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ROTENONE - POTENTIAL AND PROSPECT FOR SUSTAINABLE AGRICULTURE

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ABSTRACT

Rotenone is produced from the roots of two genera of the leguminosae family: Derris in Asia and Lonchocarpus in South America. Mode of action of rotenone is a respiratory enzyme inhibitor. Its rapid photodecomposition means that it is active only about 1 week on plant or 2-6 days in water, not expected to be a groundwater pollutant, and broken down readily by exposure to sunlight. Some its general overviews and physiochemical characteristics have been introduced in this paper. In Vietnam, during the period of 1994-2003, the biochemical characteristics of Derris elliptica Benth *and Derris trifoliata have been studied, including to identify extraction technique (protocol), crystallization of rotenone, and to set up technological process to produce different preparations for use as pesticide (or insecticide). These preparations have been tested under both fish pond and crop field conditions. Interestingly, they gained promising results. At the molecular chemistry institute belonging to the Paul Sabatier University in France, structural and spectroscopical studies of the rotenone have been recently carried out. These analyses showed that the natural rotenone crystallized for the first time from Derris trifoliata in Vietnam offered its purity met the international standard. These evidences indicate that rotenone can be used and it possibly replaces chemical pesticides in crop protection and aquaculture (shrimp culture) to obtain clean vegetable, and shrimp to meet the demand of sustainable agriculture in the forthcomimg time in Vietnam.*

INTRODUCTION

Rotenone is a selective, non-specific botanical insecticide with some acaricidal properties. Rotenone is used in home gardens for insect control, for lice and tick control on pets, and for fish eradications as part of water body management. Rotenone is a rotenoid plant extract obtained from such species as barbasco, cub, haiari, nekoe, and timbo. These plants are members of the pea (*Leguminosae*) family. Rotenone-containing extracts are taken from the roots, seeds, and leaves of the various plants. Formulations include crystalline preparations (approximately 95-97 % pure), emulsified solutions (approximately 1-50 % pure), and dusts (approximately 0.75 to 8 % pure). In Vietnam, rotenone was identified from two main species, *Derris elliptica* Benth and *Derris trifoliata* which are cultivated about ten thousand hectares in Soctrang and Binhduong provinces. Some preparations for using rotenone as a pesticide and insecticide in Mekong Delta (MD) have been reported.

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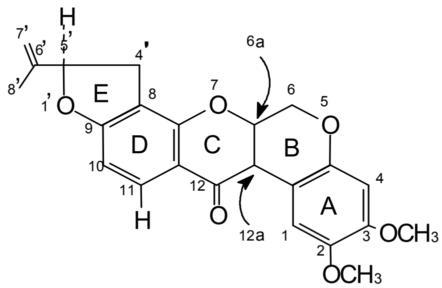
OVERVIEW OF ROTENONE IN THE WORLD
PHYSIOCHEMICAL PROPERTIES



- **Chemical name:** (2R,6aS,12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromeno[3,4-b]furo[2,3-h]chromen-6-one
- **Molecular weight:** 394.43
- **Water solubility:** 15 mg/L- 100^{0}C (Kid and James 1991), slightly soluble in water
- **Solubility in other solvents:** s. in acetone, carbon disulfide and chloroform; s.s in alcohols and carbon tetrachloride (Kid and James 1991)
- **Melting point:** 163^{0}C (Kid and James 1991)
- **Vapor pressure:** <1 mPa- 20^{0}C (Kid and James 1991)

Figure 1. Rotenone molecular formula $C_{23}H_{22}O_6$

TOXICOLOGICAL EFFECTS

- **Acute toxicity:** Local effects on the body include conjunctivitis, dermatitis, sore throat, and congestion. Ingestion produces effects ranging from mild irritation to vomiting. Inhalation of high doses can cause increased respiration followed by depression and convulsions. The compound can cause a mild rash in humans and is a strong eye irritant to rabbits (Ray 1991, U.S. Environmental Protection Agency 1988]. The oral LD_{50} of rotenone ranges from 132 to 1500 mg/kg in rats. The reported LD_{50} of rotenone in white mice is 350 mg/kg (Kidd and James 1991). A spray of 5% rotenone in water was fatal to a 100-pound pig when exposed to 250 cubic centimeters (mL) of the airborne mixture (Ray 1991). In rats and dogs exposed to rotenone in dust form, the inhalation fatal dose was uniformly smaller than the oral fatal dose (Ray 1991). Rotenone is believed to be moderately toxic to humans with an oral lethal dose estimated from 300 to 500 mg/kg (Ray 1991). Human fatalities are rare, perhaps because rotenone is usually sold in low concentrations (1 to 5% formulation) and because its irritating action causes prompt vomiting. The mean particle size of the powder determines the inhalation toxicity. Rotenone may be more toxic when inhaled than when ingested (Ray 1991), especially if the mean particle size is very small and particles can enter the deep regions of the lungs.

- **Chronic toxicity:** Growth retardation and vomiting resulted from chronic exposures of rats and dogs. Rats fed diets containing rotenone at doses up to 2.5 mg/kg for 2 years developed no pathological changes that could be attributed to rotenone (National Research Council 1983). Dogs fed doses of rotenone up to 50 mg/kg/day for 28 days experienced vomiting and excessive salivation, but no decreased weight gain (National Research Council 1983). Dogs fed rotenone for six months at doses up to 10 mg/kg/day had reduced food consumption and therefore reduced weight gain. At the highest dose, blood chemistry was adversely affected (National Research Council 1983), possibly due to gastointestinal lesions and chronic bleeding. Examination of 35 tissue types revealed only one type of lesion that might have been associated with exposure to the test chemical: lesions of the GI tract (National Research Council 1983).

- **Reproductive effects:** Pregnant rats fed 10 mg/kg/day on days 6 through 15 of gestation experienced decreased fecundity, increased fetal resorption, and lower birth weight (National Research Council 1983, U.S. National Library of Medicine 1995). Very high maternal mortality was seen at this dose. The 2.5 mg/kg/day dose produced no observable maternal toxicity or adverse effect on fetal development (Ray 1991). Fetotoxicity and failure of offspring are reported in guinea pigs at doses of 4.5 and 9.0 mg/kg/day for an unspecified period (Ray 1991). Thus reproductive effects seem unlikely in humans at expected exposures.

- **Teratogenic effects:** Pregnant rats fed 5 mg/kg/day produced a significant number of young with skeletal deformities (National Research Council 1983, U.S. National Library of Medicine 1995). The effects were not observed at the 10 mg/kg/day level, so the data do not provide convincing evidence of teratogenicity (Ray 1991, National Research Council 1983) because the effects do not appear to be dose-related. Thus, the evidence for teratogenicity is inconclusive.
- **Mutagenic effects:** The compound was determined to be nonmutagenic to bacteria and yeast and in treated mice and rats. However, it was shown to cause mutations in some cultured mouse cells (Ray 1991, National Research Council 1983). In summary, the data regarding the mutagenicity of rotenone are inclusive (U.S. Environmental Protection Agency 1983).
- **Carcinogenic effects:** Studies in rats and hamsters have provided limited evidence for carcinogenic activity of rotenone. No evidence of carcinogenic activity was seen in hamsters at oral doses as high as 120 mg/kg/day for a period of 18 months (National Toxicology Program 1984). Studies of two species of rats evidenced no statistically significant cancerous changes in any organ site, including mammary glands, at oral doses of up to 75 mg/kg/day for 18 months (National Toxicology Program 1984). Significant increases in mammary tumors have been reported in albino rats with intraperitoneal doses of 1.7 mg/kg/day for 42 days (National Toxicology Program 1984), and in Wistar rats at approximately 1.5 mg/kg/day orally for 8 to 12 months (National Toxicology Program 1984). In the latter study, however, higher dose rates (3.75 and 7.5 mg/kg/day) over the same period did not produce increased tumors (National Toxicology Program 1984). Thus, the evidence for carcinogenicity is inconclusive.
- **Organ toxicity:** Chronic exposure may produce changes in the liver and kidneys as indicated by the animal studies cited above.
- **Fate in humans and animals:** Absorption in the stomach and intestines is relatively slow and incomplete, although fats and oils promote its uptake. The liver breaks down the compound fairly effectively (Ray 1991). Animal studies indicate that possible metabolites are carbon dioxide and a more water-soluble compound that can be excreted in the urine (Ray 1991). Studies indicated that approximately 20% of the applied oral dose (and probably most of the absorbed dose) may be eliminated from animal systems within 24 hours (Ray 1991).

ECOLOGICAL EFFECTS

- **Effects on birds:** Rotenone is slightly toxic to wildfowl. The LD50 values for rotenone in mallards and pheasants are (greater than) 2000 mg/kg and 1680 mg/kg respectively [50]. A dietary LC50 of 4500 to 7000 ppm is reported in Japanese quail (Hill and Camardese 1986).
- **Effects on aquatic organisms:** Since rotenone is used as a fish toxin (piscicide), it follows that it is very highly toxic to fish. Reported 96-hour LC50s were 0.031 mg/L in rainbow trout, 0.0026 mg/L in channel catfish, and 0.023 mg/L in bluegill for the 44% pure formulation (Johnson and Finley 1986). Aquatic invertebrates have a wide range of sensitivity to rotenone with 48-hour EC50 values ranging from 0.002 to 100 mg/L (Johnson and Finley 1986). The

compound is not expected to accumulate appreciably in aquatic organisms. The bioconcentration factor for rotenone in the sunfish is 181 times the ambient water concentration. In addition the highly toxic nature of this substance to aquatic organisms means that there is little survival of the organisms that accumulate the compound.

- **Effects on other organisms:** The compound is nontoxic to bees. However, it is toxic to bees when used in combination with pyrethrum (Kidd and James 1991)
- **Breakdown in soil and groundwater:** Rotenone is rapidly broken down in soil and in water. The half-life in both of these environments is between 1 and 3 days (Augustijn-Beckers et al.1994). It does not readily leach from soil (Augustijn-Beckers et al.1994), and it is not expected to be a groundwater pollutant. Rotenone breaks down readily by exposure to sunlight (Kidd and James 1991). Nearly all of the toxicity of the compound is lost in 5 to 6 days of spring sunlight or 2 to 3 days of summer sunlight.
- **Breakdown in water:** Rotenone is rapidly broken down in soil and in water. The half-life in both of these environments is between 1 and 3 days (Augustijn-Beckers et al.1994). It does not readily leach from soil (Augustijn-Beckers et al.1994), and it is not expected to be a groundwater pollutant. Rotenone breaks down readily by exposure to sunlight (Kidd and James 1991). Nearly all of the toxicity of the compound is lost in 5 to 6 days of spring sunlight or 2 to 3 days of summer sunlight.
- **Breakdown in vegetation:** Rotenone is a highly active but short-lived photosensitizer. This means that an organism consuming the compound develops a strong sensitivity to the sun for a short time. A number of photodecomposition products are formed when bean leaves are exposed to light. It is also sensitive to heat, with much of the rotenone quickly lost at high temperatures.

REGULATORY STATUS: Rotenone is a General Use Pesticide (GUP), but uses on cranberries and for fish control are restricted uses. It is EPA toxicity class I or III - highly toxic or slightly toxic, depending on formulation. Rotenone, when formulated as an emulsified concentrate, is highly toxic and carries the Signal Word DANGER on its label. Other forms are slightly toxic and require the Signal Word CAUTION instead.

TRADE AND OTHER NAMES: Trade names for products containing rotenone include Chem-Fish, Cuberol, Fish Tox, Noxfire, Rotacide, Sinid and Tox-R. It is also marketed as Curex Flea Duster, Derrin, Cenol Garden Dust, Chem-Mite, Cibe Extract and Green Cross Warble Powder. The compound may be used in formulations with other pesticides such as carbaryl, lindane, thiram, piperonyl butoxide, pyrethrins and quassia.

RESEARCH ACTIVITIES DURING 1994-2003

Researches on the growing conditions and development of D. elliptica Benth

The suitable areas for *Derris elliptica* Benth growing and developing were concluded as coastal slopy regions with sandy soil in Mekong Delta (MD) such as Vinh Chau district, Soc Trang province. Both transplanting and harvesting are in the late rainy season. Root yielded 1.8-3.4 times in the appropriate NPK application treatments as compared to control (Hien et al 1996)

The initial **researches on the formation, translocation, and accumulation of rotenone in the *Derris elliptica* Benth** showed that rotenone content have the covariations with their precursors such as phenylalanine and methionine from the young leaves to the old leaves. In this stage, the biosynthesis of phenylalanine, methionine and rotenone have the covariations with the photosynthetic rate. By contrast, from the old leaves to stems, and roots are inverse. Actually, phenylalanine and methionine content decreased about 50% while rotenone content increased many times and offered the highest in the roots. The experiments cutting sieve-tube for blocking the transportation of solutes in the phloem vascular demonstrated that after biosynthezing in leaf, rotenone translocated downwards to the lower organs by the phloem route and eventually accumulates in the root. Observing the structure of the phloem and xylem gave an initial explaination on the mechanism of "load" at the phlohem cell source and "unload"at the xylem cell sink of rotenone in the *Derris* root. These suggested the way how to increase *Derris* root yield and rotenone content at farmer fields (Hien et al 1999, 2000).

Table 1. Existence of rotenone in *D. elliptica* Benth plant's organs determined by the different analysis methods

Derris plant's organs	By Irwin Hornstein's titration	By Colorimeter UV-VIS	By Weigh Measure D.C. Beach
Young leaf	0.788	0.39	0.54
Old leaf	1.356	0.77	1.21
Branch	1.771	1.50	1.69
Stem	2.210	1.74	1.91
Root's score	9.940	11.96	10.32
Root's bark	5.560	5.80	4.71

Table 2. Rotenone, methionine, and phenylalanine in different organs of *Derris* plant analysed by HPLC .

Derris plant's Organs	Methionine mg/100g	Phenylalanine mg/100g	Rotenone (%)
Young leaf	1.98	27.90	0.39
Old leaf	12.97	51.70	0.77
Stem	8.90	21.90	1.74
Root	5.90	26.90	11.60

Establishing the intercropping model between *D. elliptica* Benth and *Allium ascalonicum* L.

The intercropping model was recommended to overcome shortcoming due to *Derris* monoculture habit by farmers in MD. The intercropping model gained a lot of advantages such as: root's yield and rotenone content in the inter-cropping model increase 24-27% as compared to the mono culture model. By contrast, production expenditure of *Derris* in the former decreases 15-20% as compared to the latter. Eventually, total profit of the intercropping model obtained 3.15 times as compared to the control (*Derris* monoculture). The new advanced model has been applied largely and effectively in Soctrang province (Hien et al. 1999).

Study on the technology to produce preparations from the tuba root

Setting up a technical process to manufacture some products from *D. elliptica* Benth's root has been conducted. They include emulsion concentrated, water milk, and water-soluble powder preparations. The technological protocols were established by many solutions such as material preliminary treatment, stability for rotenoid bioactivity, preservation, and packing. To grind finely and keep well the intact situation of fresh root in production line, we have modified and fit up two equipments with general design as follows (Hao et al. 1998)

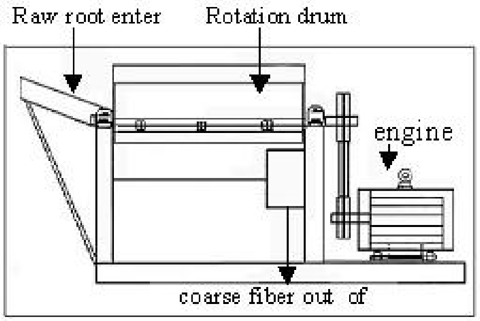


Fig 2. The first to grind raw root to wet-coarse fiber (GRR)

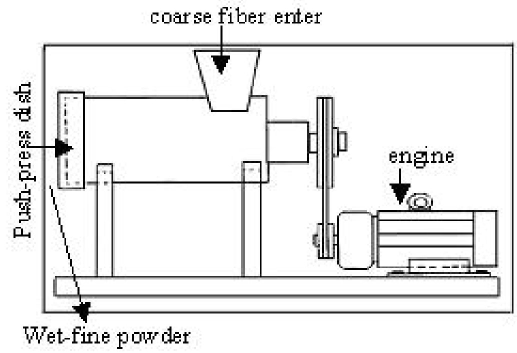


Fig 3. The second to grind, press, and push up the wet-coarse fiber to the wet-fine powder**c** (WPP) conditions and

The rotenone content of these products varied from 1.5 to 5%. Its bioactivities used as fish poisons, insect toxicants and antifeedants have been examined step by step. The effect on cruel and bastard fish has been evaluated initially along with the test on different vegetables.

Effect of rotenone in actual production

In MD Vietnam, farmers have extensively used rotenone as a fish poison for protecting shrimp against predatory fish in their fields. A few of them have effectively used rotenone as an insecticide for some vegetable crops. Based on the situation, after studying successfully the methods for extracting and isolating rotenone crystal from the root of *Derris elliptica* Benth in 1995, we have carried out manufacturing some kinds of preparation for use in both killing of predatory fish and controlling vegetable and rice insects.

Rotenone preparations produced by extraction using acetone and chloroform solvents exhibited its effect twice as that from ethanol extraction, the latter was 3-5 times as that from water extraction. Ethanol was used as a solvent largely due to its low cost and simple process. This preparation was labelled ROTND. ROTND 1.0-2.0 % had exterminated 65%-95% common insects in vegetable in MD. ROTND 200-400ppm a.i. offered the same effect as Decis (Deltamethrin) of 125ppm a.i. (Hien et al. 1999).

Table 4. Effect of rotenone on Bolworm and Diamond backed moth *(Plutella xylostera)*

ROTND concentration %	Bolworm died (%) Hours Post Spraying			Diamon backed moth died (%)		
	24	48	72	24	48	72
0.0 (OL4/§C)	0.0	0.0	0.3	0.0	1.0	1.0
1.0 (OL1)	89.2	91.6	94.1	56.0	74.0	75.6
2.0 (OL2)	91.3	94.7	95.9	62.8	82.0	83.0
4.0 (OL3)	93.4	96.6	99.1	74.0	91.0	93.4
CV%	11.74	13.57	12.50	10.79	7.47	6.34
$LSD_{0.05}$	3.579	4.321	5.213	11.761	12.501	11.769

- For controlling fish in the field, only 1ppm of ROTND was required to block the respiratory system of most fish in which trash fishes can tolerate for 30 minutes while the predatory fishes in lower class can tolerate for 180 minutes (Hien et al. 1999)

Table 5. Effect of rotenone on *Tilapia* by rotenone extracts by different solvents

Extraction solvent	Rotenone concentration (%)	ROTND treated (ppm)	Airless threshold of fish (minute)
H_2O	1.2	1	19.5
C_2H_5OH	2.8	1	9.0
$CHCl_3$	4.5	1	4.7
CH_3-CO-CH_3	5.7	1	3.7

For rice insect control

For controlling rice insects**,** ROTND exhibited the strongest effect at three-days after spraying, more powerful from 1 to 3 times as compared to commercial BT product, equally 66-71% of Karate and Trebon but its effect decreased to 75% at 10-days after spraying, equally to BT. This is a very good condition for fast rehabilitation of natural enemies, especially spiders. ROTND have exterminated 65-68% of rice skippers, similar to BT and 80% as compared to Trebon, Karate at three days after spraying. For brown plant hopper, ROTND had an effect on mortality as 72.5 times as compared to BT at one day after spraying, and only 1.5 times at 10 days after spraying (Loc et al 1999)

Table 6. Effect of rotenone on rice brown plant hopper (BHP) in rice.

Unit: % Ratio of BHP died

Treatment	Days after spraying			
	1	3	7	10
ROTND 1,5% (6,4l/ha)	59.8b	59.8b	51.8b	31.9a
ROTND 2,0% (9,6l/ha)	63.7b	63.3b	57.8bc	37.3a
BT 0.7 l/ha	0.2a	19.5a	37.4a	38.4a
BT 0.1 l/ha	1.5a	25.5a	36.7a	44.0ab
Karate 0.5 l/ha	80.0c	83.8c	65.6cd	52.3bc
Trebon 0.64 l/ha	83.4c	89.9c	73.9d	60.2c

Table 7. Influence of rotenone on the rehabilitation of spider in rice

Unit: Ratio of enemy spider died (%)

Treatment	Days after spraying			
	1	3	7	10
ROTND 1,5% (6,4l/ha)	32.6b	34.8ab	16.5a	12.9a
ROTND 2,0% (9,6l/ha)	38.0b	49.6b	14.6a	13.5a
BT 0.7 l/ha	18.5a	24.1a	28.9b	12.1a
BT 0.1 l/ha	18.7a	25.3a	30.3b	16.6ab
Karate 0.5 l/ha	90.6c	94.4d	36.0c	24.6b
Trebon 0.64 l/ha	86.8c	77.1c	25.3c	18.5ab

For golden snail, the effect of rotenone on golden snail *Pomaceae canaliculata (Ampuliariidae)* in rice field was recognized. With concentration of 1-2 ppm ROTND, over 90% *Pomaceae canaliculata* was killed at 2-4 days after treating. These initial results are going to be continued studying.

Structural and spectroscopical studies on natural rotenone crystal

In France, natural rotenone from *Derris trifoliata* roots planted in Vietnam has been extracted and crystallized by the rather particular separated technique from solvate complex $C_{23}H_{22}O_6$-CCl_4. Structural and spectroscopical studies on this rotenone were carried out by mono-crystal and powder X-ray diffraction to determine directly molecular structure.

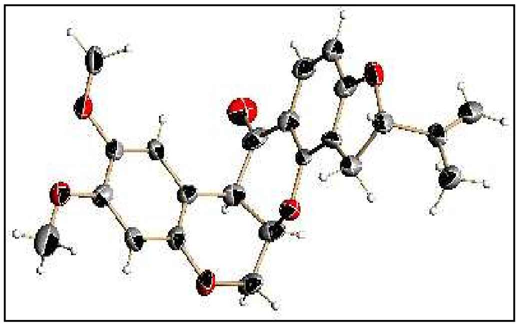


Figure 4. Rotenone molecular structure determined by X-ray mono-crystal diffraction

After that, rotenone was also analysed by ^{1}H NMR, ^{13} NMR both unidimension and bidimension on the Bruker ARX 400 MHz. For the purity, all samples were also determined by IR, MS, HPLC, LC/MS.

These results showed that the natural rotenone crystallized from *Derris trifoliata* originated from Vietnam offered the purity fit to the international standard.

CONCLUSION

Mode of action of rotenone is a respiratory enzyme inhibitor, acting between NAD+ (a coenzyme involved in oxidation and reduction in metabolic pathways) and coenzyme Q (a respiratory enzyme responsible for carrying electrons in some electron transport chains), resulting in failure of the respiratory functions. Therefore it has been generally conclude that there is little likehood that any residue left from a spray will poison people, as the amount consumed would have to be considerably it is ever likely to be in this case.

Rotenone is used as a fish poison to protect shrimps in the field in MD. It has some selectivity of action when pure, killing marine fish but not invertebrates (Gilmore et al. 1981). It is also active as a nonsystemic pesticide against a wide variety of insects, arachnids, and mollusks. Its rapid photodecomposition means that it is active only 1 week on plant or 2-6 days in water, nearly all of the toxicity of the compound is lost in 5 to 6 days of spring sunlight or 2 to 3 days of summer sunlight, not being absorded in soil,…. It does not readily leach from soil (Augustijn-Beckers 1994), and it is not expected to be a groundwater pollutant. Rotenone breaks down readily by exposure to sunlight.

Rotenone will be used and replaced gradually chemical pesticides especially in producing clean vegetable, fruit, and shrimp

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SUMMARY IN VIETNAMESE

Rotenone được tạo ra từ rễ của hai giống cây thuộc họ đậu *Leguminosea,* đó là*:* *Derris* ở Châu Á và *Lonchocarpus* ở Nam Mỹ. Nó có tác dụng như một chất ức chế enzym hô hấp và bị phân rã nhanh dưới tác dụng của ánh sáng (chỉ một tuần ở trên cây và 2-6 ngày ở dưới nước sau khi sử dụng), không thấm qua đất gây ô nhiễm nước ngầm. Một số tổng quan và tính chất hóa lý của rotenone đã được giới thiệu trong bài viết này. Ở Việt nam, trong thời kỳ 1994-2003, chúng tôi đã nghiên cứu các tính chất hóa sinh của cây *Derris elliptica* Benth *và Derris trifoliata,* nghiên cứu các kỹ thuật tách chiết, phân tích và kết tinh rotenone từ rễ của các cây này, sau đó chế biến thành các chế phẩm khác nhau để sử dụng trừ cá dữ trong ruộng nuôi tôm và phòng trừ sâu hại cây trồng. Các thử nghiệm trong nuôi trồng thủy sản cũng như trong trồng trọt đều cho những kết qủa tốt. Mới đây, ở Viện Hóa học Phân tử Trường Đại Học Paul Sabatier Cộng Hòa Pháp, chúng tôi đã nghiên cứu phân tích phân tử và phổ của tinh thể rotenone lần đầu tiên được kết tinh từ cây *Derris trifoliata* có nguồn gốc ở Bình Dương, Việt Nam. Các kết qủa phân tích này đã chứng tỏ các tinh thể rotenone của chúng ta có độ sạch đạt tiêu chuẩn quốc tế. Từ các cơ sở đó, rotenone có nhiều khả năng được sử dụng và dần dần thay thế được các loại thuốc hóa học tổng hợp cho cả trong lĩnh vực thủy sản cũng như trồng trọt, góp phần xây dựng từng bước một nền nông nghiệp sinh thái bền vững trong tương lai sắp tới cho Việt Nam.