

Chapter 4

Ascertainment of Rotenone Content in Accordance with Derris Plant Ages Cultivated in Various Container Types and Sizes Versus the Field Condition

4.1 Introduction

Rotenone is a naturally occurring chemical compound that is present in a number of plants, including the derris plant roots. These compounds are formulated in pesticides products to control insects, mites, ticks, spiders, and undesirable fish (EPA, 2002). Derris plants usually exist in natural habitats especially in the area near the riverside. Apparently this plant density is obviously declined and more difficult to collect from the natural habitats. Consequently, farmers need to cultivate derris plants under the farm ecosystem, yielding mostly unsatisfactory root parts with very low rotenone content. The objective of this investigation is maximizing of the plant root system and rotenone content with appropriate plant ages cultivated in various container types and sizes as compare to the field condition.

4.2 Material and methods

4.2.1 Preparing of stem cutting

The derris stems with 1 cm in diameter were selected and cutting into stalks of approximately 25–30 cm long. After all leaves were removed, the stalks were preplanted into the charcoal hull. All prepared plant stalks were watering twice daily in the morning and the afternoon. They were located under the shade of 50% saran roof for 45 days. After the leaf budding appeared and later the young leaves became mature leaves. Each plant stalk was transferred into 5"x 8" inches plastic bag carried with the ratio of mixture of soil to manure to hull was 2:1:1. Then they were maintained in the greenhouse or nursery for another one month.

4.2.2 Types and sizes of planting containers

Two types of containers with three different sizes were employed in the experiment: 10"x 20" and 13"x 26" of the plastic bags, and the 15"x 15" of plastic pots. The planting materials applied for each treatment was a mixture of soil to hull to cow manure as a ratio of 2:1:1. The mixture was filled up in all container sizes of each type to accommodate one plant only. Each prepared derris plant stalk was then transferred and grown in each container for a total of 15 plants per treatment. Meanwhile, an additional of 15 plants were transferred into the field plot with the plant spacing of 1x1 m provided with the same planting material mixture as supplement treatment for rotenone content comparison. All plants in all treatments were watering everyday. The

data on total fresh and dry weights, and root rotenone content were collected from each plant in each treatment at 6, 9, and 12 months after transplanting. The data on rotenone quantity were performed by HPLC method.

4.2.3 Preparation of rotenone content sample for analyzing with HPLC method

The fibrous and branch derris roots at the ages of 6, 9, and 12 months were sliced into small piece. Then they were dried under the shade for three or four days and grind to powder by mortar. Each gram of derris root powder taken from each treatment was macerated in 10 ml of dioxane and agitated for an hour. After that it was filtered by filter paper no.1 in order to get clear solution for HPLC (High Performance Liquid Chromatography) analyzation. The HPLC method is used to separate components of a mixture (Pitoyont and Sangwanich, 1997). The details on the analyzing process by HPLC as described in Chapter 3.

4.3 Result and discussion

4.3.1. Rotenone content in derris root

Percentages of rotenone quantities detected from 6-, 9-, and 12- month derris root according to treatment applications are exhibited in Table 4.1 and bar chart illustrated in Figure 4.1. At six months after transplanting, rotenone content of the fibrous root existed in 10"x 20", and 13"x 26" plastic bags, 15"x 15" plastic pot, and grown under field condition were 1.38, 1.42, 0.93, and 3.09%, respectively, while rotenone quantities recorded from the same treatment on the branch roots were 2.06, 3.05, 3.05, and 4.24%, respectively. The significantly highest rotenone content for both fibrous (3.09%) and branch roots (4.24%) were displayed on the derris plants cultivated in the field plots. The derris plants grown in 13"x 26" plastic bags provided the second highest rotenone content with 1.42% in the fibrous root and 3.05% in the branch root. In contrast, the lowest rotenone content of 2.06% was observed on the derris root grown in 10"x 20" plastic bag. These seemed to indicate that no matter of the types, bigger size containers furnished bigger space for greater root system development absorbing larger amount of humidity and mineral nutrients, thus, contributed higher level of rotenone content. Nevertheless, the field condition could provide unlimited space for the greatest development of derris plant root system; thus, the significantly highest rotenone content was inevitably acquired.

For the nine-month derris plants, the rotenone contents in accordance with the treatments detected in the fibrous root were 1.31, 3.05, 1.85, and 3.15%, respectively, while rotenone quantities observed from the same treatment on the branched roots were 2.70, 4.16, 3.74, and 4.55%, respectively. The highest rotenone content for both fibrous (3.15%) and branch roots (4.55%) were also occurred on the derris plants cultivated in the field plot, although displayed non-significant difference to the plants developed in 13"x 26" plastic bags. However, these two treatments demonstrated significantly higher levels of rotenone root contents than obtaining from all other treatments. Again,

significantly higher levels of rotenone root contents acquired from the derris plants grown either under field condition or in bigger size containers.

Among the twelve-month derris plants, the rotenone contents according to treatment applications determined in the fibrous root were 1.51, 3.29, 2.02, and 3.86%, respectively, while rotenone quantities recorded from the same treatment on the branch roots were 3.18, 4.88, 4.09, and 5.00%, respectively. The greatest rotenone content for both fibrous (3.86%) and branch roots (5.00%) were also exhibited on the derris plants cultivated in the field plots, although showed non-significant difference to the plants grown in 13"x 26" plastic bags. Nevertheless, these two treatments provided greater levels of rotenone root contents than other treatments contributed. The lowest rotenone content of both fibrous (1.51%) and branch roots (3.18%) were also observed on the derris root developed in 10"x 20" plastic bag. Once more, significantly greater levels of rotenone root contents were obtainable from the derris plants cultivated either in the field plots or bigger size containers.

Table 4.1 Percentages of rotenone quantities detected from 6-, 9-, and 12-month derris root according to treatments

Treatments	6 months		9 months		12 months	
	F	B	F	B	F	B
10"x 20" plastic bag	1.38 ^{bc}	2.06 ^c	1.31 ^b	2.70 ^c	1.51 ^b	3.18 ^b
13"x 26" plastic bag	1.42 ^b	3.05 ^b	3.05 ^a	4.16 ^a	3.29 ^a	4.88 ^a
15"x 15" plastic pot	0.93 ^c	3.05 ^b	1.85 ^b	3.74 ^b	2.02 ^b	4.09 ^{ab}
Field	3.09 ^a	4.24 ^a	3.15 ^a	4.55 ^a	3.86 ^a	5.00 ^a

*Means within columns followed by the same letter are not significantly different [P ≥ 0.05, measured by Least Significant Difference (LSD)]

Note: F= Fibrous root, B = Branch root



Figure 4.1 Derris fibrous root (left) and branch root (right)

% rotenone content

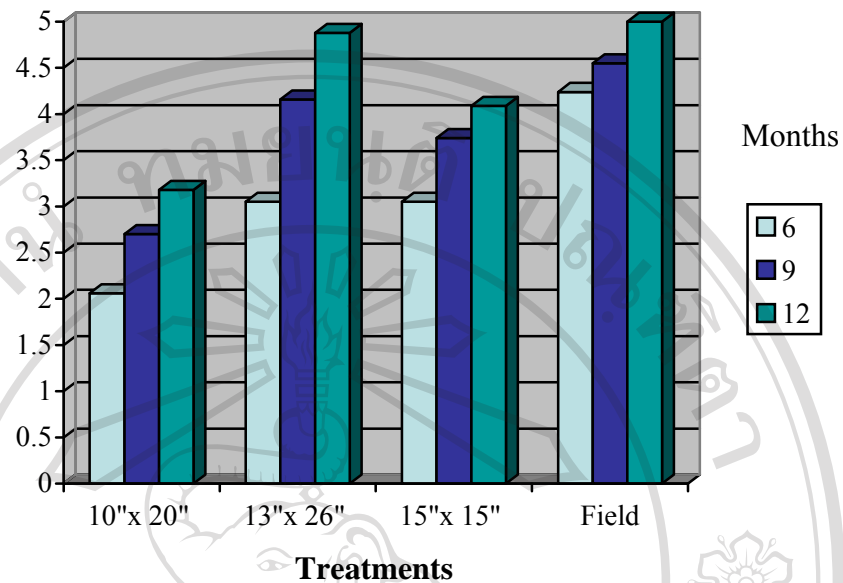


Figure 4.2 Bar chart of rotenone quantity percentages observed from 6-, 9-, and 12-month derris root according to treatments

The highest rotenone contents of derris root planted in the field were also observed by previous investigators. Srijukavan *et al.* (1988) reported that rotenone content recorded from 18-month derris root developed in the field was within a range of 6.85–7.22%. Tongma *et al.* (2004) obtained the highest level of rotenone content of 14% (w/w) from twelve-month field derris root, while 7.7% was determined from the plant root cultivated in a 40 x 80 cm circular cement container. Sangmaneedet *et al.* (2005) were also noted that rotenone content collected from 8–12 month derris root was approximately 6.69%.

The current study demonstrated the highest rotenone content for both fibrous (3.86%) and branch roots (5.00%) were also issued on the field derris plants, although displayed non-significant difference to the plants grown in 13"x 26" plastic bags. Still, these two treatments contributed greater levels of rotenone root contents than other treatments furnished. Appropriate harvest time available for maximizing rotenone content level of the derris root need to be further investigated.

4.3.2. Derris root yield

Fresh and dry weights (gm/pt) investigated from 6-, 9-, and 12-month derris root in relation to treatment applications were displayed in Table 4.2 and dry root weights were bar chart illustrated in Figure 4.2. For six-month derris roots, no significant difference of the fresh weights among treatments were observed, while the

lowest dry weight (16.29 gm/pt) was detected from the plant developed in 10"x 20" plastic bag.

Among the nine-month derris roots, plant developed in 13"x 26" plastic bags furnished significantly highest yield in both fresh (101.80 gm/pt) and dry root weights (47.20 gm/pt). In addition, the significantly lowest yield in both fresh (53.20 gm/pt) and dry root weights (24.55 gm/pt) were produced by the plants cultivated in the field plots. Similar results were also observed from the 12-month derris roots, the highest yield in both fresh (145.00 gm/pt) and dry root weights (65.53 gm/pt) were reproduced from the plant cultivated in 13"x 26" plastic bags, although they showed non-significant difference among plants grown in various sizes of containers. However, the significantly lowest yield in both fresh (76.68 gm/pt) and dry root weights (34.68 gm/pt) were still detected from the plants developed in the field plots.

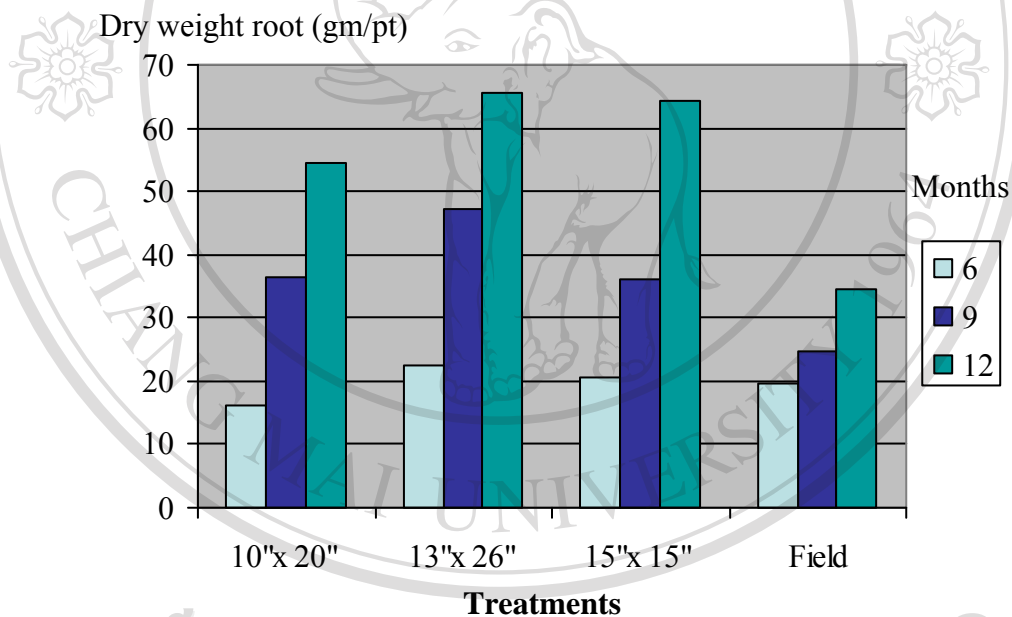
Under field condition, the derris root system was able to penetrate deeper in the ground and provided more branch and fibrous roots. To pull out the whole root system from deeper ground level was time and labor consuming and quite difficult tasks. Whereas, the derris root system was easily to harvest with less root damage when the plant developed in planting material filled in various sizes of containers, thus providing more biomass of plant root yields

Tongma *et al.* (2004) confirmed from the previous study that the twelve-month derris dry weight root grown in the field was only 65.5 gm/pt, while derris root cultivated in 40 x 80 cm (16.33" x 32.65") circular cement pot reached 192.1 gm/pt. The planting materials for the cement pot were soil mixed with rice husk alone and soil mixed with rice husk in combination with charcoal and soil mixed with powder of coconut pericarp in the ratio of 1:1. Once more, higher level of biomass of plant root yields including, branches, leaves, root length, number of root per plant, root diameter, and rotenone concentration obtained from derris plants cultivated in 40 x 80 cm cement pot than the plants developed in the field plots, though no significant difference were observed among different types of soil mixtures. Root-harvesting from the 40 x 80 cm circular cement pot filled with soil mixtures was much more convenient than harvested from the field plots. The water extraction obtained from only four-month derris root grown in cement pot was demonstrated satisfactory protection of the flea beetle (*Phyllotreta* sp.). Srijukavan *et al.* (1988) reported that eighteen-month dry weight of derris root grown in the field was 65.00 gm/pt that were closed to 12-month dry weight root derris developed in the plastic container.

Table 4.2 Fresh and dry weights (gram per plant) collected from 6-, 9-, and 12-month derris root by treatments

Treatments	6 months		9 months		12 months	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
10"x 20" plastic bag	38.35 ^a	16.29 ^b	70.00 ^b	36.28 ^b	114.00 ^a	54.38 ^a
13"x 26" plastic bag	46.52 ^a	22.39 ^a	101.80 ^a	47.20 ^a	145.00 ^a	65.53 ^a
15"x 15" plastic pot	43.02 ^a	20.46 ^{ab}	74.20 ^b	35.98 ^b	133.00 ^a	64.40 ^a
Field	41.60 ^a	19.73 ^{ab}	53.20 ^b	24.55 ^c	76.68 ^b	34.68 ^b

*Means within columns followed by the same letter are not significantly different [$P \geq 0.05$, measured by Least Significant Difference (LSD)]

**Figure 4.3.** Bar chart of dry weights of 6-, 9-, and 12- month derris roots according to treatments

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Total fresh weights recorded from 12- month derris plants cultivated in 10"x 20", and 13"x 26" plastic bags, 15"x 15" plastic pot, and grown under field condition were 460.00, 598.00, 510.00, and 837.00 gm/pt, respectively (Table 4.3). Meanwhile, total dry weights detected from the same treatments were 123.20, 286.40, 204.00, and 339.00 gm/pt, respectively, and bar chart illustrated in Figure 4.4. Both total fresh weights and total dry weights were non-significant difference among derris plants

developed in various sizes of containers; nevertheless, they demonstrated significantly highest levels with the plants cultivated in the field.

Table 4.3 Total fresh and dry weights (gram per plant) observed from 6-, 9-, and 12-month derris plants in relation to treatments

Treatments	6 months		9 months		12 months	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
10"x 20" plastic bag	189.00 ^c	52.05 ^c	317.00 ^b	78.40 ^d	460.00 ^b	123.20 ^c
13"x 26" plastic bag	275.00 ^b	81.70 ^b	388.00 ^b	149.77 ^b	598.00 ^b	286.40 ^{ab}
15"x 15" plastic pot	133.00 ^c	52.52 ^c	359.00 ^b	110.00 ^c	510.00 ^b	204.00 ^{bc}
Field	391.00 ^a	146.05 ^a	505.00 ^a	188.05 ^c	837.00 ^a	339.00 ^a

*Means within columns followed by the same letter are not significantly different [$P \geq 0.05$, measured by Least Significant Difference (LSD)]

Total derris dry weight (gm/pt)

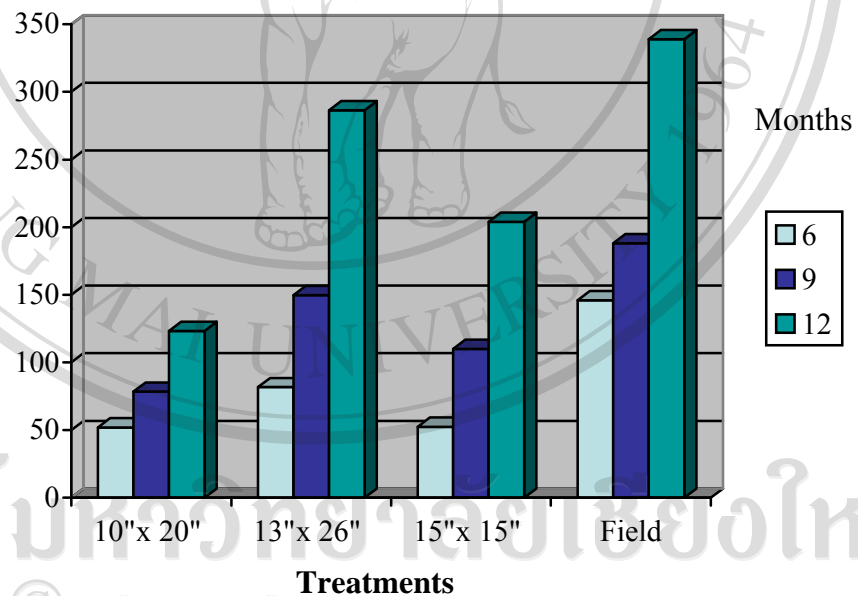


Figure 4.4 Bar chart of total dry weights collected from 6-, 9-, and 12-month derris plants in accordance with treatments

4.4 Conclusion

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.

Among the twelve-month derris plants, the plant developed in the field displayed the highest total dry weight (339.00 gm/pt), and rotenone quantity percentages in both fibrous (3.86%) and branch root (5.00%), although, exhibited non-significant difference to the same variables obtained from plants grown in 13"x 26" plastic bags. Besides, this treatment also provided highest root biomass of fresh (145 gm/pt) and dry weights (65.53 gm/pt), through they were demonstrated non-significant difference to the same variables recorded from other container sizes, while significantly lowest levels of root biomass for fresh (76.68 gm/pt) and dry weights (34.68 gm/pt) were also detected from the field plants. The only significantly different category observed on 12-month field derris plants as compare to the plants developed in the 13"x 26" plastic bags was the total fresh weight (837.00: 598.00 gm/pt). 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. Hence, the twelve-month derris plant developed in 13"x 26" container is highly recommended for better root biomass and higher rotenone quantity percentage. Recently, the highest derris root yield was observed from the derris plant grown in 40 x 80 cm(16.33" x 32.65") circular cement pot (192.1 gm/pt) with highest rotenone root content of 7.7% (Tongma *et al.*, 2004). Thus, the appropriate container size and harvesting time available for maximizing rotenone content level of the derris root need to be further investigated.



Figure 4.5 Types and sizes of planting containers 10"x 20", 13"x 26" plastic bags and 15"x 15" plastic pot



Figure 4.6 Derris plants developed in 10"x 20" plastic bags



Figure 4.7 Twelve months old of derris root removal from 10" x 20" plastic bag



Figure 4.8 Derris plants developed in 13"x 26" plastic bags



Figure 4.9 Twelve months old of derris root removal from 13" x 26" plastic bag



Figure 4.10 Derris plants developed in 15"x 15" plastic pots



Figure 4.11 Twelve month derris root system removal from 15"x 15" plastic pot



Figure 4.12 Derris plants cultivated in the field



Figure 4.13 Twelve months derris root system removal from the field



Figure 4.14 Derris plants developed in 40 x 80 cm (16.33" x 2.65") circular cement containers



Figure 4.15 Eighteen-month derris root system removal from in 40 x 80 cm (16.33" x 32.65")circular cement container

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