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Effectiveness of Soursop Leaf and Seed Extract (*Annona muricata*) and its Combination on Antifeeding Activity and Mortality of *Spodoptera frugiperda* Larvae

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Abstract
Spodoptera frugiperda is a new pest that is very influential in decreasing corn production. Bioinsecticide from soursop leaves and seeds is one alternative to control *S. frugiperda* because it contains acetogenin, alkaloids, tannins, flavonoids, and saponins. This study aimed to determine the effectiveness and interaction between soursop leaf and seed extracts and their combination against mortality and anti-feeding activity of *Spodoptera frugiperda* larvae. This study uses a 2-factor CRD, its extracts soursop leaf, soursop seeds, and their combinations; the concentration of the extract is 20%, 30%, and 40%. Negative control with aqua dest and positive control with chlorantraniliprole pesticide. The parameters of this study were larval anti-feeding activity and larval mortality. The mortality and anti-feeding activity percentage were transformed by arcsin, analyzed using 2-Way ANOVA, and further examined with the Duncan test on IBM SPSS 25. The results of this study revealed the influence and interaction between extract type and concentration on the mortality and anti-feeding activity of *S. frugiperda* larvae. The 40% concentration combination extract was the best because it produced 60.42% anti-feeding activity and 96.67% mortality. Therefore, soursop seed and leaf extracts can be recommended as bioinsecticides to replace chemical insecticides.

Keywords: bioinsecticide; crop management; *Spodoptera frugiperda*; soursop

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INTRODUCTION

Indonesia is an agrarian country with a lot of agricultural land. One of the crops that many people need is corn plants. Domestic corn demand increases by 10-15% annually (Nelly, 2022). The existence of Plant Pest Organisms (PPO) which can damage the quality and quantity of corn crops is one of the barriers to increasing national corn production (Ramadhan & Nurhidayah, 2022). In corn plantations, the fall armyworm is a new pest that significantly affects the decline in corn production (*Spodoptera frugiperda*). *Spodoptera frugiperda* is an insect originating from the tropics of the United States to Argentina then in 2016, the pest spread was first found in West and Central Africa (Nonci *et al.*, 2019). In 2019, *Spodoptera frugiperda* attacked corn fields in Lampung, Indonesia for the first time (Trisyono *et al.*, 2019). According to Trisyono *et al.* (2019), *Spodoptera frugiperda* attacks plants during the vegetative to generative phase, but the most severe damage occurs in the vegetative phase. Corn plants that are affected by *S. frugiperda* look torn on the leaves, especially young leaves; there are also results of larval secretion in the affected part (Nelly, 2022). The Central Statistics Agency (2021) reported that Indonesia's corn production in 2020 averaged 54.74 kg/ha with a percentage of land affected by PPO of 75.03%.

Farmers usually control pests using chemical pesticides. Based on Sisay *et al.* (2019), the application of synthetic insecticides with the active ingredients lambda-cyhalothrin, spinetoram, chlorantraniliprole, and spinosad effectively and significantly increases larval mortality, reduces leaf damage, and increases biomass in corn. Excessive use of chemical pesticides can create residues that harm the environment and the health of living things (Mubushar *et al.*, 2019)—based on the research results of Ulva *et al.* (2019) stated that almost half of the farmers spraying pesticides for horticultural crops in Lembah Gumanti District, Solok Regency (41.1%) experienced symptoms of pesticide poisoning in the risk category. Another alternative to replacing chemical pesticides is to use environmentally friendly biological control in the form of biopesticides.

Soursop (*Annona muricata*) is one of the plants that can be used to make biopesticides. According to Purnamasari (2021), the results of phytochemical tests on soursop leaf extract with maceration of several solvents were obtained alkaloid compounds, saponins, flavonoids, steroids, and tannins. In addition, soursop plants contain many types of acetogenin that are deadly to insects (Manikome & Handayani, 2020). Acetogenin acts as an antifeedant compound. This is because acetogenin can cause coagulation in the stomach of insects so that the digestive system of insects is disturbed (Sumantri *et al.*, 2014). Alkaloid compounds in the form of isoquinoline, aporphine, and protoberberine are the largest bioactive compounds in soursop plants (Widyastuti *et al.*, 2019). Alkaloids are neurotoxins that alter neuromuscular permeability so that new impulses are formed, which results in seizures and insect death (Boate & Abalis, 2020). In a previous study conducted by Mawuntu (2016), it was found that 20% soursop leaf extract resulted in the highest average percentage of *P. xylostella* larval mortality, which was 81.72%. This is by Dadang & Prijono (2008) who stated that extracts are said to be effective as pest control if they cause a mortality of ≥80%.

Soursop seeds can also be used as a basic ingredient for biopesticides. According to Tando (2018), secondary metabolite compounds in soursop seeds based on phytochemical screening are alkaloids, anthraquinones, flavonoids, polyphenols, coumarins, terpenoids, lactones, steroids, tannins, cyanidin, glycosides, and saponins. In a previous study conducted by Aldywaridha *et al.* (2020), it was stated that 0.5% coarse extract of soursop seeds was able to control leaf rolling pests (*L. indicata*) with a mortality percentage of 100%. The results of Ramadhan and Nurhidayah's (2022) research showed that the methanol extract of soursop seeds with a concentration of 1.6% had the highest inhibitory effect on eating activities, 32.43%. An ingredient is effective as an anti-feeding if it has a percentage of anti-feeding activity greater than or equal to 25% (Mikolajczak *et al.*, 1998).

The effectiveness of biopesticides can be increased by combining plant parts with toxic compounds for pests. The results of the study by Ningsih *et al.* (2013) stated that compared to the application of a single plant-based pesticide, the combination of filtrate of *Dioscorea hispida* tubers, soursop leaves, and earring herb had a greater influence on the mortality of *Spodoptera litura* caterpillars because it produced more secondary metabolites than a single extract treatment.

Research on the effect of soursop leaf extract, soursop seeds, and a combination of both on the mortality of *Spodoptera frugiperda* larvae needs to be carried out, due to the large losses caused by the pest attack. Therefore, the purpose of this study is to determine the effectiveness and interaction between the soursop leaf extract, soursop seeds extract, and their combinations on the mortality and anti-feeding activity of *Spodoptera frugiperda* larvae.

MATERIALS AND METHODS

This study was an experimental research with a 2-factor Completely Randomized Design (CRD), namely the type and concentration of extracts. The types of extracts in this study were soursop leaves, soursop seeds, and their combination. The concentrations of extracts in this study were 0%, 20%, 30%, and 40% (Saragih *et al.*, 2019). This research was conducted over 6 months, from January 2024 to June 2024, at the Basic biology laboratory of Building C10, Biology Study Program, State University of Surabaya.

The tools and materials used in this study were blenders, electric scales, 500 ml measuring cups, filter paper, rotary vacuum evaporators, glass jars, stirrers, scissors, 100 ml glass jars, cork drills, notebook, ballpoint pen, rulers, millimeter paper blocks, label paper, cameras, soursop leaves (*Annona muricata*), soursop seeds (*Annona muricata*), *Spodoptera frugiperda* instar 3 larvae, ethanol 96%, synthetic pesticides (chlorantraniliprole), aquadest, and corn leaves.

This research began with collecting soursop leaves and seeds, washing with running water, drying, and airing the materials. After that, the dried ingredients were mashed into simplicia powder. Maceration was carried out for 3×24 hours using 96% ethanol solvent, with the first ratio being 1:3 and the second and third ratios being 1:2. After that, filtration was carried out with filter paper, and the filtrate was evaporated using a vacuum rotary evaporator. Before applying, the extract was diluted first with aquadest according to the predetermined concentration. At a concentration of 20%, a mixture of soursop leaf/seed extracts of 20 grams and 80 ml of aquadest was carried out, a concentration of 30% was a mixture of soursop leaf/seed extracts of 30 grams and 70 ml of aquadest, and a concentration of 40% was a mixture of soursop leaf/seed extracts of 40 grams and 60 ml of aquadest.

In the combination treatment, extracts were mixed in a ratio of 1:1. The concentration of 20% consists of 10 g soursop seed extract and 10 g soursop leaf extract, the 30% concentration consists of 15 g soursop seed extract and 15 g soursop leaf extract, the 40% concentration consists of 20 g soursop seed extract and 20 g soursop leaf extract; then is continued with dilution like a single extract.

In this study, there are 2 stages of data collection, namely anti-feeding activity for the first stage and mortality for the second stage. The research units in this study are 33 units consisting of 27 treatment units, 3 negative control units (aquadest), and 3 positive control units (chlorantraniliprole pesticide 0.15 ml/ml). The extract application on *S. frugiperda* was administered using the corn leaf feed dipping methods. In each replicate, there are 10 containers, each of which contains 1 *S. frugiperda* larvae. The percentage of anti-feeding activity in 24 hours after application is calculated using the following formula (Ramadhan & Nurhidayah, 2022):

$$A = \frac{K - P}{K} \times 100\%$$

Description:
A = percentage of eating resistance (%)
P = feed weight eaten treatment (g)
K = weight of the feed eaten by the control (g)

Mortality data was obtained by counting the number of dead larvae per day. Observations were carried out every 24 hours after application for 10 days. Observation of larval mortality could be seen from changes in the body shape and color of the larvae's body (morphology). According to Siahaya & Rumthe (2014), the percentage of mortality is calculated by the following formula:

$$M = \frac{d}{N} \times 100\%$$

Description:
M = percentage of pest mortality (%)
d = number of dead pests
N = number of pests tested

The data on anti-feeding activity and mortality of *Spodoptera frugiperda* larvae obtained were transformed into arcsin first. After the data was transformed, the data was analyzed by the Kolmogorov-Smirnov test, if the data was normal, it was followed by the Two-Way ANOVA test. After that, if the test results were significant, it was continued with the Duncan test at level 0.05.

RESULTS

In this study, two data were produced, the percentage of anti-feeding activity and the percentage of mortality of *S. frugiperda* larvae. Anti-feeding activity data was collected by calculating the weight of corn leaves consumed by *S. frugiperda* using the anti-feeding activity formula. The data on the weight of corn leaves consumed by *S. frugiperda* was obtained by reducing the initial weight of the corn leaf by the weight of the remaining corn leaf. The stage of anti-feeding activity was observed and stopped after 24 hours. Mortality data was collected by counting the number of larvae that died for 10 days with observation every 1x24 hours. The feed given at each stage is young corn leaves weighing 0.08 grams.

Before analyzing using SPSS, the percentage of anti-eating activity data that has been obtained is transformed by arcsin first. The results of data analysis on SPSS show that the data is normal because a significance value of > 0.05 was obtained. The results of the 2 Way Anova test obtained a significance value of 0.00 < 0.05 for the extract type; extract concentration 0.00 < 0.05; and the interaction of concentration and type of extract, which is 0.01 < 0.05. Based on the significant results, it can be stated that there is an influence of extract application and concentration with the best result, namely a combination extract with a concentration of 40%. In addition, there was also an interaction between the concentration and type of soursop leaf extract, soursop seeds, and their combination on the anti-feeding activity of *S. frugiperda* larvae.

Table 1. Anti-feeding activity of *S. frugiperda* larvae 24 hours after application

Sample Code	Anti-feeding activity 24 hours after application (%)*		
	Concentration 20%	Concentration 30%	Concentration 40%
K- (aquadest)		0.00±0.00[a]	
K+ (chlorantraniliprole)		100.00±0.00[h]	
Soursop leaf extract	33.33±0.01[b]	39.58±0.02[cd]	42.50±0.01[de]
Soursop seed extract	36.67±0.02[bc]	41.25±0.01[de]	43.75±0.01[e]
Combination extract	44.17±0.03[e]	52.50±0.03[f]	60.42±0.03[g]

Description: Letter notation shows a noticeable difference based on the results of the Duncan test (α=0.05)

Based on Table 1, it can be seen that all extract treatments had a significantly different effect on positive control and negative control treatments. The combination of 30% and 40% extract treatments also had a noticeable difference from the other treatments. The lowest percentage of anti-feeding activity was produced by 20% soursop leaf extract, which was 33.33%, and the highest percentage was presented by the treatment of 40% concentration combination extract, which was 60.42%. The higher the concentration, the more feed leaves remain, and the higher the percentage of anti-feeding activity.

The mortality percentage data obtained is transformed arcsin first so that the data is continuous. The results of data analysis on SPSS show that the data is normally distributed because a significance value of > 0.05 was obtained. The results of the 2 Way Anova test obtained a significance value of 0.00 < 0.05 for the type of extract; extract concentrations of 0.00 < 0.05; and the interaction of concentration and type of extract by 0.03 < 0.05. Based on this, it can be stated that there is an influence on the application of extracts and concentrations with the best results, namely a combination extract of soursop leaves & seeds with a concentration of 40%. In addition, there was also an interaction between the concentration of the extract and soursop leaf and seed extract, and their combination on the mortality of *S. frugiperda* larvae.

Table 2. Mortality of *S. frugiperda* larvae after 10 Days

Sample Code	Mortality (%)*		
	Concentration 20%	Concentration 30%	Concentration 40%
K- (aquadest)		0.00±0.00[a]	
K+ (chlorantraniliprole)		100.00±0.00[e]	
Soursop leaf extract	46.67±1.15[b]	53.33±0.58[bc]	56.67±0.58[bc]
Soursop seed extract	50.00±1.00[b]	66.67±0.58[cd]	73.33±0.58[d]
Combination extract	76.67±0.58[d]	80.00±1.00[d]	96.67±0.58[e]

Description: Letter notation shows a noticeable difference based on the results of the Duncan test (α=0.05)

Based on Table 2., all the extract treatments had a significantly different effect on the negative control treatments. The 40% combination extract treatment did not differ significantly from the positive controls. While the combination extract of 20% and 30% is not significantly different from the 30% and 40% soursop seed extract. The lowest percentage of mortality was produced by the treatment of soursop leaf extract with a concentration of 20%, which was 46.67%, and the highest portion percent was presented by the treatment of combination extract with a concentration of 40%, which was 96.67%. This shows that the higher the concentration of soursop leaf and seed extracts, the higher the mortality percentage produced. Based on the Duncan test, the treatment of 40% concentration combination extract was not significantly different from the positive control treatment. However, according to Dadang & Prijono (2008), the most effective extracts for controlling pests are those that have a mortality percentage of ≥ 80%. Effective pest control can control pests with a higher mortality rate than the level of damage by the pest but also does not cause overall pest mortality (100% mortality). Therefore, the 40% concentration combination extract treatment can be said to be effective in controlling *S. frugiperda* pests because it has a mortality percentage of 96.67%, which is also close to the mortality value of positive control treatment and does not cause overall pest death.

In the results of the Two Way Anova test for the interaction between concentration and the type of soursop leaf and seed extract, as well as the combination, a significance value of 0.01 on anti-feeding activity and 0.03 on mortality was obtained. Therefore, it can be shown that there is an interaction between the type of extract and the concentration on mortality and anti-feeding activity, which is a type of extract with a combination of 40% concentration.

DISCUSSION

Based on the research, it was found that there was an influence between the type of extract and its concentration on the anti-feeding activity of *S. frugiperda* larvae. The lowest percentage of anti-feeding activity was produced by the type of soursop leaf extract with a concentration of 20%, which was 33.33%. The most effective type of extract and concentration on the anti-feeding activity of *S. frugiperda* larvae is a combination extract with a concentration of 40% with a percentage of anti-feeding activity of 60.42%. The percentage of anti-feeding activity in the single extract treatment was lower compared to the combination extract treatment.

In addition to anti-feeding activity, in this study, there was also an influence between the type of extract and its concentration on the mortality of *S. frugiperda* larvae. The lowest percentage of mortality was produced by the type of soursop leaf extract with a concentration of 20%, which was 46.67%. The most effective type of extract and concentration for the mortality of *S. frugiperda* larvae is a combination extract with a concentration of 40% with a percentage of 96.67%. The percentage of mortality in the single extract treatment was lower compared to the combination extract treatment.

The increase in the percentage of anti-feeding activity and mortality may be affected by the concentration of the extract. This is because the higher the concentration of the extract, the higher the active compounds contained in the extract, which can also cause a high percentage of anti-feeding activity and larval mortality. This is according to the statement of Baideng (2016), namely the increase in the percentage of larval mortality in line with the increase in extract concentration. Lestari *et al*. (2014) also mentioned that the higher the concentration, the greater the barrier to eating.

The compounds contained in soursop seeds and leaves based on the results of phytochemical screening according to Tando (2018) are alkaloids, anthraquinones, flavonoids, polyphenols, coumarins, terpenoids, lactones, steroids, tannins, cyanadins, glycosides, and saponins, phenols, and phytosterols. In addition, the soursop plant has the main compound in the form of acetogenin. Acetogenin has an antifeedant mechanism that causes coagulation of the insect stomach so that its digestion is disturbed (Sumantri *et al*., 2014). In addition, acetogenin can also cause inhibition of NADH ubiquinone reductase in the respiratory chain and interfere with electron transport so that ATP levels decrease and cell apoptosis (Kusumawati & Istiqomah, 2022). Another compound that plays a role in the anti-feeding activity of larvae is tannins. Tannins act as antifeedants by interfering with the activity of digestive enzymes (amylase and protease). Tannins can interact with proteins and produce complex proteins so that they can inhibit the enzyme α-amylase in insect digestion (Firdausi *et al*., 2013). The decrease in protease enzyme activity results in inhibition of the formation of amino acids so that ATP is also not formed (Hidayati *et al*., 2013).

Compounds that are toxic and play a role in larval mortality are alkaloids, flavonoids, and saponins. Alkaloids act as neurotoxins because they can alter neuromuscular permeability causing the formation of new impulses and the occurrence of death in insects (Boate & Abalis, 2020). Flavonoids act as respiratory inhibitors by entering through the larvae's respiratory system and causing wilting of the nerves so that the larvae cannot breathe and die (Cania & Setyanimgrum, 2013). Saponins can bind sterols to the food tract which act as precursors of larval ecdison hormones, causing the larval skin turnover process to be disrupted (Muta'ali & Purwani, 2015). Saponin compounds enter through the epicuticular to the target organ by causing damage to the waxy layer on the cuticle and the larvae die due to the loss of a lot of water (Rakmawati *et al*., 2018).

Based on the results of the study, the treatment of soursop leaf extract is not significantly different from soursop seed extract. This is because soursop leaves and seeds contain the same secondary metabolite compounds, namely acetogenin, tannins, alkaloids, flavonoids, and saponins. In the results obtained, soursop seed extract with several concentrations had a higher percentage of anti-feeding activity and larval mortality than soursop leaf extract. This is because although they contain the same secondary metabolite compounds, they have different levels of secondary metabolites. This is supported by the results of a study by Carole *et al*. (2022) that soursop leaf ethanol extract has a total level of flavonoids of 0.55 mg/ml, saponins of 0.33 mg/ml, tannins of 0.64 mg/ml, and alkaloids of 0.41 mg/ml. In the results of the study by Ugochi *et al*. (2019), methanol extract of soursop seeds had flavonoid levels of 0.23 mg/g, saponins of 322.85 mg/g, tannins of 5.70 mg/g, and alkaloids of 340.12 mg/g.

Based on visual observations of anti-feeding activities, the larvae treated with soursop leaf and seed extracts, and their combination, have the characteristics that the larvae tend to be still, stay away from their feed, and bend their bodies when touched. This is due to the presence of extract liquid that sticks to the surface of feed leaves that contain antifeedant compounds so that it can interfere with larval oral taste receptors and larval digestion. In the negative control treatment, the larvae continue to feed until the feed runs out. This is because the aquadest liquid in feed leaves does not contain antifeedant compounds. Meanwhile, in the positive control treatment (chlorantraniliprole), the larvae do not eat at all so the feed is still intact.

Based on morphological observations on mortality, the larvae treated with soursop leaf and seed extracts, and their combination, have the characteristics of the larval body wrinkling, changing color to blackish-brown, and secreting fluid. In addition, some larvae have failed to molt so that the head of the larva has been replaced with new skin, but the old skin is still attached to its body. This is supported by the research of Rustam & Rajani (2021), that the changes that occur after a few hours of the *S. frugiperda* larvae die are that the body changes color to dark brown until blackish, the body shape is soft and wrinkled. In the negative control treatment, the larval body remains fresh, yellowish-green in color, and does not undergo morphological changes. Meanwhile, in the positive control treatment, the morphological characteristics of the larvae are in the form of a body that changes color to deep black, wrinkled, and dry. This is due to the content of the active ingredient in the form of chlorantraniliprol in the chemical pesticide used. The way active ingredient chlorantraniliprole works by activating the ryanodine receptor (RyR) so that calcium will be released and result in irregular muscle movements, paralysis, and death (Bagariang *et al*., 2020).

The control ability of soursop leaf and seed extracts, as well as their combinations, is still under positive control, but their use is relatively safer compared to chemical pesticides because the ingredients are natural and easily decomposed. In addition, the combination extract of 40% concentration resulted in anti-feeding activity and mortality percentages of 60.42% and 96.67%. The percentage has reached a mortality rate of ≥ 80% so it can be said to be effective for the control of *S. frugiperda* larvae and can be used as an alternative substitute for chemical pesticides.

In the agricultural sector, the use of biopesticides is included in the concept of Integrated Pest Management (IPM). The use of biopesticides is safer compared to chemical pesticides. Based on the results of Yuliani's (2022) research, the application of biopesticides containing methanol extract from *Elephantopus scaber* leaves was able to increase the mortality rate of *S. litura* and *P. xylostella* to 93.35% and 96.65%, respectively. Biopesticides aim to control pests by reducing pest populations and not causing 100% deaths, so natural enemies remain sustainable and the balance of the ecosystem is maintained. Natural enemies act as biological control agents that can reduce pest populations so that the impact of pest attacks is also reduced (Nonci *et al*., 2019). In this study, the recommended single extract treatment for implementation is the use of soursop seed extract with a concentration of 40% which has a mortality percentage of 73.33%, and in the treatment of a combination of soursop leaves and seeds the recommended concentration is 40% which has a mortality percentage of 96.67%. However, from an economic point of view, the recommended treatment is a combination extract with a concentration of 30% because mortality has reached 80% and anti-eating activity ≥ 25%.

CONCLUSION

Based on the results obtained from this study, the conclusion that can be drawn is the effect of giving soursop leaf extract and soursop seed (*Annona muricata*), as well as the combination of the two on mortality and anti-feeding activity of *Spodoptera frugiperda* larvae. The combination extract of soursop leaves and soursop seeds is the most effective treatment in influencing the anti-feeding activity and mortality of *S. frugiperda* larvae. There was an effect on the concentration of soursop leaf extract and soursop seed (*Annona muricata*), as well as its combination on mortality and anti-feeding activity of *Spodoptera frugiperda* larvae. The most effective concentration was 40% on the combination extract of soursop leaves and seeds because it produced a percentage of anti-feeding activity of 60.42% and a mortality of 96.67%. There was an interaction between concentration with soursop leaf extract and soursop seeds, as well as their combination on mortality and anti-feeding activity of *Spodoptera frugiperda* larvae. The interaction between the type and concentration of the extract was a combination extract of soursop seeds and leaves with a concentration of 40%.

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CONFLICT OF INTEREST

The author declares there is no conflict of interest to disclose.

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