

## Contact activities of *Delonix regia* (Bojer ex Hook.) Raf., *Senna tora* (L.) Roxb. and *Leucaena glauca* (Willd.) Benth. seed crude extracts against maize weevil, *Sitophilus zeamais* Motschulsky

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### Abstract

Laboratory bioassays were carried out to examine the contact toxicities of seed crude hexane, dichloromethane and methanol extracts of *Delonix regia*, *Senna tora* and *Leucaena glauca* against adults maize grain weevil, *Sitophilus zeamais*. Dried seeds were ground into powder before extracting with hexane, dichloromethane and methanol at room temperature. All crude extracts were tested against 7 day-old adults of maize grain weevil. A drop of 0.5  $\mu\text{L}$  of each concentration (1, 3, 5, 7 and 9%) was dropped on the thorax of *S. zeamais* using a Burkard Arnold micro-applicator (Burkard Manufacturing Company Ltd., UK). Hexane and dichloromethane seed crude extracts of *D. regia* at 9% (v/v) concentration induced the highest mortalities of 60 and 52% where  $\text{LD}_{50}$  of 42.377 and 45.206 and  $\text{LD}_{95}$  of 64.500 and 80.371  $\mu\text{g insect}^{-1}$  were recorded, respectively at 7 days after treatment. Dichloromethane seed crude extract of *S. tora* at 9% (w/v) concentration showed the maximum mortalities of 20% at 1 day after treatment and were stable until the end of experiment. Methanol seed crude extract of *L. glauca* at 7% (w/v) concentration induced only 6% mortality at 7 days after treatment.

Keywords: crude extract, *Delonix regia*, *Senna tora*, *Leucaena glauca*, *Sitophilus zeamais*

### 1. Introduction

Thailand is an agricultural country and many people rely on cereals such as rice, maize and sorghum. The maize weevil, *Sitophilus zeamais* Mostchulsky (Coleoptera: Curculionidae) is a major insect pest of maize in the tropics (Kim and Kossou, 2003). Rice and maize seeds can be damaged in storage by *S. zeamais*, which significantly reduces the quality of seed products in several countries including Thailand (Mason and Obermeyer, 2010). Feeding by *S. zeamais* larvae and adults causes caking and mold growth, resulting in lower economic value and unsuitable products for consumption (Agboola, 1982).

Synthetic insecticides are the most effective means for controlling *S. zeamais* (Mbah and Okoronkwo, 2008). Fumigants such as phosphine and methyl bromide are commonly applied for controlling stored product pests. However, methyl bromide was banned in many countries starting in 2004 because of its ozone depleting properties (Hansen and Jensen, 2002). In addition, phosphine has been frequently failed to control insects and resistance to phosphine has developed (Bell and Wilson, 1995).

Secondary metabolites produced by plants have received more attention in stored product pest management. Plant products are less expensive than chemicals and usually can be easily processed and used by farmers and small-scale industries (Haque et al., 2000). Presently, there are few reports on insecticidal activities of *Delonix regia* (Bojer ex Hook.) Raf., *Senna tora* (L.) Roxb. and *Leucaena glauca* (Willd.) Benth. (Leguminosae) against stored product pests. Therefore, this study was conducted to assess insecticidal contact toxicity of *D. regia*, *S. tora* and *L. glauca* on *S. zeamais* adults.

## 2. Materials and Methods

### 2.1. Insect cultures

The stock colonies of *S. zeamais* were obtained from the Post-harvest Technology Research and Development Group, Department of Agriculture (DOA), Ministry of Agriculture and Cooperatives (MOAC), Thailand. Three hundred *S. zeamais* adults were cultured on 250 g brown rice at a moisture content of 12-13%. All weevils were removed after 1 day and culture media were incubated for 30-35 days in the laboratory at  $28\pm 2^\circ\text{C}$  and  $70\pm 5\%$  r.h. The adults used in the experiment were 7 days post emergence. Bioassays were conducted under the same environmental conditions as the culture.

### 2.2. Preparation of plant extracts

Dried seeds of *D. regia*, *S. tora* and *L. glauca* were grounded into powder by using a motor grinder. Dried seeds powder (2 kg) were put in the glass bottle and extracted with hexane for 2 days at room temperature. The hexane solutions were filtrated through a Whatman No.1 filter paper and concentrated by a rotary evaporator under reduced pressure to give crude hexane extract. The residual was extracted accordingly with dichloromethane and methanol to obtain crude dichloromethane and methanol extracts, respectively. The dried crude extracts were stored in the refrigerator at  $10\text{-}12^\circ\text{C}$  for further study.

### 2.3. Contact toxicity

Aliquot of 0.5  $\mu\text{L}$  of each dilutions (1, 3, 5, 7 and 9% or 5, 15, 25, 35 and 45  $\mu\text{g insect}^{-1}$ ) was applied on the thorax of *S. zeamais* adults using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd. England<sup>®</sup>). Controls were applied by using 95% ethanol and distilled water. After the solvent evaporate at room temperature, all treated insects were transferred to plastic cup (ten insects/plastic cup) (4.5 cm diameter and 2.7 cm height).

After that, the plastic lid with net cover was placed on the plastic cup. There were 10 replications for each treatment. Culture media was added to each treatment after 24 hours. Number of dead adults was recorded daily time until 7 days after treatment.

### 2.4. Statistical analysis

The percentage mortality data was subjected to analysis of variance (ANOVA) using SPSS software. Treatment means were compared by Duncan's Multiple Range Test at  $P = 0.05$ .

## 3. Results and Discussion

The contact toxicities of dried seed crude extracts of *D. regia* on *S. zeamais* adults were presented in Table 1. Crude hexane extract demonstrated moderate contact toxicity at the highest concentration. The contact toxicity tended to increase with increasing dosages. Among 5 tested concentrations, the 9% (v/v) concentration showed maximum mortality of 60.4% which differed significantly from those of lower concentrations at 4, 5, 6 and 7 days after treatment. Dichloromethane seed crude extract of *D. regia* demonstrated highest mortality (52%) at 9% (v/v) concentration which was significantly differed from other concentrations at 7 days after exposure. In addition, the crude methanol extract of *D. regia* induced low mortality of *S. zeamais* adults, where only 4.0% mortality was recorded at the 5 and 9% (w/v) concentrations at the end of experiment.

From the results, crude hexane and dichloromethane extracts showed potential contact toxicity against *S. zeamais* adults, but the adults are more susceptible to the crude hexane extract than crude dichloromethane extract (Table 2). This fact can be verified by  $\text{LD}_{50}$  and  $\text{LD}_{95}$  values where 42.377 and 64.500  $\mu\text{g insect}^{-1}$  were recorded for hexane crude extract and 45.206 and 80.371 were noticed for dichloromethane crude extract. Conversely, crude

extracts of *S. tora* and *L. glauca* seeds at all concentrations and exposure times induced low contact toxicities to *S. zeamais* adults (Tables 3 and 4).

From this study, hexane and dichloromethane seed crude extracts of *D. regia* at 9% concentration showed median efficacy to control *S. zeamais* adults via topical application method. The former study reported that crude hexane extract of some plant species such as *Dictamnus dasycarpus* Turcaz root barks, *Litsea cubeba* (Loureiro) Persoon fruits, *Narcissus tazetta* L. var. *chinensis* Roemer bulbs, *Sophora flavescens* Ait ion roots, *Stemona sessilifolia* (Miquel) Miquel root tubers, *Tripterygium wilfordii* Hook f. roots and *Torreya grandis* Fortune seeds could be used to control *S. zeamais* adults by topical application method (Liu et al., 2007). From their results and our result, it can be concluded that hexane, non-polar organic solvent, was acceptable to extract some plant species including *D. regia* seeds for controlling *S. zeamais* adults by topical application.

Hexane and dichloromethane crude extracts of *D. regia* seeds were oily in appearance but the methanol crude extract of this plant was solid. Plant oils were generally used for controlling insects due to their effect on different life stages of insects (Don-Pedro, 1989; Ahmed et al., 1999; Yalamanchilli and Punukollu, 2000; Adedire, 2003; Al-Moajel, 2006; Fekadu et al., 2012). This is also true of the results from this study. Oils of hexane and dichloromethane crude extracts of *D. regia* are toxic and able to kill *S. zeamais* adults. Hence, oils of *D. regia* from this study should contain some active chemical compounds that induced contact toxicity against *S. zeamais* adults. Oils of plant origin are extremely lipophilic and can penetrate the cuticle of insects (Don-Pedro, 1990). When insects contact oils, the spiracles of the insects are blocked, resulting in insect mortality by asphyxiation (Adedire and Ajayi, 2003).

In contrast, solid of methanol crude extract of *D. regia* seeds extracted with methanol, polar organic solvent, showed no toxicity to insects. Similar results of *D. regia* extracted with polar solvent had also been reported. For example, Ajayi (2013) described that ethanol extract of *D. regia* seeds caused low mortality of *S. zeamais* adults. However, *D. regia* flowers extracted with methanol was able to kill 3<sup>rd</sup> instar larvae of *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) (Deepa and Remadevi, 2011) and seeds of *D. regia* effectively killed 2<sup>nd</sup> instar larvae of *Anopheles gambiae* (L) (Aina et al., 2009). Hence, their results are quite difference from ours. Therefore, it can be inferred that an efficacy of *D. regia* depended on the solvents used for extraction, insect species, bioassay procedures and several factors under the laboratory conditions.

Crude extracts of *Delonix regia* extracted with other solvents or *D. regia* powder also showed insecticidal activities against some stored product insects. For example, *D. regia* seeds extracted with 0.1% of HCl containing 0.6 M of NaCl caused notable reduction in development and expanded mortality of cowpea weevil *Callosobruchus maculatus* and cotton boll weevil *Anthonomus grandis* (Alves et al., 2009). Mortality of *Trogoderma granarium* Everts larvae was also observed after application of *D. regia* powder (Al-Moajel, 2004).

Seed crude extracts of *S. tora* and *L. glauca* showed low contact toxicities on *S. zeamais* adults in this study. Consequently, more researches are needed to determine effect of these extracts on other activities such as repellent, fumigant and anti-feedant on *S. zeamais* or other stored product insects.

**Table 1** Contact toxicity of *Delonix regia* seed crude extracts applied topically to *Sitophilus zeamais*.

Solvent	Conc. (%)	Mortality (Mean%±SE) <sup>a</sup>						
		Day after treatment						
		1	2	3	4	5	6	7
n-Hexane	0	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c
	1	0.0±0.0b	1.3±1.3b	1.3±1.3b	1.3±1.3b	1.3±1.3b	1.3±1.3b	1.3±1.3c
	3	0.0±0.0b	0.0±0.0b	1.3±1.3b	1.3±1.3b	1.3±1.3b	3.3±3.3b	3.3±3.3bc
	5	0.0±0.0b	4.6±3.3b	4.6±3.3b	4.6±3.3b	4.6±3.3b	4.6±3.3b	4.6±3.3bc
	7	6.3±0.0b	6.3±0.0b	7.1±2.7b	12.5±4.2b	13.3±4.7b	22.9±7.8b	29.2±9.0b
	9	58.3±17.4a	58.3±17.4a	58.3±17.4a	60.4±16.3a	60.4±16.3a	60.4±16.3a	60.4±16.3a
	<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dichloromethane	0	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c
	1	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c
	3	1.3±1.3b	2.5±1.5bc	5.0±1.3bc	11.3±4.8bc	13.3±4.7b	13.3±4.7bc	15.4±5.5bc
	5	11.3±3.5a	11.3±3.5ab	11.3±3.5ab	11.3±3.5bc	18.8±3.9b	18.8±3.9b	21.0±5.3b
	7	4.6±3.3ab	9.2±4.6bc	9.2±4.6bc	18.8±6.1ab	18.8±6.1b	20.8±7.8b	23.0±7.8b
	9	7.9±3.8ab	18.8±3.9a	18.8±3.9a	29.2±5.1a	31.3±4.2a	45.8±9.0a	52.1±7.8a
	<i>P</i>	0.020	0.001	0.001	0.000	0.000	0.000	0.000
Methanol	0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a
	1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a
	3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	2.0±2.0a	4.0±2.5a
	7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a
	9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.0±2.0a	2.0±2.0a	4.0±2.5a
	<i>P</i>	-	-	-	-	0.439	0.561	0.096

<sup>a</sup>Values were based on 5 concentrations of content (1, 3, 5, 7, 9%), ten replicates of 10 insects in each replication. For each solvent, means in the same column followed by the same letters do not differ significantly ( $P > 0.05$ ) as determined by Duncan's Multiple Range Test.

**Table 2** Contact toxicity of n-hexane and dichloromethane crude extracts of *Delonix regia* dried seeds against *Sitophilus zeamais* at 7 days after treatment.

Concentration % ( $\mu\text{g insect}^{-1}$ )	Mortality (Mean% $\pm$ SE) <sup>a</sup>		<i>P</i>
	Hexane	Dichloromethane	
0 (control)	0.0 $\pm$ 0.0c	0.0 $\pm$ 0.0c	
1 (5)	1.3 $\pm$ 1.3cA	0.0 $\pm$ 0.0cA	0.347
3 (15)	3.3 $\pm$ 3.3cA	15.4 $\pm$ 5.5bcA	0.099
5 (25)	4.6 $\pm$ 3.3cB	21.0 $\pm$ 5.3bA	0.032
7 (35)	29.2 $\pm$ 9.0bA	23.0 $\pm$ 7.8bA	0.605
9 (45)	60.4 $\pm$ 16.3aA	52.1 $\pm$ 7.8aA	0.652
<i>P</i>	0.000	0.000	
LD <sub>50</sub>	42.377	45.206	
LD <sub>95</sub>	64.500	80.371	

<sup>a</sup>Values were based on 5 concentrations of content (1, 3, 5, 7, 9%), ten replicates of 10 insects. For each solvent, means in the same column and row followed by the same letters do not differ significantly ( $P > 0.05$ ) as determined by Duncan's Multiple Range Test. Small case letters compare means in column and capital letters compare means in row.

**Table 3** Contact toxicity of *Senna tora* seed crude extracts applied topically to *Sitophilus zeamais*.

Solvent	Conc. (%)	Mortality (Mean%±SE) <sup>a</sup>						
		Day after treatment						
		1	2	3	4	5	6	7
Hexane	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0a
	1	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0a
	3	0.0±0.0a	2.0±2.0a	2.0±2.0a	2.0±2.0ab	1.6±21.6ab	1.6±1.6ab	3.7±3.7a
	5	2.0±2.0a	2.0±2.0a	4.0±2.5a	4.0±2.5ab	4.9±2.0ab	9.0±4.1a	9.0±4.1a
	7	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	1.6±1.6ab	3.3±2.0ab	3.3±2.0a
	9	4.0±4.0a	4.0±4.0a	4.0±4.0a	8.0±3.7a	6.9±3.4a	9.0±5.2a	9.0±5.2a
<i>P</i>		0.552	0.687	0.442	0.053	0.073	0.116	0.225
Dichloromethane	0	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c
	1	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0c	0.0±0.0c	8.0±5.8bc
	3	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c
	5	0.0±0.0c	0.0±0.0c	0.0±0.0c	2.0±2.0b	6.0±2.5b	6.0±2.5b	8.0±2.0ab
	7	10.0±3.2ab	10.0±3.2b	10.0±3.2b	12.0±4.9a	12.0±4.9ab	14.0±6.8ab	14.0±6.8ab
	9	20.0±5.5a	20.0±5.5a	20.0±5.5a	20.0±5.5a	20.0±5.5a	20.0±5.5a	20.0±5.5a
<i>P</i>		0.000	0.000	0.000	0.000	0.000	0.000	0.001
Methanol	0	0.0±0.0a	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a
	1	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0a	0.0±0.0a
	3	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0a	0.0±0.0a
	5	2.0±2.0a	2.0±2.0a	2.0±2.0a	2.0±2.0a	4.0±2.5a	4.0±2.5a	4.0±2.5a
	7	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	2.0±2.0a	2.0±2.0a
	9	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0a	0.0±0.0a
<i>P</i>		0.439	0.439	0.43	0.439	0.047	0.178	0.178

<sup>a</sup>Values were based on 5 concentrations of content (1, 3, 5, 7, 9%), ten replicates of 10 insects in each replication. For each solvent, means in the same column followed by the same letters do not differ significantly ( $P > 0.05$ ) as determined by Duncan's Multiple Range Test.

**Table 4** Contact toxicity of *Leucaena glauca* seed crude extracts applied topically to *Sitophilus zeamais*.

Solvent	Conc. (%)	Mortality (Mean%±SE) <sup>a</sup>						
		Day after treatment						
		1	2	3	4	5	6	7
n-Hexane	0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	2.0±2.0a
	5	0.0±0.0	0.0±0.0	0.0±0.0	2.0±2.0a	2.0±2.0a	2.0±2.0a	2.0±2.0a
	7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
<i>P</i>	-	-	-	0.439	0.439	0.439	0.561	
Dichloromethane	0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	2.0±2.0a
	3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	4.0±2.5a
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	4.0±4.0a	4.0±2.0a
	7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	2.0±2.0a	2.0±2.0a	2.0±2.0a
	9	0.0±0.0	0.0±0.0	0.0±0.0	4.0±4.0a	4.0±4.0a	4.0±4.0a	4.0±4.0a
<i>P</i>	-	-	-	0.439	0.534	0.668	0.878	
Methanol	0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a
	1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a
	3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.0±2.0a	2.0±2.0a
	7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	6.0±6.0a
	9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a
<i>P</i>	-	-	-	-	-	0.439	0.509	

<sup>a</sup>Values were based on 5 concentrations of content (1, 3, 5, 7, 9%), ten replicates of 10 insects in each replication. For each solvent, means in the same column followed by the same letters do not differ significantly ( $P > 0.05$ ) as determined by Duncan's Multiple Range Test.

#### 4. Conclusions

The current study demonstrated the contact toxicity of dried seed crude extracts of three plant species against *S. zeamais* adults. The maximum contact toxicity was recorded on the highest dose of crude hexane extract. The crude hexane and dichloromethane extracts of *D. regia* at 9% (v/v) concentration had moderate effective contact toxicity to *S. zeamais* adults where both crude extracts at other concentrations (1, 3, 5, 7%) (v/v) showed less contact toxicity. On the other hand, all dried seed crude extracts of *S. tora* and *L. glaucae* demonstrated low mortality on *S. zeamais*. Hence, crude hexane and dichloromethane extracts of *D. regia* needed to be investigated in more detail in order to use as alternative to control *S. zeamais*.

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