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ABSTRACT

The aims of the experiment was to determine the optimum dosage of sour-sop leaves and seeds powder for decreasing *Callosobruchus* development and to maintain the quality of mungbean storaged seed. The experiment was conducted at Plant Protection Laboratory, Faculty of Agriculture, UPN "Veteran" Yogyakarta from March to August 2013. It consisted of two factors: the part of sour-sop plant's powder: leaves, rib of leaves and seeds; and the dosages of sour-sop powder: 10, 20 and 30g/100g mungbean seeds and one control treatment: no sour-sop powder application. It was arranged in Randomized Completely Design with four replications. Data collected was subjected to an analysis of variance followed by DMRT at 5% significance level. The results showed that: 1) The dosage of sour-sop seed powder 30g/100g mungbean seeds had the highest of *Callosobruchus* mortality (75%) on 96 hour after treatment and had better seed vigor than other combination treatments. 2)The sour-sop seed powder had the lowest *Callosobruchus* population and seedweight lost, on 1 and 2 month storage periods. 3) The quality of mungbean seed had decreasied on 2 month storage period.

Keywords: *sour-sop powder, Callosobruchus sp., mungbean storage seeds*

INTRODUCTION

The production of mungbean is plagued by various pests, with insects causing the worst damage. The most important storage pest of mungbean is *Callosobruchus spp.* It belongs to the family Bruchidae. The larvae bore into the pea or bean throughout most of the tropics and subtropics (Hill and Waller, 1999). They develop inside kernel and feed on starchy interior. Adults hatch and making tunnel in the grain and continue to feed voraciously on the grain. They caused 50% seed weight loss of mungbean for 3 month (Priyono and Harahap, 1995).

Losses caused by storage pests include weight loss, loss in quality and market value, promoting of mould development, reduced germination in seed material and reduced nutritional value (Lowenberg- Deboer, 2003).

Insecticides, at the moment, are the best weapon against insect pest. Insecticides are chemical that affect the biological processes of many living organism and may act as poisons to many animals' species (Hayes and Lawes, 1991). Insecticides have a wide range in mammalian toxicity, its toxic doses range from 1mg/kg in the diet of a vertebrate animal to very large amount, which are needed to kill a mammal (Hardy, 1990)

Although the pest can be effectively controlled by synthetic insecticides (Arthur 1996; Golob 1988), 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*cit.* Asmanizar *et al.*, 2012). The need for finding materials that are effectively protect rice grain which 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.

Botanical insecticides are getting the great interest, because they are natural insecticides, toxicants derived from plants. Since the use of chemicals has so many adverse effects on the environment, the botanical insecticides have been widely adopted by the farmers to control the insect pest that attack cowpea (Pereira *et al* 1982). The effectiveness of botanical insecticides has been demonstrated in many studies. 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 (Asmanizar *et al.*, 2012).

The laboratory evaluation of the repellency of two pepper varieties, *Capsicum annum* and *Caesium frutescens* (caynene pepper) to cowpea weevil, *Callosobruchus maculatus* was carried out and found effective (Egwunyenga *et al.*, 2000).The plants of *Azadirachta indica* A. Juss (common name: neem) and *Citrus sinensis* (common name sweet orange) have been reported to have some insecticidal properties against pests (Taylor, 1975). For example *C. sinensis* pea powder has proved potentially against *C. maculatus*, depressing oviposition and progeny emergence on cowpea, although at high doses (Taylor, 1975).

Grain protectants are defined as pesticides which are incorporated directly into the grain mass for protection against insect. This is also known as admixture treatment. The advantage of insecticide are: generally easy in preparing, inexpensive and a single application of an effective insecticide, correctly formulated, giving control of existing insect infestation (including, eventually, any insect stages within the kernels) and protecting the grain against re-festation for a substantial period (Proctor, 1994). More information is needed regarding the effectiveness of the soursop leaves and sour-sop seeds powder in controlling *Callosobruchus* sp. and maintaining mungbean seed quality in storage.

MATERIALS AND METHODS

The experiment was conducted at Plant Protection Laboratory, Faculty of Agriculture, UPN "Veteran" Yogyakarta from March to August 2013. It consisted of two factors: the part of sour-sop plant powder: leaves, rib of leaves and seeds and the dosages of sour-sop powder: 10, 20 and 30g/100g mungbean seeds and one control treatment: no sour-sop powder application. It was arranged in Randomized Completely Design with four replications. Data collected was subjected to an analysis of variance followed by DMRT at 5% significance level.

A. Bioassay (Mortality Test)

Each plastic glass contains, 50g seeds mixed with soursop powder, depended on the treatment. Ten (10) newly emerged adults of *Callosobruchus spp.* was introduced into plastic glass. The glasses were covered with fine fabric nets to ensure aeration. Percentage of mortality was calculated daily for four (4) days.

B. Evaluation of Seed Quality

After 2 months seed storage period, weight loss of mungbean seed was measured. For germination test, four replicated of 50 seeds from each treatment were planted on sand-filled germination bag, allowed to germinate for 7 days and then all germination test parameters were recorded.

C. Phytochemic tests

Polar fraction was analyzed by using ethanol and non-polar fraction by using n-hexan. Alcaloids were detected with Dragendorff and terpenoids with sulphate acid anisaldehide. Phitochemic tests by Thin Layer Chromatography (TLC).

RESULTS AND DISCUSSION

Comparation between treatments and control were using Least Significant Difference and contrass orthogonal at 5% level. The result showed that mortality of *Callosobruchus spp.* occured on 48 hours. Weevil mortality on mungbean seeds treated with various powder of soursop was significantly difference on 78 and 96 hours after applications. Generally, the percentage of weevil mortality increased with the increasing of powder concentration tested on mungbean seed. The highest mortality was on 78 and 96 hours after applications occured on seed powder application (Table 1). The active compound of soursop powder needed longer time to penetrate insect cuticules, that’s way significantly mortality occured on 72 hours after application. Seed powder caused higher mortality of *Callosobruchus spp.* than leaf and rib of leaf powder, because active compound of seed powder such as acetogenin, squamosin and annonain was higher than the others. Squamosin could depress respiration on mitochondria and spesificly depressed electron transfer.

The powder of *A. muricata* seed exhibited greater toxic effects against *C. chinensis* adult than *A. muricata*, indicating that the powder seeds contain chemical components that are not present in leaf. 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 range of biological activities. The acetogenin from *A. muricata* seed had been known to have substances that act as botanical insecticide (Leatemia & Isman 2004).

Table 1. Percentage of*Callosobruchus* sp mortality on 48, 72, 96 and 120 hours after application

Observation on 48 hours after application

Part of soursop plant	Dosage of soursop powder per 100 g seeds			Means
	0,5 g	1,0 g	1,5 g	
Leaf	0,00	0,00	2,50	0,83 a
Seed	0,00	0,00	0,00	0,00 a
Rib of leaf	2,50	0,00	5,00	2,50 a
Means	0,83 j	0,00 j	2,50 j	1,11 x (-)
Untreated				0,00 x

Observation on 72hours after application

Part of soursop plant	Dosage of soursop powder per 100 g seeds			Means
	0,5 g	1,0 g	1,5 g	
Leaf	2,50	0,00	2,50	1,67 c
Seed	17,50	20,00	30,00	22,00 a
Rib of leaf	15,00	12,50	17,50	15,00 b
Means	11,67 j	10,83 j	16,67 j	12,89 x (-)
Untreated				0,00 x

Observation on 96hours after application

Part of soursop plant	Dosage of soursop powder per 100 g seeds			Means
	0,5 g	1,0 g	1,5 g	
Leaf	10,00 d	12,50 d	12,50 d	11,67
Seed	42,50 c	57,50 b	75,00 a	58,33
Rib of leaf	40,00 c	40,00 c	35,00 c	38,33
Means	30,83	36,67	40,83	36,11 x (+)
Untreated				10,00 y

Observation on 120hours after application

Part of soursop plant	Dosage of soursop powder per 100 g seeds			Means
	0,5 g	1,0 g	1,5 g	
Leaf	37,50	32,50	32,50	34,17 a
Seed	80,00	75,00	77,50	77,50 a
Rib of leaf	60,00	57,50	57,50	58,33 a
Means	59,17 j	55,00 j	55,83 j	56,67 x (-)
Untreated				47,50 y

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

Table 2 showed that population of *Callosobruchus spp.* on 2 months storage on control (untreated) was higher than seed treated. Population of *Callosobruchus spp.* on seed powder treatment was lower than the others. Mortality of *Callosobruchus spp.* on seed powder treatment was higher than leaf and rib powder, so it could supress oviposition of adult female and population growth. *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, *Blatella germanica* (Alali *et al*. 1998).

Plant powders have been used to suppress the population of storage pests (Ogunleye,2000.,Ogunleye *et al*., 2004 and Onu and Baba, 2003). It has been reported that powders of plant materials are capable of blocking the spiracle of insects

(Steve,2010, Lale,2002). This can lead to suffocation and death. Secondly, these powders, when stocked under the wings of insects in the store compled with the fact that the plant has great itching effects are capable of causing great discomfort to them.This may prevent them from feeding well and eventually leads to death. It has been suggested that abrations can lead the loss of fluids and consequently, death of insects and it may also significantly reduce the rate of oviposition (Ogunwolu *et al*., 1998).

The high mortality rate could also be as a result of direct feeding of the insects on the plant materials. The insects are not able to derive enough nourishment that will support its normal growth and development from the plants and this may lead to insect mortality. It is also evident in this research work that *C. maculatus* is more susceptible to the adverse effects of the plant materials.

Table2. *Callosobruchus* sp population after 2 months seed stored

Callosobruchus sp adult population after 2 month stored				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Means
	0,5	1.0	1.5	
Leaf	117.00	165.75	217.50	166.75b
Seed	68.50	61.25	49.75	59.83a
Rib of leaf	121.75	160.25	122.25	134.75ab
Means	102.42j	129.08k	129.83k	120.44x
Untreated				253.00y

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

Table 3 showed the mungbean seed damage after 2 months in storage in the form of weight loss of seed. The mungbean seed damage increased with increasing their weight loss.

Table 3. Weight loss of mungbean seed after 2 months stored (%)

Weight loss after 2 month stored (%)				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Mean
	0,5	1.0	1.5	
Leaf	5.4576	3.9925	6.6115	5.3539a
Seed	4.4759	3.9255	4.7002	4.3672b
Rib of leaf	5.8332	7.0595	4.8667	5.9198a
Means	5.2556	4.9925	5.3928	5.2136x
Untreated				14.0365y

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

Weight loss on control was higher than treated seed because population of C. chinensis on control was higher than treated seed. Weight loss on treatment of seed powder was lower than leaf and rib of leaf powder because the active compound of seed powder such as acetogenin, squamosin and annonain was higher than the others.

There was no significant effect of the part of sour-sop plant powder on the percentage of seed moisture content (Table 4.). There was also of the dosages of sour-sop powder for 2 months seed stored. The treatment and control had no significant effect on seed moisture content. It showed that the storage condition had no change RH and temperature.

Table 4. Seed Moisture Content after 2 months stored (%)

Weight loss after 2 month stored (%)				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Mean
	0,5	1.0	1.5	
Leaf	9.33a	10.02ab	10.92ab	10.09
Seed	9.29a	9.31a	9.92ab	9.51
Rib of leaf	10.16ab	12.30b	8.49a	10.32
Means				9.97x
Untreated				10.88x

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

Seed treatment with seed sour-sop powder gave lower seed conductivity than leaves and rib sour-sop. (Table 5.) Seed sour-sop powder had higher alkaloid than their leaves and rib so can kept seed from *Callosobruchus* sp.

Conductivity test was based on the premise that as seed deterioration progresses, the cell membranes become less rigid and more water permeable, allowing the cell contents to escape into solution with the water and increasing its electrical conductivity. The conductivity of the solution reflected the general level of viability of seed (Copeland and Donald, 1995)

Table 5. Seed Conductivity after 2 months in storage (m Hos)

Weight loss after 2 month stored (%)				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Mean
	0,5	1.0	1.5	
Leaf	2.0150	2.1143	2.6500	2.2598b
Seed	1.6728	1.7243	1.8410	1.7460a
Rib of leaf	1.9040	2.7690	2.2020	2.2917b
Means	1.8639	2.2025	2.2310	2.0991
Untreated				2.2713

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

As the result of seed conductivity, seed treatment with seed sour-sop powder had higher percentage of germination than their rib leaf (Table 6.). There had been decreasing percentage of seed germination on 2 months seed storage period.

Table 6. Percentage of germination after 2 months in storage (%)

Weight loss after 2 month stored (%)				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Mean
	0,5	1.0	1.5	
Leaf	72.50	68.00	61.50	67.33ab
Seed	88.00	79.33	52.00	73.11a
Rib of leaf	80.75	50.50	32.50	54.58b
Means	80.42j	65.94k	48.47k	65.01
Untreated				59.00

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

There was interaction between the part of sour-sop plant powder and their dosages on percentage of seed vigor after 2 months seed storage period (Table 7.) The percentage of seed vigor showed its power germination velocity. The dosages 30g seed sour-sop powder had the better seed vigor than other combination treatment.

Table 7. Percentage of Seed Vigor after 2 months (%)

Weight loss after 2 month stored (%)				
Part of soursop plant	Dosage of soursop powder per 100 g seeds			Mean
	0,5	1.0	1.5	
Leaf	61.00ab	76.50a	49.50bc	62.33
Seed	78.00a	70.00a	60.00ab	69.33
Rib of leaf	70.00a	38.50c	32.50c	47.00
Means	69.67	61.67	47.33	59.55
Untreated				56.00

Note : Mean in column (P,Q,R) and row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test and Contrast Orthogonal; (-) no interaction

Table 8. Phytochemic test on etanol and n-heksana fraction on rib of leaf, seed and leaf of soursop

Fraction of	Part of plant	Phytochemic test	
		Alcaloid	Terpenoid
Ethanol	Leaf	+	+
	Rib of leaf	+	+
	Seed	++	+
	Leaf		
n-hexan	Rib of leaf	+	+
	Seed	+	+
		++	++

Note : ++ : many compound
+ : less compound

Phytochemics test have done to determine active compound on each soursop plant powder qualitatively. On ethanol and n-hexan extract showed that seed part was found many secondary metabolic such as alcaloid and terpenoid (Table 8.).

CONCLUSION

The conclusion were: 1) The dosage of sour-sop seed powder 30g/100g mungbean seeds had the highest of *Callosobruchus* mortality (75%) on 96 hour after treatment and better seed vigor than other combination treatments 2)The sour-sop seed powder had the lowest *Callosobruchus* population and seed weight loss on 1 and 2 month storage periods. 3) The quality of mungbean seed had decreased on 2 month storage period.

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