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Effect of Papaya Leaf Extract with Liquid Nutrient Supplement on Aphids (*Aphis craccivora*) in Cowpea

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ABSTRACT

Papaya leaves have potential as a source of botanical pesticides for controlling aphids, important pests in cowpea cultivation. This study aimed to evaluate the effect of papaya (*Carica papaya* L.) leaf extract as a botanical pesticide against aphid (*Aphis craccivora*) infestation on cowpea and to assess papaya leaf extract supplemented with additional compounds, including plant nutrient solutions and other additives. The experiment was arranged in a randomized complete block design (RCBD), with blocks based on environmental variation in the greenhouse. Treatments consisted of 50% papaya leaf extract and its combination with Green Tonic, Multitonik, and detergent. Each treatment was replicated seven times, resulting in 35 experimental units. Data were analysed using non-parametric methods, namely the Kruskal-Wallis test followed by the Wilcoxon test for pairwise comparisons. The results showed that papaya leaf extract had the potential to reduce aphid populations, although it did not consistently reduce damage to leaves, flowers, and pods. Treatments combining papaya leaf extract with additional substances, particularly plant nutrient solutions, tended to show different effects compared with other treatments; however, the findings should be interpreted cautiously because a nutrient-only treatment was not included.

INTRODUCTION

Cowpea (*Vigna unguiculata*) is an important vegetable crop widely cultivated in Indonesia and utilized in various food preparations, ranging from fresh consumption to cooked dishes. The edible parts of the plant include the leaves and pods. Cowpea pods are rich in essential nutrients such as vitamins A, B, and C, as well as protein [1]. In addition, cowpea contains macronutrients including protein, carbohydrates, fat, and water, along with bioactive compounds such as anthocyanins and carotenoids that are beneficial to human health [2]. The leaves of cowpea (100 g) contain approximately 6.2 mg of iron, 29 mg of vitamin C, 4.1 g of protein, and 88.10% water [3]. Therefore, cowpea is considered an important source of plant-based protein.

The national production of cowpea in 2019 reached 51,359 tons [4]. The cowpea is also cultivated in Tarakan City; however, its production has declined in recent years. Cowpea production reached 19,895 tons in 2017, decreased to 19,415 tons in 2018, and further declined to 18,685 tons in 2019, indicating an average decrease of approximately 2-6%. This decline may be associated with reduced planting area, unstable market prices, pest infestation, and other agronomic constraints. Aphids (*Aphis craccivora*) are polyphagous pests that commonly attack cowpea plants [5]. Previous research has shown that aphid infestation may cause severe damage to leaves, flowers, and pods, with damage levels reaching up to 100% under high population pressure [6].

Synthetic pesticides are commonly used to control pest attacks on cowpea plants. However, excessive and inappropriate use of synthetic pesticides may negatively affect environmental quality, human health, and agroecosystem balance. Insecticide misuse can also cause target pest resurgence and agricultural environmental pollution [7]. Therefore, botanical pesticides are considered an alternative pest control strategy because they are relatively safer for humans,

animals, and non-target organisms, and they degrade more easily in the environment [8].

Papaya leaves (*Carica papaya* L.) are a potential source of botanical pesticides. They contain several bioactive compounds, including saponins, carpaine alkaloids, papain, and flavonoids [9]. Papain may act as a contact poison by entering the insect body through mouthparts or natural openings and may inhibit the growth and development of polyphagous insects [10]. Previous research reported that papaya leaf extract at a concentration of 50% resulted in aphid mortality of 91.33% and reduced plant damage after spraying [11]. Combining papaya leaf extract with other ingredients may improve pest control effectiveness and support plant health through additional nutrient supply.

The addition of nutrients plays an important role in supporting plant growth and improving soil fertility, including through the application of liquid organic fertilizers. Multitonik POC is known to stimulate overall plant growth and improve the quality of plant products, such as leaves, flowers, and fruits, while Green Tonic is a liquid fertilizer formulated to support the growth and productivity of cowpea plants [12]. In the context of botanical pesticide application, the addition of liquid nutrients may influence the effectiveness of papaya leaf extract through several mechanisms. Improved plant nutritional status can enhance plant vigor and tolerance to pest attacks, while certain nutrient components may also affect the persistence, absorption, or distribution of active compounds from the botanical pesticide on plant surfaces. Therefore, it is hypothesized that combining papaya leaf extract with liquid nutrient solutions could enhance its effectiveness in controlling aphid infestations compared to the use of papaya leaf extract alone.

The beneficiary of papaya leaves as bioinsecticides has been widely researched, but the research was carried out on the use of those pesticides with provision of nutrition is still lacking. Therefore, this study aims to test several combinations of concentrations of several papaya leaf extracts added with additional substances in the form of liquid plant nutrients. This can provide benefits apart from controlling aphids and also supporting the growth and yield of long bean plants.

MATERIALS AND METHODS

Research Site

The research was conducted in a greenhouse at the Faculty of Agriculture, University of Borneo, Tarakan, with an area of 10 × 15 m. The temperature during the experiment ranged from 30–35°C, with natural light intensity provided through a transparent roof. The greenhouse walls were covered with approximately 80% black sarlon net to allow adequate air circulation while reducing excessive light exposure. The relative humidity inside the greenhouse ranged between 70–85%, and ventilation was maintained naturally through the netted walls to ensure continuous air exchange. The floor of the greenhouse was constructed using a concrete slab. The use of a greenhouse was intended to provide a more controlled environment by minimizing the influence of external factors such as rainfall, wind, and uncontrolled pest interference, thereby ensuring more consistent experimental conditions.

Experimental Design

This study employed a Randomized Complete Block Design (RCBD) with treatments based on a 50% concentration of papaya (*Carica papaya* L.) leaf extract [11]. The blocking factor was based on environmental variation within the greenhouse, such as differences in light intensity and temperature distribution. The treatments were arranged sequentially as follows: control without papaya leaf extract (E0), 50% papaya leaf extract (E1), 50% papaya leaf extract + 3 mL/L Green Tonic (E2), 50% papaya leaf extract + 3 mL/L Multitonik (E3), and 50% papaya leaf extract + detergent (E4). It should be noted that this study did not include a nutrient-only treatment; therefore, the individual effects of nutrient addition and papaya leaf extract could not be fully separated, and the results should be interpreted with this limitation in mind.

Research Procedures

Preparation of Planting Media

The planting process began with soil medium preparation. The soil used in this study was topsoil collected from the forest area around the Faculty of Agriculture, Universitas Borneo Tarakan. The soil was air-dried for 7 days and sieved to remove foreign materials. Each experimental unit or polybag was filled with 3 kg of soil.

Seed Treatment and Planting

Cowpea seeds of the Parade Tavi variety were soaked in clean water for 10 minutes. Soaking was conducted to select viable seeds. Three seeds were planted directly in polybags, and the two plants with the worst growth were pruned after the plants were 2 weeks old.

Plant Maintenance

Weeding and watering were carried out daily at 08:00 and 16:00 local time. Stakes were installed when the plants reached approximately 25 cm in height. During the experiment, insect pest control was performed only using the treatments evaluated in this study.

Preparation of Botanical Pesticide

Papaya (*Carica papaya* L.) leaf extract was prepared using a maceration method. Fresh papaya leaves (3 kg) were washed thoroughly with clean water to remove dirt and impurities, then chopped into small pieces. The chopped leaves were blended with 3 L of distilled water until a homogeneous mixture was obtained. The mixture was then filtered using a fine cloth to separate the liquid extract from the solid residue. The filtrate obtained was used as the stock solution, with a total volume of approximately 1.4 L (adjusted after filtration). This stock solution was then diluted to obtain a 50% concentration for application in the experimental

treatments. All extraction procedures were conducted under room temperature conditions, and the extract was used immediately after preparation to maintain its bioactive properties.

Rearing of Aphids

Aphids were collected from cultivation areas and reared on cowpea plants that were not included in the treatment units. The aphids were allowed to grow and reproduce until adults and nymphs were available for infestation of the sample plants. The rearing process was conducted for 44 days.

Aphid Infestation

Aphid infestation was conducted by introducing 50 aphids per plant in each experimental unit. Infestation was carried out when the plants were 20 days after planting (DAP). The aphids were then allowed to adapt for 3 days before treatment application. During the adaptation period, aphid populations were observed in each polybag or experimental unit up to one day before application.

Application of Treatment

The application of papaya (*Carica papaya* L.) leaf extract was carried out at 08:00 WITA using a hand sprayer. Treatments were applied four times during the growing period, specifically at 24, 31, 38, and 45 days after planting (DAP). The botanical pesticide was prepared at a concentration of 50% (v/v), consisting of 50 mL of papaya leaf extract mixed with 50 mL of water per experimental unit. For treatments involving additional substances, liquid nutrient solutions (Green Tonic and Multitonik) and detergent were added according to the specified dosage (e.g., 3 mL/L), and the final spray volume was adjusted proportionally to maintain a consistent total volume per experimental unit. All treatments, including those with added nutrients, were applied simultaneously with papaya leaf extract at each application time. No nutrient-only treatment was included. Aphid infestation overlapped with the treatment application period. Plant nutrients (Green Tonic and Multitonik) were applied at 20 days after planting using the recommended dose of 0.03%, consisting of 0.03 mL foliar fertilizer and 49.97 mL water per experimental unit. Detergent was added simultaneously during papaya leaf extract application.

Research Parameters

Number of Aphids

The number of aphids, including adults and nymphs, was counted per plant in each experimental unit using a hand counter. Observations were conducted 2 and 5 days after the first, second, third, and fourth applications. Aphid population dynamics were also recorded before application and after application until 50 days after planting (DAP).

Plant Height, Number of Flowers, and Number of Pods

Plant height was measured at 29, 36, and 40 days after planting (DAP). The number of flowers was observed at 38 and 45 DAP, while the number of pods was observed at 45 and 51 DAP. Plant height was measured from the base of the lower stem to the tip of the tallest leaf. The numbers of flowers and pods were counted for each experimental unit.

Leaf Damage

Damage intensity was assessed by visually observing symptoms on cowpea leaves. Each plant was scored directly using a 0-5 scale, ranging from healthy leaves to severe damage, as presented in Table 1.

Table 1. Criteria for Leaf Damage Due to Aphid Attacks

Score of Damage	Number of Leaves damage (%)	Criteria
0	0	Healthy Leaves (No leaf spots)
1	$0< x \leq 10$	There are leaf spots, irregularities and pale leaf veins
2	$10< x \leq 20$	There are leaf spots, irregularity and blanching of the leaf veins
3	$20< x \leq 50$	The shape of the leaves undergoes a twisting change
4	$50< x \leq 75$	Twisted leaves, growing point symptoms die, side shoots grow.
5	$X > 75$	Side shoots, and dead growth points, stunted plants, brown.

Damage intensity was calculated using the following formula [13]:

$$I = \frac{\Sigma (n \times v)}{N \times V} \times 100\ \%$$

where n is the number of plants at each damage scale, v is the damage scale value, N is the total number of observed plants, V is the highest damage scale, and I is damage intensity. Leaf damage intensity was observed when plants were 26, 33, and 40 DAP.

Damage of leaves and pods

The intensity of leaf and pod damage was calculated to quantify the level of pest attack on cowpea plants. Damage intensity was determined using the following formula:

$$DI\ (\%) = \frac{nD}{N} x100$$

where:
DI = damage intensity (%)
n_D = number of damaged leaves or pods
N = total number of observed leaves or pods

In this study, a leaf or pod was categorized as “damaged” if it showed visible symptoms of aphid infestation, such as discoloration, curling, or deformation. Observations were conducted on all sampled plants in each experimental unit.

Data Analysis

Data were analyzed using non-parametric statistical methods due to the distribution characteristics of the data. The experiment was arranged in a randomized complete block design (RCBD), where the block factor (environmental variation within the greenhouse) was incorporated into the analysis by grouping observations within each block. Because observations were conducted repeatedly over time, data from each observation period were analyzed separately to avoid violation of independence assumptions. The Kruskal–Wallis test was used to detect significant differences among treatments, followed by the Wilcoxon test for pairwise comparisons when significant effects were observed. All statistical analyses were performed at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The effects of papaya leaf extract-based botanical pesticides were evaluated based on the number of aphids, plant height, number of flowers, number of pods, leaf damage intensity, flower damage intensity, and pod damage intensity. Prior to analysis, data were tested for normality. Variables that met the assumptions of normality and homogeneity were analyzed using analysis of variance (ANOVA), whereas non-normally distributed data were analyzed using the non-parametric Kruskal-Wallis test. Plant height data were transformed before further analysis to meet parametric assumptions.

The results showed fluctuations in aphid populations, and the application of botanical pesticides was able to suppress aphid development (Figure 1). The Kruskal–Wallis test indicated significant differences among treatments at 5 days after application ($p < 0.05$) (Figure 1).

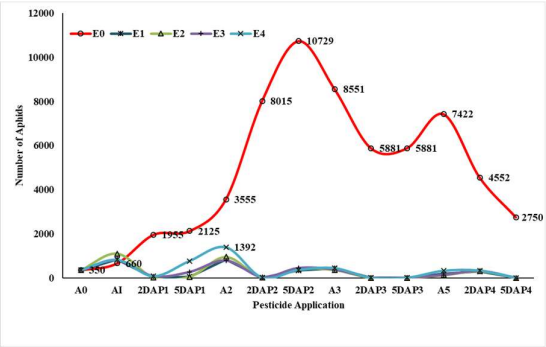


Figure 1. Fluctuation in the number of aphids before and after application of papaya leaf extract-based botanical pesticide (A0 = before application; A = first application; DAP = days after planting).

The highest number of aphids was observed in the control treatment (E0: sterile water) at 36 days after planting (DAP). In contrast, the lowest aphid populations were recorded in treatments E1 (papaya leaf extract) and E2 (papaya leaf extract + Green Tonic) at 47 DAP, corresponding to 2 days after the third application (2 DAP3). The results of the Wilcoxon test further indicated significant differences in aphid populations among treatments, particularly between E4, E3, and E1 at 5 days after the first application (5 DAP1) and after the second application (DAP2) (Figure 2).

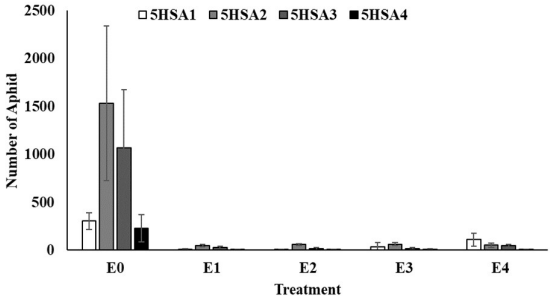


Figure 2. Number of aphids based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

Aphid populations fluctuated after the application of papaya leaf extract-based botanical pesticides. Overall, the treatments suppressed aphid population development. Papaya plants contain cysteine protease enzymes, including papain and chymopapain [14]. Papaya leaf latex also contains alkaloids, terpenoids, flavonoids, and non-protein amino acids that may be toxic to insects [15]. In addition, saponins in botanical insecticides are known to be toxic to insects, while tannins may function as feeding deterrents against herbivores [16].

The effect of botanical pesticides on aphid population fluctuations showed an increasing trend at 3 days after application (3 DAP). This increase may be associated with the declining residual activity of the pesticide, allowing aphids to become active again and enabling migration from surrounding plants. Similar findings have been reported in studies using plant-based pesticides such as Mimusops elengi, which contain alkaloids, tannins, and saponins with insecticidal properties, although these compounds degrade relatively quickly under field conditions [15].

The lowest aphid populations were observed in treatments E1 (papaya leaf extract) and E2 (papaya leaf extract + Green Tonic) at 47 DAP, corresponding to 24 hours after application across three observation periods. This reduction is likely related to physiological effects on aphids, including decreased movement and feeding activity. Aphids exposed to the treatments exhibited slower, inactive, and rigid behavior, including reduced leg movement, which became weak or passive [17]. Statistical analysis showed that all treatments resulted in significantly lower aphid populations compared to the control ($p < 0.05$). This indicates that the bioactive compounds in papaya leaves are effective in inhibiting aphid activity and reducing infestation levels. The papain enzyme is suggested to act as a contact poison,

disrupting aphid activity, and also as a stomach poison that interferes with feeding behavior [18].

Furthermore, the treatments contributed to increased aphid mortality. Previous studies reported mortality rates of up to 80% at a 30% concentration, with mortality increasing alongside higher concentrations [11]. However, treatment E4 (papaya leaf extract + liquid soap/detergent) showed a lower effect compared to E2 (papaya leaf extract + Green Tonic) and other combined applications. This may be due to reduced effectiveness of active compounds when mixed with detergent. In contrast, the application of foliar fertilizer (Green Tonic) alongside botanical pesticides appears to enhance pest control effectiveness. It should be noted that pesticides should not be mixed with alkaline substances, as most pesticides are acidic and such combinations may reduce their efficacy [19].

Plant Height, Number of Flowers, and Number of Pods

The Kruskal–Wallis analysis showed that the effects of pesticides derived from papaya leaf extract did not differ significantly among treatments ($p > 0.05$). The highest plant height at 38 days after planting (DAP) was observed in treatment E2 (papaya leaf extract + Green Tonic) (Figure 3). However, this difference was not statistically significant and should therefore be interpreted as a trend rather than a confirmed treatment effect.

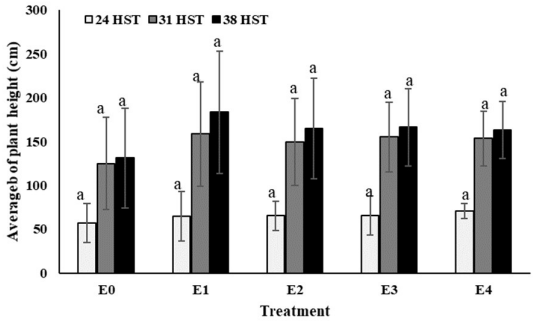


Figure 3. Average plant height (cm) based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

The Kruskal-Wallis analysis showed that papaya leaf extract treatments did not significantly affect the number of flowers ($p > 0.05$). The highest number of flowers was observed in treatment E2 at 45 DAP, corresponding to papaya leaf extract combined with Green Tonic. Pairwise analysis using the Wilcoxon test indicated differences involving treatment E2 (Figure 4).

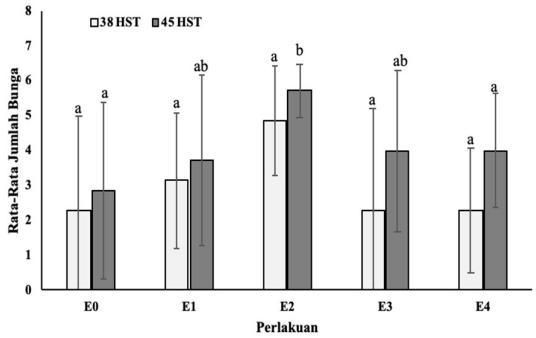


Figure 4. Average number of flowers based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

The Kruskal-Wallis analysis showed no significant difference among treatments in the number of pods ($p > 0.05$). The highest number of pods at 41 DAP was recorded in treatment E2, namely papaya leaf extract combined with Green Tonic (Figure 5).

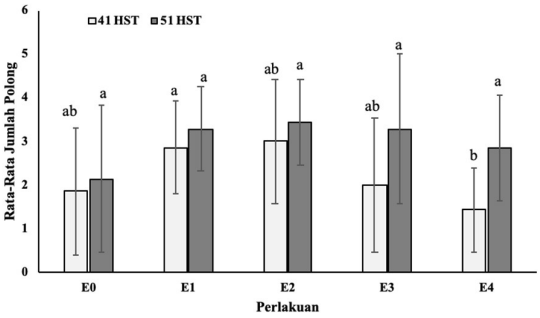


Figure 5. Average number of pods based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

Plant height did not differ significantly among treatments. This result indicates that additional liquid nutrition did not significantly support vegetative growth, especially plant height, under the conditions of this study. According to [12], liquid organic fertilizers such as Green Tonic may support the growth of branches, leaves, flowers, and fruits.

In this study, papaya leaf extract combined with Green Tonic differed from the control and detergent-combination treatments and produced the highest number of flowers. This suggests that Green Tonic may support flower development. Green Tonic is also reported to accelerate or improve flower and fruit formation, particularly in tomatoes, chili, beans, and several other crops [12].

Treatments supplemented with Green Tonic and Multitonik produced a higher number of pods than the control. However, even at lower population levels, aphids may interfere with flower development and subsequently affect fruit formation. Previous research reported no difference in flower damage between control plants and plants treated with chitosan-based pesticides because aphids resumed normal activity several days after treatment [6].

Damage intensity of leaves, flowers, and pods

The Kruskal-Wallis analysis showed no significant overall differences in the intensity of leaf, flower, and pod damage among treatments at 38 days after planting (DAP) ($p > 0.05$). However, further pairwise comparison using the Wilcoxon test indicated a significant difference between specific treatments ($p = 0.046$). Among the treatments, E2 (papaya leaf extract + Green Tonic) resulted in the lowest leaf damage intensity (Figure 6). These results suggest that although the overall treatment effect was not statistically significant, certain treatment combinations performed better in reducing damage intensity.

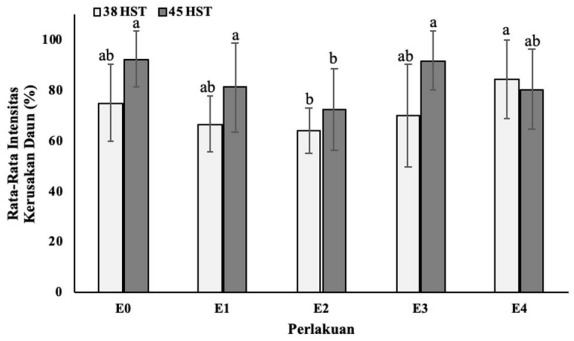


Figure 6. Average leaf damage intensity based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

The Kruskal-Wallis analysis showed no significant effect of papaya leaf extract-based botanical pesticides on flower damage intensity at 38 and 45 DAP ($p > 0.05$). Treatment E2, consisting of papaya leaf extract combined with Green Tonic, showed the lowest flower damage intensity. Wilcoxon analysis indicated differences between E2 and E1 (Figure 7).

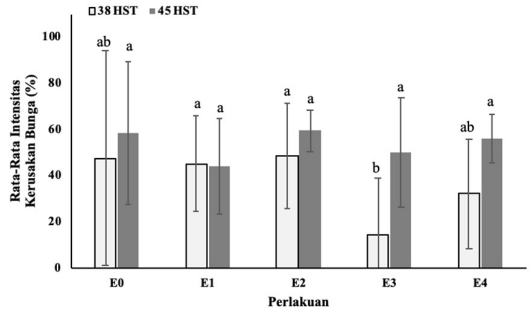


Figure 7. Average flower damage intensity based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

The Kruskal-Wallis analysis showed a significant treatment effect on pod damage intensity at 38 DAP ($p < 0.05$), but no significant difference at 45 DAP ($p > 0.05$). Treatment E4 differed from E0, E1, and E2, while treatment E2 showed the lowest damage intensity (Figure 8).

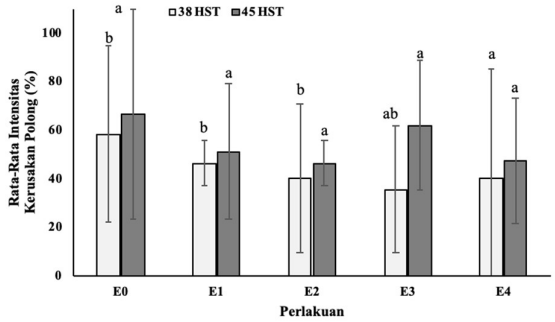


Figure 8. Average pod damage intensity based on papaya leaf extract application treatment (E0 = control, E1 = papaya leaf extract, E2 = papaya leaf extract + Green Tonic, E3 = papaya leaf extract + Multitonik, E4 = papaya leaf extract + detergent).

The highest leaf damage intensity occurred in the control treatment at 45 DAP and differed from the papaya leaf extract + Green Tonic treatment (E2). This indicates that Green Tonic may support plant growth, particularly leaf development. Leaf damage intensity reached 92.6% in the control treatment and 72.4% in the E2 treatment. One application of Green Tonic may not have been sufficient to support plant growth and resistance against aphid attack, even when aphid population density was relatively low. According to [12], concentration and spraying interval influence the effectiveness of Green Tonic application. Furthermore, the amount and frequency of organic fertilizer application may significantly affect the number of pods produced [20].

Flower damage intensity at 45 DAP did not differ among treatments. This indicates that papaya leaf extract-based botanical pesticides were not able to consistently reduce

flower damage caused by aphids. Similar findings were reported by [6], who found no differences between chitosan-based pesticide treatments and the control. In addition to having a rapid life cycle, aphids can move quickly from one location to another through intermediaries such as ants.

At 45 DAP, pod damage intensity did not differ among treatments, indicating that papaya leaf extract-based botanical pesticides were not consistently able to reduce pod damage. According to [21], aphids not only have a rapid life cycle and high mobility but also produce honeydew that can cover plant organ surfaces. This may reduce the effectiveness of botanical pesticides applied to plants. Aphids can also act as vectors of Bean common mosaic virus strain Black eye cowpea (BCMV-BlC), leading to leaf damage that may subsequently contribute to flower and pod damage [22]. Therefore, even at low population levels, aphids can cause substantial damage to cowpea plants.

CONCLUSION

The conclusions of this study are as follows: (1) botanical pesticides derived from papaya leaf extract showed potential in suppressing aphid populations; however, their effects on reducing damage intensity in leaves, flowers, and pods were not consistently significant across treatments; (2) the highest leaf damage intensity was observed in the control treatment at 45 days after planting (DAP). Although treatment E2 (papaya leaf extract + Green Tonic) showed lower leaf damage intensity than the control, this effect cannot be attributed solely to Green Tonic because the experimental design did not include a Green Tonic-only treatment. Therefore, the observed differences may reflect the combined effects of botanical pesticide and additional inputs rather than the distinct contribution of Green Tonic to plant growth or resistance.

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