

Toxicity and Repellency Activities of the Crude Methanol Extract of *Duabanga grandiflora* (Lythraceae) Against *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract.- Laboratory bioassays were carried out to examine the toxicity and repellency of a crude methanol extract of *Duabanga grandiflora* against adult rice weevil, *Sitophilus oryzae*. Toxicity was evaluated using a no-choice test with treated filter paper and rice grains while repellency evaluated using a choice test with treated filter paper. The crude methanol extract was found to possess low toxicity against *S. oryzae* with maximum mortality of 33% at 32 mg/mL (w/v) 7 days after exposure. Toxicity depended on both concentration and duration. The crude methanol extract was also repellent against *S. oryzae* ranging from 37 and 83% at 5 min to 2 h after exposure and from 60 to 100% at 4 to 24 h after exposure. The exposure period appeared to be the most important factor affecting the repellent effect of crude methanol extract of *D. grandiflora* ($P < 0.05$) while the tested concentrations showed no different in repellent activity ($P > 0.05$).

Keywords: Crude extract, *Duabanga grandiflora*, *Sitophilus oryzae*, toxicity, repellency.

INTRODUCTION

Sitophilus oryzae L. (rice weevil, Coleoptera: Curculionidae) is one of the most important pests of stored products in the world. Females deposit eggs into grain, larvae are legless and remain in the grain kernel for their entire duration. Newly emerged adults may spend several days within the grain, before chewing exit holes to emerge (Benhalima *et al.*, 2004). Feeding by *S. oryzae* larvae and adults can reduce grain weight by as much as 75% (Dal Bello *et al.*, 2001), and also decreases nutritional and aesthetic value of the grain. The weevils reduce germination resulting in lower prices for seed grain (Moino *et al.*, 1998).

Synthetic insecticides such as chlorpyrifos-methyl, pirimiphos-methyl, deltamethrin, methyl bromide and phosphine are effective in controlling *S. oryzae*. However, heavy use of synthetic insecticides has resulted in the development of

resistance of stored-grain insects to many of these insecticides. There is concern about exposure to workers of these chemicals and insecticide residues are unwanted by consumers (Champ and Dyte, 1976; Richard and Bruce, 1990; Subramanyam and Hagstrum, 1995; White and Leesch, 1995). Finally, these chemicals are also toxic to natural enemies that help control stored-product insect pests (Subramanyam and Hagstrum, 1995; Srivastava and Singh, 2002).

Due to these concerns, there is an increased interest in alternatives to chemical insecticides. There is a long history of using plants to control insect pests (Belmain *et al.*, 2001), and an extensive knowledge on plants to control stored-product insects (Golop *et al.*, 1999) and the compounds responsible for activity (Nawrot and Harmatha, 1994; Isman, 2006). Many plant extracts and essential oils contain several bioactive chemicals which are toxic to stored-product insects including *S. oryzae*. For instance, ethanol extract of *Clerodendrum inerme* L. (Verbenaceae) and *Withania somnifera* L. (Solanaceae) can be used for the protection of stored wheat from infestations of *S. oryzae* (Yankanchi and Gadache, 2010). Crude

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extracts of *Cinnamomum cassia* Blume, *Cinnamomum sieboldii* Meissner, cinnamon oil, horseradish oil, and mustard oil induced 100% mortality of *S. oryzae* adults after 1 day (Kim *et al.* 2003), whereas insecticidal action of extracts from *Acorus calamus* var. *angustatus* Besser, *Acorus gramineus* Solander, *Illicium verum* Hooker fil, *Eugenia caryophyllata* Thunberg and *Foeniculum vulgare* Gaertner induced 90% mortality after 4 days (Kim *et al.*, 2003).

Duabanga grandiflora (Roxb. ex DC.) Walp. belongs to subfamily Duabangoideae, family Lythraceae, and it is a member of a tropical African and Southeast Asian trees (Graham *et al.*, 1998, 2005). The hill tribe people in the Northern Thailand use the poultices from its leaves to treat stomach pains (Anderson, 1986). The leaf extract is used for skin whitening, anti-aging and anti-inflammation, and contains eugenin, which showed strong dose dependent activity for type III collagen production (Tsukiyama *et al.*, 2010). Stem bark of *Duabanga sonneratioides* Ham., (synonymous with *D. grandiflora*), showed anti-cancer activity against Walker Carcinosarcoma 256 in rats (Sharma *et al.*, 1972). Recently, *D. grandiflora* inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* (Othman *et al.*, 2011). Extracts from *D. grandiflora* stem branches have phenolic, triterpenoid, flavonoid and steroid compounds (Auamcharoen *et al.*, 2009). The stem bark of *D. sonneratioides* has hentriacontane, hentriacontanol, β -sitosterol, α -amyrin, epioleanolic acid, epifriedelinol, lignoceryl ferulate, betulinic acid, acacetin, ellagic acid and its tetramethyl ether, quercetin 3-glucoside and 3-galactoside, genkwanin and gentianin 4'-galactoside (Bhakuni *et al.*, 1971; Sharma *et al.*, 1972, 1974).

Given that *D. grandiflora* crude extracts have shown biological activity we tested the toxicity and repellency of crude methanol extract from the stem branches of *D. grandiflora* on *S. oryzae* under laboratory conditions.

MATERIALS AND METHODS

Test insects

Sitophilus oryzae were obtained from Food

Entomology Laboratory in Food Safety Division of the National Food Research Institute, Tsukuba, Japan. Insects were cultured on brown rice kernels at $24\pm 1^\circ\text{C}$ and 65-70% relative humidity (r.h.) with a 16 L:8D photoperiod. All experiments were conducted at these environmental conditions.

Plant crude extract

Duabanga grandiflora stem branches were collected from Kanchanaburi province located in Western Thailand in January 2007. The plant material was identified by Mr. Pranai Penchit and the herbarium specimen (CHKU 00010) was deposited at the Bangkok Herbarium Botanical Research Unit, Plant Variety Protection Division, Department of Agriculture, Bangkok, Thailand.

The stem branches were machine-cut into small pieces and dried at 40°C in a hot air oven before grinding into powder by a grinding machine. Dried powder (5 kg) was extracted with methanol 3×20 L at room temperature. The methanol solution was filtered with a Whatman #1 filter paper and concentrated by a rotatory evaporator under reduced pressure to give the crude methanol extract (190 g) which was used for bioassays.

Toxicity test

The toxicity of *D. grandiflora* extract against *S. oryzae* was conducted with two bioassays; 1) treated filter paper with rice grains, 2) treated filter paper bioassay without rice. Whatman#1 filter paper discs (9 cm diameter) and 10 rice grains which were placed at the center of each filter paper were treated with 1 mL of *D. grandiflora* extract at the concentrations of 1, 2, 4, 8, 16 or 32 mg/mL, whereas controls received the same volume of absolute methanol. The solvent was allowed to evaporate completely at room temperature. Each filter paper disc with rice grains was then placed on a glass Petri dish (9 cm diameter) and 10 unsexed *S. oryzae* adults (0-14 days old) were introduced into the center of each Petri dish before wrapping with plastic wrap and covered up with its lid (10 Petri dishes/replication, 3 replications/ concentration). The number of dead insects was recorded after 1, 2, 3, 4 and 7 days. The entire experiment was repeated but without rice.

Repellency test

The repellency of *D. grandiflora* extract was determined in glass Petri dishes (9 cm in diameter). Whatman #1 filter paper of 9 cm diameter was divided into two equal parts. Test solutions were prepared by diluting the crude extract in absolute methanol to various concentrations (1, 2, 4, 8, 16 and 32 mg/mL, and applying 500 μ L of each solution which produced the equivalent to 0.031, 0.063, 0.126, 0.252, 0.503 and 1.007 mg/cm². The control half was treated with the same volume of absolute methanol. Both treated and control parts were allowed to dry completely at room temperature. Then, the treated and control parts were connected with clear adhesive tape and placed alternately on each Petri dish. Ten rice grains were then placed on both treated and control parts and ten unsexed *S. oryzae* adults were released at the center of the filter paper disc. Plastic wrap was used to wrap each Petri dish before its cover was placed (10 Petri dishes/ replication, 3 replications/concentration). The number of insects presented in the control (N_{control}) and treated (N_{treated}) sides of the disc was counted at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after the beginning of exposure.

Statistical analyses

For toxicity test, *S. oryzae* mortality was expressed as a percentage, whereas repellency test was evaluated using the following formula: Percentage repellency (PR) = $(N_c / (N_c + N_t)) \times 100$ where N_c was the number of *S. oryzae* on the control side and N_t was the number of *S. oryzae* on the treated side. The data for percentage of repellency were square root arcsin transformation before running the ANOVA using SAS program (SAS Institute Inc, 1999 Cary, NC), and treatment means were compared by the Least Significant Difference (LSD) at $P = 0.05$.

RESULTS AND DISCUSSION

The *D. grandiflora* extracts had low toxicity against *S. oryzae* adults with rice (Table I). When the insects were held on filter paper without rice there was no mortality at any of the concentrations after 7 days (data not shown). Therefore, the mortality of the *S. oryzae* is probably caused by

feeding on the rice grains treated with crude extract. There were no significant differences in mortality between the controls and 1, 2, 4, 8 and 16 mg/mL. At the highest concentration (32 mg/mL), the crude methanol extract induced significantly higher mortality of 23-33% than other doses. Higher doses of the extract for a relatively short period are more effective than the lower doses for longer periods. These findings are in agreement with Yankanchi and Gadache (2010).

The toxicity of *D. grandiflora* extract increased with both concentration and time. This phenomenon has also been reported for most insecticides and botanical insecticides from *Clerodendrum inerme* L. (Verbenaceae), *Withania somnifera* L. (Solanaceae), *Gliricidia sepium* L. (Fabaceae), *Cassia tora* L. (Caesalpinaceae) and *Eupatorium odoratum* L. (Asteraceae) against *S. oryzae* (Yankanchi and Gadache, 2010). Interestingly, *D. grandiflora* extract showed relatively lower insecticidal activity than pomegranate (*Punica granatum* Linn.), even though both plants belong to the same family. Gandhi *et al.* (2010) found that leaf powder of pomegranate caused high mortality, delayed growth and reduced populations of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

The toxicity of *D. grandiflora* extract could be caused by the bioactive compounds present in the extract. Auamcharoen *et al.* (2009) reported that chloroform extracts of *D. grandiflora* stem obtained from the crude methanol extract, contained three triterpenes (betulinic acid, oleanolic acid and arjunolic acid); three flavones (acacetin, apigenin and acacetin 7-*O*-glucoside); four phenolics (*p*-hydroxybenzaldehyde, vanillic acid, 6*H*-dibenzo[*b,d*]pyran-3,9-dihydroxy-6-one and 4-*O*- α -L-rhamnopyranosyl-3'-methoxyellagic acid) and one steroid (3-glycosyl- β -sitosterol). Some of these compounds and their derivatives have been previously reported for their insecticidal properties against stored-product pests. Pungitore *et al.* (2005a, 2005b) reported that oleanolic acid, a major component of *Junellia aspera* (Gillies ex Hook) (Verbenaceae), exhibited toxic effects by ingestion on *S. oryzae* and direct application on the cuticle of *T. castaneum* adults. On the other hand, benzaldehyde, which occurs in peach and almond

Table I.- Mortality (%) caused by crude methanol extract of *Duabanga grandiflora* against *Sitophilus oryzae* adults, using treated filter paper and rice grains (Temperature = 24±1°C, relative humidity = 65-70%, 16L:8D photoperiod). The values are Mean±SE.

| Concentration (mg/mL) | Days after treatment | | | | |
|-----------------------|----------------------|------------|--------------|--------------|--------------|
| | 1 | 2 | 3 | 4 | 7 |
| 0 | 0.0±0.0 a | 0.0±0.0 b | 3.3±3.3 b | 3.3±3.3 b | 6.7±3.3 bc |
| 1 | 0.0±0.0 a | 0.0±0.0 b | 3.3±3.3 b | 6.7±6.7 b | 6.7±6.7 bc |
| 2 | 0.0±0.0 a | 0.0±0.0 b | 3.3±3.3 b | 6.7±3.3 b | 10.0±0.0 bc |
| 4 | 0.0±0.0 a | 0.0±0.0 b | 6.7±3.3 ab | 10.0±5.8 ab | 10.0±5.8 bc |
| 8 | 0.0±0.0 a | 3.3±3.3 ab | 3.3±3.3 b | 10.0±5.8 ab | 10.0±5.8 bc |
| 16 | 0.0±0.0 a | 3.3±3.3 ab | 16.7±12.0 ab | 20.0±11.6 ab | 23.3±14.5 ab |
| 32 | 0.0±0.0 a | 6.7±3.3 a | 23.3±8.8 a | 30.0±10.0 a | 33.3±8.8 a |

Means within the column followed by the same letters are not significantly different ($P = 0.05$; LSD). Means±SE of untransformed data are reported.

Table II.- Repellency (%) of the crude methanol extract of *Duabanga grandiflora* to *Sitophilus oryzae* adults, using treated filter paper (Temperature = 24±1°C, relative humidity = 65-70%, 16L:8D photoperiod). The values are Mean±SE.

| Concentration of extract (mg/cm ²) | Duration of exposure | | | | | | | |
|--|----------------------|----------|---------|----------|-----------|-----------|----------|----------|
| | 5 min | 15 min | 30 min | 1 h | 2 h | 4 h | 8 h | 24 h |
| 0.031 | 43±9 b | 37±3 b | 53±3 ab | 47±7 ab | 67±12 ab | 63±22 ab | 80±15 a | 80±12 a |
| 0.063 | 43±12 a | 37±9 a | 67±19 a | 60±12 a | 73±17 a | 60±21 a | 70±21 a | 70±17 a |
| 0.126 | 50±12 b | 60±17 ab | 67±7 ab | 60±6 ab | 73±9 ab | 60±12 ab | 67±9 ab | 83±9 a |
| 0.252 | 63±27 ab | 37±15 b | 67±7 ab | 47±20 b | 43±13 b | 73±18 ab | 77±3 ab | 90±6 a |
| 0.503 | 43±12 d | 53±17 cd | 83±9 ab | 67±7 bcd | 80±6 bcd | 73±18 bcd | 87±3 ab | 100±0 a |
| 1.007 | 57±12 c | 67±13 bc | 63±3 bc | 50±10 c | 73±12 abc | 90±6 a | 83±12 ab | 80±0 abc |

Means within the same row followed by the same letters are not significantly different ($P = 0.05$; LSD). Means±SE of untransformed data are reported.

kernels, was found to have a potent fumigant toxicity against *S. oryzae* whereas 4-methoxybenzaldehyde is less toxic towards the same weevils (Lee *et al.*, 2001). Flavonoids (flavone, rutin, quercetin, myricetin, fisetin, quercitrin and flavonoids from *Calotropis procera* (Ait.) R. Br.) were toxic to adult *Callosobruchus chinensis* (L.) via contact on the filter paper bioassay (Salunke *et al.*, 2005). Hence, these compounds play an important role in compound-insect relationship.

The *D. grandiflora* extract was repellent to *S. oryzae* (Table II). There were significant difference between times (ANOVA $F = 7.02$, $P < 0.0001$) however no significant effect of concentrations ($F = 1.68$, $P = 0.1466$) or interaction between concentration and time ($F = 0.62$, $P = 0.9470$). The crude methanol extract at all concentrations showed repellency effect against *S. oryzae* ranging from 43-

63% 5 min after releasing insects. At 15 min to 2 h after release, the extract showed repellency ranging from 37-83%. As the time progressed, the extract showed fluctuation in repellency between 60-100% at 4 to 24 h after release. At the end of experiment (24 h), the concentration of 0.503 mg/cm² showed strongest repellency effect (100%) which was significantly different from that after 5 min, 15 min, 1 h, 2 h and 4 h. The other concentrations showed repellency ranging from 70-90%.

Repellent effect of the crude methanol extract of *D. grandiflora* could be attributed to the mixture of compounds detected by the *S. oryzae* adults. The bioassays of this crude extract using four-arm olfactometer showed no repellency against *S. oryzae* (Fig. 1). The *S. oryzae* was exposed to the vapor of the crude extract without touching for 5 min under the air flow within the tested machine. The obtained

result can be explained by the fact that the constituents of the crude methanol extract of *D. grandiflora* are high molecular weight compounds with low volatility. Another possibility is that the exposure time used in the experiment may be too short for the tested materials to exhibit their repellency effect. Thus, the tested extract did not show the repellency to *S. oryzae* adults under the limited time.

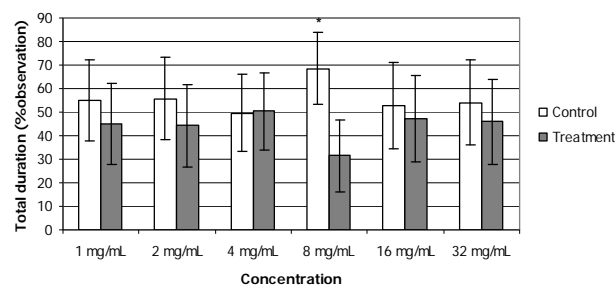


Fig. 1. Total duration in the control and treated sides of the four-arm olfactometer (5 min observation). Concentrations with * indicated a significant difference between the treatment and control sides ($P < 0.05$).

It is evident from this experiment that time is the main factor for repellency of *S. oryzae* by the *D. grandiflora* extract. Similar result was also found for the hexane leaf extract of *Solanum argentinum* Bitter et Lillo (Solanaceae) where 10 mg/mL or 0.31 mg/cm² showed repellency of 15, 60, 70, 80 and 90% at 1, 2, 3, 4 and 5 h after exposure, respectively on *S. oryzae* adults by using the filter paper test (Viglianco *et al.*, 2008).

This study showed that toxicity and repellency of the *D. grandiflora* extract against *S. oryzae* adults depended on several factors including chemical constituents of the extract, insect species and exposure time. This extract may act as a repellency rather than oral toxicity against *S. oryzae* which may be a better way for managing this insect. The fact that larvae of this weevil stay inside the grain for a long time before emerge to adult, it is difficult to control them with the insecticide while the chemical repellent can protect the grains and diminish the initial infestation, as was mentioned by Highland and Cline (1986). Moreover, low toxicity of this crude extract also leads to less subsequent

resistance of pest. However, the fact that this mixture has low toxicity needs more work. In addition, more researches are required to determine stability, duration of effectiveness, effect on end use quality, toxicity to human, contact toxicity with treated grain and effect of extract on different species of stored-product insects.

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