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Short Communication: Papaya (*Carica papaya*) seed extract test againts *Spodoptera litura* mortality

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Abstract. *Bahuwa IC, Lamondo D, Katili AS. 2022. Short Communication: Papaya (*Carica papaya*) seed extract test against* Spodoptera litura *mortality. Cell Biol Dev 6: 1-5.* This study aims to determine the effect of papaya (*Carica papaya* L.) seed extract on mortality, the most influential concentration of papaya seed extract, Lethal Concentration (LC_{50}) 24 hours, and Lethal Time (LT_{50}) 24 hours armyworm (*Spodoptera litura* Fabricius, 1775) larvae instar III. The research was carried out at the Biology Department, Universitas Negeri Gorontalo, Indonesia, in June 2021. The method used was an experiment with a Completely Randomized Design consisting of 9 treatments and 3 replications, there are A (aquadest control), B (CMC control), C (1 ppm), D (50 ppm), E (100 ppm), F (200 ppm), G (400 ppm), H (800 ppm), and I (1600 ppm). The data were analyzed by probit analysis LC_{50}, LT_{50}, and One Way Anova. The results showed that the LC_{50} value was at a dose of 489 ppm. The value of LT_{50} 24 hours at a concentration of 1600 ppm is 10.61 hours, with the fastest time to kill 50% of armyworm larvae. Papaya seed extract affects the mortality of armyworm larvae; the higher the concentration, the less time it takes to kill 50% of the larvae.

Keywords: *Carica papaya,* caterpillar, mortality, papaya seeds, *Spodoptera litura*

INTRODUCTION

Insects are animal species belonging to the phylum Arthropoda that have habitats almost everywhere. One that belongs to the insect group is the armyworm or grayak caterpillar (*Spodoptera litura* Fabricius, 1775). That follows Rusdy (2009), who said that *S. litura* belongs to the order Lepidoptera, a polyphagous pest that causes damage to cultivated plants. Armyworm (*S. litura*) is a pest that often causes decreased productivity and reduced yields or crop failure in food crops. Plants commonly attacked by armyworm pests are corn, tomatoes, chilies, kale, cabbage, eggplant, spinach, soybeans, and mustard greens.

Farmers have made efforts to overcome the problem of armyworms that attack plants are controlled by using synthetic pesticides. However, the negative impact of synthetic pesticides is that pests become resistant, fertilizing chemical residues and killing natural enemies of pests. Therefore, it is necessary to control the armyworm that is friendly to the environment (Bedjo 2017; Saputra 2019). Asmaliyah et al. (2010) reported various types of plants that contain vegetable pesticides that can be used in pest control, namely, neem, papaya, duku, durian, tobacco, jatropha, cloves, garlic, belimbingwuluh, brotowali.

The community still considers papaya seeds waste and are not optimally used. Besides, the community still considers papaya seeds waste and have not been used optimally. According to research by Utomo et al. (2010), papaya seeds contain alkaloids that are toxic to larvae when used in large quantities and can result in nerve paralysis, cessation of the nervous system, and heart disease suppression, causing death in larvae.

The use of botanical pesticides is strongly recommended to replace the action of synthetic pesticides. Vegetable insecticides are insecticides whose basic ingredients are plant or natural ingredients. For example, the papaya plant (*Carica papaya* L.) has the potential as a vegetable insecticide because it contains alkaloids, terpenoids, and flavonoids that are highly toxic to insects (Julaily et al. 2013).

MATERIALS AND METHODS

Procedure

This study was conducted from June to July 2021 to manufacture extracts at the Chemistry Laboratory. The treatment was carried out at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo, Indonesia. The tools used are a blender, measuring cup, beaker, Erlenmeyer, glass beaker, funnel, jar, aluminum foil, rotary evaporator, hand sprayer, filter paper, label paper, and writing utensils. The materials used were papaya seeds (*C. papaya*), 96% ethanol, and instar III larvae of armyworm (*S. litura*). Instar III larvae of armyworms were obtained from the Germination Center in Malang, East Java, Indonesia, then reared for acclimatization to the laboratory environment for 2 days and fed cabbage leaves before the treatment.

Papaya seeds were washed, cleaned, and dried until the water content was reduced. Next, dried papaya seeds were mashed using a blender and weighed 500 g. Then the smooth papaya seeds were extracted according to the Harborne method in Rasyid (2012). First, the smooth papaya seeds were macerated in a jar using 96% ethanol solvent and stirred using a stirring rod, then covered with aluminum foil and allowed to stand for 24 hours. Next, the maceration results were filtered using a funnel lined with filter paper. Then the pulp from the fine powder of papaya seeds from the results of the first maceration was macerated again using 96% ethanol. Finally, the results of filtering the papaya seed filtrate are put into a rotary evaporator at a temperature of 60^0C so that the ethanol evaporates and gets a thick extract. Furthermore, the extract was measured according to the concentration of 1 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm.

The test animal was placed in a glass container or covered with gauze containing cabbage (cabbage) leaves. Each container contains one concentration of papaya seed extract with concentrations of 1 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm; each contains 5 mL of papaya seed extract concentration. Each container contains 10 armyworms instar III and is sprayed on glass walls using a hand-held spray bottle containing papaya seed extract (*C. papaya*). Spraying was carried out 2 times, the first spraying and the second for 12 hours.

Data analysis

This study used experimental methods and a Completely Randomized Design with treatment determined by the formula t (n-1) 15 (Hanafiah and Nanang 2009) obtained 7 treatments and 3 replications. The papaya seed extract treatment concentration consisted of 0.1 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm.

The collected data were analyzed using probit analysis to determine Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) values. ANOVA analysis was used to determine the effect of papaya seed extract and continued with Duncan's test to determine the significant difference in papaya seed filtrate concentration on the mortality of armyworm larvae effect. The percent mortality value is obtained from the formula:

$$\text{Mortality} = \frac{a}{b} x100\%$$ (Nurhudiman et al. 2018)

Where:
a= Number of dead larvae
b= Total Number of larvae tested

RESULTS AND DISCUSSION

The mortality percentage value of *Spodoptera litura* larva in each treatment

The results showed that the administration of papaya (*C. papaya*) seed extract affected the mortality of armyworm larvae (*S. litura*). The results of the calculation of the mortality percentage of armyworm larvae applied with papaya seed extract are described in Table 1.

The results of the study Table 1 obtained the mortality rate in each treatment within 24 hours. The details of the mortality control treatment were 0 individuals of armyworm larvae, a concentration of 1 ppm obtained a mortality percentage of 3%, a concentration of 50 ppm obtained a mortality percentage of 7%, a concentration of 100 ppm obtained a mortality percentage of 10%, a concentration of 200 ppm obtained a mortality percentage of 17%, a concentration of 400 ppm obtained a mortality percentage of 40%, a concentration of 800 ppm obtained a mortality percentage of 60%, a concentration of 1600 ppm obtained a mortality percentage of 87%. Based on this, the higher the dose of papaya seed extract given, the higher the mortality rate obtained.

Analysis of probit LC_{50} and LT_{50} on *Spodoptera litura* larvae in each treatment for 24 hours

Probit analysis aims to see at what concentration can kill armyworm larvae (*S. litura*) as much as 50%. The LC_{50} value using probit analysis obtained values of x and y= 1.5727+0.7675 with a regression value of 0.863; the results obtained LC_{50}= 489 ppm within 24 hours after application. Based on probit analysis that at a concentration of 489 ppm, it can kill 50% of larvae. The results of the LT_{50} value in the probit analysis in each treatment can be seen in Figure 2.

Table 1. The concentration of papaya seed extract on mortality of caterpillar larvae

Treatment	Total Dead Individuals	Mortality Percentage
A: Aquadest control	0	0 %
B: CMC control	0	0 %
C: 1 ppm	1	3 %
D: 50 ppm	2	7 %
E: 100 ppm	3	10 %
F: 200 ppm	5	17 %
G: 400 ppm	12	40 %
H: 800 ppm	18	60 %
I: 1600 ppm	26	87 %

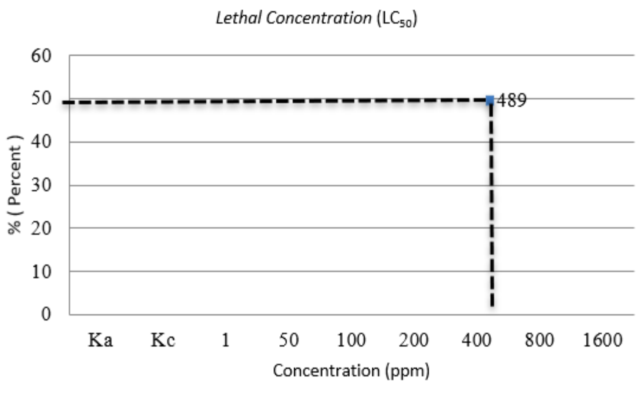

Figure 1. Graph of LC_{50} mortality of *Spodoptera litura* larvae

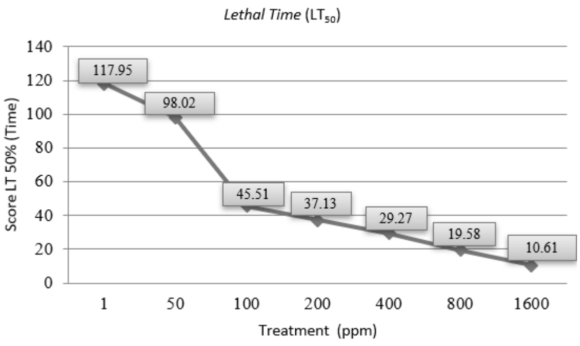

Figure 2. Graph of LT_{50} value in each treatment

The results obtained in the control showed no mortality activity, so the LT_{50} value was 0 because the x and y equation values were 0. The 1 ppm treatment had the highest LT_{50} value compared to other treatments, at 117.95 hours, while the lowest LT_{50} value was in the treatment of 1600 ppm at 10.61 hours. So it can be concluded that the most effective on the mortality of *S. litura* larvae is the treatment of 1600 ppm at 10.61 hours can kill the larvae as much as 50%. Based on the above, the higher the dose of papaya seed extract given, the faster the time used to kill armyworm larvae.

Statistic analysis

Based on Table 2, the One Way ANOVA statistical analysis results, the F count value is greater than the F table, namely 27.109>2.39 with a value of sig = 0.00 < = 0.05, which means H0 is rejected, and H1 is accepted. That shows the effect of giving papaya seed extract on the mortality of armyworm larvae and continued with the Duncan test.

Duncan's test aims to see a significant difference between papaya seed extract treatments on the mortality of armyworm larvae; the results of Duncan's test are shown in Table 3. Table 3 Duncan's analysis results show that treatment A was not significantly different from treatments B, C, D, E, and F but significantly different from treatments G, H, and I. Treatment B was not significantly different from treatments A, C, D, E, and F but significantly different from treatments G, H, and I. Likewise, treatments C, D, E, and F. Treatment G, was significantly different or significantly different from treatments A, B, C, D, E, F, and H, I. Treatment H was not significantly different from treatment I, but significantly different from treatment A, B, C, D, E, F, and G. The highest percent mortality value was found in the treatment. The value of I (1600 ppm) is 87%. This event differed significantly from all treatments, but treatment H differed from treatment I.

Discussion

The results showed the effect of giving papaya (C. papaya) seed extract on armyworm larvae (S. litura) instar III mortality. The highest armyworm mortality was found at a concentration of 1600 ppm, with as many as 26 individuals with a percentage value of 87%, while the lowest mortality was found in the 1 ppm treatment with 1 individual with a percentage value of 3%. The Aquadest and CMC control treatments did not show any deaths. The concentration that can kill 50% of the instar III armyworm (*S. litura*) is 489 ppm. Therefore, the mortality of *S. litura* instar III larvae was influenced by concentration; the higher the concentration of papaya seed extract (*C. papaya*), the death toll of the instar III armyworm larvae have increased.

Table 2. One-way ANOVA statistical analysis

Anova					
Mortality	**No. of squares**	**Free degrees**	**Average square**	**F Count**	**Sig.**
Between groups	116,202	7	16,600	27.109	.000
In grub	9.798	16	.612		
Total	126.000	23			

Table 3. Duncan's mortality test of caterpillar larvae given papaya seed extract

Treatment	Mortality percentage	Notation
(A) Aquadest control	0%	a
(B) CMC control	0%	a
(C) 1 ppm	3%	a
(D) 50 ppm	7%	a
(E) 100 ppm	10%	a
(F) 200 ppm	17%	a
(G) 400 ppm	40%	b
(H) 800 ppm	60%	c
(I) 1600 ppm	87%	c

Note: Different letter notations show significant differences between Duncan's test treatments = 0.05

Figure 3. *Spodoptera litura* infected with papaya seed extract (*Carica papaya*): A. The larvae's body shrinks and is black; B. The larvae' legs are black (Primary data 2021)

The results of observing the time required for the death of the armyworm showed that 1 hour after the application, the larva's body rolled up and moved very fast. After 4 hours of observation, some larvae were far away from the affected area and smeared with papaya seed extract. Observed 8-12 hours after use, the size of the larvae seems to be shrinking, moving slowly, and not so active. Under the treatment of 1600 ppm, the fastest time to kill the armyworm larvae was 10.61 hours, so it also showed that the higher the concentration, the faster the death time.

Larvae mortality in this study was also influenced by condition factors where the larvae used were instar III larvae. Haryono (2015) said that the digestive system is fully formed in this instar III larvae. Therefore, the saponins and alkaloids in papaya seeds can work optimally as poisons in the digestive tract.

The mortality of *S. litura* larvae obtained from each treatment showed no larval death in the control treatment, so the larvae continued to experience the development process. The characteristics of non-poisoned larvae are blackish-brown bodies marked by yellow spots, yellowish legs, and a body with fine hairs, and they do not emit a foul odor. Treatment of papaya seed extract on *S. litura* larvae showed mortality activity at 1 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm. The larval mortality activity of each treatment was seen at 8 hours of observation, and the mortality value increased until the observation was 24 hours.

Changes in body shape characterize larval mortality, and the larva's body color becomes black and shrinks (Figure 3 A&B). The death of armyworm larvae indicated the influence of the chemicals in the applied papaya seed extract. Based on research by Arismawati et al. (2017), papaya seed extract (*C. papaya*) has a larvicidal effect characterized by the death of the larvae. The results of the phytochemical test of papaya seed extract contain secondary metabolic compounds of flavonoids, alkaloids, saponins, and tannins. These secondary metabolic compounds can inhibit larval growth. Taufiq et al. (2015) explained that the saponin content in papaya seeds works as a stomach poison where the substance enters the larva's body through the digestive system (mouth) and then poisons the larvae. Aside from that, Kartina et al. (2019) explained that the saponin content in papaya seeds works as a stomach poison where these substances enter the larva's body through the digestive system (mouth) and then poison the larvae. In addition, saponins are very influential as a contact poison seen in the external physical disorder (cuticle) in larvae, namely washing the waxy layer that protects the larval body parts so that it can cause death to the loss of a lot of body fluids.

Tannins are plant components that are phenols with a bitter taste. Tannins can interfere with the process of digesting food because tannins will bind to proteins in the digestive system (Utami et al. 2010). According to Azlansah et al. (2019), tannins work on the larval body to bind to proteins in the digestive system so that the process of protein absorption in the digestive system is disrupted.

According to Cania and Setyaningrum (2013), alkaloid compounds can be used as larvicides by working as contact poisons (contact poisoning). Alkaloids enter the body through absorption, degrade skin cell membranes, damage cells, and interfere with larval nerve work. Alkaloid compounds in the form of salts can degrade cell membranes. In comparison, the workings of flavonoid compounds influence the work of the respiratory system or act as respiratory poisons (fumigants).

Ahmad and Adriyanto (2019) reported that the use of papaya seed extract (*C. papaya*) has great potential as a bio larvicide because it contains secondary metabolic compounds in the form of alkaloids, saponins, flavonoids, and tannins that can inhibit and kill larvae in 3 ways, namely as (contact poisoning), stomach poison (stomach poisoning), and as a respiratory poison (fumigant).

In conclusion, based on the analysis test results, papaya seed extract (*C. papaya*) significantly affected the mortality of armyworm larvae (*S. litura*) with a calculated F value of 27.109. The dose that can kill the armyworm larvae (*S. litura*) is as much as 50%, namely at a dose of 489 ppm. The best time needed to kill 50% of larvae is at 1600 ppm treatment, which is 10.61 hours.

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