

Effect of Four Selected Plant Powder as Rice Grain Protectant Against  
*Sitophilus zeamais* (Coleoptera: Curculionidae)  
(Kesan Empat Serbuk Tumbuhan Terpilih Sebagai Pelindung Beras Terhadap  
*Sitophilus zeamais* (Coleoptera: Curculionidae))

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ABSTRACT

A study to evaluate the effect of four selected plant powder as rice grain protectant against *Sitophilus zeamais* adult mortality, F1 progeny production, weight loss and rice grain damaged was conducted. The plant powders used were made from seed of *Annona muricata*, *Jatropha curcas*, *Azadirachta indica* and from leaf of *J. curcas* at 0.5, 1, 1.5, 2 and 2.5% (w/w) concentrations. Probit analysis showed that *J. curcas* seed powder was highly toxic ( $LC_{50} = 0.28\%$ ) to *S. zeamais* adult followed by *A. muricata* seed ( $LC_{50} = 0.33\%$ ), *J. curcas* leaf ( $LC_{50} = 1.15\%$ ) and *A. indica* seed ( $LC_{50} = 3.63\%$ ). The *Annona muricata* and *J. curcas* seed had caused the highest mean mortality (100 and 98.85%) at 2% concentration, while the *A. indica* seed and *J. curcas* leaf powder had only caused 32.32 and 77.84%, respectively at 2.5% concentration. There was no progeny produced, no weight loss recorded and no rice grain damaged on treated rice grain with *A. muricata* and *J. curcas* seed at 1% concentration. In contrast, *J. curcas* leaf and *A. indica* seed powder had the least toxicity effect on the weevil as shown by number of progeny produced (167 and 228), total of weight loss (10.04 and 10.49%) and rice grain damaged (19.35 and 21.14%) even at the highest powder concentration (2.5%) tested. Results of this study revealed the potential of *J. curcas* and *A. muricata* seed powder to be used in controlling *S. zeamais* on stored rice grain.

**Keywords:** *Annona muricata*; *Azadirachta indica*; *Jatropha curcas*; plant powder; *Sitophilus zeamais*

ABSTRAK

Satu kajian telah dijalankan untuk menilai kesan daripada empat serbuk tanaman dipilih sebagai pelindung beras terhadap kematian *Sitophilus zeamais* dewasa, progeni F1, kehilangan berat beras dan beras rosak. Serbuk tanaman yang digunakan berasal daripada biji *Annona muricata*, *Jatropha curcas*, *Azadirachta indica* dan daun *J. curcas* pada kepekatan 0.5, 1, 1.5, 2 dan 2.5% (w/w). Keputusan analisis probit menunjukkan bahawa serbuk biji *J. curcas* sangat beracun ( $LC_{50} = 0.28\%$ ) terhadap *S. zeamais* diikuti dengan biji *A. muricata* ( $LC_{50} = 0.33\%$ ), daun *J. curcas* ( $LC_{50} = 1.15\%$ ) dan biji *A. indica* ( $LC_{50} = 3.63\%$ ). Serbuk biji *Annona muricata* dan *J. curcas* telah menyebabkan min kematian tertinggi (100 dan 98.85%) pada kepekatan 2%, sementara serbuk biji *A. indica* dan *J. curcas* masing-masing hanya 32.32 dan 77.84% pada kepekatan 2.5%. Tidak ada progeni dihasilkan, kehilangan berat beras dan beras rosak pada perlakuan dengan serbuk biji *A. muricata* dan *J. curcas* pada kepekatan 1%. Sebaliknya, serbuk daun *J. curcas* dan serbuk biji *A. indica* mempunyai kesan toksisiti paling rendah terhadap *S. zeamais*, dengan jumlah progeni yang dihasilkan (167 dan 228), kehilangan berat beras (10.04 dan 10.49%) dan beras rosak (19.35 dan 21.14%) meskipun pada perlakuan kepekatan serbuk tertinggi (2.5%). Keputusan kajian ini menunjukkan potensi serbuk biji *J. curcas* dan *A. muricata* untuk digunakan dalam kawalan *S. zeamais* pada beras yang disimpan.

**Kata kunci:** *Annona muricata*; *Azadirachta indica*; *Jatropha curcas*; serbuk tumbuhan; *Sitophilus zeamais*

INTRODUCTION

*Sitophilus zeamais* is one of the most destructive insect pests on rice grain. Infestations can result in reduced quantity and quality of grain as a result of the larvae feeding on rice grain. The percentage of weight loss on milled rice due to *S. zeamais* infestation was 14.8% in 3 months of storage (Sidik & Pranata 1988). Although the pest can be effectively controlled by synthetic insecticides (Arthur 1996; Golob 1988), but these insecticides

cause serious problems of toxic residues, health and environmental hazards, in addition to development of insect resistance (Fishwick 1988; Golob et al. 1982; Yusof & Ho 1992). The need to find materials that effectively protect rice grain that are readily available, affordable, relatively less poisonous and less detrimental to the environment had stimulated interest in the development of alternative method of control such as using of botanical insecticide.

The effectiveness of botanical insecticides has been demonstrated in many studies (Haque et al. 2000; Malik & Nafqi 1984; Prakash & Rao 1997; Rahman & Schmidt 1999; Tripathi et al. 2000). Many of the plant species concerned have also been used in traditional medicine by local communities and have been collected from the field or specifically cultivated for these purposes. Leaves, roots, twigs and flowers have been admixed as protectant with various commodities in different parts of the world, particularly India, China and Africa (Golob et al. 1999). Belmain et al. (2001) studied the effect of six plants powder traditionally used in Ghana on four storage insects pests. Ogendo et al. (2003) had evaluated the insecticidal effect of *Lantana camara* and *Tephrosia vogelii* powder on *S. zeamais* in stored maize grain.

Grain protectants are defined as pesticides which are incorporated directly into the grain mass for protection against insect and mite attack. This is also known as admixture treatment (Rejesus & Rejesus 1992). The advantages of insecticide admixture treatments are that they are generally easy in preparing, inexpensive and a single application of an effective insecticide, correctly formulated, will give control of existing insect infestation (including, eventually, any insect stages within the kernels) and will protect the grain against re-infestation for a substantial period (Proctor 1994).

Our previous study showed that the crude extract of *J. curcas* (seed and leaf), *A. muricata* (seed) and *A. indica* (seed) caused high mortality effect against *S. zeamais* (Asmanizar et al. 2008). More information is needed regarding the effectiveness powder form in controlling *S. zeamais*. This paper presents effect of four plant powder as rice gain protectant against *S. zeamais* (Coleoptera: Curculionidae).

## MATERIALS AND METHODS

### INSECT CULTURE

The initial population of *S. zeamais* was obtained from rice grain in the open market. The culture was maintained in rice grain as growth medium throughout the study (Cooms & Porter 1986). The rice grain and all apparatus equipments were sterilized by heating to 60 °C for 1 h to protect stock culture from natural enemies (insects, mites and pathogens). The *S. zeamais* was cultured for 6<sup>th</sup> generation prior to the experiment (to adapt the weevil to the laboratory condition). A total of 50 adults were fed on 150 g rice grain filled up 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. The weevil were then removed and rice grains were kept at 29 ± 2 °C and 80 ± 10% R.H.) until adult emergence (ca. 4 weeks).

### SEED AND LEAF POWDER PREPARATION

The *Jatropha curcas* seed and leaves, *Annona muricata* seed and *Azadirachta indica* seeds were collected during

of April 2005 from around Medan City and Kabupaten Deli Serdang, Indonesia. The seeds which were collected from fruit were hulled to get the kernel. Whilst, *J. curcas* leaves were washed up with tap water. All plant materials were air-dried for 1 week before grinding with an electric grinder and then sieved through a 30 mesh to obtain the powder.

Forty gram rice grain was mixed with five concentrations (treatments) of plant powder 0.5, 1, 1.5, 2 and 2.5% (w/w = weight of powder/weight of rice) in the 250 mL flask and shook manually by hand for 30 s (Belmain et al 2001). The treated rice grain were placed into the plastic cup (11 cm height and 6 cm diameter), after which 10 pairs of 4-5 days old adult weevil were released and the cup were then covered with a piece of muslin cloth held by rubber band to prevent adults from escaping. The untreated rice grain was used as control treatment. The treatments were replicated five times and arranged following Completely Randomized Design (CRD). The number of dead insects in each cup was counted daily from 1 to 21 days after treatment. The remaining released adults were removed at 21 days after treatment. Emerging adults emerge were counted and removed from the cup daily to avoid further mating egg laying egg. The weight loss and rice grain damaged were recorded if no more adult emerge. To determine the percentage of rice grain damage, the undamaged rice grain was counted. The number of damaged rice grain was the difference between undamaged rice before treatment minus the undamaged rice grain after treatment. To determine the weight loss and the rice grain were sieved with a 12 mesh sieve to remove the frass and powdery material, and then weighed to assess their % of weight loss (Adams & Schulten 1976; Tionson 1992).

The insect mortality data was corrected by Abbott's formula (1925), then normalized using  $\sqrt{x}$  before analysis. Whilst, data of progeny produced was normalized using  $\log x + 1$ , and  $\sqrt{x + 0.5}$  for weight loss and rice grain damaged (Gomez & Gomez 1984). Data were analyzed using 2-way ANOVA (plant powder and concentration as variable factors) to determine the differences in mean mortality (%), number of progeny produced, weight loss (%) and rice grain damaged (%) among plant powder and/or concentrations. If ANOVA results were significant, Fishers Protected Least Significant Differences ( $p < 0.05$ ) were used to separate the means. Probit analysis (Finney 1971) was conducted to find toxicity effect ( $LC_{50}$ ) of the powder on the weevil. It was run on POLO-PC Program (LeOra Software 1987). Whilst, all other statistical analyses were run on the MINITAB Statistical Package (Minitab Vol. 14 2003).

## RESULTS

The probit statistics estimates of  $LC_{50}$ , their 95% fiducial limits (FL) and the slopes of regression lines are presented in Table 1. The  $LC_{50}$  of the *J. curcas* seed and *A. muricata* seed powder were significantly lower than the  $LC_{50}$ s of *J. curcas* leaf and *A. indica* seed. However, there was no significant difference in  $LC_{50}$  value for *A. muricata* seed

and *J. curcas* seed, as indicated by overlapping 95% FL. The highest  $LC_{50}$  was for the powder of *A. indica* seed. The slope of the regression line of *A. indica* seed powder was the steepest, while for *J. curcas* leaf powder was the least one. The slope of *A. muricata* seed and *J. curcas* seed were not significantly different with that of *A. indica* seed, but the slope of both were significantly steeper than that of *J. curcas* leaf. The value of all calculated  $x^2$  was less than value of tabulated  $x^2$  (Table 1 and Figure 1).

There was a significant interaction between the effect of different plant powder and concentrations on the *S. zeamais* adult mortality ( $F = 15.2$ ,  $df = 15$  &  $96$ ,  $p < 0.05$ ), progeny produced ( $F = 100.38$ ,  $df = 15$  &  $96$ ,

$p < 0.05$ ), weight loss ( $F = 26.24$ ,  $df = 15$  &  $96$ ,  $p < 0.05$ ) and rice grain damaged ( $F = 53.59$ ,  $df = 15$  &  $96$ ,  $p < 0.05$ ) (Table 2).

Mortality of weevil was significantly different ( $F = 313.83$ ,  $df = 3$  &  $96$ ,  $p < 0.05$ ) among rice grain treated with different plant powder (Table 2). The mortality of weevil on rice grain treated with *A. indica* seed powder was significantly lower ( $p < 0.05$ ) than that of *A. muricata* seed and the *J. curcas* seed and leaf powder (Table 3). However, there was no significant difference ( $p > 0.05$ ) in percentage mortality of weevil fed on the rice grain treated with *A. muricata* seed and those fed on *J. curcas* seed powder.

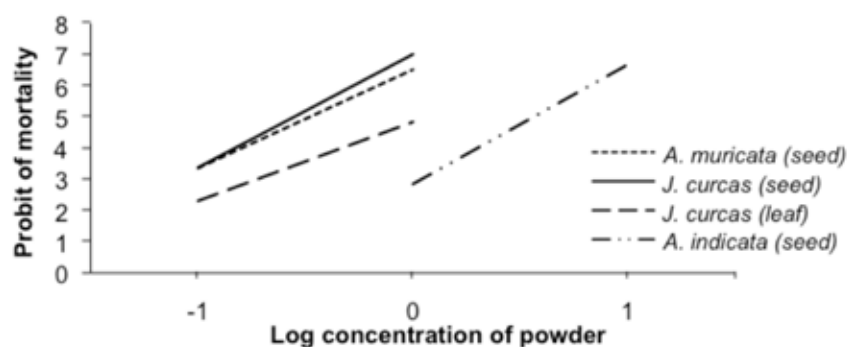


FIGURE 1. Log concentration-probit mortality graph for four plant powder against *S. zeamais*

TABLE 1. Toxicity of plant powder to *S. zeamais* adults

Plant Powder	Insects (N)	$LC_{50}$	95% fiducial limit	Slope $\pm$ SE	Chi-square (df, $\chi^2$ )
<i>A. muricata</i> (seed)	600	0.33 a	0.022 - 0.423	$3.16 \pm 0.32$ a	3.85 (3, 7.81)
<i>J. curcas</i> (seed)	500	0.28 a	0.142 - 0.403	$3.63 \pm 0.38$ a	3.95 (2, 5.99)
<i>J. curcas</i> (leaf)	600	1.15 b	1.015 - 1.295	$2.53 \pm 0.28$ b	2.23 (3, 7.81)
<i>A. indica</i> (seed)	600	3.63 c	2.901 - 8.060	$3.83 \pm 1.14$ ab	1.00 (3, 7.81)

TABLE 2. Two-way ANOVA statistics for effect of plant powder on *S. zeamais* adult mortality, progeny production, weight loss and rice grain damaged

Factor (Dependent variable)	Source	df	Sum of square	F- value	p - value
Mortality	Powder	3	64715.8	313.83	< 0.05
	Conc.	5	62972.0	183.23	< 0.05
	Powder x Conc.	15	15667.0	15.2	< 0.05
	Error	96	6598.8		
Progeny production	Powder	3	94.2687	1957.14	< 0.05
	Conc.	5	29.1294	362.86	< 0.05
	Powder x Conc.	15	24.1738	100.38	< 0.05
	Error	96	1.5413		
Weight Loss	Powder	3	137.7710	602.49	< 0.05
	Conc.	5	39.3053	103.13	< 0.05
	Powder x Conc.	15	29.9998	26.24	< 0.05
	Error	96	7.3174		
Rice grain damaged	Powder	3	277.028	1105.72	< 0.05
	Conc.	5	79.189	189.64	< 0.05
	Powder x Conc.	15	67.136	53.59	< 0.05
	Error	96	8.017		

Percent weevil mortality on rice grain treated with various of plants powder was significantly difference among concentrations ( $F = 183.23$ ,  $df = 5 \& 96$ ,  $p < 0.05$ ) (Table 2). Generally, the percentage of weevil mortality increased with increasing powder concentration tested on rice grain (Figure 2). There was a significant difference ( $p < 0.05$ ) of weevil mortality at 0.5% concentration between *J. curcas* seed and *A. muricata* seed powder with 93.45 and 76.65 mortality, respectively. However, the increasing concentration thereafter had caused no significant difference of weevil mortality of both plant powder, with the maximum mortality achieved 98.85 and 100%, respectively at 2% concentration. The *Jatropha curcas* leaf powder showed moderate effect on mortality compared to the other three and the increasing of the concentration at 2.5% showed only 77.84% of mortality. The *A. indica* seed powder had caused the lowest mortality on weevil (<35%) as compared to other powders (Figure 2).

There was a significant difference among weevil progeny produced from rice grain treated with different plant powder ( $F = 1957.14$ ,  $df = 3 \& 96$ ,  $p < 0.05$ ), weight loss of rice grain ( $F = 602.49$ ,  $df = 3 \& 96$ ,  $p < 0.05$ ) and rice grain damaged ( $F = 1105.72$ ,  $df = 3 \& 96$ ,  $p < 0.05$ ) (Table 2). There was a significantly ( $p < 0.05$ ) higher number of progeny produced, weight loss and rice grain damaged from rice grain treated with *J. curcas* leaf and *A. indica*

seed powder than *A. muricata* seed and *J. curcas* seed powder (Table 3).

Number of progeny produced, weight loss and rice grain damaged were significantly different ( $F = 362.86$ ,  $df = 5 \& 96$ ,  $p < 0.05$ ;  $F = 103.13$ ,  $df = 5 \& 96$ ,  $p < 0.05$ );  $F = 189.64$ ,  $df = 5 \& 96$ ,  $p < 0.05$ ) among concentration tested on rice grain (Table 2). Generally, those variable values decreased as the concentration of powder increased (Figures 3, 4 and 5).

The *J. curcas* and *A. muricata* seed powder at 0.5% concentration significantly ( $p < 0.05$ ) reduced progeny production of the weevil, weight loss and rice grain damage (Figures 3, 4 and 5). Interestingly, there was also no progeny produced, weight loss and rice grain damage at 1% concentration tested. Whilst, the *J. curcas* leaf and *A. indica* seed powder caused the least effect on the number of progeny produced (167 and 228), weight loss (10.04 and 10.49%) and rice grain damaged (19.35 and 21.14%) even at the highest concentration tested (2.5%). The powder of *A. indica* seed and *J. curcas* leaf could only reduced progeny production up to 25.58 and 39.55%, 18.36 and 20.25% of weight loss, 10.84 and 15.76% of rice grain damaged, respectively, when compared with control. But, *J. curcas* and *A. muricata* seed powder had caused 100% reduction of progeny production, weight loss and rice grain damaged at 1% concentration (Figures 3, 4 and 5).

TABLE 3. Mean of *S. zeamais* mortality, progeny produced, weight loss and rice grain damaged on treated rice grain with different plant powder

Treatment (Powder)	Mortality of weevil (% Mean $\pm$ SEM)	Number of progeny produced (Mean $\pm$ SEM)	Weight loss (% Mean $\pm$ SEM)	Rice grain damaged (% Mean $\pm$ SEM)
<i>A. muricata</i> (seed)	77.41 $\pm$ 6.82 a	47.37 $\pm$ 18.93 a	2.25 $\pm$ 0.83 a	4.66 $\pm$ 1.43 a
<i>J. curcas</i> (seed)	81.19 $\pm$ 6.78 a	59.40 $\pm$ 23.18 a	2.69 $\pm$ 0.96 a	5.37 $\pm$ 1.80 a
<i>J. curcas</i> (leaf)	45.99 $\pm$ 5.79 b	223.57 $\pm$ 12.58 b	11.43 $\pm$ 0.35 b	21.54 $\pm$ 0.56 b
<i>A. indica</i> (seed)	9.68 $\pm$ 2.33 c	278.67 $\pm$ 10.37 b	12.02 $\pm$ 0.41 b	22.74 $\pm$ 0.35 b

Means in a column followed by different letters are significantly different ( $P < 0.05$ ) by LSD Test. SEM = Standard Error of Mean

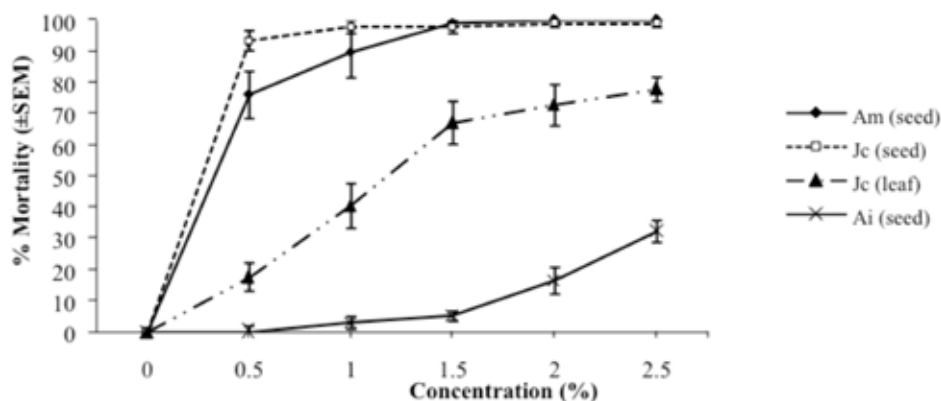


FIGURE 2. Effect of plant powder and concentrations on *S. zeamais* adult mortality

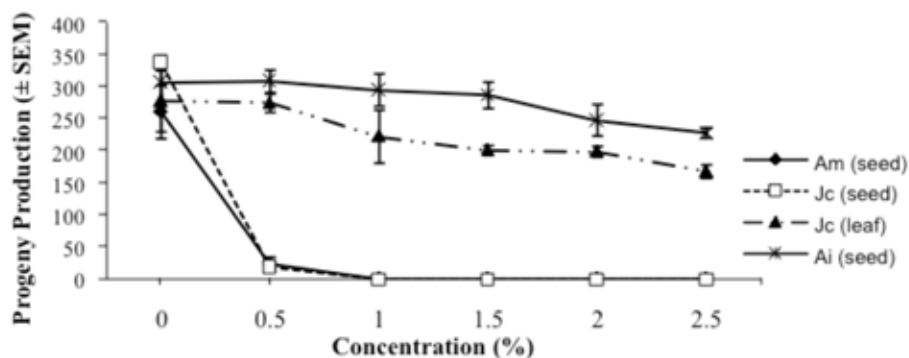


FIGURE 3. Effect of plant powder and concentrations on progeny production of *S. zeamais* adult

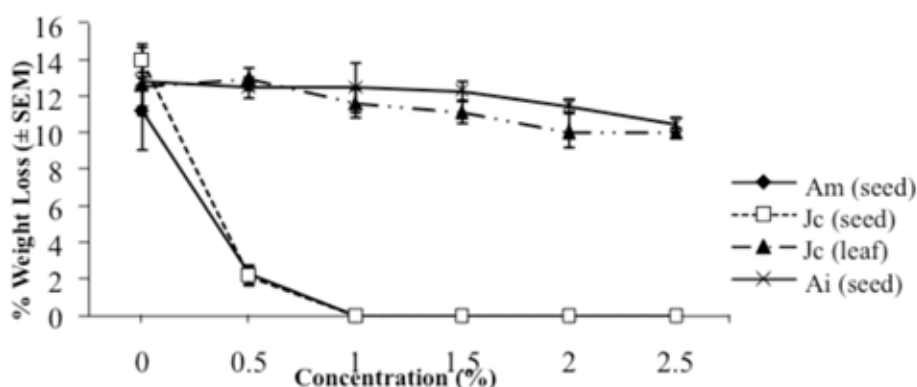


FIGURE 4. Effect of plant powder and concentrations on weight loss of rice grain (%)

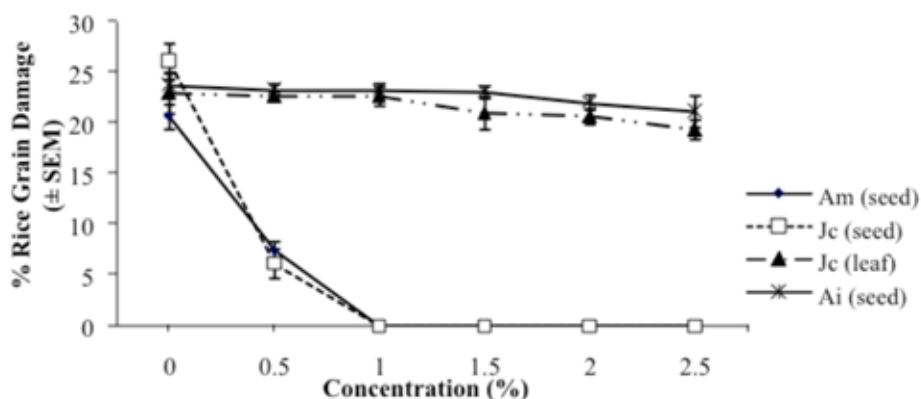


FIGURE 5. Effect of plant powder and concentrations on rice grain damage (%)

#### DISCUSSION

The *A. muricata* seed powder and *J. curcas* seed powder had high toxicity effect on *S. zeamais* adult ( $LC_{50}$  was 0.33% and 0.28%, respectively). The powder from both *A. muricata* seed and *J. curcas* seed exhibited greater toxic effects against *S. zeamais* adult than *J. curcas* leaf and *A. indica* seed ( $LC_{50}$  was 1.15% and 3.63%, respectively), indicating that the powder seeds contain chemical components that are not present in leaf in increasing their toxicity. Dos Santos and Sant'Ana (2001) and Isman (2006) reported that the Annonaceous species such as *A. muricata* had the Annonaceous acetogenin, a class

of natural compound with a wide varieties of biological activities. The acetogenin from *A. muricata* seed had been known to have substances that act as botanical insecticide (Leatemia & Isman 2004). On the other hand, the *J. curcas* seed contain curcin and phorbol esters (Adolf et al. 1984; Stripe et al. 1976). Sauerwein et al. (1993) found that phorbol esters from *J. curcas* seed have insecticidal properties. The steeper of slope *J. curcas* seed and *A. indica* seed powder ( $3.63 \pm 0.38$  and  $3.83 \pm 1.14$ ) indicates that the weevil has somewhat more sensitive to the seed powder of the plant than the powder of *J. curcas* leaf and *A. muricata indica* seed ( $2.53 \pm 0.28$  and  $3.16 \pm 0.32$ ). The

low chi-square value obtained as compared to chi-square tabular indicating that there was no significant variation among individual of weevil within treatment, which also means that genetically the weevil individual are somewhat similar (Table 1 and Figure 1).

The *J. curcas* seed and *A. muricata* seed powder caused significantly high mortality of weevil than *J. curcas* leaf and *A. indica* seed powder (Table 3). It could be due to the toxic effect (low  $LC_{50}$ ; Table 1) of the compounds contained in seeds of these plants. The toxic effect of *J. curcas* seed could be due to the presence of several sterols and terpen alcohols which have been known to exhibit insecticidal properties to *Callosobruchus maculatus* (Adebowale & Adedire 2006). Whilst, *A. muricata* seed contains acetogenins which could be contributed to the weevil mortality. The acetogenins from the family Annonaceae was reported to cause high mortality of German cockroach, *Blattella germanica* (Alali et al. 1998). The moderate effect of *J. curcas* leaf powder on the *S. zeamais* mortality indicated that leaf has bioactive substances less than that of seed. It could also be due to the secondary metabolite differ at different part of plant. According to Schmutterer (1992) activity of plant extract depend on plant part use. In contrast, the low mortality caused by *A. indica* seed could be resulted from the immature seed admixed with matured one used this study. This is because of the difficulties to obtain the fully mature fruit in large number during the study period. Mane (1968) (in Jotwani & Srivastava 1983) revealed that fully mature fruits of *A. indica* was found to be superior to that from yellow or green fruits.

*Jatropha curcas* and *A. muricata* seed powder at 0.5% concentration caused 93.45 and 76.05% weevil mortality (Figure 2) and effectively reduced progeny production, weight loss and rice grain damage (Figures 3, 4, 5). Both *J. curcas* and *A. muricata* powder showed somewhat similar effect on *S. zeamais*, with the 1% (w/w) concentration had achieved good control. High mortality of adults had resulted in no progeny produced, weight loss and rice grain damaged (Figures 2, 3, 4 and 5).

The *J. curcas* leaf showed moderate effect in killing *S. zeamais* adult with the mortality ranging from 17 to 70% (Figure 2), and the remaining adult still producing progeny (Figure 3), causing significant weight loss (Figure 4) and rice grain damaged (Figure 5). This indicates that these plant powders especially those from the seeds, had acted as an effective insecticide in controlling *S. zeamais*. A similar result was observed when the dried ground leaves of *Ricinus communis* (also Euphorbiaceae), at 16 g/kg admixed with cowpea was reported to cause 100% mortality of adult *Callosobruchus maculatus* within seven days and reduced F1 emergence (Okonkwo & Okoye 1992). Sakthivadivel and Daniel (2008) reported that extract of *J. curcas* leaf tested on fourth instar larvae of *Culex quinquefasciatus* caused 50% mortality at concentration 53.86 ppm. They also stated that the bioactive compound in these leaves were mainly glycosides, tannis, phytosterols, flavonoids and steroidal sapogenins.

In conclusion, this study showed that using *J. curcas* and *A. muricata* seed powder were comparatively more effective in controlling *S. zeamais* than using other plant powder. Powder formulations have commonly been used for the preservation of stored grains. In Nepal, dried powdered rhizome of *Acorus calamus* (sweet flag) has been found to be effective in almost eliminating insect damage when applied to maize cobs in traditional storage barn (Golob et al. 1999). *Eupatorium odoratum*, *Nicotiana tabacum* leaf powder are used by farmer to protect beans against *Acanthoscelides obtectus* and *Callosobruchus maculatus* (Delobel & Malonga 1987). Our result also showed that seed powder of *J. curcas* and *A. muricata* are good protectant of stored rice grain from the attack of *S. zeamais*. Because rice grain is a staple-food, estimation of human hazard potential is required to ascertain the toxicity of plant material as botanical insecticide. In other related study conducted by Amanizar (2011) showed that  $LD_{50}$  acute oral toxicity value for the *J. curcas* seed crude extract obtained by soaking with acetone and by Soxhlet extraction was relatively non toxic ( $LD_{50} = 10.66$  and  $12.33$  g/kg body weight of mice) to mammals. Moreover, as powder form, both *J. curcas* and *A. muricata* seed settled at the bottom of rice grain mass. It is easy to remove the powder before cooking the rice.

Finally, results of this study indicated the potential of using powder form of *J. curcas* and *A. muricata* seed powder at 1% concentration is good enough for controlling *S. zeamais*.

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