Yield and Rotenone Content in Derris Root as Affected by Plant Age and Growing Conditions

Aroon Sottikul[1/] *and Pittaya Sruamsiri*[2/]
อรุณ โสตถิกุล[1/] ***และ พิทยา สรวมศิริ***[2/]

Abstract: Percentages of rotenone in derris root were investigated at six months after transplanting. Rotenone content of the fibrous root cultivated in 10"x 20", 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, whereas rotenone quantities of the branch roots from the same treatment increased significantly to be 2.06, 3.05, 3.05, and 4.24%, respectively. However, no significant difference of the fresh weights among treatments were observed. At nine months, rotenone contents, in the fibrous root in accordance with the above mentioned treatments were 1.31, 3.05, 1.85, and 3.15%, respectively, Whereas the branch roots contained 2.70, 4.16, 3.74, and 4.55%, respectively. The nine-month plant grown in 13"x 26" plastic bags produced the highest fresh root (101.80 g/pt) and dry root weights (47.20 gm/pt). The lowest fresh root(53.20 g/pt) and dry root weights (24.55 g/pt) were harvested from the plants cultivated under field conditions. For the twelve months old derris plants, the rotenone contents, according to the treatments, were 1.51, 3.29, 2.02, and 3.86% in fibrous root, and 3.18, 4.88, 4.09, and 5.00% in branch roots, respectively. At one year old the highest yield in both fresh (145.00 g/pt) and dry root weights (65.53 g/pt) were also obtained 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 yields in both fresh (76.68 g/pt) and dry root weights (34.68 g/pt) were still detected from the plants raised in the field.

Keywords: Rotenone content, derris plant, container

[1/]สถาบันวิจัยและฝึกอบรมการเกษตรลำปาง ตู้ ปณ 89 อำเภอเมือง จังหวัดลำปาง 52000

[2/]ภาควิชาพืชศาสตร์และทรัพยากรธรรมชาติ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ จังหวัดเชียงใหม่ 50200

[1/]Lampang Agricultural Research and Training Center, P.O. Box 89 Amphur Muang, Lampang 52000, Thailand

[2/]Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

บทคัดย่อ: ในกรณีของรากหางไหลที่มีอายุ 6 เดือน ซึ่งปลูกในภาชนะต่าง ๆ ได้แก่ ถุงพลาสติกดำ ขนาด 10 x 20 นิ้ว และ 13 x 26 นิ้ว ถังพลาสติกดำขนาด 15 x 15 นิ้ว และปลูกในแปลง พบว่ารากฝอย มีปริมาณโรทีโนน 1.38, 1.42ม 0.93 และ 3.09% ตามลำดับ สำหรับรากแขนง มีปริมาณโรทีโนนเพิ่มขึ้นเป็น 2.06, 3.05, 3.05 และ 4.24% ตามลำดับ สำหรับน้ำหนักรากสดที่ได้จากทุกกรรมวิธีไม่มีความแตกต่างกัน รากหางไหลที่มีอายุ 9 เดือน ปริมาณโรทีโนนที่พบในรากฝอยมีค่าเท่ากับ 1.31, 3.05, 1.85 และ 3.15% ตามลำดับ และรากแขนงมีค่าเท่ากับ 2.70, 4.16, 3.74 และ 4.55% ตามลำดับ ต้นหางไหลที่ปลูกในถุงพลาสติกดำ ขนาด 13 x 26 นิ้ว มีน้ำหนักรากสด (101.80 กรัม/ต้น) และน้ำหนักรากแห้ง (47.20 กรัม/ต้น) ซึ่งมากที่สุดและมากกว่าน้ำหนักรากสด (53.20 กรัม/ต้น) และน้ำหนักรากแห้ง (24.55 กรัม/ต้น) ที่ได้จากต้นหางไหลที่ปลูกในแปลง สำหรับรากหางไหลที่มีอายุ 12 เดือน ปริมาณโรทีโนนที่พบในรากฝอยมีค่าเท่ากับ 1.51, 3.29, 2.02 และ 3.86% ตามลำดับ และในรากแขนงมีค่าเท่ากับ 3.18, 4.88, 4.09 และ 5.00% ตามลำดับ และน้ำหนักรากสด (145.00 กรัม/ต้น) และน้ำหนักรากแห้ง (65.53 กรัม/ต้น) ของต้นหางไหลที่ปลูกในถุงพลาสติกดำ ขนาด 13 x 26 นิ้ว ยังคงมีค่ามากที่สุด แต่ไม่มีความแตกต่างจากต้นหางไหลที่ปลูกในภาชนะอื่น ส่วนน้ำหนักรากสด (76.68 กรัม/ต้น) และน้ำหนักรากแห้ง (34.68 กรัม/ต้น) ของต้นหางไหลที่ปลูกในแปลงมีค่าน้อยที่สุด แตกต่างจากทุกกรรมวิธี

คำสำคัญ: สารโรทีโนนในรากหางไหล ภาชนะปลูกต้นหางไหล

Introduction

Rotenone is a organic compounds produced naturally in a number of plants, including the derris plant roots. These compounds are extracted and formulated as pesticides to control insects, mites, ticks, spiders, and undesired fishs (EPA, 2002). Derris plants usually grow wild in natural habitats especially as the riverside. However, the plant number is decreasing and no more economic to collect from the natural habitats. Consequently, farmers have to cultivate derris plants under farm ecosystem, yielding mostly unsatisfactory root parts with very low rotenone content.

The objective of this investigation is to studying derris root production, when grow in various container types and sizes and compare to under field conditions.

Materials and methods

1. Preparing of stem cutting

The derris stems with 1 cm in diameter were selected and cut into stalks of approximately 25–30 cm. long. After all leaves were removed, the stalks were preplanted into the rice hull charcoal and watered twice daily in the morning and the afternoon. The plots were covered with 50% saran roof for 45 days. After the leaf budding appeared and later the young leaves became mature, each plant stalk was transferred into 5"x 8" inches plastic bag filled with soil : manure : rice hull charcoal at the ratio of 2:1:1. Seedlings were kept moist in the greenhouse for one month.

2. Types and sizes of planting containers

Two types of containers with three different sizes were employed 10"x 20" and 13"x 26" plastic bags, and 15"x 15" plastic pots (Figure 2). Mixture of soil with rice hull charcoal and cow manure at a ratio of 2:1:1 was filled into all containers. Each derris plant stalk was transferred and grown in each container for a total of 15 plants per treatment. At the same time another 15 plants were transferred into the

field plot with the plant spacing of 1x1 m. Planting beds were provided with the same soil mixture as supplement treatment for rotenone content comparison.

All plants were irrigated everyday and each experiment was completely randomize design with 4 treatments and 4 replications. The data on total root fresh and dry weights, as well as root rotenone content were collected from each plant in each treatment at 6, 9, and 12 months after transplanting. Rotenone quantity were extracted and determined by HPLC (High Performance Liquid Chromatography) method.

3. Preparation of sample for rotenone content analyzing with HPLC method

The fibrous root and branch roots at the age of 6, 9, and 12 months were sliced into small pieces before drying under shade condition for three to four days and ground by mortar. Each gram of derris root powder taken from each sample was soaked in 10 ml of dioxane and shook for one hour. Extraction was filtered using filter paper no.1 in order to get clear solution for HPLC analysis. (Pitiyont and Sangwanich, 1997).

4. Rotenone analysis by HPLC method

4.1. Principle of the method

Root samples are extracted with dioxane and rotenone analysis is obtained by reversed phase HPLC and UV detector at 280 mm.

4.2. Apparatus and reagents

1) HPLC with variable UV detector

2) HPLC column ODS-3 4.6 x 150 mm, Prontosil C18 H, 5-micron with guard column

3) Mobile phase, Phosphoric acid 1ml/water 100ml and degas before use by methanol HPLC grade.

4) Pure rotenone standard.

4.3.Standard curve construction

Weight 0.1005 gm (99.5%) pure rotenone in 100 ml volumetric flask, dissolve with dioxane and shake for 30 minutes. Solution was then diluted to be stock solution at 1,000 ppm rotenone concentration. Further dilution was made with dioxane to 750, 500 and 250 ppm (calculate with formula $N_1V_1 = N_2V_2$). Based on HPLC value. The linear regression derived from peak area and concentration was Y = 17438X + 187783. Where Y = peak area from HPLC of each concentration, X = concentration of the unknown (ppm) (Figure 1).

Results and discussion

1. Rotenone content in derris root

Percentages of rotenone content detected from 6, 9, and 12 months old derris roots as affected by each treatment are exhibited in Table1. At six months after transplanting, rotenone content of the fibrous root collected from 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 in the branch roots of the same treatment were 2.06, 3.05, 3.05, and 4.24%, respectively. The significantly highest rotenone content for both fibrous root (3.09%) and branch roots (4.24%) were found on the derris plants cultivated under 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

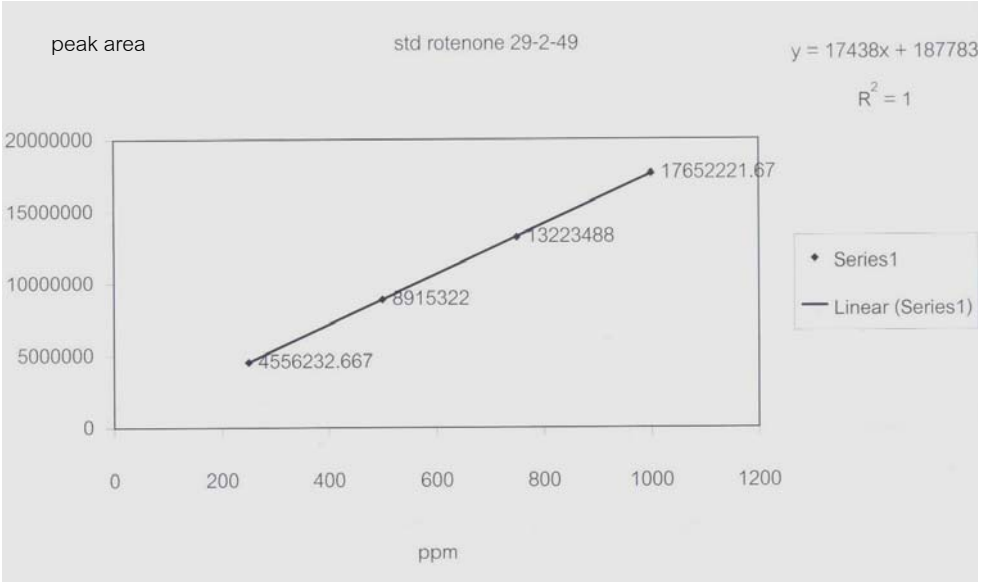

Figure 1 Standard curve of pure rotenone.

Table 1 Percentages of rotenone extracted from 6-, 9-, and 12-month derris root.

Treatment	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 plot	3.09^{a}	4.24^{a}	3.15^{a}	4.55^{a}	3.86^{a}	5.00^{a}

*Means within the same column followed by the same letter are not significantly different at LSD 0.05

Note: F= Fibrous root, B = Branch 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 larger space for greater root system development, with lips efficiency in absorbing larger amount of humidity and mineral nutrients, thus, produced higher level of rotenone content. Nevertheless, the field condition could provide unlimited space for the development of root system; thus, the significantly highest rotenone content was accordingly acquired.

For the nine-month old derris plants, the rotenone contents, in accordance with the treatments, detected in the fibrous roots were 1.31, 3.05, 1.85, and 3.15%, respectively, while rotenone quantities

Figure 2 Types and sizes of planting containers 10"x 20"(right), 13"x 26" plastic bags and 15"x 15" plastic pot (left).

Figure 3 Twelve months old derris root in 10" x 20" plastic bag.

observed from the same treatment on the branch 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 they displayed non-significant difference to the plants grown 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 old derris plants (Figure 3), the rotenone contents, according to the treatments, 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 they showed no-significant to the plants grown in 13" x 26" plastic bags. Nevertheless, these two treatments provided greater levels of rotenone contents than other treatments. The lowest rotenone content of both fibrous (1.51%) and branch roots (3.18%) were also observed on the derris root from 10"x 20" plastic bag. Once more, significantly greater levels of rotenone root contents were obtained from the derris plants cultivated either in the field plots or bigger size containers.

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 old derris root grown in the field was 6.85–7.22%. Tongma *et al*. (2004) obtained the highest level of rotenone content of 14% (w/w) from twelve-month old field grown derris root, while 7.7% was determined from the plant root cultivated in a 16" x 32" (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%.

Table 2 Fresh and dry root weights (gram per plant) collected at 6-, 9-, and 12-month of age treatments.

Treatment	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 plot	41.60[a]	19.73[ab]	53.20[b]	24.55[c]	76.68[b]	34.68[b]

*Means within the same column followed by the same letter are not significantly different at LSD 0.05

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

2. Derris root yield

Fresh and dry weights of root (g/pt) from 6-, 9-, and 12-month old plants were displayed in Table 2. At six-month, no significant difference of the fresh weights among treatments were observed, while the lowest dry weight (16.29 g/pt) was detected form the plant grown in 10"x 20" plastic bag.

Among the nine-month plants, those grown in 13"x 26" plastic bags gave the highest yield of both fresh (101.80 g/pt) and dry root weights (47.20 g/pt). In addition, the significantly lowest yield of both fresh (53.20 g/pt) and dry root weights (24.55 g/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 g/pt) and dry root weights (65.53 g/pt) were collected from the plant cultivated in 13"x 26"plastic bags, although they showed no-significant difference among plants grown in various sizes of containers. However, the significantly lowest yield in both fresh (76.68 g/pt) and dry root weights (34.68 g/pt) were still detected from the plants cultivation 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 grown 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 old derris grown in the field produced dry root weight of only 65.5 g/pt, while derris root cultivated in16" x 32" (40 x 80 cm) circular cement pot reached 192.1 g/pt. The planting materials in the cement pot were soil mixed with rice husk alone and soil mixed with rice husk in combination with charcoal and soil mixed with

Table 3 Total fresh and dry weights (gram per plant) observed from 6-, 9-, and 12-month derris plants in relation to treatments.

Treatment	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^{a}	837.00^{a}	339.00^{a}

*Means within the same column followed by the same letter are not significantly different at LSD 0.05

coconut pericarp powder 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 16" x 32" (40 x 80 cm) circular cement pot filled with soil mixtures was much more convenient than harvested from the field plots. The insecticide formulated from water extraction obtained from only four-month derris root grown in cement pot 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 g/pt which closed to 12-month dry weight root derris developed in the plastic container.

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 g/pt, respectively (Table 3). Meanwhile, total dry weights detected from the same treatments were 123.20, 286.40, 204.00, and 339.00 g/pt, respectively. Both total fresh weights and total dry weights were non-significant difference among derris plants cultivated in various sizes of containers; nevertheless, they demonstrated significantly highest levels with the plants cultivated in the field.

Conclusion

Overall, the plant biomass in terms of total fresh and dry weights of each plant (g/pt); the root biomass in terms of root fresh and dry weights (g/pt); and rotenone content in fibrous and branch roots are normally increased with age, hence, the twelve-month derris plants exhibited significantly the highest levels. Types of containers either plastic bags or plastic pots showed no significant effect, although the biggest values of tested variables were recorded in the plant cultivated in the bigger size container (13"x 26"), they demonstrated no-significant difference to certain variables of plants developed in smaller size containers.

Among the twelve-month derris plants, the plant cultivated in the field displayed the highest total dry weight (339.00 g/pt), and rotenone content in both fibrous (3.86%) and branch root (5.00%), although, they exhibited no-significant difference to the same variables obtained from plants grown in 13"x 26" plastic bags. Besides, these treatment also provided the highest root biomass of fresh (145 g/pt) and dry weights (65.53 g/pt), while the significantly lowest levels of root biomass for fresh (76.68 g/pt) and dry weights (34.68 g/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 grown 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 convenient harvest with less time and labor consuming and with less root damage than collecting 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. Recently, the highest derris root yield was observed from the derris plant grown in16" x 32" (40 x 80 cm) circular cement pot (192.1 g/pt) with 7.7% rotenone content.(Tongma *et al.,* 2004). Thus, the appropriate container size and harvesting time for maximizing rotenone content level of the derris root need to be further investigated.

Acknowledgement

The authors would like to express their sincere gratitude to Dr. Sanit Ratanabhumma, Associate Professor in Entomology, for his constructive criticism and invaluable advice in preparation of this manuscript.

References

EPA, 2002. Controlling pests with rotenone. (Online). Environment Protection Agency. Fact Sheets, Office of Pesticide Programs, U.S.A. Available:http://www.epa.gov/oppsrrd1/REDs/facsheets/rotenone_fs.pdf (November 17, 2007)

Pitiyont, V. and A. Sangwanich. 1997. Extraction, isolation and identification of pesticidal compounds from *D. elliptica*. The Second Conference of Agricultural Toxic Substance Division, Department of Agriculture, Bangkok. pp. 84-92.

Sangmaneedet, S., K. Kanistanon, P. Papirom, and T. Tessiri. 2005. Uses of Thai medicinal herb (*Derris elliptica* (Roxb.) Benth) in control of fly larva population and its application in the treatment of cutaneous myiasis in animals. KKU. Res. J. 10(1): 22-30.

Srijukavan, S., A. Kasampasart, P. Kaidsiri and N. Yudjan. 1988. Growth and rotenone content in various age of derris. Kasetsart Sci. J. 21(3): 166 – 175.

Tongma, S., A. Sottikul, Y. Kaosumain, and N. Tiengburanaturm. 2004. Appropriate cultural practice for derris root production. LARTC. Rajamongala Institute of Technology. Report. 48 p.