



Joint Action of *Azadirachta indica* and *Barringtonia asiatica* Seed Extracts against *Crocidolomia pavonana*

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ABSTRACT

A high population of *Crocidolomia pavonana* larvae reduces Brassicaceae crop productivity. To control the pest population, mixed plant extract as botanical insecticides is one of the alternatives. The purpose of this research is to evaluate the joint action between mixed extracts of *Azadirachta indica* and *Barringtonia asiatica* seed extracts against *C. pavonana* larvae and the effect on feeding behavior. The seeds are extracted with ethanol using the maceration method. Bioassays are conducted by a feeding method. Each level of concentration tested and controlled is repeated five times. The concentration-mortality relationship is analyzed using probit. Feeding behavior assayed by choice and no-choice at concentrations equivalent to LC_{25} , LC_{50} , and LC_{75} . The results show the mixture of *A. indica* and *B. asiatica* seed extract at a ratio of 3:2.3 has a strongly synergistic action with an LC_{50} of 0.04% and a combination index of 0.27. The extract mixture at a concentration of 0.02-0.08% is reduced feeding activity 77.16-92.84%. Further research is needed to evaluate the extract mixture in the field.

INTRODUCTION

Azadirachta indica and *Barringtonia asiatica* have been domesticated in Asia. The drought-resistant *A. indica* is widespread across India and Indonesia and grows well in drained and sandy soil. Roughly speaking, *A. indica* showed an anticancer activity by promoting the antioxidant enzyme, and modifying the intracellular components essential for cancer growth and development (Nagini, Nivetha, Palrasu, & Mishra, 2021). *Barringtonia asiatica* are widely grown in Indonesia. This plant is often found in botanical gardens and is rarely planted by the public. The fruit is dispersed in the same way as a coconut and is extremely water-resistant and buoyant. The seeds are ground to a powder and used to stun or kill fish (Umaru, I.J., Ahmed, Umaru, H.A., Umaru, K. I., & Samling, 2018). *A. indica* and *B. asiatica* seeds can be used for the manufacture of botanical insecticides (Dono, Natawigena, & Majid, 2012; Syahputra, 2010).

Using insecticides has become a part of our agricultural production system to control insect pests. Residues, pollution, and the killing of non-

target organisms are the impact of using synthetic insecticides. Alternative insecticides need to be found relatively safer, and botanical insecticides become an alternative (Biondi, Desneux, Siscaro, & Zappalà, 2012; Khater, 2012; Martinou, Seraphides, & Stavrinides, 2014; Miresmailli & Isman, 2014). The impact of botanical insecticides is less than synthetic insecticides. Botanical insecticides are easily degraded, do not pollute nature, and are safer for non-target animals and humans (Arnason, Sims, & Scott, 2012).

Botanical insecticides from plant extract can be used singly or in mixed form. A mixture of botanical insecticides minimizes the dependence of raw materials on one plant species to overcome the limitations of natural materials shortage. Mixing insecticides that action differently can delay the emergence of pest resistance and can increase application efficiency, especially when the mixture is synergistic (Lina, Dadang, Manuwoto, Syahbirin, & Prijono, 2015; South & Hastings, 2018)

Many plants have been known to possess insecticidal activity against insects, including seeds

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Edy Syahputra and Minarti : *Synergistic of A. indica and B. asiatica Seed Extracts*

of *Azadirachta indica* (Meliaceae) and seeds of *Barringtonia asiatica* (Lecythidaceae). The activity of *A. indica* plant extracts against several insect pests has been widely reported. The seed extracts *B. asiatica* were reported to have insecticidal activity against *C. pavonana* larvae (Dono, Natawigena, & Majid, 2012; Syahputra, 2010). Plants extract have a broad biological activity as well. In addition, plants extract can also have other bioactivities simultaneously, such as the feeding deterrent (Arivoli & Tennyson, 2013; Paul & Sohkhet, 2012). A mixture of seed extracts of *A. indica* and *B. asiatica* against *C. pavonana* larvae has not been reported. The purpose of this research is to evaluate the joint action mix seed extract of *A. indica* and *B. asiatica* against *C. pavonana* larvae and their effect on feeding behavior.

MATERIALS AND METHODS

Plant Extract Material

This research was performed in the Laboratory of Pesticides, Faculty of Agriculture, Universitas Tanjungpura from 2017 to 2018. Larvae of *Crociodolomia pavonana* larvae were used as test insects. Pesticide-free broccoli leaves feed the larvae.

Plant extract materials used in this test were seeds of *A. indica* (Meliaceae) and *B. asiatica* (Lecythidaceae) from West Kalimantan. The extraction was performed by cutting the seeds into small pieces and dried for several days (Syahputra, 2010). After drying, the material was blended and sieved with a gauze sieve (0.5 mm). The fine powder was extracted with ethanol (p.a) with a ratio of 1:10 (w / v) for 24 hours. The extract obtained was filtered with Whatman filter paper (No. 42). The filtering was evaporated with a rotavapor at a 55-60°C temperature and 580-600 mmHg pressure. The evaporated extract is stored in a refrigerator (\pm 4°C) and ready for use.

Insecticidal Activity Test

Insecticidal activity test was assayed using the residual method on leaves. The preparations were assayed tested at five levels of concentration which were expected to result from 1-99% in mortality of test insects through preliminary tests. The extracts were dissolved with acetone: methanol (1:1). The extract solution was applied evenly 25 μ l micro to each broccoli leaf disc surface (3 cm diam.). After the solvent evaporates, two treatment

leaf discs are placed on a 9 cm diameter petri dish that gives a tissue base. Subsequently, 15-second instar larvae were placed in each petri dish. As a control, larvae were fed with leaves smeared with acetone:methanol (1:1). Each level of concentration tested, and the control was repeated five times. The treated given for 48 hours, and then the larvae were fed leaves without treatment until they reached instar IV. Mortality of larvae was calculated and processed by probit analysis using the SAS program (SAS Institute Inc., 2011). The LC_{50} value obtained from every single extract was used to determine the ratio of the extract mixture. Based on the LC_{50} value, the percentage of the quote in the mix was 3:2.3 (LC_{50} 0.18% and 0.14%). The mixed extract assay was performed in the same manner as the single extract assay.

Analysis of Joint Action of Extract Mixture of *A. indica* and *B. asiatica*

The joint action of the mixed extract was analyzed based on an independent model. The combination index (CI) calculated at the LC_{50} level according to Chou & Talalay (1984):

$$CI = \frac{LC_{50}^{1(m)}}{LC_{50}^1} + \frac{LC_{50}^{2(m)}}{LC_{50}^2} + \left(\frac{LC_{50}^{1(m)}}{LC_{50}^1} \times \frac{LC_{50}^{2(m)}}{LC_{50}^2} \right) \dots\dots 1)$$

Where: LC_{50}^1 and LC_{50}^2 are LC_{50} extracts of *A. indica* and extract of *B. asiatica* in a separate test; $LC_{50}^{1(cm)}$ and $LC_{50}^{2(cm)}$ of each LC_{50} extract of *A. indica* and extract of *B. asiatica* in the mixture. LC_{50} level is gained by multiplying the LC_{50} mixture proportions of concentration extract *A. indica* and *B. asiatica*. The interaction was categorized base on the inverse co-toxicity ratio values: (1) $CI < 0.5$, strongly synergistic, (2) $CI 0.5$ to 0.77 , less synergistic, (3) $CI > 0.77$ to 1.43 , additive and, (4) $CI > 1.43$, antagonistic (Gisi, 1996; Kosman & Cohen, 1996).

Feeding Deterrent

The feeding deterrent was assayed using the *C. pavonana* third instar larvae with the leaf-discs choice method and leaf-discs no-choice method. Treatment procedures of extract application in this test were similar to the mortality test above.

Choice Method

In this test, the concentration extract tested was equivalent to LC_{25} , LC_{50} , and LC_{75} . Each treated and control leaf disc was alternately placed on the Petri dish (9 cm diam.). Before use, Petri dish lined with damp filter paper. Healthy larvae tests

were put into containers. Five larvae per dish were maintained in treatment for 12 hours. The test insect did not feed the dry weight of the broccoli leaf disc on the treatment, and the control was recorded. Data were analyzed using paired *t*-test on a 5% significant level.

No-choice Method

The procedure in this method was somewhat similar to the choice method above. In this method, treated leaf disc and control were put in other dishes. A completely randomized design with five replications was performed in this study. Data analysis was conducted with ANOVA and mean of leaf consumption by larvae analyzed with Tukey's range test at 5% significance level using a computer with the SAS program package (SAS Institute Inc., 2011). Percentage of feeding deterrent activity (FDA) were calculated by the formula 2 and 3.

$$FDA (\%) = [(C - T) / C] \times 100\% \dots\dots\dots 2)$$

for choice test and

$$FDA (\%) = [1 - (T / KC)] \times 100\% \dots\dots\dots 3)$$

for No-choice test

Where: T = average dry weight of the treated fed by test larvae, C = average dry weight of controls fed by test larvae.

RESULTS AND DISCUSSION

Synergistic Action Mix Extracts of *A. indica* and *B. asiatica* Seed Extracts

Based on the preliminary test, the concentration range of the single extract test is higher than the mixed extract (Table 1). The single seed extracts of *A. indica* and *B. asiatica* are tested at a concentration range of 0.05-0.30% and 0.05-0.20%, respectively. At the same time, the concentration range of mixed was tested at 0.01-0.15%. In the range of test concentrations, the two single extracts show LC₅₀ of 0.18% and 0.14%, respectively, while the mixed extract is 0.04% (Table 2). It indicates that the activity of the mixed extract possessed a stronger action than the insecticidal activity of the single constituent against *C. pavonana* (Table 2). This is in line with the calculation results of the combination index (IC), which is 0.27, indicating that the mixed extract is strong synergistic (Table 3). The LC₅₀ level of the mixed extract is not less than 4.5 times compared to the LC₅₀ level of the single extract of *A. Indica* and not less than 3.5 times compared to the LC₅₀ level of extract of *B. asiatica*. It means that the mixed extracts at a ratio of 3:2.3 are no less deadly 4.5 times and 3.5 times compared to the *A. indica* and *B. asiatica* extracts against *C. pavonana* larvae.

Table 1. Mortality of *C. pavonana* larvae treated with *A. indica* seed extract, *B. asiatica* seed and their mixed extract

<i>A. indica</i> seed extract		<i>B. asiatica</i> seed extract		Mixed extract (3 : 2.3)	
Concentration (%)	Mortality (%)	Concentration (%)	Mortality (%)	Concentration (%)	Mortality (%)
0.05	1.3	0.05	9.3	0.01	12
0.08	5.2	0.07	14.7	0.02	14.7
0.13	33.8	0.10	18.7	0.04	25.3
0.20	66.7	0.14	54.7	0.08	74.6
0.30	70.1	0.20	86.7	0.15	96

Remarks: ^a The number of early second instar larvae treated was 375, and control was 75.

Table 2. Parameters of the concentration-mortality relationship of *A. indica* seed extract, *B. asiatica* seed, and their mixed extract against *C. pavonana* larvae

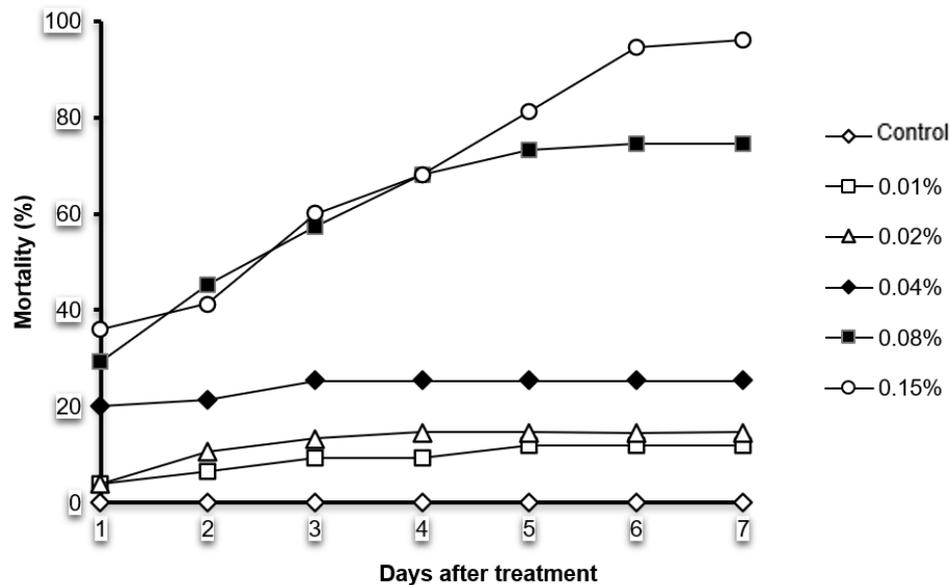
Extract	a ± SE	b ± SE	LC ₅₀ (CI 95%)(%)
<i>A. indica</i> seed extract	2.66 ± 0.48	3.58 ± 0.58	0.18 (0.13-0.26)
<i>B. asiatica</i> seed extract	-2.24 ± 0.26	16.12 ± 2.50	0.14 (0.13-0.16)
Mixed extract (3 : 2.3)	3.42 ± 0.78	2.57 ± 0.55	0.04 (0.02-0.11)

Remarks: a = interception of the regression line; b = slope of the regression line; SE = standard error; CI = confidence Interval

Table 3. Combination index and joint action of mixed seed extract of *A. indica* and *B. asiatica*

LC ₅₀	Combination index	Joint action*
0.04	0.27	Strongly synergistic

Remarks: * Joint action category (Gisi, 1996)

**Fig. 1.** The pattern of mortality of *C. pavonana* larvae treated with a mixture of *A. indica* and *B. asiatica* seed extracts

Several mixed insecticides from plant extracts or active compounds and their activities have been reported. Azadirachtin is a pure substance from *A. indica*, widely known as an active ingredient of insecticides. On the other hand, information on the insecticidal activity of extracts or purified compounds of *B. asiatica* and their mixtures is limited. Azadirachtin, due to their lower toxicity to mammals, their relatively less residue in the environment, and the common ability of insect pests to develop resistance against them, make these materials attractive and increase research interest (Isman, 2006). In addition, Azadirachtin has various other bioactivities, including antifeedant and inhibiting growth bioactive (Esparza-Díaz et al., 2010; Morgan, 2009; Wang & Shen, 2007). Azadirachtin has also possessed synergistic activity with biological pesticides and with other substances. The action of Azadirachtin and their mixtures on insect pests, for example, diamondback cabbage caterpillar *Plutella xylostella* and the armyworm *Spodoptera litura* have been reported (Mohan, Reddy, Devi, Kongara, & Sharma, 2007; Warnock & Cloyd, 2005).

The ethanol and methanol seed extracts of *B. asiatica* have insecticidal activity to *C. pavonana* larva and reduce oviposition of female *C. pavonana*. *B. asiatica* seeds ground in water were also active against larva of *C. pavonana* and effectively suppressed *C. pavonana* larvae in the field (Dono, Natawigena, & Majid, 2012; Syahputra, 2010). Saponin is the main active compound in the extract of *B. asiatica* acted as stomach poison (Agrell, Anderson, Oleszek, Stochmal, & Agrell, 2004). It reduces the activity of protease enzymes in insects' digestive tract, which disrupts the absorption process of insect food (Gershenson & Croteau, 1991). Arisanti & Dono (2015) reported that the activity of a mixture of *B. asiatica* seed extract and *A. indica* latex at 95% LC was antagonistic against *S. litura* larvae.

The mortality of *C. pavonana* larvae at one day after treatment (DAT) is low for all treatments (Fig. 1). Mortality at 2 DAT began to appear and generally increased with the greater the concentration tested. In 2 DAT, treatments at a concentration of 0.01-0.15% shows larval mortality of 6.7-41.33%. For all treatments, larval mortality generally increased only

slightly after 3 DAT. The pattern of mortality of *C. pavonana* larvae shows that the mixed seed extract of *A. indica* and *B. asiatica* work relatively slowly. Based on visual observations, among larvae treated with the extract mixture of *A. indica* and *B. asiatica* seed extracts show similar poisoning symptoms, namely developmental disorders and barriers to molting. The test larvae begin to lose mobility, the larval body shrinks and is black compared to the control. In the dead larvae, the insect's hormone system malfunctioning, and the larvae fail to molt.

The joint action of several mixed plant extracts at a certain mixing ratio against a particular insect pest can vary. A mixture of *A. indica* stem sap and *B. asiatica* seed extract at a ratio of 4.9:4.1 is antagonistic to the larvae of *Spodoptera litura* on the LC₉₅ level with ecotoxicity ratio value of 0.23%. A mixture of *Piper aduncum* leaf extract and *Tephrosia vogelii* at a concentration ratio of 1:2. synergistic against the larvae of *Scirpophaga incertulas* (Arisanti & Dono, 2015; Susanto & Prijono, 2015). The extract mixture *Brucea javanica*, *P. aduncum*, and *T. vogelii* has synergistic joint action to the larva of *C. pavonana* at LC₅₀ and LC₉₅ levels with IC of 0.2 and 0.19, respectively. Mixed extract between *Tephrosia vogelii* and *Piper aduncum* in emulsifiable concentrate formulation have synergistic action to the larva of *C. pavonana* with LC₅₀ were 0.15 and IC 0.2 (Lina, Dadang, Manuwoto, Syahbirin, & Prijono, 2015). The extract in a 1:1 ratio had LC₅₀ 0.056% and was synergistic to *Scirpophaga incertulas* larvae (Susanto & Prijono, 2015).

The efficiency of insecticide application can increase from using synergistic action of botanical insecticide and their mixture. A mixture of insecticides is used at a lower dose compared with the quantity of a single substance. Usually, the mixed botanical insecticides, with their synergistic action, reduce the raw materials used. The use of mixed botanical insecticides can overcome the limitation of raw materials in the field because their sources are not always abundant. Lower doses of mixed botanical insecticide also reduce the effect on non-target insects and the environment.

In addition, mixed botanical insecticides manage to delay the occurrence of insect resistance. Non-specific methods of mixed botanical insecticides are active on multiple target sites in the insect body, which means a non-specific way of action. According to this nature, insects would need

to counter some process adaptation to develop resistance to these extracts. *Myzus persicae* has developed resistance to Azadirachtin compared to neem seed extract. A mixture of active substances in botanical insecticide such *A. indica* (neem) might reduce insect resistance development compared with a single active substance (Tangtrakulwanich & Reddy, 2014).

Effect of Mixed Extract of *A. indica* and *B. asiatica* on Feeding Behavior

In the two methods of testing the feeding behavior, it appears that the mixture of *A. indica* and *B. asiatica* seed extracts has feeding deterrent activity (Table 4 and Table 5). In the equivalent concentration interval of LC₂₅, LC₅₀ and LC₇₅ tested, in the choice test, the extract mixture could suppress the feeding activity of *C. pavonana* larvae 67.4%, 69.2%, and 71.6%, respectively. While in the no-choice test, at LC₂₅, LC₅₀, and LC₇₅, the extract mixture could suppress the feeding activity by 20.6%, 42.2%, and 53.8%. This feeding deterrent or antifeedant activity is probably caused by the presence of foreign compounds in the mixed extract as a feeding inhibitor which can shorten feeding activity or stop feeding. Azadirachtin (limonoid) has marked antifeedant activity against a large number of insect species. Alkaloid capsaicin and nicotine from Solanaceae (*Solanum capsicum* and *Nicotiana tobacum*) possess antifeedant activity against some insect pests. Although antifeedants belong to various phytochemical classes, most are alkaloid, flavonoid, and terpene secondary compounds (Purrington, 2003).

Table 4. Feeding deterrent of mixed seed extract of *A. indica* and *B. asiatica* (3:2.3) by choice test

Treatment	Average weight of leaves fed (mm ² ± SD)		FDA (%)
	Control	Treatment	
0.02 (LC ₂₅)	1.23 ± 1.05	0.40 ± 0.28	67.4
0.04 (LC ₅₀)	1.15 ± 0.97	0.35 ± 0.20	69.3
0.08 (LC ₇₅)	0.96 ± 0.64	0.27 ± 0.27	71.6

Remarks: SD = Standard Deviation, FDA = Feeding Deterrent Activity. The average followed by the same letter is not significantly different for each concentration by paired *t*-test ($\alpha=5\%$).

Table 5. Feeding deterrent of mixed seed extract of *A. indica* and *B. asiatica* (3:2.3) by no-choice test

Treatment	Average weight of leaves fed (mm ²)	FDA (%)
Control	3.64 a	
0.02 (LC ₂₅)	2.89 ab	20.6
0.04 (LC ₅₀)	2.10 a	42.2
0.08 (LC ₇₅)	1.68 b	53.8

Remarks: FDA = Feeding Deterrent Activity. The average followed by the same letter is not significantly different for each concentration by Tukey's range test ($\alpha = 5\%$).

Compounds may also cause feeding deterrents in mixed extract preparations that cover or disrupt the stimulating food signals in the feed, as implications that larva of *C. pavonana* could distinguish between treated and untreated plant parts in the field. In general, it is concluded that in either choice or no-choice tests, the mixture of *A. indica* and *B. asiatica* seed extracts has feeding inhibition activity against the larva of *C. pavonana*. The mortality of this larva in the insecticidal activity test above is caused by the active compounds that have a deadly function. The active compounds contained in the mixed extract can also function as a feeding inhibition activity.

Acceptance of plants for feeding involves the central nervous system, which response to various factors that are attractive (stimulant) and inhibitory (deterrent). In this experiment, the inhibitory components in the mixed extract seemed to be sufficient to prevent the larvae from eating much of the leaves treated with various mechanisms. Insect pest control uses compounds that have food-inhibiting activity. Apart from being easily degradable and relatively non-toxic against non-target insects, this compound has several advantages, including not causing resistance (even helping to solve resistance problems) and having high selectivity. These advantages make feeding retardant compounds used in insect control, and their application could be combined with other control methods in Integrated Pest Control (IPC). Although the research results on feeding deterrents have been widely reported, the exact mechanism of feeding deterrents against insects has not been registered as a feeding barrier from various plant extracts or active components has been written by Paul & Sohkhet (2012) and Szczepanik, Grudniewska, Zawitowska, & Wawrzeńczyk (2014). For IPC, using a substance

with feeding deterrents is exhibited because of their selectivity (Nawrot & Harmatha, 2012). Mixtures of extract will also have amount activity, growth inhibitor activity, and feeding deterrent activity. The complexity of the active components of a mixed extract is needed because it can expand the target spectrum. The extract mixture may also reduce the potential for developing genetically resistant insects or lessen the potential for behavioral desensitization. These compounds could be used for IPC and integrated with other insect control techniques in IPC (Koul, 2008; Koul et al., 2004).

CONCLUSION AND SUGGESTION

The mixture of *A. indica* and *B. asiatica* seed extracts at a ratio of 3:2.3 in this test possess strongly synergistic against larva of *C. pavonana*. The mix of *A. indica* and *B. asiatica* seed extracts also has acted as a feeding inhibitor.

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