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Insecticidal effect of volatile compounds from fresh plant materials of *Tephrosia vogelii* against *Solenopsis invicta* workers

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Abstract

The effect of volatile compounds from the mashed fresh bean pods (B) as well as the branches and leaves (L) of *Tephrosia vogelii* on the behavior of *Solenopsis invicta* workers was investigated by fumigation toxicity bioassay. Gas chromatography–mass spectrometry analysis was used to identify and quantify the volatile compounds. α -pinene, thujene, caryophyllene, and d-limonene were identified as major components of the volatile compounds, which were found toxic to workers when applied by fumigation. Responses varied according to worker size, exposure time, and plant material. An increase in exposure time from 1h to 12h led to increases in mortality from 18.33% to 100.00% (B) and 13.33% to 100.00% (L) in minor workers as well as increases from 1.67% to 95.00% (B) and 15.00% to 98.33% (L) in major workers. The volatile compounds were also found to exert a behavioral effect against *S. invicta* in an A4 paper test. Walking and grasping abilities decreased at exposure times ranging from 40 min to 280 min. These findings suggest that the volatile compounds of *T. vogelii* can be used to control *S. invicta*.

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta*, is one of over 280 species in the widespread genus *Solenopsis*. Although RIFA is native to South America, it has become a pest in the southern United States, Australia, Thailand, Taiwan, the Philippines, Hong Kong, and the southern Chinese Provinces of Guangdong, Guangxi and Fujian. There are also reports of ant hills in Macau. RIFA are known to have a strong, painful and persistent irritating sting that often lead a pustule on the skin (Laura & Rudolf, 2001). The ant stings humans, pets, farm animals and wildlife, as well as damaging farm, electrical equipments and irrigation systems. Moreover, besides destroying crops and fruits directly or indirectly, they negatively affect the local biodiversity and cause approximately US\$5 billion losses in urban and agricultural areas yearly in the USA (Cheng et al., 2008). Many botanical insecticidal

compounds possessed good toxicity against RIFA, and it is potential for applying botanical insecticides to control RIFA.

Tephrosia vogelii is native to West Africa, but is found in India, Asia and other tropical regions (Dalziel, 1937; Lambert et al., 1993). It is widespread in tropical Africa from Sierra Leone and Ethiopia southwards to Angola, the Flora Zambesiaca area and the Comoro Islands, also from Assam to Indonesia. It was introduced into China by 1986 and planted over a large area in Guangdong province. It is a shrubby plant used as a fallow plant to improve soil fertility and to reduce erosion, particularly in higher areas. The leaf macerate is purgative and emetic (Walker, 1961; Burkill, 1995). Powders of *T. vogelii* are effectively used in the Congo against the stored ground nut pest *Caryedon serratus* (Delobel, 1987). It is also applied directly to treat head lice, fleas, scabies and other ectoparasites (Klaassen, 1996; Nwude, 1997). The water extract of the dried leaf possessed molluscicidal activity



against *Bulinus globosus* (Chiotha, 1986). Water extract of oven dried stem, leaf and seed showed weak molluscicidal activity against *Biomphalaria pfeifferi* (Kloos, 1987). Acetone extract of the leaf showed feeding deterrent activity against the insect *Pieris rapae* (Shin, 1989). This study is the first to report on the toxicity of the volatile compounds released from the mashed fresh bean pods (B) as well as the branches and leaves (L) of *T. vogelii* against RIFA workers. In addition, this study aims to investigate the effects of the volatile compounds from the fresh plant materials of *T. vogelii* on the RIFA workers and analyze the volatile compounds by gas chromatography–mass spectrometry (GC–MS).

Materials and Methods

Plant materials

T. vogelii (Family: Legume, Papilionacea; Genus: *Tephrosia*) was planted in the sample region of insecticidal plants at South China Agricultural University in April 2010. The B and L were cut off in April 2013 and immediately sent to the laboratory.

Insects

S. invicta colonies were obtained from a suburb in Guangzhou and maintained in the laboratory for bioassays in plastic containers under the following conditions: 25 ± 2 °C, 65%±5% relative humidity and constant darkness. Colonies were fed with a mixture of live insects (*Tenebrio molitor*) and 10% honey. A test tube (25mm × 200 mm) partially filled with water and plugged with cotton was used as the water source. Experiments were performed under similar environmental conditions.

Fumigation toxicity bioassay

Indoor test of the toxicity of mashed fresh bean pods (B) as well as branches and leaves (L) of *T. vogelii* against RIFA workers

B (500 g) and L (500 g) *T. vogelii* were mashed with a high-speed organization stamp mill for 5 min. Mashed plant materials (5 g) were weighed and placed at the bottom of the beaker (1000 mL). Red imported fire ants were classified into minor workers (body length = 2.8 mm to 3.0 mm, head width = 0.6 mm to 0.7 mm) and major workers (body length = 4.3 mm to 4.5 mm, head width = 1.0 mm to 1.1 mm). Twenty workers settled on the bottom of the beaker (100 mL), which had an inside vertical wall coated with Fluon emulsion that was allowed to dry for 24 h to prevent the ants from escaping. The 100 mL beaker was allowed to settle on the bottom of the 1000 mL beaker without covering the mashed plant materials. The 1000 mL beaker was sealed with a plastic wrap. The

worker was maintained at 25 ± 2 °C and 65%±5% relative humidity. Experiments 1 (fumigant toxicity bioassay), 2 (walking ability test), and 3 (grasping ability test) were conducted. All treatments were replicated thrice. The contrast was the absence of mashed plant materials in the 1000mL beaker.

Outdoor test of the toxicity of live bean pods (B) as well as branches and leaves (L) of *T. vogelii* against RIFA workers

Twenty workers settled on the bottom of the beaker (100mL) sealed with gauze. One live branch of *T. vogelii* (not cut off from the shrub) with about 10 bean pods or 100 leaves was slipped into a transparent plastic bag (40cm × 29 cm × 10 cm) with two open ends. The beaker (100 mL) was tied upside down in the bag, and the other open end was sealed. Experiments 1 (fumigant toxicity bioassay), 2 (walking ability test), and 3 (grasping ability test) were conducted. The average temperature was set between 25°C and 30°C, average humidity was 70% to 90%, and average wind velocity was <0.5m/s during the test.

Physiological index observation of RIFA workers

The cumulative mortalities of fumigant toxicity were determined 1, 2, 4, 6, 8, 10, and 12h after testing. Behavioral observation on the walking ability of the workers was determined 40, 80, 120, 160, 200, 240, and 280min after testing. The workers were placed on an A4 paper. If the workers could walk continuously for 10 cm without falling, these workers were regarded to possess walking ability. We used the following equation: walking rate = (number of workers possessing walking ability/number of workers per replicate) × 100.

Behavioral observation on the grasping ability of the workers was determined 40, 80, 120, 160, 200, 240, and 280 min after testing. The workers were placed on an A4 paper, which was softly rotated in 180° after 10s. The workers that did not fall from the A4 paper were regarded to possess grasping ability. We used the following equation: grasping rate = (number of workers possessing grasping ability/number of workers per replicate) × 100.

Chemical analysis of the volatile compounds

Isolation, identification, and quantification of the component of the volatile compounds from the mashed fresh plant materials of *T. vogelii* were analyzed using GC–MS. The indoor test method was conducted following the procedure in 2.3; however, the 20 workers were replaced with a 2g adsorbents. The adsorbents were silicone (200 mesh to 300 mesh). The adsorbent was inserted into the injector (length = 20cm, diameter = 0.5cm) 6h after the treatment. The volatile

compounds were absorbed by the silicone, which was eluted with 5ml petroleum ether or acetone. The analytical conditions of the GC–MS are shown in Table 1. Most compounds were identified based on GC–MS retention times, Kovats indices, and mass spectra.

Statistical analysis

Data were transformed into arcsine square root values for a three-way analysis of variance (ANOVA) to determine the significance of the effects of plant material, exposure time, and worker size on the mortalities, walking rate, and grasping rate of the minor and major workers as well as various interactions. The differences in the data were also assessed using Duncan's multiple range test, with $P < 0.05$ considered statistically significant. The figures were generated using the Microsoft Office Excel 2007 program.

Table 1. The analytical conditions of GC-MS

Gas chromatography		HP 6890N-5957 series plus (Agilent)	
Carrier Gas	Helium		
Injector Temp	280°C		
Detector Temp	230°C		
Capillary column		HP-5MS (Agilent) or DB-5 (J&W)	
Head Pressure	100 kPa		
Oven Program			
Initial Temp	60°C		
Initial Time	1 min		
	Rate	Final Temp.	Final Time
	(°C/min)	(°C)	(min)
	25	210	5
	10	280	10
Mass spectrometer		HP 5972 (Agilent)	
EI voltage	70 Ev		
Mass spectrum database		NBSLI-BRARY	

Results

Indoor test

When B and L of *T. vogelii* were bioassayed, the mortalities, walking rate, and grasping rate of minor and major workers varied significantly according to plant material, exposure time, and worker size (ANOVA, $P < 0.05$). The results reveal that the three main effects and the partial interactions were significant (Table 2). Marked variations in the mortality of fumigant toxicity as well as the walking and grasping abilities were observed at different plant material, exposure time, and worker size (Table 3). The mortality of RIFA was significant at different plant material ($F = 10.272$, $P = 0.0022$), exposure time ($F = 235.652$, $P < 0.0001$), and worker size ($F = 54.450$, $P = 0.0001$). However, there was no difference in the percentage of mortality between the interaction exposure time×worker size ($F = 1.341$, $P = 0.2547$) and the interaction plant material×exposure time×worker size ($F = 1.230$, $P = 0.3052$). In addition, significant differences were found at walking rate and grasping rate of RIFA: developing from different exposure time ($F = 312.108$, $P < 0.0001$; $F = 286.326$, $P < 0.0001$) and worker size ($F = 49.371$, $P = 0.0001$; $F = 9.496$, $P = 0.0032$).

Fumigant toxicity

The indoor test of the mashed B and L of *T. vogelii* showed effective fumigant activity against the RIFA workers; by contrast, the outdoor test showed no such activity (Fig. 1). The volatile compounds released from the live B and L of *T. vogelii* caused no mortality in the fumigant toxicity assay, even after 12h exposure, there were no dead individuals. However, in all cases of the indoor test, a marked variation in insect mortalities was observed with the increase in exposure time. A significant difference in the mortalities between the minor and the major workers was indicated, with the volatile compound of the B and L of *T. vogelii* being significantly more toxic to the RIFA minors than the majors. After a 1h exposure, the volatile compounds of B and L resulted in 13.33% (B) and 18.33% (L) mortality rates in minor workers as well as

Table 2. ANOVA for the main factors of the indoor test affecting the behaviors of RIFA

Factors	Mortality			Walking ability			Grasping ability		
	DF	F values	P values	DF	F values	P values	DF	F values	P values
A	1	10.272	0.0022	1	0.610	0.4383	1	4.845	0.0319
B	6	235.652	0.0001	6	312.108	0.0001	6	286.326	0.0001
C	1	54.450	0.0001	1	49.371	0.0001	1	9.496	0.0032
A×B	6	3.000	0.0130	6	0.876	0.5183	6	1.390	0.2347
A×C	1	5.339	0.0246	1	0.343	0.5605	1	0.628	0.4315
B×C	6	1.341	0.2547	6	2.660	0.0243	6	0.796	0.5772
A×B×C	6	1.230	0.3052	6	0.521	0.7902	6	1.008	0.4296

A: Plant material, B: Exposure time, C: Worker size ($P=0.05$)

1.67% (B) and 15.00% (L) mortality rates in major workers. However, the mortality rates were 100% (B) and 100.00% (L) for the minors as well as 95.00% (B) and 98.33% (L) for the majors at 12h of treatment.

Walking and grasping abilities

The volatile compound from the mashed B and L exhibited good fumigant activity observed at different exposure times against the RIFA workers. However, the volatile compounds released from the live B and L of *T. vogelii* caused no reduction in the walking and grasping abilities of the RIFA workers (Figs. 2 and 3). The minor and major workers showed good walking and grasping abilities with the live plant materials in outdoor test. And the walking and grasping abilities of the RIFA workers decreased with longer exposure in indoor test (Table 3). At exposure times ranging from 40 min to 280 min, the walking abilities decreased from 98.33% to 20.00% (B) and 96.67% to 21.67% (L) for the major workers and from 96.67% to 13.33% (B) and 95.00% to 16.67% (L) for the minor workers; the grasping abilities was reduced from 96.67% to 15.00% (B) and 95.00% to 13.33% (L) for the major workers and from 91.67% to 15.00% (B) and 96.67% to 15.00% (L) for the minor workers (Table 3).

Chemical components of the volatile compounds

The data from the GC-MS analysis demonstrated that the volatile compound from the mashed B and L of *T. vogelii* contains 17 and 14 major constituents, respectively (Table 4). The major components comprising 88.46% (B) and 75.37% (L) of the total volatile compound were identified as α -pinene, thujene, caryophyllene, and d-limonene. Other major components of the volatile compound included eucalyptol, α -cubebene, β -pinene, sabinene, and α -guaiene. α -Pinene is the major component, accounting for 63.44%(B) and 65.82%(L).

Discussion

T. vogelii is a shrubby plant used as a fallow plant in southern China. Our data show that the volatile compounds released from live B and L show no toxicity against RIFA workers and can not reduce the walking and grasping abilities of the RIFA workers despite abundant insecticidal contents. However, the volatile compounds released from the mashed B and L exhibited high toxicity against the RIFA workers and evidently reduced the walking and grasping abilities of the RIFA workers. This reduction indicated that the volatile

Table 3. Influences of mashed B and L of *T. vogelii* on mortality, walking and grasping abilities of workers (Mean \pm SE, %)

Material	Worker size	Mortality		Walking ability		Grasping ability	
		Time (h)	Percentage	Time (min)	Percentage	Time (min)	Percentage
B	Major	1	1.67 \pm 1.67b	40	98.33 \pm 1.67a	40	96.67 \pm 1.67ab
	Minor		13.33 \pm 1.67a		96.67 \pm 1.67a		91.67 \pm 1.67 b
L	Major		15.00 \pm 2.89a		96.67 \pm 1.67a		95.00 \pm 2.89ab
	Minor		18.33 \pm 1.67a		95.00 \pm 2.89a		96.67 \pm 3.33ab
B	Major	2	6.67 \pm 1.67b	80	91.67 \pm 1.67b	80	90.00 \pm 2.89ab
	Minor		30.00 \pm 5.77a		86.67 \pm 1.67bc		83.33 \pm 4.41b
L	Major		30.00 \pm 7.64a		88.33 \pm 3.33bc		85.00 \pm 5.77b
	Minor		35.00 \pm 2.89a		81.67 \pm 3.33c		81.67 \pm 3.33b
B	Major	4	16.67 \pm 4.41b	120	80.00 \pm 2.89bc	120	76.67 \pm 4.41b
	Minor		40.00 \pm 5.77a		73.33 \pm 1.67bc		71.67 \pm 1.67b
L	Major		35.00 \pm 7.64a		81.67 \pm 4.41b		80.00 \pm 5.00b
	Minor		45.00 \pm 2.89a		71.67 \pm 3.33c		73.33 \pm 4.41b
B	Major	6	36.67 \pm 4.41b	160	65.00 \pm 5.77b	160	60.00 \pm 2.89b
	Minor		61.67 \pm 4.41a		58.33 \pm 3.33b		58.33 \pm 3.33b
L	Major		51.67 \pm 4.41a		70.00 \pm 5.77b		70.00 \pm 5.77b
	Minor		60.00 \pm 5.77a		61.67 \pm 4.41b		61.67 \pm 4.41b
B	Major	8	66.67 \pm 4.41a	200	60.00 \pm 2.89b	200	43.33 \pm 4.41bc
	Minor		76.67 \pm 6.67a		40.00 \pm 2.89c		41.67 \pm 4.41c
L	Major		63.33 \pm 4.41a		60.00 \pm 5.77b		53.33 \pm 3.33b
	Minor		76.67 \pm 3.33a		45.00 \pm 2.89c		48.33 \pm 1.67bc
B	Major	10	85.00 \pm 5.77ab	240	41.67 \pm 1.67b	240	23.33 \pm 1.67c
	Minor		95.00 \pm 2.89a		23.33 \pm 4.41d		21.67 \pm 4.41c
L	Major		76.67 \pm 1.67b		40.00 \pm 2.89b		36.67 \pm 1.67b
	Minor		91.67 \pm 4.41a		31.67 \pm 1.67c		20.00 \pm 2.89c
B	Major	12	95.00 \pm 2.89ab	280	20.00 \pm 2.89b	280	15.00 \pm 2.89b
	Minor		100.00 \pm 0.00a		13.33 \pm 1.67c		15.00 \pm 2.89b
L	Major		98.33 \pm 1.67ab		21.67 \pm 1.67b		13.33 \pm 3.33b
	Minor		100.00 \pm 0.00a		16.67 \pm 1.67bc		15.00 \pm 2.89b

Values sharing same letters means they are not significantly different from each other ($P > 0.05$, Duncan's Multiple Range Test).

Table 4. The volatile compounds from mashed B or L (5 g) of *T. vogelii* adsorbed on silicon(2g) of indoor test.

The volatile compounds from 5 g mashed fresh bean pods(B)		The volatile compounds 5 g mashed fresh branches and leaves(L)			
Volatile compound	Retention time (min)	Relative content (%)	Volatile compound	Retention time (min)	Relative content (%)
thujene	3.379	0.88	1R- α -pinene	3.459	65.82
1R- α -pinene	3.454	63.44	β -terpinene	3.746	0.23
β -phellandrene	3.741	2.21	2-propyl pyridine	3.827	0.69
4-methylene-1-(1-methylethyl)-cyclohexene	3.784	2.56	d-limonene	4.145	0.74
β -pinene	3.822	5.66	eucalyptol	4.178	1.28
d-limonene	4.146	0.96	decamethylcyclopentasiloxane	4.842	3.99
tridecane	5.799	0.17	2,3-dihydro-5-methyl-1H-indene	4.945	0.50
cyclohexene	6.123	0.39	azulene	5.285	0.41
α -cubebene	6.371	1.59	copaene	6.366	0.21
aristolene	6.436	1.07	α -guaiene	6.431	0.11
(-)-isocaryophyllene	6.560	0.44	caryophyllene	6.636	0.69
caryophyllene	6.641	5.77	calarene	6.674	0.16
1H-cyclopenta[1,3]cyclopropa[1,2] benzene, octahydro-					
7-methyl-3-methylene-4-(1-methylethyl)]3aS(3a. α ,3b. β ,4, β ,7. α ,7aS*)]	6.679	0.64	germacrene	6.955	0.39
(-)-g-cadinene	6.820	0.79	hexadecane	7.376	0.15
β -sesquiphellandrene	6.955	1.30			
germacrene	7.036	0.31			
10s,11s-himachala-3(12),4-diene	7.063	0.28			

compounds could produce and release insecticidal compounds that could be defensive compounds, with functions varying from those in the B and L. The volatile compounds can be used to ward off pests when branches and leaves were harmed. The fumigant activity as well as walking and grasping abilities were influenced by the worker size, exposure time, and plant material.

To the best of our knowledge, no previous studies have been reported regarding the effects of volatile compounds from fresh plant materials of *T. vogelii* on the fumigant activity as well as the walking and grasping abilities of RIFA workers. However, earlier studies indicated that the insecticidal activity of the acetone extract of the leaf showed feeding deterrent activity against the insect *Pieris rapae* (Shin, 1989).

Plant-derived essential oils may contain hundreds of

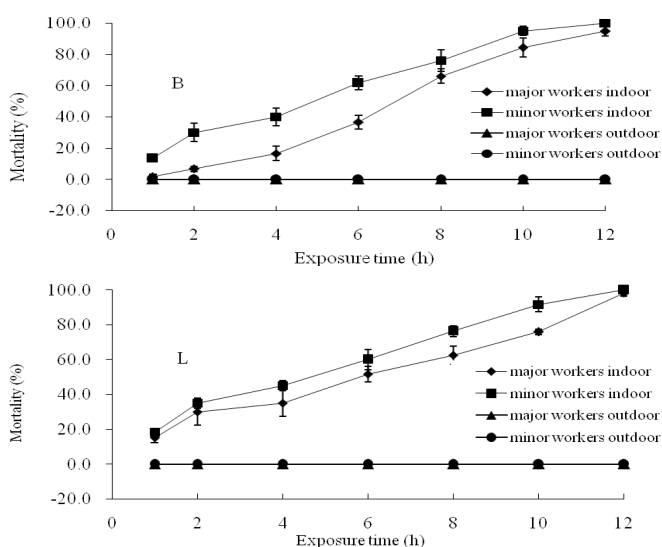


Fig. 1 Mortalities of workers after treated with fresh B or L of *T. vogelii* (Indoor test: 5g mashed fresh plant material, Outdoor test: one live branch with about 10 bean pods or 100 leaves)

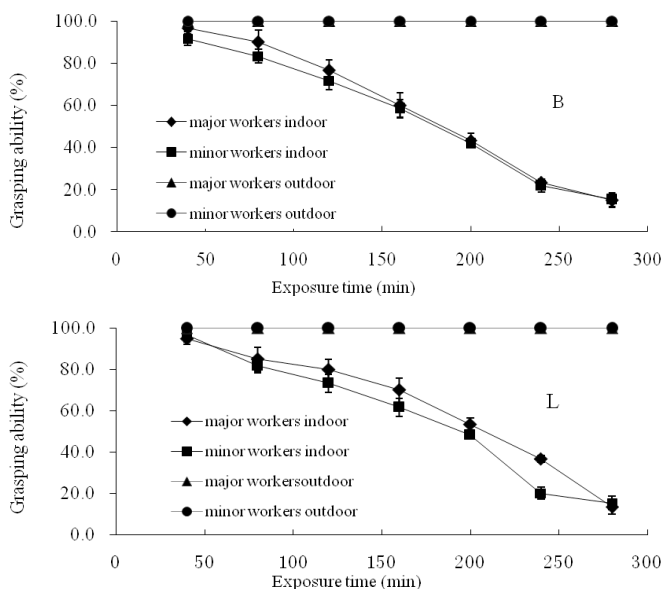


Fig. 2 - The grasping abilities of workers after treated with fresh B or L of *T. vogelii* (Indoor test: 5g mashed fresh plant materials, Outdoor test: one live branch with about 10 bean pods or 100 leaves)

different constituents but only few components predominate (Rajendran & Sriranjini, 2008). The results of the GC-MS analysis showed thujene, α -pinene, caryophyllene, eucalyptol, and d-limonene were found in the compounds from the mashed B and L. Eucalyptol, one of the monoterpenoids of the volatile compounds, is widely known for its high insecticidal property. α -pinene (64.63%) as its major components, is also found in other plants exhibiting biological activity against various insect species (Ojimelukwe & Alder, 1999; Choi et al., 2006).

The L of *T. vogelii* could have abundant insecticidal compounds and aboveground biomass (dry matter) produced by *T. vogelii* during the six-month growth period of 5.3 t/ha with an L:stem ratio = 1:2 (Venant, 1999). This finding indicates that *T. vogelii* can potentially control RIFA. The dry matter of *T. vogelii* is often prepared in the production of rotenone-based insecticides. However, this study suggests that the mashed B and L of *T. vogelii* are more suitable than dry matter for producing insecticides.

The volatile compounds from the mashed B and L of *T. vogelii* possess high toxicity against the RIFA workers and can reduce the walking and grasping abilities of these workers. Compared with dry matter, the mashed B and L of *T. vogelii* exhibit greater potential for producing insecticides.

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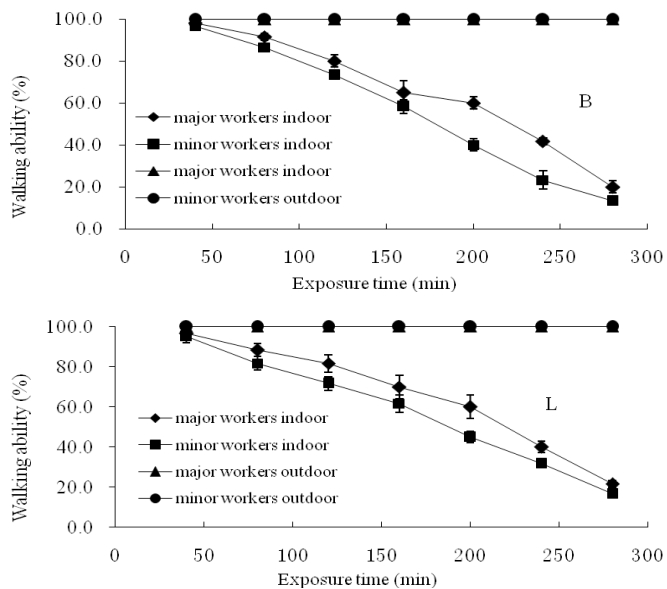


Fig. 3 - The walking abilities of workers after treated with fresh B or L of *T. vogelii* (Indoor test: 5g mashed fresh plant materials, Outdoor test: one live branch with about 10 bean pods or 100 leaves)

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