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Toxicity Tests of Botanical Pesticide Made of Tuba Root Extract on *Spodoptera frugiperda*

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ABSTRACT

One of the main pests that attack corn plants is *Spodoptera frugiperda* J. E. Smith. The usual control of this pest is by using synthetic insecticides, but the continuous or unwise use of synthetic insecticides will have negative impacts on humans and the environment, therefore alternative insecticides that are safe and environmentally friendly, such as tuba root (*Derris elliptica* Benth) are needed. This study aims to determine the effect of tuba root extract phytopesticide on *S. frugiperda* larvae and to determine the concentration of tuba root extract that is effective in controlling *S. frugiperda* larvae, as well as the eating inhibition power of *S. frugiperda* larvae after administration. The research was conducted at the Applied Entomology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta from September to November 2022. The treatment concentrations used were 0, 2, 4, and 8 $mL.L^{-1}$ of water. The experiment employed Completely Randomized Design (CRD) with four treatments and five replications. The results showed that the concentration of 4 $mL.L^{-1}$ of water is an effective concentration for controlling *S. frugiperda* larvae and can cause a total mortality of 82% with an initial death of 19 hours, Lethal Time 50 at 39.20 hours after application. In addition, the concentration of tuba root powder extract of 8 $mL.L^{-1}$ of water appeared to be the highest in reducing the appetite of larvae compared to other treatments with a rate of 17.03%. This showed the highest reduction in larval appetite compared to other treatments. A small amount of feed eaten means a high level of antifeedant activity.

Keywords: *Derris elliptica; Effective concentration*; *Lethal dosage*; Larvae

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PENDAHULUAN

Corn is the second most important food commodity after rice (Panikkai et al. 2017). Aside from being a source of carbohydrates and protein, corn is also rich in vitamins A, B, E, and many minerals (Iskandar, 2007). It is estimated that more than 55% of domestic corn needs are used for animal feed, while only about 30% is used for food consumption and the rest is for industrial needs and seeds (Pratama et al. 2015). The challenge in the future is how to meet the demand for corn as a raw material for feed, food, and industry (Zakaria 2011). According to data from the Pusdatin Kementan (2020), corn production in Indonesia in 2015 reached 19,61 million tons; in 2016 it reached 23,19 million tons and in 2017 it came to 28,92 million tons. The expected targets often cannot be achieved due to various obstacles. According to Fattah and Hamka (2010), one of the obstacles to developing corn in Indonesia is pest attacks.

The armyworm is a pest that often troubles agriculture in Indonesia, including corn cultivation. Currently, a new type of armyworm that is endemic in the world, i.e. the Fall Armyworm (FAW) or *Spodoptera frugiperda* J. E. Smith (Herlinda et al. 2022), is an insect pest that can attack more than 80 plant species. These pests belong to the order Lepidoptera, family Noctuidae (Nadrawati et al. 2019). *S. frugiperda* is an insect originating in America and has spread to various countries, including Indonesia (Trisyono et al. 2019). In Indonesia, this pest was first detected in West Pasaman District, West Sumatra in early March 2019 with a severe attack rate of 2-10 per plant (Nonci et al. 2019). According to (Trisyono et al. 2019), *S. frugiperda* attacked all stages of the maize plant from the vegetative phase to the generative phase and caused the highest damage in the vegetative phase. Adult *S. frugiperda* is a strong flier and has a high cruising range of up to 100 km in one night with the help of wind (FAO-CABI 2019).

Symptoms of *S. frugiperda* larvae attack can be seen on the tops of the corn plants, where there is coarse powder resembling brown sawdust. The larvae of *S. frugiperda* damage corn plants by making drill holes and eating the leaf tissue from the edge to the inside leaving a transparent epidermis layer, causing severe damage

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to the corn plants. Yield losses due to this pest attack can reach 15-73% if the attacked plant population is at 55-100% (Nonci et al. 2019).

The pest control strategy that has been carried out by farmers so far is the use of synthetic insecticides. Farmers deem it as the first choice because it controls pests quickly and practically. However, continuous or unwise uses of synthetic insecticides will have negative impacts, such as environmental pollution, death of natural enemies, the emergence of secondary pests, death of non-target organisms, pest resistance, and resurgence (Novizan 2002). An alternative control that is quite appropriate to be applied in reducing those negative impacts is using botanical insecticides.

Botanical insecticides are insecticides made from plants' active secondary metabolites that can provide one or more biological activities, influencing both physiological aspects and the behavior of plant pests and meeting the requirements for use in pest control (Dadang and Prijono 2008). According to Kardinan (2004), botanical insecticides have the advantage of controlling pests, for they quickly decompose into materials that are harmless to the environment, and their residues disappear with ease. One type of plant that has great potential as a source of botanical insecticides is the tuba plant (*Derris elliptica* Benth.) (Hien et al. 2003).

The tuba plant belongs to the class Fabaceae (*Leguminosae*) whose leaves, roots, and twigs have the potential to become botanical insecticides (Rahmawasiah 2017). According to Kaufman et al. (2016), the effectiveness of a plant as a source of botanical insecticides has something to do with certain parts of the plant. Different parts of the plant have different toxicity to pests. The active compounds contained in tuba roots include dehydrotenone, dequelin, elliptone, and rotenone (Utomo et al. 2017). Rotenone compounds are spread throughout all parts of tuba root plants such as in twigs, stems, and leaves (Kuncoro 2006), and mostly in the roots (Ginting et al. 2015). The rotenone compound contained in tuba roots is 0.3-12% (Kardinan 2004). The rotenone compound has been widely reported in agriculture as an insecticide (Isman 2006) because it acts as a contact and stomach poison against insect pests (Kardinan 2004).

Several research results have reported on the effectiveness of tuba root extract in controlling insect pests that attack agricultural crop commodities. The research results of Ibrahim and Rustam (2020) stated that the application of tuba root extract at a concentration of 0.75% with organic solvents was effective in controlling *H. armigera* larvae and was able to cause total mortality of 85%. In addition, Sitohang (2019) also reported that tuba root powder extract can control *S. litura* F. caterpillars on acacia plants (*Acacia crassicarpa* A. Cunn. Ex Benth), with a concentration of 0.8% with organic solvents and capable of causing total mortality by 85%. This shows that tuba root botanical insecticides are effective in controlling insect pests. This is in accordance with the statement of Dadang and Prijono (2008) that botanical insecticides are said to be effective if they can cause the death of pests greater than 80% using organic solvents at concentrations not exceeding 1%. This study is aimed at determining the effect of tuba root extract botanical insecticides on *S. frugiperda* larvae, figuring out the effective concentration of tuba root extract to control *S. frugiperda* larvae, and finding out the feeding inhibition of *S. frugiperda* larvae after application.

BAHAN DAN METODE

This research was carried out experimentally using a Completely Random Design (CRD) consisting of four treatments. Each treatment was repeated five times resulting in 20 treatments units at the Applied Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta Province. The research was conducted from September to November 2022. The average room temperature was 25 ºC and the average humidity was 73.65%.

The materials used in this research were third-instar *S. frugiperda* larvae, tuba roots, young corn cobs, cream soap, distilled water, honey, methanol, sawdust, and water. Meanwhile, the tools used in this study were plastic cups with a height of 9.5 cm, a top diameter of 7 cm, and a bottom diameter of 4 cm; plastic jars with a height of 20 cm, a top diameter of 19 cm, and a bottom diameter of 17 cm; rubber bracelet; gauze; stir sticks; rotary evaporators; beakers 500 mL; measuring cup; cotton; paintbrush; thermo-hygrometers; hand sprayers; analytical balances; blenders; pounding stones; sieve; label; stationary; and cameras.

Propagation and Maintenance of *S. frugiperda*

The *S. frugiperda* pest used for the experiment was obtained from the maize plantation in Condongcatur Village, Depok District, Sleman Regency, Yogyakarta Province. Furthermore, the breeding is done by putting the larvae into a plastic cup with a height of 9.5 cm and a diameter of 7 cm using a brush. Then the cup was covered with gauze. Young corn cobs were cut into small pieces with a size of 3 cm and put into the glass as the larvae feed and continue to be given until the larvae became pupae. The pupae were then transferred to a plastic jar with a height of 20 cm, a top diameter of 19 cm, and a bottom diameter of 17 cm filled with sawdust and maintained until they became imagos or adults. Those imagos were given food in the form of a solution of honey and water with a ratio of 1:10 using a cotton swab dipped in honey solution, then placed on the top of the cage. Five strands of corn leaves were also put in as a place to lay eggs. The eggs were taken care of until they hatched into larvae.

The Making of Tuba Root Extract

The tuba root plant was taken from Indrapuri Village, Tapung District, Kampar Regency, Riau Province. The roots of the tuba plant were cleaned under running water and cut into 2 cm pieces and then dried in the air for one week. The pieces were then pounded to form fibers. Next, the fibers were ground in a blender until smooth and then sifted to become flour. The refined tuba root flour was filtered using a sieve (Dadang and Prijono 2008). The size of the sieve used is 0.5 mesh. After that, the tuba root flour obtained was stored in a jar.

Tuba root flour was weighed as much as 500g, then macerated with methanol solution in 1000 ml Erlenmeyer until completely soaked. The soaking was carried out at room temperature (28-30 °C) for 24 hours. After 24 hours, the maceration results were filtered using a *Buchner* funnel layered with filter paper. After that, the extract was evaporated using a rotary evaporator at 45°C until a pure tuba root extract was produced (Sae-Yun et al. 2006). The pure extract was diluted using distilled water so that the volume became 1 liter to obtain each concentration of the treatment to be given (0, 2, 4, and 8 $mL.L^{-1}$ of water).

Data Analysis

Data on daily mortality and feed inhibition obtained from the research results are presented in graphical charts and analyzed descriptively. The data of initial mortality, lethal time 50 (LT50), and total mortality were statistically analyzed using Analysis of Variance (ANOVA) with the *F* test at a 5% alpha level. If the treatment has a significant effect, it is continued with the Duncan's New Multiple Range Test (DNMRT) using the SAS application version 9.1.3.

HASIL DAN PEMBAHASAN

Time of initial death of *S. frugiperda* (hour)

The results of the initial mortality observation through analysis of variance showed that the treatments of various concentrations of tuba root powder extract have significant effects on the time of initial death of *S. frugiperda* larvae. Observations were made by calculating the time needed to kill one of the *S. frugiperda* larvae after being given tuba root extract in each treatment. Observations are made one hour after application and observed once an hour. The mean results of the follow-up DNMRT test of the initial death of *S. frugiperda* larvae at a 5% level can be seen in Table 1.

Table 1. Time of Initial death of S. *frugiperda* larvae after being given several concentrations of tuba root (*D. elliptica* Benth) extract (hour)

Tuba root extract ($mL.L^{-1}$) of water	Time of Initial death of *S. frugiperda* (hour)	
0	120.00	c
2	21.60	b
4	19.00	b
8	14.40	a

Note: The numbers in the column followed by lowercase letters have a significant difference after being tested by DNMRT at the 5% level after being transformed into $\sqrt{y}$

Table 1 shows that administering several tuba root extract concentrations cause a difference in the time of the initial death of *S. frugiperda* larvae with a time range of 14.40–21.60 hours. The concentration of tuba root extract of 0 $mL.L^{-1}$ of water resulted in no dead larvae until the fifth day (120 hours). This is due to the absence of the rotenone compound given, so it does not have any significant effects on killing *S. frugiperda* larvae.

The treatment of tuba root extract with a concentration of 2 $mL.L^{-1}$ of water caused the time of the initial death of *S. frugiperda* larvae to occur at 21.60 hours after application and was not significantly different with a concentration of 4 $mL.L^{-1}$ water with an initial death time of 19.00 hours after application. When the concentration was increased to 8 $mL.L^{-1}$ water, it resulted in a faster initial death time of *S. frugiperda* larvae (14.40 hours after application) and was significantly different from all the other treatments. It is suspected that tuba roots contain toxic secondary metabolites. The content of the toxic secondary metabolites in tuba roots entered the bodies of *S. frugiperda* larvae cusing the insects tend to die faster than when being treated with the concentration of 0 $mL.L^{-1}$ of water in which the concentration gave no effects until 120 hours after application. This assumption is supported by Ente et al. (2020) that states the higher the concentration given, the faster the death of the pest will be.

Early symptoms of death of *S. frugiperda* larvae are shown by changes in behavior: the larvae become less active, have less appetite, look weak or move passively due to paralysis caused by the reaction of the rotenone compound contained in the tuba root extract that eventually results in death. Other changes that occur after a few hours of death of *S. frugiperda* larvae are morphological changes, i.e. changes in body color and shape. The larvae of *S. frugiperda* which are initially light brown in color become dark brown to blackish. Whereas the changes in the body shape of the larvae are that their body becomes soft and wrinkled and they have swelling at the end of the abdomen because the larva's digestive system is damaged. This shows that tuba root extract treatments with several concentrations do work. Changes that occur in *S. frugiperda* larvae after application can be seen in Figure 1.

Lethal Time$_{50}$ (LT$_{50}$) *S. frugiperda* (hour)

The results of lethal time 50 (LT50) data were analyzed probit using SPSS application version 25 against the time needed to kill 50% of *S. frugiperda* larvae. Observations were made one hour after treatment until there were 50% dead *S. frugiperda* larvae from each experimental unit. The results of probit analysis can be seen in Table 2.

Table 2. Average lethal time50 of S. *frugiperda* larvae after being treated with several concentrations of the tuba root (*D. elliptica*) extract (hour)

Tuba root extract of water ($mL.L^{-1}$)	LT$_{50}$ (Day)	95% Confidence Limits for days	
		Lower Bound	Upper Bound
0	0.00	0.00	0.00
2	2.64	0.00	5.35
4	2.09	0.02	3.61
8	1.86	0.04	3.21

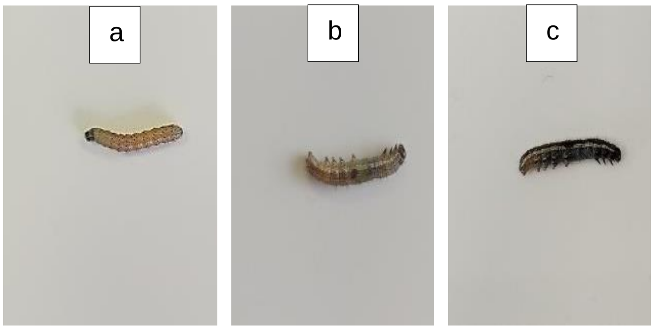

Figure 1. The process of the morphology changes of *S. frugiperda* larvae after the application of the tuba root extract (a) third-insar *S. frugiperda* larva was healthy, (b) *S. frugiperda* larva at initial death of 14,40 hours, (c) dead *S. frugiperda* larva after 24

Table 2 shows that the application of several concentrations of tuba root powder extract caused LT_{50} of *S. frugiperda* larvae in the range of 1.86 – 2.64 days (44.64 – 63.24 hours). The concentration of tuba root powder extract of 0 mL.L^{-1} of water was not able to result in death *lethaltime*$_{50}$ of *S. frugiperda* larvae until the end of the observation (120 hours) and was significantly different from other treatments. The treatment with the concentration of tuba root powder extract of 8 ml/L of water made the time tend to be faster in killing 50% of *S. frugiperda* larvae (1,86 days (44, 64 hours) after application); not significantly different with the concentration of 4 ml/L of water causing LT_{50} of *S. frugiperda* larvae at 2,09 days (50,16 hours) after application and significantly different from other treatments. This also occurred at the time of the initial death using the concentration of 8 ml/L of water (Table 1) which showed that the initial mortality of *S. frugiperda* larvae tended to be faster, resembling the results of LT_{50} (Table 2). This is because *S. frugiperda* larvae absorb more rotenone compounds at high concentrations. The higher the concentration given, the faster the time needed to kill 50% of the test insects will be. This is supported by Hasyim et al. (2019) that state the type of plant extract used is essential; and the higher the concentration of the extract, the faster the time of initial death and the greater the LT_{50} value will be.

Haryuningtyas et al. (2012) state that the rotenone compound is a strong inhibitor of respiratory enzymes so that electron transport in the respiratory system is inhibited and ultimately the synthesis of adenosine triphosphate (ATP) as an energy source is inhibited. In addition, rotenone also works as a poison against the nervous system by inhibiting the glutamate oxidase enzyme which results in nerve conduction failure.

Feeding Inhibition of *S. frugiperda* Larvae

Observing the feeding inhibition of *S. frugiperda* larvae by treating them with several concentrations of tuba root powder extract showed that the percentage of the feeding inhibition of *S. frugiperda* larvae fluctuated. The fluctuation in the feeding inhibition of *S. frugiperda* larvae can be seen in Figure 2.

Figure 2 shows that applying several concentrations of tuba root powder extract significantly affected the feeding inhibition of *S. frugiperda* larvae. It can reduce the appetite of *S. frugiperda* larvae for the feed. Of all treatments, the highest appetite rate was found in the treatment using the concentration of 0 mL.L^{-1} of water (50.2%). This means that there is no level of antifeedant activity in the treatment. While the lowest appetite rate was found in the treatment using the concentration of 8 mL.L^{-1} of water (17.03%). It shows that it is the highest in reducing the appetite of the larvae compared to the other treatments. The small amount of feed eaten means that the level of antifeedant activity is high. The appetite rate of *S. frugiperda* larvae can be seen in Figure 2.

Relevant research by Yanuwiadi et al. (2013) proved that applying tuba root powder extract inhibited the feeding process of *S. frugiperda* larvae. The application of the bioactive substances contained in the extract inhibits the activities of the larvae, characterized by the movement of the larvae slowing down until no movements at all, thus it leads to a stage where the larvae stop feeding.

Daily mortality (%)

The results of daily mortality observations of *S. frugiperda* larvae treated with several concentrations of tuba root powder extract showed that the percentage of *S. frugiperda* larvae mortality fluctuated from the first day to the fifth day. The daily mortality fluctuation of *S. frugiperda* larvae can be seen in Figure 3.

Figure 3 shows that administering several concentrations of tuba root powder extract caused different daily mortality rates of *S. frugiperda* larvae from the first day to the fifth day of observation. On the first day, all treatments resulted in the death of *S. frugiperda* larvae in the range of 10-14% except for the treatment using the concentration of 0 mL.L^{-1} of water. The peak mortality of *S. frugiperda* larvae occurred on the second day with the range of 38−50%. On the third, fourth and fifth day, the mortality of *S. frugiperda* larvae decreased in the range of 10−22.5%, 8−10%, and 2−6%, respectively.

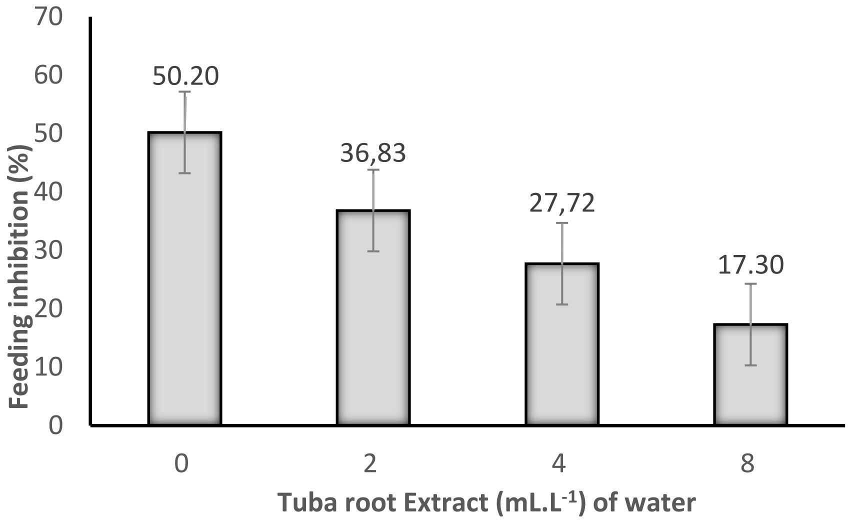

Figure 2. Fluctuation in the feeding inhibition of *S. frugiperda* larvae after applying several concentrations of tuba root extract

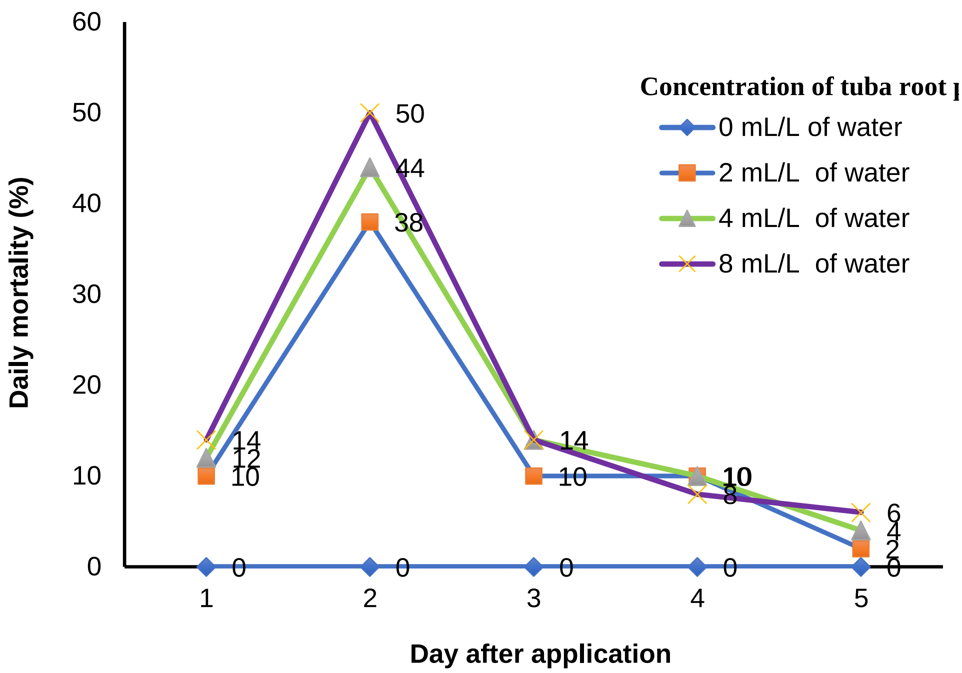

Figure 3. Fluctuation of the daily mortality of *S. frugiperda* larvae after applying several concentrations of tuba root powder extract

On the first day after the treatment, it was seen that the treatment of 2 mL.L^{-1} of water caused a mortality of 10%, the treatment using the concentration of 4 mL.L^{-1} of water caused a mortality of 12%, and the treatment using the concentration of 8 mL.L^{-1} of water caused a mortality of 14%. The difference in daily mortality of *S. frugiperda* larvae in each treatment is thought to be due to the different active ingredient content in each treatment of tuba root powder extract. The higher the concentration given; the more active substance enters the body of *S. frugiperda* larvae. Mortality will occur faster at high concentrations because more and more active ingredients enter the insect's body and vice versa (Yunianti 2016).

The second day after the application was the peak mortality of *S. frugiperda* larvae with a range of 38−50%. The highest mortality was in the treatment using the concentration of 8 mL.L^{-1} of water which caused the death of the test insects by 50% followed by treatment with the concentration of 4 mL.L^{-1} of water (44%), and the concentration of 2 mL.L^{-1} of water (38%). This is because the active ingredient in tuba root powder extract has accumulated in the bodies of *S. frugiperda* larvae so they work optimally. In general, botanical insecticides will work optimally 24 hours after application (Tukimin dan Rizal).

Factors that affect the death of *S. frugiperda* larvae in each treatment are the active ingredients contained in the tuba roots which are toxic to *S. frugiperda* larvae. After the application of tuba root powder extract, the consumption rate of the larvae decreased, and they became less active until ultimately, they died. Tuba root contains rotenone, deguelin, ellipton and toxicarbol compounds (Wu et al. 2012). Rotenone is an inhibitor of cellular respiration, affecting nerve tissues and muscle cells which causes insects to stop eating so that the larvae die in a suffocating condition (Suganya dan Thangaraj 2014). In addition, tuba root powder extract was able to reduce the digestibility of the test larvae by inhibiting the work of digestive enzymes so that the digestion of the larvae was disturbed.

On the third, fourth, and fifth days there was a decrease in the daily mortality of *S. frugiperda* larvae. This was due to the lower number of tested S. frugiperda larvae in each treatment because it had experienced a peak in mortality on the second day. In addition, the decrease in mortality is believed to have occurred due to the decomposition of the active ingredient from the tuba root powder extract given to the treatment, thus it is no longer effective in poisoning pests and is difficult to cause death. This is in accordance with the statement by Dadang and Prijono (2008) saying that the disadvantages of botanical pesticides include their low persistence. The active ingredients contained in botanical pesticides decompose quickly, even requiring more frequent or repeated applications so that the test insect population decreases.

Total Mortality of *S. frugiperda* (%)

The results of observing the total mortality of *S. frugiperda* larvae after the analysis of variance showed that treatments with various concentrations of tuba root powder extract had a significant effect on the total mortality of *S. frugiperda* larvae. The average results of the DNMRT follow-up test on the total mortality of *S. frugiperda* larvae at the 5% level can be seen in Table 3.

Table 3. Average lethal time50 of S. *frugiperda* larvae after being treated with several concentrations of the tuba root (*D. elliptica*) extract (hour)

Tuba root extract (mL.L^{-1}) of water	Mortality of *S. frugiperda* (%)
0	0.00 a
2	70.00 b
4	82.00 bc
8	90.00 c

Note: The numbers in the column followed by lowercase letters have a significant difference after being tested by DNMRT at the 5% level after being transformed with the arcsin formula or $\sin^{-1}\sqrt{y}/100$

Table 3 shows that the total mortality of *S. frugiperda* larvae by administering several concentrations of tuba root extract ranged from 70-90%. The treatment using the concentration of tuba root powder extract of 0 ml/L of water until the end of the observation (120 hours) showed no dead *S. frugiperda* larvae and was significantly different from other treatments. The concentration of 8 mL.L^{-1} of water resulted in a high total mortality of *S. frugiperda* larvae (90%), which was not significantly different from the treatment with the concentration of 4 mL.L^{-1} of water (82%), but significantly different from the concentration of ml/L of water (70%). This is due to the active ingredient rotenone compounds with high concentrations hitting the bodies of the larvae more and more resulting in high mortality of the larvae. This is in accordance with the statement by Ardiansyah and Mahajoeno (2002) which says that the higher the concentration given, the higher the content of botanical insecticide compounds thus increasing effectiveness and inhibiting growth and causing faster death of insects. In addition, according to (Dewi 2010), the toxicity contained in botanical insecticides will increase if the concentration used is higher so that physiological processes are disrupted and insect development is hampered.

Utomo et al. (2017) states that the ability of tuba root powder extract to kill *S. frugiperda* larvae is because tuba root powder extract contains toxic compounds including rotenone, dehydrorotenone, dequelin and elliptone. Rotenone contained in tuba root extract is at 0.3 - 12% (Kardinan 2003).

The treatment of tuba root powder extract with a concentration of 4 mL.L^{-1} of water was able to kill *S. frugiperda* larvae by 82% so the concentration used can be said to be effective in controlling insect pests. According to Dadang dan Prijono (2008), botanical insecticides are said to be effective if they are able to kill large insect pests greater than or equal to 80% with a water solvent not exceeding 10% (100 g.L^{-1} of water) and organic solvents not exceeding 1% (10 mL.L^{-1} of water).

CONCLUSIONS

Based on the results of toxicity tests of botanical pesticide made of tuba root extract on *S. frugiperda* larvae, it was concluded that tuba root extract is effective for controlling *S. frugiperda* larvae. The concentration of 4 mL.L^{-1} of tubal root extract water is an effective concentration for controlling *S. frugiperda* larvae because it was shown to cause total mortality of 82% with an initial death of 19 hours, Lethal Time 50 at 39.20 hours after application. The concentration of tubal root extract of 8 mL.L^{-1} of water caused a loss of insect appetite of 17.03%, meaning it was the highest in reducing appetite compared to other treatments.

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