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Organic Extract Effectiveness of Chaya Leaf (*Cnidoscolus aconitifolius*) to Corn Cob Borer Mortality

Rusli Rustam, Farha Safhira Puti*

Department of Agrotechnology, Faculty of Agriculture, University of Riau, Pekanbaru, Riau 28293, Indonesia

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ABSTRACT

The decrease in corn crop yields in Riau Province was possibly caused by several factors, one of which was the infestation of plant pests such as the corn cob borer (*Helicoverpa armigera* Hubner). The primary choice for farmers to control *H. armigera* was using synthetic insecticides, but this could lead to various negative impacts. A solution to reduce the use of synthetic insecticides was the utilization of botanical insecticides, such as those derived from the leaves of chaya (*Cnidoscolus aconitifolius* I. M. Johnst). This research was conducted with the aim of determining the effective concentration of chaya leaf organic extract against corn cob borer larvae mortality in laboratory conditions. The experiment was conducted in a completely randomized design (CRD) with 5 treatments, each replicated 4 times, totaling 20 experimental units. Each experimental unit consisted of 10 third-instar *H. armigera* larvae. The concentrations of chaya leaf organic extract used were 0%, 0.25%, 0.50%, 0.75%, and 1%. Parameters observed included initial mortality time, lethal time 50 (LT_{50}), lethal concentration 50 and 95 (LC_{50} and LC_{95}), daily mortality rate and total mortality. The results indicated that the concentration of 0.75% of chaya leaf organic extract was effective in controlling *H. armigera* larvae. It caused a total mortality of 82.50%, with an initial mortality time of 6.25 hours and a lethal time 50 (LT_{50}) of 29.75 hours after application.

Keywords: Botanical insecticide; *Cnidoscolus aconitifolius*; *Helicoverpa armigera*; Lethal concentration; Total mortality

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INTRODUCTION

Corn (*Zea mays* L.) is the most important food crop in the world besides wheat and rice. It is estimated that more than 55% of domestic corn needs are used for feed, 30% for food consumption and the rest for other industrial needs and seeds (Amrezi 2018). The increasingly widespread role of corn causes the need for corn to also increase. Efforts to meet domestic corn needs must be accompanied by increasing production.

The production of corn plants in Riau Province in 2021 was 18,852 tons, while in 2022 it decreased to 8,156 tons (Indonesian Ministry of Agriculture 2023). A decrease in corn crop production can be caused by pest attacks on corn plants. A pest that often attacks corn plants is the corn cob borer caterpillar (*Helicoverpa armigera* Hubner).

H. armigera is a polyphagous insect that attacks corn plants during its larval stage. Symptoms of *H. armigera* larval infestation include severed cob silks, feeding/scratch marks on the tip of the cob, and the presence of the larvae themselves. Infestation by this corn cob borer pest *(H. armigera)* can reduce yields by up to 80%.

The use of synthetic insecticides is the main choice of farmers in controlling *H. armigera*. The unwise and excessive use of synthetic insecticides can cause pest resistance, the emergence of pest resurgence, secondary pest explosions, killing non-target insects, environmental pollution, interfering with human health and producing agricultural products that contain pesticide residues (Saragih and Ahmad 2021). The solution to suppress the use of synthetic insecticides is to look for other alternatives such as the use of plant-based insecticides. Vegetable insecticides have advantages such as biodegradable residues in nature and are easily lost, can be made at a low cost so that they do not pollute the environment and are relatively safe for humans and livestock (Kusumawati and Istiqomah 2022).

The Japanese papaya plant (*Cnidoscolus aconitifolius* I. M. Johnst) is a traditional medicinal plant that is widely found in Indonesia. Japanese papaya leaves have the potential as a plant-based insecticide because they contain several bioactive compounds such as cyanogenetic glycosides. Cyanogenic glycosides are hydrocarbon compounds that bind to CN groups and

****Corresponding Author***:
E-Mail: farha.safhira2526@student.unri.ac.id

sugars that have the potential to decompose into cyanide acid (HCN) (Gleadow and Moller 2014). The mechanism of action of HCN is to prevent the utilization of oxygen by cells, so that there is an inhibition of electron transport in brain cells which results in loss of consciousness, respiratory arrest, and eventually death (National Research Council 2002). Cyanogenic glycosides enter the respiratory system of insects or the trachea as respiratory toxins (Easson 2021), acting as a nerve agent through its mechanism as *an anticholinesterase* (Iftita 2016).

Previous research has shown that Japanese papaya leaf extract can be used as a plant-based insecticide. The administration of Japanese papaya leaf extract with a concentration of 75 g.l^{-1} water is an effective concentration because it is able to cause the total mortality of *Spodoptera frugiperda* J.E Smith pests by 85% (Rustam and Fitri 2022). Dadang and Prijono (2008), stated that vegetable insecticides are said to be effective if they can cause the death of large pests or equal to 80% at the concentration of water solvents not more than 10%, and organic solvents of no more than 1%.

The extraction process of vegetable insecticides by the maceration method using organic solvents is more efficient than water solvents because it requires less plant material. The choice of maceration method using 70% ethanol solvent is because ethanol is relatively non-toxic compared to acetone and methanol, low cost, can be used in various extraction methods, is easy to obtain, efficient, and safe for the environment (Hakim and Saputri 2020). Hidayati et al. (2023), stated that Japanese papaya leaves extracted with 70% ethanol solvent are the best concentration that can optimally attract total flavonoids.

Until now, there has been no reported research on Japanese papaya leaf extract to control corn cob borer caterpillar pests, so the author has conducted a study on the effectiveness of the concentration of organic extract of Japanese papaya leaves (*Cnidoscolus aconitifolius* I. M. Johnst) on the mortality of corn cob borer caterpillars (*Helicoverpa armigera* Hubner) in the laboratory.

MATERIALS AND METHODS

This research was carried out at the Plant Pest Laboratory, Faculty of Agriculture and the Laboratory of Organic Chemistry and Natural Materials, Faculty of Engineering, University of Riau, Bina Widya Campus km 12.5, Simpang Baru Village, Bina Widya District, Pekanbaru. This research was carried out for 3 months, starting from January to March 2024.

This research was conducted experimentally using a complete random design (RAL) consisting of five treatments, each treatment was repeated four times so that 20 experimental units were obtained. Each experimental unit consisted of 10 larvae of *H. armigera* instar three. The treatment given is a concentration of 0%, 0.25%, 0.5%, 0.75% and 1% organic extract of Japanese papaya leaves. Observation parameters consisted of early death time, lethal time 50 (LT_{50}), lethal concentration 50 and 95 (LC_{50} and LC_{95}), daily mortality and total mortality.

Daily mortality data obtained from the results of the study were analyzed descriptively using graphs. Data on early death time (hours), lethal time 50 (LT_{50}) (hours) and total mortality *of H. armigera* (%) were statistically analyzed using multiple fingerprints and follow-up tests using the SAS 9.0 application. Lethal *concentration data* (LC_{50} and LC_{95}) were probit-analyzed using the POLO-PC program.

Materials and tools include; *H. armigera* instar 3 larvae, Japanese papaya leaves, 70% ethanol, corn cobs, Aquades, gauze, honey, cotton, and sawdust, plastic cups, plastic jars, *beaker glass*, measuring cups, dark-colored bottles, blenders, label paper, analytical scales, Whatman No. 41 filter paper, stirring rods, sieves, steaming flasks, rotary evaporators, hand sprayers, thermohygrometers and stationery.

Larval reproduction of *H. armigera larvae*

The collected larvae are then kept in plastic containers with a diameter of 5.5 cm and a height of 9.5 cm. The larvae are kept individually and covered with gauze. The larvae are fed with young corn until they develop into pupae. The pupa is transferred to a plastic container 16 cm in diameter and 7 cm high which has been sawdust and preserved until it becomes an imago. A pair of male and female imago is transplanted into jars 22 cm in diameter and 24 cm high. The jar is covered with gauze. Imago is fed in the form of cotton that has been dipped in a honey solution by hanging it on the top of the confinement. Young corn cobs and their hair are placed at the bottom of the cage as a place to lay eggs. The eggs that have hatched into larvae are then transferred into plastic containers with a diameter of 5.5 cm and a height of 9.5 cm individually. The larvae are fed with young corn pieces to be kept up to instar 3.

Manufacture of Japanese papaya leaf extract

The manufacture of Japanese papaya leaf organic extract began by collecting Japanese papaya leaves weighing 5 kg wet weight from a garden on Jl. Naga Sakti, Kel. Japanese papaya leaves are washed with clean water and air-dried for 7 days. The papaya leaves are blended until smooth and sifted. Japanese papaya leaf flour is soaked using 70% ethanol in a ratio of 1:4 (500 g of Japanese papaya leaf flour: 2000 ml ethanol solvent). Soaking is carried out for 48 hours. The extract is filtered using Whatman filter paper and funneled into a beaker glass. The filter results are evaporated using a rotary evaporator with a temperature of 50°C. Organic extract of Japanese papaya leaves is stored in the refrigerator at a temperature of 4°C.

Infestation of *H. armigera larvae*

The larvae *of H. armigera* instar three were infested into a plastic container 1 hour before application, each plastic container filled with one larva. Young corn cobs measuring 3 cm are also put in the plastic container as feed for *H. armigera larvae*. Plastic containers are used in 200 containers.

Organic extract of Japanese papaya leaves

Organic extracts of Japanese papaya leaves are diluted using sterile Aquades when they are to be administered. Dilution of pure extract is carried out by taking pure extract according to the concentration of treatment given, namely 0 ml, 2.5 ml, 5 ml, 0.75 ml, and 1 ml then each of them is mixed into 1000 ml of sterile Aquades and stirred until homogeneous. The organic extract of Japanese papaya leaves obtained is then transferred into *a 100 ml hand sprayer*. Japanese papaya organic extract is given at 17.00 WIB with a spraying method according to the spray volume obtained, which is 10 times the spray pressure or equivalent to 2 ml. Observation is carried out until the larvae die or a maximum of 7 days.

RESULTS AND DISCUSSIONS

Early time of death

The results of the observation of the concentration of organic extract of Japanese papaya leaves (*Cnidoscolus aconitifolius* I.M. Johnst) after being analyzed by various fingerprints had a significant effect on the early death time *of H. armigera larvae*. The average results of BNT follow-up tests at the initial death of *H. armigera* at the level of 5% can be seen in Table 1.

Table 1. Average time of early death *H. armigera* after administering some concentration of Japanese papaya leaf organic extract (JAM)

Organic extract concentration of Japanese papaya leaves (%)	Initial time of death (hours)
0.00	120.00 to
0.25	14.00 b
0.50	11.50 bc
0.75	6.25 cd
1.00	4.50 d
Significance (*p*)	0.05
Coefficient Variation (%)	7.14

Remarks: The numbers in the column followed by the lower case letters are significantly different according to the BNT test at the level of 5% after being transformed by $\sqrt{y}$

Table 1 shows that the administration of some concentration of Japanese papaya leaf organic extract caused an early death time in *H. armigera* larvae with a time range of 4.50-120.00 hours after application. Treatment with 0% concentration of Japanese papaya leaf organic extract showed that no *H. armigera* larvae died until the last day of observation (120 hours).

The administration of Japanese papaya leaf organic extract with a concentration of 1% causes the initial death time *of H. armigera* larvae tends to be faster, namely 4.50 hours after different applications is not real with a concentration of 0.75% which causes the initial death time *of H. armigera* larvae to tend to be longer, which is 6.25 hours. This is suspected because at a high concentration of organic extracts, Japanese papaya has more active compounds of cyanogenic glycosides, which if broken down produce HCN compounds that can interfere with breathing to the nervous system, causing the death *of H. armigera* larvae to tend to be faster. Alfaizal et al. (2021), stated that the increased concentration given can increase the amount of active ingredients accumulated in the insect's body so that the killing power is faster.

The administration of Japanese papaya leaf organic extract with a concentration of 0.25% causes the initial death time *of H. armigera larvae* tends to be longer, namely 14.00 hours after different applications is not real with a concentration of 0.50% which causes the initial death time *of H. armigera larvae* tends to be faster, which is 11.50 hours. This is suspected because the treatment of 0.25% concentration of organic extract of Japanese papaya leaves contains a small amount of active ingredients so that the initial death time *of H. armigera* larvae tends to be longer. Iftita (2016), stated that the concentration is directly proportional to the death of the test larvae.

Lethal time 50 (LT_{50}) (Hours)

The results of the observation of the concentration of organic extract of Japanese papaya leaves (*Cnidoscolus aconitifolius* I.M. Johnst) after being analyzed for fingerprints had a significant effect on *lethal time* 50 (LT_{50}) *of H. armigera*. The average results of BNT follow-up tests at the level of 5% can be seen in Table 2.

Table 2. Average lethal time 50 (LT_{50}) *H. armigera* after administering some concentration of Japanese papaya leaf organic extract (JAM)

Organic extract concentration of Japanese papaya leaves (%)	*Lethal time* 50 (hours)
0.00	120.00 to
0.25	44.75 b
0.50	34.25 c
0.75	29.75 c
1.00	24.50 c
Significance (*p*)	0.05
Coefficient Variation (%)	10.46

Remarks: The numbers in the column followed by the lower case letters are significantly different according to the BNT test at the level of 5% after being transformed by $\sqrt{y}$

Table 2 shows that the administration of the concentration of Japanese papaya leaf organic extract causes a lethal time of 50 (LT_{50}) in *H. armigera larvae* in the range of 24.50-120.00 hours after application. The treatment of 0% Japanese papaya leaf organic extract concentration could not reach LT_{50} until the end of observation (120 hours).

The treatment of 1% Japanese papaya leaf organic extract concentration was able to cause *a 50 faster lethal time* of 24.50 hours after application, which was different from the concentration of 0.75% and 0.50% which caused LT_{50} to be slower which was 29.75 hours and 34.25 hours after application, but it was significantly different from the 0.25% concentration treatment. This is suspected because in LT_{50} the concentrations of 0.5%, 0.75%, and 1% organic Japanese papaya extract enter the larvae body in two ways, namely respiratory poison and stomach poison. This causes its toxicity to be high so that with different concentrations it causes an intangible but different difference in the time it takes to kill 50% of *H. armigera* larvae. Higher concentrations can speed up the time it takes to kill 50% of test larvae.

Cyanogenic glycoside compounds and tannins enter the larvae's body as respiratory toxins and stomach toxins. Cyanogenic glycoside compounds are high and can form cyanide acid toxin (HCN) which can attack the respiratory system to the nervous system of insects, causing insects to die slowly (Wati et al. 2020). Tannins have a bitter taste that causes insects to tend not to consume feed, so that insects become hungry until they die. Tannins can bind to proteins, minerals and carbohydrates present in the insect's body, so they can interfere with the digestive process (Pradana et al. 2023).

Lethal concentration (LC_{50} and LC_{95}) (%)

Based on the results of probit analysis using the POLO-PC application, several concentrations of organic extracts of Japanese papaya leaves showed LC_{50} and LC95, respectively, which were 0.19% and 1.71%. The results of the probit analysis can be seen in Table 3.

Table 3. Lethal concentration Japanese papaya leaf organic extract against *H. armigera*

Lethal concentration (LC)	Concentration (%)	SK 95% (%)
LC_{50}	0,19	0,11 – 0,26
LC_{95}	1,71	1,12 – 3,51

Remarks: SK = Trust interval

Table 3 shows that the right concentration of Japanese papaya leaf organic extract to kill 50% of *H. armigera* pests (LC_{50}) is at a concentration of 0.19% of Japanese papaya leaf organic extract or equivalent to 1.9 $ml.l^{-1}$, with a confidence interval of 0.11-0.26%, while the appropriate concentration to kill 95% of *H. armigera* pests (LC_{95}) is at a concentration of 1.71% of Japanese papaya leaf organic extract or equivalent to 17.1 $ml.l^{-1}$, with a confidence interval of 1.12-3.51%. Munandar and Halim (2020) mentioned that the smaller the confidence interval, the higher the level of accuracy, on the other hand, the larger the confidence interval, the lower the level of accuracy.

Organic extract of Japanese papaya leaves can be classified as a plant insecticide with high toxic power. This is because the concentration of Japanese papaya leaf organic extract that is right to kill 50% of *H. armigera* pests (LC_{50}) is 0.19%. According to Obichi et al. (2015), the high toxicity of Japanese papaya leaf organic extract is suspected to be due to various active compounds in the form of flavonoids, alkaloids, tannins, saponins and cyanogenic glycosides. Tannin compounds will bind to proteins in the digestive system so that the larvae's digestive process is disrupted. Saponin compounds act as contact toxins that will damage the larvae's bodies. Flavonoid compounds will attack the nerve organs in the respiratory system and digestive system, thereby damaging the nerves and slowly causing death (Kurniawan et al. 2020). Cyanogenic glycosides enter the respiratory system of insects or trachea as respiratory toxins, working by disrupting the nervous system (Easson 2021).

Daily mortality

The results of the observation of daily mortality of *H. armigera* larvae after testing several concentrations of organic extracts of Japanese papaya leaves showed that the percentage of *mortality of H. armigera* larvae fluctuated from the first to the fifth day. The fluctuations in the daily mortality of *H. armigera* larvae can be seen in Figure 1.

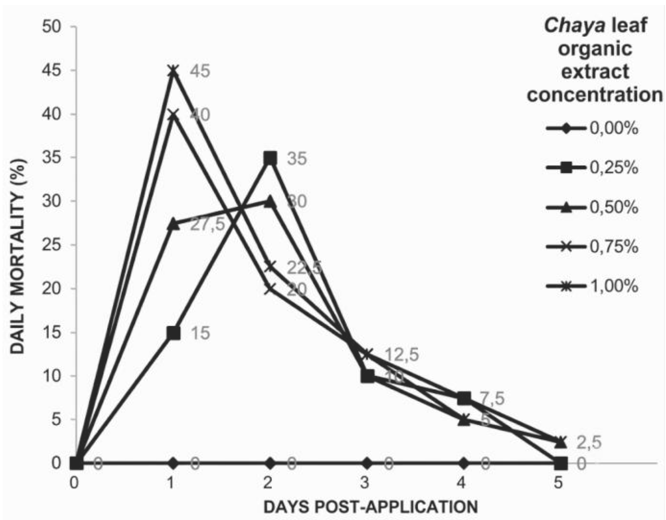

Figure 1. Daily mortality of larvae *H. armigera* after administering some concentration of Japanese papaya leaf organic extract

Figure 1 shows that the daily mortality of *H. armigera larvae* after several treatments with a concentration of organic extract of Japanese papaya leaves. The administration of several concentrations of Japanese papaya leaf organic extracts, namely concentrations of 1%, 0.75%, 0.50% and 0.25%, caused a daily mortality percentage *of H. armigera* except for the concentration of 0%. The 0% concentration did not cause death in the test larvae until the end of observation because there was no organic extract of Japanese papaya leaves contained in it.

On the first day of observation of the treatment of organic extract of Japanese papaya leaves, *it was found that H. armigera* larvae died at each concentration. At a concentration of 1% it causes a daily mortality of 45%, a concentration of 0.75% causes daily mortality of 40%, a concentration of 0.50% causes a daily mortality of 27.5%, and a concentration of 0.25% causes a daily mortality of 15%. The higher concentration of organic extracts of Japanese papaya leaves leads to a higher percentage of daily mortality. On the first day the concentrations of 1% and 0.75% reached the peak of daily mortality, while the concentrations of 0.25% and 0.50% did not reach the peak of daily mortality. This happens because high concentrations have a high toxic effect so that mortality is also higher, while low concentrations have low toxic effects so that mortality is low. The higher the concentration used, the higher the increase in the effects of toxins and mortality, and vice versa. This is also suspected because the high concentration has a high content of cyanogenic glycosides. HCN is poisonous if inhaled by insects, causing poisoning to the respiratory system and nervous system to death.

Observations on the second day showed that the concentrations of 0.25% and 0.50% of organic extracts of Japanese papaya leaves reached the peak of daily mortality of *H. armigera* larvae of 35% and 30%. This is suspected because at the concentrations of 0.25% and 0.50% organic Japanese papaya leaf extracts contain low active ingredients so that it takes time to kill test larvae. The low content of active ingredients in plant pesticides will affect the ability to kill pests. The concentration of 0.75% and 1% of organic extract of Japanese papaya leaves showed that the daily mortality of *H. armigera* larvae decreased by 20% and 22.5%. This is suspected because the concentration of 0.75% and 1% organic extracts of Japanese papaya leaves has passed the peak of mortality, and the number of pest populations has decreased compared to the previous day so that the percentage of daily mortality is lower compared to other treatments.

Observations on the third day showed that the concentrations of 0.25% and 0.50% of organic extracts of Japanese papaya leaves caused a mortality of *H. armigera larvae* of 10%, respectively, and at concentrations of 0.75% and 1% caused mortality of 12.5%, respectively. The daily mortality percentage of test larvae decreased at all concentrations. This is suspected because the toxicity of Japanese papaya leaf organic extracts is starting to decrease and the number of test larvae is decreasing. Alfaizal et al. (2021), stated that the degradation process in the content of plant insecticides causes their toxicity and killing power to be reduced, so that the reaction to pests is reduced.

The fourth and fifth observations showed a decrease in daily mortality of *H. armigera larvae* at all concentrations of Japanese papaya leaf organic extract concentrations. On the fourth day of treatment of Japanese papaya leaf organic extract concentrations of 0.25%, 0.50%, 0.75% and 1% were able to kill *H. armigera* larvae with mortality of 7.5%, 5.%, 7.5% and 5%. On the fifth day of treatment of Japanese papaya leaf organic extract with concentrations of 0.50%, 0.75% and 1% killed *H. armigera* larvae with the same mortality of 2.5%. This is due to the smaller population of *H. armigera larvae* that are still alive than the previous day and the decrease in the active compounds contained in the organic extract of Japanese papaya leaves, so that the performance and effectiveness of the poison against the death of *H. armigera larvae* is reduced. This is in accordance with the opinion of Dadang and Prijono (2008), who stated that plant-based pesticides have disadvantages such as low persistence, so that the active ingredients contained in plant-based pesticides decompose quickly and require repeated application in order to reduce the population of test insects.

Total mortality

The results of the observation of total mortality *of H. armigera* after multivariate fingerprint analysis showed that the treatment of several concentrations of organic extracts of Japanese papaya leaves (*Cnidoscolus aconitifolius* I.M. Johnst) had a significant effect on the total mortality of *H. armigera*. The average results of BNT follow-up tests at the level of 5% can be seen in Table 4.

Table 4. Total mortality *H. armigera* after testing with several concentrations of Japanese papaya leaf organic extract

Organic extract concentration of Japanese papaya leaves (%)	Total mortality (%)
0.00	0.00 c
0.25	67.50 b
0.50	75.00 ab
0.75	82.50 to
1.00	87.50 a
Significance *(p)*	0.05
Coefficient of variation (%)	12.56%

Remarks: The numbers in the column followed by the lower case letters are significantly different according to the BNT test at the level of 5% after being transformed by $\sqrt{y}+0.5$

Table 4 shows that the administration of the concentration of organic extract of Japanese papaya leaves caused total mortality in *H. armigera* larvae in the range of 67.50%-87.50%. Treatment with 0% Japanese papaya leaf organic extract concentration did not cause death in *H. armigera* larvae until the end of observation, because no Japanese papaya leaf organic extract was given so that there were no toxic compounds at work.

The results of observation of the 1% concentration treatment caused the total mortality of *H. armigera larvae* to tend to be higher, namely 87.50%, not significantly different from the treatment of 0.75% and 0.50% which caused a total mortality of 82.50% and 75.00%, respectively, but it was significantly different with a concentration of 0.25% which caused a total mortality of 67.50%. This happens because the active ingredients that enter as respiratory toxins and stomach toxins with high concentrations are hitting the body *of H. armigera* larvae, causing the death of H. armigera *larvae* to be high. Cyanogenic glycosides enter the respiratory system of insects or the trachea as respiratory toxins (Easson 2021), acting as a nerve agent through its mechanism as *an anticholinesterase* (Iftita 2016). *Anticholinesterase* causes inactivation of the enzyme cholinesterase (an enzyme in the blood that regulates nerve function), causes muscle spasms in the respiratory tract and causes death in insects (Iftita 2016). Tannin compounds enter as stomach toxins and work as digestive toxins, causing a decrease in the activity of protease enzymes in converting amino acids so that the work of these enzymes can be inhibited and cell metabolic processes are disrupted (Rahmaningtyas et al. 2022). Larval mortality is directly proportional to the concentration, namely the higher the concentration of the extract, the percentage of mortality will also increase.

The results of research by Rustam and Fitri (2022) reported that the concentration of 75 $g.l^{-1}$ of Japanese papaya leaf extract water caused the highest total mortality rate *of Spodoptera frugiperda*, which was 85% after 120 hours of application. The research that has been carried out has shown that the concentration of 7.5 $ml.l^{-1}$ of Japanese papaya leaf organic extract water caused *a mortality of H. armigera* of 82.50% after 120 hours of application. The extraction process of vegetable insecticides by the maceration method using organic solvents is more efficient than water solvents because it requires less plant material.

Organic extract of Japanese papaya leaves with a concentration of 0.75% is an effective concentration in killing *H. armigera* larvae because it has reached a mortality of 82.50%. Dadang and Prijono (2008), stated that vegetable insecticides are said to be effective if they can cause large pest deaths or equal to 80% in organic solvents of no more than 1%. The administration of Japanese papaya leaf organic extract with a concentration of 1% showed the best mortality percentage of *H. armigera* larvae , which was 87.50%.

CONCLUSIONS AND SUGGESTIONS

Conclusion

The concentration of Japanese papaya leaf organic extract of 0.75% is an effective concentration because it has reached a mortality of *H. armigera* of 82.50% with an initial death time of 6.25 hours and a lethal time of 50 (LT_{50}) at 29.75 hours after application.

Suggestion

Larval control *of Helicoverpa armigera* Hubner with organic extract of Japanese papaya leaves can use concentrations of 0.50%, 0.75%, 1% because there is no significant difference in mortality, lethal time 50 (LT_{50}), but further research is needed for application in crop cultivation.

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