



Effect of *Castanopsis megacarpa* Extract on Mortality, Longevity Development and Feeding of *Crocidolomia pavonana* Larvae

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ABSTRACT

As several reports of impacts arise due to the use of synthetic insecticides, the investigation for safer alternative insecticides should be continued. Using plant material that has several bioactivities is a promising way out to control insect pest. The objective of this study was to evaluate the effect of leaf and seed extract of *Castanopsis megacarpa* on mortality, longevity development and feeding preference of *Crocidolomia pavonana* larvae. Extraction conducted by infusion method. The bioassay assesment was carried out using the residual method. The concentration-mortality relationship was analyzed by probit. The antifeedant test was tested using choice and no-choice methods. Both extracts showed the same strong insecticidal activity, LC_{50} of 0.18% (leaf) and 0.12% (seed). Leaf and seed extracts at concentrations equivalent to LC_{50} and LC_{75} in both methods used showed consistent antifeedant properties. The antifeedant activity of extracts in the no-choice method at both concentrations showed a range of activities of 40.1%-53.7% (leaf) and 74.4%-82.7% (seed). The leaf and seed extracts of *C. megacarpa* did not significantly extend the larval development period. Plant material that has more than one bioactivity will be better for suppress the insect pest populations in the field.

INTRODUCTION

The use of synthetic insecticides becomes an integral of an agricultural production system to control insect pests attack in many economically important crops up to this moment. The application of broad spectrum synthetic insecticides has induce several undesired side effects, such as soil and water pollutions and also the destruction of non-targeted (Biondi, Desneux, Siscaro, & Zappalà, 2012; Martinou, Seraphides, & Stavrindes, 2014). The alternative insecticides that are relatively safer to the environment were urgent to be investigated through the Integrated Pest Management Program scheme. One promising alternatives is that using plant materials that have insecticidal activity or botanical insecticides (Amoabeng, Johnson, & Gurr, 2019; Dubey, Shukla, Kumar, Singh, & Prakash, 2010; Khater, 2012; Miresmailli & Isman, 2014; Pretty et al., 2018).

The use of botanical insecticides in pest control has several advantages. Botanical insecticides are easily decomposed in nature so they less pollute the environment. They were also considered safe for humans and non-targeted animals (Arnason, Sims, & Scott, 2012). Botanical insecticides have a broad spectrum of the insects activity. In addition to have a lethal effect, botanical insecticides also have other simultaneous impacts such as the anti feedant effects (Arivoli & Tennyson, 2013; Koul, 2008; Nawrot & Harmatha, 2012; Paul & Sohkhet, 2012; Syahputra, Prijono, Dadang, Manuwoto, & Darusman, 2006; Zapata, Budia, Viñuela, & Medina, 2009).

Several plant species have been reported to have insecticidal activities, especially those under the families of Asteraceae, Fabaceae, Lamiaceae, Meliaceae and Annonaceae (Amoabeng, Johnson, & Gurr, 2019). Other plants from the family of Lecythidaceae have also been observed to exhibit

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similar feature (Syahputra, 2013). Most likely, many potential species of plants still yet unexplored. With the high diversity of flora in Indonesia's forests, the opportunity to find a plant species that has the insecticidal properties is widely possible.

Castanopsis megacarpa is a potential plant species that has been observed to have insecticidal properties. Our preliminary observation revealed that bark extract of *C. megacarpa* (Fagaceae) possess an insecticidal activity against the larvae of *Crociodolomia pavonana*. To elucidate deeper potential of the plants, then this research was conducted to evaluate the lethal effects and antifeedant activity of *C. megacarpa* leaf and seed extracts on *C. pavonana* larvae.

MATERIALS AND METHODS

Plant Material and Extraction

The leaves and seeds of *C. megacarpa* (Fagaceae) used in the study were taken from forests in Sajingan Besar District (Sambas, West Kalimantan) on February 2015. The extraction process was carried out through the following procedures. The seeds and leaves of *C. megacarpa* were cut into pieces and dried under direct sunlight for several days. After drying the cut leaves and seeds were then blended separately and filtered with 0.5 mm gauge sieve. 100 g of fine powder from the sieve dissolved with 1 L of 99.9% ethanol and then shaken using a magnetic stirrer for 24 hours. The filtered solution was then evaporated with a rotary evaporator to separate the extract from the solvent. The extract (crude) produced is stored in a refrigerator ($\pm 4^{\circ}\text{C}$) until used.

Test of Lethal Effects and Longevity of Development of *C. pavonana* Larvae

Lethal effect testing was carried out using the residual method on leaves. The test was conducted in Pesticides Laboratory, Agriculture Faculty, University of Tanjungpura. The evaluation was dedicated to find the rate of insect death from 0–100% in response to the concentration of the extract solutions. Leaf and seed extracts were tested at concentration of 0.05%; 0.1%; 0.2%; 0.3%; 0.5% and 0.025%; 0.05%; 0.1%; 0.2%; 0.4%, respectively.

The extracts were dissolved with acetone: methanol (1:1, v/v) at various concentrations that have been determined. The extract solution was applied evenly to each surface of 3 cm diameter broccoli leaf-disc using 25 μl per surface. After the

solvent evaporated, two leaf-disc of treatment were placed on a 9 cm diameter petridish with a tissue paper at the base. Subsequently, 15 larvae instar II were placed in each petridish. Control larvae were fed with leaves which were only smeared with acetone: methanol (1:1, v/v) 25 μl per surface. Each level of extract treatments and control were repeated 5 times. After 48 hours, all the larvae were maintained by feeding the leaves without any extract treatments until they reached instar IV stages. The number of death larvae and the longevity development of the survived larvae were recorded. Data on larval mortality was processed by probit analysis using the SAS program (SAS Institute, 2008). Longevity development data are expressed as mean \pm standard deviation.

Feeding Test

The test was conducted in choice and no-choice arrangements, using broccoli leaf-disc treated with extract suspension and exposed to third instar larvae of *C. pavonana*. The application of the extracts and the test procedures were similar with those in lethal effect experiment. Concentrations of the extract tested in this experiment were equivalent to LC_{25} , LC_{50} , and LC_{75} of mortality test. In choice test, two treated and two untreated (control) leaf-discs were arranged alternately around the edge of the dish (9 cm diameter) lined with moisten towel paper. Five third instar larvae (three hours old) were released into each dish and allowed to feed for 12 hours. A total of 25 larvae (5 larvae per dish) were used in each treatment. The dry weight of the remaining leaf-disc of the treatment and control that were fed by the larvae were recorded. Data were analyzed using a paired *t*-test on 5% significance level. Data on the choice test was processed by probit analysis using the SAS program (SAS Institute, 2008).

In no-choice test, treated and untreated leaf discs were placed in separate dishes. As in choice test, 5 larvae were placed into each dish and allow to feed for 12 hours. Each treatment was replicated 5 times and all the experiment units were arranged in a completely randomized design. The amount of leaf disc consumed by the larvae was measured after 12 hours and the data was analyzed statistically using Analysis of Variance to compare the effect of extract concentration on leaf consumption. Tukey's range test was applied to see the means difference between concentrations using the SAS program (SAS Institute, 2008).

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Percentage of antifeedant activity (AA) was calculated by the formula:

$$AA(\%) = [(C - T) / C] \times 100\% \quad \text{for choice test}$$

$$AA(\%) = [1 - (T / C)] \times 100\% \quad \text{for no-choice test}$$

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

RESULTS AND DISCUSSION

Effects on Mortality and Longevity of Development of *C. pavonana* Larvae

The results showed leaf and seed extracts of *C. megacarpa* were toxic to *C. pavonana* larvae. From probit analysis, the LC₅₀ leaf and seed extracts were 0.18%, and 0.12% respectively (Table 1). Both LC₅₀ values were not significantly different; indicated by the slope (b) of the regression line of both extracts that were overlapped. The small LC₅₀ value of leaf and seed extracts indicating both extracts possess a strong insecticidal activity against *C. pavonana*; slightly stronger than LC₅₀ of *Azadirachta indica* seed extract in our previous study (Syahputra, 2013).

The potential of seed and leaf of *C. megacarpa* as source of botanical insecticides

against *C. pavonana* as indicated by low LC₅₀ value, opens a wide possibility to be deeply and widely explored, including the range of targeted insects pests and investigation on the chemical properties. This finding also provides a new information on the variety of plant or plant parts that contain insecticidal properties against *C. pavonana*. Previously, several plant extracts have been known to pose a lethal effect against this larvae, including *Barringtonia sarcostachys* (Lecythidaceae) and *Piper retrofractum* (Piperaceae). The bark extract of *B. sarcostachys* possessed a strong lethal effect against *C. pavonana* larvae with LC measured at 0.14%. Mixture formulation between extract of *Piper aduncum* and *Tephrosia vogelii* in form of emulsifiable concentrate had strong insecticidal activity against *C. pavonana* with LC₅₀ formulation of 0.15% (Lina, Syahbirin, & Dadang, 2017; Syahputra, 2013). The methanol extract of *Castanopsis costata* stems bark showed activity against *Artemia salina* shrimp larvae with LC₅₀ of 71.2 ppm. These plant materials contained in flavonoid that have antimicrobial activity against *Staphylococcus aerens*, *Bacillus* sp. and *Serratia marcescen* (Nurtjahja et al., 2013; Sitepu & Bahar, 2019).

Table 1. Parameters of concentration-mortality relationship of *C. megacarpa* extract against *C. pavonana* larvae

Extracts	a±SE	b±SE	LC ₅₀ (CI 95%) (%)
Leafs	1.91 ± 0.19	2.59 ± 0.24	0.18 (0.16 - 0.21)
Seed	1.89 ± 0.55	2.10 ± 0.52	0.12 (0.05 - 0.57)

Remarks: a = interception; b = slope; y = ax + b; SE = standard error; CI = confidence interval

Table 2. Longevity of development of *C. pavonana* larvae treated *C. megacarpa* extract

Extracts	Concentrations (%)	Longevity ± SD (days)		Longevity ± SD (days)		Longevity ± SD (days)	
		Instar II	N	Instar II - III	N	Instar II - IV	N
Leaf	Control	2.14 ± 0.36	75	2.19 ± 0.46	75	4.07 ± 0.30	75
	0.05	2.36 ± 0.50	72	2.15 ± 0.49	71	4.11 ± 0.32	70
	0.1	2.29 ± 0.49	70	2.09 ± 0.37	69	4.77 ± 1.01	62
	0.2	2.26 ± 0.44	64	2.41 ± 0.73	59	4.44 ± 0.74	48
	0.3	2.39 ± 0.56	58	2.43 ± 0.78	58	5.00 ± 1.14	33
	0.5	2.15 ± 0.43	42	2.44 ± 0.58	42	4.82 ± 0.91	20
Seed	Control	2.05 ± 0.23	75	2.25 ± 0.47	75	4.48 ± 0.64	75
	0.025	2.16 ± 0.47	71	2.32 ± 0.58	72	4.67 ± 0.77	70
	0.05	2.06 ± 0.23	70	2.46 ± 0.57	69	4.78 ± 0.67	63
	0.1	2.11 ± 0.32	70	2.64 ± 0.61	69	4.44 ± 0.74	59
	0.2	2.11 ± 0.32	47	2.87 ± 0.50	43	5.20 ± 0.78	41
	0.4	2.33 ± 0.52	3	3.67 ± 0.58	3	7.50 ± 2.12	2

Remarks: SD = standard deviation; N = the number of larvae that were survived in the intended developmental period

Leaf and seed extracts of *C. megacarpa*, in general, did not prolong the development longevity of the survived larvae (Table 2). Both extracts tested in 48 hours showed larval mortality (Fig. 1 and Fig. 2) and continued to increase in subsequent observation. Based on the pattern

of larval development and mortality, where the mortality of larvae was high at the beginning of the observation and relatively constant in subsequent observations, the results indicates that the active compound of the extracts have fast work in causing larval mortality.

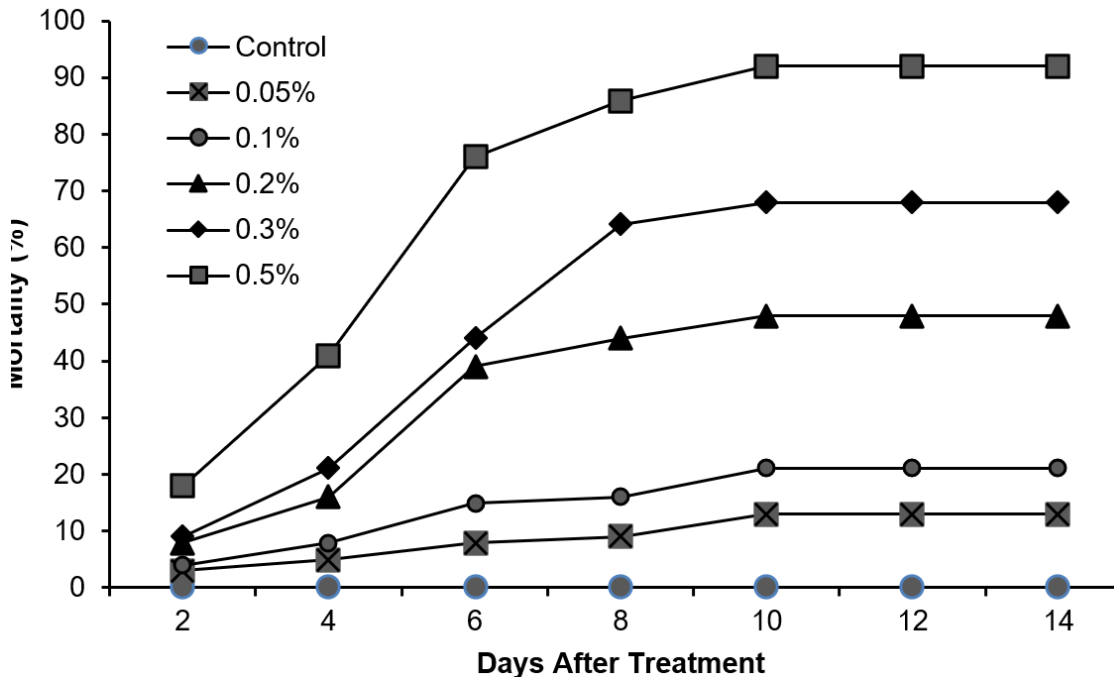


Fig. 1. Mortality of *C. pavonana* larvae treated with *C. megacarpa* leaf extract

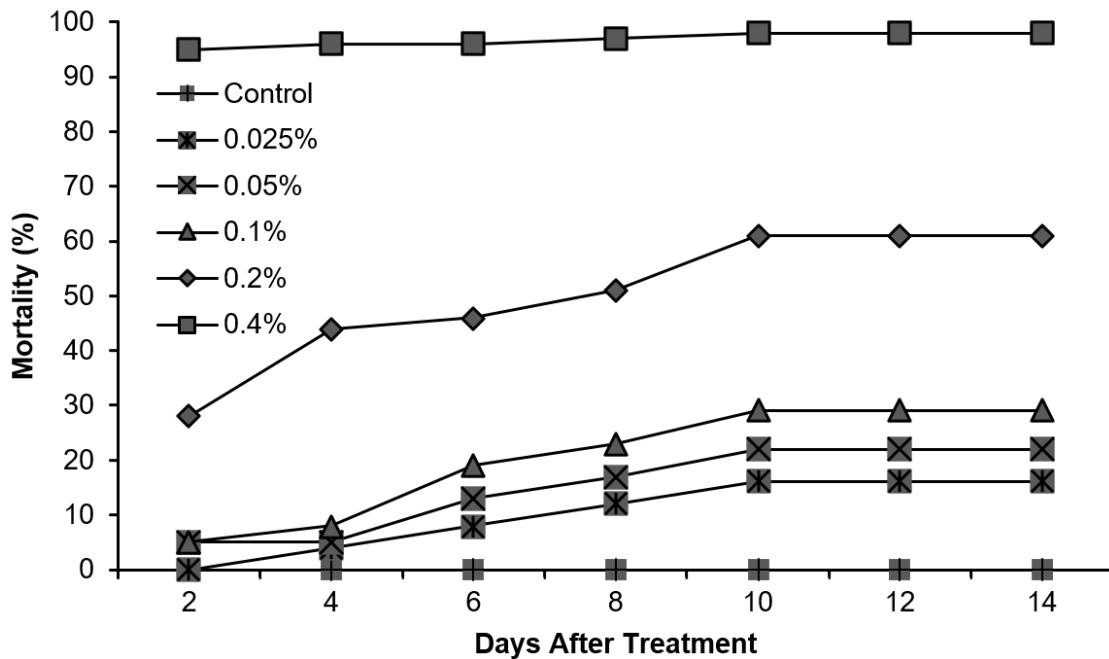


Fig. 2. Mortality of *C. pavonana* larvae treated with *C. megacarpa* seed extract

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Effect on Feeding

The results showed that leaf and seed extracts in all concentration tested in choice test suppressed the larvae feeding activity with has antifeedant activity of 34.4%-89.4%; 49.8%-76.1%, respectively (Table 3). In this test, the larvae consumed untreated leaves more than leaves treated with the extract. This result showed the presence of active substances on leaf-disc surface that can be detected and deterred larvae *C. pavonana* to feed. Yet, further studies are required to explore these active substances that act as feeding deterrent. Inhibitory compounds contained in the extract of *C. megacarpa* seem to disrupt feeding stimulation signals or to be able to cover. Similar results occur in the no-choice test. In the no-choice tested both of extract suppressed the larvae feeding with antifeedant activity of 15.5%-53.7%; 53.8%-82.7%, respectively (Table 4).

The data from choice-test and no-choice test complement each other in demonstrating antifeedant activity of *C. megacarpa* leaf and seed extracts. This implies that in the field, *C. pavonana* larvae able to distinguish between the treated and untreated crops. Indirectly, the *C. pavonana* larvae might die due to starvation. Some other *C.*

pavonana still exist for a certain period due to low feeding, before finally they died. In this experiment, at concentrations tested, the inhibitor component in the leaf and the seed extract of *C. megacarpa* seemed adequate to deter larval feeding. For both extracts, their feeding mechanisms should be examined more deeply after the pure component has been identified.

Feeding mechanisms are known for some active compounds. Various extracts and active components reported that work as feeding inhibitors (Paul & Sohkhet, 2012; Szczepanik, Grudniewska, Zawitowska, & Wawrzeńczyk, 2014), such as flavonoids isolated from cabbage was reported to act as feeding stimulants for *Plutella xylostella* (Lepidoptera: Xponomeutidae) (van Loon, Wang, Nielsen, Gols, & Qiu, 2002). For IPM, utilization of pest control materials contained antifeedant properties was considered safer for beneficial insects and non-target organisms, as these properties usually exhibit high selectivity (Nawrot & Harmatha, 2012). In the field, these compounds can be used for pest control and applied in combination with other control measures within integrated pest management (IPM) framework (Koul, 2008).

Table 3. The antifeedant activity of extract of *C. megacarpa* by choice test

Extracts	Concentrations (%, w/v)	Average weight of leaves fed (mg) ± SD ^a		Antifeedant Activity (%)
		Treatment	Control	
Leaf	0.09 (LC ₂₅)	1.2 ± 0.8 a	1.9 ± 0.4 a	34.4
	0.18 (LC ₅₀)	0.6 ± 0.3 b	1.8 ± 0.2 a	69.7
	0.33 (LC ₇₅)	0.2 ± 0.3 b	2.1 ± 0.8 a	89.4
Seed	0.06 (LC ₂₅)	1.4 ± 0.1 b	2.9 ± 0.6 a	49.8
	0.12 (LC ₅₀)	0.8 ± 0.6 b	3.0 ± 0.3 a	76.1
	0.26 (LC ₇₅)	0.6 ± 0.3 b	2.4 ± 0.5 a	73.9

Remarks: ^a SD = standard deviation; The average followed by the same letter for each concentration is not significantly different by paired *t* - test ($\alpha = 0.05$)

Table 4. The antifeedant activity of extract of *C. megacarpa* by no-choice test

Extracts	Concentration (%, w/v)	Average weight of leaves fed (mg)±SD	Antifeedant Activity (%)
Leaf	Control	0.8 ± 0.4 a	
	0.09 (LC ₂₅)	0.7 ± 0.2 ab	15.5
	0.18 (LC ₅₀)	0.5 ± 0.1 bc	40.1
	0.33 (LC ₇₅)	0.4 ± 0.3 c	53.7
Seed	Control	2.1 ± 0.7 a	
	0.06 (LC ₂₅)	1.0 ± 0.5 b	53.8
	0.12 (LC ₅₀)	0.6 ± 0.3 c	74.4
	0.26 (LC ₇₅)	0.4 ± 0.3 c	82.7

Remarks: SD = standard deviation; The average followed by the same letter for concentration in the same extract is not significantly different by Tukey's range test ($\alpha = 0.05$)

CONCLUSION AND SUGGESTION

Leaf and seed extracts of *C. megacarpa* possessed a strong insecticidal activity against *C. pavonana* larvae that are categorized as toxic with antifeedant properties to *C. pavonana* larvae. The efficacy of these extracts needs to be evaluated in the field.

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