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Repellent activity of *Glycosmis* plant extracts against two stored product insects

[Actividad repelente de extractos de plantas *Glycosmis* contra dos insectos de productos almacenados]

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Abstract: In the present study, the repellent activities of the leaf and/or stem crude extracts of *Glycosmis lucida* Wall. ex Huang, *G. craibii* var. *glabra*, *G. craibii* Tanaka, *G. oligantha* Huang, *G. pentaphylla* (Retz) Correa. and *G. esquirolii* (Levl.) Tanaka were analyzed by using assays on petri dishes against *Tribolium castaneum* and *Liposcelis bostrychophila*. The leaf and stem extracts of *G. lucida*, *G. craibii* var. *glabra*, *G. craibii* Tanaka, *G. oligantha* and *G. esquirolii* possessed significant repellent activities against *T. castaneum*, the same level repellent with the positive control, DEET. However, the extracts of *G. pentaphylla*, no repellency but some insect attractant was observed. Moreover, they also showed repellent activities against *L. bostrychophila*. These results indicate that extracts from *G. lucida* and *G. oligantha* leaf could be a source of novel repellent against insects.

Keywords: extraction, *Glycosmis,* repellency, *Tribolium castaneum, Liposcelis bostrychophila*

Resumen: En el presente estudio, las actividades repelentes de la hoja y/o tronco de los extractos crudos de *Glycosmis lucida* Wall. ex Huang, *G. craibii* var. *glabra*, *G. craibii* Tanaka, *G. oligantha* Huang, *G. pentaphylla* (Retz) Correa y *G. esquirolii* (Levl.) Tanaka se analizaron mediante el uso de ensayos en placas de Petri contra *Tribolium castaneum* y *Liposcelis bostrychophila*. Los extractos de las hojas y tallo de *G. lucida*, *G. craibii* var. *glabra*, *G. craibii* Tanaka, *G. oligantha* y *G. esquirolii* poseían actividades repelentes significativas contra *T. castaneum*, el mismo nivel repelente del control positivo, el DEET. Sin embargo, los extractos de *G. pentaphylla*, no se observó la repelencia pero sí actividad atrayente de insectos. Por otra parte, también se mostraron las actividades repelentes contra *L. bostrychophila*. Estos resultados indican que los extractos de hojas de *G. lucida* y *G. oligantha* podrían ser una fuente de repelente contra los insectos.

Palabras clave: Extracción, *Glycosmis,* repelencia, *Tribolium castaneum, Liposcelis bostrychophila*.

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INTRODUCTION

Antagonistic storage has been used as one of traditional Chinese medicinal materials conservation methods. It mainly utilizes some traditional Chinese medicinal materials having special volatile odor to store with medicinal materials vulnerable to insects, so as to prevent the insects. According to our survey of literatures, many cases which use this method to prevent the insects were reported (Xia *et al.*, 2000; Wang, 2003; Han *et al.*, 2004; Luo *et al.*, 2012). In order to inherit and develop the traditional method of prevention and control of stored grain insects, we took five Glycosmis species as research object and two traditional Chinese medicinal materials storage insects (*Tribolium castaneum* and *Liposcelis bostrychophila*) as the target insects. It was expected that this research work would provide some theoretical basis for the conception of antagonistic storage.

The red flour beetle (*Tribolium castaneum* Herbst) (Coleoptera: Tenebrionidae) is world-widely distributed and among the most economically important stored product pests (Garcia *et al.*, 2005). Infestations can also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (Magan *et al.*, 2003). The booklouse (*Liposcelis bostrychophila* Badonnel) (Psocoptera) is an important emerging pest of stored products. It feed on a wide variety of foods, but they are particularly associated with amylaceous products, such as grain and flour (Nayak, 2006; Throne *et al.*, 2006; Zhou *et al.*, 2012). It is widely distributed in several tropical and sub-tropical countries in Asia, Europe, North America, South America, Africa and Australia (Turner, 1999; Li *et al.*, 2011). This insect's parthenogenic mode of reproduction coupled with its short life cycle in favourable conditions make it particularly troublesome, as it can rapidly infest susceptible commodities (Fisher, 1985; Mills *et al.*, 1992; Phillips & Throne, 2010). Infestations of stored product insects could be controlled by insecticidal treatment of commodities and surfaces (Zettler & Arthur, 2000). However, increasing attention is being given to the development of more ecologically and economically sustainable control methods for stored-product pests, mainly because of the problems already mentioned, for example, the management of the habitat and the use of semiochemical-baited traps, repellents or of biological control (Phillips & Throne, 2010).

It is well known that Rutaceae are used in many countries for their repellent properties against several insects (Venkatachalam & Jebanesan, 2001; Ngassoum *et al.*, 2007; Fogang *et al.*, 2012; Mehmood *et al.*, 2012; Conti *et al.*, 2013; Ali *et al.*, 2013). The *Glycosmis* genus is a species of shrub or dungarunga of the Rutaceae family and distributes in south and southest of Aisa, northest of Australia and mainly located in south of nanling, south of Yunnan and southeast of Tibet in China (Huang, 1997). A survey of literature has shown that the chemical composition of genus *Glycosmis* possessed the insecticide activities against several insects (Greger *et al.*, 1996; Latha & Joseph, 1999). Among the *Glycosmis* genus, the repellency of *G. pentaphylla* essential oil against the insect was evaluated (Khan *et al.*, 1983; Pandey *et al.*, 2013). However, little is known about the effectiveness of other *Glycosmis* species extract against stored product insects.

To our knowledge, nothing was reported on the repellent activity of *G. lucida*, *G. craibii* var. glabra, *G. craibii* Tanaka, *G. oligantha*, *G. pentaphylla* and *G. esquirolii* extracts against *T. castaneum* and *L. bostrychophila*. In this study, their repellent activity against the red flour beetle and the booklouse were investigated for the first time.

MATERIAL AND METHODS

Plant materials

The leaf and stem samples of *G. lucida*, *G. craibii* var. *glabra*, *G. craibii* Tanaka, *G. oligantha*, *G. pentaphylla* and *G. esquirolii* plants were collected in Xishuangbanna, Yunnan province, China (northern latitude: 21°08′~22°36′; east longitude: 99°56′~101°50′), between 2011 June and 2013 June. The specimens were identified by Dr. LX Zhang, Yunnan branch of the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences, Yunnan, China, and the voucher specimens were deposited at the Herbarium (BNU) of the College of Resources Science and Technology, Beijing Normal University.

Extraction

The plant samples were dried naturally for a week. The dried plant samples (100 g) were ground to powders using a grinding mill (High speed grinder 6202, Beijing huanya tianyuan machinery technology co. LTD, Beijing, China) and extracted thrice with methanol (800 mL, 30 min each) at room temperature by using ultrasonic irradiation. All extracts were filtered through Whatman Nº 1 filter paper, evaporated to dryness through rotary evaporator at 45° C under vacuum and further dried under high vacuum overnight. The dried extracts were stored in parafilmed glass vials at 4° C until required for analysis.

Insects

The red flour beetle, *T. castaneum* was obtained from laboratory cultures maintained for the last 2 years in the dark, in incubators at 28-30° and 70-80% relative humidity. The beetles were reared on wheat flour mixed with yeast (10:1, w/w) at 12-13% moisture content. Unsexed adult beetles used in the experiments were about 2 weeks old.

The booklouse, *L. bostrychophila* was obtained from laboratory cultures maintained for the last 2 years in the dark, in incubators at 29-30° and 70-80% relative humidity and was reared on a 1:1:1 mixture, by mass, of milk powder, active yeast and flour. All containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, *Sino-Rich*, Beijing, China). Insects used in the experiments were 1 week old.

Repellent activity bioassay

The repellent effects of the essential oil against *T. castaneum* and *L. bostrychophila* were assessed by using assays on petri dishes (Chaubey, 2007). Petri dishes 9 cm in diameter were used to confine beetles during the experiment. The extracts of *G. lucida*, *G. craibii* var. glabra, *G. craibii* Tanaka, *G. oligantha*, *G. pentaphylla* and *G. esquirolii* were prepared in ethanol (157.26, 31.45, 6.29 nL/cm^2), and absolute ethanol was used as the control. Filter paper 9 cm in diameter was cut in half and 500 µL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 µL of absolute ethanol. Both the treated half and the control half were then air-dried to evaporate the solvent completely (2 min). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Each reassembled filter paper after treatment with solid glue was placed in a petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the petri dish. Five replicates were used, and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil was then calculated using the formula:

$$\mathbf{PR\ (\%) = [(N_c - N_t) / (N_c + N_t)] \times 100}$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. The averages were then assigned to different classes (0 to V) using the following scale (percentage repellency) (Liu & Ho, 1999). Class, % repellency: 0, >0.01 to <0.1; I, 0.1-20.0; II, 20.1-40.0; III, 40.1-60.0; IV, 60.1-80.0; and V, 80.1-100.

As for the booklouse, petri dishes and filter papers were changed to 6 cm in diameter and the concentration of the oil used in the experiments were 126.32, 25.26, 5.05 nL/cm^2. The half filter paper was treated with 150 µL of the solution. As a positive control, a commercial repellent DEET (*N*, *N*-diethyl-3-methylbenzamide), was used under the conditions as the oil. Analysis of variance (ANOVA) and LSD test were conducted by using SPSS statistics 20 for Windows 2007. Percentage was subjected to an arcsine square-root transformation before ANOVA and LSD tests.

RESULTS AND DISCUSSION

Methanol extracts were obtained before carrying out each specific biological assay. The plant extracts yields of six Glycosmis species were 1.84% to 7.80% (Table 1). The repellent activity of the leaf and/or stem extracts of medicinal plants against *T. castaneum* and *L. bostrychophila* was determined by using assays on petri dishes (Table 2 and Table 3).

According to our results, except for *G. pentaphylla*, other five *Glycosmis* species extracts possessed strong repellency against *T. castaneum*, especially, the *G. lucida* leaf extract displayed the highest repellency against *T. castaneum* (Table 2). When at the lowest tested concentrations of 6.29 nL/cm^2, the extract of *G. lucida* leaf still showed stronger repellency (94% and 84%, class V) than the positive control, DEET (78% and 68%, class IV) at 2 h and 4 h after exposure (Table 2). The extracts of *G. oligantha* leaf, *G. esquirolii* leaf displayed the same level repellency against *T. castaneum* compared with the positive control. When at the concentration of 31.45 nL/cm^2, the extracts of *G. craibii* var. glabra leaf showed strong repellency (88%, class V) at 2 h after exposure and the *G. craibii* Tanaka leaf extracts also exhibited strong repellency (90% and 88%, class V) against *T. castaneum* at 2 h and 4 h after exposure (Table 2). With the concentration decreasing, the repellency of the *Glycosmis* species extracts also weakened gradually. However, the extracts of *G. pentaphylla* leaf and stem especially the *G. pentaphylla* stem, no repellency but some insect attractant properties against the red flour beetle were observed at 2 and 4 h after exposure (Table 2).

Table 1
Methanol extracts of the 6 Glycosmis species

Species	Source	Yield（%）	Family
G. lucida	**Leaf**	**5.48**	**Rutaceae**
G. craibii* var. *glabra	**Leaf**	**7.80**	**Rutaceae**
***G. craibii* Tanaka**	**Leaf**	**1.84**	**Rutaceae**
G. oligantha	**Leaf**	**2.66**	**Rutaceae**
	Stem	**2.86**	
G. pentaphylla	**Leaf**	**2.79**	**Rutaceae**
	Stem	**4.34**	
G. esquirolii	**Leaf**	**6.22**	**Rutaceae**
	Stem	**5.08**	

At the same time, the extracts of the six *Glycosmis* species also possessed repellency against *L. bostrychophila*. When at the highest concentration of 126.32 nL/cm^2, the extracts of *G. lucida* leaf, *G. oligantha* leaf possessed the same level repellency against *L. bostrychophila* at 2 h after exposure and the extracts of *G. craibii* Tanaka leaf, *G. oligantha* leaf, *G. pentaphylla* leaf, *G. esquirolii* leaf also displayed strong repellency against *L. bostrychophila* at 4 h after exposure (Table 3). While other Glycosmis species extracts possessed moderate repellency at 2 h and 4 h after exposure. With the concentration decreasing, the repellency of the six *Glycosmis* species extracts weakened gradually. Compared with the positive control, at the concentrations of 25.26, 5.05 nL/cm^2, the extracts of all species displayed lower level repellency against *L. bostrychophila*. At the concentration of 5.05 nL/cm^2, the extracts of *G. pentaphylla* leaf and *G. esquirolii* stem, also no repellency but some insect attractant properties against the booklouse were observed at 4 h after exposure (Table 3). Some extracts were evaluated for repellency against insects (Lou *et al.*, 2013). In China, the extracts derived from Chinese medicinal herbs were also evaluated for insecticidal activity and repellency against the red flour beetle and other insects (e.g., Huang *et al*., 2007; Yao et al., 2007; Tang *et al*., 2009a; Tang *et al*., 2009b; Tang *et al*., 2009c; Huang *et al*., 2010; Wei *et al.*, 2014). However, no reports are on bioactivity to the booklouse. In this article, the extracts of 6 *Glycosmis* species were evaluated for repellency against the two grain storage insects, *T. castaneum* and *L. bostrychophila*.

Table 2
Percentage repellency of the *Glycosmis* species extracts against *Tribolium castaneum* after two exposure times

Treatment	Plant part	2h			4h		
		157.26*	31.45*	6.29*	157.26*	31.45*	6.29*
G. lucida	leaf	90±6ab V	86±10abc V	94±6ab V	88±9ab V	76±21ab IV	84±13a V
G. craibii var. *glabra*	leaf	94±9ab V	88±10ab V	60±19cd III	100±0a V	74±16ab IV	58±14bc III
G. craibii Tanaka	leaf	58±18c III	90±8ab V	60±18cd III	72±20c IV	88±11a V	34±19c II
G. oligantha	leaf	80±15bc IV	76±16bc IV	76±21bc IV	86±14b V	68±18b IV	64±23b IV
	stem	84±14ab V	82±18abc V	42±21d III	92±9ab V	72±16ab IV	30±21c II
G. pentaphylla	leaf	54±19c III	76±16bc IV	-58±17e -	48±21d III	66±19b IV	-8±13d -
	stem	-36±20d -	-62±16d -	-76±15e -	-64±12e -	-76±21c -	-84±10e -
G. esquirolii	leaf	82±18ab V	68±15c IV	64±9cd IV	76±15c IV	90±12a V	42±11bc III
	stem	78±18bc IV	88±18ab V	52±12d III	80±13bc IV	80±8a IV	44±17bc III
DEET		100±0a V	98±3a V	78±14bc IV	96±3a V	82±8a V	68±5b IV

*** Concentration (nL/cm^2);**
a-e Means in the same column followed by the same letters do not differ significantly (P < 0.05) in ANOVA and LSD tests. PR was subjected to an arcsine square-root transformation before ANOVA and LSD tests.

To our knowledge, the repellent activity of *G. lucida*, *G. craibii* var. glabra, *G. craibii* Tanaka, *G. oligantha*, *G. pentaphylla* and *G. esquirolii* has not been reported before. Previous studies have isolated carbazole alkaloid, acridone alkaloids from *G. pentaphylla* (Govindachari *et al.*, 1966; Yang *et al.*, 2012), hydroquinone diglycoside acyl esters, isoflavone diglycosides from *G. pentaphylla* (Wang *et al.*, 2006a; Wang *et al.*, 2006b) and flavanones, flavanonols from *Glycosmis* species (Lukaseder *et al.*, 2009). Some compounds from *Glycosmis* species with antitumor, antifungal, antibacterial and insecticidal have been observed (Bhattacharyya *et al.*, 1985; Greger *et al.*, 1996; Quader *et al.*, 1999; Ito *et al.*, 2004; Sripisut *et al.*, 2012). However, their association with repellency activity is currently unknown. The results pointed out the potential value of the compounds from the extracts used to prevent the stored products insects.

CONCLUSIONS

The results have shown that the extracts of *G. lucida*, *G. craibii* var. glabra, *G. craibii* Tanaka, *G. oligantha* and *G. esquirolii* exhibit strong repellent activity against *T. castaneum*. They show potential to be developed as possible natural repellent for control of stored product insects. However, for the practical application of the extracts, further studies on the safety of the extracts to humans and on the effect of the compounds from the plant extracts are necessary to improve the efficacy.

Table 3
Percentage repellency of the Glycosmis species extracts against *Liposcelis bostrychophila* after two exposure times

Treatment	Plant part	2h			4h		
		126.32*	25.26*	5.05*	126.32*	25.26*	5.05*
G. lucida	leaf	84±10ab V	58±8b III	34±12bc II	76±6cd IV	48±18bc III	20±12cd I
G. craibii var. glabra	leaf	78±17bc IV	70±8ab IV	38±11bc II	78±10bcd IV	68±12abc IV	18±13cd I
G. craibii Tanaka	leaf	76±14bc IV	66±13ab IV	34±11bc II	86±13bc V	76±13ab IV	46±12bc III
G. oligantha	leaf	88±8ab V	58±11b III	40±12bc II	100±5a V	74±11ab IV	32±18c II
	stem	80±9abc IV	62±15ab IV	48±8bc III	62±12d IV	56±15bc III	60±8b III
G. pentaphylla	leaf	70±17bc IV	52±6b III	16±19de I	84±14bc V	58±15bc III	-24±24e -
	stem	70±13bc IV	50±7b III	50±9bc III	76±10cd IV	38±6c II	10±14cd I
G. esquirolii	leaf	74±12bc IV	54±13b III	68±17ab IV	88±10bc V	76±13ab IV	36±18bc II
	stem	66±13c IV	28±20c II	6±7e I	58±14d III	38±8c II	-6±12de -
DEET		94±6a V	82±5a V	86±8a V	92±5ab V	84±3a V	82±8a V

*** Concentration (nL/cm^2);**
a-e Means in the same column followed by the same letters do not differ significantly (P < 0.05) in ANOVA and LSD tests. PR was subjected to an arcsine square-root transformation before ANOVA and LSD tests.

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