

EFFECT OF SELECTED PLANT EXTRACT ON MORTALITY OF ADULT *Sitophilus zeamais* MOTSCHULSKY (COLEOPTERA: CURCULIONIDAE), A PEST OF STORED RICE GRAINS

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ABSTRACT

A preliminary study was conducted to test 16 crude extracts of selected plants on the mortality of *Sitophilus zeamais* adult. The bioassay was carried out by mixing crude extracts with rice grain at 0.5 and 0.25% concentrations by weight. It was found that crude extracts of *Jatropha curcas* (seed) and *Annona muricata* (seed) caused 100% mortality on weevil at 0.5% concentrations while at 0.25% they caused 96.7 and 98.3% mortality respectively. Crude extract of *J. curcas* (leaf) and *Azadirachta indica* (seed) at 0.5% caused 81.7 and 51.7% mortality respectively, while at 0.25% the mortality was 55% and 25%, respectively. Crude extracts of *J. curcas* seed and leaves, *A. muricata* (seed) and *A. indica* (seed) seem to have potential as botanical insecticides but further studies need to be conducted against *S. zeamais*.

ABSTRAK

Satu kajian awal untuk menguji kesan 16 ekstrak kerd tumbuhan terpilih terhadap kematian *S. zeamais* dewasa telah dijalankan. Bioassey dilakukan dengan mencampurkan ekstrak kerd dengan beras pada kepekatan ekstrak kerd 0.5 dan 0.25% (mengikut berat). Didapati ekstrak *Jatropha curcas* (biji) dan *Annona muricata* (biji) telah menyebabkan 100% kematian kumbang pada kepekatan 0.25%, sementara pada kepekatan 0.25% kematian adalah masing-masingnya 96.7 dan 98.3%. Pada kepekatan 0.5% ekstrak kerd *J. curcas* (daun) dan *Azadirachta indica* (biji) kematian kumbang masing-masingnya adalah 81.7 dan 51.7, sementara kepekatan ekstrak 0.25% telah menyebabkan kematian kumbang masing-masingnya 55% dan 25%. Kajian ini menunjukkan bahawa biji dan daun *J. curcas*, biji *A. muricata*, dan biji *A. indica* berpotensi di bangunkan sebagai racun serangga botanical, namun kajian selanjutnya perlu dilakukan.

Key word: *Sitophilus zeamais*, rice grain, stored product pest, Coleoptera, Curculionidae

INTRODUCTION

Rice grains in huge quantity are continuously stored in Indonesia for food security. They are infested by many stored pests especially the *Sitophilus zeamais* (Motschulsky) (Sidik and Pranata, 1988). Hitherto, the warehouses are constantly fumigated with fenitrothion or with aluminium phosphide tablet if *S. zeamais*

population is too high (B. Pinem BULOG SUMUT, Medan, Pers. Comm, 2004). However, over relying on pesticides may result in insecticide resistance development, and hazards when handling the toxic compounds (Golob *et al.*, 1982). Hence, there is a need to search of botanical insecticides to substitute or supplement the synthetic chemical.

The effectiveness of botanical insecticides like *Azadirachta indica* has been demonstrated in many studies (Malik and Nafqi, 1984; Rahman

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and Schmidt, 1999; Prakash and Rao, 1997; Tripathi *et al.*, 2000; Haque *et al.*, 2000; Adebowale and Adedire, 2006). This paper present the results of effect of plant extracts on the mortality of *S. zeamais*. This is a preliminary study of series of experiments, which will be conducted to find out the suitable botanical or insecticides for controlling stored product pests such as *S. zeamais*.

MATERIALS AND METHODS

Stock Culture of *S. zeamais*. The initial population of *S. zeamais* was obtained from rice grain in the market. The culture was maintained in rice grain as growth medium through out the study (Cooms and Porter, 1986). The rice grain and all apparatus equipments were sterilized by heating to 60°C for 1 hour to protect stock culture from natural enemies (insect, mites and pathogens). The *S. zeamais* was cultured for six generations prior to the experiment. A total of 50 adults (mated male and female) were placed in 150 g rice grain in a transparent plastic cup (as egg oviposition arena) of 9.5 and 8.5 cm top and bottom diameter, respectively, and 7 cm high for 1 week. They were then removed and rice grains were incubated under laboratory environment (29 ± 2°C and 90 ± 5% RH) until adult emergence (ca. 4 weeks). Adults were temporary reared in a plastic cup under laboratory condition as mentioned above before being used in next experiment.

Collection of Plant Materials. Twelve plants species were collected from around Medan City and Kabupaten Deli Serdang – Indonesia during the months of November to December 2004. Those plants were *Azadirachta indica* (Meliaceae), *Annona muricata* (Annonaceae), *Jatropha curcas* (Euphorbiaceae), *Lantana camara* (Verbanaceae), *Ageratum conizoydes* (Asteraceae), *Chromolaena odorata* (Asteraceae), *Alpinia galanga* (Zingiberaceae), *Zingiber officinale* (Zingiberaceae), *Centella asiatica* (Apiaceae), *Andrographis paniculata* (Acanthaceae), *Tamarindus indica* (Caesalpiniaceae), *Eugenia polyantha* (Myrtaceae). The plants were washed thoroughly, air dried under shade, ground using electric grinding machine and finally passed through a 30 mesh sieve to obtain the powder.

Extraction of Plant Materials. Extraction was done using 'Soxhlet Extractor' (Pandey *et al.*, 1986) where 50 g of the powdered material was placed in a paper thimble and then placed in the extractor. Then 200 ml acetone was poured in to the receiving flask. The process of extraction took about 10 hours. Crude extract was obtained after

complete removal of the solvents with vacuum evaporation at temperature <40°C until it becomes a semi solid material. The extract was weighted and diluted with fresh acetone.

Testing Method. Method used was adopted from Talukder and Howse (1994) with some modification. Each crude extract were separately mixed with rice grain at 0.5 and 0.25% (300 and 150 mg of crude extract per 60 g of rice grain respectively). Sixty gram of rice grain was then placed into a 250 ml flask. The crude extract was dissolved in 1.2 ml acetone and then poured into the flask containing rice grain and shook thoroughly for 40 seconds to ensure uniform distribution over grain surface. Twenty grams of the treated rice grain was placed into a plastic cup (11 cm height, and 6 cm diameter), then 20 weevils were placed into the plastic cup and covered with a piece of muslin cloth held by rubber band to prevent escapes. The rice grain treated with acetone was used as control. The experiment was arranged following a complete randomized design by having 16 crude plant extracts (treatments) and replicated three times. The experiment was conducted under laboratory environment as mentioned above. Mortality of insects was recorded everyday for 15 days after treatment. The percentage of insect mortality in each replicate was corrected using Abbott's formula to obtain the actual percentage of insect mortality in the treatments (Busvine, 1971) before analyzed with one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to separate the means of the treatments.

RESULTS AND DISCUSSION

There was a significant difference in the percentage of mortality of *S. zeamais* among plant extracts at both crude extract concentrations (concentration 0.5%; F = 86.1 df = 15 & 32, P < 0.05; concentration 0.25% F = 54.4, df = 15 & 32, P < 0.05) (Table 1). In all treatments, mortality was relatively higher in higher extract concentration (0.5%) than lower extract concentration (0.25%). Percent mortality was significantly (P < 0.05) higher (100%) in seed extract of *A. muricata* and *J. curcas* at 0.5% concentration while 98.3% and 96.7% mortality, respectively, were observed at 0.25% concentration. All other seed extracts caused less than 50% mortality at 0.5% concentration except for *A. muricata*, *J. curcas* and *A. indica*. Although no mortality was observed when extract of *Ageratum conizoydes* (flower) and *Azadirachta indica* (leaf) at 0.25% concentration were used, the mortality was still very low at 0.5%.

Table 1. Mean percent mortality of *Sitophilus zeamais* fed on rice grain treated with crude extracts of 16 plant species

Crude Plant Extract	Family	Mortality (%) \pm S.E	
		0.5 %	0.25 %
<i>Ageratum conizoides</i> (leaf)	Asteraceae	38.33 \pm 8.49 cd	8.33 \pm 11.78 de
<i>Ageratum conizoides</i> (flower)	Asteraceae	3.33 \pm 2.35 fg	0 f
<i>Alpinia galanga</i> (rhizome)	Zingiberaceae	25.86 \pm 2.35 d	12.07 \pm 7.07 d
<i>Andrographis paniculata</i> (leaf)	Acanthaceae	12.07 \pm 4.08 e	10.34 \pm 2.35 d
<i>Anona muricata</i> (seed)	Annonaceae	100 a	98.33 \pm 2.35 a
<i>Azadirachta indica</i> (leaf)	Meliaceae	3.45 \pm 4.71f	0 f
<i>Azadirachta indica</i> (seed)	Meliaceae	51.67 \pm 10.27 c	25 \pm 4.08 c
<i>Centella asiatica</i> (leaf)	Apiaceae	10.34 \pm 2.35 ef	1.72 \pm 4.08 ef
<i>Chromolaena odorata</i> (leaf)	Asteraceae	3.33 \pm 2.35 fg	1.67 \pm 2.35 ef
<i>Eugenia polyantha</i> (leaf)	Myrtaceae	0 g	0 f
<i>Jatropha curcas</i> (leaf)	Euphorbiaceae	81.67 \pm 6.23 b	55 \pm 8.16 b
<i>Jatropha curcas</i> (seed)	Euphorbiaceae	100 a	96.67 \pm 2.88 a
<i>Lantana camara</i> (leaf)	Verbanaceae	0 g	0 f
<i>Lantana camara</i> (flower)	Verbanaceae	13.8 \pm 4.71 e	6.9 \pm 1.0 de
<i>Tamarindus indica</i> (leaf)	Caesalpiniaceae	8.63 \pm 2.35 ef	3.45 \pm 4.71 ef
<i>Zingiber officinale</i> (rhizome)	Zingiberaceae	6.67 \pm 4.71 ef	1.67 \pm 2.35 ef

Within a column, means with the same letter are not significantly different ($P < 0.05$, DMRT).

No mortality was observed when *E. polyantha* and *L. camara* leaves was used as treatment at both concentrations (Table 1).

The effectiveness of *A. indica* against storage pest has been reported by many authors (Jotwani and Sircar, 1965 in Jotwani and Srivastava 1983; Pandey *et al.*, 1986; Makanjuola, 1989; Jilani and Saxena, 1988 in Saxena, 1995). In the present study, seed extract of *A. indica* gave better mortality effects than leaf extract (Table 1). These results are in agreement with those of other. Jilani and Malik (1973, in Makanjuola, 1989) found that extract of neem seeds exhibited maximum repellency when compared with the leaves and flowers extracts. In another study, Makanjuola (1989) reported that neem seed extract was clearly more effective in suppressing the population of *S. zeamais* than the leaf extracts. It would appear that the effect of the neem depends on the part of the plant used. The differential effects may be due to the presence of higher proportion of active chemical components in the seeds than the leaf.

Although the effectiveness of neem tree has been reported by many authors, in the present study, the crude seed extract of *A. indica* caused lower mortality on *S. zeamais* than crude seed extract of *A. muricata* and *J. curcas* (Table 1). The differential effects may be due to the maturity status of seeds. The seeds *A. indica* used were not yet fully matured (collected from a green-yellow fruit that manually detached from the tree) as for the seeds of *A. muricata* and *J. curcas* (collected from the matured fruit). Mane (1968, in Jotwani and Srivastava, 1983) reported that the suspension produce from fully mature fruits of neem was

found to be superior to that of yellow or green fruits (less matured fruits). They also reported that the kernel of the fallen and matured fruits of the neem tree should be taken to get superior effect against larvae *Euproctis lunata* (Walker).

There has been no study on effectiveness of *J. curcas* against *Sitophilus* spp. However, *J. curcas* oil was reported to give significant protection on cowpea seeds from *Callosobruchus maculatus* (Adebowale and Adedire, 2006). In the Phillipines, Morales (In ILEIA newsletter, 1995) found that *J. curcas* caused abnormalities to bollworm and flowerweevil. Shelke *et al.* 1987, in Prakash and Rao, 1997) reported that seed extract of *J. curcas* reduced oviposition of potato tuber moth, *Pthorimaea operculella* by 32%. In contrast to the results obtained in the present study, Jadhav and Jadhav (1984) (in Golob *et al.* 1999) stated that 0.2% *J. curcas* oil admixed with stored grain did not cause adult mortality but reduced the number of eggs laid by adult *C. maculatus* and prevented egg hatch 33 days after treatment. Duke (1985); Adolf *et al.* (1984) in Golob *et al.* (1999) reported that the seed oil of *J. curcas* contains a diterpenoid (12-deoxy-16-hydroxyphorbol). According to Adebowale and Adedire (2006) the insecticidal activity of this seed oil could be due to the presence of several sterols and terpen alcohols which have been known to exhibit insecticidal properties. Result of this study also showed that crude extract of *J. curcas* seed caused high mortality on *S. zeamais* at both 0.25 and 0.5% concentrations. The *Jatropha gossypifolia*, which is also Euphorbiaceae, was reported to cause 100% mortality on adult *Tribolium*

castaneum at 24 hours after treatment (Chatterjee *et al.*, 1980 in Dev and Koul, 1997).

Percent mortality of *S. zeamais* was significantly ($P < 0.05$) higher when seed extract of *A. muricata* were used at both 0.5% and 0.25% concentrations (mortality was 100% and 98.33%, respectively) compared to other plants extract except extract of *J. curcas* (Table 1). Some study on the insecticidal effects of Annonaceae group reported that some plants extract have significant effects to the insect pest survival, for instance *Callosobruchus chinensis* (Linnaeus) (Priyono, 1999), the stored product pests of pulses. In addition, the aqueous seed extract or seed powder of some of the Annonaceae group was reported to be injurious to the pea aphid, *Acyrtosiphum pisum* (Harris) and the southern armyworm *Spodoptera eridania* (Cramer) (Jacobson, 1958 in Prakash and Rao, 1997). Extracts of *A. muricata* exhibited bioactivity to *Spodoptera litura* (Fabricius) and showed larval growth declined from 18% to 96% compared to control (Leatemia and Isman, 2004). This could be due to starvation resulting from antifeedant effect of the extract, as the weevils find it difficult to feed on the treated rice grain treated with extract of *A. muricata*. Kusharyanto *et al.* (2004) reported that acetogenin substances in *A. muricata* seed acts as antifeedant to the insect caused mortality of the insect.

Crude extracts of *A. conyzoides* leaf and *A. galanga* rhizome cause moderate toxicity to *S. zeamais* (Table 1). *A. conyzoides* possesses bioactive compounds, the terpenics, mainly precocenes with antijvenile hormonal activity that may affect the growth and development of the insect rather than the direct cause to insect mortality. Vyas and Mulchandani (1980 in Ming, 1999) reported the action of cromenes (precocenes I and II) isolated from *Ageratum* plants that accelerate larval metamorphosis resulted in maintaining the juvenile forms or producing weak and small adults. Whilst, an isolated kavikol from *A. galanga* caused mortality to *Callosobruchus chinensis* and *Plutella xylostella* L. (Priyono, 1999). Its rhizome powder admixed with stored grain 1% (w/w) was reported to protect the grain against infestation of rice weevil *S. oryzae* (Linnaeus) and rice moth, *Corcyra cephalonica* (Stainton) (Ahmad and Ahmad, 1981, in Prakash and Rao, 1997); they reported that a 100% mortality to both insect when observation continued up to 45 days. Our results showed that the rhizome crude extract of *A. galanga* caused only 25.9% and 12.1% mortality to *S. zeamais* at 0.5 and 0.25% concentrations of crude extract respectively (Table 1). This difference may due to the geographic

variation in locations where *A. galanga* are grown and also time of observation that was only 15 days. As natural products, the bioactive components of plant extracts can be varied with environmental conditions (i.e. type of soil, soil nutrients, temperature, humidity) as well as genetic factor, which could be responsible for the variability. Additionally, its effects on different insect species may also vary.

Result showed that the seed extract of *J. curcas* and *A. muricata* at 0.5% took only 3 and 4 days after treatment (DAT) to cause 100% mortality on *S. zeamais* (Fig. 1). However, mortality of *S. zeamais* caused by 0.25% concentration of extracts of *A. muricata* and *J. curcas* was maximum at 5 DAT and 8 DAT, respectively. The cumulative percentage of mortality of *S. zeamais* caused by crude extract of *A. indica* seed at 0.5% and 0.25% concentration was 51% and 25% and maximum at 8 DAT and 4 DAT respectively (Fig. 1). As explain above, this result may not truly reflect the actual bioactivity of *A. indica* extract, as it was extracted from immature fruits. Except for *J. curcas* and *A. muricata* seed extracts, all other plant extracts resulted in maximum mortality on weevil more than 5 DAT. Comparatively, mortality of weevil fed on rice grains treated with extract of *J. curcas* leaf at both concentrations was very much lower than those fed on rice grains treated with seed of *J. curcas* (Fig. 1). This indicates that leaf extract of *J. curcas* has less bioactive substances than that of seed extract.

The cause of high mortality of *S. zeamais* adult within 3 to 4 DAT could be due to acetogenin substances in *A. muricata* seed while acts as antifeedant and stomach poison (Kusharyanto *et al.*, 2002, Isman, 2006). There is a possibility that the active components of the seed extract of *A. muricata* was at the highest concentration that resulted in rapid mortality on *S. zeamias* adults.

CONCLUSION

In conclusion, crude extracts of *J. curcas* seed and leaves, and *A. muricata* (seed) seem to have potential to be used as botanical insecticides even though further studies need to be conducted against *S. zeamais*. Although the extract of *A. indica* did not show a high mortality on *S. zeamais* as previously reported on other insects (Jotwani & Srivastava 1983), it could be as effective as extract of *J. curcas* and *A. muricata* if it is extracted from matured seed. However, it is intriguing that the seeds of *A. muricata* and *J.*

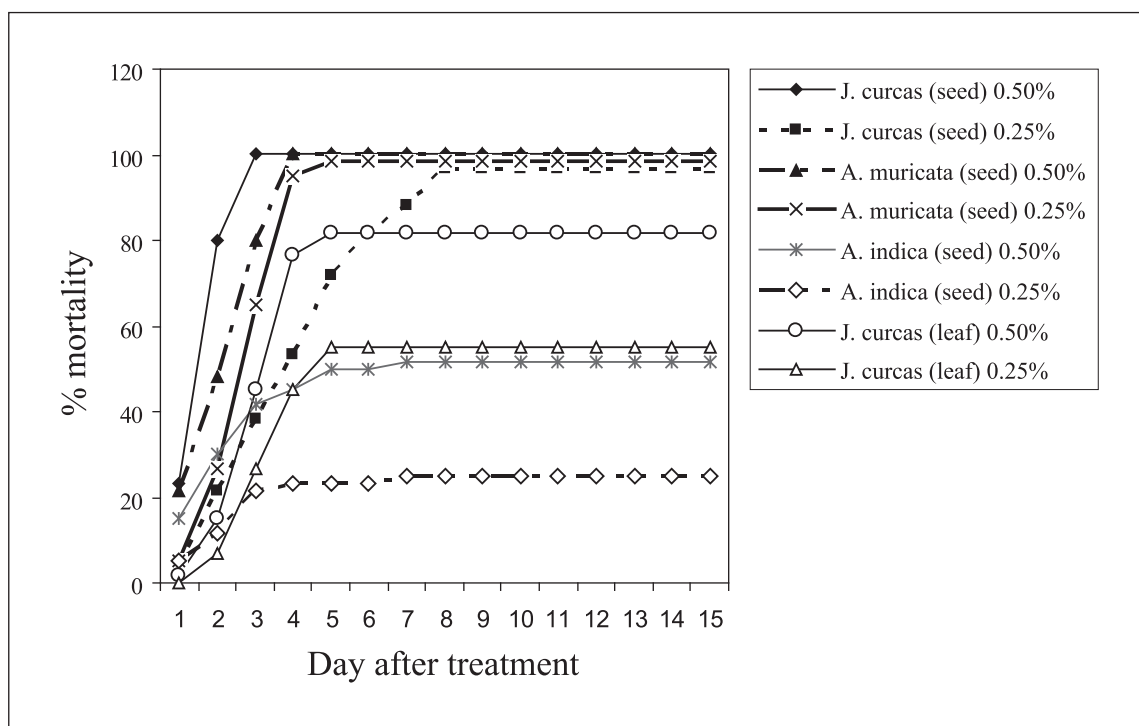


Fig. 1. Cumulative percent mortality of *S. zeamais* adult fed on rice grain treated with extract of three plant species (*Jatropha curcas*, *Annona muricata*, *Azadirachta indica*).

curcas produced higher weight of crude extracts and percent extract from 50 g plant powder than the other plant species (Asmanizar, unpublished data). This suggests that we do not need a lot of materials from this plant species to produce the same amount of extract compared to the other plant species. Additionally, the *J. curcas* and *A. muricata* are easy to grow and their seed or leaf crude extract are cheaper to be produced.

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