 1-3 days (Caldwell *et al.*, 2005). Since rotenone adsorbs strongly to organic matter in soil and is rapidly degraded and detoxified, contamination of rotenone treatment to ground water is improbable (Dawson *et al*., 1991). Nearly all of the toxicity of the rotenone compound is lost in 5 to six days of spring sunlight and 2 to 3 days of summer sunlight (Extoxnet, 1996). U.S. Environmental Protection Agency (2007), recently accepted that currently registered piscicidal uses of rotenone are eligible for reregistration. The current experiment is aimed to prevent possible rotenone application contamination in the human food chains to ensure the food safety practice and environment protection.

6.2 Materials and Methods

The experiment was conducted at the Lampang Agricultural Research and Training Center, Rajamongala University of Technology Lanna, during June to October, 2005. Trials on rotenone degradation for **indoor, outdoor, and the cabbage field** conditions were observed at 6, 12, 24, and 30 hours after sprayed the derris root extract treatments into the Petri dish and on the cabbage field with 4 treatments in 3 replications. The first treatment was prepared from 20 gm of squeezed fresh sliced derris root macerated in distilled water 1,000 ml and agitated for 24 hours. The second treatment was prepared from 10 gm of squeezed dried sliced derris root macerated in distilled water 1,000 ml and agitated for 24 hours. The third treatment was prepared from 100 gm of derris root powder macerated in ethanol 95% 1,000 ml and agitated for 72 hours then

dilute the obtained extract with water to acquire 1 % concentration before treatment application. The fourth treatment was prepared with the same procedure of the third treatment, diluted the obtained extract with 90 ml of 95% ethanol followed by dry evaporated step and finally diluted the obtained crude extract with water to acquire 1 % concentration before treatment application. Measured the rotenone content in all treatments before spray applications by HPLC (High Performance Liquid Chromatography) method as represented by Pitiyont and Sangwanich, 1997. The state of HPLC instruments as described in Chapter 3.

The fields of 4 plots, 2 m^2 each, were planted to cabbage with 30 x 30 cm plant spacing, for a total of 36 plants per plot. After one month, all plants in each plot were sprayed with 2 liters of the assigned treatment by knapsack sprayer. After 6, 12, 24, and 30 hours randomly collected 3 leaves from 3 plants in each plot and the leaves were finely sliced. One gm of the sliced fresh weight was macerated in 10 ml dioxane and agitated for 1 hour. After filtered through filter paper the solutions were analyzed for rotenone contents by HPLC method.

6.3 Results and discussion

Rotenone contents in extracts before treatment applications of fresh root, dry root, non evaporation crude, and evaporation crude were 287.43, 366.26, 111.45, and 192.32 ppm, respectively (Table 1), after spray treatment applications on dry plates (Petri dishes) and washed the plates immediately by dioxane 10 ml and analyzed rotenone concentrations by HPLC revealed rotenone concentration degraded immediately with the records of 176.46, 232.60, 54.33, 54.33, and 114.43 ppm, respectively.

For indoor condition with the light density measured was 374–560 lux, 30 hours after sprayed percentages of rotenone degradation were 90.12, 75.15, 62.34, and 59.60 %, respectively (Table 2), the regression equations of rotenone degradations were y = 157.68-4.93 (x), y = 203.07-5.36 (x), y = 49.08-1.03 (x) and y = 109.98-2.14 (x), respectively (Table 1 and Figure 1).

For outdoor condition with the light density measured were 45,000 – 56,000 lux in the morning and 56,000 – 65,000 lux in the afternoon, 30 hours after sprayed percentage of rotenone degradation were 95.76, 96.05, 91.72, and 94.52 %, respectively (Table 2), the regression equations of rotenone degradations were y =120.67-4.41 (x), y=164.32-6.05 (x),y=45.93-1.50 (x),and y=91.16-3.15 (x), respectively (Table 1 and Figure 2).

For the field condition, rotenone content concentrations before spray applications in all assigned treatments were 351.28, 383.51, 111.45, and 132.32 ppm, respectively, rotenone residue concentrations on cabbage leaves immediately after sprayed (0 hour), were 1.29, 1.62, 1.40, and 1.74 ppm, respectively, 12 hours after treatment applications rotenone concentrations were enormously decreased to 0.16, 0.35, 0.13, and 0.22 ppm, respectively, and percentages of rotenone degradations were 87.60, 78.4, 90.72, and 87.36 %, respectively (Table 3).

Rotenone is rapidly degraded after spray especially for outdoor condition because the ultraviolet irradiation of sunlight. One of the most important factors affecting the rate of sunlight degradation of pesticides and other organic chemicals is the presence of photosensitizers, compounds that facilitate the transfer of the energy of light into the receptor chemicals. Rotenone to be sensitizers for degradation of various insecticides. In addition to rotenone, good photosensitizers were some aromatic amines, anthraquinone and benzophenone (Cheng *et al*., 1972). Four basic types of photochemical reactions may take place when aromatic pesticides are exposed to ultraviolet: ring substitution, hydrolysis, oxidation, and polymerization. Oxidative and reductive photochemical reactions are also important degradation processes of pesticides. Thus chlorobenzoic acid gives rise to benzaldehyde on ultraviolet irradiation (Crosby, 1966). Exposure of parathion to sunlight and ultraviolet radiation results in the production of paraoxon and oxo analogues of parathion (Matsumura, 1985). Duration of insect contact with the sprayed surface was crucial for the effectiveness of rotenone. Residues of both 10% and 20% rotenone solutions were monitored in olive oil extracted from fruit harvested immediately, and 4, 8 and 25 days after spraying. The rate of rotenone degradation was linear and fast in both treatments, depending on doses. Residue in olive oil was also monitored during storage in a tank in the dark at 20-26°C for 5 months followed by storage in transparent glass bottles in darkness or light at 20-26°C or darkness at -20°C for 3 more months. Monitoring of the residual activity in olive oil during storage (December 1996-August 1997) showed that light plays a dominant role in rotenone degradation. Rotenone residues were relatively stable in the dark at both temperatures. The rate of degradation after a period of 8 months was very slow (16%). However, olive oil samples stored for 5 months in the dark, followed by

three-month storage in the light showed a 44% decline in rotenone content (Stavroulakis *et al*., 2001).

6.4 Conclusion

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 (treatments 1 and 2) and rotenone residue concentrations was within a range of 17.44-57.80 ppm, while ethanol extractions (treatments 3 and 4) 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, 30 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 as compared with European food exposure limits of 0.04 and 0.1 mg/kg (40–100 ppm) (WHO, 1992). 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.

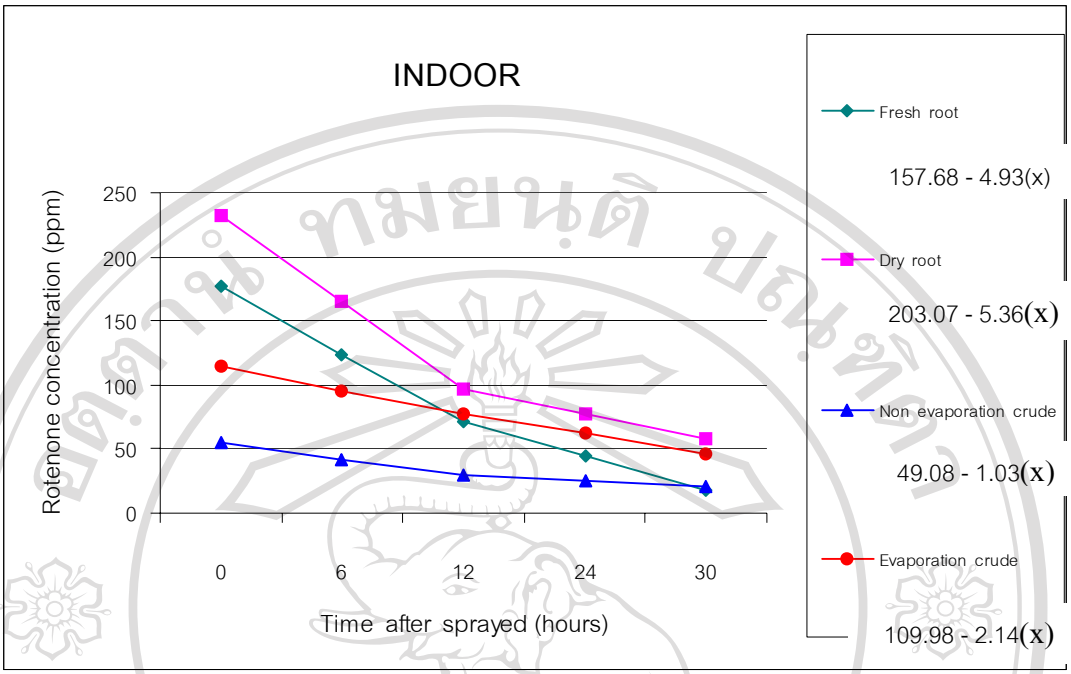

Figure 6.1 Rotenone degradation by treatments for indoor condition

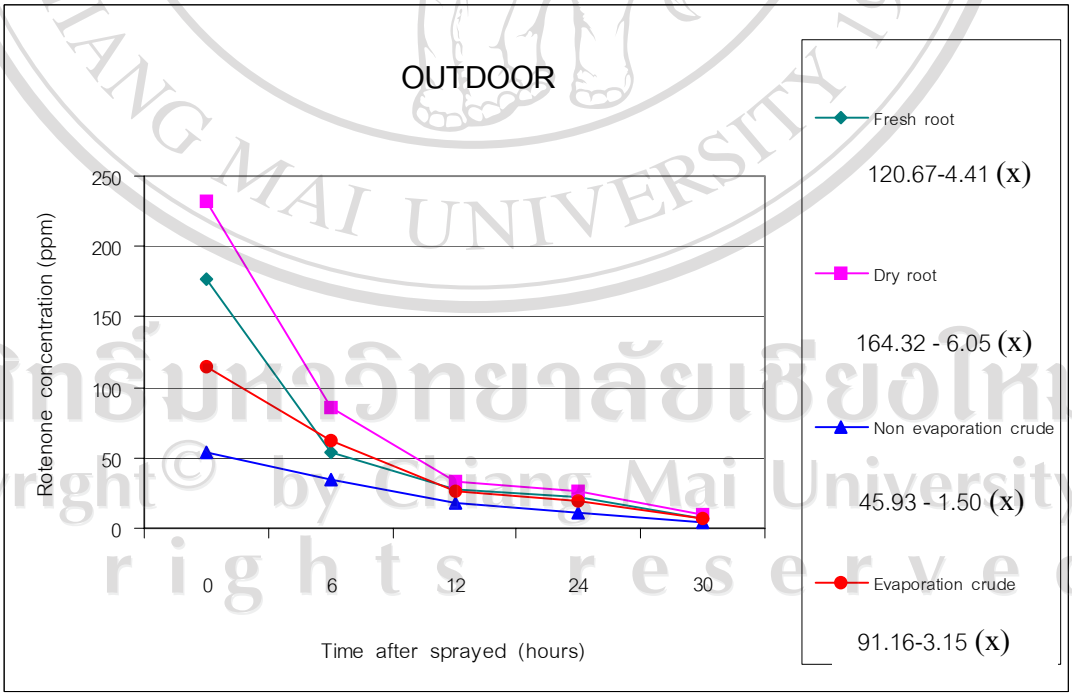

Figure 6.2 Rotenone degradation by treatments for outdoor condition

Table 6.1 Rotenone contents in solutions and on Petri dishes by treatments before and after sprayed at 0, 6, 12, 24 and 30 hours,
for indoor and outdoor conditions with regression equations of rotenone degradations.

Treatment	Rotenone concentration (ppm)										Regression equation (y = a + bx)	
	Time after sprayed(hours)											
	Before sprayed	0	6		12		24		30		Indoor	Outdoor
			Indoor	outdoor	Indoor	outdoor	Indoor	outdoor	Indoor	outdoor		
Fresh root	287.49 ±15.89	176.46 ±8.54	123.83 ±4.82	53.30 ±1.42	71.20 ±3.74	27.12 ±1.43	44.32 ±1.97	21.62 ±1.02	17.44 ±1.62	7.49 ±0.32	157.68-4.93 (x) r = - 0. 96	120.67-4.41 (x) r = - 0.80
Dry root	366.26 ±18.49	232.60 ±11.52	164.70 ±4.92	85.24 ±4.53	96.80 ±4.28	32.54 ±1.57	77.80 ±2.79	26.68 ±1.34	57.80 ±2.10	9.19 ±0.83	203.07-5.36 (x) r = - 0.93	164.32-6.05 (x) r = - 0.82
Non evaporation crude extract	111.45 ±7.28	54.33 ±2.78	41.44 ±2.26	33.89 ±1.63	29.56 ±1.69	17.48 ±0.89	25.76 ±2.48	11.54 ±0.79	20.46 ±0.92	4.50 ±0.41	49.08-1.03 (x) r = - 0.94	45.93-1.50 (x) r = - 0.93
Evaporation crude extract	192.32 ±8.64	114.43 ±3.61	95.93 ±4.19	62.25 ±3.42	77.44 ±3.22	26.62 ±1.37	61.83 ±3.14	19.12 ±1.47	46.23 ±1.23	6.27 ±0.30	109.98-2.14 (x) r = - 0.99	91.16-3.15 (x) r = - 0.90

Note: Y = rotenone concentration (ppm) X = time after sprayed (hours) r = x and y relationship in linear regression equation

Table 6.2 Percentages of rotenone degradation after sprayed on Petri dish at 6, 12, 24, and 30 hours, for indoor and outdoor conditions.

Treatments	Percentage of rotenone degradation (%)							
	Time after sprayed (hours)							
	6		12		24		30	
	Indoor	outdoor	Indoor	outdoor	Indoor	outdoor	Indoor	outdoor
Fresh root	29.83	69.79	59.65	84.63	74.88	87.75	90.12	95.76
Dry root	29.19	63.35	58.38	86.01	66.55	88.52	75.15	96.05
Non evaporation root powder	23.72	37.62	45.59	67.83	52.59	78.76	62.34	91.72
Evaporation root powder	16.17	45.60	32.32	76.74	45.97	83.29	59.60	94.52

Table 6.3 Percentages of rotenone degradation and rotenone concentration by treatments before and after sprayed on cabbage at 0, 6, and 12 hours.

Treatments	Rotenone concentration (ppm)					
	Before sprayed	Times after sprayed (hours) and % degradation				
		0	6	% degrade	12	% degrade
Fresh root	351.28 ± 6.73	1.29 ± 0.29	0.27 ± 0.02	79.07	0.16 ± 0.02	87.60
Dry root	383.51 ± 8.01	1.62 ± 0.36	0.83 ± 0.04	48.77	0.35 ± 0.04	78.40
Non evaporation root powder	111.45 ± 4.92	1.40 ± 0.13	0.15 ± 0.03	89.29	0.13 ± 0.01	90.72
Evaporation root powder	132.32 ± 3.21	1.74 ± 0.34	0.24 ± 0.10	86.21	0.22 ± 0.01	87.36

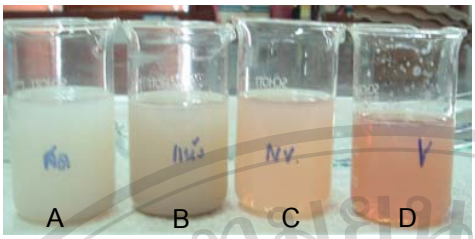

Figure 6.3 A = Derris fresh root extract (20gm/L)
B = Derris dry root extract (10gm/L)
C = Non evaporate ethanol derris root powder extract (1%)
D = Evaporate ethanol derris root powder extract (1%)

Figure 6.4 Field Spraying of derris extract solution (L/m^2)

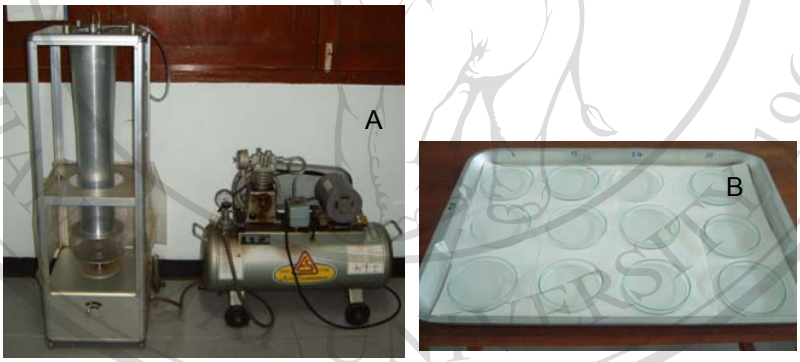

Figure 6.5 A = Topical tower sprayer (5 ml, 40 lb/$inch^2$)
B = Derris extract sprayed on dry plates (Petri dishes)