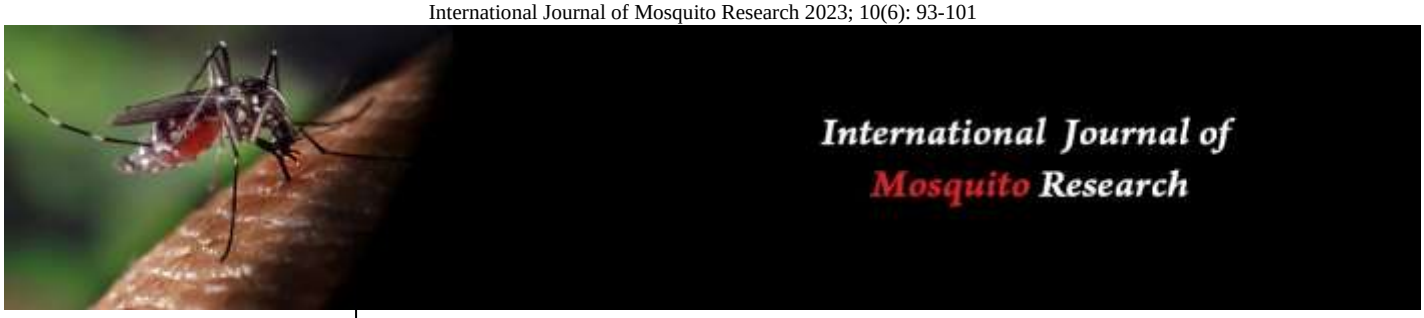

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Toxicity of *Acacia arabica* leaf extract for the control of *Aedes aegypti* mosquito larvae-A laboratory study

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Abstract
The primary objective of this research was to test the effectiveness and LC_{50} value of babool (*Acacia arabica*) leaf extract as a bio larvicide for *Aedes aegypti*. This research was a laboratory experimental study consisting of 6 treatments and 4 replications. The treatment groups consisted of babool leaf extract concentrations of 0.25%, 0.50%, 0.75%, and 1.0%. Next, the extract was tested for its effectiveness as a larvicide on test animals in the form of *Aedes aegypti* larvae in third instar. The results of the research were data analysed using Analysis of Variance (ANOVA) followed by Probit Analysis and determining the LC_{50} value. The research results showed that babool leaf extract (*Acacia arabica*) was effective as a bio larvicide with an LC_{50} of 3303 ppm or equivalent to a concentration of 0.33%. The Insect Growth Regulator test findings indicate that it has the same impact as pyriproxyfen at all fatal concentrations. The study's findings indicate that the ethanol extract of babool leaves inhibits the growth of insects and acts as an ovicide against the *Aedes aegypti* mosquito.

Keywords: *Aedes aegypti* L., *Acacia arabica* extract, bio larvicide

1. Introduction
Dengue is a disease that has become a serious public health problem worldwide. The number of cases of this disease reported by the World Health Organization (WHO) has multiplied by eight in the last 20 years: in 2000, 505,430 cases were reported, more than 2.4 million in 2010 and 4.2 million in 2019 [1]. The high number of cases and incidence of dengue fever in endemic areas has led to many prevention efforts being carried out. However, until now no vaccine or cure for dengue has been found. So, controlling this disease depends on controlling the vector, namely the *A. aegypti* mosquito [2]. Various efforts to control the *A. aegypti* mosquito population have been carried out, including physical, chemical and biological control. However, until now the problem of dengue fever has not been resolved.
The *Aedes* (Stegomyia) *aegypti* (Linnaeus) mosquito is considered the main vector of viral diseases such as dengue, which have caused the loss of millions of human lives [3]. In India [4], *Ae. Aegypti* is the main vector of dengue virus (DENV), Chikungunya (CHIKV) and Zika virus (ZIKAV); Furthermore, this vector is associated with the transmission of yellow fever virus (YFV), and Mayaro (MAYV) in other regions of the world [5]. Therefore, measures aimed at controlling the populations of this species are important to reduce the number of infections of these diseases in countries with the highest incidence.
The most effective and efficient control of *A. aegypti* is by eradicating the larval stage. The control of *A. aegypti* mosquito larvae that has often been carried out is chemical control using insecticides. This can reduce the vector population quickly. Until now, the community's efforts to overcome dengue fever have been by using chemical insecticides in accordance with the health program implemented by the government. One of the chemical insecticides used is temephos to suppress the larval population [6]. People still continue to use these chemical insecticides for several reasons, including cheap, easy to obtain and practical to use. However, incorrect administration, such as the ratio of insecticide levels and water volume, causes unpleasant odors in the water reservoir. Apart from polluting the environment, repeated use can cause resistance to these chemical insecticides.

However, according to the WHO, if its use is not wise it will have negative impacts, including causing the death of non-target organisms, environmental problems and resistance to vectors.

Even research by Kasai *et al*. (2014) [7] have reported the resistance of *A. aegypti* mosquitoes to various insecticides. Because of the many negative impacts they cause, the use of plant-based insecticides (bio larvicides) can be used as an alternative to chemical insecticides. Apart from that, generally botanical insecticides have low toxicity to mammals. Because of this characteristic, it is possible for plant-based insecticides to be applied to human life [8]. The use of larvicides over a long period of time will cause vector resistance to the larvicides. As an alternative, larvicides from the Insect Growth Regulator (IGR) [9] group can be used in accordance with WHO recommendations. Insect Growth Regulators (IGR) are insecticides that contain juvenoid compounds that affect Mosquito morphogenesis is characterized by the failure of larvae to develop into pupae. Based on the previous explanation, *A. arabica* leaves contain secondary metabolite compounds which can act as vegetable insecticides. Research on the activity of metabolite compounds from *Acacia arabica* leaf extract as an ovicide and Insect Growth Regulator (IGR) has never been carried out before [9]. Based on phytochemical tests conducted by Srivastava *et al*. (2014) [10] positive (+) babool leaf extract contains secondary metabolite compounds of alkaloids, flavonoids, saponins, tannins, steroids and terpenoids which are capable of providing a larvicidal effect on *A. aegypti* mosquito larvae.

Species of the genus Acacia may contain dimethyltryptamine derivatives and cyanogenic glycosides (Prunasin, sambunigin, acacipetalin) in the seeds, leaves and bark [11]. Dimethyltryptamine was synthesized for the first time in 1931 and its hallucinogenic effect was proven in 1956. Unfortunately, only a limited number of studies have evaluated the larvicidal activity of plant extracts against *Ae. aegypti* in places where water is stored; such as tanks and wastewater bodies, elucidating the effectiveness of these extracts in the field. It is necessary to reduce the use of chemical insecticides, and promote the use of plant extracts and their derivatives as sustainable practices, so the objective of this study was to evaluate the extract of the fruit of *Acacia arabica* for the control of *Ae* larvae *aegypti* under controlled conditions (laboratory) and in field conditions.

The aim of this research is to test the concentration of babool leaf extract (*Acacia arabica*) which is effective as a bio larvicide for the *Aedes aegypti* mosquito and to explain the effect of the concentration of babool leaf extract (*Acacia arabica*) on the death of *Aedes aegypti* mosquito larvae.

2. Materials and Methods: This research was carried out in our college laboratory for the extraction process of babool leaves (*Acacia arabica*) and at the Parasitology Laboratory for bio larvicide tests on *Aedes aegypti* L. instar III mosquito larvae. The research was conducted in July – September 2022. The tools used in this research were buckets, scissors, winnowing pans, ovens, blenders, analytical balances, a set of rotary evaporators, storage containers, laboratory coats. The materials used were 550 g of wet babool (*A. arabica*) leaves, 134 g of dry babool (*A. arabica*) leaves, 3 liters of 70% ethanol (CH_3CH_2OH) separator liquid, filter paper, handscoon, mask.

Eggs, larvae and pupae of *Ae. aegypti* directly from puddles of water, artificial containers and surfaces where water could accumulate. Subsequently, they were placed in a plastic tray for development in the laboratory. Once the pupae had developed, the adult mosquitoes were collected and placed in an entomological cage to continue breeding *Ae. aegypti* in order to have individuals for future studies, adult males were fed with sugar water and females with blood from a common poultry.

2.1 Identification of *A. arabica* leaf Plants

Plant identification or determination is carried out in research that uses natural plants as samples, with the aim of knowing the truth of the type of plant used in the research. Identification of *A. arabica* leaves is carried out in the Laboratory of the Faculty of Natural Sciences, Nagarjuna University. *A. arabica* Leaf samples are submitted to the laboratory along with a letter of introduction to the research. The identification results show that the samples used are indeed *A. arabica* leaves referred to in the research samples.

2.2 Research design

This research used a laboratory experimental research method, which was carried out in a laboratory room to test the biolarvicide effect of babool leaf extract (*A. arabica*) on *A. aegypti* larvae, after treatment for 24 hours. Based on WHOPES larvicide testing guidelines (2005), 25 larvae were used per replication. The number of repetitions was 4 times with 5 treatment groups and 1 positive control group (abate 1.0%). Each repetition consists of 2 replications. So overall it can be seen as follows:

Table 1: Extract Treatment of Larvae

Treatment 1	Control (-) 0% + 25 larvae
Treatment 2	Extract 0.25% + 25 Larvae
Treatment 3	Extract 0.50% + 25 Larvae
Treatment 4	Extract 0.75% + 25 Larvae
Treatment 5	Extract 1.0% + 25 Larvae
Treatment 6	Control (+) temephos + 25 Larvae

Table 2: Number of Larvae Required

Treatment		Number of larvae x (number of repetitions x replication)	Amount
Control (-)	0%	25 larvae x (4x2)	200 larvae
Treatment 1	0.25%	25 larvae x (4x2)	200 larvae
Treatment 2	0.50%	25 larvae x (4x2)	200 larvae
Treatment 3	0.75%	25 larvae x (4x2)	200 larvae
Treatment 4	1.00%	25 larvae x (4x2)	200 larvae
Control (+)	temephos 1%	25 larvae x (4x2)	200 larvae
		Total	**2** larvae

2.3 Research procedure

Making Simplicia: According to Prasetyo & Inoriah (2013) [17], there are several stages in making simplicia, namely:

- **Wet Sorting:** The collected babool (*A. arabica*) leaves are cleaned of dirt and foreign materials such as soil, gravel, grass and damaged leaves.
- **Arterial Washing:** The babool leaves are washed with clean running water to remove any remaining dirt that is still attached.
- **Chopping:** Before chopping, babool leaves are first dried in the sun intact for one day. After that, the babool leaves are chopped using a knife to make the drying process easier.
- **Drying:** Next, the babool leaves are dried using an oven at a temperature of 40 – 60 ºC. Drying is carried out until a constant weight is obtained. A sign that simplicia is dry is that it crumbles easily when squeezed or breaks easily.
- **Preparing Simplicia:** Extract from babool leaves, weigh 500 grams and grind with a blender until smooth, then put in a container/bottle. Soak in 70% ethanol solution until the simplicia is completely submerged. Beat until completely mixed (± 30 minutes). Leave it for 24 hours until it settles. The top layer of the 70% ethanol mixture with the mixed active substance was taken using filter paper. During 3 days, the simplicia was replaced with 70% ethanol solvent every 24 hours and stirred occasionally. The soaking solution is collected and allowed to settle. The precipitate is separated from the solution that does not settle; the solution that does not settle is placed in an evaporation flask. The extract obtained was then evaporated using a rotary evaporator at a temperature of 40 – 50 ºC until a thick extract was obtained with a concentration of 100%.
- **Biolarvicide Effect Test:** The process of testing the biolarvicidal effects of babool leaf extract consists of several stages, namely:
- Pipette the babool leaf extract according to the concentration to be used then put it in a 100 ml measuring flask. The concentrations used were 0.0%, 0.25%, 0.50%, 0.75% and 1.0% positive control (+) temephos, and distilled water is negative control
- Add distilled water to a 100 ml measuring flask which has been filled with extract until the volume limit mark is 100 ml then put it into a 250 ml Erlenmeyer flask.
- In each Erlenmeyer flask, 25 larvae were placed *A. aegypti* third instar.
- Count the number of larvae that die after 1x24 hours of treatment.

Preliminary tests were carried out with several concentrations, namely 0.25%, 0.50%, 0.75% and 1.0%. Which were given to each of the 25 test larvae in 100 ml of water with two replications and then observed every 24 hours. After that, observations were made and the number of dead larvae was counted. The data was analysed using probit and used as a benchmark for the concentration range to determine the concentration series (C1, C2, C3, C4) for the IGR test.

To create the required concentration a formula can be used

$$V_1 M_1 = V_2 M_2$$

Where

- **V1:** volume of extract to be diluted (ml)
- **V2:** desired volume of solution (water+extract) (100 ml)
- **M1:** concentration of available moonflower leaf extract (100%)
- **M2:** concentration of babool leaf extract to be made (%), namely C1, C2, C3, C4

For the negative control group (0%) use 100 ml of distilled water. Meanwhile, the positive control group used pyriproxyfen. For the treatment group, 4 doses of starfruit extract solution were used, namely C1%, C2%, C3%, C4%, where each dose was dissolved in distilled water until it reached 100 ml.

Ae mosquito eggs. *Aegypti* used in this research was obtained from the Entomology Laboratory ANU. Eggs are hatched in a plastic tub filled with water. The eggs will become larvae for 1-3 days at a conditioned room temperature.

Larvae that have been obtained from hatching eggs are fed chicken liver that has been dried and cut into thin pieces. Larvae are maintained until they reach the third instar at a conditioned room temperature.

Test the activity of babool leaf extract as an ovicide on *Ae* mosquito eggs. *Aegypti*, namely using 24 glass beakers. This amount is adjusted to the number of concentrations multiplied by the number of repetitions. Then the test solution used was babool leaf extract with concentrations namely C1, C2, C3, C4, 0% (negative control) and positive control, dissolved in distilled water, where the test solution for babool leaf extract was made by making a stock solution for each Concentrate the treatment by taking the distilled water into a measuring cup, then taking the Moonflower leaf extract from the storage bottle using a 3 ml dropper, then putting it into the measuring cup. Then each solution with the treatment concentration was poured into a beaker glass, and 25 Ae eggs were added to each. *Aegypti* in beaker glass. Each treatment was repeated 4 times. Then, during the observation, pay attention to the condition of the eggs that hatch into larvae, then count the number of larvae to find out the number of eggs that do not hatch, once every 24 hours until the 72nd hour. Then after 72 hours the eggs that have not hatched will be counted and accumulated in a table observation.

The IGR activity test was carried out by preparing third instar *Ae* larvae. *Aegypti* as many as 25 each, prepared in a small glass filled with water. The larvae were taken from a small glass using a sieve and put into a plastic cup that was ready for the 6 treatment doses, each treatment was repeated 4 times. The experiment was carried out by conducting assessments every 24 hours for 7 days, then counting the number of larvae, pupae and adult mosquitoes that emerged.

IGR Activity Formula:

$$\%IE = 100 - \left(T \times \frac{100}{C}\right)\%$$

2.4 Data Analysis Techniques

In this study, primary data was used obtained from the number of deaths of *A. aegypti* larvae after treatment for 1x24 hours at babool leaf extract concentrations of 0.0%, 0.25%, 0.50%, 0.75% and 1.0%. Negative control (-) 0% uses distilled water and positive control uses abate 1.0%.

Next, a one-way Analysis of Variance (ANOVA) was carried out at the 1% level to test whether there were significant differences between the several treatments. If the ANOVA results show a difference in meaning, then proceed with

Probit analysis to obtain the Lethal Concentration 50 (LC_{50}) value [8].

3. Results

This type of research is experimental using the Completely Randomized Design (CRD) method. The RAL method is an experimental design that is applied if you want to study treatments using experimental units for each treatment or using the total units in the experiment. The first stage of this research was a preliminary test, then continued with further tests, namely insect growth regulator (IGR) and ovicide. Preliminary tests were carried out to determine the effective concentration range that could kill 10-95% of test larvae.

3.1 Morphology of *Aedes aegypti* Larvae

Observations of *Aedes aegypti* larvae were carried out at the Parasitology Laboratory of the Muhammadiyah University of North Sumatra using a light microscope at 10x magnification. Larvae are characterized by one row of comb teeth at the end of the abdomen, a short, fat chiffon surrounded by chiffon hairs and having spines (spinae). The morphology of *Aedes aegypti* larvae can be seen in figure 1 and 2.

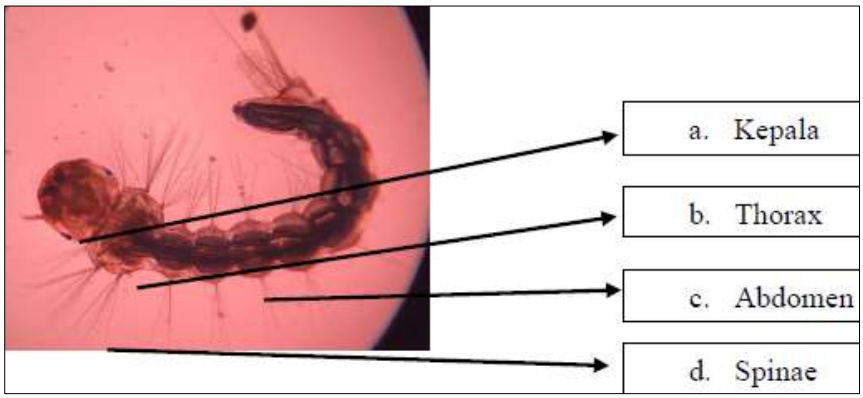

Fig 1: III instar *Aedes aegypti* larvae with 10x magnification

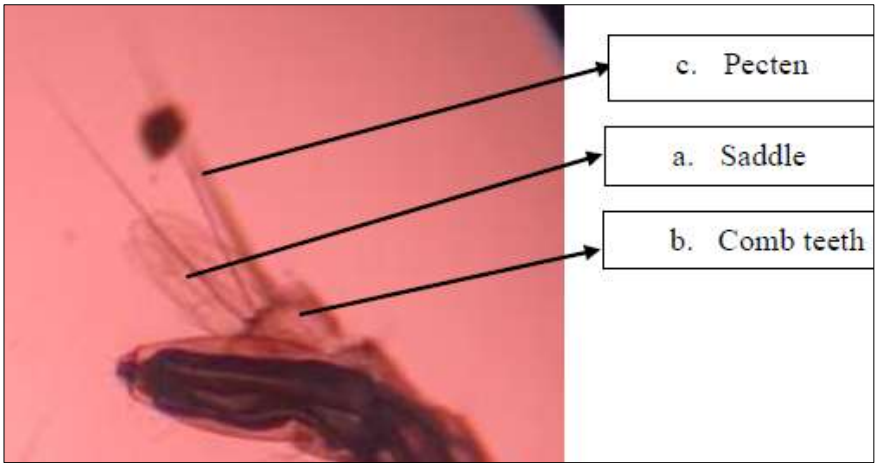

Fig 2: Tail of *Aedes aegypti* instar III with 10x magnification

3.2 Bio larvicide Effect Test

The results of the bio larvicide test showed that the concentration of *A. arabica* leaf extract had a larvicidal effect on *A. aegypti* mosquito larvae. Data from research regarding the number of deaths of *A. aegypti* mosquito larvae at various concentrations of *A. arabica* leaf extract are presented in the following table 3.

Based on the research results obtained, analysis of variance (ANOVA) was then carried out at the 1% level to test whether there were significant differences between the several treatments. If f count > f table, then H_0 is rejected or there is a significant difference. However, if f count < f table, then H_0 is accepted or there is no significant difference. ANOVA results can be seen in Table 4.

Table 3: Number of deaths of *A. aegypti* larvae after 24 hours of treatment

	Number of Larval Deaths								
	Deuteronomy 1		Deuteronomy 2		Deuteronomy 3		Deuteronomy 4		Average
Concentration Variations	1	2	1	2	1	2	1	2	
Control (-)	0	1	2	0	0	0	0	0	0.375
0.25%	14	13	10	13	14	12	12	12	12.5
0.50%	15	14	12	15	16	13	14	17	14.5
0.75%	18	16	17	18	16	17	18	18	17.25

1.00%	21	20	19	20	20	22	21	19	20.25
Control (+)	25	25	25	25	25	25	25	25	25

The ANOVA results show that the calculated f value is greater than the f table, namely f calculated 411.33 > f table 4.24., which means there is a significant difference between treatments of babool leaf extract concentration on the death of third instar *A. aegypti* larvae. Determination of Lethal Concentration 50 (LC_{50})

Table 4: One Way ANOVA Results

SK	DB	JK	KT	*f* −count	*f*-table
Treatment	5	1413.958	282.7916667	411.333	4.2478
Error	18	12.375	0.6875	-	-
Total	23	1426.333	-	-	-

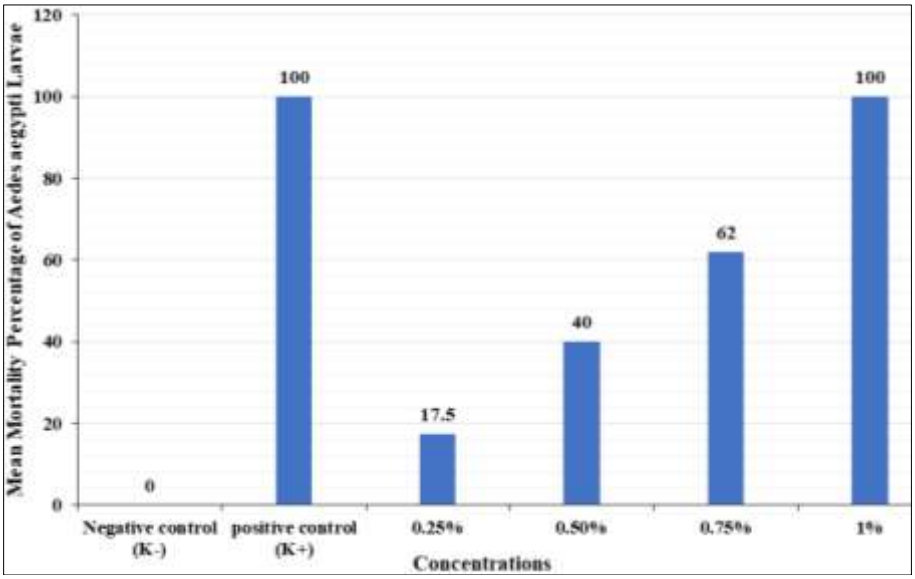

Fig 3: Preliminary Test Results Mean Mortality Percentage of *Aedes aegypti* Larvae after 24 hours of exposure to Ethanol Extract of babool leaves.

Figure 3 shows the results that at a concentration of 0.01%, larval mortality of 18% was observed. In a series of concentrations of ethanol extract of *A. arabica* leaves ranging from 1% to 3%, the activity was equivalent to the positive control (K+), namely 100%, which could cause larval death. Based on the results of the preliminary test at a concentration of 0.1%, the result was 40% larval death so that five types of concentration series could be determined to be used in the next test. The concentrations used were concentrations of 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5% and 1%. As well as water as a negative control and temephos 1% as a positive control.

In the next test, observations and calculations of the number of larval deaths were carried out after 24 hours of exposure. The results of research on the larvicidal activity of the ethanol extract of *A. arabica* leaves against the larvae of *Ae. aegypti* instar III can be seen in Figure 4

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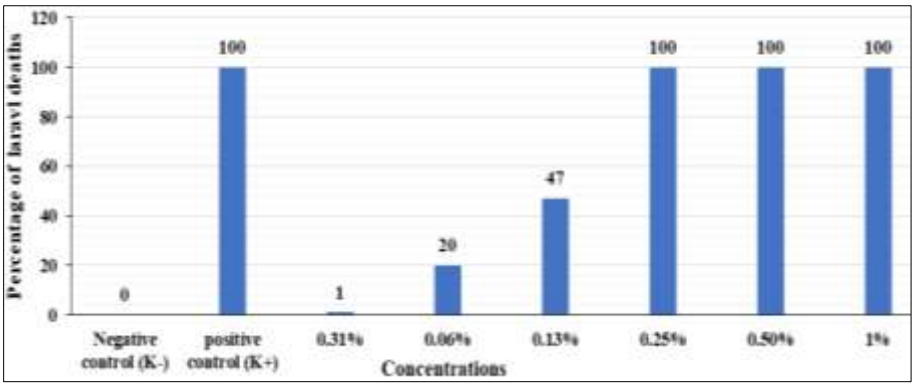

Fig 4: Average Larvicidal Activity Test Results of Ethanol Extract of *A. arabica* Leaves against *Aedes aegypti* Larvae for 24 hours.

Figure 4 shows a graph of the percentage of death of third instar *Ae. aegypti* larvae after 24 hours of exposure to ethanol extract of *A. arabica* leaves. In the positive control using 1% temephos there was larval death with an average percentage of 100%.

In Figure 4, there is an increase in the larval death rate as the amount of extract concentration given increases. The average percentage of larval deaths in the serial concentration of

0.03125% was 1%, at a concentration of 0.0625% it was 20%, at a concentration of 0.125% it was 47%, at a concentration of 0.25% it was 95%. at a concentration of 0.5% it is 99%, and at a concentration of 1% it is 100%.

The research results were analysed using a probit test in the SPSS program to determine the values of LC_{10}, LC_{25}, LC_{50}, LC_{90}, and LC_{99} in the ethanol extract of *A. arabica* leaves against *Ae* larvae. *aegypti*. From the results of the probit test, the LC_{10}, LC_{25}, LC_{50}, LC_{90}, and LC_{99} values are shown in figure 5.

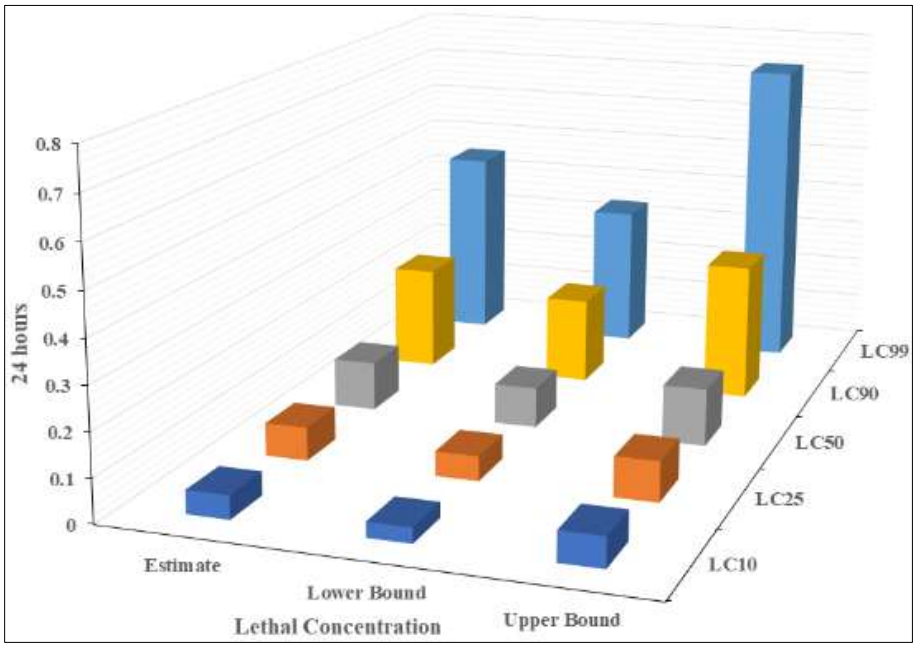

Fig 5: LC_{10}, LC_{25}, LC_{50}, LC_{90}, and LC_{99} values from Larvicide Test Results for Ethanol Extract of babool Leaves. Death of *Aedes aegypti* Instar III Larvae for 24 hours.

- The LC10 value for 24 hours of exposure is the concentration of ethanol extract of *A. arabica* leaves which can cause 10% larval death at 24 hours of exposure in a concentration range of 0.035% to 0.069% with an estimate of 0.054%.
- The LC25 value on exposure for 24 hours is the concentration of ethanol extract of A. arabica leaves which can cause larval death of 25% on exposure for 24 hours in a concentration range of 0.057% to 0.093% with an estimate of 0.077%.
- The LC50 value for 24 hours of exposure is the concentration of ethanol extract of *A. arabica* leaves which can cause 50% larval death at 24 hours of exposure in a concentration range of 0.094% to 0.134% with an estimate of 0.114%.
- The LC90 value for 24 hours of exposure is the concentration of ethanol extract of *A. arabica* leaves which can cause 90% larval death at 24 hours of exposure in a concentration range of 0.202% to 0.321% with an estimate of 0.243%.
- The LC99 value for 24 hours of exposure is the concentration of ethanol extract of *A. arabica* leaves which can cause 99% larval death at 24 hours of exposure in a concentration range of 0.337% to 0.731% with an estimate of 0.451%.

Followed by the insect growth regulator test using the concentration results from probit analysis LC_{10}, LC_{25}, LC_{50}, LC_{90}, and LC_{99}, serial concentrations of 0.05%, 0.08%, 0.12%, 0.24%, 0.45% were obtained. ethanol extract of babool leaves exposed to *Ae. aegypti* third instar. The treatment was repeated 4 times. Observations on the effects of exposure to ethanol extract of babool leaves were carried out every 24 hours for 7 days. In the IGR test, what was observed was the appearance of adult mosquitoes in the concentration series, apart from that, the number of larval deaths, pupa emergence and pupa death were also observed. Figure 6 shows the percentage of larval deaths during observation. Figure 3 shows the percentage of larval deaths during observation.

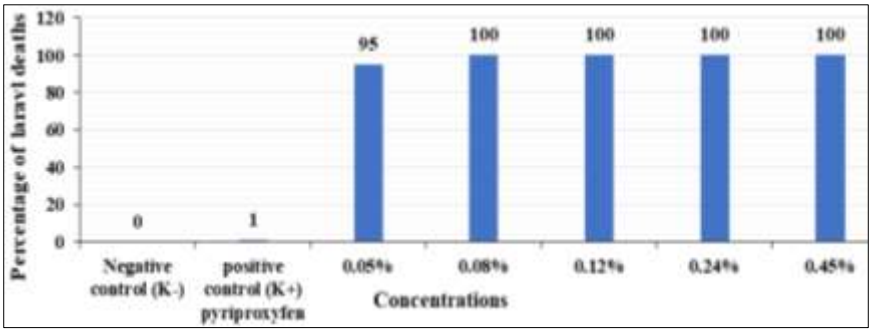

Fig 6: Percentage of Death of *Aedes aegypti* Larvae during Exposure to Ethanol Extract of babool Leaves with 7 treatments for 7 days.

In Figure 6, the negative control group after exposure for 7 days did not show any larval death. In the positive control with administration of pyriproxyfen after exposure for 7 days, larval mortality was observed with an average of 1%. At the lowest concentration, namely 0.05%, after exposure for 7 days, larval mortality was found to be an average of 95%. At a concentration of 0.12%, after exposure for 7 days, larval mortality was found to be an average of 99%. At concentrations of 0.08%, 0.24%, and 0.45% there was 100% larval death.

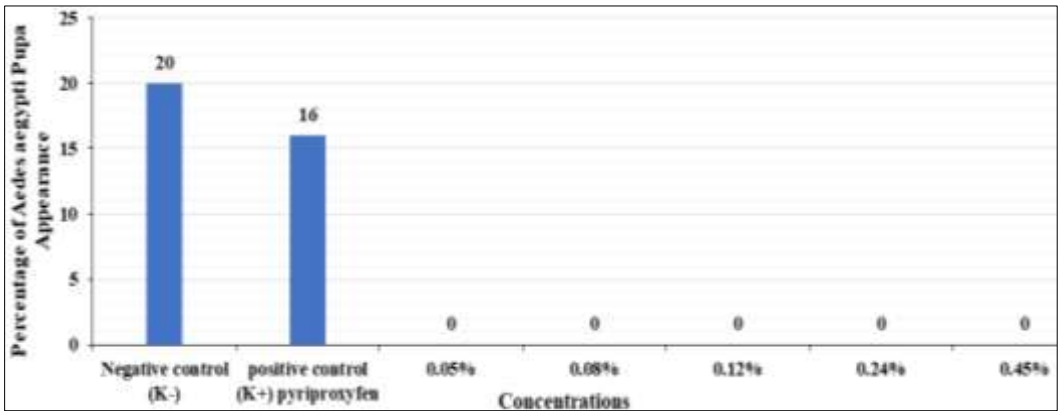

Fig 7: Percentage of appearance of *Aedes aegypti* pupae during exposure to ethanol extract of *A. arabica* leaves with 7 treatments for 7 days.

In Figure 7, the negative control group after exposure for 7 days found 20% pupa emergence, while in the positive control with pyriproxyfen administration, pupa emergence was 16%. At concentrations of 0.05%, 0.08%, 0.12%, 0.24% and 0.45%, no pupae appeared.

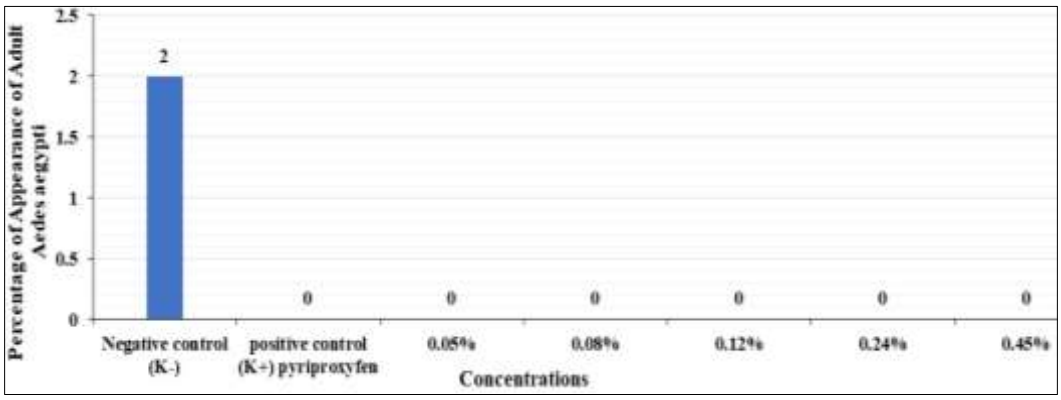

Fig 8: Percentage of appearance of adult *Aedes aegypti* mosquitoes during exposure to ethanol extract of *A. arabica* leaves with 7 treatments for 7 days.

In Figure 8, the negative control group after exposure for 7 days found the appearance of adult mosquitoes at 2%, while in the positive control no adult mosquitoes appeared. At concentrations of 0.05%, 0.08%, 0.12%, 0.24%, and 0.45%, no adult mosquitoes appeared. Based on the research above, the activity of *A. arabica* leaf extract as an Insect Growth Regulator is calculated using the IGR activity formula. The percentage of emergence of adult mosquitoes at concentrations of 0.05%, 0.08%, 0.12%, 0.24% and 0.45% was 0%. It can be concluded that at all concentrations the IGR activity is 100%, this is due to the content of secondary metabolites contained in A. arabica leaves such as flavonoids, saponins, tannins and alkaloids [12].

Flavonoids have a mechanism of action by entering the larvae's body through the respiratory system which will cause nerve paralysis and damage to the respiratory system, making it difficult for the larvae to breathe and eventually die [13].

Alkaloids act as poison through the larvae's mouth and have a bitter taste. Colour changes occur in the larva's body to become more transparent and its movements slow down when stimulated by touch and its body always bends, this is also caused by alkaloid compounds. Just like alkaloids, saponins are mouth toxic to larvae and have a bitter taste which reduces the larvae's appetite. Saponins can also reduce digestive enzyme activity and food absorption and irritate the gastrointestinal mucosa [14].

Tannin works by binding the protease enzyme, by binding the enzyme to the tannin, the work of the protease enzyme is inhibited. Inhibition of the protease enzyme results in disruption of cell metabolism and nutritional deficiencies in the larvae. Lack of nutrition can inhibit the growth of larvae and if it continues continuously it will lead to death. There was a positive control using pyriproxyfen which did not show the appearance of pupae or adult mosquitoes, this is because pyriproxyfen is an IGR larvicide which can kill *Aedes aegypti* larvae and pupae due to stunted growth. larvae due to failure to molt and damage to the digestive system [15].

The results of the ovicide activity test of the ethanol extract of *A. arabica* leaves using concentrations of 0.05%, 0.08%, 0.12%, 0.24%, 0.45%, 0% as negative controls (water) and

pyriproxyfen and temefos as positive control. The results of exposure for 72 hours can be seen in Figure 9.

After 72 hours there was no egg death, whereas at a concentration of 0.45% egg death was 48%. In the positive controls used, namely pyriproxyfen and temephos, no ovicide activity was found, this is because pyriproxyfen is an IGR larvicide which can kill *Aedes aegypti* larvae and pupae due to inhibition of larval growth due to failure to molt and damage to the digestive system. Temephos is an organophosphate insecticide that binds to the enzyme acetylcholinesterase (AChE) which overstimulates the nervous system, thereby stimulating muscle fibers repeatedly and resulting in death [15].

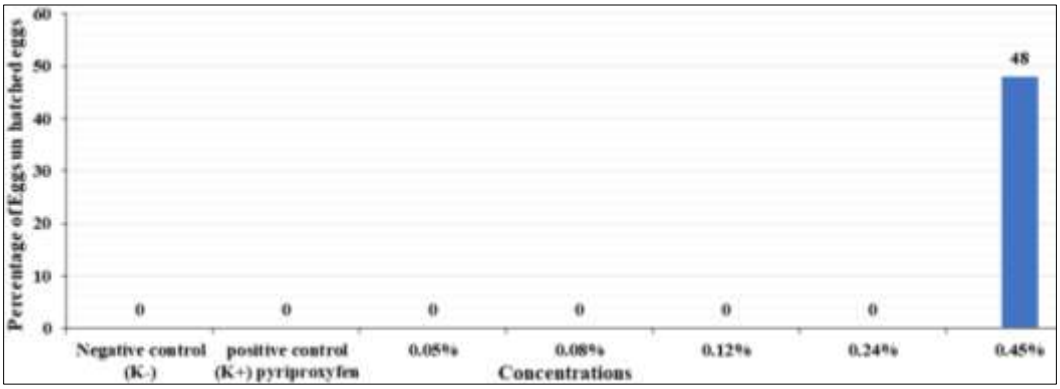

Fig 9: Percentage of Eggs un hatched eggs.

In Figure 9, the negative control group after 72 hours of exposure did not find any eggs that did not hatch (dead eggs). In the positive control using temefos and pyriproxyfen, no eggs were found that did not hatch (dead eggs). At concentrations of 0.05%, 0.08%, 0.12%, and 0.24% after exposure

Egg death at a concentration of 0.45% is due to the presence of metabolite compounds such as saponin which can damage the egg cell membrane thereby inhibiting egg hatching, while the tannin compounds contained in the extract can inhibit the process of egg cell division [16].

The percentage of eggs that did not hatch during 72 hours of exposure was tested for normality and homogeneity using the Shapiro-Wilk and Levene tests. In this statistical test, the concentration of 0.05% is symbolized as 1, 0.08% as n2, 0.12% as n3, 0.24% as n4, and 0.45% as n5. The results of the Shapiro-Wilk test show significance results of p=0.001 for n1, p value=0.001 for n2, p value=0.001 for n3, p value=0.001 for n4, p value=0.577 for n5. Based on these results it can be concluded that the data is not normally distributed. In the results of the homogeneity test using the Levene method, a significance value of p=0.007 was obtained, which means the data is not homogeneous. The results of the normality and homogeneity tests still show that the data is not normally distributed and not homogeneous, so the statistical test is continued with the Kruskal-Walli's test.

4. Discussion

The biolarvicide effectiveness test of babool leaf extract (*A. arabica*) is a test of the phytochemical compounds contained in babool leaf extract against third instar *A. aegypti* larvae which are divided into various concentrations. This test aims to determine how much effect babool leaf extract has on the death of the test larvae within 24 hours.

The biolarvicide effectiveness test was carried out on babool leaf extract using distilled water as a solvent. The use of distilled water as a solvent aims to ensure that the larval growth process occurs in clean and clear water free from contaminants such as chlorine which can interfere with larval growth. A total of 25 *A. aegypti* larvae were put into an Erlenmeyer flask containing babool leaf extract with concentrations of 0.25%, 0.50%, 0.75% and 1.0%. Control (-) uses distilled water and control (+) uses 1% Temephos. The results of the effectiveness test of babool leaf extract biolarvicide against third instar *A. aegypti* larvae can be seen in Table 1. In the control (-) the average number of deaths of *A. aegypti* larvae was 0.375 individuals. Meanwhile, in the control (+) all *A. aegypti* larvae died.

The test results showed that the four variations in the concentration of babool leaf extract had a larvicidal effect on third instar *A. aegypti* larvae. As the concentration increases, the number of deaths of *A. aegypti* larvae increases. The greater the concentration of babool leaf extract, the greater the toxicity to *A. aegypti* larvae so that the number of deaths increases. This is because babool leaf extract contains secondary metabolite compounds which can have a larvicidal effect on *A. aegypti* larvae [17].

From the results of the Mann-Whitney test, comparisons were made between the two controls with a concentration of 0.05% (n1), a concentration of 0.08% (n2), a concentration of 0.12% (n3), a concentration of 0.24% (n4) and a concentration of 0.45% (n5). When comparing negative controls with concentrations of 0.05%, 0.08%, 0.12%, and 0.24%, a significant difference was obtained with a significance value of 0.046 (p<0.05), while the negative control had a concentration of 0.45% there was no significant difference with a significance value of 0.157 (p>0.05). In comparing the positive control with concentrations of 0.05%, 0.08%, 0.12%, and 0.24%, a significant difference was obtained with a significance value of 0.046 (p<0.05), while the positive control had a concentration of 0.45% there was no significant difference with a significance value of 0.157 (p>0.05).

5. Conclusion

Based on the results of research testing the effectiveness of babool leaf extract (*Acacia arabica*) biolarvicide against third instar *Aedes aegypti* mosquito larvae, it can be concluded that babool leaf extract has a larvicidal effect on mosquito larvae. *A. aegypti* and has the potential to be developed as a larvicide against *A. aegypti* mosquito larvae. The concentration of babool leaf extract that provides the greatest larvicidal effect is the highest concentration in the study, namely a

concentration of 1.0% and the LC_{50} value of babool leaf extract which can kill 50% of *A. aegypti* mosquito larvae is 3.303 ppm or at a concentration of 0.33%. The number of deaths of *Aedes aegypti* mosquito larvae in the *A. arabica* leaf extract group was the same as the number of larval deaths in the 1% abate treatment group after 24 hours of treatment. There was no difference in the number of larval deaths at various concentrations of *A. arabica* leaf extract.

5.1 Suggestions

- It is hoped that there will be further research to determine the optimal dose of *A. arabica* leaf extract which can have a larvicidal effect on *Aedes aegypti* mosquito larvae.
- There is further research to find the right treatment time to kill 100% of the test larvae.
- Further research is needed on other plants that can have a larvicidal effect on mosquito larvae

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7. Conflict of Interest

The author has no conflicts of interest regarding this investigation.

8. References

1. Zeng Z, Zhan J, Chen L, Chen H, Cheng S. Global, regional, and national dengue burden from 1990 to 2017: A systematic analysis based on the global burden of disease study 2017. E Clinical Medicine. 2021 Jan 6;32:100712.
2. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin. Microbiol. Rev. 1998 Jul;11(3):480-96.
3. Dahmana H, Mediannikov O. Mosquito-Borne Diseases Emergence/Resurgence and How to Effectively Control It Biologically. Pathogens. 2020 Apr 23;9(4):310.
4. Soni S, Gill VJS, Anusheel, Singh J, Chhabra J, Gill GJS, *et al*. Dengue, Chikungunya, and Zika: The Causes and Threats of Emerging and Re-emerging Arboviral Diseases. Cureus. 2023 Jul 11;15(7):e41717.
5. Tidiane Diagne C, Bengue M, Choumet V, Hamel R, Pompon J, y Missé D, *et al*. Mayaro Virus Pathogenesis and Transmission Mechanisms. Pathogens. 2020;9(9):738.
6. Meier CJ, Rouhier MF, Hillyer JF. Chemical Control of Mosquitoes and the Pesticide Treadmill: A Case for Photosensitive Insecticides as Larvicides. Insects. 2022 Nov 28;13(12):1093.
7. Kasai S, Komagata O, Itokawa K, Shono T, Ng LC, Kobayashi M, *et al*. Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: target site insensitivity, penetration, and metabolism. PLoS. Negl. Trop Dis. 2014 Jun 19;8(6):e2948.
8. Pratiwi A. Descriptive Study of Community Acceptance of Natural Larvacides. Unnes. Journal of Public Health. 2014:3(2):1-10.
9. Solichah N, Martini, Susanto HS. The effect of administering Insect Growth Regulator (IGR) larvicide containing the active ingredient pyriproxyfen on changes in larvae-free rates (ABJ) in Bulusan sub-district, Semarang city. Journal of Public Health. 2016;4(1):168.
10. Srivastava, Manjoosha, Kumar G, Mohan R, Malhotra, Swadesh, *et al*. Phytochemical studies and antimicrobial activity of babool seeds. Journal of Scientific and Industrial Research. 2014:73:724-728.
11. EFSA. Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. In Scientific Report of European Food Safety Authority. 2012:10(5):126-132.
12. Iskandar I, Horiza H, Fauzi N. Effectiveness of Papaya seed powder (*Carica papaya* Linnaeaus) as a natural larvicide against the death of *Aedes aegypti* larvae in 2015. EXACT. 2017;18(1):13-18.
13. Eka CB, Endah S. Testing the Effectiveness of Legundi Leaf Extract Larvicides (*Vitex trifolia*) against *Aedes aegypti* Larvae. Medical Journal of Lampung University. 2013;2(4):58.
14. Roni K, Hanny HP. Test of Mahogany Seed Extract (*Swietenia macrophylla*) against *Aedes aegypti* Larvae Vector Dengue Fever. The Indonesian Journal of Public Health. 2016;12(4):221.
15. Nur S, Martini, Henry SS. The Effect of Providing Insect Growth Regulator (IGR) Larvicide with the Active Ingredient Pyriproxyfen on Changes in Larvage Free Rates (ABJ) in Bulusan Subdistrict, Semarang City. Journal of Public Health. 2016;4(1):168.
16. Maretta G, Kuswanto E, Septikayani NI. Effectiveness of Patikan Kebo (*Euphorbia hirta* L) Leaf Extract as an Ovicide against Dengue Haemorrhagic Fever Mosquitoes (*Aedes aegypti*). Biosphere. 2019;10(1):1-9.
17. Widawati M, Prasetyowati H. Effectiveness of *Beta vulgaris* L. (Beetroot) Fruit Extract with Various Solvent Fractions on *Aedes aegypti* Larva Mortality. Aspirator. 2013;5(1):23-29.