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RESEARCH ARTICLE - ANTS

Toxicities comparison of rotenone and acetone extract of *Tephrosia vogelii* and *Derris trifoliata* against *Solenopsis invicta*

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Abstract

The high rotenone content and the rotenone crude extract of *Tephrosia vogelii* and *Derris trifoliata* were evaluated for its efficacy in the control of red imported fire (RIFA), *Solenopsis invicta* under both laboratory and field conditions. The acetone extracts of *D. trifoliata* roots and *T. vogelii* leaves exhibited strong toxicity to macroergate and micrergate of RIFA. When active ingredients of the crude extracts were convert to rotenone, the activity of the acetone extracts were higher than that of rotenone technical material. At the same time, the extracts showed significant inhibitory effect on walking ability and grasping ability of worker ants and stronger than the effect of 98.6% rotenone technical material. Under field conditions, the 0.01% rotenone-bait, formulated with the acetone extract of *D. trifoliata* roots and *T. vogelii* leaves, had the same control effect on RIFA as that of 0.01% fipronil-bait when treated after 30 d. The bait formulated with the extract of *D. trifoliata* exhibited quicker and higher effect on RIFA than that of rotenone technical material. It was showed that the acetone extracts of *D. trifoliata* roots and *T. vogelii* leaves are able to control *S. invicta* under both laboratory and field conditions and can be used as an effective agent against RIFA.

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is one of over 280 species in the widespread genus *Solenopsis*. RIFA is a voracious consumer of numerous other arthropod species and often is the most abundant predaceous arthropod in crop fields throughout the United States, New Zealand, and Australia (Nattrass, 2001; Ascunceet et al., 2011). RIFAs were introduced to mainland China in 2005 (Zheng et al., 2007) and became widely distributed in South China, eventually causing severe damage to humans, animals, and the environment. RIFA stings humans, pets, farm animals, and wildlife and can damage farms, electrical equipment, and irrigation systems. Many botanical insecticidal compounds exhibit good toxicity against RIFA, and botanical insecticides can potentially be used to control RIFA (Li et al., 2014). Rotenone-based bait is a good botanical insecticide used to control RIFA.

Tephrosia vogelii and *Derris trifoliata* are resource plants containing rotenone that can be used to control RIFA. *T. Vogelii* is native to West Africa but is also found in India, Asia, and other tropical regions (Dalziel, 1937; Lambert et al., 1993). *T. vogelii* was introduced into China in 1986 and has been planted over a large area in Guangdong Province (Huang et al., 2006). The leaf macerate has purgative and emetic properties (Walker, 1961; Burkill., 1995). The acetone extracts of the *T. vogelii* leaf exhibited insecticidal activity against *Pieris rapae* (Shin, 1989). The volatile compounds released from the mashed fresh bean pods, branches, and leaves

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of *T. vogelii* exhibited high toxicity against the RIFA workers and evidently reduced the walking and grasping abilities of the RIFA workers. *D. trifoliata* is an ever green mangrove plant belonging to the family Fabaceae (Leguminosae), which is commonly known as the legume family. *D. trifoliata* is a perennial climber or a highly branching climbing shrub that can reach a length of 8 m or less. The leaves of *T. vogelii* and the roots of *D. trifoliata* contain rotenone, a poison that kills a wide range of creatures from insects to earthworms and fish. *T. vogelii* and *D. trifoliata* are commercially cultivated as sources of insecticidal rotenoids in China. *D. trifoliata* is used to produce rotenone in commercial quantities as an insecticide.

The insecticidal raw material of rotenone-based bait is usually pure rotenone. Raw material can also be obtained from *T. vogelii* leaf extract and *D. trifoliata* root extract, which are cheaper. This study aimed to demonstrate which one of the above mentioned three sources is more applicable to be used as the raw material of rotenone based bait.

Materials and Methods

Insects

S. invicta colonies were collected from the campus of South China Agricultural University. Ant workers were reared in plastic containers (50 cm diameter × 20 cm height) coated with Teflon emulsion on the top under ambient conditions (temperature 25 ± 2º C and a relative humidity of 60% to 80%). A test tube (25 mm × 200 mm) partially filled with 10% honey water and plugged with cotton was used as the water source. The tested ants were fed with ham sausage. The tested macroergates ranged from 5 mm to 6 mm in length, and the micrergates ranged from 3 mm to 4 mm in length.

Plant

T. vogelii (Family: Legume, Papilionacea; Genus: *Tephrosia*) was planted in the sample region of insecticidal plants at South China Agricultural University in April 2012. The size of the plant area was 20 m×20 m. The height of the trees was about 2 m, and the basal stem diameter was about 3 cm. The leaves were cut off in February 2014 and immediately sent to the laboratory. *D. trifoliata* (Family: Fabaceae, subfamily: Faboideae, Genus: *Derris*) was planted in May 2010 in Fengshun County, Meizhou City, Guangdong Province, China. The size of the plant area was 90 m×20 m. The roots were collected in February 2014.

Apparatus and Chemicals

High-performance liquid chromatography (HPLC): LC-20 A equipped with UVD and an auto sampler, interfaced to LC solution data processing software (Shimadzu, Japan). Zorbax TC-C18column (250 mm4.6 mm ID) with film thickness of 5 μm (Agilent, American) was used. Rotenone technical material (98% purity) was purchased from Tangxijiaxing Welfare Chemical Factory (Fengshun County, Meizhou City, Guangdong Province, China). Rotenone standard substance (99.5% purity) was purchased from Sigma Co. Ltd., United States (www.sigmaaldrich.com). HPLC-grade acetonitrile and acetone were purchased from Guangzhou Chemical Reagent Factory, China (www.chemicalreagent.com). Fipronil technical material (96% purity) was purchased from Bayer Crop Science China Co., Ltd (www.bayercropscience.co.th).

Preparation of plant extracts

The dried leaves of *T. vogelii* and roots of *D. trifoliata* were powdered by using an electric blender. Dried plant powder (200 g) was prepared by passing the leaves through a 1 mm sieve and extracted thrice (60 min each time) with 3000 mL of acetone. The extracts were concentrated using a rotary vacuum evaporator at 40º C, and the residue obtained was stored at 0º C. The rotenone content was analyzed using reverse-phase HPLC (Zhou et al., 2014).

Preparation method of rotenone or fipronil-based bait

Rotenone or fipronil was mixed with maize powder, peanut oil, fishbone powder, and carbohydrates and was prepared into granules (700 mm to 1500 mm particles) according to the method previously described by Lekhnath (2010) and Zhong (2012), with slight modifications. Rotenone, fipronil, and the acetone extracts of *T. vogelii* leaves and *D. trifoliata* roots were dissolved separately with small amounts of acetone. The solution was mixed uniformly with the bait. The rotenone-or fipronil-based bait was prepared after the acetone volatilized completely.

Insecticidal toxicity bioassay

The method was similar with those described by Zeng (2006). A water test tube, 0.5 g of rotenone-based bait, and worker ants were placed in a disposable plastic cup (top/bottom/height: 62 mm/40 mm/60 mm) whose vertical wall was coated with a fluon emulsion. Each treatment was replicated three times, and each replicate included 30 worker ants. The contrast group was the acetone-based bait. The workers were maintained at 24º C to 26º C and 60% to 80% relative humidity. During the tests, the mortalities were recorded at 24, 48, and 72 h after treatment.

Behavior observation on walking ability of worker ants

Worker ants were treated as insecticides toxicity bioassay. Behavioral observation on walking ability of worker ants was performed using methods similar with those of Li (2014) and Zheng (2007). Worker ants were placed on a square graph paper (side:20 cm), and the traveling paths were filmed using Canon digital camera (EOS6D), after which the camera was connected to a computer. The total distance covered by the worker ant was calculated. If the worker ants could walk continuous for 10 cm without falling, they were regarded to possess walking ability. The following equation was used for calculations:

Grasping rate (%) = (number of workers possessing walking ability/number of workers per replicate)x100

Behavior observation on grasping ability of worker ants

Worker ants were treated as insecticides toxicity bioassay. Worker ants were placed in the disposable plastic cup, and the cup was shook gently. After 10 s, when the ants were distributed uniformly in the bottom, after which the cup was turned 180 degrees gently. The ants were regarded to possess grasping ability if they would not fall from the cup. The following formula was used for calculation:

Grasping rate(%)=(number of workers possessing grasping ability/number of workers per replicate)x100

Control effect of rotenone-based bait against RIFA in the field

To determine the control effect of 0.01% rotenone-based bait against RIFA in the field, a method previously reported by Zhong (2012) was used with some modifications. The field studies were conducted in the lawn of Zengcheng farm, South China Agricultural University, Guangzhou City. The experiment was designed in single factor random block. The following five treatments were employed: D: 0.01% rotenone-based bait, formulated with the acetone extract of *D. trifoliata* roots; E: 0.01% rotenone-based bait, formulated with the acetone extract of *T. vogelii* leaves; F: 0.01% rotenone-based bait, formulated with 98.6% rotenone technical material; G: 0.01% fipronil-based bait as control treatment; H: the blank bait mixed with only acetone was used as a negative control treatment. RIFA mounds that were scattered randomly in the lawn were selected, and each treatment had three replicates. Poison bait (10 g) was placed in the periphery of each active RIFA mound. The RIFA population numbers of each treated mound were investigated 1 d before treatment and 1, 5, 10, 20 and 30 d after treatment. The mounds were designated as active when at least30 adult workers exited the mounds of excavated soil when probed with a metal rod (5 mm in diameter) (Furman et al., 2006). The control effect was determined using the following equation: $P = [(S_{CK} - S_{treatment}) / (100 - S_{CK})] \times 100$

Statistical analysis

Data were reported as means ± SE based on three independent experiments. The percentage values were transformed into arc sin of square root of the percentages prior to the analysis and three-factor ANOVA. Worker size and concentration were considered as the main effects. Moreover, the differences between the data were assessed using Duncan's multiple range test (SAS 1989), with $P < 0.05$ regarded as statistically significant. The figures were produced using Microsoft Excel 2007.

Results

Rotenone contents of T. vogelii and D. trifoliata

The rotenone contents of *T. vogelii* leaves and *D. trifoliata* roots were measured by HPLC method (Fig 2 and 3). Compared with the HPLC figure of rotenone standard substance (Fig 1), the rotenone content of the acetone extract of *D. trifoliata* roots was 18.14%, whereas that of *T. vogelii* leaves was 2.11% (Table 1). When the rotenone content was converted into the percentage of dry plant powder, the obtained values were 1.86% and 0.19%, respectively.

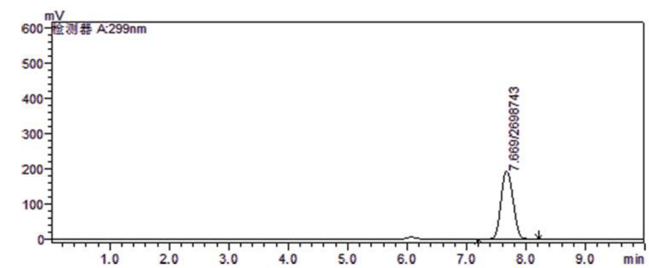

Fig 1. The HPLC Figure of rotenonestandard substance.

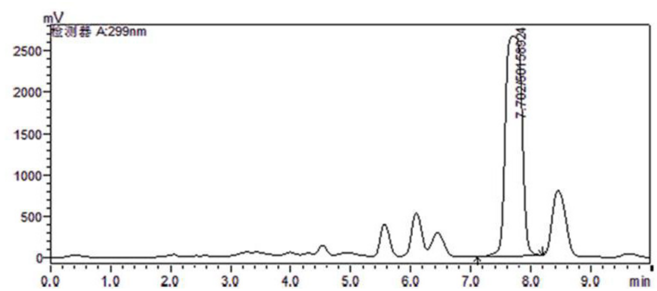

Fig 2. The HPLC Figure of rotenone in acetone extract of *Derris trifoliata* roots.

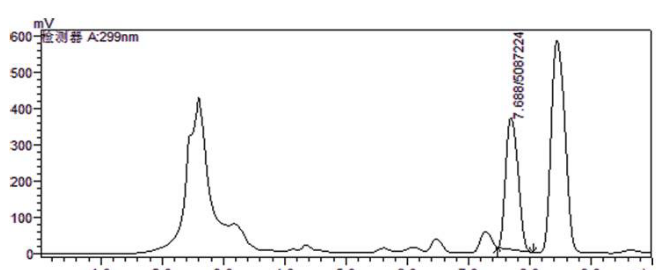

Fig 3. The HPLC Figure of rotenone in acetone extractof *Tephrosia vogelii* leaves.

Table 1. Determination of rotenone content.

Plant material	Rotenone content (%)	
	dry plant powder	acetone extracts
Derris trifoliata roots	1.86±0.25	18.14±2.37
Tephrosia vogelii leaves	0.19±0.11	2.11±0.30

The toxicity of the extract

Toxicities of rotenone, extracting solution of *D. trifoliata*, and *T. vogelii* against RIFA were determined (Table 2 and 3). The acetone extracts of *D. trifoliata* roots and *T. vogelii* leaves exhibited strong toxicity to macroergates of RIFA and were treated for 24 h. The LC_{50} values were 30.27 and 48.63 mg/kg, which are higher than those of 98.6% rotenone technical material. The toxicity of the acetone extracts and rotenone technical material increased as treatment time increased. The LC_{50} of *D. trifoliata* roots extract and *T. vogelii* leaf extract were respectively 19.28 and 31.91 mg/kg when treated for 48 hand were 14.67 and 26.40 mg/kg when treated for 72 h. The bioactivities of the two extract baits to macroergates of RIFA were apparently higher than that of rotenone technical material.

Experimental results indicated that the toxicity of *D. trifoliata* root extracts and *T. vogelii* leaf extracts was higher than that of 98.6% rotenone technical material under the same treatment time. The LC_{50} values of the two extracts to microergates of RIFA were respectively 15.12 and 26.28 mg/kg when treated for 24 h; 9.94 and 17.19 mg/kg when treated for 48 h; and 6.92 and 12.53 mg/kg when treated for 72 h. The LC_{50} values of 98.6% rotenone technical material were 65.24, 50.30, and 36.85 mg/kg, respectively.

Table 2. LC50(mg/kg) of *D. trifoliata* roots estract, *T. vogelii* leaves extract, and rotenone against macroergates of RIFA.

Treatment	Time	Regression equation	LC_{50}(mg/kg)	95% Fiducial limit	Correlation coefficient
A	24h	Y=3.5233+0.9970x	30.27	18.32~50.03	0.9945
B	24h	Y=3.1531+1.0948x	48.63	31.35~75.43	0.9918
C	24h	Y=2.3158+1.2592x	135.41	91.89~199.54	0.9928
A	48h	Y=3.5911+1.0963x	19.28	12.36~30.06	0.9930
B	48h	Y=3.0618+1.2887x	31.91	20.91~48.70	0.9959
C	48h	Y=2.1394+1.4465x	94.99	66.39~135.89	0.9893
A	72h	Y=3.5294+1.2607x	14.67	9.57~22.49	0.9863
B	72h	Y=3.0477+1.3733x	26.40	16.99~41.01	0.9874
C	72h	Y=2.0125+1.5476x	85.20	59.89~121.21	0.9907

A：The acetone extract of *D. trifoliata* roots；B：The acetone extract of *T. vogelii* leaves；C：98.6% rotenone technical material. Active ingredients in rotenone.

Behavior observation on walking ability and grasping ability of worker ants

The extracts of *D. trifoliata* roots and *T. vogelii* leaves exhibited good toxic activity against the RIFA workers and at the same time showed significant inhibitory effect on walking ability and grasping ability of worker ants (Tables 4 and 5), which is stronger than the effect of 98.6% rotenone technical material. The macroergates and microergates showed poor walking and grasping abilities after treatment with the extracts and rotenone. The walking and grasping abilities of RIFA workers significantly decreased as treated time increased. The walking rates of microergates of RIFA treated by *T. vogelii* leaf extracts were 43.33%, 21.67%, and 5.00%, and the grasping abilities were 20.00%, 8.33%, and 0.00%, respectively, which were significantly lower than the walking ability and grasping ability of *D. trifoliata* root extracts and rotenone. Compared with the control, the treatments by extracts and rotenone could reduce the walking ability and grasping ability of microergates of RIFA. Within these treatments, *T. vogelii* leaf extract, *D. trifoliata* root extract, and rotenone had the same effect on the walking ability and grasping ability of macroergates of RIFA.

Table 3. LC50 (mg/kg) of *D. trifoliata* roots extract, *T. vogelii* leaves extract, and rotenone against micrergates of RIFA.

Treatment	Time	Regression equation	LC_{50}(mg/kg)	95 % Fiducial limit	Correlation coefficient
A	24h	Y=3.7725+1.0309x	15.52	9.49~25.36	0.9917
B	24h	Y=3.0373+1.3826x	26.28	18.41~37.50	0.9913
C	24h	Y=2.7892+1.2184x	65.24	43.11~98.73	0.9915
A	48h	Y=3.9148+1.0883x	9.94	6.37~15.51	0.9926
B	48h	Y=3.2200+1.4239x	17.79	12.27~25.78	0.9922
C	48h	Y=3.0368+1.1537x	50.30	33.11~76.41	0.9946
A	72h	Y=3.8289+1.3937x	6.92	4.63~10.36	0.9932
B	72h	Y=3.3601+1.4934x	12.53	8.19~19.17	0.9946
C	72h	Y=2.9653+1.2990x	36.85	24.78~54.79	0.9868

A:The acetone extract of *D. trifoliata* roots; B: The acetone extract of *T. vogelii* leaves; C: 98.6% rotenone technical material. Active ingredients in rotenone.

Table 4. Influences of *D. trifoliata* roots extract, *T. vogelii* leaves extract, and rotenone on walking and grasping abilities of micrergates of RIFA (Mean ± SE, %).

Treatment	rotenone content (mg/kg)	Walking ability (%)			Grasping ability (%)		
		1 d	3 d	7 d	1 d	3 d	7 d
A	5	51.67±4.41c	28.33±4.41c	10.00±2.89c	35.00±5.77c	11.67±3.33c	3.33±1.67c
B	10	43.33±3.33d	21.67±4.41c	5.00±2.89c	20.00±2.89d	8.33±1.67c	0.00±0.00d
C	20	81.67±1.67b	63.33±6.01b	28.33±4.41b	70.00±2.89b	40.00±2.89b	16.67±4.41b
CK	0	100.00±0.00a	100.00±0.00a	96.67±1.67a	100.00±0.00a	98.33±1.67a	95.00±2.89a

A：The acetone extract of *D. trifoliata* roots；B The acetone extract of *T. vogelii* leaves；C：98.6% rotenone technical material. Active ingredients in rotenone.

Control effect of rotenone-based bait on RIFA in the field

The 0.01% rotenone-based bait, formulated with the acetone extract of *D. trifoliata* roots, showed significant controlling effect on RIFA in the field and worked faster than the other three treatments. The control effect was 57.78% for 5 d after treatment. The control effect sequence of other three treatments was 0.01% for fipronil-based bait, 0.01% for rotenone-based bait (formulated with the acetone extract from *T. vogelii* leaves), and 0.01% for rotenone-based bait (formulated with 98.6% rotenone technical material) 5 d after treatment. The control effect of 0.01% rotenone-based bait (formulated with the acetone extract of *D. trifoliata* roots) was higher than the other treatments 10 d after treatment and approached 100% within 20 d. At the end of the study (30 d), all four treatments achieved perfect controlling effect.

Table 5. Influences of *D. trifoliata* root extract,*T. vogelii* leaf extract, and rotenone on walking and grasping abilities of macroergates of RIFA (Mean ± SE, %)

Treatment	Rotenone content (mg/kg)	Walking ability (%)			Grasping ability (%)		
		1 d	3 d	7 d	1 d	3 d	7 d
A	5	66.67±6.01c	45.00±2.89c	23.33±1.67c	56.67±4.41c	35.00±2.89c	16.67±3.33c
B	10	50.00±5.77d	33.33±3.33d	15.00±2.89d	36.67±4.41d	25.00±2.89d	10.00±2.89c
C	20	91.67±3.33b	76.67±1.67b	46.67±4.41b	83.33±3.33b	63.33±4.41b	41.67±4.41b
CK	0	100.00±0.00a	98.33±1.67a	95.00±2.89a	98.33±1.67a	96.67±1.67a	91.67±4.41a

A：The acetone extract of *D. trifoliata* roots；B The acetone extract of *T. vogelii* leaves；C：98.6% rotenone technical material. Active ingredients in rotenone.

Discussion

Scientists have previously reported that natural products derived from plant extracts were tested in a discovery program for effective, environmental-friendly pesticide control agents. Among the natural products, rotenoids is a type of important plant source pesticide and has been widely applied for insect control (Zhang et al., 2005). Rotenone from natural sources easily degrades and has high environment compatibility that can be used in organic agriculture. In China, the rotenone is extracted using different organic solvents, such as dimethylbenzene, methylbenzene, and the major product of rotenone, is an emulsifiable concentrate (Xu et al., 2001).

The extraction process and traditional product application could harm the environment and endanger public health, thereby lowering the merit of the rotenone. The results obtained in this study demonstrate that the acetone extract with rotenone of *D. trifoliata* roots showed higher biological activity than rotenone technical material against RIFA at same doses and treatment times. Results of the study showed that rotenone crude extracts could be directly used to control pests and serve as a powerful insecticide for controlling RIFA.

Table 6. The control effect of rotenone-based bait on RIFA.

Treatment	Dose (g/nest)	Average nest number before treatment	Control effect (%)				
			1 d	5 d	10 d	20 d	30 d
D	10	15±0	22.22±2.22a	57.78±2.22a	93.33±3.85a	100.00±0.00a	100.00±0.00a
E	10	15±0	17.78±2.22a	35.56±2.22b	68.89±4.44b	88.89±4.44b	95.56±4.44ab
F	10	15±0	20.00±3.85a	31.11±4.44c	40.00±3.85d	73.33±3.85c	88.89±4.44b
G	10	15±0	22.22±2.22a	40.00±3.85b	53.33±3.85c	86.67±3.85b	97.78±2.22a

Note: D:0.01% rotenone bait, formulated with the acetone extract of *D. trifoliata* roots. E: 0.01% rotenone bait, formulated with the acetone extract *T. vogelii* leaves. F: 0.01% rotenone bait, formulated with 98.6% rotenone technical material. G:0.01%fipronil bait.

Resource plants containing rotenone and its analogues belong to the legume family, including *Derris, Tephrosia, Lonchocarpus,* and *Millettia* (Xu, 2001). *Derris* plant is one of the most famous insecticidal plants and has a long history in China. Some of the more common *Derris* plants are *D. trifoliata, D. elliptica, D. malanccensis,* and *D. ferruginea*. Various plants of *Tephrosia* with rotenone exist, and *T. vogelii* leaves are rich in rotenone and its analogues (Li et al., 2010).

High activity and rich plant resources of rotenone are conducive to develop new products by crude extract to control RIFA. The *D. hancei* root power mixture had good field control efficacy against RIFA (Tian et al., 2010). The control efficacy of worker ants reached up to 98.25%, and the nest elimination averaged 95.32% 21 days after applying the powder mixture that contains 20% *D. hancei* root power. After which, all ants within nests eventually died. The results indicated that plant dry power containing rotenone could be directly used to control red fire ants. The toxicity bait with the active ingredient of rotenone showed an obvious trapping and killing effect on *Pheidole yeensis* (Wang et al., 2010). Li et al (2014) had studied the effect of volatile compounds from the mashed fresh bean pods, as well as the branches and leaves of *T. vogelii* on the behavior of *S. invicta* workers. Results showed that the volatile compounds possess high toxicity against the RIFA workers and can reduce the walking and grasping abilities of these workers. Compared with dry matter, the mashed branches and leaves of *T. vogelii* exhibit greater potential for producing insecticides.

The results of this study showed that rotenone can be developed into a new variety that is effective against *S. invicta*. Aside from the direct utility of the rotenone compound, results of the study illustrated that rotenone crude extract and the dried powder and mashed branches and leaves of rotenone resource plants could be directly used to control pests and could serve as a powerful insecticide for controlling *S. invicta*.

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