THE *Callosobruchus spp.* CONTROLLED USING *Piper cubeba* and *Annona muricata* SEED EXTRACTS FOR INCREASING THE QUALITY OF MUNGBEAN STORAGED SEEDS

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ABSTRACT

The aims of the experiment was to find the optimum composition and concentration of *Piper cubeba* and *Annona muricata* seed extracts for decreasing *Callosobruchus spp.* 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 one factor: the composition and concentration of *P.cubeba* and *A.muricata* seed extracts : 2/0-5%, 2/0-7,5%, 2/0-10%, 2/1-5%, 2/1-7,5%, 2/1-10%, 1/1-5%, 1/1-7,5%, 1/1-10%, 1/2-5%, 1/2-7,5%, 1/2-10%, 0/2-5%, 0/2-7,5%, 0/2-10%, and one control treatment: no extracts application. It was arranged in Randomized Complete 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 the composition and concentration of *P. cubeba* and *A. muricata* seed extracts: 2/1-10% ;1/2-10% dan 1/1-10% had the highest of *Callosobruchus spp.* mortality and better seed vigor than other treatments 2) There had been decreasing mungbean seed quality on 2 month storage period.

Keywords: *Piper cubeba* and *Annona muricata seed extracts , Callosobruchus sp., mungbean storage seeds*

INTRODUCTION

The production of mungbean is plagued by many different pests, with insects causing the worst damage. Field insect pests are the pests that affect these crops in field before harvest. 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 eat their way out of 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, promotion of mould development, reduced germination in seed material and reduced nutritional value (Hill and Waller, 1999 *cit*. Asmanizar *et al*., 2012).

Synthetic chemical insecticides have been instrumental in the evolution of modern agriculture. Nevertheless, overuse and misuse of pesticides has sometimes resulted in problem of environmental contamination, poisoning, pesticides resistance and test resurgence. This issues, consumer demands for low-risk product and legislative with drawal of older chemistries in many jurisdiction has resulted in increased attention towards reduced risk tactics for pest management (Faraone *et al*.,2015)

Although the pest can be effectively controlled by synthetic insecticides, but these insecticides cause serious problems of toxic residues, health and environmental hazards, in addition to development ofinsect resistance (Fishwick, 1988; Golob *et al*., 1982; Yusof& Ho, 1992 *cit.* Asmanizar *et al.,* 2012). 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.

Botanical insecticides are of great interest to many, 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 has been adopted by the farmers to control the insect pest that attack cowpea. 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).

Extract from plants material were tested in choice and no choice tests as oviposition deterrents for cowpea bruchid, *C. maculatus* on chickpea, cicer arietinum. Seed treatments with 0.1% crude extract of materials resulted in a significant reduction in ovipositional preverence of the bruchid (Elhag, 2010). *P. cubeba* was essential oils were investigated for repellent, insecticidal, antiovipositional, egg hatching, persistence of its insecticidal activities against rice weevils *Sitophylus oryzae* (Coleoptera: Curculionidae) (Su, 1990).

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 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-festation for asubstantial period (Proctor, 1994). More information is needed regarding the effectiveness of the composition and concentration of *P. cubeba* and *A. muricata* extracts in controlling *Callosobruchus spp*. and mungbean seed quality in storage.

MATERIALS AND METHODS

The experiment was conducted at Plant Protection Laboratory, Faculty of Agriculture, UPN "Veteran" Yogyakarta. It consisted of one factor: the composition and concentration of *P. cubeba* and *A. muricata* seed extracts : 2/0-5%, 2/0-7,5%, 2/0-10%, 2/1-5%, 2/1-7,5%, 2/1-10%, 1/1-5%, 1/1-7,5%, 1/1-10%, 1/2-5%, 1/2-7,5%, 1/2-10%, 0/2-5%, 0/2-7,5%, 0/2-10%, and one control treatment: no extracts application. It was arranged in Randomized Complete Design with four replications. Data collected was subjected to an analysis of variance followed by DMRT at 5% significance level.

Bioassay (Mortality Test)

Into each of the plastic glass, 50g of seeds was mixed with *P.cubeba* and *A.muricata* seeds extracts; the composition and concentration of them depend of the treatment. Ten (10) newly emerged adults of *Callosobruchus spp.* was introduced into plastic glass. The glass were covered with nets to ensure aeration. Percentage mortality was calculated on daily basis for four (4) days.

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 germination bag with sand-filled, allowed to germinated for 7 days and then all germination test parameters measured.

RESULTS AND DISCUSSION

Statistical analysis showed that the highest mortality of *Callosobruchus spp.* on 24 hours after treatment was 2/1-5% treatment. The observation on 48, 72 and 96 hours the highest mortality occured on 1/1-7,5% dan 10% treatments but not significantly difference with 2/1-10% and 1/2-10%. This showed that mixing *A. muricata* seeds extract with *P. cubeba* seed extract could increas insect mortality than was separated (Table 1).

The extract from *A. muricata* seed exhibited great toxic effects against *Callosobruchus spp.* adult. Londershausen *et al*. (1991) 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.Acetogenins are mitochondrial poisons, inhibiting cellular energy production through a mode of action identical to that of the well-known botanical insecticide and fish poison, rotenone (More specifically, acetogenins block the respiratory chain at NADH ubiquinone reductase (complex I) and cause a decrease in ATP levels, directly affecting electron transport in the mitochondria, causing apoptosis. Acetogenins also inhibit insect development and behavior.

The content of *P. cubeba* fruits was lignan compound those were clusine, dihidroclusine and yatein, they had great activity to inhibit P450 cytochrome enzyme for degradating stranger compound including insecticide. Inhibiting that enzyme caused the combining insecticide with lignan had synegyze effect potentcially (Usia *et al*., 2005).. This research also showed that the combining *A. muricata* and *P. cubeba* extract could increase *Callosobruchus spp* mortality.

Table 1. Percentage of *Callosobruchus spp.* mortality on 24, 48, 72 and 96 hours after application

Composition and concentration of *P.cubeba:A.muricata*	Mortality on ... hours after application			
	24	48	72	96
2/0-5%	10,00 ab	13,33 b	23,33 ab	50,00 ab
2/0-7,5%	13,33 ab	23,33 ab	40,00 ab	63,33 ab
2/0-10%	0,00 b	0,00 b	10,00 b	33,33 b
2/1-5%	23,33 a	26,67 ab	43,33 ab	50,00 ab
2/1-7,5%	0,00 b	10,00 b	13,33 b	53,33 ab
2/1-10%	3,33 b	20,00 ab	50,00 ab	80,00 a
1/1-5%	6,67 ab	13,33 b	33,33 ab	70,00 ab
1/1-7,5%	13,33 ab	40,00 a	53,33 ab	73,33 ab
1/1-10%	13,33 ab	30,00 a	60,00 a	70,00 ab
1/2-5%	3,33 b	10,00 b	30,00 ab	46,67 b

1/2-7,5%	3,33 b	6,67 b	16,67 b	63,33 b
1/2-10%	10,00 ab	30,00 a	53,33 ab	80,00 a
0/2-5%	3,33 b	10,00 b	26,67 ab	43,33 b
0/2-7,5%	3,33 b	10,00 b	20,00 ab	60,00 ab
0/2-10%	6,67 b	10,00 b	30,00 ab	56,67 ab
Control	3,33 b	13,33 b	20,00 b	46,67 b

Note : Mean in row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test

The highest population of *Callosobruchus spp* was occured on 0/2-5% and not significantly different with control. This showed that *P. cubeba* extract didn't give effect in supressing *Callosobruchus spp*. population growth. Increasing *Callosobruchus spp.* population caused higher seed damaged and seed moisture content. (Table 2).

Table 2. *Callosobruchus spp*. population, Seed moisture content (%) and mungbean seed damaged (%) after 2 months seed stored

Composition and concentration of *P.cubeba:A.muricata*	*Callosobruchus* spp.population	Seed moisture content (%)	Seed damaged (%)
2/0-5%	69,33 a	11,27 cd	4,23 ab
2/0-7,5%	25,67 a	10,40 cd	1,19 a
2/0-10%	13,67 a	9,73 d	1,31 a
2/1-5%	34,33 a	10,40 cd	1,60 a
2/1-7,5%	10,67 a	10,13 cd	0,87 a
2/1-10%	0,00 a	9,07 d	0,00 a
1/1-5%	28,00 a	10,73 cd	1,96 a
1/1-7,5%	16,67 a	10,47 cd	0,87 a
1/1-10%	0,00 a	8,77 d	0,00 a
1/2-5%	82,00 a	11,67 cd	6,43 ab
1/2-7,5%	33,67 a	10,00 cd	2,25 a
1/2-10%	8,00 a	8,90 d	0,32 a
0/2-5%	473,33 b	16,93 a	37,84 c
0/2-7,5%	90,33 a	12,03 c	7,65 b
0/2-10%	13,33 a	10,57 bc	2,24 a
Control	492,67 b	16,47 a	48,98 c

Note : Mean in row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test

Table 3 showed the mungbean seed damaged after 2 months in storage connecting with the weight loss of seed. The mungbean seed damaged increased with increasing their weight loss. Weight loss on control and 0/2-5% treatment was higher than other treatments because the population of *Callosobruchus spp* was high so many material that was consumed.

Seed treatment with compotition and concentration of *P.cubeba* and *A.muricata* seed extracts 0/2-5% and control no pesticides gave higher seed conductivity than another composition and concentration. They had low concentration of *A.muricata* (5%) and no extracts as biopesticides so can not kept seed from *Callosobruchus* spp.

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). Seed germination and seed vigor showed that on control and 0/2-5% treatment was lower than another treatment.

Table 3. Weight loss of mungbean seed (%); seed conductivity (m Hos); Percentage of seed germination and seed vigor after 2 months stored (%)

Composition and concentration of *p.cubeba:a.muricata*	Weight loss	Seed conductivity	Seed Germination	Seed Vigor
2/0-5%	1,27 bc	1,509 b	88,67 ab	82,67 ab
2/0-7,5%	0,66 c	1,495 b	93,33 a	88,67 ab
2/0-10%	0,48 c	1,450 b	95,33 a	90,67 a
2/1-5%	0,71 bc	1,437 b	94,67 a	88,00 ab
2/1-7,5%	0,41 c	1,499 b	95,33 a	89,33 ab
2/1-10%	0,14 c	1,382 b	84,00 ab	82,67 ab
1/1-5%	0,9 bc	1,571 b	85,33 ab	82,00 ab
1/1-7,5%	0,61 c	1,448 b	94,67 a	85,33 ab
1/1-10%	0,57 c	1,533 b	92,67 ab	83,33 ab
1/2-5%	1,85 bc	1,685 b	85,33ab	82,00 ab
1/2-7,5%	0,49 c	1,415 b	92,00 ab	89,33 ab
1/2-10%	0,47 c	1,461 b	93,33 a	86,67 ab
0/2-5%	8,13 a	2,927 a	72,67 b	70,00 b
0/2-7,5%	1,91 b	1,689 b	82,67 ab	80,00 b
0/2-10%	0,87 bc	1,493 b	90,67 ab	86,67 ab
Kontrol	6,42 a	2,484 a	64,00 b	54,00 b

Note : Mean in row (a,b,c) followed by the same letters are not significantly different at 5% level Duncan test

CONCLUSION

The conclusion were: 1) The the composition and concentration of *Piper cubeba* and *Annona muricata* seed extracts: 2/1-10% ;1/2-10% dan 1/1-10% had the highest of *Callosobruchus* spp. mortality and better seed vigor than other combination treatments 2) There had been decreasing mungbean seed quality on 2 month storage period.

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