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RESEARCH ARTICLE

Soursop leaf extract (*Annona muricata* L) as a biochemical pesticide against fruit flies (*Bactrocera* sp)

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Abstract

Background: The citrus cultivation industry is commonly operated by the government, private sector, or individual owners from small, medium, to large scales. The fruit flies (*Bactrocera* sp) insect was reported to attack citrus crops in almost every region in Indonesia. **Objective:** to determine the effectiveness of soursop leaf extract (*Annona muricata* L) as a plant-derived insecticide against fruit flies. **Methods**: This study used true experimental with Posttest Only Control Design to analyse the difference in the average mortality of fruit flies for 24 hours. **Results:** The data generated through the Kruskal Wallis test showed that at a concentration of five per cent and 10% the effect of the mortality in fruit flies equals $p > 0.939$. However, at a concentration of 15%, the number decreased to $p < 0.001$. This finding showed that the compound has evolved its function to antifeedant only or as a secondary metabolite that does not result in immediate death but rather gives discomfort to insects. **Conclusion:** the use of an antifeedant compound as a biopesticide can inhibit fruit flies' attack on citrus crops.

Introduction

The success of citrus cultivation practices is generally determined by several factors, including location, seedlings, planting, and pest management (Endarto & Martini, 2016). The most common pest that attacks citrus plants is fruit flies (*Bactrocera* sp.) (Indriyanti *et al*., 2014). There is great potential for the use of biopesticides for long-term development and growth, in guiding the field of pest management science (Copping & Menn, 2000).

Exploiting soursop leaves has proven to be a promising idea as a plant-derived insecticide (*Annona muricata* L) (Nurjannah *et al.*, 2013). The soursop leaf contains acetogenin compounds, including asymycin, integer, and squamosin. In high concentrations, acetogenin compound can work as an antifeedant that disturbs fruit flies appetite. But at low temperatures (less than 20^{o}C), acetogenin compounds can be toxic to fruit flies thereby causing their death (Widihastuty, Desi & Dafni, 2021). Therefore, the main objective of this study is to determine the effectiveness of soursop leaf extract as a biochemical insecticide against the mortality of fruit flies.

Methods

The research method used was True experimental with a Posttest Only Control design to analyse the difference in the average mortality of fruit flies (*Bactrocera* sp.) for 24 hours. The samples used were 240 fruit flies. Flies were put into 24 container tanks with ten tails in each container. The extract was made at the Jember University Pharmacy Laboratory. The refined soursop leaves were macerated using 96% ethanol at a ratio of 1:7.5 for three days and stirred two times within 12 hours. The result of the pure extract from the maceration process is dark green as much as 30 g. The finished soursop (*Annona muricata*) leaf extract is divided into several concentrations, namely 5 g, 10 g, and 15 g.

This study used a completely randomised design (CRD) which consisted of four treatments with six repetitions,

each using ten animals for each treatment with an observation time of 24 hours and concentrations of 5%, 10%, and 15%. This study got the Ethical clearance No.189/UN25.8/KEPK/DL/2018 from the KEPK Density Faculty of Jember.

The pest control intervention using soursop leaf extract to fruit flies was carried out at Tulungrejo Tulungagung, Indonesia.

a. Making soursop leaf extract

The soursop leaves were extracted. Exactly two kilograms of leaves were washed and cut into small pieces. It was air-dried until the moisture content was 10%, after which it was mashed and macerated. The maceration process yielded up to a 30 g powder of the leaf extract. Several concentrations and times of observation were decided.

b. Mortality of fruit flies

The pest control intervention using soursop leaf extract to fruit flies was carried out on 15 December 2018. Following the pest control intervention, an investigation was carried out to determine the effect of soursop leaf extract on the mortality of fruit flies (see Figure 1).

c. Difference in average mortality of fruit flies

The difference in the average mortality of fruit flies (*Bactrocera* sp.) in each variable was determined by carrying out several tests. First, the Kolmogorov-Smirnov test was carried out to find out which groups of data were normally distributed or not, then proceeded with the Kruskal-Wallis test.

Results

The amount of pure extracts from the maceration process was as much as 30g.

After each intervention, the mortality of fruit flies differed depending on the amount of concentration and the length of observation after the intervention. The following is a description of mortality of fruit flies at concentrations of 0% (control), 5%, 10%, and 15% with observation time every two hours for 24 hours (Table I).

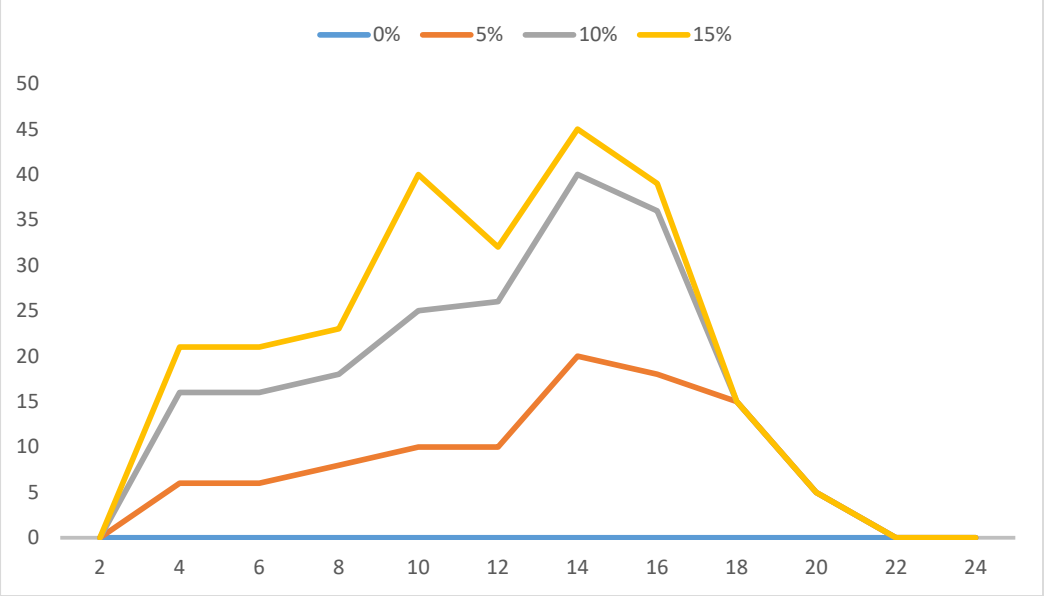

Figure 1: Average mortality of fruit flies at a concentration of 0%, 5%, 10 % and 15 %

Table I: Mortality of fruit flies

Concentration	Repeats	Mortality of fruit flies per two hours												Total
		2	4	6	8	10	12	14	16	18	20	22	24	
0%	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
5%	1	0	0	1	1	1	1	2	2	2	0	0	0	10
	2	0	1	1	0	1	1	2	2	1	1	0	0	10
	3	0	1	0	1	1	1	2	2	2	0	0	0	10
	4	0	0	1	1	1	1	2	2	1	1	0	0	10
	5	0	1	0	1	1	1	2	2	1	1	0	0	10
	6	0	1	1	1	1	1	2	1	2	0	0	0	10
Total		**0**	**4**	**4**	**5**	**6**	**6**	**12**	**11**	**9**	**3**	**0**	**0**	**60**
10%	1	0	1	1	1	2	2	2	1	0	0	0	0	10
	2	0	1	1	1	1	2	2	2	0	0	0	0	10
	3	0	1	1	1	2	1	2	2	0	0	0	0	10
	4	0	1	1	1	1	2	2	2	0	0	0	0	10
	5	0	1	1	1	2	1	2	2	0	0	0	0	10
	6	0	1	1	1	1	2	2	2	0	0	0	0	10
Total		**0**	**6**	**6**	**6**	**9**	**10**	**12**	**11**	**0**	**0**	**0**	**0**	**60**
15%	1	0	1	1	1	1	1	1	1	0	0	0	0	7
	2	0	1	0	0	1	0	1	0	0	0	0	0	3
	3	0	0	0	1	2	1	1	1	0	0	0	0	6
	4	0	0	0	0	2	1	0	0	0	0	0	0	3
	5	0	1	1	0	1	1	0	0	0	0	0	0	4
	6	0	0	1	1	2	0	0	0	0	0	0	0	4
Total		**0**	**3**	**3**	**3**	**9**	**4**	**3**	**2**	**0**	**0**	**0**	**0**	**27**

Based on the data (Table II), a significance value of 0.0001 was obtained. The significance value <0.05 indicated that there was a significant difference in the mortality of fruit flies (*Bactrocera* sp.) by administering the extract at concentrations of 5%, 10%, and 15% because the $p < 0.05$ value.

Table II: Kruskal Wallis test results

Concentration (g)	Mean rank	Sig	Information
5 g/0.1 L	120.56	0.0001	Meaningful to $p<0.05$
10 g/0.1 L	119.13	0.0001	Meaningful to $p<0.05$
15 g/0.1 L	85.81	0.0001	Meaningful to $p<0.05$

Based on the results (Table III) The extract concentration of five per cent does not show any significant difference from the 10% one. The$p > 0.05$ means there is no significant difference. However, the concentration of the 10% group shows a significant difference from the 15% group. The $p < 0.05$ means there is a significant difference. Furthermore, the concentration of five per cent shows a significant difference from the 15% one. The $p < 0.05$ means there is a significant difference.

Table III: POC host test results at concentrations of 5%, 10% and 15%

Conc(g) / Sig	5%	10%	15%
5%	-	0.939	0.0001
10%	0.939	-	0.001
15%	0.001	0.0001	-

The extract concentration of five per cent does not show any significant difference from the 10% groups.

The $p > 0.05$ means there is no significant difference. However, the concentration of 10% shows a significant difference from the 15% group. The $p < 0.05$ means there is a significant difference. Furthermore, the concentration of five per cent shows a significant difference from the 15% group. The $p < 0.05$ means there is a significant difference.

Discussion

In this study, soursop leaf extract (*Annona muricata*) was used as a biochemical insecticide using the filter Simplicia which contains active substances (Nuhu and Aisha, 2001). Maceration was employed because it is the mostpractical extraction method and uses a simple tool. In addition, Simplicia contained in soursop leaves is easilyabsorbed (Pai *et al.*, 2016). This immersion of Simplicia used 96% ethanol with a ratio of 1:7.5 which could attract addictive substances contained in polar and nonpolar Simplicia (Saputri & Marcellia, 2021). The results of the research conducted showed that *M. jalapa* has 30 types of specific compounds (Maulina *et al.*, 2018). Soursop leaf extract has effective killing power against *Aedes aegypt* larvae, head lice, and pests on cabbage plants (Mawuntu, 2016; Kolo, 2018; Tee & Badia, 2019).

Decreased fruit yields due to fruit fly attacks vary between 30-100% depending on environmental conditions and the vulnerability of the types of fruit they attack (Maung *et al.*, 2019). The intensity of fruit fly attacks in several regions in Indonesia shows quite large variations. In the Bali area, fruit fly pest attacks on jackfruit, star fruit, water guava and guava can reach 100% (Astriyani, Supartha and Sudiarta, 2016). In Central Maluku Regency, the intensity of fruit fly attacks on starfruit plants can reach 70% and on cayenne pepper plants around 41-49% (Sahetapy, Uluputty & Naibu, 2019).

The results of the study reported that the effectiveness of using the GF-120 Fruit Fly Bait spray proved to be very effective in preventing protein-deficient females from landing on cucumbers (23% of released females died on sorghum-sprayed bait; 0% were observed alive on cucumbers), but proved less effective in suppressing females fed protein (14% of released females were observed to die when sprayed with sorghum bait; 11% were observed alive on cucumbers) (Prokopy *et al.*, 2009). Chemical analysis showed that the extract contained alkaloids and flavonoid compounds. *Annona muricata* extract caused high mortality rates for both mosquito species compared to *Annona squamosa* extract (Ravaomanarivo *et al.*, 2014). The ethanol extract of soursop leaves has antioxidant activity by capturing DPPH IC50 radicals of 141.127 g/m (Hasmila, Natsir & Soekamto, 2019).

The content contained in soursop leaves, namely acetogenin, functioned as an antifeedant at high concentrations and could be toxic at low temperatures. At the concentration of 0%, there was no mortality of fruit flies due to the absence of the extract. The extract concentration of five per cent caused mortality with a total of 60 fruit flies in 24 hours. The extract concentration of 10% caused mortality with a total of 60 fruit flies, and the extract concentration of 15% caused mortality with a total of 27 fruit flies. The mortality of fruit flies in all repetitions was different due to the immune condition of fruit flies which affected the time of death. The results showed that the use of soursop leaf extract with a concentration of five per cent affected the mortality of fruit flies (*Bactrocera* sp.) in the first four hours after the intervention. Based on the results of the study, the mortality rate of fruit flies at a concentration of five per cent exceeded 50% in 24 hours. At this concentration, the chemical compounds in soursop leave entering the body of fruit flies were the highest. The time of mortality of fruit flies on each repetition was almost the same where the number of dead fruit flies amounted to ten fruit flies on each repetition occurred in 20 hours. At a concentration of 10%, the time of mortality was faster at each observation because the acetogenin content in soursop leaves functioned more strongly as a poison. In the extract concentration of ten per cent fruit flies were more silent and did not fly much because they had been exposed to chemical compounds and got tired quickly (Manullang, Marbun & Nurjannah, 2020). At a concentration of 15% the mortality rate of fruit flies had not reached 50% in 24 hours because the compound contained in soursop leaf extract no longer functioned as a poison, instead, it functioned just as an antifeedant.

A study stated that the low consumption of food is caused by the intervention that functions as an antifeedant and affects flies' appetite (Sumampouw, 2012). Antifeedants are compounds which cause secondary metabolites to insects by disturbing flies' appetite. Research states that the soursop leaf plant can act as an insecticide, by acting as a food inhibitor or antifeedant in a certain amount and as a stomach poison at low concentrations (Hartini and Yahdi, 2015). Research studies found that the death of flies on exposure to soursop leaf extract (97.25%) with the highest level of effectiveness at a dose of 25%, for 15 minutes. Flavonoids are believed to be the compounds responsible for the death of flies (Ahyanti, Yushananta & Usman, 2023).

Conclusion

There is a significant difference in the mortality of fruit flies starting at four hours after intervention and the highest mortality occurred at 16 hours after intervention. Among the concentrations of five per cent, 10%, and 15% observed in a period of 24 hours, the most effective concentration was a concentration of 10% with $p < 0.05$.

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