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Bioactivity of Sweet Flag (*Acorus calamus* Linnaeus) Essential Oils Against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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ABSTRACT

The study aims to determine the chemical compounds, toxicity, and antifeedant activity of sweet flag (*Acorus calamus*) essential oils against third instar larvae of *Spodoptera litura*. The study was conducted using a completely randomized design (CRD) using various concentration of the essential oils (10^3, 2×10^3, 3×10^3, 4×10^3, 5×10^3 ppm). Mortality and antifeedant activity was observed 24 hours after treatment. Toxicity and antifeedant activity values were 92.5% and 79.3%, respectively, with an LC_{50} value 586.96 ppm. Gas chromatography-mass spectrometry analysis showed that essential oil of *A. calamus* consists of five chemical compounds: methyl isoeugenol, 3.9-decadien-ol-1,3-methyl-6-(1-methylethenyl), 4-pentyl-1-(4propylcyclohexyl)1cyclohexene, γ-asarone and β asarone.

Keywords: *Acorus calamus, antifeedant, essential oils, mortality, Spodoptera litura*

INTRODUCTION

Asian armyworm (*Spodoptera litura*) is a polyphagous insect that is abundant in tropical countries. It atttacks more than 120 species of cultivated crops at vari - ous phases of growth causing serious yield loss [1]. The spread of *S. litura* in Indonesia covered 22 provinces with an area of attack reaching 11.163 ha/year [2].

Secondary metabolites of plants or botanical insecticides can be used to *S. litura*. There are over 2400 species of plants that are potential sources of bioinsecticide [3]. Bioinsecticides provide benefits for both environment and agriculture product consumers. For example, the application of botanical insecticide can cause mortality at the time of application and residue left behind will immediately decompose, so the plant is safe for consumption. Botanical insecticides also can kill or disturb insect pests through separately or sinergistically combination, their works very specific like disturbing the development of eggs, larvae, and pupae, inhibits change of skin, disrupting the communication of insect, inhibiting reproduction of female insects, reducing appetite (antifeedan), block the ability of eating insects and repelent insects [4]. Botanical insecticide can even inhibit insects already resistant to active ingredients from conventional pesticides because there are different compounds in biopesticides that act simultaneously [5].

Sweet flag (*Acorus calamus)* rhizome, a member of family Acoraceae, contains saponins, flavonoids and essential oils. *A. calamus* essential oil has been documented to have insect-repellent, antifeedant and chemosterilant activities [6-7]. Its essential oil is toxic to third-instar larvae of *Plutella xylostella* [8] and has an antifeedant activity against the third instar larvae of *S. litura* and *Peridroma saucia* [9]. *A. calamus* essential oil exhibits larvicidal activity against third instar larvae of *Aedes aegypti* with LC_{50} value 150 ppm [10], and early fourth instar larvae of *Culex quinquefasciatus* with LC_{50} value 63.4 mL/L [11]. Hexane and methanolic fraction of *A. calamus* rhizome also has termiticidal activity against *Coptotermes curvignathus* [12]. Given the potential of *A.calamus* as a candidate

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biopesticide, in this paper, we analyzed the bioactivity of its essential oil against *S. litura* larvae and characterized the bioactive components.

MATERIALS AND METHODS

Plant materials

The rhizome of sweet flag (*A. calamus)* were collected from Semambung Village, Purwodadi District, Pasuruan. The rhizomes were around 1 year old and had the dimensions: 5-10 cm (length) and 1-2 cm (diameter).

Essential oil distillation and gas chromatography- mass spectrometry (GC-MS) Analysis

The rhizomes were washed after collection to remove dust and other contaminants, sliced and dried under a shade at room temperature (30 ± 2°C) for 4 days. The plant material was hydro-distilled for 6 hours. The oil obtained was desiccated with anhydrous Na_2SO_4 to remove excess water and stored in a refrigerator at 4°C prior to chemical analyzed and bioassay.

The volatile constituents were analyzed by gas chromatography- mass spectrometry (GC-MS) using a Shimadzu QP 2010S. The parameters used were: temperature range, 80-250 °C, rate of temperature increase, 10°C; helium carrier gas pressure, 100 kPa; the total injection rate, 588.8 mL/min; and split ratio, 1:400. The identification of the components was based on a comparison of their mass spectra with those of WILEY8.LIB. Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area percentages [11].

Rearing of *Spodoptera litura*

Egg mass of *S. litura* was collected from Indonesian Sweetener and Fiber Crops Research Institute (ISRFI), Malang. The eggs were reared and allowed to hatch under laboratory conditions 28°C and 74% relative humidity. After hatching, the neonate larvae were fed castor leaves (*Ricinus communis*) in the laboratory until third instar larvae emerged [13].

Larvicidal activity

Larvacidal activity of the essential oil was determined using the leaf dipping method [14]. The experiment was conducted at laboratory temperature of 28°C and 74% relative humidity.

The stock solution was prepared by dissolving the essential oil with Tween 80 as an emulsifier. A small square disc (4 × 4 cm^2) was cut from the fresh castor leaves. The leaves were dipped for 10 seconds in various concentrations of the essential oil (10^3, 2 × 10^3, 3 × 10^3, 4 × 10^3, 5 × 10^3 ppm) and then air-dried [15]. The assay was performed in a humidified jar (11 cm (diameter) × 20 cm (high)). To avoid early drying of the leaf disc, moist filter paper was placed inside the jar. Twenty early third-instar larvae *S. litura,* that have been pre-starved for 6 hours were introduced to the treated and control leaf disc. The larvae were continously maintained on untreated fresh castor leaves for 24 hours. Four replicates with one control were used for every treatment concentration tested. Larval mortality was calculated after 24 hours until 96 hours. The lethal concentration LC_{50} were calculated by probit analysis. Percentage larval mortality was calculated using formula:

$$\frac{Number\ of\ dead\ larvae}{Number\ of\ treated\ larvae} \times 100\% \quad (1)$$

Antifeedant activity

Antifeedant activity was tested by no-choice leaf-disc method [14]. Leaf weight consumed by the larva in the control and the treatments were determined after 24 hours until 96 hours. Four replicates with one control were maintained for each concentration. The percentage antifeedant activity was calculated by formula:

$$1 - \frac{Leaves\ weight\ treatment\ consumed}{Leaves\ weight\ control\ consumed} \times 100\% \quad (2)$$

Statistical analysis

The analysis of variance (ANOVA) was calculated using *SPSS ver. 18* for *Windows* using level of significance (α) 0.05. Significant differences between treatment were determined by Duncan Multiple Range Test (DMRT). The data mortalities were corrected using Abbott's formula [16] and LC_{50} values were calculated using probit analysis [17].

RESULTS AND DISCUSSION

Essential oil chemical composition

The yield of *A. calamus* essential oil was 0.88% (V/W). The essential oil was yellow in color and has a strong aroma. GC-MS analysis of the essential oil of *A. calamus* rhizomes led to identify and quantify of 5 (five) components (Figure 1).

The chemical compound of GC-MS essential oils of

Table 1. Chemical compound of sweet flag (*A. calamus*) essential oils

Peak	*Retention time (min)*	*Molecular formula*	*Estimated Name*	*Molecular mass (M^+, g/mol)*	*Relative area (%)*
1	11.199	$C_{11}H_{14}O_2$	Benzene, 1,2 dimethoxy-4-(1-propenyl) (Methyl isoeugenol)	178	0.63
2	11.845	$C_{14}H_{24}O$	3,9-Decadien-1-Ol, 3-Methyl-6-(1-Methylethenyl)	208	0.05
3	12.107	$C_{20}H_{36}$	4-Pentyl-1-(4-Propylcyclohexyl)-1-Cyclohexene	276	0.85
4	12.711	$C_{12}H_{16}O_3$	1-Allyl-2,4,5-Trimethoxy-Benzene (γ-asarone)	208	0.31
5	13.361	$C_{12}H_{16}O_3$	Benzene, 1,2,4-Trimethoxy-5-(1-propenyl)-(Z) (β- asarone)	208	98.16

Tabel 2. Percentage mortality of *S. litura* larvae

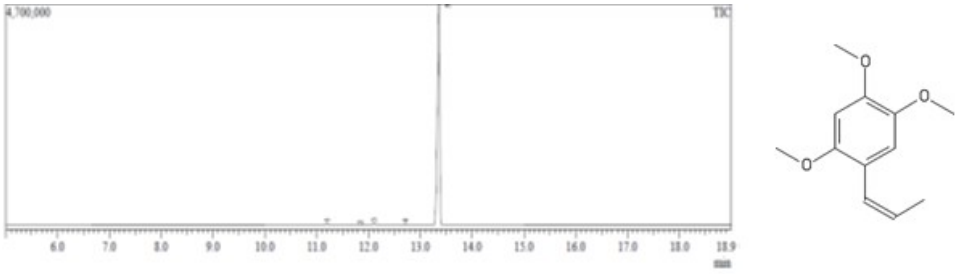
Treatment	*Mortality of larvae (%)*			
	24 HAT	*48 HAT*	*72 HAT*	*96 HAT*
(P0K0) Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
(P1K1) Essential oil 10^3	10.00 ± 4.08^b	35.00 ± 7.07^b	56.25 ± 8.53^b	72.50 ± 2.88^b
(P1K2) Essential oil 2 × 10^3	15.00 ± 4.08^b	41.25 ± 4.78^b	56.25 ± 10.30^b	78.75 ± 13.14^{bc}
(P1K3) Essential oil 3 × 10^3	17.50 ± 6.45^b	43.75 ± 2.50^{bc}	62.50 ± 2.88^{bc}	83.75 ± 6.29^{cd}
(P1K4) Essential oil 4 × 10^3	27.50 ± 6.45^c	51.25 ± 7.50^{cd}	68.75 ± 4.78^c	91.25 ± 6.29^d
(P1K5) Essential oil 5 × 10^3	33.75 ± 6.29^c	53.75 ± 7.50^d	68.75 ± 7.50^c	92.50 ± 2.88^d

Note:

- HAT: hours after treatment
- Figures followed by the same letters in the same column are not significantly different based on DMRT test (α = 0.05%)

Table 3. Percentage antifeedant activity of *S. litura* larvae

Treatment	*Mortality of larvae (%)*			
	24 HAT	*48 HAT*	*72 HAT*	*96 HAT*
(P0K0) Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
(P1K1) Essential oil 10^3	10.00 ± 4.08^b	35.00 ± 7.07^b	56.25 ± 8.53^b	72.50 ± 2.88^b
(P1K2) Essential oil 2x10^3	15.00 ± 4.08^b	41.25 ± 4.78^b	56.25 ± 10.30^b	78.75 ± 13.14^{bc}
(P1K3) Essential oil 3x10^3	17.50 ± 6.45^b	43.75 ± 2.50^{bc}	62.50 ± 2.88^{bc}	83.75 ± 6.29^{cd}
(P1K4) Essential oil 4x10^3	27.50 ± 6.45^c	51.25 ± 7.50^{cd}	68.75 ± 4.78^c	91.25 ± 6.29^d
(P1K5) Essential oil 5x10^3	33.75 ± 6.29^c	53.75 ± 7.50^d	68.75 ± 7.50^c	92.50 ± 2.88^d



(a) (b)

Figure 1. Chromatogram of sweet flag (*A. calamus*) essential oils (a). β-asarone structure (b)

sweet flag (*A. calamus*) can be show in Table I. The chromatogram shows chemical compounds with the highest relative area 98.16%. These are benzene, 1,2,4-trimethoxy-5-(1-propenyl)- (Z) or (β-asarone) (Figure 1b). There are 4 other chemical constituents that have percentage relative areas < 1%.

Larvacidal activity of Spodoptera litura larvae

The application of essential oils affect led to a dose-dependent increases in mortality of *S. litura* with DMRT at 5% level (Table 2). Based on Table 2, P1K4 and P1K5 are not significally different and P1K5 shows the highest percentage mortality (92.5%) with LC50 values of 586.96 ppm. Treated larvae exhibit both a decrease in mobility and feeding activity. The larvae have become shriveled and smaller, and the body color has changed from brown to brown-black (Figure 2b). The change in the pigments may be due to the melanization process which is a defense mechanism of the insect against foreign compound [18] Melanization is influenced by phenoloxidase that play roles in wound healing and cuticle formation [19].

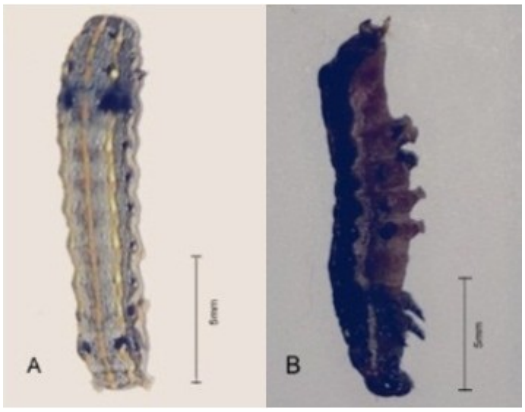


Figure 2. *S. litura* larvae normal (A) and after exposure by *A. calamus* essential oil (B)

Mortality of *S. litura* larvae is affected by the chemical compounds in essential oils, particularly by β-asarone (Figure 1b). β-asarone serves a contact and stomach poison [6]. β-asarone is a contact poison that penetrates the body through the cuticle layer towards hemolymph affecting the nervous system by targeting acetylcholinesterase [20]. While the acetylcholinesterase enzyme degrades acetylcholine into acetyl co-A and choline at the synapes, the build up of acetylcholine can disturb delivery system impulses to the muscle cells. The insect becomes spastic and paralyzed, and eventually dies [21]. When ingested, β-asarone can damage the insect's intestinal wall which can lead to death. β-asarone penetrates and disrupts the function of the mesenteron, a tissue layer composed of epithelial cells that absorb nutrients and secrete digestive enzymes in insects. The late-instar *S. litura* larvae treated with 500 and 1000 ppm essential oil orally have been earlier demonstrated to have abnormal hemocyte profile [22]. The cell counts of different types of hemocytes (plasmatocytes, prohemocytes, oenocytoids, spherulocytes, granular hemocytes) decreased after 48-72 hours after treatment. Total hemocytes decreased 29.15 and 49.05% at 500 and 1000 ppm after 72 hours.

Antifeedant activity of Spodoptera litura larvae

Application of essential oils in various concentration level shows a dose-dependent increase in antifeedant activity of *S. litura* larvae (Table 3). Based on Table 3, the antifeedant activities of P1K4 and P1K5 are not significally different from each other. Also, P1K5 showed the highest percentage antifeedant activity (79.3%). Antifeedants are defined as substances that can reduce consumption or feeding by an insect through a direct action on peripheral sensilla [23]. Antifeedant activity was induced by the chemical compounds in the essential oil that inhibitted appetite and of the larvae eventually leading to starvation and death [15]. The inhibitory chemical compounds have an effect on the transmission of signals to the taste receptors in insect gustatory sensilium [24]. Essential oil derived from *A. calamus* at 0.5-1% concentrations was found to significantly induce antifeedant activity and inhibition of growth third instar larvae of *S. litura* [8]. Asarone isolated from *A. calamus* also has potential as a growth inhibit and antifeedant of *Peridroma saucia* Hubner. asarone leads to 100, 83 and 40% mortality in *Nilaparvata lugens* at concentrations of 1000, 500 and 250 ppm. In third instar larvae of *Plutella xylostella*, the mortality after asarone treatment is 83 and 50% at 1000 and 500 ppm. It is not effective against adult females of *Myzus persicae* and the third instar larvae *S. litura* even at 2000 ppm [25].

CONCLUSION

A. calamus essential oil has potential as a biological insecticide against *S. litura* larvae with β-asarone as the major bioactive constituent. The application of *A. calamus* essential oil was to kill and reduce feeding activity in *S. litura* larvae by 92.5% and 79.3% respectively with LC50 of 586.96 ppm.

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