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LETHAL CONCENTRATION AND LETHAL TIME BOTANICAL INSECTICIDE TUBA ROOT EXTRACT AGAINST *Spodoptera frugiperda* J. E. Smith LARVAE

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Abstract. *Utilization of botanical insecticides is an option to control environmentally friendly Spodoptera frugiperda pests. The purpose of this study was to determine the lethal concentration (LC) and lethal time (LT) of botanical insecticides on the third instar larvae of S. frugiperda in the laboratory. The experiment was conducted at the Applied Entomology Laboratory, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, over a period of 3 months, from September to November 2022. The concentration treatments used were 0, 2, 4, and 8 ml/L of water. The experiment was designed using a completely randomized design (CRD) with 4 treatments and 5 replications. Larval mortality data was processed using probit analysis in order to determine the value of Lethal Concentration (LC_{50} and LC_{95}) and Lethal Time 50 (LT_{50}). The results showed that the tuba root powder extract concentration showed LC_{50} and LC_{95} namely 0.07% equivalent to 0.7 ml/L of water and 1.53% equivalent to 15.3 ml/L of water. Meanwhile the concentration of 8 ml/L of water extract of tuba root flour causes LT_{50} of S. frugiperda larvae causing time to tend to be faster at 1.86 days or 44,64 hours after application. Botanical insecticides of the tuba root extract concentration show high efficacy against S. frugiperda larvae so that they can be recommended as a component for integrated pest management (IPM).*
Keywords: *tuba root; botanical insecticide; $LC_{50\ 95}$; LT_{50}*

1. Introduction

Armyworm is a pest that often disturbs agriculture in Indonesia, including corn cultivation. Currently, a new type of armyworm that is endemic in Asia, the Fall Armyworm (FAW) or *Spodoptera frugiperda* J. E. Smith (Setiawan *et al.*, 2021) is an insect pest that can attack more than 80 plant species. This pest belongs to the Lepidoptera Order, 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, it was detected in West Pasaman District, West Sumatra for the first time in early March 2019 with a fairly high attack rate (Kementan, 2019). According to Trisyono *et al.* (2019), *S. frugiperda* attacked all stages of the maize plant from the vegetative 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 (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

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by making drill holes and eating the leaf tissue from the edge to the inside leaving a transparent epidermis layer, causing severe damage to the corn plants. Yield losses due to this pest attack can reach 15 - 73% if the attacked plant population is at 55 -100% (Kementan, 2019).

One of the pest control strategies 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, the synthetic insecticides basically can have several negative impacts like the pest resistance tend to be happened toward chemical insecticides (Che *et al.*, 2013), pest resurgence (Ueno, 2015), the likeliness of secondary pest explosions (Gross & Rosenheim, 2011), environmental pollution (Guruprasad & Pasha, 2014), the death of non-target insects such as natural enemies including parasitoids and predators as well as pollinating insects (Mallinger *et al.*, 2015), environmental and health problems for humans (Mokhtar *et al.*, 2015), as well as high costs. Thus, an alternative control by using vegetable insecticides derived from plant extracts that are more environmentally friendly is necessary.

One alternative to replace synthetic insecticides is by obtaining natural chemicals derived from plants (Sarwar, 2015). Plants that have the potential as a source of insecticides generally have the characteristics for controlling pests, i.e. containing antifeedants (Arivoli & Tennyson, 2013), having the ability to kill pests or inhibit the growth of insects (Jeyasankar *et al.*, 2014), containing repellents, killing eggs or ovicidal (Packiam *et al.*, 2012), preventing insect activity to lay eggs or oviposition deterrent activities (Radha & Susheela, 2014) and disrupting the insect reproductive system (Gokce *et al*., 2018). One of the plants that have that potential is Tuba plant (*Derris elliptica* Benth.) (Hien *et al.*, 2003).

Tuba plant belongs to the Fabaceae (*Leguminosae*) type (Rahmawasiah, 2017) whose leaves, roots and branches have the potential to be botanical insecticides. According to Kaufman *et al.* (2006), As botanical insecticides, the plant effectiveness is affected by the plant parts used. Different parts of plants exhibit varying toxicity rates against pests. The active compounds found in tuba roots comprise dehydrorotenone, dequelin, elliptone, and rotenone (Utomo *et al.*, 2017). Rotenone levels are present in various parts of the tuba root plant, including twigs, stems, and leaves, distributed uniformly (Kuncoro, 2006). The rotenone compound contained in tuba roots is 0.3-12% (Kardinan, 2004). The rotenone compound has been extensively utilized in agriculture as an insecticide (Isman, 2006) because rotenone acts as contact and stomach poison against insect pests (Kardinan, 2004).

Expected benefits that will be obtained by alternative pest control with botanical insecticides include: (1) reducing the use of synthetic pesticides, (2) improving the efficiency of resource use through environmentally-friendly based pest management, (3) increasing awareness of the need to

organize farming systems on corn that is in line with the principles of sustainable agriculture, (4) reducing environmental pollution and thus increasing populations of useful organisms, and (5) yielding corns that are safe for consumption.

The toxicity of insecticides to organisms is usually described as LD_{50} (lethal dose to kill 50% of insects). In some cases, lethal concentrations LC_{50} and LC_{95} are used to express insecticide concentrations that will kill half of the population of the test insects. Meanwhile the LT_{50} value is the time (hours) needed to kill 50% of the test insects (Hasyim *et al.*, 2016).

Several research findings regarding the effectiveness of tuba root extract in controlling insect pests that attack agricultural crop commodities have been reported. A study by Ibrahim and Rustam research (2020) It was discovered that the application of tuba root extract at a concentration of 0.75% with organic solvents effectively controls *H. armigera* larvae, as it can result in total mortality of 85%. This finding aligns with the statement of Dadang and Prijono (2008); botanical insecticides are deemed effective if they can induce pest mortality exceeding 80% when applied with organic solvents at concentrations not exceeding 1%.

The hypothesis put forward in this study is to obtain tuba root extract contains compounds that have the potential as insecticides that can cause the death of *S. frugiperda* larvae. This study was aimed at determining the sub lethal concentration insecticide of the tuba root extract concentration and prolethal time of the botanical insecticide on the third instar larvae of *S. frugiperda*.

2. Methods

2.1. Place

The study was conducted at the Applied Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Gadjah Mada University, located in Yogyakarta Province. The research was conducted from September to November 2022. The average room temperature was 25ºC and the average humidity was 73.65%.

2.2. Research Methods

This research was experimentally conducted using a completely randomized design (CRD), comprising 4 treatments. Each treatment was replicated 5 times in order to get 20 experimental units. Each experimental unit consisted of 10 third instar *S. frugiperda* larvae. The concentration of tuba root extract given is based on research by Ibrahim and Rustam research (2020) are as follows:

A0 = concentration of tuba root extract of 0 ml/L of water

A1 = concentration of tuba root extract of 2 ml/L of water

A2 = concentration of tuba root extract of 4 ml/L of water

A3 = concentration of tuba root extract of 8 ml/L of water

2.3. Research Procedures

2.3.1. 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 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 measuring 3 cm and placed in the container for the larvae to feed on. This feeding continued until the larvae progressed into the pupae stage. The pupae were subsequently moved to a plastic jar with dimensions of 20 cm in height, a top diameter of 19 cm, and a bottom diameter of 17 cm. The jar was filled with sawdust, and the pupae were kept there until they developed into imago or adult stages. 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 breeding of *S. frugiperda* larvae can be seen in Figure 1.

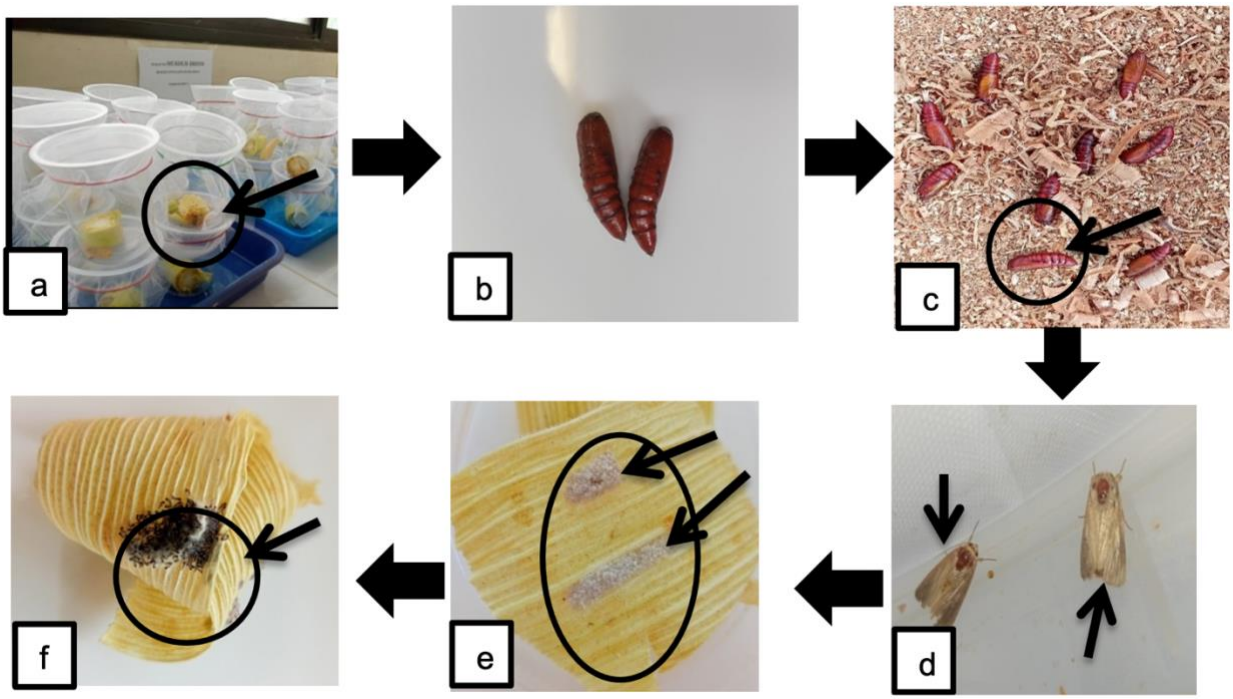

Figure 1. Breeding of *S. frugiperda* larvae, (a) rearing individual larvae, (b) larvae that have turned into pupae, (c) pupae are put into plastic jars containing sawdust, (d) pupae that have turned into adults, (e) *S. frugiperda* eggs, (f) *S. frugiperda* eggs that have hatched into larvae.

2.3.2. The Making of Tuba Root Extract

The tuba root plant was taken from Indrapuri Village, Tapung District, Kampar Regency, Riau Province. The tuba plant roots were cleaned under running water, cut into 2 cm pieces, and subsequently air-dried 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 & Prijono, 2008). The size of the sieve used is 0.5 mesh. After that, the tuba root flour obtained was stored in a jar.

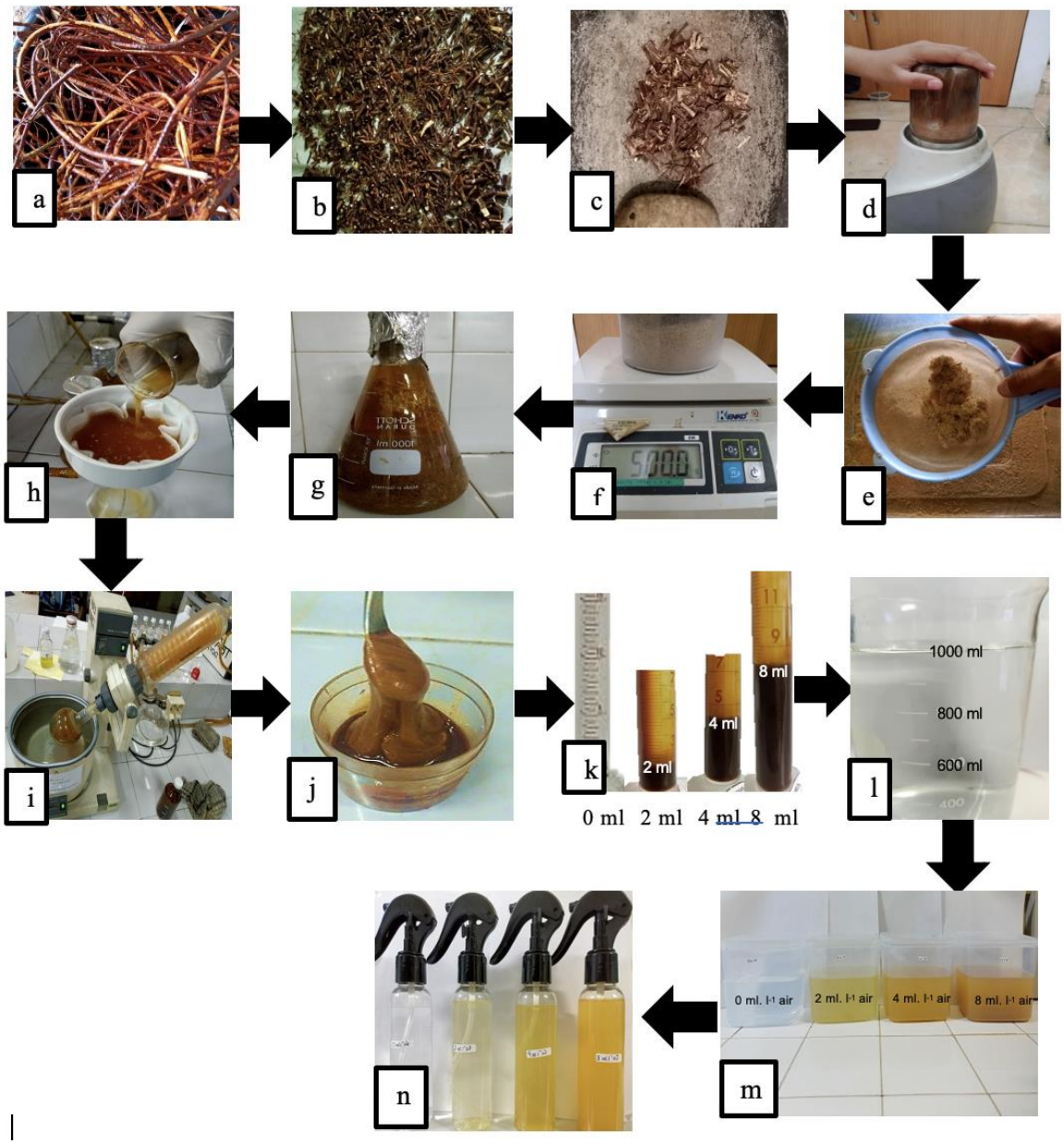

Figure 2. Preparation of tuba root extract, (a) tuba root that has been washed, (b) tuba root cut into small pieces, (c) tuba root pounded, (d) tuba root blended, (e) tuba root sieved, (f) tuba root powder was weighed, (g) tuba root powder mixed with methanol solvent, (h) filtering the solution using filter paper (i) evaporation of the solution using a rotary evaporator, (j) pure tuba root extract, (k) measurement of each treatment concentration, (l) addition of 1000 ml of distilled water for each treatment, (m) Tuba root extract was put into a hand sprayer

Tuba root flour was weighed as much as 500 g, 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 (Yenie *et al.*, 2013). After 24 hours, the macerated mixture underwent

filtration using a Buchner funnel lined with filter paper. Following filtration, the extract was evaporated using a rotary evaporator at 45°C until a pure tuba root extract was obtained (Yun *et al.*, 2006). The pure extract was diluted using distilled water to achieve a final volume of 1 liter, resulting in each concentration of the treatment to be given (0, 2, 4, and 8 ml. l^{-1} of water). The preparation of tuba root extract can be seen in Figure 2.

2.3.3. The Tuba Root Extract Application

The application of tuba root extract is carried out by spraying the tuba root extract using a 100 ml hand sprayer which has been filled according to the treatment of tuba root extract concentrations, namely 0, 2, 4, and 8 ml/L of water. The hand sprayer containing the tuba root concentration is shaken before spraying so that the solution does not precipitate. It is then sprayed on the *S. frugiperda* larvae and corn cobs until evenly wet according to the calibration results obtained, i.e. 5 ml for each treatment. The application time is at 18.00 WIB (Western Indonesian Time) or 06.00 p.m.

2.4. Data Analysis

The effect of the toxicity of each tested vegetable insecticide on *S. frugiperda* larvae was calculated by determining the lethal concentration (LC_{50} and LC_{95}) and lethal time 50 (LT_{50}), calculated based on mortality data obtained using probit analysis. Probit analysis used SPSS 25.

3. Results and Discussion

The estimation of the toxicity value of some insecticides against insect pests is done by looking at the lethal concentration and lethal time . The lethal concentration is the concentration that can cause the death of 50% of the insect pests tested at certain observations (Hasyim *et al.*, 2016), while the lethal time is the time (hours) needed in order to kill 50% of the test insects.

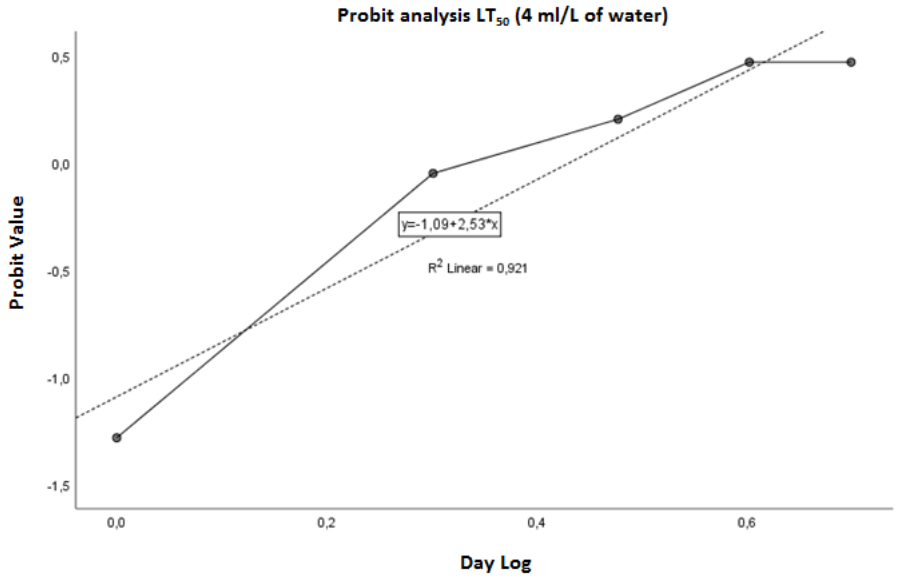

Figure 3. Probit analysis LT_{50} (2 ml/L of water)

3.1. Lethal Time 50 (LT_{50}) *S. frugiperda* (hour)

The concentrations of tuba root extract exhibited Lethal Time 50 (LT_{50}). The probit analysis of LT_{50} can be observed in Figure 3, Figure 4, and Figure 5.

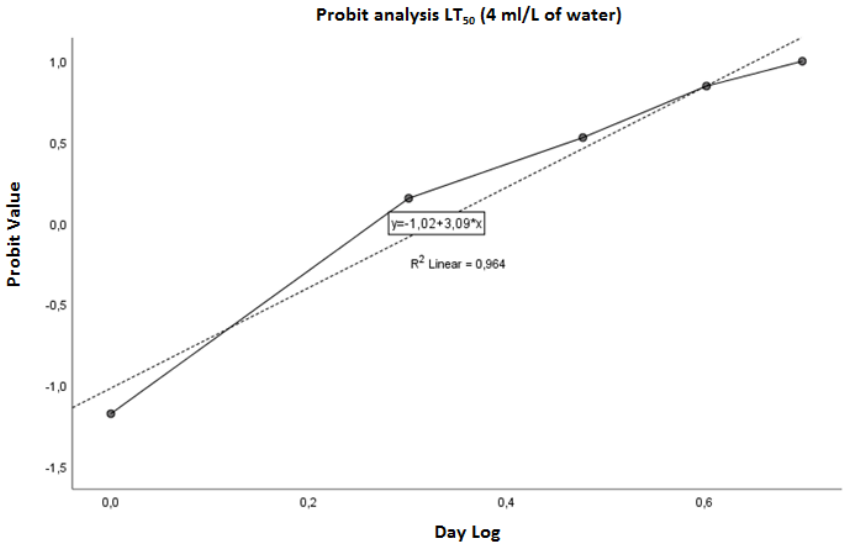

Figure 4. Probit analysis LT_{50} (4 ml/L of water)

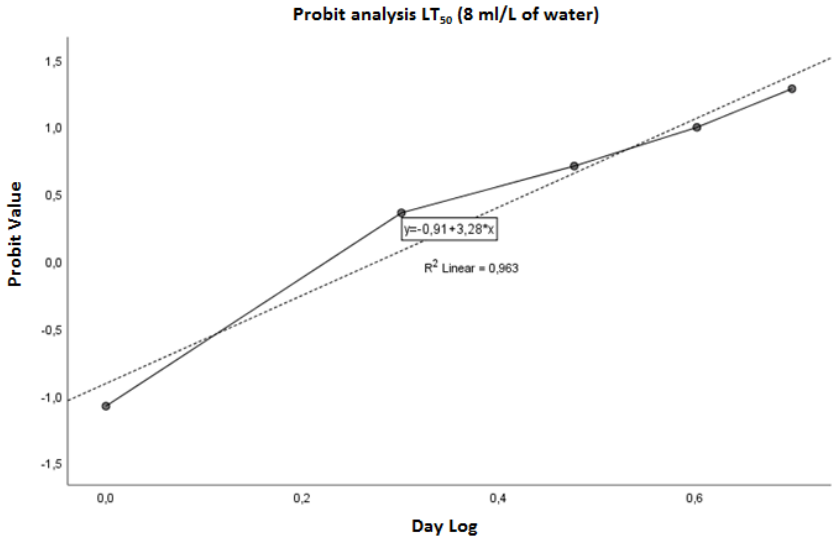

Figure 5. Probit analysis LT_{50} (8 ml/L of water)

Table 1 indicates that the application of various concentrations of tuba root extract led to a range of LT_{50} values for *S. frugiperda* larvae of 1.86 – 2.64 days (44.64 – 63.24 hours). The treatment involving 0 ml/L tuba root extract concentration did not result in mortality or achieve an LT_{50} value for *S. frugiperda* larvae within the observation period of 120 hours this outcome was significantly different from the other treatments. The application of 8 ml/L tuba root powder extract resulted in a relatively quicker mortality rate, with an LT_{50} of 1.86 days (44.64 hours) after application. Interestingly, this LT_{50} value was not significantly different from that observed with

the concentration of 4 ml/L of water, which yielded an LT_{50} of 2.09 days (50.16 hours) post-application. However, both of these treatments exhibited a notable difference compared to the other treatment conditions. This is due to *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 it is supported by Hasyim *et al.* (2019) The statement emphasizes the importance of the type of plant extract used and suggests that higher concentrations of the extract lead to faster initial death times and greater LT50 values.

Table 1. The average lethal time50 (LT50) of *S. frugiperda* larvae after treatment with various concentrations of tuba root (*D. elliptica*) extract (hour)

Tuba root extract Concentration (ml/L of water)	LT_{50} (day)	95% Confidence Limits for day	
		Lower Bound	Upper Bound
0	0	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

CI = Confidence Interval

Haryuningtyas *et al.* (2011) indicates that the rotenone compound acts as a potent inhibitor of respiratory enzymes, causing a disruption in the electron transport within the respiratory system. Consequently, the synthesis of adenosine triphosphate (ATP), a crucial source of energy is hindered. Furthermore, rotenone can act as a neurotoxin by inhibiting the enzyme glutamate oxidase, leading to a failure in nerve conduction.

3.2. Lethal Concentration 50 and 95 (LC_{50} dan LC_{95}) (%)

The concentrations of tuba root extract exhibited LC_{50} and LC_{95} values of 0.07% and 1.53%, respectively. The probit analysis for LC_{50} and LC_{95} can be observed in Figure 6.

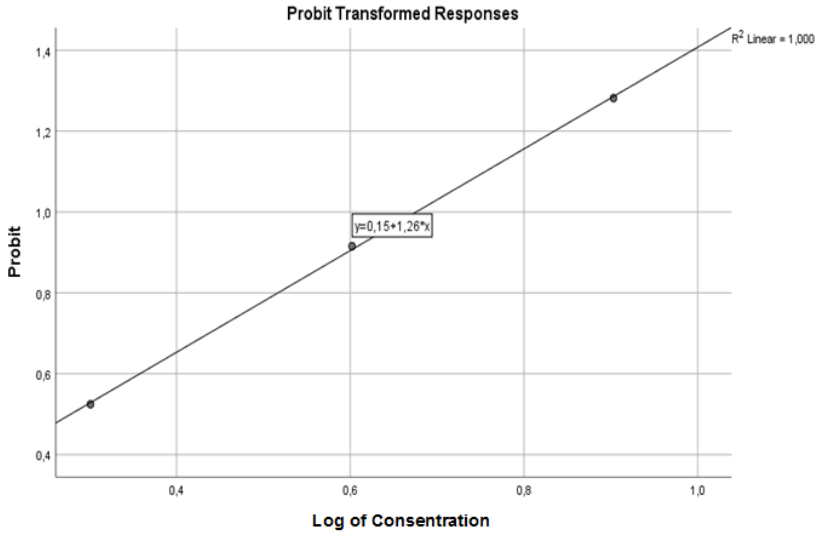

Figure 6. Probit analysis LC_{50} and LC_{95}

Table 2 suggests that the concentration needed to induce 50% mortality in the population of *S. frugiperda* larvae is 0.07%, which is equivalent to 0.7 ml/L of water of tuba root extract. The concentration effective in killing 95% of *S. frugiperda* larvae is 1.53%, equivalent to 15.3 ml/L of root extract.

Table 2. Lethal concentration 50 and 95 (LC_{50} and LC_{95})

Lethal concentration (LC)	Concentration (%)	CI Range 95%	
		Lower Limit	Upper Limit
LC_{50}	0.07	0.03	0.16
LC_{95}	1.53	0.74	297.07

CI = Confidence Interval

Based on the findings in Hasyim *et al.* (2019) It was observed that the tuba root extract treatment yielded the lowest LC_{50} value (1,256.07 ppm with fiducial limits 944.19-2045.45) in comparison to the kirinyuh (*Chromolaena odorata*) extract treatment (1304.35 ppm with fiducial limits 859.12-2,072.47). In addition, the World Health Organization (1992) found out that the LC_{50} of tuba root extract dissolved in chloroform was around 0.02-0.2 mg/L for fish. The smaller the LC value of a botanical insecticide, the more toxic the ingredient is.

The findings from this study suggest that the concentration of tuba root extract is not appropriate to achieve a 95% mortality rate in S. *frugiperda* larvae. To achieve such mortality, a tuba root extract concentration of 1.53%, equivalent to 15.3 ml/L of water is necessary. This is in accordance with the opinion of Prijono (1999) who suggests that the LC95 (lethal concentration for 95% mortality) of botanical insecticide with organic solvents is effective when the concentration needed to kill the test insects does not exceed 1% (10 ml/L of water). The high concentration needed to kill 95% of the *S. frugiperda* population is thought to be due to the high survival rate of *S. frugiperda* larvae.

4. Conclusion

The concentration of tuba root powder extract required to achieve 50% mortality in *S. frugiperda* larvae is 0.07%, equivalent to 0.7 ml/L of water. Meanwhile, the appropriate concentration to eliminate 95% of *S. frugiperda* larvae is 1.53%, equivalent to 15.3 ml/L of water.

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