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Toxicity of *Barringtonia asiatica* L. (KURZ.), *Melia azedarach* L. and *Annona muricata* L. Seed Extract Mixture Against Larvae *Crocidolomia pavonana* F. (Lepidoptera:Pyralidae)

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Abstract

Crocidolomia pavonana is the main pest of Brassicaceae family. *Barringtonia asiatica, Melia azedarach* and *Annona muricata* are plants that have a potency for insect pest control. Their insecticidal activities include poisoning, antifeeding and inhibiting the growth of larvae. This study aimed to determine insecticidal activities of seed extracts of *B. asiatica, M. azedarach* and *A. muricata* and their mixtures against *C. pavonana* F. Experiment was carried out in the Laboratory of Pesticide and Application Techniques, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran from August to November 2014. The experiment was arranged in a completely randomized desain (CRD) with 13 treatments and 3 replications. The treatments were the seed extract of *B. asiatica, M. azedarach, A. muricata* and their mixtures (1:1) at the concentrations of 0.1%, 0.5% and a control treatment. The results showed that the mixture of *B. asiatica* and *A. muricata extract* was effective to control *C. pavonana* larvae. At the concentration of 0.1%, it caused 100% larval mortality at 3 days after application. It had also antifeedant activity. It seems there is a synergistic effects between *B. asiatica* and *A. muricata* extract. Therefore, seed extracts of *B. asiatica*, *M. azedarach*, *A. muricata* and their mixtures have a potency to control *C. pavonana* larvae.

Keywords: *Annona muricata*, *Barringtonia asiatica, Melia azedarach, Crocidolomia pavonana*, extract mixture, mortality.

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1. Introduction

Cabbage head caterpillar, *Crocidolomia pavonana* F., is one of the most important pests on Brassicaceae family such as cabbage, broccoli, cauliflower, mustard greens, and turnip [1]. The yield losses caused by this pest can reach up to 100% [2]. The common method to control the insect pests of cabbage plants is the use of synthetic insecticides.

However, the use of synthetic insecticides may cause several negative effects such as pest resistance and resurgence, the emergence of secondary pests, the death of non-target organism, the presence of insecticide residues on the agricultural products, pollution on environment, and also endanger the user [3]. An important alternative to synthetic insecticides is botanical pesticide. There are several plants known to have insecticidal activities such as *Barringtonia asiatica* (Lecythidaceae), *Melia azedarach* (Meliaceae), and *Annona muricata* (Annonaceae).

B. asiatica or also known as Bitung, has been widely studied and known to contain toxic compounds such as Saponin. Dono *et al*. [4] showed that the methanol extract of *B. asiatica* seeds had a strong insecticidal activity against larvae of *C. pavonana.* It had also detrmental effects to the insect such as feeding inhibition, delaying the formation of eggs, lowering egg production, reducing fertilization, causing abnormality and shortening the life span of adult insects. Another example of plant that has been utilized as a botanical pesticide is *M. azedarach* or known as Mindi. Generally, insect pests that can be well controlled by extract of *meliaceae* plants are those with chewing and biting mouth types. Botanical pesticides with antifeedant and repellent activities can prevent insect pests to eat and lay eggs on the treated plants. *A. muricata* or known as *sirsak* is a plant from the family Annonaceae which was reported to have acetogenin compound [5]. Extract of this plant was effective against insect pests from the order of Lepidoptera, Coleoptera, Homoptera and Diptera [6]. Acetogenin can also be used as acaricides, bactericides and fungicides.

Farmers often mix several types of insecticides with the aim to increase their effectiveness in controlling insect pests. The use of botanical insecticide mixtures can have an advantage when there are synergistic effects among the components. A mixture of insecticides is categorized as synergistic if the mixture toxicity is higher than the toxicity of each component. This study aimed to evaluate insecticidal activities of *B. asiatica, M. azedarach* and *A. muricata* and their mixture against *C. pavonana*.

2. Materials and Methods

Experiment was arranged in a completely randomized design with 13 treatments and three replications. The treatments consisted of *B. asiatica* seed extract, *M. azedarach* seed extract, *A. muricata* seed extract and the mixtures of *B. asiatica* and *M. azedarach*, *B. asiatica* and *A. muricata*, and also *M. azedarach* and *A. muricata* at the concentrations of 0.1% and 0.5% and control.

2.1. Extraction of *B. asiatica, M. azedarach,* and *A. muricata*

The seeds of *B. asiatica*, *M. azedarach* and *A. muricata* were obtain from Jatinangor, Sumedang. Later, the seeds were cut into small pieces, dried, and blended into powder. Powdered seeds were soaked with organic solvent methanol at the ratio of 1:10 (w/v) for 72 hours, then filtered by using filter paper. The filtrate was then evaporated with a *rotary evaporator* at temperatures of 55-60°C and vacuumed at the pressures of 580-600 mmHg.

2.2. Rearing of Larvae *C. pavovana*

The test insects were early second instar of *C. pavonana*. The insect was obtained from cabbage grown in Jatinangor which was then reared in rearing room of Plant Pests and Diseases Department, Agriculture Faculty, Universitas Padjadjaran using the following procedures. The larvae obtained from the field were maintained in a plastic box measuring 34 × 28 × 7 cm containing soil. After that, the pupa were transferred into a cage of 44.5 × 44.5 × 49.5 cm and fed with 10% liquid honey absorbed in a cotton. Pesticide-free lettuce leaves were put in the plastic-cage as a place for egg laying. The eggs in the lettuce leaves were then collected and placed in a ventilated plastic container measuring 10 × 9 × 4.5 cm lined with filter paper at the bottom. After the eggs hatched, the larvae were transferred into a plastic box measuring 34 × 28 × 7 cm covered with the blotting paper and fed with the pesticide-free lettuce leaves. The hatched eggs were maintained until they became early second instar for the mortality tests.

2.3. Bioassay

Seed extracts of *B. asiatica*, *M. azedarach*, *A. muricata* and their mixtures were tested at 0.1% and 0.5% concentration. The method used in this test was feeding assay. Each extract tested was dissolved in a mixture of methanol (50 ml), agristick 0.5 ml/L, and distilled water until the final volume of 1000 ml. Two pieces of lettuce leaf measuring of 4 × 4 cm were dipped into each solution until wet at the both sides of leaf' surface, and then they were air dried on tissue paper. The leaves were placed in a petri dish which has been lined with filter paper. Furthermore, by using a brush, ten early second instar *C. pavonana* were placed in each petri dish. Control larvae were fed with the leaves which were only dipped with the solvent. The treated leaves were exposed to the larvae for 72 hours. After that, the larvae were fed with untreated leaves until they became instar IV. Each treatment was repeated for three times. The data of larval

mortality and feeding activity were statistically analysed by using analysis of variance, at confidence level of 95% using the SPSS version 17.

2.4. Observation

The variables observed were the mortality of Instar II and Instar IV, the developmental time of larvae, the weight of leaves consumed by larvae, and the weight of Instar IV. The mortality of larvae was measured by using the following formula:

$$\text{Mortality (\%)} = \frac{\text{number of dead } \textit{C. pavonana}}{\text{number of } \textit{C. pavonana} \text{ tested}} \times 100\%$$

The observation of larval growth was performed every day. The observation was begun starting from a day after application (DAA) to early Instar IV. The observation on leaves consumed larvae was carried out at 1-3 day after application. The leaves were weighed before being given to the larvae and then being weighed again in the following day. After treatment, the leaves were weighed and then dried in oven at the temperature of 95°C for 24 hours to gain the dry weight. The observation on larval weight was performed to the larvae reaching Instar IV stage. The larvae were then dried in oven at the temperature of 95°C for 24 hours to get the dry weight.

3. Result and Discussion

3.1. Effect of *B. asiatica, M. azedarach, A. muricata* extract and the mixtures on the mortality of *C. pavonana* larvae

Effect of *B. Asiatica, M. azedarach, A. muricata* extract and their mixtures at 1% concentration on the mortality of *C. pavonana* can be seen in Fig. 1. The most effective single extract was *A. muricata* as at 1 DAA it was able to cause 60% mortality and even reached 100% mortality at 3 DAA. The treatment of *B. asiatica* extract caused 83.3% larval mortality at 4 DAA. The treatment with *M. azedarach* extract did not cause mortality at 1 DAA, but the mortality reached 73.3% mortality at 7 DAA. The most effective treatment was the mixture of *B. asiatica* and *A. muricata*, and also *M. azedarach* and *A. muricata*. Both of these mixtures were able to cause 100% mortality after 3 DAA.

The visual observation on the dead larvae of *C. pavonana* treated by *B. asiatica* extract showed that the larvae had smaller size, blackening and dry. The compounds contained in *B. asiatica* which probably caused the death of larvae *C. pavonana* was saponins and alkaloids [4]. Saponins contained in the methanol extract of *B. asiatica* seed can reduce the activity of protease enzymes in the insects' digestive tract, thereby it affects the absorption of nutrient from food. This disruption causes the

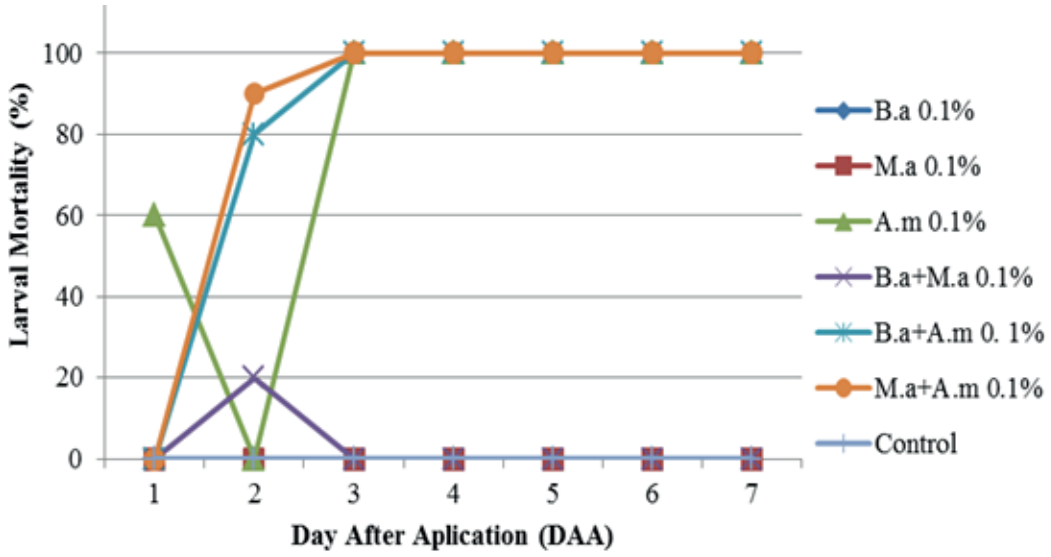

Figure 1: Mortality of larvae *C. pavonana* feeding on leaves treated with *B. asiatica, M. azedarach, A. muricata* extract and their mixtures at a concentration of 0.1%.

nutrients supply needed by the insect body become decreasing and may cause the death of the insects [7]. Herlt *et al.* [8] and Cannon *et al.* [9] reported that saponins as the main bioactive component from *B. asiatica* extract.

Larvae which feed on leaves treated with *M. azedarach* extract showed the symptoms of agility loss, the decrease of feeding activity, the change of the body colour (became black, and eventually they died). From the mortality rate graph (Fig. 1 & 2), it can be seen in the treatment of *M. azedarach* that all the concentration levels at 5 to 7 day after application there was still an increase of larval mortality although in small rate, while on the other single extract treatment which was approximately at 3 or 4 day after application, the mortality did not increase. Meliaceae plants are known to contain various compounds which can be utilized as insecticide, antifeedant, and also growth inhibitor that work slowly [10].

C. pavonana larvae which were treated by *A. muricata* extract had a characteristic of body color became black and very soft. This symptoms is likely due to the act active compounds in the extract which influence insect respiration. This resulted in the insects becoming paralyzed because the muscles and the tissues were lack of energy so that the cells and the tissues were dead. The death of cells and tissues caused the larvae' body turned into black and then they died. Annonain and squamosin contained in the seed of *sirsak* (soursop) can cause the death of insects' cells due to their cytotoxic and neurotoxic effect. Annonain and squamosin contained in *A. muricata* can cause the death of insects' cells, hence if these compounds are in contacts or enter the insects' body, it will deter the enzyme to bond with Nicotinamide Adenine Dinucleotide Hydrogen (NADH), with cytochrome c-reductase and cytochrome complex unit I inside mitochondria insects. As the result, cell respiration will be stopped due to loss of the

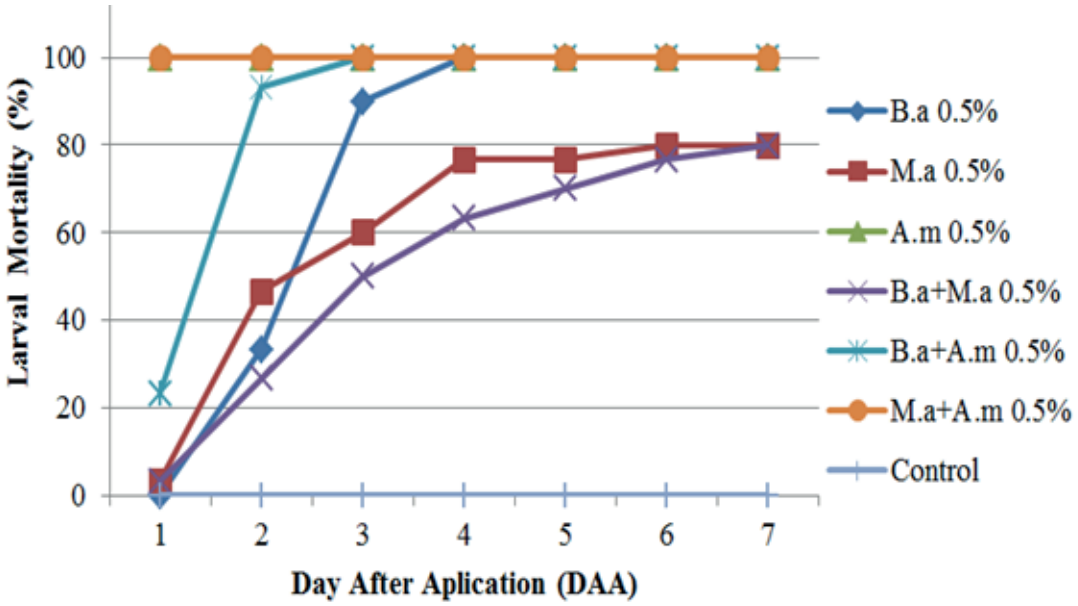

Figure 2: Mortality of larvae *C. pavonana* feeding on leaves treated with *B. asiatica, M. azedarach, A. muricata* extract and their mixtures at a concentration of 0.5%.

Table 1: The content of extract yield of *B. asiatica, M. azedarach,* and *A. muricata*.

Plants material	Dried Weight (g)	Extract Weight (g)	Rendement (%) W/W
B. asiatica	450	142.63	28.5
M. azaderach	465	21.65	4.3
A. Muricata	460	17.48	3.5

energy [11]. Extraction of *B. asiatica* seed had the highest yield value which was 28.5%. While the extraction of *M. azedarach* and *A. muricata* seeds had the lowest yield values which were 4.3% and 3.5% respectively (Table 1). The smaller the extract yield, the higher the amount of raw materials if the extract will be applied in the field. The synergistic effect of extract mixing is expected to be able to overcome the problem of raw material provision.

3.2. Effect of *B. asiatica, M. azedarach, A. muricata* extract and their mixtures on feeding activity of *C. pavonana* larvae

The average weight of leave consumed in each treatment is showed in Table 2. At 3 day after application, there was a significant difference in the amount of leave consumed in control, which was 41.7%, and the treatment of extract *B. asiatica, M. azedarach, A. muricata* and its mixture at 0.1% and 0.5% concentration. The treatment of *M. azedarach* and *A. muricata* mixture at 0.5% concentration had the lowest amount of leave consumed which was 2.71%. This may due to the high toxicity of the extract as it caused 100% mortality at one day after application. It is estimated that at the lower the concentration, the lower the toxicity. However, with the antifeedant effect

Table 2: The weight of leave consumed by *C. pavonana* larvae after treatment by *B. asiatica, M. azedarach, A. muricata* ekstract and their mixtures.

Treatment	Leaf consumed (%) ± SD
B.a 0.1%	17.87 ± 8.23 b
B.a 0.5%	15.72 ± 4.24 b
M.a 0.1%	15.19 ± 4.16 b
M.a 0.5%	12.48 ± 4.15 b
A.m 0.1%	15.99 ± 4.39 b
A.m 0.5%	2.83 ± 0.67 d
B.a + M.a 0.1%	9.04 ± 6.01 c
B.a + M.a 0.5%	8.93 ± 4.44 c
B.a + A.m 0.1%	18.99 ± 4.79 b
B.a + A.m 0.5%	14.26 ± 1.91b
M.a + A.m 0.1%	7.30 ± 1.06 c
M.a + A.m 0.5%	2.71 ± 0.19 d
Kontrol	41.70 ± 6.50 a
Note: Using The Scott Knott Test, Level 0.05%	
SD: Standard deviation, B.a: *B. asiatica,* M.a: *M. azedarach,* A.m: *A. muricata*	

of *M. Azedarach* and the extract mixture, the extracts had resulted in 100% mortality at 3 day after application. The mixture of *B. asiatica* and *M. azedarach* extract caused less amount of leave consumed compared to that of the treatment of single extract.

3.3. Effect of *B. asiatica, M. azedarach, A. muricata* extract and their mixtures on the developmental time of *C. pavonana* larvae

Effect of *B. asiatica, M. azedarach, A. muricata* and their mixture on the development of *C. pavonana* larvae could not be observed on all treatments since there were several treatments which caused 100% mortality. In general, each treatment caused slower developmental time (Table 3). Treatment of *B. asiatica* and *M. azedarach* extract mixture at 0.5% lengthened the developmental time of *C, pavonana*. Dadang and Prijono [12] stated that the growth and development of insect is influenced by the quality and quantity of the food consumed. The addition of particular compounds to the insects' food leads to the disruption of growth and development of the larvae.

3.4. Effect of *B. asiatica, M. azedarach, A. muricata* extract and their mixtures on the growth of *C. pavonana* larvae

From the observation of the weight of larvae that survived until instar IV, the treatment of 0.1% *B. asiatica* extract showed the lowest weight. The second lowest weight of

Table 3: Larval developmental time from instar II to IV of C. *pavonana* after being treated with *B. asiatica, M. azedarach, A. muricata* extracts and their mixtures.

Treatment	Developmental period (Day) ± SD			
	Instar II - III	(N)	Instar II - IV	(N)
B.a 0.1%	2.33 ± 0.67	18	4.8 ± 0.98	5
B.a 0.5%	-	-	-	-
M.a 0.1%	3.32 ± 0.75	11	4.63 ± 0.86	8
M.a 0.5%	3.57 ± 0.49	7	5.29 ± 1.28	7
A.m 0.1%	-	-	-	-
A.m 0.5%	-	-	-	-
B.a + M.a 0.1%	2.23 ± 0.52	22	4.06 ± 0.24	17
B.a + M.a 0.5%	3.5 ± 0.82	14	6.5 ± 1.12	6
B.a + A.m 0.1%	-	-	-	-
B.a + A.m 0.5%	-	-	-	-
M.a + A.m 0.1%	-	-	-	-
M.a + A.m 0.5%	-	-	-	-
Kontrol	2.13 ± 0.34	30	3.2 ± 0.4	30

Note: SD: Standard Deviation, B.a: *B. asiatica,* M.a: *M. azedarach,* A.m: *A. muricata*

larvae occurred at the treatment of 0.5% and 0.1% *M. azedarach* extract (Table 4). Saponin contained in *B. asiatica* extract is considered to contribute to the disruption of the metabolism of *C. pavonana* larvae. According to Widodo [13], saponins are able to bind to phospholipids which create cell membranes which can disturb the cell membrane permeability. The decrease in cell membrane permeability is possible to cause the toxic substances enter and interfere the larval metabolism process and the ATP formation process, so that the larvae will face energy deficiency and lead to the death [14].

4. Conclusion

The mixture of *B. asiatica* and *A. muricata* extracts was effective in controlling *C. pavonana* larvae since it caused 100% mortality at 3 DAA and influenced larval feeding activity.

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Table 4: The weight of *C. pavonana* larvae fed on leaves treated with *B. asiatica, M. azedarach, A. muricata* and their mixtures.

Treatment	Larval weight (g) ± SD	N
B.a 0.1%	0.0007 ± 0.0002	5
B.a 0.5%	-	-
M.a 0.1%	0.0013 ± 0.0006	8
M.a 0.5%	0.0010 ± 0.0006	6
A.m 0.1%	-	-
A.m 05%	-	-
B.a + M.a 0.1%	0.0018 ± 0.0005	17
B.a + M.a 0.5%	0.0017 ± 0.0015	6
B.a + A.m 0.1%	-	-
B.a + A.m 0.5%	-	-
M.a + A.m 0.1%	-	-
M.a + A.m 0.5%	-	-
Control	0.0035 ± 0.0012	30
SD: Standard deviation, B.a: *B. asiatica,* M.a: *M. azedarach,* A. m: *A. muricata*, and		
N: Number of larvae which survive in developmental period		

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