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Toxicity Test of *Annona Muricata* L. Leaf Extract as a Biopesticide Against Mortality of *Spodoptera Frugiperda* J. E. Smith

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ABSTRACT

Spodoptera frugiperda is a new invasive pest that can cause losses in corn production, so control measures are needed to suppress its population. The purpose of this study was to obtain the best concentration of botanical pesticide from soursop leaves extract against the fall armyworm (S. frugiperda). The method used is experimental method using a single factor Completed Randomized Design (RAL) consisting of 7 treatments with 4 replications: A (Control); B (Insecticide deltamethrin 1 ml/lt); C (A. muricataleaves extract 20 gr/lt); D (A. muricataleaves extract 30 gr/lt); E (A. muricataleaves extract 40 gr/lt); F (A. muricataleaves extract 50 gr/lt); G (A. muricataleaves extract 60 gr/lt). The treatment effect was analyzed with Analysis of Variance (ANOVA). If the 5% level F test showed significant results, then it proceeded with the DMRT (Duncan Multiple Range Test) further test at a 5% significance level to determine the best treatment. The results achieved from this study were the concentration of soursop leaves extract 60 gr/lt was able to cause 50% mortalityof S. frugiperdain less than one day based on the analysis of probit LT50, inhibiting the larvae from stopping eating with a percentage of 75% at 1 Day After Application (DAA), gave the highest mortality of 85% in 3 DAA. A. muricataleaves extract with a concentration of 60 g/lt was recommended to be used because it gave the best results that were not significantly different from the use of synthetic insecticides against S. frugiperda.

Keywords: *LT50, Mortality, Annona muricataleaves extract concentration, S. frugiperda*

INTRODUCTION

Fall armyworm (*Spodoptera frugiperda* J.E. Smith) is an invasive pest that attacks maize (*Zea mays*) as its primary host. *S. frugiperda* is a crop pest native to the Americas and has spread to various countries. This pest attacks the growing point of the plant, which can result in the failure of the formation of young shoots/leaves of plants. *S. frugiperda* larva hasa high feeding ability that will actively feed inside the plants once they enter it, making them difficult to detect in a low population. *S. frugiperda* Imago is a robust aviator with a high cruising range (CABI 2019). *S. frugiperda* attack caused 40% yield losses in Honduras (Wyckhuys & O'Neil, 2006) and 72% in Argentina (Murúa et al., 2006). From the negative impacts caused, the population development of *S. frugiperda* is necessary to be cautious of (Lubis et al., 2020).

S. frugiperda is polyphagous, besides maize, *S. frugiperda* also attacks other essential crops, including rice, sugarcane, sorghum, beets, tomatoes, potatoes, and cotton (Day et al., 2017). With the rapid spread of the population of *S. frugiperda*, monitoring and observation regarding its control ne ALE to be carried out as a form of strategy to suppress the population. In an action of controlling *S. frugiperda*, the use of synthetic pesticides is still widely considered as the main choice because it is considered easier, faster, and more practical. In fact, excessive use of pesticides will increase control costs, increase the death of non-target organisms and has the potential to reduce environmental quality (Laba, 2010). In addition, excessive use of synthetic pesticides will trigger a rapid increase of *S. frugiperda* resistance (Afandhi et al., 2022). In considering the negative impact of synthetic pesticides on human health and the environment, it is necessary to develop a more sustainable control strategy to control *S. frugiperda*.

One environmentally friendly solution

in controlling pests and diseases is the use of botanical pesticides (Zaidun, 2004). The use of plant extracts as botanical pesticide is based on the idea that there are chemical compounds from plants that has the potential to cause toxicity against pests. One of the compounds produced by plants is a metabolite compound that has the ability to act as repellent (repellent), food inhibitor (antifeedant), secondary development inhibitor and egg laying inhibitor (oviposition), and kills insects (Prijono, 1999). Botanical pesticides are proceeded from plants that contain active ingredients that are able to control pests. The use of botanical matter is relatively safer for humans and livestock, because it is easier to decompose and the residue is easily bio-degradedto minimize environmental problems (Sutriadi et al., 2020). One of the plants that can be used as a botanical pesticide is *A. muricata* plant.

The leaves of the *A. muricata* plant can be used as ingredients for botanical pesticides. *A. muricata* leaves extract contains acetogenin compounds that can cause coagulation in the stomach of insects, causing the insect's digestive system to malfunction. The acetogenin compound contained in *A. muricata* leaves also acts as a repellent so that it can reduce the palatability of *Spodoptera litura* by 41.6% (Tohir, 2010). *A. muricata* leaves contain acetogenin compounds, including acimicin, bulatacin, and squamocin (Arimbawa et al., 2018). *A. muricata* leaves also contain secondary metabolites that function as self-defense. The content of this substance acts as an active ingredient in botanical pesticide. This secondary metabolite compound has the characteristic of giving a bitter taste because it contains terpenes and alkaloids. These substances also emit an unpleasant odor and spicy to minimize pest attacks (Hasyim et al., 2010).

Based on the study of (Moniharapon et al., 2018), the application of *A. muricata* leaves affected the mortality of *Sitophylus oryzae* imago. The increase in the number of *A. muricata* leaves linearly increased imago mortality, with an average mortality index of 78.31% with 35 g of *A. muricata* leaves. This illustrates that the more *A. muricata* leaves are given, the higher the mortality rate. This phenomenon is because the more the number of *A. muricata* leaves are given, the higher the anonian and resin compounds can work as stomach poisons and contact poisons on insects.

Yanuwiadi et al. (2013) reported in their research that *A. muricata* leaves extract positively affected the activity of stopping eating on Spodoptera litura by 33.3% at the 16 and increasing to 46.7% at the 24 hours after application. The mortality rate of larva with the application of *A. muricata* leaves extract started at 48 hours after application, which was 10% and continued to increase until 168 hours after application of 50%. Another effect of the application of *A. muricata* leaves extract was the failure of the formation of pupa and imago. The percentage of failure to form pupa with the application of *A. muricata* leaves extract was 64% while the imago was 67.7%.

This study aimed to determine the effectiveness of *A. muricata* leaves extract against mortality of *S. frugiperda*, and to obtain the best concentration against mortality of *S. frugiperda*. Thus, the results of this study are expected to be useful and become a reference for the use of *A. muricata* leaves extract as a biopesticide as an alternative form of environmentally friendly *S. frugiperda* pest control.

MATERIALS AND METHOD

This research was carried out at the Botanical Pesticide Laboratory, Pest and Disease Forecasting Institute in March 2022 – May 2022. The main materials and tools used in this study were *S. frugiperda*, *A. muricata* leaves, deltamethrin, aquadest, ethanol 70%, tween 80, cup, analytical balance, digital microscope, hand sprayer, blender, scissors, filter cloth, petri dish, dropper, and stationery. The research method used was a single factor Completed Randomized Design (CRD) consisting of 7 treatments with 4 replications. *A. muricata* leaves extract (ALE) concentration was obtained from calculations based on the dilution formula :

$$\mathbf{C_1V_1 = C_2V_2}$$

Where :

C1 = Concentration of the starting solution
V1 = Volume of the starting solution
C2 = Concentration of the final solution
V2 = Volume of the final solution
So that the following results are obtained.
A = Control
B = Deltamethrin (Recommended concentration is 1 ml/lt)
C = 20 gr/lt (20 gr*A. muricata*leaves + 333,3 ml aquadest)
D = 30 gr/lt (30 gr*A. muricata*leaves + 500 ml aquadest)
E = 40 gr/lt (40 gr*A. muricata* leaves + 666,67 ml aquadest)
F = 50 gr/lt (50 gr*A. muricata* leaves + 833,33ml aquadest)
G = 60 gr/lt (60 gr*A. muricata* leaves + 1000ml aquadest)

Rearing of S. frugiperda

The adult instar larvae were bred in trays and fed with babycorn until they molt to pupa. The larvae that have turned into pupae were then separated and placed into trays with a pile of sawdust. Trays containing pupae were transferred to copulation boxes containing corn plants. Pupae in the copulation box will hatch into imago in approximately 7 days, and then the imago will mate to produce eggs on corn leaves. The larvae used as test larvae were the 2nd instar larvae.

Producing A. muricata Leaves Extract

Fresh green coloured *A. muricata* leaves were cleaned with running water and drained. After that, *A. muricata* leaves were separated from the leaves bones, cut into small pieces, put into a blender, and added ethanol 70%, tween 80, and aquadest according to the concentration of each treatment based on the dilution formula. The results of the mixture of *A. muricata* leaves with ethanol, tween 80, and aquadest were then filtered using a filter cloth, and the filtered solution were stored in an erlenmeyer and ready to be used after being stored for 24 hours.

Biopesticide Toxicity Test

The larvae used as the test larvae were 2nd instar of *S. frugiperda* larvae. 5 test larvae were put into a container. The test larvae were sprayed according to the concentration variations in each treatment by contact as much as 1 ml. Parameters observed for the biopesticide toxicity test of *A. muricata* leaves extract were larval mortality which was observed at 1-7 days after application (DAA), the percentage of test larvae stopped eating which was observed at 6, 9, 12, 24, 48, 72 hours after application (HAA), and Lethal Time 50 (LT50).

Data Analysis

The data obtained from the observation parameters of mortality and the percentage of larvae stopped eating were analyzed by analysis of variance. If the results of the F test for the treatment in the variance were significantly different, then the average for each different treatment was proceeded using the DMRT (Duncan Multiple Range Test) advanced test at a significant level of 5% to determine the best treatment. Data analysis for the LT50 parameter was tested by probit analysis.

RESULT AND DISCUSSION

Mortality of S. frugiperda Larvae

Based on observations, the application of *A. muricata* leaves extract had a significant effect against mortality of *S. frugiperda* larvae. The results of the percentage of mortality is provided in (Table 1).

Table1. Mortality of *S. frugiperda* larvae in 1 – 7 DAA due to *A. muricata* leaves extract

Treatment	Mortality (%)						
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	6 DAA	7 DAA
A	0,0c	0,0c	0,0c	0,0e	0,0d	0,0c	0,0b
B	100,0a	100,0a	100,0a	100,0a	100,0a	100,0a	100,0a

C	25,0b	35,0b	55,0b	55,0d	60,0c	75,0b	95,0a
D	25,0b	35,0b	55,0b	55,0d	65,0c	90,0b	95,0a
E	30,0b	40,0b	65,0b	75,0c	85,0b	100,0a	100,0a
F	35,0b	45,0b	65,0b	80,0bc	90,0b	100,0a	100,0a
G	60,0ab	75,0a	85,0a	95,0ab	100,0a	100,0a	100,0a

a) Treatment : A (Control), B (Deltamethrin 1 ml/lt), C (ALE 20 gr/lt), D (ALE 30 gr/lt), E (ALE40 gr/lt), F (ALE 50 gr/lt), G (ALE 60 gr/lt)

b) Means followed by the same letters within each column are not significantly different at 5% level according to DMRT

The application of *A. muricata* leaves extract with a concentration of 60 gr/lt (G) showed mortality results that were not significantly different from the application of the synthetic insecticide deltamethrin (B) since 1 DAA with a mortality rate of 60% and continued to increase to 85% in 3 DAA, until it reached 100% in 7 DAA. In addition, the application of *A. muricata* leaves extract at a concentration of 50 gr/lt (F) showed larval mortality rates that were not significantly different from concentrations of 40 gr/lt (E) and 60 gr/lt (G), but were significantly different from other concentrations. Application of *A. muricata* leaves extract with the highest concentration of 60 g/lt (G) resulted in faster mortality of *S. frugiperda* larvae compared to other concentrations. This is supported by (Saragih et al., 2019)in their research on the effect of *A. muricata* leaves extract on *Setothosea asigna*, which showed that the higher the concentration of *A. muricata* leaves extract, the faster the time needed to kill *S. asigna*.

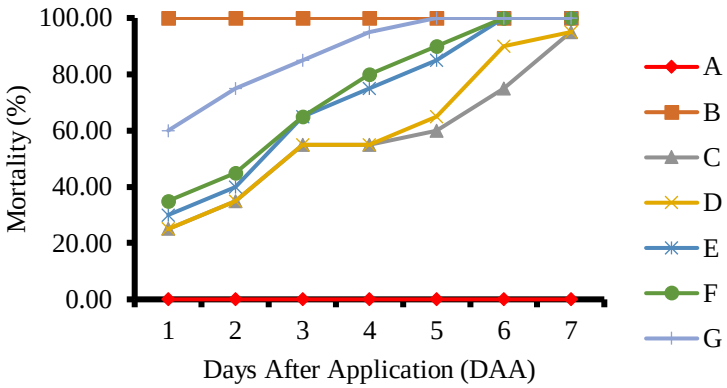

Figure 1. Mortality of *S. Frugiperda* graph in 1 – 7 DAA due to *A. muricata* leaves extract. Treatment : A (Control), B (Deltamethrin 1 ml/lt), C (ALE 20 gr/lt), D (ALE 30 gr/lt), E (ALE40 gr/lt), F (ALE 50 gr/lt), G (ALE 60 gr/lt)

The mortality rate of *S. frugiperda* larvae in (Figure 1) shows that the application of *A. muricata* leaves extract at a concentration of 60 g/lt (G) gave the highest mortality rate compared to other concentrations. In line with the research by (Arimbawa et al., 2018), application of *A. muricata* leaves extract at a concentration of 40% with a target application to larvae was able to cause the death of the *Crocidolomia pavonana* with an average mortality of 52%. The death of *S. frugiperda* can be caused by the content of Alkaloid compounds in *A. muricata* leaves in the form of salt so that it can degrade cell membranes to enter and damage cells and can also disrupt the larval nervous system by inhibiting the work of the acetylcholinesterase enzyme (Anggraini & Kamalliyah, 2018).

The occurrence of mortality of *S. frugiperda* larvae was caused by specific compounds contained in *A. muricata* leaves which were able to inhibit the development of *S. frugiperda* larvae. *A. muricata* leaves extract has specific substances that can cause

larval death, including flavonoids. According to (Cania & Setyaningrum, 2013), flavonoids are compounds that work by entering the respiratory system of the larvae, which can then cause the nerves in the larvae to wither and cause damage to the respiratory system, which causes the larvae to not breathe and causes death eventually.

Percentage of Larvae Stop feeding

Based on observations, the application of *A. muricata*leaves extract significantly affected the percentage of *S. frugiperda* larvae stop feeding. The results of the percentage of *S. frugiperda* larvae stop feedingare provided in (Table 2).

Table2. Percentage of *S. frugiper da*stop feeding in 6 – 72 HAA

Treatment	Larvae Stop Feeding (%)					
	6 HAA	9 HAA	12 HAA	24 HAA	48 HAA	72 HAA
A	0,0c	0,0c	0,0d	0,0c	0,0c	0,0c
B	40,0a	95,0a	95,0a	100,0a	100,0a	100,0a
C	0,0c	5,0b	10,0cd	30,0b	55,0b	55,0b
D	0,0c	10,0b	15,0cd	40,0b	55,0b	55,0b
E	0,0c	10,0b	25,0b	45,0b	60,0b	65,0b
F	5,0bc	15,0b	25,0b	50,0b	65,0b	65,0b
G	15,0b	25,0b	60,0ab	75,0a	85,0a	85,0a

a) Treatment : A (Control), B (Deltamethrin 1 ml/lt), C (ALE 20 gr/lt), D (ALE 30 gr/lt), E (ALE40 gr/lt), F (ALE (50 gr/lt), G (ALE 60 gr/lt)
b) Means followed by the same letters within each column are not significantly different at 5% level according to DMRT

Larvae stop feeding is a condition where the test larvae do not eat the leaves of the feed after the application of botanical pesticides (Batubara et al., 2021). Based on (Table 2), larvae began to stop feeding at 6 HAA due to the application of *A. muricata* leaves extract with concentrations of 50gr/lt and 60gr/lt (F and G). At 6 – 12 HAA showed that application of *A. muricata* leaves extract at a concentration of 50 gr/lt (F) gave results that were not significantly different from 60 gr/lt (G) but significantly different from other concentrations. Meanwhile, at 12-72 HAA, application of *A. muricata* leaves extract at a concentration of 60 g/lt gave larvae stopping feeding results which were not significantly different from the application of the insecticide deltamethrin (B).

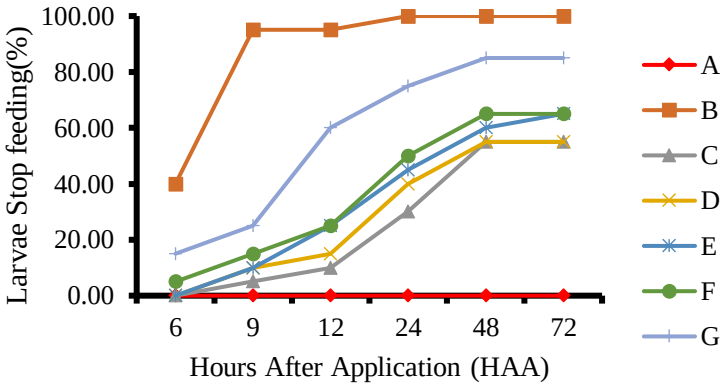

Figure2. *S. frugiperda* larvae stop feeding graph in 6 – 72 HAA due to *A. muricata* leaves extract. Treatment : A (Control), B (Deltamethrin 1 ml/lt), C (ALE 20 gr/lt), D (ALE 30 gr/lt), E (ALE40 gr/lt), F (ALE 50 gr/lt), G (ALE 60 gr/lt)

The application of *A. muricata* leaves extract with the highest concentration of 60 g/lt (G) gave the results of *S. frugiperda* larvae stop feeding the fastest by 15% at 6 HAA, 75% at 24 HAA, and 85% at 72 HAA compared to the application of *A. muricata* leaves extract at other concentrations (Figure 2). According to (Harahap et al., 2019), *A. muricata* leaves contain secondary metabolites such as flavonoids, phenolics, saponins, tannins that are cytotoxic. This is in line with the research of (Batubara et al., 2021) which states that phenolics as larvicides work by suppressing food consumption because they contain a bitter taste, causing inhibition and reduced appetite in larvae.

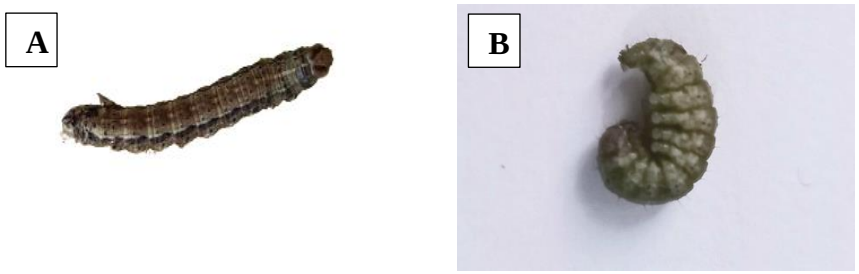

Figure3. (A) Healthy *S. frugiperda* larvae with normal body color (B) *S. frugiperda* larvae with symptoms of poisoning with dark green body color.

A. muricata leaves extract significantly affected the activity of larvae stop feeding due to the secondary metabolite compounds contained in it. Larvae treated with *A. muricata* leaves extract showed symptoms of poisoning which were indicated by a dark green color change on their bodies (Figure 3). Changes in body color due to indications of poisoning symptoms are commonly referred to as knockdown (Turhadi et al., 2020). There is no standard limit regarding the effective concentration of anti-feeding compounds. Some researchers say that a compound that has anti-eating activity appears to have an effect on concentrations that can inhibit feeding up to 50% (Bernays & Chapman, 1978 and Rose et al., 1981 in Schoonhoven, 1982). However, several other researchers say that anti-feeding compounds are effective when they can inhibit feeding about 80-100% (Schoonhoven, 1982). Application of *A. muricata* leaves extract at a concentration of 60 g/lt inhibited Spodoptera frugiperda larvae feeding by 70% at 24 HAA, so it can be said that the concentration was effective in causing *S. frugiperda* larvae to stop feeding.

***Lethal Time* 50 (LT50)**

Toxicity test of several concentrations of *A. muricata* leaf extract on larvae of *S. frugiperda* test was carried out by analyzing the LT50 results. Calculating of LT50 value is needed to determine the average time required by a treatment to kill 50% of the test insect population (Hasyim et al., 2016). Analytically, the average time needed to kill 50% of *S. frugiperda* due to *A. muricata* leaf extract is provided in (Table 3).

Table3. *Lethal Time* 50 Value of*S. frugiperda*due to *A. muricata* leaves extract

Lethal Time	Concentration	Average Hours
LT50	ALE 20 gr/lt	73,17
	ALE 30 gr/lt	61,20
	ALE 40 gr/lt	47,04
	ALE 50 gr/lt	42,72
	ALE 60 gr/lt	22,56

Based on the data in (Table 3), the LT50 value decreased as the concentration of *A. muricata* leaf extract increased, which means that the higher the concentration of *A. muricata* leaf extract, the faster the time required to cause death in *S. frugiperda*

larvae. This is in line with the research by (Junaidi et al., 2016)which reported that application of *A. muricata* leaf extract as much as 1gr/ml with ethyl acetate fraction gave the fastest death to test pests based on LT50 results compared to application of *A. muricata* leaf extract with concentrations below it.

The results of the probit LT50 analysis showed differences in the LT50 value of several concentrations of *A. muricata* leaf extract given, where the higher the concentration of *A. muricata* leaf extract given, the better the LT50 value produced. This is presumably due to differences in the concentration of *A. muricata* leaf extract, affecting the amount of toxic compounds in *A. muricata* leaf extract. This is supported by the results of the research by(Dzulhijja et al., 2019) which stated that the difference in the LT50 value obtained is due to differences in a toxic content possessed by the treatment. The treatment of *A. muricata* leaf extract containing acetogenin which acts as a poison for *S. frugiperda* larvae had a quick effect on killing the test insects.

CONCLUSIONS

1. *A. muricata* leaf extract has a significant effect on the mortality of *S. frugiperda* larvae
2. *A. muricata* leaf extract with a concentration of 60 g/lt is recommended to be used as a biopesticide for controlling *S. frugiperda* larvae because it is effective in providing the fastest results in killing *S. frugiperda* larvae by 60% at 1 DAA, inhibiting *S. frugiperda* larvae feeding by 75% at 24 HAA, and killed 50% of test larvae in less than 24 hours based on the LT50 value.

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