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Insecticidal Activity of Gamal (*Gliricidia sepium*) Leaf Extracts Against the Darkling Beetle *Alphitobius diaperinus*

Priyantini Widiyaningrum*, Wibowo Faria Fadillah Nur, Dyah Rini Indriyanti, Ning Setiati

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

* Corresponding author: wiwiedeka@mail.unnes.ac.id

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Abstract. *Alphitobius diaperinus* is a well-known insect pest in broiler farms. Larvae and adults of *A. diaperinus* act as disease vectors for poultry, and their presence has the potential to increase the risk of carcass contamination in slaughterhouses. Chemical insecticide control is still considered ineffective and environmentally unfriendly. Plant-based insecticide formulations offer a more environmentally friendly approach to pest control compared to chemical insecticides. The ethanol extract of Gamal leaves was tested on the darkling beetle *A. diaperinus* to determine its insecticidal activity. Five pairs of *A. diaperinus* were treated at five levels of extract concentration and then maintained in an insect container with wheat bran (pollard) food for 5 weeks. The insecticidal efficacy was measured based on the difference in mortality in the toxicity test and the reproductive ability of adult *A. diaperinus* by counting the number of larvae produced during the rearing period. The results showed that differences in extract concentrations significantly affected toxicity and the number of larvae produced. The more concentrated the extract, the higher the insect mortality, with the estimated LC_{50} value of the extract being 20.80% concentration within 72 hours. Reproductive ability showed that the higher the concentration of the extract, causing a decrease in the number of larvae produced, with the optimum concentration found in the 80% treatment. Thus, Gamal leaf extract has potential as a botanical insecticide for biological control of *A. diaperinus* pest.

Keywords: *Alphitobius diaperinus*; *Gliricidia sepium*; insecticidal activity; reproductive ability; toxicity.

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INTRODUCTION

The *Alphitobius diaperinus* beetle is a troublesome and difficult-to-control insect on poultry farms. It emerges and reproduces rapidly during the production period and disappears when the house is empty. In high populations, *A. diaperinus* larvae and adults have been shown to be vectors of several pathogens, including *Salmonella* spp. and *Escherichia coli*, and can increase the risk of carcass contamination in slaughterhouses (Crippen et al., 2018). The presence of this species has been linked to the transmission of bacterial infections from *Escherichia coli*, and poultry viral diseases such as Marek's disease, Newcastle disease, Gumboro disease, and avian influenza; and parasitic diseases such as coccidiosis and chicken tapeworm (Dinev, 2013); Krinsky, 2018), as birds often feed on the abundant insects on the cage floor. Decreased chicken health due to *A. diaperinus* infestation on farms has the potential to stunt growth, resulting in economic losses for farmers (Calla-Quispe et al., 2021; Dupont, 2018)

Insecticide fumigation during breaks in production is usually used to control this pest. However, due to the facultative nature of this pest, chemical control is usually ineffective. Control of *A. diaperinus* using synthetic insecticides over long periods of time has also been shown to induce resistance (Sammarco et al., 2023). Plant-based insecticide formulations (bioinsecticides) offer a more environmentally friendly approach to pest control than chemical insecticides. Active compounds in plant organs have multiple biological activities against insect pests. One plant with the potential as a bio insecticide is the Gamal plant (*Gliricidia septum*). This plant is often used as a hedge or shade plant in a conventional agricultural environment. However, the biological activity of Gamal leaf extract is found to have potential as a bioinsecticide, so this plant can have dual benefits as a hedge plant and as a source of bioinsecticide in the control of *A. diaperinus*.

Several previous studies have shown that Gamal leaf extract has antifeedant effects on

Helicoverpa armigera larvae attacking maize plants (Jose & Sujatha, 2017), *Paracoccus marginatus* mealybugs on papaya plants (Pratami et al., 2018); Nukmal et al., 2019), and as a larvicide for *Aedes aegypti* mosquitoes (Hillary et al., 2024). Research by Rajkumar et al., (2022) states that Gamal leaf contains alkaloids, flavonoids, phytosterols, steroids, tannins, terpenoids, and coumarins. This study aims to test the toxicity of Gamal leaf extract and analyze its effect on the reproductive ability of *A. diaperinus*.

METHODS

The research was conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University between January and March 2024, with an experimental approach. The research stages included toxicity tests of Gamal leaf extracts and reproductive ability tests against the darkling beetle *A. diaperinus*.

Extract Preparation.

Gamal leaves were collected from the herbal medicine garden of Temu Gesang (Magelang, Indonesia). The wind-dried leaves were powdered and then macerated with 95% ethanol solvent at a ratio of 1:5 (w/v) for 3 x 24 hours. The mixture was stirred daily to avoid precipitation. On the 3rd day, the mixture was pressed and filtered through filter paper. The resulting substrate was evaporated using a vacuum rotary dryer and a water bath heater at 70 ºC. The concentrated extract was considered as 100% ethanol extract.

Insect Collection

Adult *A. diaperinus* were collected from the rearing of 3rd instar larvae obtained from a small mealworm farmer in Banyumanik, Semarang, Indonesia. Larvae were reared in insect containers with pollard (wheat bran) as food, carrots as a water source, and polystyrene sheets as pupation medium. After 4 weeks of rearing, larvae that successfully complete pupation metamorphose into adult beetles. These adult *A. diaperinus* will be used as test insects in this study. The resulting beetles will then be separated into males and females for reproductive tests. For toxicity testing, the test insects will be unsexed.

Phytochemical Analysis

Phytochemical analysis of the extracts was performed using GC-MS Perkin Elmer method to quantitatively determine the active compounds contained in the extract (Kathirvel & Sujatha, 2016). The main active components obtained are presented in tables.

Toxicity Test.

Toxicity tests are required to determine the LC_{50} value of the extract and are used as the basis for determining the sublethal concentration in the reproductive performance test. In this study, toxicity is assessed by the percentage of insect mortality due to exposure to the extract within a specified time period. Mortality tests were conducted at six levels of extract concentration (0, 20, 40, 60, 80 and 100%). The experiment used plastic cups measuring 5 x 5 x 7 cm, each containing 50 test insects. Each container was dripped with 200 µL of extract at the specified concentration, and then 50 adult *A. diaperinus* were added. To ensure that all beetles were in direct contact with the extract, the containers were gently shaken for a few seconds. Pollard ± 3 g and a 4.5 x 4.5 cm polystyrene sheet were also added to the container for protection. Each treatment was repeated five times. Observations were made for 3 x 24 hours by calculating beetle mortality at 24, 48, and 72 hours after exposure to the extract. Distilled water was used to dilute the extract. Mortality was calculated according to the following formula (Widiyaningrum et al., 2019).

$$\mathbf{M = r/n \times 100\%}$$

M = mortality
r = number of dead larvae
n = total number of larvae

Reproductive Ability

The study was designed using a one-way completely randomized design with six levels of extract concentration. The upper limit of the sublethal concentration used was 50%. Each treatment was replicated 5 times, with each replicate consisting of five pairs of adult *A. diaperinus*. This required 30 experimental containers with a total of 150 male and 150 female beetles tested. The experimental containers were 5 x 5 x 7cm plastic cups with black gauze covers. The volume of extract exposed for the treatment group was 100µL. First, the extract was dripped into the container according to the treatment up to 100 µL, and then five pairs (male and female) of adult *A. diaperinus* were added. The container was shaken slowly so that the beetles came into direct contact with the extract, then 3 g of pollard powder was added to the five experimental containers as food and polystyrene sheets measuring 0.5 x 4 x 4 cm as protection. Carrot pieces were placed on the polystyrene sheets as a water source for the beetles. All treatment containers were covered with black gauze and placed on the experimental rack to be maintained until *A. diaperinus* larvae emerged. During the maintenance period, checks were made every 2-3 days to ensure safe maintenance and to replace the carrots if they were dry or used up. Maintenance lasted approximately 6 weeks.

Reproductive ability was measured by the number of larvae produced in five observations since the first larval emergence. The criteria for counting larvae are live larvae that are already reddish in color and clearly visible on the surface. The lower the number of larvae found in each treatment, the lower the reproductive ability of the test insects.

Data Analysis

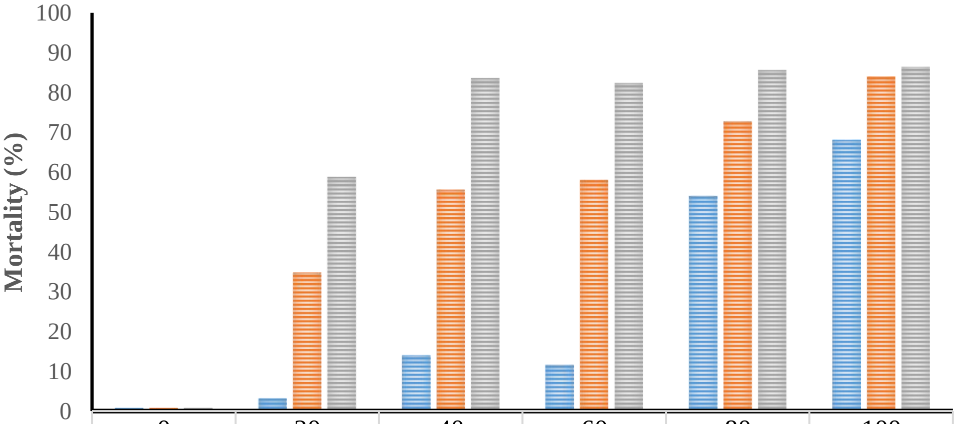
Mortality data in the toxicity study were analyzed descriptively and presented as bar graphs, while the LC_{50} value was determined by probit analysis. Reproductive ability data were analyzed by ANOVA statistics due to normal and homogeneous distribution, Both one-way ANOVA and probit analysis were performed using SPSS for Window 25.

RESULTS AND DISCUSSION

Toxicity test

Mortality data of adult *A. diaperinus* exposed to Gamal leaf extracts at various concentrations are tabulated and presented as shown in Figure 1, while Table 2 shows the results of the probit analysis to determine the LC_{50} value of the extract.

Observations of *A. diaperinus* exposed to extracts at different concentrations showed that the time of death was not simultaneous, but the number of deaths in each treatment was different (Figure 1). There was a tendency that the higher the concentration of the extract, the greater the number of insects that died. In the 24-hour observation, more than 50% mortality was found only in the 100% concentration, but after 48 hours of exposure, more than 50% mortality was found in the 40%, 60%, 80% and 100% treatment groups. On the third day of observation (72 hours), insect mortality had not reached 50% in the 20%, 40%, and 60% concentration groups. This condition indicates that the higher the concentration of the extract, the greater the number of insect deaths, and the time of death was also faster. The results of phytochemical analysis by GC-MS method conducted before the study recorded 10 active compounds from flavonoids, steroids, terpenoids, saponins, and tannins group, which were suspected of having anti-insect activity (Table 1).



	0	20	40	60	80	100
24h	0,8	3,2	14,0	11,6	54,0	68,0
48h	0,8	34,8	55,6	58,0	72,8	84,0
72h	0,8	58,8	83,6	82,4	85,6	86,4

24h 48h 72h

Extract concentration (%)

Figure 1. Mortality of adult *A. diaperinus* at 24, 48, and 72 hours.

Table 1. Ten major active compounds were identified from Gamal (*G. sepium*) extract by GC-MS method*)

No.	RT (min)	Compound Name	Chemical Formula	Peak Area (%)
1.	11.02	*Caryophyllene*	$C_{15}H_{24}$	5.97
	11.13	*2(3H)-Naphthalenone, 4,4a,5*	$C_{10}H_{12}O$	3.37
2.	11.95	*Coumarin*	$C_9H_6O_2$	13.18
3.	15.80	*17-Octadecynoic acid*	$C_{18}H_{32}O_2$	2.78
4.	17.35	*Hexadecanoic acid, ethyl ester*	$C_{18}H_{36}O_2$	2.22
5.	18.49	*Phytol*	$C_{20}H_{40}O$	22.67
6.	18.96	*6,9,12,15-Docosatetraenoic*	$C_{23}H_{38}O2$	3.97
7.	24.12	*Ethyl iso-allocholate*	$C_{26}H_{44}O_5$	3.11
8.	25.07	*Piperine*	$C_{17}H_{19}NO_3$	10.05
9.	29.70	*Sitosterol*	$C_{29}H_{50}O$	11.35
10.	31.01	*Lupeol*	$C_{30}H_{50}O$	7.82

*) Analysis was performed at the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, February 2024

The results of quantitative analysis by GC-MS method (Table 1) showed that phytol compounds were found at 22.67%, followed by coumarin, sitosterol and piperine. Phytol compounds belong to the group of terpenoids and are the main constituents of many EOs from plant extracts (Popova et al., 2020). Phytol compounds are also known as aroma producers, which are thought to have antifeedant or antifeedant activity (Azeem et al., 2020). Coumarin occurs naturally in various plants and has a distinctive aroma, but can be toxic at high doses. According to Tang et al. (2024), coumarin and its derivatives have physiological activities including insecticides, antifungals, antibacterials, herbicides, and antivirals. Research by Ma et al. (2024) showed that most of the coumarin derivative compounds showed good insecticidal activity against *Mythimna separata*. Coumarin compounds and their derivatives have similarities to pesticides, so they have the potential to be developed as bioinsecticides. Sitosterol is a derivative of phytosterol, a natural sterol compound found in plants. Steroids are growth hormones that affect larval molting (Banne et al., 2021; Masih & Ahmad, 2019). Piperine is an active compound of the alkaloid group commonly found in plants. Piperine has a special character that is responsible for its spicy taste. When piperine evaporates, it produces a spicy aroma (Chopra et al., 2017). Based on the characteristics of the four active compounds, it is further strengthened that Gamal leaf extract has a broad activity in influencing the life of insects, both at the cellular, tissue, and organ levels. Chowański et al., (2016) stated that there are physiological effects on insects, including sublethal changes in various tissues and organs, which ultimately cause death. Based on the probit analysis, the estimated LC_{50} of Gamal leaf extract against *A. diaperinus* will be achieved at a concentration of 20.80% within 72 hours, 63.9% within 48 hours, or 87.2% within 24 hours (Table 2). This means that the estimation of LC50 in a short time requires a more concentrated extract concentration so that the toxic effect is also greater.

Table 2. Estimation of LC_{50} at 24, 48, and 72 hours of observation based on probit analysis

Estimation	Observation time (hours)		
	24	48	72
LC_{50}	87.205	63.964	20.802
Upper Bound	90.251	69.636	23.625
Lower Bound	84.382	60.763	17.562

Reproductive Ability

The reproductive ability of *A. diaperinus* exposed to gamal leaf extract in this study is indicated by the number of larvae produced during the observation period. Table 3 shows the average number of larvae produced.

Table 3. Reproductive ability of adult *A. diaperinus* exposed to Gamal extract

Treatment (%)	Replication					Average
	1	2	3	4	5	
0	595	497	593	567	644	579.2[a]
20	493	624	429	470	491	501.4[a]
40	402	275	313	327	403	344.0[b]
60	271	269	287	263	233	264.6[b]
80	170	144	157	196	180	169.4[c]
100	127	141	148	145	159	144.0[c]

Notes; different superscript letters in the column with the mean values indicate significant differences ($p < 0.05$; Tukey's test).

In this study, the reproductive ability of the groups exposed to extract concentrations of 40%, 60%, 80%, and 100% was significantly different from the control group, and no difference was found at a concentration of 20%. This is because the more concentrated the extract is, the more anti-insect compounds it contains. Of the active compounds identified in Table 2, there are several dominant compounds that may act synergistically to affect insect survival. (Jayakumar et al., 2017) reported that the synergistic effect of a mixture of phytochemical compounds in plant extracts, both separately and synergistically, will affect insect survival, such as antifeedant properties, toxicants, or acting as precursors of the physical defense system. According to Jing et al., (2020), plant-derived sterols, when consumed in excess by insects, have a negative effect on insect reproduction. Plant-derived steroids can also cause disturbances in taste and hormone receptors (Das et al., 2021), molting, and reproductive processes (Taj et al., 2021). Flavonoid compounds can affect the respiratory system by damaging the spiracles and witting the insect's nervous system, resulting in a reduced oxygen supply (Sunarti, 2019); Kasman et al., 2020). Saponins have the potential to disrupt insect survival through the digestive system (Subahar et al., 2020). In addition, saponins are irritating upon direct contact and can disrupt the insect's hemolymph system (Jiang et al., 2018). Tannins are antifeedants through a protein binding mechanism catalyzed by the protease enzyme, making it difficult for insects to digest the food they consume (Sari & Isworo, 2020) . Thus, Gamal leaf extract has active compounds that do not kill directly, but rather first disrupt various body systems and ultimately affect reproduction.

CONCLUSIONS

Gamal leaf extract (*G. sepium*) was found to have insecticidal activity. The results of the toxicity test showed that the higher the concentration of the extract, the higher the mortality rate, with an estimated LC_{50} value that can be achieved using a concentration of 20.80% within 72 hours, 63.9% within 48 hours, or 87.2% within 24 hours. The results of the reproductive ability test showed that the higher the concentration of the extract, the lower the number of larvae produced, with the optimum concentration at 80%. Based on GC-MS analysis, it is known that the dominant elements that have the potential to be bioinsecticides against *A. diaperinus* are derived from phytol, coumarin, sitosterol, and piperine compounds. This finding can be further explored, especially in efforts to utilize Gamal plants as hedgerows as well as sources of bioinsecticides in poultry farming areas.

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