The Effect of Soursop and Betel Leaf Extracts as Organic Pesticides in Pest Control of *Spodoptera exigua* (Lepidoptera: Noctuidiae) on Onion (*Alium ascalonicum L.*) Cultivation

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Abstract

Spodoptera exigua is a major pest in onion cultivation. S.exigua larvae have a rapid spread and can cause yield losses of up to 20-100% if not controlled. Soursop leaf extract and betel leaf pesticides can cause mortality in S.exigua pests. This is due to secondary metabolites found in soursop leaf extract and betel leaf such as acetogenin, alkaloids, flavonoids, terpenoids, steroids, saponins and tannins. The purpose of this study was to determine the effectiveness of soursop leaf extract and betel leaf and the combination of both in controlling S.exigua pests. This study used a non-factorial Complete Randomized Design method with 7 treatments, namely P0 = Control, P1 = 30% soursop leaf extract, P2 = 60% soursop leaf extract, P3 = 30% betel leaf extract, P4 = 60% betel leaf extract, P5 = 15% soursop leaf extract + 15% betel leaf extract, P6 = 30% soursop leaf extract + 30% betel leaf extract. The results showed that the P2 treatment had the highest mortality rate with 67.50% mortality and the P3 treatment had the lowest mortality rate of 25.00%. Symptoms of attack show that the larvae are initially actively eating and then begin to stay away from feed, the larvae then show a change in color to brown and slowly remain still and die. The duration of pest mortality showed the fastest mortality occurred in 24 hours with the death of 20 larvae, larval mortality continued to increase with each observation. Testing on natural enemies showed results that the use of soursop leaf extract did not show any death

Keywords: *Alium ascalonicum, Spodoptera exigua, Vegetable pesticides, Soursop leaves, Betel leaves*

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INTRODUCTION

Onion (*Allium ascalonicum L*) is one of the important export commodities in Indonesia since 2017 (Lubis *et al.*, 2022). Onion are one of the main vegetable commodities in Indonesia and have many benefits. Shallots are flavoring vegetables that are needed by people in Indonesia. Because shallots have a distinctive aroma, therefore these vegetables are widely used as flavoring spices or with the term "Spice Vegetables"

(Sulardi & Zulbaidah, 2020). This crop has a large contribution to regional economic development, amounting to approximately Rp 2.7 trillion per year, and can provide benefits for farmers of Rp 42 million / ha in one planting season (Herlita *et al*., 2016). BPS data shows that onion production in the North Sumatra region has increased production in 2021 by 537,754 tons, of which in 2020 it was only 292,221 tons (BPS, 2022).

Problems that often occur in the demand for shallot supply continue to increase among the community. Meanwhile, for the production of onion that are annuals, so that under certain conditions it can cause turmoil between demand and supply that continues to occur (Tharmizi & Sukma, 2019). One of the obstacles in onion production is due to pest attacks. *Spodoptera exigua Hubner* (Lepidoptera; Noctuidae) is an important insect pest on onion plants. *S.exigua* has the ability to spread quickly on onion plants both in lowlands and highlands, besides this pest attacks onion plants during the dry and rainy seasons (Marsadi & Sunari, 2017). The potential yield loss by pests in old and young plant stadia can reach 20-100% depending on the management of onion cultivation (Sipahutar, 2023). The main part of the attacked plant is the leaves, both young and old. Young larvae hatched from eggs make holes in the tips of the leaves and get into the onions, making the tips of the leaves damaged or cut off. The larvae hoist inside part of the leaf, leaving behind the outer epidermal layer. As a result of this attack, leeks look white or clear spots. Other attacks cause the leaves to dry out. At first the larvae gather together, then spread as the inside of the leaf wears off. If the larval population is high then the larvae will also attack the bulbous part of the onion (Wiraningrum, 2022).

Pest control carried out by farmers cannot be separated from the use of chemical pesticides, but the use of chemical pesticides can cause environmental damage in the long term and cause resistance to target pests. Chemical pesticides do not decompose naturally so residues accumulate in the soil and adhere to vegetables. Synthetic chemical compounds consumed together with vegetables will cause degenerative diseases such as cancer. Today, pesticides that accumulate in the soil can cause pest resistance, as well as damage the soil (Astuti & Widyastuti, 2017).

Vegetable pesticides can be used as one of the environmentally friendly control techniques Vegetable pesticides have advantages over chemical pesticides. Vegetable pesticides are more environmentally friendly because of the nature of organic substances that are easily decomposed, vegetable pesticide residues do not last long in the plant, looking at it from an economic point of view, the use of vegetable pesticides provides added value to the products produced, the use of pesticides with integrated pest control does not cause resistance to pests (Astuti & Widyastuti, 2017). Vegetable pesticides also have several disadvantages, including less practical. Vegetable pesticides cannot be stored for a long time and the results obtained from spraying vegetable pesticides are not as fast as the results obtained from synthetic chemical pesticides.

However, along with the development of ecological agricultural technology, there have been many innovations to overcome these obstacles such as using active substances containing secondary metabolite groups such as alkaloids, terpenoids, phenols and other chemicals. This active ingredient can attack pests in various ways, for example as a repellent, anti-feedant, growth regulator, attractant, and deadly poison. Organic pesticides made from animal body parts usually come from urine. Some microorganisms can also control pests, so they can be used to make pesticides (Astuti & Widyastuti, 2017).

Soursop and betel plants are plants that can be used as vegetable pesticides. Soursop plant (*Annona muricata*) is a plant that is widely known to the public. Plant parts that are suitable as raw materials for extracts are on the leaves, which can be used as insecticides because they contain substances that can cause repellent and anti-feedent properties. According to Sumantri *et al.*, (2014), Soursop leaf extract contains acetogenin compounds (*antifeedent*) which can cause impaired function in the digestive organs of insects. Betel (*Piper betle* L) is one type of plant included in the Piperaceae family whose leaves can be a source of vegetable pesticides (Fissabililah & Rustam, 2020). Active compounds contained in the Piperaceae family belong to the piperidine group, such as piperine, pipericide, piperlonguminine, and guinincine. This compound is widely reported to have insecticidal properties. Betel leaf also contains compounds such as hexane, cyanide, saponins, tannins, flavonoids, steroids, alkaloids and essential oils that are believed to act as insecticides (Taufik *et al.*, 2021).

Several ways are usually used to reduce pest attacks, including using biological agents (parasitoids, predators and microbial agents or insect pathogens). Several pathogens (fungi, bacteria, viruses and nematodes) have been widely used to control armyworm pest attacks (Ginting *et al.*, 2014). This study aims to test the results of Soursop leaf extract, Betel leaf extract and a combination of both that causes the highest mortality.

MATERIALS AND METHODS

This research was carried out in the laboratory of the Panca Budi Medan Development University. The study was conducted from November to December 2023. The ingredients used aquades, soursop leaves, betel leaves, red onions, *S.exigua* larvae, *Trichogramma sp*, *Cheilomenes sexmaculata*, *Phenacoccus solenopsis*, label paper, honey, tape, filter paper and cotton. Tools used plastic cups, gauze, sprayers, tweezers, scissors, measuring pipettes, ovens, beakers, test tubes, filters, stirrers, mashers / blenders, black glass bottles, rubber bands, markers, planel cloth, cellphone cameras and stationery.

The method used is a Nonfactorial Complete Randomized Design (RAL) with 7 treatments and 4 repeats so that there are 28 experimental units. Each experimental unit contained 10 instar II larvae applied with vegetable pesticides as follows: P 0 = 0% (0ml leaf extract + 100ml a quades) P 1 = 30% (30ml Soursop leaf extract +70ml aquades)) P2 = 60% (60ml soursop leaf extract + 40ml aquades) P 3 =30% (30ml extract betel leaf +70ml aquades) P 4 = 60% (60ml extract betel leaf +40 ml aquades) P5 = 30% (15ml extract soursop leaf +15ml extract Betel leaf +70ml aquades) P6 = 60% (30ml soursop leaf extract +30ml betel leaf extract +40ml aquades).) Treatment using *the Dipping* Method is given through feed.

Making Soursop Leaf Extract and Betel Leaf Extract

Soursop and betel leaves are collected and washed thoroughly and then dried for ±2 weeks. After drying, the leaves are blended into powder. Soursop and betel leaf powder are then macerated using aquades. Maceration is carried out for 3 days, then the results will be filtered and the filter results will be stored in the glass of the beak. The filter results are evaporated using an oven at a temperature of 50º. The evaporated extract is then bottled and stored in the refrigerator.

Phytochemical Screening Test

The results of soursop leaf extract and betel leaf that have been macerated and evaporated using an oven are then tested to the Biochemistry laboratory of the University of North Sumatra. The test aims to determine the content of secondary metabolite compounds contained in soursop leaf extract and betel leaf.

Propagation of *S.exigua*

S.exigua larvae are obtained directly from the field of UPT Onion plants. Horticultural Mother Seed (BIH) Kutagadung, Berastagi. The obtained larvae are put into plastic cups separately, the larvae are fed with leeks. The larva is maintained until it enters the prepupa phase, in this phase the larva's body will wrinkle and bend slightly, the prepupa phase lasts 1-2 days until it becomes a pupa. After the larva becomes a pupa, the pupa is separated into a dark place and then waited until it becomes an imago, the pupa phase lasts 8-10 days. *S.exigua* imago are placed in groups into modified maintenance containers. Imago is fed using cotton wool soaked in a 10% honey solution and changed once a day. Imago will lay eggs in 2-4 days, after the imago lays separate the eggs in groups and put them into the enlargement cup. Eggs take 3-5 days to hatch, eggs that have hatched into larvae will be reared again for 5-6 days until instar 2 larvae are used as test larvae.

Natural Enemy Propagation

Imago *C. sexmaculata* is obtained from eggplant fields. 10 pairs of male and female imago are put into a jar and fed with mealybugs (*P.solenopsis*), Imago lays eggs after 2-3 days. After laying, the eggs will be separated from the imago. The eggs will hatch in 2-4 days and will become larvae, the larvae are reared again 6-10 days until they become pupae and fed mealybugs. The pupa phase lasts 2-4 days, the imago that appears will be pale reddish-orange and ready for testing.

Trichogramma sp was obtained from Bogor Agricultural University (IPB) which was propagated using *Crocidolomia pavonana* eggs glued to pias paper. Pias paper that has eggs that have been parasitized is inserted into a test tube. After 7 days the parasitoids hatch and are put into test tubes to be maintained and given a 10% honey solution deposited on a cotton swab.

Application to *S.exigua*

The test larvae used are instar 2 larvae. Before testing the larvae are starved first for 6 hours. testing using the *Dipping* method. Feed dipped in each extract solution for 15 minutes and dried aerated. The feed is then given to the test larvae.

Application of Natural Enemies

Application to natural enemies is carried out by the Residue method. The test tube/container to be used is dripped with an extract solution of 1 ml using the treatment with the highest mortality rate, then the test tube/container is air-dried to dry. Predators and parasitoids are then put into test tubes/containers and then observed for 24 hours.

Test Parameters

Phytochemical Screening

The test used soursop leaf extract and betel leaf extract. Phytochemical screening tests were carried out at the biochemistry laboratory of the University of North Sumatra.

Mortality of S.exigua

The mortality of *S. exigua* was observed after application. Observations were made up to 96 hours after application (HAA) : 24 Hours, 48 Hours, 72 Hours, and 96 Hours. According to Siregar (2017) analyzed using the following formula,

$$M=\frac{a}{a+b}x100\%$$

Description
M : Mortality percentage of *S.exigua*
a : Total dead *S.exigua* (tail)
b : Total living *S.exigua* (tail).

According to Warsa *et al.*, (2019) Benchmark standards in determining the effectiveness of vegetable pesticides on caterpillar pest mortality are,

0-30 % = ineffective
31-50 % = less effective
51-70 % = effective
71-100% = highly effective

Symptoms of Pest Attack and Behavior

Symptoms of attacks are observed for 96 hours after application. The larvae are observed and recorded symptoms of attack and behavior until the larvae die.

Duration of Death

The duration of death is observed after application until the larvae experience death. Observations were made ±96 JSA.

Biosafety Test

Biosafety testing was conducted using predators and parasitoids from *S.exigua*. Natural enemy testing is carried out for 24 hours. Where natural enemies will be put in containers that have been exposed to vegetable pesticide residues with the highest percentage of *S.exigua* mortality. The survival rate of natural enemies is calculated using the formula of Supono *et al.*, (2022).

$$SR = \frac{Nt}{N0}100\%$$

Information,
SR = Survival rate(%);
Nt = Number of final predators/parasitoids (tails);
N0 = Number of initial predators/parasitoids (tails).

Data Analysis

Data on the percentage of *S.exigua* deaths were analyzed by fingerprint (analysis of variance = ANOVA), then continued with Duncan's Multiple Range Test DMRT at a real level of 5%.

RESULTS AND DISCUSSION

Phytochemical Screening

Based on phytochemical results in table 1. It was found that vegetable pesticides of soursop leaf extract contain secondary metabolites, namely alkaloids, flavonoids, terpenoids, steroids, saponins, and also tannins. The above results are in line with research Fitriani (2015) and Tando (2018) which states that soursop extract contains alkaloid compounds, flavonoids, terpenoids, steroids, saponins and also tannins. The results of vegetable pesticide screening tests of betel leaf extract also found that betel leaves contain alkaloid compounds, flavonoids, terpenoids, steroids, saponins and tannins. The above results are also in accordance with the research (Nisyak & Haqqo, 2022) which states similar results.

Table 1. Phytochemical Screening Test Results of Soursop Leaf and Betel Leaf Extracts

Compound	Reagent	Soursop Leaves	Betel Leave	Color Change
Alkaloids	*Bouchardart*	+	+	Brick red deposits
	Maeyer	+	+	Yellowish-white precipitate
Flavonoids	*Fecl3*	+	+	Colloidal Black
	Mg.Hcl	+	-	Pink Solution
	H2So4	-	-	Yellowish-Orange Solution
Terpenoids	*Liebermann-Bouchard*	+	+	Bluish-green solution
	Salkowsky	-	-	Red Solution
Steroids	*Liebermann-Bouchard*	+	+	Bluish-green solution
	Salkowsky	-	-	Red Solution
Saponins	*Aquadest + Shuffle*	+	+	Foaming
Tannins	*Fecl3*	+	+	Colloidal Black

Mortality of *S.exigua*

Based on the data in Table 2. The test results of applying vegetable pesticides of soursop leaf extract, betel, and combination obtained significant results on the mortality percentage of *S.exigua*. In the vegetable pesticide treatment above, the highest mortality result was obtained which was 67.50% from the P2 treatment of 60% soursop leaf extract. This can be caused because the P2 treatment is thought to contain secondary metabolites of acetogenin, alkaloids, flavonoids, terpenoids, steroids, saponins and also tannins contained in soursop leaf extract so that it can cause mortality in *S.exigua* larvae. This is according to research Kusuma (2015) which states the influence of soursop leaf extract is caused by secondary metabolite compounds, such as acetogenin, alkaloids, flavonoids, saponins, and tannins. Hambali (2018) states that acetogenin compounds of the *Annonaceae* type are reported to have effective toxicity to control some insects such as Lepidoptera, Coleoptera, Hemiptera and Diptera. The content of acetogenin compounds from soursop plants is also thought to affect mortality in insects through contact poisons, so that insects experience respiratory problems through pores in the body of insects. According to Azlansah *et al.*, (2019), that alkaloid compounds are anti-eating so that these alkoid compounds can damage the nervous and digestive systems, decreased growth rate, failure of the pupa to become imago. Flavonoid compounds present in soursop leaves act as digestive inhibitors in insects. Hambali (2018) argues that flavonoid compounds have an effect on reducing the activity of protease and amylase enzymes so that the ability to digest food in *S.exigua* larvae will decrease. Saponin compounds in soursop leaves can damage the wax layer on the cuticle layer which causes caterpillars to lack a lot of water and eventually die. While tannin compounds are antifeedant, shrink and dry caterpillar skin tissue (Qin *et al.*, 2021).

The mortality outcome is second highest in Table 2. It is found in the P6 Combination treatment with a mortality rate of 55%. This is thought to be due to the active substances contained in both extracts complementing each other's deficiencies. However, because the amount of concentration combined is lower than that of a single treatment, the results of the P6 combination are no better than the P2 treatment.

Table 2. Mortality percentage of S.exigua due to administration of soursop leaf extract, betel nut and a combination of both

Treatment		Observation			
		24 HAA	48 HAA	72 HAA	96 HAA
P0	Control	0,00^{l}	0,00^{l}	0,00^{l}	0,00^{l}
P1	Soursop Extract 30%	10,00^{kl}	22,50^{jk}	35,00^{gh}	45,00^{de}
P2	Soursop Extract 60%	17,50^{kl}	37,50^{fg}	57,50^{ab}	67,50^{a}
P3	Betel Extract 30%	7,50^{kl}	10,00^{kl}	22,50^{jk}	25,00^{ij}
P4	Betel Extract 60%	10,00^{kl}	22,50^{jk}	37,50^{fg}	40,00^{ef}
P5	Combination 15%+15%	2,50^{kl}	15,00^{kl}	32,50^{gh}	32,50^{gh}
P6	Combination 30%+30%	15,00^{kl}	30,00^{hi}	47,50^{cd}	55,00^{bc}

Remarks : Numbers followed by the same letter indicate no real difference in the Duncan 5% test

Symptoms of Pest Attack and Behavior

The lowest mortality rate of 25% was found in the P3 treatment. The occurrence of mortality in P3 treatment is due to the content of secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins and tannins. In research Mahera *et al.*, (2015) states that the active compounds contained in betel leaves are included in the piperamide group such as piperine, pipericide, piperlongumin and guininene. These compounds enter the body of insects as contact poisons and work as nerve poisons, disrupting the flow of nerve impulses in nerve axons (Irawan & Rustam, 2018). According to Anisah & Sukesi (2018) Tannin compounds are the most abundant compounds found in betel leaves. Based on the data above, it was found that the higher the concentration of betel leaf extract, the higher the mortality rate produced. The lack of compounds in betel leaves compared to soursop causes the resulting mortality rate is quite low compared to the P2 treatment.

Figure 1. Symptoms of *S.exigua* attacks
a. The state of the larvae before application b. Larvae of *S.exigua* after application with the larval state stay away from feed c. Larvae after application, the body looks blackened and thin d. The bodies of the larvae rot and some look dry out.
Source : Live Photo.

Control treatment (P0) found no mortality. Vegetable pesticides of soursop leaf extract, betel, and combination had no real effect on 24 HAA, but on 48, 72, and 96 HAA obtained significantly different control treatment (P0). The initial symptoms found in soursop and betel leaf extract show that larvae that initially look active eating begin to look away from feed and climb up to the observation container, larvae then experience a change in body color which was originally yellowish-green began to change color to brown, after that the larvae looked just still and died. This is according to research Setiawan *et al.*, (2021), *Spodoptera fugiperda* experiencing color changes due to soursop leaf extract from yellowish-green to blackish-brown. According to Sundari *et al.*, (2021) expressed symptoms of death in the larvae of *Spodoptera litura* experience anxiety, convulsions, weight loss due to tannin compounds and finally death. The above statement is in accordance with the research (Syah & Purwani, 2016).

Table 3. Number of Deaths of *S.exigua*

Treatment	Number of Deaths			
	24 HAA	**48 HAA**	**72 HAA**	**96 HAA**
P0	0	0	0	0
P1	3	5	4	6
P2	6	5	7	9
P3	2	1	4	3
P4	2	3	6	5
P5	2	5	3	3
P6	5	4	7	6
Total	20	23	31	32
Average	2,8	3,2	4,4	4,5

Duration of Death

The length of death is calculated by the number of dead larvae in each observation. The fastest mortality duration was found at 24-hour observation with a total number of deaths of 20 test larvae, mortality results increased at 48, 72, and 96 hour observations. According to Astuti & Widyastuti (2017) The results obtained from spraying vegetable pesticides are not as fast as the results obtained from synthetic chemical pesticides. It takes time and more frequent spraying to be effective.

Biosafety Test

Testing of natural enemies using extracts with the highest mortality, namely in P2 treatment with a mortality rate of 67.50% applied and observed for 24 hours. The test results in (Table 4), P2 treatment showed that 60% soursop leaf extract did not cause mortality in natural enemies (predators and parasitoids). The above results are in accordance with the research Ilmi *et al*., (2016) which states that the highest number of natural enemies is found in vegetable pesticide treatment, while the lowest number of natural enemies is due to chemical pesticide treatment. According to Rohmah *et al*., 2022) The use of plant-based insecticides will not affect the abundance and diversity in predators. There is no mortality in natural enemy insects due to the application of vegetable pesticides because some secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, and tannins are anti-feedants that affect the feeding activities of herbivorous insects.

Table 4. Test results against natural enemies

Parasitoid/Predator	24 HAA (%)			
	1	2	3	4
Trichogramma sp.	100	100	100	100
Cheilomenes sexmaculata	100	100	100	100

CONCLUSION

The treatment of vegetable pesticides of soursop leaf extract and betel leaf extract has a significant influence on the control of *S.exigua.* Soursop leaf extract 60% in P2 treatment has the highest mortality rate with 67.50% mortality and betel leaf with a concentration of 30% in P3 treatment has the lowest mortality rate of 25%. Testing of natural enemies also shows that pesticides of soursop leaf extract and betel leaf extract are proven safe in maintaining the abundance of natural enemies.

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