

Journal of **Entomology**

ISSN 1812-5670



Journal of Entomology 9 (1): 13-22, 2012 ISSN 1812-5670 / DOI: 10.3923/je.2012.13.22 © 2012 Academic Journals Inc.

Evaluation of *Jatropha curcas* and *Annona muricata* Seed Crude Extracts Against *Sitophilus zeamais* Infesting Stored Rice

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ABSTRACT

A laboratory study to test the bioactivity of Jatropha curcas and Annona muricata seed crude extract against Sitophilus zeamais (Coleoptera: Curculionidae) was conducted by using dipping and surface protectant methods. Both J. curcas and A. muricata seed crude extract had contact and stomach poison against S. zeamais. By dipping method, the weevil mortality were 90 and 70%, respectively at concentration 20% (v/v), whilst by surface protectant method, the weevil mortality was 100% at 0.4% (v/w) concentration for both crude extracts. The J. curcas and A. muricata seed crude extracts applied on rice grain (surface protectant method) can reduced the F₁ progeny production, weight loss and rice grain damaged. This result suggests that the J. curcas and A. muricata seed crude extracts may have the potential to be used in controlling S. zeamais infesting stored rice grain.

Key words: Stored product pest, *Sitophilus zeamais, Jatropha curcas, Annona muricata*, rice grains

INTRODUCTION

The rice weevil, Sitophilus zeamais, is one of most destructive stored product pest world-wide. It is one of the major primary pests of cereal seeds. In Indonesia, rice grains are continuously stored in huge quantity for food security. It is infested by many insect pests, especially S. zeamais (Sidik and Pranata, 1988). At present, pest control measures for stored product insect pest rely heavily on the use of synthetic insecticide and fumigants (Bengs, 2005). However, over relying on pesticides may result in insecticide resistance development, contamination of toxic residues in stored product grain, hazards when handling the toxic compounds as well as polluting the environment (Yusof and Ho, 1992; Golob et al., 1999). Hence, there is a need to search the alternative method of control such as using botanical insecticides to substitute or supplement the synthetic chemicals. The effectiveness of many botanicals compound against stored grain insects has already been demonstrated (El-Nadi et al., 2001; Koona and Njoya, 2004; Roy et al., 2005).

The plant of *Jatropha curcas* is well distributed in tropical and subtropical areas. It is planted as 'living fences' around fields and settlements in North Sumatra, Indonesia. It can easily be grown on almost any soil and therefore prevent erosion. Most part of the plant and their extracts are used

in traditional medicine, e.g., for their antimicrobial properties (Thomas, 1989). It has been reported to have the molluscicidal (Liu et al., 1997) and insecticidal activities (Li et al., 2004). The seed kernel contain up to 60% oil. In the last few years the potential of J. curcas seed for the production of bio-fuels have been actually researched (Gubitz et al., 1999). Jatropha curcas is easily available and used for various purposes in many tropical areas (Abulude et al., 2007) and as such exploitation of its insecticidal properties appears to be highly viable (Kumar and Sharma, 2008). This plant could be able to survive more than 20 years after sowing or planting (Ratree, 2004).

The plant of Annona muricata are abundant as fruits tress in North Sumatra, Indonesia. The annonaceous acetogenins extracted from tree leaves, bark and seed of annonaceae tree have been reported to possess pesticidal and/or insect antifeedant properties (Leatemia and Isman, 2004a). These trees are sources of fresh fruits and/or fruits juices and that could generate of wasted seeds. These waste products might potentially be developed into simple and locally available botanical insecticides.

The present study was carried out to evaluate the bioactivity of *J. curcas* and *A. muricata* seed crude extracts on the *S. zeamais*.

MATERIALS AND METHODS

Culture of Sitophilus zeamais: The initial population of S. zeamais was obtained from rice grain in the open market. The insect culture was maintained on rice grain as growth medium throughout the study period (Coombs and Porter, 1986). The rice grain and all apparatus or equipments were sterilized by heating at 60°C for 1 h to protect stock culture from its natural enemies (insect, mites and pathogen). The S. zeamais was cultured for 6th generations before used in the experiment to avoid external factors and to acclimatize the stock with the laboratory condition. A total of 50 adults were fed on 150 g rice grain filled up in a transparent plastic cup (7 cm high, 9.5 top and 8.5 cm bottom diameter, respectively) for 1 week. They were then removed and the rice grains were incubated at 29±2°C and 80±10% R.H. until adult emergence (ca. 4 weeks).

Source of plant materials: The *J. curcas* and *A. muricata* seeds were collected from around Medan City and Kabupaten Deli Serdang, Indonesia in May 2006. The seeds collected from mature fruit were hulled to get the kernel and ground with electric grinder to obtain the powder for extraction process.

Extraction of plant materials: Extraction was done using 'Soxhlet Extractor' (Pandey *et al.*, 1986). To do this, 50 g of the powdered material was placed in a paper thimble and then put into the extractor after which 200 mL acetone was poured into the receiving flask. The process of extraction took about 10 h. Crude extract was obtained after complete removal of the solvents with vacuum evaporation at <40°C for 2 h.

Dipping method: Direct toxicity by dipping method was conducted following Rahman and Talukder (2006) with some modification.

Effect on *S. zeamais* mortality: The crude extracts of *A. muricata* and *J. curcas* were diluted with acetone (60%) to make 0.5, 1, 2.5, 5, 10 and 20% (v/v) concentrations (treatments) of crude extract after which 1% Teepol was added to yield a homogenous solution. Twenty 4-5 days old *S. zeamais* adult were placed into a cylinder screen cage (3 cm height and 3 cm in diameter) then

dipped in a respective diluted extract solution or control solution for 10 sec. The insects were then removed, air-dried and released into a plastic cup (11 cm height and 6 cm in diameter) containing 20 g of rice grain, covered with a piece of muslin cloth held by rubber band to prevent adults from escaping. Mortality was recorded everyday for one week. Each treatment was replicated five times.

Effect on *S. zeamais* progeny, weight loss and rice grain damage: The crude extract concentrations (treatments) of 0.05, 0.1, 0.2, 0.4 and 0.8% (v/v) and five pairs weevil were used. The same procedure of the first study was followed in this study but the weevils were removed after 5 days in plastic cup and rice grain was held until progeny (adult) emergence. Number of adults emerge was counted daily. All emerging adults were removed from the cup daily to avoid further mating and oviposition. The weight loss and the number of seed rice damaged were taken if no more adult emerged. The experiments were conducted at 29±2°C and 80±10% R.H. arranged following a Complete Randomized Design (CRD).

Surface protectant method: In this study, the application of crude extract on rice as grain protectant was tested following (Lale and Abdulrahman, 1999) with some modifications.

Effect on *S. zeamais* mortality: Five crude extract concentrations, i.e., 0.025, 0.05, 0.1, 0.2 and 0.4% (v/w) (treatments) were employed. Each concentration was diluted in 2 mL acetone (to ensure application to all exposed surfaces of the rice grain) and poured into the flask (1000 mL) containing 100 g rice grain. The flask was shaken manually until the rice grains were uniformly coated. The coated rice grains were then taken out and air-dried for 30 min to evaporate the acetone. A total of 20 g rice grains were put into each plastic cup (replicate). Rice grains treated with only acetone were used as control. Twenty 4-5 days old adults were released in each cup and covered with a piece of muslin cloth held by rubber band to prevent adults from escaping. Each treatment was replicated five times. Mortality was recorded daily at 1000 h until 21 days after treatment.

Effect on *S. zeamais* progeny, weight loss and rice grain damaged: The crude extract concentrations (treatments) of 0.0025, 0.005 and 0.01% (v/w) were employed. The experiment was set up as above except only a total of 5 males and 5 females of weevil per treatment were used and they were removed after 5 days in the plastic cup. Experiment was arranged following Complete Randomized Design (CRD).

Both dipping and surface protectant methods, the data (mortality, progeny, weight loss and rice grain damage) were analyzed by 2-way ANOVA (crude extracts and concentrations as two independent variables). When ANOVA was significant means were separated using LSD Test at p = 0.05. All analyses were run on the MINITAB (Release 14) Statistical Software (Minitab Inc, 2003).

RESULTS

Dipping method: There was a significant interaction between type of crude extracts and it concentrations in influencing the percentage mortality of S. zeamais (F = 4.48, df = 6,56, p<0.05). The S. zeamais adult mortality was difference significantly among type of extracts (F = 22.11, df = 1,56, p<0.05) and concentrations (F = 499.12, df = 6,56, p<0.05) (Table 1). Sitophilus zeamais

Table 1: Two-way ANOVA statistics for effect of crude extract on S. zeamais adult mortality, progeny production, weight loss and rice grain damage by dipping method

Factor (Dependent variables)	Source	$\mathrm{d}\mathrm{f}$	Sum of square	F-value	p-value
Mortality	Extract	1	363.5	22.11	< 0.05
	Conc.	6	49239.5	499.12	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	6	441.8	4.48	< 0.05
	Error	56	770.6		
Progeny production	Extract	1	0.039015	7.19	< 0.05
	Conc.	5	0.368895	13.60	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	5	0.22055	0.81	>0.05
	Error	48	0.56402		
Weight loss	Extract	1	0.20300	4.74	< 0.05
	Conc.	5	1.77161	8.28	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	5	0.04947	0.23	>0.05
	Error	48	10.7255		
Rice grain damage	Extract	1	0.25873	5.36	< 0.05
	Conc.	5	3.15558	13.07	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	5	0.08227	0.34	>0.05
	Error	48	2.31728		

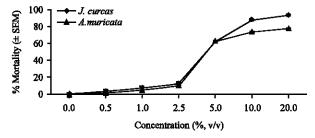


Fig. 1: Percent mortality of S. zeamais treated with different concentrations of J. curcas and A. muricata seed crude extracts using dipping method

adult mortality was not different significantly (p>0.05) when treated with low concentration (0.5-5%) of both extracts. However, at 10 and 20% concentrations, the *J. curcas* crude extract was significantly (p<0.05) more toxic than *A. muricata* to *S. zeamais* (Fig. 1).

There was no significant interaction between types of crude extract and it concentration in influencing F_1 progeny production of S. zeamais, weight loss and rice grain damaged (F = 0.81, df = 5,48, p > 0.05; F = 0.23, df = 5,48, p > 0.05 and F = 0.34, df = 5,48, p > 0.05). However, the types of crude extracts and concentrations alone had significant effect in influencing F_1 progeny production of S. zeamais, weight loss and rice grain damaged (F = 7.19, df = 1,48, p < 0.05 and F = 4.74, df = 1,48, p < 0.05; F = 5.36, df = 1,48, p < 0.05 for crude extracts; F = 13.6, df = 5,48, p < 0.05; F = 8.28, df = 5,48, p < 0.05 and df = 5,48, df = 5,

Table 2: Two-way ANOVA statistics for effect of crude extract on *S. zeamais* adult mortality, progeny production, weight loss and rice grain damage by surface protectant method

Factor (Dependent variables)	Source	$\mathrm{d}\mathrm{f}$	Sum of square	F-value	p-value
Mortality	Extract	1	12725.1	455.21	< 0.05
	Conc.	5	51817.5	370.73	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	5	7372.8	52.75	< 0.05
	Error	48	1341.8		
Progeny production	Extract	1	0.059290	12.92	< 0.05
	Conc.	3	0.045130	3.28	< 0.05
	$Extract \times Conc.$	3	0.017490	1.27	>0.05
	Error	32	0.146800		
Weight loss	Extract	1	0.20164	8.46	< 0.05
	Conc.	3	0.50973	7.13	< 0.05
	$Extract \times Conc.$	3	0.10454	1.46	>0.05
	Error	32	0.76260		
Rice grain damage	Extract	1	0.82369	13.54	< 0.05
	Conc.	3	0.75403	4.13	< 0.05
	${\bf Extract}{\bf \times}{\bf Conc.}$	3	0.09019	0.49	>0.05
	Error	32	1.94604		

Table 3: F1 progeny production of S. zeamais, percent weight loss and percent of rice grain damage as affected by various concentrations of seed crude extracts of J. curcas and A. muricata by dipping method

		Conc. (%, v/v)	-						
Dependent									
variable (SEM)	Extract	0	0.05	0.1	0.2	0.4	0.8		
Progeny	J. c	90.20±4.64ª	84.60±8.34ª	80.00±5.84ª	78.80±5.31ª	77.80±6.06ª	45.80±3.11ª		
	A.m	91.80±7.94ª	90.80±5.81ª	89.80±5.34ª	88.40±4.92ª	83.00±6.15ª	62.20 ± 6.27^{b}		
Weight loss	J. c	8.96±0.42ª	8.72±0.49ª	8.39±0.84 ^a	8.08±0.73ª	7.63 ± 0.52^{a}	5.95±0.33ª		
	A.m	9.82±0.59ª	9.63±0.75ª	8.86 ± 0.20^{a}	8.32±0.65ª	7.94 ± 0.22^{a}	7.02 ± 0.15^{b}		
Rice damage	J. c	16.94±0.46ª	16.57±0.92ª	14.97 ± 0.72^{a}	14.67±0.90ª	14.09±1.11ª	11.43±0.48ª		
	A.m	18.04±0.68ª	17.75±0.57ª	16.38 ± 0.78^{a}	14.93±0.78ª	14.47 ± 0.66^a	13.17 ± 0.53^{b}		

Means in a column for each dependent variable followed by different letters are significantly different at p<0.05 by Least Significant Difference. J.c: Jatropha curcas, A.m: Annona muricata

Surface protectant method: There was a significant interaction (F = 52.75, df = 5,48, p<0.05) between crude extracts and their concentrations in influencing S. zeamais mortality. Percent mortality was also significantly affected by the concentrations (F = 370.07, df = 5,48, p<0.05) and type of crude extracts (F = 455.21, df = 1,48, p<0.05) alone (Table 2). There was no significant difference on weevil mortality at control. At low concentration (0.025-0.2%), the crude extract of J. curcas caused significantly (p<0.05) higher mortality to S. zeamais than by the crude extract of A. muricata. It was 100% weevil mortality achieved when rice grain treated at 0.1% concentration of J. curcas seed crude extract. However, the weevil mortality increased when rice grain treated with 0.1% or more of A. muricata seed crude extract. There was no significant difference in mortality observed at 0.4% concentration of both extracts (Fig. 2).

There was no interaction effect between types of crude extract and their concentrations on F_1 progeny production of S. zeamais, weight loss and rice grain damaged (F = 1.27, df = 3,32, p>0.05; F = 1.46, df = 3,32, p>0.05 and F = 0.49, df = 3,32, p>0.05). However, the type of crude extract alone had significant effect on progeny production, weight loss and rice grain damaged (F = 12.92,

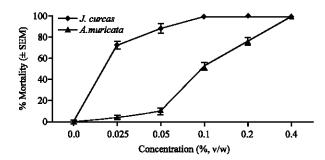


Fig. 2: Percent mortality of *S. zeamais* treated with different concentrations of *J. curcas* and *A. muricata* crude seed extracts on rice grain using by surface protectant method

Table 4: F1 progeny production of *S. zeamais*, percent weight loss and percent of rice grain damage as affected by various concentrations of crude extracts of *J. curcas* and *A. muricata* by surface protectant method

		Conc. (%, v/w)						
Dependent								
variable (SEM)	Extract	0	0.0025	0.005	0.01			
Progeny	J. c	64.60±4.84ª	53.60±4.57ª	51.80±3.81ª	48.00±2.98ª			
	A.m	65.60±1.63ª	65.20±4.46 ^b	61.60 ± 4.15^{b}	61.00 ± 2.34^{b}			
Weight loss	J. c	8.33±0.34ª	6.94 ± 0.25^{a}	6.61 ± 0.19^{a}	6.27±0.29ª			
	A.m	8.19±0.28ª	7.63±0.36 ^b	7.43 ± 0.33^{b}	7.26 ± 0.28^{b}			
Rice damage	J. c	14.33±0.89ª	12.80±0.59ª	12.23 ± 1.17^{a}	10.82±0.51ª			
	A.m	15.32±0.80a	15.03±0.90 ^b	$12.23 \pm 1.17^{\mathrm{b}}$	13.28 ± 0.50^{b}			

Means in a column for each dependent variable followed by different letters are significantly different at p<0.05 by Least Significant Difference. J.c: $Jatropha\ curcas$, A.m: $Annona\ muricata$

df = 1,32, p<0.05; F = 8.46, df = 1,32, p<0.05 and F = 13.54, df = 1,32, p<0.05). Similarly, the concentration also had significantly affected the progeny production, weight loss and rice grain damaged (F = 3.28, df = 3,32, p<0.05; F = 7.13, df = 3,32, p<0.05 and F = 4.13, df = 3,32, p<0.05) (Table 2).

The effect by both extracts on progeny produced, weight loss and rice grain damaged seemed to be decreasing with increasing in concentration. At the highest concentration tested (0.01%), *J. curcas* seed crude extract caused significantly (p<0.05) less progeny produced, weight loss and rice grain damaged (48, 6.27 and 10.82%, respectively) than *A. muricata* seed crude extract (61, 7.26 and 13.28%, respectively) (Table 4).

DISCUSSION

The mortality effect of *J. curcas* and *A. muricata* seed crude extracts on *S. zeamais* for both dipping and surface protectant methods are showed in Fig. 1 and 2, respectively. Generally, percent mortality increases with the increasing concentration of both plant's crude extracts.

By dipping method, both crude extracts did not cause a significant difference on *S. zeamais* mortality at low concentrations (0.5-5%) but at high concentrations (10-20%), the *J. curcas* extract was significantly more toxic (higher mortality) to *S. zeamais* than by the *A. muricata* extract. The *S. zeamais* mortality reached 90% when insects were exposed to >10% concentration of *J. curcas* while at the same concentration the *A. muricata* could only cause ca. 70% mortality (Fig. 1). Nevertheless, result of this study indicates that both *J. curcas* and *A. muricata* crude extracts had

contact effect (poison) against *S. zeamais* adults. This is supported by Li *et al.* (2004), who reported that the seed oil of *J. curcas* had strong contact toxicity against mustard aphid, *Lipaphis erysimi*. In another study, Sinchaisri *et al.* (1991) found *A. muricata* crude seed extract exhibited 100% mortality on Diamondback Moth, *Plutella xylsotlla* L., larva when cabbage leaf was coated with the extract at 20 mg mL⁻¹ concentration where the larvae died with no eating lesions on the cabbage leaf.

In surface protectant method, the *J. curcas* seed crude extract inflicted a significantly higher mortality on *S. zeamais* than that of *A. muricata* irrespective of concentrations (Fig. 2). This result suggests that both type of seed extracts had both contact and stomach poison but the *J. curcas* crude extract had a stronger stomach poison than *A. muricata* against *S. zeamais. Jatropha curcas* probably contains some chemical components that contributed to higher toxicity effect on *S. zeamais* which that *A. muricata* does not have. Adolf *et al.* (1984) stated that the kernels of *J. curcas* contain between 0.03 and 3.4% of phorbol esters and that experiment with phorbol ester isolated from its oil indicated that the derivative of 12-deoxy-16-hydroxyphorbol was being one of the toxic principles.

The A. muricata crude extract only exhibited the same effect with the J. curcas only when applied to the rice grain at 0.4% concentration (Fig. 2). This shows that the stomach poison effect of A. muricata crude extract was lower than that of J. curcas, as high concentration of A. muricata crude extract needed to get similar effect as for J. curcas (Fig. 2). Annonaceous species was reported to contain the 'Annonaceous acetogenins', a class of natural compound with a wide varieties of biological activities (Dos Santos and Sant'Ana, 2001; Isman, 2006). Maryam et al. (2004) reported that the leaf of Jasminum sp., sprayed with A. muricata seed crude extract and fed on the larvae of Palpita unionalis, a pest on Jasminum sp., resulted in larval mortality up to 95.6%. Therefore, the mortality of S. zeamais in our study could be due to the acetogenins action as contact and stomach poison but the toxic effect of Annonaceous acetogenins could be less than that of 12-deoxy-16-hydroxy phorbol. Alali et al. (1998) reported that acetogenins from the plant family Annonaceae (bark of Goniothalamus giganteus, leaves of Rollina mucosa, seeds of Asimia triloba) caused high percentages of mortality and delayed development of the 5th instars of German cockroach, Blattella germanica when used in dietary toxicities. Leatemia and Isman (2004b) reported that the crude seed extract of A. squamosa (Annonaceae) have both toxic and antifeedant properties against P. xylostella larva as high concentration of extracts caused high mortality on larva even though only very small portions of the treated leaf were consumed. Acetogenins which are slow-acting stomach poisons, identical to the action of rotenone (Seffrin et al., 2010) could also contribute to lower percent mortality compared to J. curcas.

Result verified that types of crude extract and their concentrations significantly affect the number of progeny produced, weight loss and rice grain damaged by *S. zeamais* by dipping method. Significantly (p<0.05) lower numbers of progeny produced, total weight loss and percentage of rice grain damaged when *S. zeamais* treated with *J. curcas* seed crude extract at 0.8% (v/v) concentration than when they were treated with *A. muricata*. However, there was no significant difference (p>0.05) on progeny production, weight loss and rice grain damage when treated with lower concentration (0.05 to 0.4%) of both types *J. curcas* and *A. muricata* crude extracts (Table 3).

By surface protectant method, the types of crude extracts significantly (p<0.05) affected the number of progeny produced, weight loss and rice grain damaged (Table 4). The bioactive components of *J. curcas* and *A. muricata* may have affected the ability of *S. zeamais* to produce

offspring. This is because it protected and prevented *S. zeamais* from oviposition and possibly adult emergence. Adebowale and Adedire (2006) reported that the cowpea seed when treated with *J. curcas* seed oil reduced the number egg laid by *Callosobruchus maculatus* and prevented adult emergence at concentration between 0.5 and 2% (v/w). Jadhav and Jadhav (1984) also reported that at 0.2% (v/w) concentration *J. curcas* seed oil had significantly reduced the numbers of eggs laid by adult *C. maculatus* and prevented egg hatch 33 days after treatment.

CONCLUSION

The present study showed that crude seed extract of *J. curcas* and *A. muricata* are the promising candidate as botanical insecticide for controlling *S. zeamais*. This is especially true as the plants are locally available and method for preparation of the extracts is simple which can be recommended for use in the management of weevil in stored grains especially in the 3rd world country. Again, further test on residual contact effect on rice bag with crude extract are desirable as the rice grain in countries like Indonesia is stored and packaged in bag. Moreover, this study showed that crude extract of *J. curcas* seed exhibited strong contact effect against *S. zeamais*.

ACKNOWLEDGMENTS

Author would like to thank the Laboratory of Entomology, Department of Agroecotechnology, Faculty of Agriculture, Islamic University of North Sumatra for the facilities provided. This study was supported by Islamic University of North Sumatra, Indonesia.

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