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BIOINSECTICIDE TEST OF CRUDE STEM BARK EXTRACTS OF SOME MELIACEOUS PLANTS AGAINST *SPODOPTERA LITURA*

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Abstract

In the study of screening for bioinsecticides from plants, the activity of the stem bark extracts of some Meliaceous plants growth in Indonesia, namely *Aglaia odorata* Lour, *Aglaia odoratissima* Blume, *Aglaia elaeagnoidea* A.Juss, *Sandoricum koetjape* Merr. and *Xylocarpus moluccensis* (Lamk.) M.Roem was investigated. Solvent residues of these stem bark of plants were obtained from different solvent extracts (hexane, chloroform and methanolic extracts). All extracts dissolved in distilled water and added tween 80 (a few drops) as emulsifying agent were separately tested at various concentration (mg/L) continuously for 1, 2 and 3 days on the third instar larvae of the armyworm, *Spodoptera litura*. The results indicated the presence of bioinsecticide effect which was maximum of *Sandoricum koetjape.* This plant extracts (hexane and methanolic extracts) gave enough sensitive effects to the third instar larvae with LC_{50}s of 104.24 and 170.23 mg/L, respectively after 3 days of application. Meanwhile, other plant extracts showed much less sensitive and relatively insensitive after 3 days of application because their LC_{50} values were more than 200 and 1500 mg/L, respectively.

Keywords: *Bioinsecticide, Lethal Concentration* (LC_{50}), *Meliaceae,* Spodoptera litura.

1. Introduction

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous insect pest (Holloway, 1989). It is an indigenous pest of a variety of crops in South Asia and was found to cause more than 26-100% yield loss in groundnut (Dhir *et al*., 1992 as stated by Muthusamy *et al*., 2011). *Spodoptera litura* is one of the major lepidopteran pests of tobacco in Sumatra (Indonesia), but it is an important pest also on other crops such as groundnuts, potatoes, onions and cabbage (Kalshoven, 1981). Because of these reasons, the insect was used to screen bioinsecticide from some Meliaceous plants growth in Indonesia. An extensive programme of screening the Meliaceous plant extracts and isolates for biological activities (bioinsecticide) has also been done for sometime in our laboratory.

As known that the Meliaceae or the Mahogany family is a flowering plant family of mostly trees and shrubs (and a few herbaceous plants, mangroves) in the order Sapindales. Meliaceae is a tropical family of woody plants comprising approximately 51 genera and 550 species (Cronquist, 1993). Effective insecticidal properties were investigated in several plant species of various families, including Meliaceae. As reported that Meliaceae is known as plant sources of insecticides (Simanjutak *et al*., 1999). The genera belonging to Meliaceae used in this investigation are genus of *Aglaia*, *Sandoricum* and *Xylocarpus*.

The genus of *Aglaia* consists of more than 105 woody plant species, and more than 65 of them are found in Indonesia. The plants are important component of Indo-Malesian tropical forest because some of this genus provide edible fruit and the woods can be used in traditional medicines (Heyne, 1987).

On the other side, *Sandoricum* is a genus of plant in Meliaceae family that contains at least the following species *Sandoricum vidalii*, Merr. and *Sandoricum koetjape* Merr. But, the genus *Sandoricum* cultivated and growth in Indonesia is *Sandoricum koetjape* Merr. This plant having syns. *S. indicum* Cav., *S. nervosum* Blume, *Melia koetjape* Burm. f., is also known as *sentieh, sentol, setol, sentul, setul, setui, kechapi* or *ketapi,* in Malaya; *saton, satawn, katon,* or *ka-thon* in Thailand; *kompem reach* in Cambodia; *tong* in Laos; *sau chua, sau tia, sau do, mangoustanier sauvage,* or *faux mangoustanier in* North Vietnam. In the Philippines, it is *santor* or *katul;* on Sarawak and Brunei, it is *klampu.* In India, it may be called *sayai, sevai, sevamanu* or *visayan.* In Indonesia, this plant is called as *ketjapi* or *sentool*. It is commonly cultivated throughout these regions and the fruits are seasonally abundant in the local markets. It has also been introduced into China, Taiwan, Australia and into a few locations in Central America and Southern Florida. The plant is a medium-sized fruit tree native to southeast Asia and has been used in traditional medicine by local populations (Burkill, 1966 as stated by Pancharoen *et al*., 2005).

Furthermore, *Xylocarpus* is a genus of plant in the mahogany family (Meliaceae). It includes two or three species of mangroves, native to coastal mangrove forests of the Western and Central Indo-Pacific, from eastern Africa to Tonga (Raju, 2003). The genus *Xylocarpus* found in Indonesia is *Xylocarpus moluccensis* (Lamk.) M.Roem. The plant is distributed in the coastal regions of India, Ceylon, Burma, Malaya, and Indonesia. The fruit of plant is a green color, lemon fruit sized, hard and heavy, leading to the common name 'cannon ball tree'. Several biological properties have been attributed to *Xylocarpus moluccensis*, such as: aphrodisiac, fever, malaria, hair preservatives, astringent, antidiarrhoea, antiemetic and haemostatic properties. In Indonesia, the use of medicinal plants such as mangrove to cure several illnesses has been used routinely by coastal native (Prihanto, 2011).

By the way, the main objectives of the research was to do the bioinsecticide test of the plants above (see Table 1). The insecticidal bioactivity of crude stem bark extracts of these five species against the Asian armyworm, *Spodoptera litura* was investigated in this study and reported as follows.

2. Material and Methods

2.1 Collection of Plants

Stem barks of *Aglaia odorata*, *Aglaia odoratissima*, *Aglaia elaeagnoidea*, *Sandoricum koetjape* and *Xylocarpus moluccensis* were collected from different locations in different years (2010-2012) in East Java, Indonesia. The plants were identified by the Herbarium LIPI Staff, Purwodadi, East Java, Indonesia and specimens are held in the Herbarium.

Table 1. Plant Used for the Preliminary Bioinsecticide Screening

No.	Local name	Botanical name	Family	Part used
1.	Pacar Cina	*Aglaia odorata* Lour	Meliaceae	Stem Bark
2.	Pancal Kidang	*Aglaia odoratissima* Blume	Meliaceae	Stem Bark
3.	Kemubang	*Aglaia elaeagnoidea* A. Juss.	Meliaceae	Stem Bark
4.	Kecapi	*Sandoricum koetjape* Merr.	Meliaceae	Stem Bark
5.	Nyiri Batu	*Xylocarpus moluccencis* (Lamk.) M.Roem	Meliaceae	Stem Bark

2.2 Plant Extracts

Stem barks of the plants were air-dried, ground with a coffee grinder. Each powdered plant materials were sieved using a strainer. Three - five kilograms of each powdered plant materials was sequentially extracted with hexane, chloroform and methanol on *Aglaia odorata*; hexane and chloroform on *Aglaia odoratissima*; chloroform on *Aglaia elaeagnoidea*; hexane and methanol on *Sandoricum koetjape*; and hexane and chloroform on *Xylocarpus moluccensis* for a period of 24 hours each and then filtered, respectively. This extraction process was conducted with three replications, respectively. The filtered content was then subjected to vacuum rotary evaporator until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained were stored in sterilized bottles and maintained at room temperature. Furthermore, preparation of standard stock solution (ex. 6400 mg/L) was carried out by dissolving 1.6 g of crude extract in 100 mL of distilled water and added tween 80 (3 – 5 drops), the mixture then was shaken and filtered (if necessary). The solution was then entered into 250 mL volumetric flask and added distilled water until level sign to get the stock solution.

2.3 Obtained Insects

Asian armyworms (*Spodoptera litura*) used in this study were obtained from Balittas (Balai Penelitian Tanaman Tembakau dan Serat), Malang, East Java, Indonesia and laboratory-reared colony of *S. litura* has been maintained at the State University of Surabaya (Unesa), Indonesia for 3 years.

2.4 Bioinsecticide Test

Bioassay: Leaf discs (3 cm x 3 cm) of *Ricinus communis* L. (local name: *Jarak kepyar*) were used for bioassay tests, after washing it with tap water. The leaf discs were sprayed with various concentration of each of the plant extracts for twenty seconds, air dried at room temperature and kept in petri plates (9 cm dia). Bioassays were performed on 3rd instars larvae using the standard topical application procedure followed by J.A. Leatemia and M.B. Isman (2004). Different concentration of bioinsecticide was prepared and control with water. After treatment the larvae was released in 9 mm diameter Petri plate. Fifteen third instar larvae were released and each treatment as replicated four times. Observations for mortality were recorded for 24, 48 and 72 hours. Larvae were considered dead if unable to move in a coordinated way when prodded with a fine haired brush. The corrected mortality was calculated by using Abbott's formulation (1925) and formula data were subjected to probit analysis as described by Finney (1971).

3. Result and Discussion

Various concentrations of crude plant extracts used in this study included six concentrations started from 0 to over 1000 mg/L. These used concentrations that were subjected to 3rd instar larvae is almost similar to be reported by Leatemia and Isman (2004). The results of bioinsecticide test were shown in Table 2 as follows.

Table 2. Bioinsecticide Test of the Stem Bark of Meliaceous Plant Extracts After 1, 2 and 3 Days of the Insecticide Applications (DAA)

No.	**Extracts**	**Various Concentration (mg/L)**	**LC_{50} (mg/L)/ DAA**		
			1	**2**	**3**
1.	*n*-Hex. of AO	0, 200, 400, 800, 1600, 3200, and 6400	18094.14	15577.08	11624.74
2.	$CHCl_3$ of AO	0, 200, 400, 800, 1600, 3200, and 6400	11169.86	9629.06	10243.27
3.	MeOH of AO	0, 1600, 3200, 6400, 12800, and 51200	75372.24	50191.75	41705.11
4.	*n*-Hex. of AOs	0, 200, 400, 800, 1600, 3200, and 6400	19005.28	17376.31	11583.78
5.	$CHCl_3$ of AOs	0, 200, 400, 800, 1600, 3200, and 6400	11216.97	10480.50	7478.82
6.	$CHCl_3$ of AE	0, 100, 200, 400, 800, and 1600	1272.56	931.71	701.63
7.	*n*-Hex. of SK	0,100, 300, 500, 700, and 1000	1133.95	656.82	104.24
8.	MeOH of SK	0,100, 300, 500, 700, and 1000	564.77	370.50	170.23
9.	*n*-Hex. of XM	0,100, 300, 500, 700, and 1000	4734.40	938.90	237.60
10.	$CHCl_3$ of XM	0, 100, 200, 400, 800, and 1600	1272.56	971.12	701.63

AO: *Aglaia odorata,* AOs: *Aglaia odoratissima*, AE: *Aglaia elaeagnoidea*, SK: *Sandoricum koetjape*, and XM: *Xylocarpus moluccencis.*

As shown in the table 2, it can be reported several important notes as follows. **First**, it seems that the higher the concentration of the extracts of all species, the higher death rate of the larva related to the dose of the extract.

Second, all of hexane extracts of each different plant yielded LC_{50} value that are lower than the other extracts. Additional notes that chloroform extract of the selected plant gave LC_{50} value that are higher than the other crude solvents. It was predicted that chloroform is solvent extracting phenolic compounds where these compounds mostly gave strong insecticidal activity.

Third, all of *Aglaia* species selected in this reseach gave the lowest LC_{50} value after 3 DAA, especially for hexane and methanlic extracts of *Aglaia odorata*. The plant were least sensitive and relatively insensitive after 3 days of application because their LC_{50} values were very small i.e. 11624.74 and 41705.11 ppm, respectively. As reported in many literatures, genus *Aglaia* contained aromatic compounds with a cyclopenta[*b*]benzofuran skeleton (rocaglamide) representing a typical chemical character of it. This class of compounds was named flavaglines. The genus has received increasing scientific attention due to its bioactive potential. The biologically very active cyclopenta[*b*]benzofurans are mainly accumulated in the roots and stem bark (Brader *et al*., 1998). For instance, bioassays with lipophilic crude extracts of *Aglaia edulis* against *Spodoptera littoralis* displayed strong insecticidal activity. The insect toxicity of *A. basiphylla* was caused by the well known benzofuran flavaglines rocaglamide, desmethylrocaglamide, and aglafoline (Netnapis *et al*., 2006). But, these information seemly do not supported to the results in the investigations. This may be caused in methanolic extracts of it and is not single compounds.

Fourth, from five different plant species, LC_{50} value of *Sandoricum koetjape* was higher than that of the others. This plant extracts (hexane and methanolic extracts) gave enough sensitive effects to the third instar larvae with LC_{50}s of 104.24 and 170.23 mg/L, respectively after 3 days of bioinsecticide application (DAA). Even, LC_{50} value of the methanolic extract of the plant is the highest compared with the others.

Fifth, furthermore, *Xylocarpus moluccencis* gave LC_{50} value that is quite low after 2 and 3 DAA compared with all of selected *Aglaia* plants above. Talking about this plant, the stem bark of the plant has not been much researched in relation to be either insecticides or chemical contents. In this opportunity, we need to report that three compounds have been isolated from the stem bark of the plant, namely 2-ethylhexyl 4-methoxycynnamate, β-sitosterol and stigmasterol (Tukiran *et al*., 2012). The parts of this plant which has much been investigated to be either bioinsecticide (i.e. using larvae of *Brontispa longissima* (Gestro)) (Jun Li *et al*., 2010) or chemical constituents (Jing Zhang *et al.*, 2010 and Taylor, 1983) are their seed and timber.

Further studies are still now needed to investigate the other component on the part and also try to look for the exact mechanism of action for bioinsecticide action of the plant extracts.

Conclusion

The results indicate the presence of bioinsecticide effect which was maximum of *Sandoricum koetjape.* This plant extracts (hexane and methanolic extracts) gave enough sensitive effects to the third instar larvae with LC_{50}s of 104.24 and 170.23 mg/L, respectively after 3 days of application. Meanwhile, other plant extracts showed much less sensitive and relatively insensitive after 3 days of application because their LC_{50} values were more than 200 and 1500 ppm, respectively. The present study showed that crude stem bark extract of *Sandoricum koetjape* is a promising candidate as a botanical insecticide.

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