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The Effectiveness of Basil (*Ocimum basilicum*) and Tobacco (*Nicotiana tabacum*) Leaf Extracts on the Mortality and Antifeedant Activity of *Spodoptera litura*

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Abstract
Spodoptera litura is a pest in Indonesia that can reduce agricultural and horticultural yields by up to 80%. Biopesticides from basil and tobacco leaf extracts contain eugenol and nicotine which can be an alternative to control *S. litura*. This study aims to determine the effect of various types of extracts, concentrations, and interactions between the two, on the mortality rate and antifeedant activity of *S. litura*. This study used a 2-factor Completely Randomized Design (CRD) with treatments of extract types (basil leaves, tobacco, and a combination of both), extract concentration (30%, 40%, 50%, 60%), using negative control of distilled water and positive control of cypermethrin. Data on the percentage of mortality and feeding activity were analyzed using two-way ANOVA, followed by the Duncan test. The results showed that the type of extract, extract concentration, and interaction between the two, affected the mortality and feeding activity of *S. litura*. The combined extract at a concentration of 60% was the most effective treatment at mortality rate of 86.67% and antifeedant activity of 41.67%. Therefore, the combination of basil and tobacco leaf extracts can be applied as a biopesticides against *S. litura*.

Keywords: agricultural innovation, basil, botanical pesticides, *S. litura*, tobacco

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INTRODUCTION

Indonesia has a large area of agricultural land and the majority of the population works in the agricultural sector. This indicates that the agricultural sector has a significant role in improving the economy and welfare of the people in Indonesia. However, efforts to increase production yields are often faced with problems such as pest attacks, one of which is *S. litura* (Budi *et al.*, 2013). *S. litura* (armyworm) is a polyphagous leaf pest that attacks a variety of host plants, including food crops, vegetables, and fruits (Zestyadi *et al.*, 2018). In its life cycle, the larval phase of *S. litura* causes varied damage. Dirgayana *et al.*, (2023) explained that *S. litura* attacked tomato plants with the highest percentage of attack at 40%. According to Vijayalakshmi *et al.*, (2016) *S. litura* damages chili plants, causing a 40%-50% reduction in yield.

Farmers currently rely on synthetic pesticides to control *S. litura* attacks due to their quick effectiveness and ease of use. However, continued use has the potential to cause pest resistance and environmental pollution (Muhidin *et al.*, 2020). In addition, pesticide residues can cause health problems in humans, such as symptoms of nausea, vomiting, dizziness, and skin irritation, which are a direct result of pesticide exposure (Amilia *et al.*, 2016). Therefore, a solution is needed to replace synthetic pesticides that are safe for the environment and health, one of which is the use of natural pesticides by utilizing the potential of active compounds from natural ingredients. The use of natural ingredients is more environmentally friendly because it is easily dissolved and decomposed, not dangerous like synthetic pesticides, and the production costs are more affordable (Ariyanti *et al.*, 2017). Potential plants as natural pesticides include basil (*O. basilicum*) and tobacco (*N. tabacum*).

Basil leaves contain compounds such as saponins, flavonoids, tannins, terpenoids, and essential oils that have potential as natural pesticides (Surahmaida and Umarudin, 2019). Essential oil of basil leaves contains 46% eugenol, which functions as a nerve and respiratory poison (Soedarso, 2013). Eugenol inhibits the action of the enzyme acetylcholinesterase and affects larval breathing through the spiracles, making it difficult for them to breathe (Sutikno and Anggraini, 2023). Research by Mefta and

Fauzana (2021) stated that essential oil from basil has potential as a natural insecticide to control fruit flies.

Tobacco is an economical plant that is widely cultivated in Indonesia, especially for its leaves, which are the main ingredient for making cigarettes. Tobacco extract with a concentration of 5% can cause mortality of *Plutella xylostella* pests by 34% on mustard plants (Haryanti *et al*., 2017). The main content of tobacco leaves is nicotine. Afifah *et al*., (2015) explained that nicotine is a neurotoxin that can interact quickly. The accumulation of nicotine in the larval body can inhibit the nervous system and cause permanent blockage of receptors. (Khater, 2012).

Indonesia has the highest smoking rate in ASEAN with 36.3% of smokers aged 25-64 years (Lian and Dortheo, 2018). This increases the need for tobacco for sustainable cigarette production. The use of plant-based pesticide combinations is an important alternative due to the limited availability of tobacco for cigarettes. The combination of several plants with compounds toxic to pests can produce stronger effects than using a single plant. Previous research results by Ali *et al*. (2023) showed that the combination of tobacco leaf extract and neem leaves at a dose of 20 ml can cause 50% mortality of *S. litura* larvae on the second day. Research on the effectiveness of tobacco leaf extract as a biopesticide has been studied before. However, the combination of basil and tobacco leaf extracts has never been studied. The purpose of this study was to examine the effect of extract type, concentration difference and interaction between extract type and concentration of basil leaf extract, tobacco and their combination on mortality and anti-feeding activity of *S. litura*.

MATERIALS AND METHODS

This type of research is experimental research conducted at the Basic Biology Laboratory and Plant Physiology Laboratory from December 2023 to July 2024. This research was as completely randomized design (CRD) study with two treatment factors; type of extract and extract concentration. The extracts used were basil leaf extract, tobacco, and their combinations. The extract concentrations used consisted of 30%, 40%, 50%, and 60% (Manikome, 2021; Azzahra, 2023).

This study began with the collection of 10 kg of fresh basil leaves, which were then washed and dried. The tobacco leaves used were 2.5 kg of *grosok* tobacco leaves. After drying, the leaves are then mashed until they become simplisia powder. The maceration process was carried out three times for three days in a ratio of 1:3, 1:2, 1:2 using 96 ethanol. After that, the filtrate from maceration was extracted using a rotary evaporator until 100% extract was obtained.

The thick extracts of basil leaves and tobacco leaves that have been obtained respectively were diluted in distilled water according to the concentration for the treatment test. In this study, the concentrations used were 30%, 40%, 50%, and 60% (w/v). Combination treatment was carried out at 1:1 ratio of basil leaf and tobacco leaf extracts. Treatments applied were as presented in Table 1.

Table 1. Treatments applied to *S. litura*

No	Treatment	Concentration (w/v)
1	EDK (Basil Leaf Extract)	30%
		40%
		50%
		60%
2	EDT (Tobacco Leaf Extract)	30%
		40%
		50%
		60%
3	EDKT (Combination of Basil Leaf and Tobacco Leaf Extracts)	15% EDK + 15% EDT (30%)
		20% EDK + 20% EDT (40%)
		25% EDK + 25% EDT (50%)
		30% EDK + 30% EDT (60%)

The study was divided into two stages. Phase one involved observing the anti-feeding activity of *S. litura* by measuring the consumed leaves and weighing them after 24 hours of application. The second phase was the observation of *S. litura* mortality rate. Each phase of the study involved 42 sample units, consisting of 36 treatment units, three positive control units, and three negative control units. The positive control used a synthetic pesticide (cypermethrin 0.1/100ml), while the negative control used distilled water. The extract was applied to *S. litura* larvae by immersion in 0.1 g of mustard leaf feed. Each repetition had 10 test containers, with each container containing one *S. litura* larva. The anti-feeding activity was observed based on the number of leaves consumed after 24 hours and evaluated using the following formula (Baskar *et al*., 2010):

$$A=\frac{C-T}{T}\times 100\%$$

Description:
A = Anti-feeding activity of *S. litura*
C = Weight of leaves consumed in the control
T = Weight of leaves consumed in the treatment

The initial symptoms of larval death are characterized by changes in behavior from active to passive, as well as changes in body color and shape where the body of *S. litura* turns brown and shrinks. The percentage of larval mortality is determined by determining the number of dead larvae every 24 hours until before the prepupa stage. The mortality percentage was examined using the following formula (Mawuntu, 2016):

$$M=\frac{a}{b}\times 100\%$$

Description:
M: Percentage of larvae mortality
a: Number of larvae deaths
b: Total number of larvae tested

The resulting data, such as the percentage of antifeedant activity and mortality of *S. litura*, were converted into arcsine values using SPSS software. Data normality was tested first before the data homogeneity test. Two-way ANOVA analysis was conducted on the data, followed by Duncan's post hoc test.

RESULTS

Based on the results of the research that has been carried out, two data were obtained, namely the percentage of anti-feeding activity and mortality of *S. litura*. In stage one, data on the anti-feeding activity of *S. litura* was generated by calculating the amount of feed consumed for 24 hours. Phase I ended 24 hours after application. In stage two, data were obtained by counting the number of dead larvae observed every 24 hours for up to 10 days.

The results of the *S. litura* anti-feeding activity test on various types of extracts and extract concentrations after 24 hours of application is presented in Table 2.

Table 2. Effect of extracts on the antifeedant activity of *S. litura* after 24 hours.

No	Sample Code	24-hour Antifeedant Activity (%)*			
		30% Concentration	40% Concentration	50% Concentration	60% Concentration
1	Basil Leaf Extract (EDK)	9.67±0.01[b]	14.00±0.01[d]	15.00±0.01[d]	18.00±0.01[e]
2	Tobacco Leaf Extract (EDT)	11.67±0.01[c]	14.33±0.02[d]	16.67±0.02[e]	20.67±0.02[f]
3	Combination of Basil Leaf and Tobacco Leaf Extracts (EDKT)	22.00±0.01[f]	27.00±0.02[g]	31.00±0.01[h]	41.67±0.02[i]
4	Negative Control (K-)	0.00±0.00[a]			
5	Positive Control (K+)	100.00±0.00[j]			

Notes: *) Different letters indicate that the results obtained were significantly different based on Duncan's test (a=0.05).

Based on Table 2, it can be seen that the extracts of basil leaves, tobacco leaves, and their combination affected the anti-feeding activity of *S. litura*. In addition, there was a significant interaction between the type of extract and the concentration of extract in affecting the anti-feeding activity of *S. litura*. Table 2 showed that each treatment had significant difference from K- and K+. Basil extract treatment at 40% concentration was not significantly different from 50% concentration, but significantly different from other treatments. The lowest percentage of anti-feeding activity was 9.76% in the treatment of basil leaf extract with a concentration of 30%, and the highest was 41.67% in the combination of basil leaf extract and tobacco leaf. The higher the concentration, the greater the anti-feeding activity of *S. litura*, as shown in the graph in Figure 1. The increase in concentration caused *S. litura* to consume less feed leaves. However, in the negative control (distilled water), *S. litura* continued to eat without inhibition. In the combined extract treatment, the remaining feed was more than the single treatment.

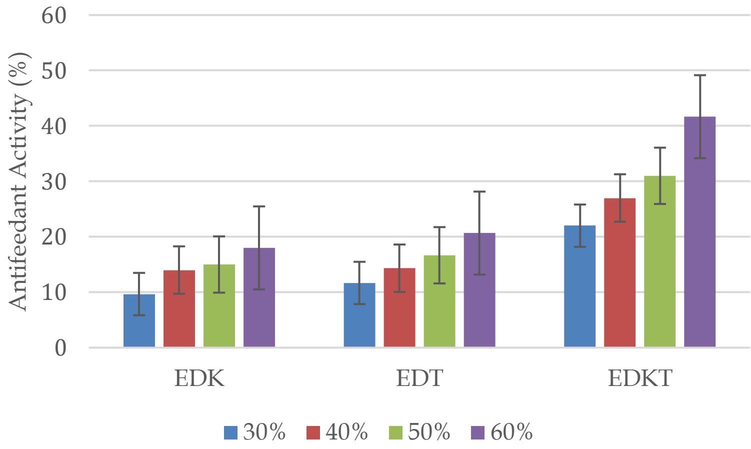

Figure 1. Percentage of antifeedant activity of *S. litura*. EDK: Basil Leaf Extract, EDT: Tobacco Leaf Extract, EDKT: Basil Leaf and Tobacco Leaf Extract (combination)

Based on Table 3, it can be seen that extracts of basil leaves, tobacco leaves, and their combinations affected the mortality of *S. litura*, with the best results in the combination of extracts with a concentration of 60%. There was a significant interaction between the type of extract and extract concentration in affecting *S. litura* mortality. The analysis in Table 3 shows that K- and K+ were significantly different from all other treatments. Duncan test results showed the lowest mortality percentage in basil leaf extract (36.67%) and the highest in the combination of basil leaf and tobacco leaf extract (86.67%). An increase in concentration increased the percentage of mortality of *S. litura*, as seen in Figure 2.

Table 3. Effect of extracts on the mortality of *S. litura* for 10 days.

No	Sample Code	Mortality (%)*			
		30% Concentration	40% Concentration	50% Concentration	60% Concentration
1	Basil Leaf Extract (EDK)	36.67±0.58[b]	43.33±0.58[bc]	46.67±0.58[bc]	53.33±1.53[cde]
2	Tobacco Leaf Extract (EDT)	56.67±0.58[de]	60.00±1.00[e]	66.67±0.58[efg]	73.33±0.58[fg]
3	Combination of Basil Leaf and Tobacco Leaf Extracts (EDKT)	63.33±0.58[ef]	66.67±0.58[efg]	76.67±0.58[g]	86.67±0.58[h]
4	Negative Control (K-)	0.00±0.00[a]			
5	Positive Control (K+)	100,00±0.00[i]			

Notes: *) Different letters indicate that the results obtained are significantly different based on Duncan's test (a=0.05).

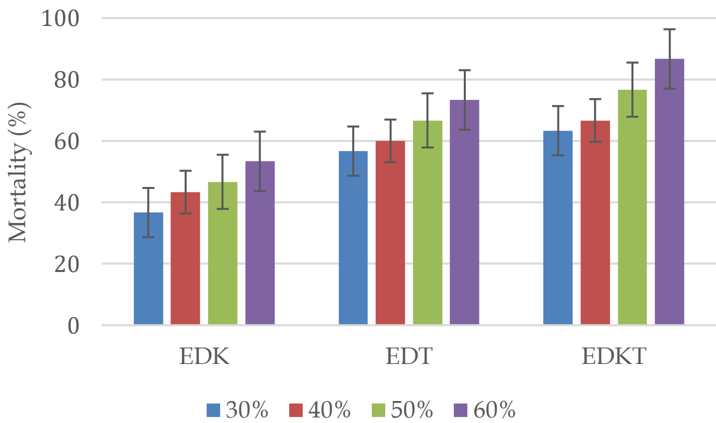

Figure 2: Percentage graph of *S. litura* mortality. EDK: Basil Leaf Extract, EDT: Tobacco Leaf Extract, EDKT: Basil Leaf and Tobacco Leaf Extract (combination)

DISCUSSION

Extracts of basil leaves, tobacco leaves, and a combination of both at various concentrations (30%, 40%, 50%, and 60%) showed an impact on the antifeedant activity and mortality rate of *S. litura*.

The combined extract with a concentration of 60% proved to be the most effective, with a percentage of antifeedant activity of 41.67% (see Table 3) and a mortality percentage of 86.67%.

The results of the study on the effectiveness of basil leaf extract, tobacco leaf, and the combination of both as a biopesticide on anti-feeding activity and mortality of *S. litura* showed that the combination extract was the most effective, especially at a concentration of 15%. This combination is effective because the diverse bioactive compounds in basil leaves and tobacco leaves produce stronger effects in controlling pests compared to single extracts. This finding is in line with previous research by Ali *et al*., (2023) which showed that a combination of tobacco leaf and neem leaf extracts can result in 50% mortality of *S. litura* larvae on the second day.

The increase in the anti-feeding effect and mortality rate of *S. litura* depended on the concentration level of the extract administered. At higher concentrations, the amount of active compounds in the extract increased, which resulted in more effectiveness to interfere with the physiological and biochemical processes of the pest. This increase in concentration also increased the dose of toxic compounds received by the pest, causing more severe disregulations in physiological processes and accelerated systemic failure and increase mortality rates. This finding is in line with Mutaali and Purwani (2016) who indicated that the higher the concentration used in the treatment, the more secondary metabolite compounds contained in the extract.

Research on the anti-feeding activity and mortality of *S. litura* was conducted using third instar larvae and the dip-feeding method. Third instar larvae are active eaters that eat the entire surface of the leaves and only leave the bones of the leaves (Uge *et al*., 2021). In addition to attacking young leaves, larvae also attack plants by eating young pods in the generative phase (Budi *et al*., 2013). Third instar larvae already have a complete organ system so they are relatively stable to environmental influences (Utomo *et al*., 2010). The feed dipping method is a method used to ensure that the plant-based insecticidal compounds contained therein are effective in killing the test larvae. These compounds control anti-feeding activity and pest mortality with different mechanisms such as in tobacco leaves containing nictoin compounds that function as contact poisons, saponins as stomach poisons, flavonoids as nerve and respiratory poisons, while in basil leaves eugenol functions as a nerve poison. This method is done by immersing the feed leaves into a container containing extracts that have been diluted so that the extract sediment sticks to the leaves to be consumed. Supported by Wahyuni and Yuliani (2023) research, that the treatment of 15% combination of lamtoro leaf and papaya leaf extract using the feed dipping method can cause mortality of *S. litura* up to 80%.

The growth and development of larvae requires adequate nutrition, if there are obstacles, it will affect larvae feeding activity. In the negative control treatment, it can be seen that the larvae tend to be active and have a high appetite. In contrast, larvae treated with the extract tended to look passive and consumed less feed. Physiological changes when anti-feeding activity occurs are that larvae tend to be less active, stay away from food sources, and do not increase body weight. These changes are due to secondary metabolite compounds contained in the feed leaves due to the immersion of feed leaves into extracts of basil leaves, tobacco leaves, and a combination of both which are antifeedant. Antifeedant compounds in basil and tobacco leaf extracts are terpenoids that work by inhibiting taste receptors around the mouth, causing larvae to be unable to detect taste stimuli from the surrounding food, reducing feeding activity and disrupting larval growth due to lack of nutrients, which in turn can cause death (Afifah *et al*., 2015). In addition, nicotine compounds contained in tobacco leaves also act as repellents and antifeedants by producing a sharp aroma and bitter taste that prevents larvae from eating plants (Tobing *et al*., 2023). Nasir *et al*., (2022) explained that antifeedant compounds can stop larval feeding activity either temporarily or permanently, depending on the potency of the substance.

In the negative control treatment, zero *S. litura* larvae died because it only used distilled water without toxic compounds. The positive control treatment used a chemical pesticide made from cypermethrin. During the treatment, the larvae were placed on the leaves that had been dipped in cypermethrin liquid, causing contact between the larval cuticle and the treated leaves. The positive control treatment resulted in the highest mortality of 100%. This was due to the active ingredient cypermethrin is a pyrethroid which is included in a group of chemicals that are neurotoxic (Yamani and Ciptono, 2022). The mechanism of action of the active ingredient is by entering the central nervous system directly through contact and attacking the larval nervous system, causing interference with the transmission of nerve impulses. Neurotoxic causes paralysis and death in larvae (Prusty *et al*., 2015). Research by Kurniawan *et al*., (2020) showed that the use of cypermethrin insecticide is effective in controlling *S. litura* with application as a contact poison.

In the treatment using basil leaf extract, tobacco leaf, and a combination of both, the larvae were inhibited from feeding. This inhibition supports larval mortality. In this study, larval mortality is the result of a combination of the effect of anti-feeding activity and toxicity of active compounds contained in basil and tobacco leaf extracts so that as anti-feeding activity increases, mortality also increases. This is in line with the research of Suroto *et al.*, (2021) which explains that the high mortality of larvae is caused by the unwillingness of larvae to consume the given leaf feed, while at low mortality levels the test leaf feed is consumed a lot.

Basil leaves contain active compounds such as saponins, flavonoids, tannins, terpenoids, and essential oils containing 46% eugenol and have potential as a biopesticide (Surahmaida and Umarudin, 2019). Eugenol inhibits the enzyme acetylcholinesterase, causing accumulation of acetylcholine which results in muscle contractions, convulsions, paralysis, and death in larvae (Sutikno and Anggraini, 2023). Tannins in basil leaves interfere with metabolism and synthesis of proteins and digestive enzymes, affecting larval development and reproduction (Tan *et al*., 2022). Tobacco leaves contain nicotine, flavonoids, terpenoids, and saponins that are toxic (Sharma *et al*., 2016). Nicotine functions as an antifeedant and contact poison, damaging the larval nervous system and causing paralysis and death. Saponins in tobacco leaves act as stomach poisons that reduce the work of digestive enzymes and inhibit nutrient absorption, causing larval death (Hasyim *et al*., 2019).

Flavonoids function as neurotoxins that enter through the larval spiracles, disrupting breathing and damaging the digestive and nervous systems, which ultimately causes larval death (Khalia, 2016). Terpenoids act as repellents with a distinctive odor that makes larvae unable to recognize food, inhibits feeding activity, and causes a lack of nutrients that can lead to death (Lina and Suryadarma, 2016).

Symptoms of *S. litura* death include changes in body color and shape, shrinkage, shrinkage, the body becomes soft and changes color to brownish to blackish. Research by Faizah and Dewi (2024) also explained that the symptoms of death are characterized by a shrunken body, oozing a lot of fluid, and black in color.

Extracts of basil leaves, tobacco leaves, and their combination were effective on mortality and anti-feeding activity of *S. litura*. The combination extract was most effective at 60% concentration with 86.67% mortality and 41.67% anti-feeding activity. According to Supriadi (2013), a biopesticideis considered effective if it can kill the tested larvae with a mortality of 80%-90%. According to Mandana *et al*. (2013) an ingredient is categorized as active antifeedant if the percentage of antifeedant activity is above 25%. When using a single extract, the type of extract that has the highest mortality is tobacco leaf extract with a concentration of 60%, which is 73.33%. The use of this biopesticide is certainly safer for the environment and human health and can reduce environmental pollution because the residue is easily decomposed so that it can improve food safety and the quality of agricultural products (Yuliani, 2022). In addition, the use of natural pesticides also plays a role in the application of the concept of integrated pest control which maintains the balance of the larval population and minimizes the impact on predators and other non-target organisms.

CONCLUSION

The study showed that extracts of basil leaves, tobacco leaves, and their combination affected the mortality and anti-feeding activity of *S. litura*, with the combination extract as the best biopesticide. A 60% concentration of the combined extract was effective in reducing feeding activity by 41.67% and increasing *S. litura* mortality by 86.67%. There was a significant interaction between extract type and concentration, especially the combined extract at 60% concentration, on the anti-feeding activity and mortality of *S. litura*.

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CONFLICT OF INTEREST

The authors declares no conflict of interest to disclosure.

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