DOI:10.1021/jf901862z

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

# Development and Evaluation of Granule and Emulsifiable Concentrate Formulations Containing *Derris elliptica* Extract for Crop Pest Control

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*Derris elliptica* Benth. extracts containing rotenone have long been used as natural insecticides, but time-consuming preparation processes and the short shelf life of the extract limit their use in pest control. In this study, stable water-dispersible granules and emulsifiable concentrate liquids containing *Derris* extract (equivalent to 5% w/w of rotenone) were developed with simple techniques. Accelerated degradation kinetics of rotenone in the *Derris* extract, and in both formulations, indicated that its degradation followed first-order kinetics. The predicted half-life ($t_{1/2}$) and shelf life ($t_{90\%}$) at 30 °C of rotenone in *Derris* extract were 520 and 79 days, respectively. *Derris* granules and emulsifiable concentrate clearly prolong the stability of rotenone 8-fold ($t_{90\%}$ = 633 days) and 1.4-fold ($t_{90\%}$ = 110 days), respectively. The study of rotenone degradation after application onto plants indicated that both formulations would be effective for up to 3 days after spraying. Preliminary efficacy testing indicated that the *Derris* emulsifiable concentrate was clearly more effective than *Derris* water-dispersible granules in controlling *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae).

**KEYWORDS: *Derris elliptica*; rotenone; natural insecticide; water-dispersible granules; emulsifiable concentrate**

## INTRODUCTION

Pest control in recent years has become a major problem in almost all agricultural countries. The use of large amounts of synthetic pesticides by farmers worldwide to protect crops has resulted in some cases in the accumulation of potentially toxic residues in soil, water, and agricultural products. This concern over the excessive use of pesticides has led farmers to turn to the use of alternative methods that are environmentally friendly and are of relatively lower cost compared to the chemical pesticides. Plant extract preparations are examples of such pest control alternatives, and they are of increasing interest due to their proven efficacy and rapid decomposition in the environment after usage.

*Derris elliptica* Benth. has previously been known as an important source for compounds with broad-spectrum pesticidal properties (*1*). *Derris* is a genus of the family Papillionaceae. It is locally known in Southeast Asian countries as "derris" or "tuba" and in Thailand as "lotin". Extracts from the stem and root of this plant have been used over centuries as fish poisons and as insecticidal preparations (*2*, *3*). These properties are mainly due to the presence of rotenone (**Figure 1**), a compound that is highly toxic to cold-blooded animals, especially fish and insects. Rotenone is classified by the World Health Organization as a moderately hazardous Class II pesticide. There are some reports indicating that an exposure to rotenone may cause toxicity to mammals. However, rotenone is usually used in small quantities, and it is rapidly broken down in soil and water. Rotenone is therefore good for the environment and safe for agriculturists and other users (*4*−*7*). Recently, we have reported on the rotenone content of extracts from different parts of the plants using optimized extraction methods to afford maximum yields (*8*).

Rotenone is available as technical grade solution at concentrations of 35, 90, or 95%, as wettable powders containing 5 or 20% of active substance, and as 0.75−5% dusts. It is also available as a 5% emulsifiable concentrate. These formulations are mostly marketed in the United States and Europe (*6*, *9*). However, most products containing rotenone have relatively high cost, and the formulation ingredients are commercial secrets. *D. elliptica* is easy to grow and provides high root yield within 2 years (*10*). There is a single Thai study on growth patterns of this plant and accumulation of rotenone and other rotenoids in *Derris* sp. harvested at different ages. The quantity of rotenoids in the roots accumulates until the age of 27 months, after which it decreases, the highest quantity being found when derris is 26 months old (*11*). Therefore, it is possible to grow this plant on a large scale with relative ease for commercialization of the dried root or its products within

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 Published on Web 11/11/2009 

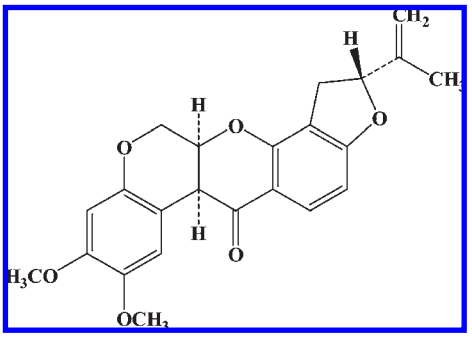


**Figure 1.** Chemical structure of rotenone.

2−3 years after planting. Farmers in Thailand and other developing countries usually macerate the *Derris* root in water and use the resulting milky suspension for spraying their crops. However, this method of preparation and use is inconvenient because the amount of active compound may vary from application to application. Therefore, there was a need for the development of effective, stable, and standardized formulations of *Derris* extracts. *Spodoptera litura*, which is a serious pest in agricultural areas and causes damage to many cultivated plants such as vegetables, field crops, and many kinds of fruit trees, was used for the preliminary efficacy test in this study. A common vegetable host of *S. litura* is Chinese kale (*Brassica alboglabra* Bailey), which is a popular leafy vegetable in Thailand. The objectives of this study were (1) to develop two types of pesticide formulations containing *Derris* extract [water-dispersible granules (WG) and emulsifiable concentrate (EC)], (2) to study the physicochemical properties and stability of rotenone in the developed formulations, and (3) to carry out a preliminary efficacy test of the formulations against *S. litura* in laboratory experiments, prior to future field trials.

Water-dispersible granules were chosen as one of the formulations because they potentially offer significant advantages in packaging, ease of handling, stability, and safety. Because the surface area of granules is lower than that of comparable volumes of powder formulations, granules are usually more stable to the effects of the atmosphere (*12*, *13*). Granules are associated with a much lower inhalation hazard compared to dusts and wettable powders. Granules can readily be dispersed in water to form fine suspensions in the spray tank and require only gentle agitation to maintain a uniform mixture.

Emulsifiable concentrates are widely used for formulating pesticides. However, most currently used pesticide formulations require the use of organic solvents and other additives, which may present a problem in terms of user and environmental safety (*13*). In this study, emulsifiable concentrate formulations, using mixtures of mineral oil or soybean oil solution and emulsifier, were prepared. The plant extract was miscible in the liquid mixture, and the system formed a fine oil-in-water (o/w) emulsion when introduced into an aqueous phase under conditions of gentle agitation (*14*). The concentrate liquid formulation was preferred, rather than the emulsion formulation, due to ease of handling and delivery.

## MATERIALS AND METHODS

**Materials.** The roots of *D. elliptica* were collected from the botanical garden of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand, in April 2007. Authentication of plant materials was carried out at the herbarium center of the Department of Forestry, Bangkok, Thailand, where a herbarium voucher has been kept identifying the plant species. A voucher specimen of this plant was also kept in the Herbarium of Southern Center of Thai Traditional Medicine at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The roots were washed with tap water, dried in air, cut into small pieces, and dried in a hot air oven at 40 °C for 24 h. The dried plants were then ground to a powder and stored in plastic bags in the dark at room temperature (30 ± 2 °C).

HPLC grade acetonitrile and analytical grade methanol and acetone were purchased from Labscan Asia (Bangkok, Thailand). Commercial grade dichloromethane was obtained from High Science distributor (Songkhla, Thailand). Water was purified by passing it through a Milli-Q purification system. Standard rotenone was purchased from Sigma-Aldrich (Steinheim, Germany). The stock solution (0.1 mg/mL) was prepared by dissolving 10 mg of rotenone in 100 mL of methanol. Working solutions were freshly prepared on the day of any study by suitable dilution of the stock solution with methanol. The stock solution was kept in the dark at 4 °C and used within 1 month.

Commercial grade polyvinyl pyrrolidone K-30 (PVP K-30), sodium alginate, sorbitan monooleate (Span 80), and polyethylene sorbitan monooleate (Tween 80) were purchased from Srichand United Dispensary (Bangkok, Thailand). Pharmaceutical grade lactose monohydrate was obtained from DMV International Distributor (Thailand). Microcrystalline cellulose (Avicel PH101) and commercial grade light mineral oil were from PC Drug Center Co., Ltd. (Bangkok, Thailand). Food grade soybean oil was purchased from Thai vegetable oil public company limited (Bangkok, Thailand), and analytical grade butylated hydroxytoluene (BHT) was purchased from Sigma (St. Louis, MO). Cypermethrin, 35% w/v of emulsifiable concentrate (Starship 35), was from Hi-tech Group Chemical Supply Co., Ltd. (Bangkok, Thailand).

**Extraction of the Dried Root Powder of *D. elliptica*.** The dried root powder was macerated with dichloromethane at room temperature (30 ± 2 °C) for 3 days with occasional stirring. The solvent was then filtered and evaporated in a rotary evaporator (Eyela, Tokyo, Japan) under vacuum at 40 °C. The residue was placed in a vacuum oven (Napco, Chicago, IL) at room temperature until dry. The crude extract was stored in a well-closed container and protected from light and kept in a desiccator at 4 °C. Rotenone content in the crude extract was determined by HPLC. Approximately 1 mg of the extract was accurately weighed and quantitatively transferred to a 25 mL volumetric flask, the volume was made up to 25 mL with methanol, and the mixture was sonicated for 5 min. The methanolic sample was then filtered before analysis. The analyses were performed in triplicate, and calibration standards were analyzed on the same day as the samples.

**HPLC Analysis.** The analysis of rotenone in the *Derris* extract and *Derris* formulations was performed using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) with a Restek C18 5 μm column (250 × 4.6 mm). A linear gradient was used for the separation of rotenone with the initial mobile phase composition of acetonitrile/water 50:50 (% v/v) reaching 70:30 (% v/v) in 30 min. Before each injection, the HPLC column had to be stabilized for 10 min with the initial mobile phase composition of acetonitrile/water mobile phase 50:50 (% v/v). The injection volume was 20 μL, and the flow rate was 1 mL/min. Detection was by UV spectroscopy at a wavelength of 294 nm. In our previous study (*8*), standard calibration curves of rotenone were constructed by plotting concentrations against peak areas. A good linearity was achieved with a correlation coefficient of 0.9999 over the concentration range of 1.2−20 μg/mL. The limit of detection (LOD) value for rotenone was 0.24 μg/mL, and the limit of quantitation (LOQ) value was 0.71 μg/mL, respectively. The reproducibility of the method was demonstrated by repeated injections of rotenone standards. Five daily injections over a 5 day period gave intraday relative standard deviation (RSD) ranging from 0.59 to 1.18%, whereas interday relative standard deviation ranged from 2.18 to 3.22%, respectively (*8*).

**Preparation of *Derris* Water-Dispersible Granules.** The *Derris* water-dispersible granules (containing 5% w/w rotenone), consisting of *Derris* extract, PVP K-30, sodium alginate, microcrystalline cellulose, Tween 80, and lactose, were prepared by a wet granulation method. The *Derris* extract (4.15 g), which contained 1.5 g of rotenone (36.13% w/w), was mixed with 1.5 g of PVP K-30, 1.5 g of sodium alginate, 1.5 g of microcrystalline cellulose, and 21.05 g of lactose by a geometric dilution technique to give a homogeneous powder. Tween 80 (0.30 g) was dissolved in 5 mL of distilled water and added dropwise to the powder with continuous mixing to produce a damp mass, which was then passed through a no. 14 sieve and dried in a hot air oven at 40 °C for 2 h. After that, the dried particles were screened through a no. 16 sieve. The granules were stored in a well-closed container at room temperature (30 ± 2 °C) and

protected from light. Water-dispersible granules without *Derris* extract were also prepared and are hereafter referred to as negative control.

**Evaluation of the Physical Properties of *Derris* Water-Dispersible Granules.** *Rotenone Content*. Approximately 10 mg of the granule sample was accurately weighed and quantitatively transferred to a 25 mL volumetric flask, the volume was made up to 25 mL with methanol, and the mixture was sonicated for 30 min. The methanolic sample was then filtered and the rotenone content determined by HPLC as described above. The analyses were performed in triplicate, and calibration standards were analyzed on the same day as the samples.

*Friability*. Granules (10 g) were placed in the drum of a Roche friabilator with 25 steel balls. The samples were rotated at 25 rev/min for 16 min, each rotation causing the granules to fall a distance of 15 cm inside the drum. Afterward, each sample was sieved through a 40 mesh screen, and the weight of sample remaining above the screen was determined; the percentage friability index calculated. Three replicates were performed for each test, and the data are given as means (±SD).

*Disintegration Time, Viscosity, and pH*. Granule samples (2.5 g) were dispersed in 50 mL of distilled water (1:20 dilution) and stirred at 500 rpm at room temperature (30 ± 2 °C). The time required for complete disintegration of the granules was recorded. The viscosity of the resulting suspension (equivalent to 0.25% rotenone) was measured by a Brookfield model DV III rheometer (spindle no. 31 at 250 rpm). The pH of the suspension was also determined using a pH-meter (Mettler-Toledo Co. Ltd., Switzerland). Three replicates were performed for each test, and the data are given as means (±SD).

**Studies of Emulsifiable Concentrate Formulations of *Derris* Extracts.** *Study To Determine Optimum Ratios of Oils and Surfactants*. The oils (soybean oil or light mineral oil) and surfactants (Tween 80 and Span 80) were mixed in different proportions to find the most suitable formulations that gave a clear homogeneous liquid and provided stable emulsions after 1:20 dilution with water. The formulation that showed the best characteristic was selected for the preparation of the *Derris* emulsifiable concentrate.

*Preparation of Derris Emulsifiable Concentrate*. The emulsifiable concentrate containing *Derris* extract was prepared by simple mixing. The *Derris* extract (6.92 g), which contained 2.5 g of rotenone (36.13%w/w), was ground and mixed with Tween 80 (17.5 g) and Span 80 (12.5 g) using a mortar and pestle. Soybean oil (13.03 g) and BHT (0.05 g) were then added and mixed to give a homogeneous concentrate mixture that contains 5% w/w of rotenone. The resulting formulation was stored in a glass bottle at room temperature (30 ± 2 °C) and protected from light. An emulsifiable concentrate without *Derris* extract was also prepared and is hereafter referred to as the negative control.

**Evaluation of the Physical Properties of *Derris* Emulsifiable Concentrate.** *Rotenone Content*. Approximately 10 mg of emulsifiable concentrate sample was accurately weighed and quantitatively transferred to a 25 mL volumetric flask, the volume was made up to 25 mL with methanol, and the mixture was sonicated for 30 min. The methanolic sample was then filtered and the rotenone content determined by HPLC as described above. The analyses were performed in triplicate, and calibration standards were analyzed on the same day as the samples.

*Particle Size, Viscosity, and pH*. Emulsifiable concentrate (2.5 g) was dispersed in 50 mL of distilled water by stirring with a magnetic stirrer at 500 rpm for 5 min to complete emulsification, at room temperature (30 ± 2 °C). The resulting emulsion (equivalent to 0.25% rotenone) was evaluated for oil droplet size by the Mastersizer E (Malvern Instrument Ltd.). The viscosity of the emulsion was measured by a Brookfield model DV III rheometer (spindle no. 31 at 250 rpm). The pH of the emulsion was also determined using a pH-meter (Mettler-Toledo Co. Ltd.). Three replicates were performed for each test, and the data are reported as means (±SD).

**Chemical Stability of Rotenone in *Derris* Extract and *Derris* Formulations.** The stability of rotenone during storage of the *Derris* extract and the two *Derris* formulations was tested under accelerated conditions. Samples of each (1 g) were stored in closed glass containers at 45, 60, and 70 °C with 75% relative humidity (RH) and protected from light. The amount of rotenone in each stored sample ($n = 3$) was analyzed at 0, 1, 3, 7, 14, 21, 28, 56, 84, 112, 140, and 168 days after storage by HPLC. Approximately 1 mg of the extract and 10 mg of the two *Derris* formulations were accurately weighed and quantitatively transferred to a 25 mL volumetric flask, the volume was made up to 25 mL with methanol, and the mixture was sonicated until complete solubilization. The methanolic samples were then filtered and rotenone content determined by HPLC as described above. The analyses were performed in triplicate, and calibration standards were analyzed on the same day as the samples. The amount of rotenone was plotted against the storage time to calculate the half-life ($t_{1/2}$) and shelf life ($t_{90\%}$). A study of the real-time stability of the *Derris* extract and the two *Derris* formulations was also compared to that calculated from accelerated studies. Samples of *Derris* extract and the *Derris* formulations (1 g) were stored in closed glass containers at room temperature (30 ± 2 °C) and protected from light for 6 months; rotenone content remaining was determined by HPLC after 6 months of storage.

**Stability of Rotenone in *Derris* Formulations after Spraying onto Plants.** The stability of rotenone in *Derris* formulations after spraying onto plants was studied under greenhouse conditions. Ten pots of Chinese kales were placed in a greenhouse, which was exposed to sunlight. Granule or emulsifiable concentrate samples (5% w/w) were mixed with water (1:20) by gentle stirring to form a suspension or an emulsion that contained 0.25% w/v of rotenone. The prepared suspensions or emulsions were sprayed onto the leaves (50 mL/plant) of 60-day-old Chinese kales. Ten replicates were performed for each treatment. The leaves of Chinese kales were sampled at 0, 1, 3, 5, 7, 10, and 14 days after spraying. Rotenone residues on the both sides of foliage (80 $cm^2$) ($n = 3$) were determined by rinsing the leaves with acetone, and the acetone washings were evaporated to dryness. The resulting samples were then reconstituted with methanol and quantitatively transferred to a 25 mL volumetric flask, the volume was made up to 25 mL with methanol, and the mixture was sonicated for 10 min. The methanolic samples were then filtered, and rotenone content was determined by HPLC as described above. The analyses were performed in triplicate, and calibration standards were analyzed on the same day as the samples.

**Preliminary Efficacy Testing of the Two *Derris* Formulations.** The efficacy of each formulation against *S. litura* was investigated under laboratory conditions. Each formulation (5% w/w of rotenone) was mixed with water by gentle stirring to form a suspension or an emulsion that contained 0.25% w/v of rotenone. The leaves of Chinese kale were dipped in the resulting suspension or emulsion (1 min), air-dried (15 min), and placed in plastic boxes as feed for the larvae. Five second-instar larvae of *S. litura* were placed in each box. Ten replicates were performed for each treatment. Larvae mortality was recorded every 24 h for 3 days, and any growth abnormalities of surviving larvae were also investigated. The larvae in boxes containing leaves treated with control formulations (not containing *Derris* extract) were used as the negative controls. For positive controls, the leaves were dipped into a solution of the pesticide cypermethrin at the concentration suggested for field applications (0.0175% w/v).

## RESULTS AND DISCUSSION

**Preparation of *Derris* Root Extract.** The extract of *Derris* root was a yellow powder (yield = 10.03% w/w relative to the weight of dried plant), and the amount of rotenone in the *Derris* root was 3.62% w/w calculated as absolute yield of rotenone (g) in 100 g of dried plant. Because the crude extract was hygroscopic, it was kept in a desiccator at 4 °C.

From the previous papers, the cultivated *D. elliptica* root contained about 13% of rotenone, and the black resinous extract that is sold in the market contained about 30% of rotenone (*2*, *3*). The rotenone content of the extract from *Derris* root (36.13% w/w) obtained in our study, from locally obtained fresh roots, is considerably higher than reported in earlier studies. It is clear that future studies need to examine how cultivation and soil conditions and geographical location affect rotenone formation and accumulation in *Derris* root, particularly if there is any hope for successful commercialization of the formulations we have developed.

Although it is possible to isolate pure rotenone from *Derris* extract, or indeed develop methods for its chemical synthesis, this would not be cost-effective for commercialization of any formulated products, because the primary aim is to provide agriculturists

in developing countries with cheaper, locally produced, and safer alternatives to chemically based pesticides. Additionally, it may be that the efficacy, and perhaps the overall safety, of *Derris* extract is not only due to rotenone content but also due to a delicate balance of a variety of other ingredients in the *Derris* extract. It should be relatively easy to grow *D. elliptica* on a large scale for regular harvesting of root and preparation of standardized extracts with known rotenone content.

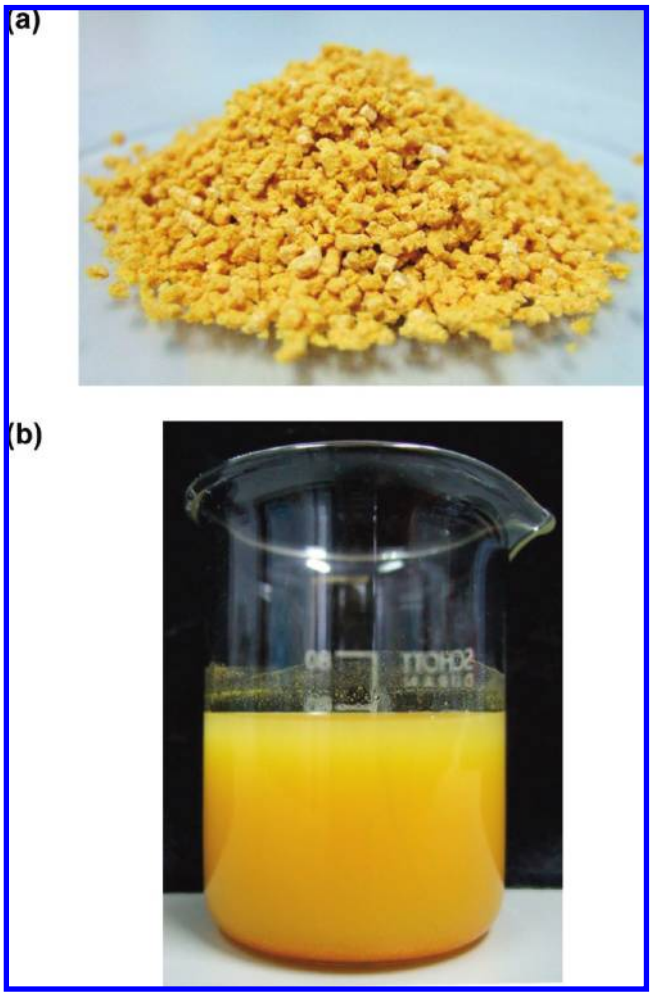


**Figure 2.** Appearance of *Derris* water-dispersible granules (**a**) and the obtained aqueous suspension (**b**). The former contains 5% w/w rotenone, and the latter contains 0.25% w/v rotenone.

**Table 1.** Physical Properties of *Derris* Water-Dispersible Granules

| | |
|---|---|
| disintegration time[a] (min) | 5.67 ± 0.41 |
| pH[a] | 6.87 ± 0.15 |
| viscosity[a] (cP) | 11.0 ± 0.1 |
| friability index | 95.8 ± 0.8 |
| rotenone content (% w/w) | 5.08 ± 0.13 |

[a] Data refer to aqueous suspensions of granules (equivalent to 0.25% w/v rotenone).

**Physical Properties of *Derris* Water-Dispersible Granules.** *Derris* water-dispersible granules containing 5% w/w rotenone were successfully developed and yellow granules obtained as shown in **Figure 2a**. A rotenone content of 5% w/w is usually used in commercially available products (*15*). A range of granule formulations containing different proportions of PVP K-30, sodium alginate, microcrystalline cellulose, Tween 80, and lactose were prepared as part of this study, and the formulation eventually selected yielded granules with a high friability index and fast disintegration time compared to other test formulations. The physical properties of the granules are shown in **Table 1**. The granule friability index was high (95.8 ± 0.8), and the granules completely disintegrated in water within 6 min; the fine suspension obtained is shown in **Figure 2b**. The viscosity and pH of aqueous suspensions were 11.0 ± 0.1 cP and 6.9 ± 0.2, respectively.

**Effect of Oils and Surfactants on Physical Properties of Emulsifiable Concentrate.** Soybean oil and mineral oil are widely used in liquid agricultural products, especially for those containing biocontrol microorganisms (*16*−*20*). Four emulsifiable concentrate formulations that provided stable aqueous emulsions after 1:20 dilution are shown in **Table 2**. The viscosity and pH values of the obtained emulsions were in the range of 3.3−4.6 cP and 5.6−6.3, respectively (**Table 2**). The results clearly showed that the mean particle sizes of emulsions prepared from formulations containing soybean oil were smaller than those of formulations containing mineral oil. This may be due to the composition of soybean oil, which contains emulsifiers, for example, long-chain polyunsaturated fatty acids including, oleic acid, linoleic acid, and linolenic acid (*21*). It is known that droplet size in emulsions is one of the most important factors governing its stability; reduction of droplet size usually leads to formation of stable emulsions (*14*). The small droplet size may also improve overall efficacy by enhancing the spread of the formulation over the plant foliar surfaces. Therefore, formulation 2 (**Table 2**), which produced the smallest droplet size (16.9 ± 0.4 μm), was selected for production of the *Derris* emulsifiable concentrate.

**Physical Properties of *Derris* Emulsifiable Concentrate.** Dark brown viscous *Derris* emulsifiable concentrate containing 5% w/w rotenone was successfully developed as shown in **Figure 3a**. The emulsifiable concentrate, when diluted with water (1:20), rapidly provided a stable yellow emulsion (0.25% w/v rotenone) (**Figure 3b**). Very little (< 0.1%) upward creaming layers occurred after 24 h, and any creaming that did occur could be redispersed again with gentle stirring. The viscosity of the 5% w/v aqueous emulsion (0.25% w/v rotenone) obtained from the emulsifiable concentrate (4.3 ± 0.1 cP) was slightly higher than that from the corresponding aqueous control emulsifiable concentrate (3.4 ± 0.0 cP). The pH value of the aqueous emulsion obtained from the emulsifiable concentrate (4.8 ± 0.0) was lower than that from the corresponding aqueous control emulsifiable concentrate (5.9 ± 0.0). The formulations containing *Derris* extract gave bigger mean particle size of emulsion (22.0 ± 1.8 μm) than that from control formulations (16.9 ± 0.4 μm). The bigger mean inner phase diameter may be caused by the constituents in the crude extract, which consists of many components that might affect emulsion formation and droplet size therein.

**Table 2.** Emulsifiable Concentrate Formulations[a] and the Physical Properties of the Obtained Emulsion after 1:20 Dilution with Water

| no. | soybean oil (%) | mineral oil (%) | Tween 80 (%) | Span 80 (%) | viscosity (cP) (±SD) | pH (±SD) | mean particle size (μm) (±SD) |
|---|---|---|---|---|---|---|---|
| 1 | 50 | | 25 | 25 | 3.28 ± 0.07 | 6.33 ± 0.02 | 63.3 ± 1.6 |
| 2 | 40 | | 35 | 25 | 3.36 ± 0.00 | 5.93 ± 0.01 | 16.9 ± 0.4 |
| 3 | | 50 | 25 | 25 | 4.56 ± 0.12 | 5.82 ± 0.02 | 224 ± 3 |
| 4 | | 40 | 35 | 25 | 4.32 ± 0.00 | 5.58 ± 0.01 | 67.3 ± 4.8 |

[a] These preliminary formulation trials do not contain *Derris* extract.

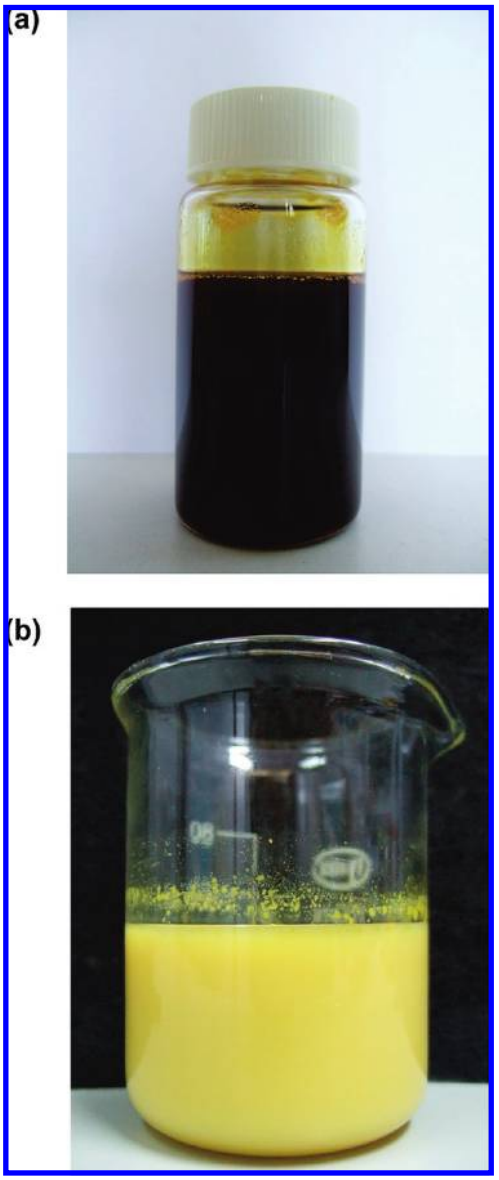


**Figure 3.** Appearance of *Derris* emulsifiable concentrate (**a**) and the obtained aqueous emulsion (**b**). The former contains 5% w/w rotenone, and the latter contains 0.25% w/v rotenone.

**Chemical Stability of Rotenone in *Derris* Extract and *Derris* Formulations.** The accelerated degradation kinetic studies of rotenone in the *Derris* extract were performed at three elevated temperatures, 45, 60, and 70 °C (75% RH). At the end of sampling periods (1−168 days), the extent of rotenone degraded was determined and found to follow first-order kinetics (**Figure 4**). The rate constant ($k$) for each temperature was determined to be 0.0025, 0.0044, and 0.0063 day$^{-1}$ for 45, 60, and 70 °C, respectively. Extrapolation of the Arrhenius plots (**Figure 7**) obtained from a relationship between ln $k$ and the reciprocal of the absolute temperature led to an estimated $k$ of 0.0013 day$^{-1}$, at 30 °C ($k_{30°C}$). Under the conditions used $t_{1/2}$ and $t_{90\%}$ were predicted to be 520 and 79 days, respectively (**Table 3**).

The degradation of rotenone in water-dispersible granule and emulsifiable concentrate formulations also followed first-order kinetics. In the case of water-dispersible granule formulation, the rate constant ($k$) for each temperature was determined to be 0.0007, 0.0033, and 0.0061 day$^{-1}$ for 45, 60, and 70 °C, respectively (**Figure 5**). In the case of emulsifiable concentrate formulation, the rate constant ($k$) for each temperature was determined to

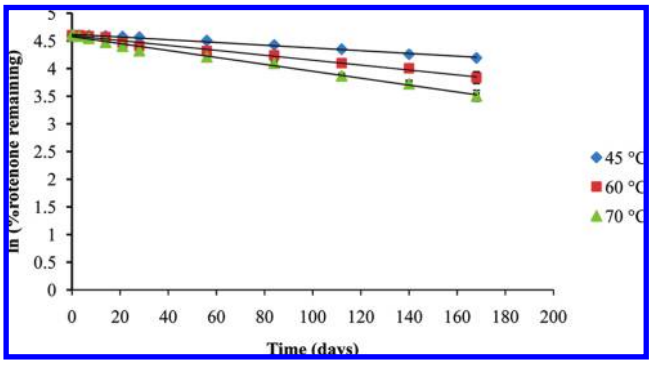


**Figure 4.** First-order plots for degradation of rotenone in *Derris* extract at 45, 60, and 70 °C (75% RH). Rate equations for each temperature are expressed as ln $c$=4.6203 − 0.0025$t$, $r^2$=0.9905; ln $c$=4.5969 − 0.0044$t$, $r^2$ = 0.9877; and ln $c$ = 4.5768 − 0.0063$t$, $r^2$ = 0.9912, respectively.

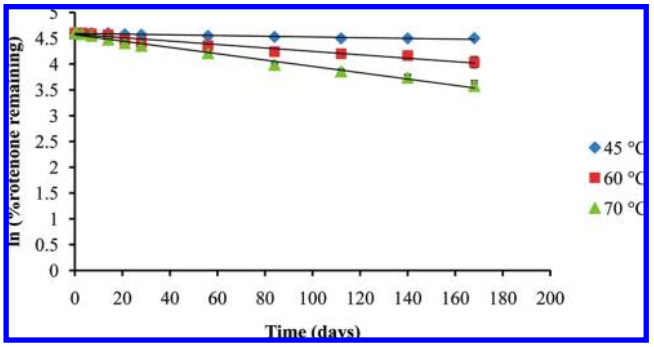


**Figure 5.** First-order plots for degradation of rotenone in water-dispersible granules at 45, 60, and 70 °C (75% RH). Rate equations for each temperature are expressed as ln $c$ = 4.5974 − 0.0007$t$, $r^2$ = 0.9218; ln $c$ = 4.5808 − 0.0033$t$, $r^2$ = 0.9590; and ln $c$ = 4.5702 − 0.0061$t$, $r^2$ = 0.9904, respectively.

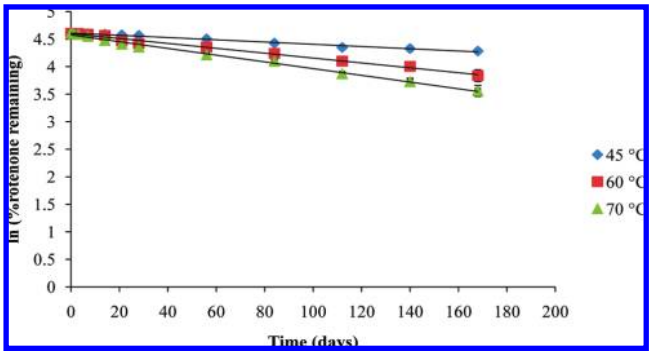


**Figure 6.** First-order plots for degradation of rotenone in emulsifiable concentrate at 45, 60, and 70 °C (75% RH). Rate equations for each temperature are expressed as ln $c$ = 4.6111 − 0.0020$t$, $r^2$ = 0.9879; ln $c$ = 4.6012 − 0.0044$t$, $r^2$ = 0.9904; and ln $c$ = 4.5766 − 0.0061$t$, $r^2$ = 0.9937, respectively.

be 0.0020, 0.0044, and 0.0061 day$^{-1}$ for 45, 60, and 70 °C, respectively (**Figure 6**). Extrapolation of the Arrhenius plots (**Figure 7**) led to estimated $k$ values at 30 °C ($k_{30°C}$) of 0.00017 and 0.00095 day$^{-1}$ for rotenone in water-dispersible granules and emulsifiable concentrate, respectively.

The predicted $t_{1/2}$ and $t_{90\%}$ at 30 °C of rotenone in granule and emulsifiable concentrate formulations are shown in **Table 3**. Both types of formulations were clearly able improve the stability of rotenone, compared to stability in the unformulated crude extract.

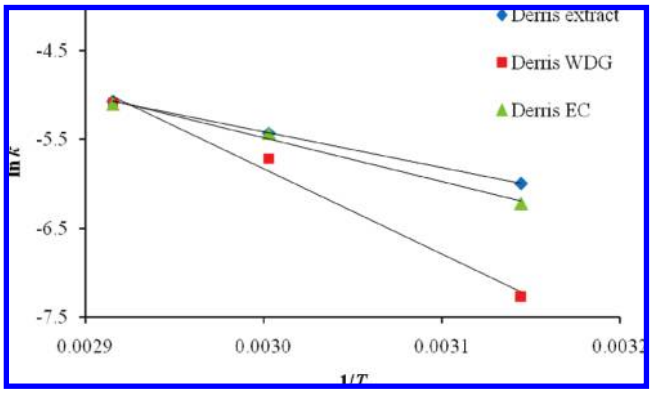


**Figure 7.** Arrhenius plots for degradation of rotenone in *Derris* extract (◆), water-dispersible granules (WDGs) (■), and emulsifiable concentrate (EC) (▲). The Arrhenius relation for rotenone in *Derris* extract can be expressed as $\ln k = 6.6754 - (4028.5/T)$; $r^2 = 0.9999$. The Arrhenius relation for rotenone in water-dispersible granules can be expressed as $\ln k = 22.943 - (9589/T)$; $r^2 = 0.9881$. The Arrhenius relation for rotenone in emulsifiable concentrate can be expressed as $\ln k = 9.3204 - (4932.2/T)$; $r^2 = 0.9902$.

**Table 3.** Predicted $t_{1/2}$ and $t_{90\%}$ at 30 °C of *Derris* Extract and *Derris* Formulations

| sample | $t_{1/2}$ (days) | $t_{90\%}$ (days) |
|---|---|---|
| extract | 520 | 79 |
| water-dispersible granules | 4176 | 633 |
| emulsifiable concentrate | 728 | 110 |

**Table 4.** Predicted Rotenone Remaining after Storage at 30 °C for 6 Months and Rotenone Remaining after 6 Months of Real-Time Storage at Room Temperature

| sample | predicted rotenone remaining (%) | rotenone remaining (%) |
|---|---|---|
| extract | 78.7 | 66.7 ± 1.4 |
| water-dispersible granules | 97.0 | 83.1 ± 2.8 |
| emulsifiable concentrate | 84.3 | 73.8 ± 0.7 |

In this study, the stability of rotenone in solid granule formulation was compared with the liquid emulsifiable concentrate formulation, and the rotenone in granules ($t_{1/2}$ and $t_{90\%}$ were 4176 and 633 days, respectively) showed a much slower degradation rate than in emulsifiable concentrate formulation ($t_{1/2}$ and $t_{90\%}$ were 728 and 110 days, respectively) (**Table 3**). This is as expected, because compounds in solid form are usually more stable than in solutions or suspensions.

In real-time stability studies, *Derris* extract stored at room temperature (30 ± 2 °C) and protected from light for 6 months still contained 66.7 ± 1.4% of the starting amount of rotenone. The water-dispersible granules were found to contain 83.1 ± 2.8% rotenone, and the emulsifiable concentrates were found to contain 73.8 ± 0.7% rotenone. These real-time values were lower than those predicted (by about 10−15%) when compared to estimated values (**Table 4**). Nonetheless, the real-time stability data for the extract and the two formulations showed patterns similar to those of data from the accelerated stability. The results from an accelerated stability study at elevated temperatures and humidity gave useful information on the long-term stability of rotenone in *Derris* extract and *Derris* formulations, and therefore the Arrhenius equation could be used to predict the half-life and shelf life of formulations with a certain amount of accuracy.

The degradation of rotenone in *Derris* extract and *Derris* formulations may occur by oxidation and hydrolytic reactions (*5*). Surface exposure is important with degradation processes (*22*).

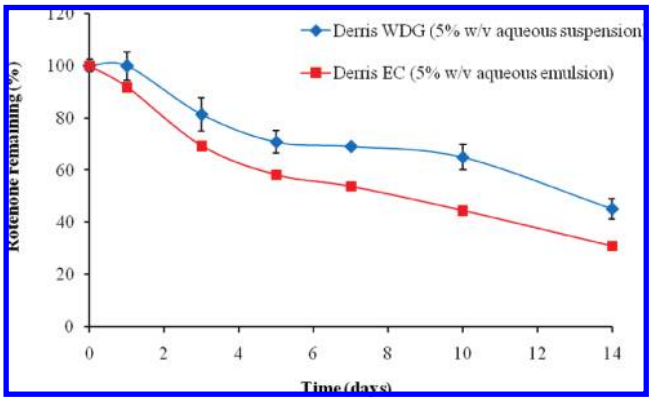


**Figure 8.** Stability of rotenone after spraying an aqueous suspension of *Derris* water-dispersible granules (WG) and emulsion of the emulsifiable concentrate (EC) formulations on the foliage of Chinese kales. Both application sprays contained the equivalent of 0.25% w/v rotenone.

When *Derris* extract is incorporated into water-dispersible granules, the ingredients in the formulation are most likely able to protect rotenone from exposure to oxygen and humidity. The stability of rotenone was also improved in the *Derris* emulsifiable concentrate because water was not used in the formulation, and the rotenone in emulsifiable concentrates is therefore protected from exposure to humidity. In our previous studies, water-dispersible granule formulation has been shown to provide better stability of pyridostemin, the main component of *Stemona curtisii* extract, than in the crude extracts (*23*). The results indicated that both types of developed formulations could prevent the degradation of rotenone during storage.

**Stability of Rotenone in *Derris* Formulations after Spraying onto Plants.** One of the advantages of natural pesticides is their rapid degradation in the environment. In this study, the stability of rotenone on plants was monitored after the formulations had been sprayed under controlled greenhouse conditions. The results show that the degradation of rotenone from aqueous suspensions (equivalent to 0.25% w/v rotenone) of *Derris* water-dispersible granules was significantly slower than the degradation of rotenone from aqueous emulsions (equivalent to 0.25% w/v rotenone) of *Derris* emulsifiable concentrate ($p < 0.05$) (**Figure 8**). Rotenone is decomposed by photooxidation due to exposure to air and light (*24*, *25*). The presence of waxes on the foliage surface affects both the degradation rate and the degradation pathway of rotenone (*26*). In this study, the solid particulate residues of the suspension of *Derris* water-dispersible granules on the foliage appear to protect rotenone from light and oxygen, when compared to the emulsion of *Derris* emulsifiable concentrate. At day 3, 81.3 ± 6.5 and 69.1 ± 0.5% of rotenone remained on the foliage surface after spraying of the suspension of the *Derris* water-dispersible granules and the emulsion of the *Derris* emulsifiable concentrate, respectively, suggesting that the formulations would have the best efficacy up to 3 days after spraying. At days 10 and 14, rotenone on the leaves after spraying of the suspension of the *Derris* water-dispersible granules decreased to 65.0 ± 4.9 and 45.3 ± 3.9%, respectively, whereas rotenone on the leaves at 10 and 14 days after spraying of the emulsion of the *Derris* emulsifiable concentrate decreased to 44.5 ± 0.9 and 31.1 ± 0.9%, respectively. These results have implications for effective concentrations of rotenone and frequency of spraying and suggest that repeated applications might be necessary when the concentration drops to 50% or below. Clearly, guidelines have to be drawn on when the spraying has to stop prior to harvesting of the crop, because residues of rotenone have to be minimal in the harvested crop.

**Table 5.** Efficacy of *Derris* Formulations (Aqueous Suspensions or Emulsions, 0.25% w/v Rotenone) against Second-Instar Larvae of *S. litura*

| | no. of deaths | | | | |
|---|---|---|---|---|---|
| treatment | 24 h | 48 h | 72 h | mortality (%) | no. of survivals after 72 h |
| *Derris* WDG[a] | 1 | 0 | 0 | 2 | 49[b] |
| *Derris* EC[c] | 48 | 0 | 1 | 98 | 1[b] |
| control WDG | 0 | 0 | 0 | 0 | 50 |
| control EC | 0 | 0 | 0 | 0 | 50 |
| water | 0 | 0 | 0 | 0 | 50 |
| cypermethrin (0.0175% w/v)[d] | 49 | 1 | 0 | 100 | 0 |

[a] WDG, water-dispersible granules. [b] The larvae that survived from treatment with *Derris* formulations showed abnormal growth. [c] EC, emulsifiable concentrate. [d] The concentration of cypermethrin was that commonly used for pesticidal activity.

**Preliminary Efficacy Testing of the Two *Derris* Formulations.** The efficacy of emulsions of the *Derris* emulsifiable concentrate against second-instar larvae of *S. litura* was comparable to that of cypermethrin, whereas the emulsion of the control emulsifiable concentrate, as expected, did not cause death of *S. litura* in this test (**Table 5**). The suspensions of the *Derris* water-dispersible granules showed remarkably lower efficacy against the larvae of *S. litura* in the 3 day experiment, although the concentrations were equivalent to 0.25% w/v rotenone for both formulations. However, all of the larvae that survived treatment with *Derris* water-dispersible granules exhibited abnormal growth. This may be due to the sublethal initial amount of rotenone on leaves dipped in aqueous suspensions of *Derris* water-dispersible granules ($17.2 \pm 1.5\ \mu g/cm^2$) compared to that of rotenone on leaves dipped in aqueous emulsion of *Derris* emulsifiable concentrate ($23.9 \pm 2.5\ \mu g/cm^2$). The application of an aqueous suspension of *Derris* water-dispersible granules results in a 20% lower initial loading of rotenone, when compared to dipping into aqueous emulsions of *Derris* emulsifiable concentrate. The initial load of an agrochemical on foliar surfaces may depend on a variety of factors, including adhesion properties, droplet size, velocity and angle of incidence of the applied spray, and plant cuticle and spray droplet interaction and extent of feeding (*27*, *28*). Furthermore, it is important that "rain-resistance" is built into the foliar application fungicide and pesticide formulations, in order not to lose the entire initial load after the first rainfall (*29*). Therefore, the total initial load of rotenone on foliage after application of rotenone formulations (by dipping or spraying) may depend on a combination of the amount physically adhered to the leaf cuticle surface, the amount of rotenone absorbed into the cuticle waxy layer, and the amount ingested. The former (leaf surface) is easy to measure by solvent washing of the leaves, but that absorbed/transported into the leaves is not easy to assay by the HPLC methods utilized in this study, due to the large amount of endogenous components in leaf homogenates that interfere with the HPLC assay of rotenone. The most likely factors accounting for the higher efficacy of the emulsifiable concentrate formulation are those that affect the bioavailability of rotenone in *S. litura*. Rotenone is insoluble in water, whereas it is solubilized in the oil/water (o/w) emulsion foliar application. This could mean that rotenone from the o/w emulsion is transported into the leaves, whereas the insoluble rotenone in the aqueous suspensions of the water-dispersible granules is not transported via the foliar cuticle. The actual bioavailability of rotenone after the application of the emulsifiable concentrate formulation might therefore be considerably higher than that for the granules, thus accounting for the lower efficacy of the latter. The remaining small particles of *Derris* water-dispersible granules on the leaves are less likely to be ingested than emulsifiable concentrates that sometimes mix with leaf epicuticular waxes (*30*). The suspension of *Derris* water-dispersible granules may have an effect on the bioavailability of rotenone in the larvae after ingestion, may discourage feeding, and may account for the slow action of rotenone. In contrast, the emulsion of *Derris* emulsifiable concentrate is in an oil-soluble form that may lead to better bioavailability after leaf ingestion by the larvae; rotenone may also be absorbed through the skin of the larvae, and therefore rotenone could act effectively as both a stomach and a contact poison (*4*, *5*). The efficacy of *Derris* water-dispersible granules may be improved by repeated application, or addition of synergists to increase the effectiveness of rotenone, or inclusion of components that might improve adhesion and initial load on the foliar surfaces. In a previous paper, a higher mortality of third-instar larvae of the diamondback moth was observed by adding triphenyl phosphate (TPP) and piperonylebutoxide (PB) to *Derris* extract (*31*).

**Conclusion.** Ready-to-use stable formulations of *Derris* extract in water-dispersible granules and liquid-emulsifiable concentrate using commercially feasible technology were developed. Both types of formulations were able to clearly prolong the shelf life of rotenone during storage. The study of rotenone degradation on plants gives useful data for the consideration of use of effective concentrations for spraying and frequency of applications that might be necessary. The preliminary efficacy testing of the formulations under laboratory conditions demonstrated the higher efficacy of *Derris* emulsifiable concentrate in controlling *S. litura*, compared to *Derris* water-dispersible granules. However, the drawback of the emulsifiable concentrate formulation is that the stability of rotenone is only 110 days, compared to 633 days for the water-dispersible granule formulations. We are currently working on modified emulsifiable concentrate formulations that might increase the stability of rotenone.

## ACKNOWLEDGMENT

We thank the Faculty of Pharmaceutical Sciences and the Faculty of Natural Resources, Prince of Songkla University, for personal and laboratory assistance. Professor L. A. Damani is acknowledged for his critical reading of the manuscript.

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**Received for review June 1, 2009. Revised manuscript received October 4, 2009. Accepted October 23, 2009. This research was supported by Prince of Songkla University.**