Eastern Journal of Agricultural and Biological Sciences (EJABS)
ISSN: 2753-3247
https://qabasjournals.com/index.php/ejab

Utilization Of Bandotan (*Ageratum conyzoides* L.) Leaf Extract Compounds As A Botanical Pesticide Of Wandering Grasshopper (*Locusta migratoria*) In Rice (*Oryza sativa* L.)

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ARTICLE INFO

Received: : May 15, 2022
Accepted: : : June 1, 2022
Volume: 2
Issue: 2

KEYWORDS

Bandotan Leaves, Migratory Locust , Organic Botanical Pesticides,

ABSTRACT

Organic botanical pesticides are an alternative to reduce the use of chemical pesticides that have a negative impact on the environment and agricultural crops. Bandotan leaf (*Ageratum conyzoides* L.) contains bioactive compounds that function as insecticides and nematicides, namely saponins, flavonoids, tannins, and phenols that can prevent pests from approaching plants and inhibit insect development. The purpose of this study was to determine the active compound content of bandotan leaves through phytochemical tests, to determine the effect of bandotan leaf extract as an antifeedant compound and to determine the optimal concentration of bandotan leaf extract which affects the mortality of traveling locusts. Parameters observed were feeding activity and mortality rate of locusts after being given the test treatment. This study used a completely randomized design (CRD) consisting of 4 test treatments, namely, concentrations of bandotan leaf extract 3%, 6%, 9% and aquadest as control with 6 replications observed for 12, 24, 36, and 48 hours. The data were analyzed by ANOVA using SPSS software and if the results were significantly different ($P < 0.05$) followed by Duncan's test. The results showed that bandotan leaf extract contained alkaloids, flavonoids, saponins, and tannins. Different concentrations of bandotan leaf extract had a statistically significant effect as an antifeedant compound, and a concentration of 9% bandotan leaf extract had the most optimal effect on the mortality of the wandering locust pest.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important and main agricultural products in Indonesia. As the main food ingredient, it is hoped that a sustainable level of production will be maintained so that the quality and quantity are maintained. One of the obstacles often faced by farmers in the presence of plant pest organisms (OPT) that can reduce rice productivity [1]. Rice plant pests can be insects, mites, and mollusks. One of the pests that often causes rice harvest failure is the locust attack (*Locusta migratoria*). Plants that are attacked by this pest have symptoms

of tearing in the leaves, and in severe attacks, almost all of the leaves are exhausted, including the leaf bones [2]. The use of chemical pesticides with high or excessive frequency over time can cause several negative effects on health, disturbance of the ecological balance, and the environment contaminated with chemical pesticides [3]. The use of botanical pesticides is one alternative that can be used for these problems without having a negative impact on the environment, as well as being cheap (because the basic ingredients in the form of plants are available in nature), and easy to use [4].

Pest control using various natural ingredients has been started, one of which is using bandotan leaves (*Ageratum conyzoides* L.) as a botanical insecticide [5]. The part of the bandotan plant that is used as a botanical pesticide is the leaf because the bandotan leaf contains saponins, flavonoids, polyphenols, and essential oils, which are quite toxic to insects so that they can inhibit the growth of insects into cocoons [6].

2. Methodology

Place and time of research

The study was conducted in June 2021 - August 2021. Bandotan leaf sampling and observations of bandotan leaf extract tests on the mortality of the wandering locust were carried out in Bedulu Village, Blahbatuh District, Gianyar Regency. Phytochemical tests were carried out at the Organic Laboratory of the Chemistry Study Program, Udayana University, and the manufacture of bandotan leaf extract was carried out at the Biochemistry Laboratory of Udayana University and the Laboratory of Genetic Resources and Molecular Biology at Udayana University.

Research procedure

Preparation of Extracts

The extract is made from mature bandotan leaves (dark green) because they have the potential to contain secondary metabolites [7], bandotan leaves are obtained from bandotan plants in the rice fields of Bedulu Village, Blahbatuh District, Gianyar Regency. Bandotan leaves were obtained, weighed as much as 500 grams, then washed with water until clean, then dried until dry. The dried bandotan leaves were then blended until smooth, and the crushed leaves were then macerated with methanol for 72 hours (methanol was used to extract secondary metabolites in plants). Extract material that has been macerated using a solvent, then filtered using Whatman filter paper no. 4 size 110 mm, so that it can be separated between the solvent and the solute. The solvent obtained is then evaporated with a vacuum rotary evaporator to obtain a crude extract which is considered a concentrated concentration (100%). Then 4 ml of the extract is taken for phytochemical tests, and the remainder is diluted with distilled water to reach a volume of 100 mL with a concentration of extract. That is, 3%, 6%, 9%, and as a control, only 100 mL aquadest was used.

Antifeedant compound test and insect mortality rate

Nymphs from the wandering locust used as test insects in each jar (4 jars) were fed 5 g of rice leaves for each treatment and were sprayed with bandotan leaf extract in accordance with the concentration that had been determined as the test treatment, namely 3%, 6 %, 9% and as a control used aquadest (previously, the test insects fasted for one day). Each jar is filled with 5 wandering grasshopper nymphs. Observation of the antifeedant compound test (inhibiting feeding activity) was carried out by calculating the weight loss of rice leaves 48 hours after being given treatment, and the mortality rate of the test insects was indicated by the immobility of the test insects for 12, 24, 36, and 48 hours after being given the test treatment according to the concentration given extract [8]

Statistic analysis

The data processing method was carried out quantitatively, namely by observing the effect of bandotan leaf extract with different concentrations on feeding activity and the number of nymph mortality of traveling locusts using Analysis of Variance (ANOVA) and if significantly different results were obtained ($P < 0.05$) it would be continued with the test. Duncan [8]

3. Results and Discussion

Phytochemical Test of Bandotan Leaf Extract

The results of the bandotan leaf extract's phytochemical test showed that bandotan leaf extract contained alkaloids, flavonoids, saponins, and tannins (Table 1.).

Table 1. Phytochemical test results of bandotan leaf extract

No.	Type of Examination	Method of Examination	Results
1.	Alkaloids	Chloroform+Mayer	+(there is a white precipitate)
2.	Flavonoids	Mg/HCl	+(yellow color)
3.	Saponins	The sample is heated, shaken	+(foam is formed)
4.	Tannins	FeCl3+H2SO4	+(greenish black color)

Note : + (indicates a positive result)

Antifeedant Compound Test

The results of statistical analysis of the antifeedant compound test of bandotan leaf extract showed that the antifeedant compound of bandotan leaf extract against wandering locust nymphs in control up to a concentration of 9% showed significantly different results (Table 2.). The control (aquadest) showed that the appetite for wandering locust nymphs was still high, so the rice leaves eaten for 48 hours were still in large quantities, with an average weight of 2.83 grams of rice leaf. At concentrations of 3%, 6%, and 9%, the appetite for wandering locust nymphs began to decrease, so that the average weight of rice leaves eaten for 48 hours was 1.5 grams, 0.83 grams, and 0, respectively. 16 grams. (Table 2).

Table 2. The average weight of rice leaf eaten by wandering locust nymphs after being given treatment

Treatment	Average weight of rice leaves eaten for 48 hours (grams)
Control	2.83 ± 0.40^{a}
Concentration 3%	1.50 ± 0.54^{b}
Concentration 6%	0.83 ± 0.75^{c}
Concentration 9%	0.16 ± 0.40^{d}

Note: Numbers followed by different letters in each column show significantly different results ($\alpha<0.05$).

Insect Mortality Test Results

The results of statistical analysis showed that the treatment factors with different concentrations of bandotan leaf extract had a significant effect on the mortality of the traveling locust nymphs. The mortality of the wandering locust nymph in the control treatment for 12, 24, and 36 hours was 0, while at 48 hours, the average mortality was 6.67%. The locust mortality at 3% concentration treatment for 12, 24, 36, and 48 hours was 20%, 20%, 30%, and 40%, respectively. The locust mortality at 6% treatment for 12, 24, 36, and 48 hours was 20%, 30%, 50% and 60%, respectively. The concentration of bandotan extract was 9%, and the mortality data of the traveling locust for 12, 24, 36, and 48 hours were 40%, 50%, 80%, and 100%, respectively. (Table 3).

Table 3. The average mortality of traveling locust nymphs

Treatment	Percentage of test insect mortality			
	12 HAA	24 HAA	36 HAA	48 HAA
Control	0 ± 0	0 ± 0	0 ± 0	$6,67 \pm 10,33^{a}$
Concentration 3%	20,00 ± 0,00	20,00 ±0,00	30,00 ± 10,95	$40,00 \pm 0,00^{b}$
Concentration 6%	20,00 ± 0,00	30,00 ± 10,95	50,00 ± 10,95	$60,00 \pm 0,00^{c}$
Concentration 9%	40,00 ± 0,00	50,00 ± 10,95	80,00 ± 0,00	$100,00 \pm 0,00^{d}$

Note: Numbers followed by different letters in each column show significantly different results ($\alpha<0.05$).
HAA = Hours after application

Based on statistical analysis, the activity of antifeedant compounds (appetite inhibitors) of bandotan leaf extract against wandering grasshopper nymphs at concentrations of 3% to 9% concentration showed a significant difference or significantly different ($\alpha < 0.05$) from the control (Table 2.). The data showed that the higher the concentration of bandotan leaf extract given, the less rice leaf eaten by the wandering locust nymphs. This is in accordance with research conducted by [6] that the optimal treatment was obtained by giving a concentration of 9%. The low feeding activity of wandering locust nymphs on rice leaves treated with bandotan leaf extract with a higher concentration was caused by the increasing number of active substances on the surface of the rice leaves, which caused a stronger antifeedant effect [9]

The antifeedant effect of bandotan extract comes from secondary metabolites found in the leaves, including alkaloids, flavonoids, saponins, and tannins [10]. Alkaloid compounds that enter through the mouths of pests can trigger a mechanism for inhibiting pest eating activity which causes pests not to get the energy to move, causing starvation and resulting in pest mortality. Alkaloid compounds also inhibit insect growth and cause metamorphosis failure [11]. Flavonoid compounds, which are toxic to insects, can inhibit growth and work as respiratory inhibitors; if there is contact with insect skin it will damage the insect skin mucosa [12]. Saponin compounds that enter the insect's body can bind free sterols in the digestive tract of food, this situation results in a decrease in free sterols, which can interfere with the process of skin turnover in insects [13]. The molting of the skin is not only needed by insects to grow but is also needed to reach the imago so that they can reproduce [14]. Tannin compounds are able to inhibit the activity of digestive enzymes in animals by interacting with the substrate side that should bind to the enzyme so that the substrate will form other molecules that are not specific to the enzyme, and enzyme performance will be inhibited [15]. The process of entering secondary metabolites into the body of the wandering locust nymph occurs through the digestive system when eating rice leaves that have been sprayed with bandotan leaf extract and through breathing, namely breathing in the gas released from the active ingredients in the extract [16].

4. Conclusion

Based on the results and discussion above, it can be concluded that the active compounds contained in bandotan leaf extract include alkaloids, flavonoids, saponins, and tannins. Bandotan leaf extract has an effect as an antifeedant compound at all levels of extract concentration and causes mortality. The optimal concentration of bandotan leaf extract that affects the mortality of the wandering locust nymph is a concentration of 9%, with the highest mortality at each observation time.

Acknowledgments: Thank you to the biochemistry Laboratory, Department of Biology, Faculty of mathematics and natural sciences, Udayana University, so that this research can be carried out.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflicts of interest

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