

The Potency of *Ageratum conyzoides* as Biopesticide

Yuliani^{1,*}, Yuni Sri Rahayu²

¹ Department of Biology Universitas Negeri Surabaya Surabaya, Indonesia

² Department of Biology Universitas Negeri Surabaya Surabaya, Indonesia

*Corresponding author. Email: yuliani@unesa.ac.id

ABSTRACT

Ageratum conyzoides, which contains various secondary metabolites such as alkaloids, flavonoids, phenols, hydroquinone, tannins, and essential oils, can be used as herbicide or insecticide. The purposes of this study were to describe the effect of methanol extract of *A. conyzoides* leaves on mortality of *Spodoptera litura* larvae and determine LC₅₀ and LC₈₀ of *A. conyzoides* methanol extract. This study used completely randomized design with two factors; concentration of *A. conyzoides* leaves extract (0%, 6%, 8%, 10%, 12%) and altitude from where *A. conyzoides* leaves were collected (highland, medium land, and lowland). Experiment was replicated six times. Larvae mortality rate was analyzed using ANOVA followed by Tukey multiple range tests, while LC₅₀ and LC₈₀ was analyzed using probit analysis. Results showed that there was significant differences of *S. litura* larvae mortality rate between *A. conyzoides* extract concentration (0%, 6%, 8%, 10%, and 12%), but no difference was found in the mortality rate between different locations. The higher the concentration of extract, mortality rate of *S. litura* larvae was also increased. Extract at 12% concentration resulted in highest mortality of *S. litura* larvae at 95%. LC₅₀ of *A. conyzoides* extract was found to be 3.45-3.87%, while LC₈₀ was 7.76-8.18%.

Keywords: Potency, *Ageratum conyzoides*, Biopesticide

1. INTRODUCTION

Plant secondary metabolites, such as tannins, alkaloids, flavonoids, saponins, and phenols, are produced by plants and used for defense mechanism against insects, because these compounds are able to inhibit insect metabolism process [1]; [2]. Insecticidal secondary metabolites effects including early death, lowering growth rate, shortening lifespan, abnormality in morphology, and restlessness and other abnormal behaviors.

Secondary metabolites, such as phenols, that act as feeding deterrents to inhibit insect development. Some phenols also attract natural enemies of the insect pests so the predators of insect will come and kill the insect [3]. Phenols can also dissolve lipids from cell walls, which affect cytoplasmic membrane integrity, thus inducing cell lysis and inhibit ATP-ase binding to cell membrane. Saponins can be used as insecticide; in addition to its ability to control *Aedes aegypti* larvae, saponins can also induce mortality in adult mosquitoes and act as repellent that prevent them from approaching.

Ageratum conyzoides, or locally known as babadotan, is a plant traditionally used to control insect pest [4] [5]. This plant contains various secondary metabolites, such as saponins, polyphenols, coumarins, eugenols, essential oils, alkaloids, tannins, sulfur, flavonoids, alkaloids, chromenes, benzofurans, and terpenoids (monoterpenes and sesquiterpenes) [6]. Study of *A. conyzoides* leaf oil as insecticide showed that at 0.5% concentration, it could induce mortality in more than 90% *Spodoptera litura caterpillar* [7]. Jaya and his colleagues reported that Essential oils (EOs) from *A. conyzoides* L., could be used against *Tribolium castaneum* [5].

A. conyzoides Linn contain alkaloids compounds with chemical structures in the form of Lycopsamin and Echinatin, which have been developed as natural pesticides. Both of these compounds are toxic against Lepidoptera insects, *Aedes aegypti* mosquito larvae and able to eradicate pests Mahogany shoot [8]. Renuga and Sahayaraj who studied ethanol extract of *Ageratum* genus concluded that *A. conyzoides* and *A. vulgaris* extract could significantly lower total protein of *S. litura* at concentration of 0.01 µL/insect [6]. Sari and

Armayanti also reported the similar results that *A. conyzoides* extract has a high antifeedant effect and can be used for controlling *Spodoptera* sp. [9]

Plant extract was known to have pharmacology effect beside insecticidal and nematocidal activities [10]; [11]. Okunade explained that bioactivity of *A. conyzoides* was highly varied. Methanol extract of its leaves could be applied as antimicrobial agent, while essential oils of *A. conyzoides* had anti-inflammatory, analgesic, and antipyretic effect [10]. Extract of this plant was also able to induce abnormal morphogenetic in mosquito larvae. Based on this elaboration, *A. conyzoides* could possibly be applied as botanical insecticide to control insect pest.

Secondary metabolites are formed within plants as part of defense mechanism against threat in their ecosystem. Because of that, level of secondary metabolites in each plant are affected by its environment, such as elevation, rainfall, and temperature [12]. Furthermore, environment factor is thought to interact with genetic factor in the expression of secondary metabolites; thus, production and secretion of secondary metabolites are affected by temperature, photoperiod, soil condition, microorganism, and nutrient status [13]. This implies that plants applied as biopesticide must be cultured in accord with its habitat as to maximize secondary metabolites production.

A. conyzoides extract in the current study was applied to *S. litura* larvae. The attack of this caterpillar caused damage up to 12.5% and more than 20% cultivated plants at age more than 20 days after planting [14]. Up to today, *S. litura* was controlled widely using synthetic pesticide. However, because of excessive use, insect resistance towards insecticide emerged. Because of that, to control *S. litura* population, biopesticide from secondary metabolites was applied [2] [15]. Based on this background, the purposes of this study were to describe the effect of *A. conyzoides* leaf methanol extract on *S. litura* larvae mortality and determine LC50 and LC80 of *A. conyzoides* leaf extract.

2. METHODS

A. conyzoides leaves collected from three different altitude (lowland (28.4-31.7 m above sea level) in Bangkalan, medium land (725-937 m above sea level) in Trawas, and highland (1300-1323 m above sea level) in

Batu) were dried for 10 days at room temperature, then grounded and sieved with a 40-mesh sized sieve into dried leaf powder. Powdered leaf was extracted using modified procedure of Dorman and Hiltunen (16). *Simplicia* was macerated with petroleum ether (1: 4 w/v) at room temperature for 24 hours, then dried residue was extracted with methanol (1:15 w/v) using Soxhlet extraction at 65°C for 3 hours. The solvent was evaporated under low pressure using a rotary evaporator to obtain methanol extract.

The target organism used in the test was instar 2 larvae of *S. litura*. The concentration of plant extracts biopesticide was 0% (negative control), 6%, 8%, 10%, and 12%. Each concentration was replicated three times with 10 larvae for respective replication. Methanol extract was diluted to using 10% DMSO until all desired concentration was obtained. Larvae was fed cabbage leaves during experiment. Cabbage leaves was cut into circular shape, weighed 0.1 g, and placed in prepared container. After larvae had acclimated, 0.2 mL of methanol extract were administered into each container. Container was closed and observed daily for 7 days. Parameter recorded was larvae mortality (number of dead caterpillars per day). Data of mortality number was analyzed statistically using two-way ANOVA, while LC50 and LC80 were analyzed using probit analysis.

3. RESULTS

3.1. Mortality Rate

Biopesticide of *A. conyzoides* leaf methanol extract was tested against *Spodoptera litura* larvae to observe the effect in larvae mortality. Result of test is presented in Table 1.

Percentage of mortality was recorded 24 hours after the start of treatment up to 7th day of experiment (Table 1). Administration of *A. conyzoides* extract at various level showed that 12% concentration resulted in highest mortality rate of *S. litura*. Extract concentration of 8% from lowland and highland *A. conyzoides* could induce mortality of *S. litura* at 79.58%. At 10% concentration, medium land *A. conyzoides* extract was able to cause 88.33% larvae mortality. Highest rate of *S. litura* mortality was found from extract of *A. conyzoides* leaves collected from medium land, followed by highland and lowland.

Table 1. Percentage of *Spodoptera litura* larvae mortality (in day 7) given various concentration of *Ageratum conyzoides* leaf methanol extract from various altitude of habitat

Altitude of habitat	Mortality rate of <i>S. litura</i> at extract concentration (%)				
	0 %	6 %	8 %	10 %	12 %
Lowland	18.33±7.5	75.51±0.0	79.58±8.1	83.67±8.1	91.83±8.1
Medium land	28.33±11.6	61.67±4.1	76.67±5.2	88.33±11.7	95.00±5.4
Highland	18.33±14.7	71.43±15.1	79.58±16.3	85.71±11.7	91.83±8.2

Two-factor variant analysis showed that there was significant difference among *A. conyzoides* extract concentration administered (0%, 6%, 8%, 10%, and 12%) with F value (148.80) > F statistic (2.493). However, no significant difference of larvae mortality rate between different altitudes from where *A. conyzoides* were collected, in which F value (0.473) < F statistic (3.110).

3.2. LC50 and LC80

Based on mortality rate of *S. litura*, probit analysis was performed to determine LC50 and LC80 of *A. conyzoides* leaf methanol extract, which is presented in Table 2.

Table 2. LC50 and LC80 of *A. conyzoides* methanol extract on *Spodoptera litura* larvae

Habitat altitude	<i>Ageratum conyzoides</i> extract concentration (%)	
	LC ₅₀	LC ₈₀
Lowland	3.45±0.48	7.76±0.46
Medium land	3.87±0.46	8.18±0.45
High land	3.51±0.48	7.81±0.46

Based on *S. litura* mortality, LC₅₀ of *A. conyzoides* extract collected from lowland was 3.45 %, implying that at that level, extract could induce 50% larvae mortality. Extract LC₅₀ of *A. conyzoides* collected from medium land was 3.87%, while from highland was 3.51%. Extract LC₈₀ of *A. conyzoides* collected from lowland was 7.76%, from medium land was 8.18%, and from highland was 7.81%, meaning that at those levels, *A. conyzoides* could induce mortality in 80% of *S. litura* larvae.

4. DISCUSSIONS

Plants have evolved various mechanisms to defend themselves against insects, including the production of specialized metabolites that act as natural insecticides [2]. Anti-insect chemicals such as secondary metabolites that produced by can be effective in the battle against insects through different modes of action, ranging from damaging the exoskeleton of the insect, to preventing the insect from feeding [2].

Active compounds in plants, such as coumarins, phenols, terpenoids, polyphenols, saponins, and alkaloids, are known from various previous studies to have effect as antifeedant and growth inhibitor in insect that cause chronic toxicity and mortality in larvae [17]. Secondary metabolites such as furanocoumarins (in particular, the major substance imperatorin) are

compounds that toxic for *S. litura* larvae, caused by inhibition of their growth. This larval growth inhibition could be caused not only by the antifeedant effect, but also by possible histological changes in the alimentary canal of the insect [18,19].

Various studies showed that plant secondary metabolites could be used as pesticide to control *S. litura*. For example, study by Yooboon and his colleagues that study about A plant-based extract mixture for controlling *S. litura* (Lepidoptera: Noctuidae). Based on those studies, it was found that the ethanolic crude extracts of *P. retrofractum* and *A. calamus* mixtures could be used as the pesticidal compound and to develop a binary mixture formulation for controlling lepidopteran pests [18]. Pavela [20] observed the effect methanol extracts from 134 plants in inducing mortality of instar 4 *S. litura* larvae. The result showed that three plants from family Asteraceae, *Artemisia compresis*, *Camelia sinensis* and *Eupatorium cannabinum*, applied respectively as biopesticide at dose of 15 mg/g were able to cause 100% mortality of *S. litura* larvae (observation performed after 5th day application).

The application of botanical pesticides on organic farming is expected to conserve natural resources and agricultural productivity in long term, ensure minimal environmental impact, produce optimum crops with minimal chemical input, and provide proportionate economic benefits for farmers. Controlling pests permanently is expected to help create balanced agricultural ecosystem and sustainable agriculture.

5. CONCLUSIONS

Based on results and discussions, it can be formulated some conclusions such as followed:

1. Application of *A. conyzoides* extract at 12% concentration produced the best result in inducing *S. litura* mortality, at 95%. However, no difference was found in *S. litura* mortality given extract of *A. conyzoides* from different altitude of habitat; highland, medium land, and lowland.
2. Methanol extract of *A. conyzoides* had LC50 level of 3.61% and LC80 level of 7.91% in inducing *S. litura* larvae mortality.

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