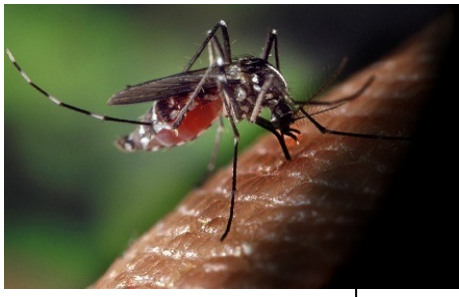

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Larvicidal and mortality activities of ethanolic extract from *Euphorbia indica* against *Aedes aegypti* and *Culex vishnui* larvae

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Abstract
This study aimed to ascertain the larvicidal and mortality activities of crude ethanolic extract derived from *Euphorbia indica* against larvae of *Aedes aegypti* and *Culex vishnui*. A preliminary test was conducted in order to ascertain the mortality rate ranging from 10% to 90%. The concentrations for the final test encompassed a range from 0.0998 g/L to 1.498 g/L alongside a single negative control. The LC_{50} and LC_{90} values of the ethanolic extract derived from *Euphorbia indica* leaf were determined to be 466.90 g/L and 868.42 g/L, respectively. The larvicidal effect of *Euphorbia indica* leaf is attributed to a chemical component that acts as a positive allosteric modulator of the GABA pathway. The collected data was subjected to analysis using the Kruskal-Wallis statistical test. Probit analysis was employed to examine the LD_{50} and LD_{90} values. The minimal lethal dose that resulted in 100% mortality of *Culex vishnui* larvae was determined to be 0.2 g/100 ml (p=0.01). The probit analysis yielded LD_{50} and LD_{90} values of 0.01 g/100 ml and 0.06 g/100 ml of aquades in *Culex vishnui*, respectively. The larvicidal efficacy of the dose was determined to have an LD_{90} value of 0.06 g/100 ml of aquades, but the LD_{50} value was found to be 0.01 g/100 ml of aquades. The assessment of *Culex vishnui* treatment involved comparing the number of mortality larvae and the number of living larvae from each treatment to the control group. In conclusion, the ethanolic extract derived from the leaves of *Euphorbia indica* exhibits promising potential for developing mosquito larvicides.

Keywords: *Aedes aegypti*, *Culex vishnui*, ethanolic extract, *Euphorbia indica*, LC_{50}, LC_{90}, LD_{50} and LD_{90}

1. Introduction
Mosquitoes are one of the major public health problems in India, especially diseases carried by *Aedes* mosquitoes, such as the new strain of dengue fever that is more severe and chikungunya [1]. The report on the Bureau of Epidemiology's epidemiological surveillance situation found that dengue fever is on the rise. It is expected that there will be approximately lakhs of patients throughout the year [2], with the highest number of cases occurring during the rainy season. (June - August) Currently, there is no medicine or vaccine that directly treats both dengue fever and chikungunya-prevention of dengue fever encephalitis, elephantiasis, and malaria. There is still an emphasis on controlling disease-carrying mosquitoes as the main measure [3].
Chemicals commonly used to kill mosquito larvae include the organophosphate group. (Organophosphates) such as Temphos (Abate®), Malathion and Fenthion, etc. Insect repellent and mosquito repellent products on the market contain the important active ingredient N, N-diethyl-m-toluamide (DEET), which is an effective synthetic substance [4]. High and broad-spectrum effect in repelling mosquitoes widely used in mosquito repellent products sold in the market. But there is a disadvantage. It has a rather pungent smell and may cause allergic reactions to substances and be toxic to consumers. Therefore, to reduce the effects, the use of natural substances from medicinal plants is another option to replace the use of chemicals. This is because medicinal plant substances have low toxicity to humans and pets [5].
Malaria is a disease caused by infection with the Plasmodium parasite, which is transmitted through the bite of the Anopheles mosquito [6].

The four types of Plasmodia that are capable of infecting humans are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale* [6]. Malaria is a disease that is still a health problem in several regions. Various efforts to break the chain of transmission through vectors, both mosquitoes and larvae, have been continuously carried out, but their application still faces many obstacles. Previous research has found that synthetic larvicides kill malaria larvae, but resistance develops in the larvae's bodies after some time. Apart from that, there are still obstacles to this larvicide, such as the high price and the fact that it cannot be degraded by nature, which causes environmental pollution, so a natural larvicide that is safe for the environment is needed.

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus. The *Aedes aegypti* mosquito is the main vector of dengue fever. This mosquito goes through 4 stages of development: egg, larva, pupa and adult mosquito. The eggs will turn into larvae in two days, the larvae will actively eat microorganisms within five days to become pupae, and the pupae will turn into adult mosquitoes in two days [7].

Crude extracts from plants have the effect of killing mosquito larvae and repelling mosquitoes using different extraction solvents, such as the methanol solvent used to extract crude extracts from neem (*Azadirachta indica*) from India good effect in killing *Ae. Aegypti* larvae [8]. Used to extract crude extract from lotus leaves (*Nelumbo nucifera*), it has a good effect in killing Anopheles mosquito larvae, *An. stephensi* and price mosquitoes. *C. pseudovishnui*. Using crude extracts from the under-leaves (*Phyllanthus amarus*) has good effects in killing the larvae of the *Anopheles* mosquito *An. stephensi* and the mosquito *C. pseudovishnui* [8-9]. Ethanol solvent used to extract crude extracts from the leaves of the *Datura stramonium* tree from India at a concentration of 1% had no effect on repelling *Ae. Aegypti* mosquitoes. It has a moderate impact on repelling *Anopheles* mosquitoes [10]. Dirus and was effective. Good for repelling the mosquito *C. pseudovishnui* and the ethyl acetate solvent used to extract the crude extract from pepper seeds has the effect of killing mosquito larvae. *An. stephensi* and the mosquito *C. pseudovishnui* [9]

Past research reports of *Euphorbia* species indicate that the crude *Euphorbia indica* extract extracted with water, methanol, and ethanol is suitable for killing mosquito larvae and repelling mosquitoes well [11]. In addition, from past research [11-12], it has been found that plants from different sources are found. It has different effects on killing mosquito larvae, and the leaves and roots contain substances found in crude extracts. Research analysing the effect of *E. indica* leaves on *Anopheles and Ae. Aegypti* mosquito larvae have never been carried out. Therefore, researchers want to research to determine the effect of *Ae. Aegypti* leaf extract on the mortality of third instar *Culex vishnui* larvae. Therefore, this research has an idea to use crude ethanolic extract from the *E. indica* L. plant leaves. This is to promote the use of many local herbs and bring weeds to benefit and develop them into products to reduce the cost of importing synthetic chemicals that are harmful to the environment and haveresistant to chemicals.

2. Methodology

2.1 *Euphorbia indica L* leaves collection: *Euphorbia indica* L, which belongs to the genus Euphorbia and the family Euphorbiaceae [13], is depicted in Figure 1. The *Euphorbia indica* L. plant has an annual growth pattern with heights ranging from 6 to 60 cm. Occasionally, the botanical specimen is harvested from its natural habitat for utilisation in indigenous medicinal practices. The latex present in most, if not all, species of Euphorbias exhibits caustic and toxic properties. Direct contact with the skin often leads to irritation and the formation of blisters. Eye contact, on the other hand, can cause temporary or even permanent blindness. Ingestion of this latex can result in purging or more severe health complications. Nevertheless, there needs to be more detailed information about this particular plant.

To address the condition of oedema, the submerged portions of plants are included in the bathing water. The treatment of diarrhoea and dysentery involves the administration of a plant decoction. *Euphorbia indica* L. is relatively less prevalent in comparison to *Euphorbia hirta*. Upon close examination, both objects appear similar when observed from a distance, yet they can be readily differentiated [14]. The geographical dispersion of South India encompasses several regions, including Kerala, Andhra Pradesh, Tamil Nadu, Iran, Afghanistan to Pakistan, Sri Lanka, Bangladesh, Myanmar, and India etc.,

Fig 1: *Euphorbia indica* Lam (Euphorbiaceae family) plant, leaf extract

2.2 Preparation of *Euphorbia indica* L. Extract: Extraction was carried out using the maceration method. *Euphorbia indica L* was collected from an agricultural field in Nuzvid, Krishna district, Andhra Pradesh, India. The collected leaves were plucked from their branches, washed with tap water, then distilled water, and left to dry. After drying with a water content of 5%, it is blended finely and then macerated in stages using ethanol. The solvent to leaf powder ratio is 1:4, or 50 g of *Euphorbia indica* L leaves is extracted with 200ml of solvent. The filtrate obtained is then subjected to a rotary evaporator to obtain a thick extract. The thick extract was then labelled as ethanol extract of *Euphorbia indica* L leaves. The extract is stored in the refrigerator.

2.3 Collection of bioassays (mosquitoes): Mosquito Catching in the Field and Maintenance in the Laboratory: The technique for catching mosquitoes in the field uses the method ofColucci *et al.* (2018) [15], which was modified. 10 Mosquitoes were obtained from their natural habitats, including residential waterways around residential areas wetlands with little intensity of sunlight. Adult mosquitoes obtained using the capture technique were immediately identified for their species position using Reid's (1968) [16] mosquito determination book. The first generation of IV instar larvae was used to test the toxicity of the extract.

Individually rearing mosquitoes by placing them separately to lay eggs. The eggs that hatch into larvae are moved to a

rearing area in the form of a tray measuring 26 cm long and 15 cm wide. The larvae are given food in the form of fish pellets. Stage 3 and stage 4 *Anopheles* sp mosquito larvae were used for toxicity tests. Adult mosquitoes are fed a powdered mixture of rice bran and meat in a ratio of 10:4, amounting to 75 mg-200 mg

2.4 Larvicidal Effect on *Aedes aegypti* Larvae: This research is experimental to see the larvicidal effect of ethanol extract of *E. indica* leaves on third and fourth instar larvae of the *Aedes aegypti* mosquito. 2 kg of *E. indica* leaves are washed until clean. Dried *E. indica* leaves are crushed and soaked in 96% ethanol. Thickening was carried out using a rotary evaporator, and a thick extract of 17 grams was obtained. To make a stock solution of 6.992 g/L, 7 grams of thick *E. indica* leaf extract is dissolved in 1 litre of water. Making test concentrations by dilution. Using the dilution formula, a certain amount of mother liquor is taken and diluted using distilled water to 100 ml.

Preliminary tests were carried out by inserting 20 larvae each at concentrations of 0.0999 g/L, 0.199 g/L, 0.399 g/L, 0.799 g/L, 1.598 g/L, 3.196 g/L, 6.392 g/L and negative control. In the preliminary test, 2 repetitions were carried out. Through probit analysis[17], concentrations that cause 10-90% mortality in the preliminary test will be used in the final test, namely 0.2996 g/L, 0.3995 g/L, 0.5993 g/L, 0.7990 g/L, 0.9988 g/L, 1.1986 g/L, 1.3984 g/L and negative control. Dead larvae sink and do not move after being stirred using a stirrer after 24 hours of administering the test material. The final test was carried out 3 times. Data on the number of larvae that died from ethanol extract exposure within 24 hours was processed in probit analysis.

2.5 Larvicidal Effect on *Culex vishnui* Larvae: The research was conducted using a pure experimental type of research with a post-test-only control group design. The population in this study were *Culex vishnui* larvae obtained from hatching results by the Parasitology Laboratory, Rajahmundry, Andhra Pradesh, India. The time for conducting the research was in December 2021. The samples in this research were *Culex vishnui* larvae instar III (6-7 days old). This research used 20 larvae/100 ml.

This research was carried out by preparing *E. indica* leaf extract and preparing third instar *Culex vishnui* larvae. Preliminary research was carried out to determine the core larvicide test. Preliminary research on *E. indica* leaves began with a dose of 1.4, 1.3, 1.2, 1.1, 1, 0.8, 0.6, 0.4, 0.2 gr/ 100 ml is reduced to reach the minimum dose of LD_{100}. Each was put into a plastic bowl containing 100 ml of distilled water. For control, a plastic bowl was filled with 100 ml distilled water. Enter 20 larvae in each treatment. Air temperature and water pH were measured and recorded during the experiment. Larval observations were carried out after 24 hours, and the number of dead was counted. The minimum dose that can kill all LD_{100} larvae was sought and used as a benchmark in the core test. After getting a minimum dose of LD_{100}, carry out core larvicide research. Various doses of *E. indica* leaf extract is provided, starting from the LD_{100} dose according to the results of preliminary investigation, the dose being reduced gradually until a dose is found that cannot kill the larvae. Put various doses of *E. indica* leaf extract into each plastic bowl and add water up to 100 ml. The control group contained 100 ml of water without giving *E. indica* leaf extract. Larval observations were carried out after 24 hours, and then the number of dead larvae was recorded so that data on the number of dead larvae in each bowl was obtained, and the experiment was repeated three times. Determine LD_{50} and LD_{90} after treatment using probit test analysis.

The data obtained in this study were analysed using comparative statistical tests for the means of numerical variables for more than 2 unpaired groups. Data analysis was done using the Shapiro-Wilks normality test [18] with abnormal data results. The study was continued with the Kruskal-Walli's test [19] with significance > 0.05 and CI 95%. Probit analysis was carried out to analyse LD_{50} and LD_{90}.

3. Results

3.1 Larvicidal Effect on *Aedes aegypti* Larvae: Data from preliminary test results of ethanol extract of *E. indica* leaves on *Aedes aegypti* larvae are shown in Table 1. It shows that 30% of the larval population died at 0.3995 g/L and 100% died at a concentration of 1.598g/L.

Table 1: Number and percentage of *Aedes aegypti* larvae that died at various concentrations of *E. indica* leaf ethanol extract in the preliminary test

			Repetition			
S. No	**Concentration (g/L)**	**Number of test larvae (*Aedes aegypti*)**	**1**	**2**	**Average**	**Mortality (%)**
1	0.0000	20	0	0	0	0
2	0.0999	20	0	0	0	0
3	0.1998	20	0	0	0	0
4	0.3995	20	8	6	7	35
5	0.7991	20	12	8	10	50
6	1.5982	20	20	20	20	100
7	3.1963	20	20	20	20	100
8	6.3927	20	20	20	20	100

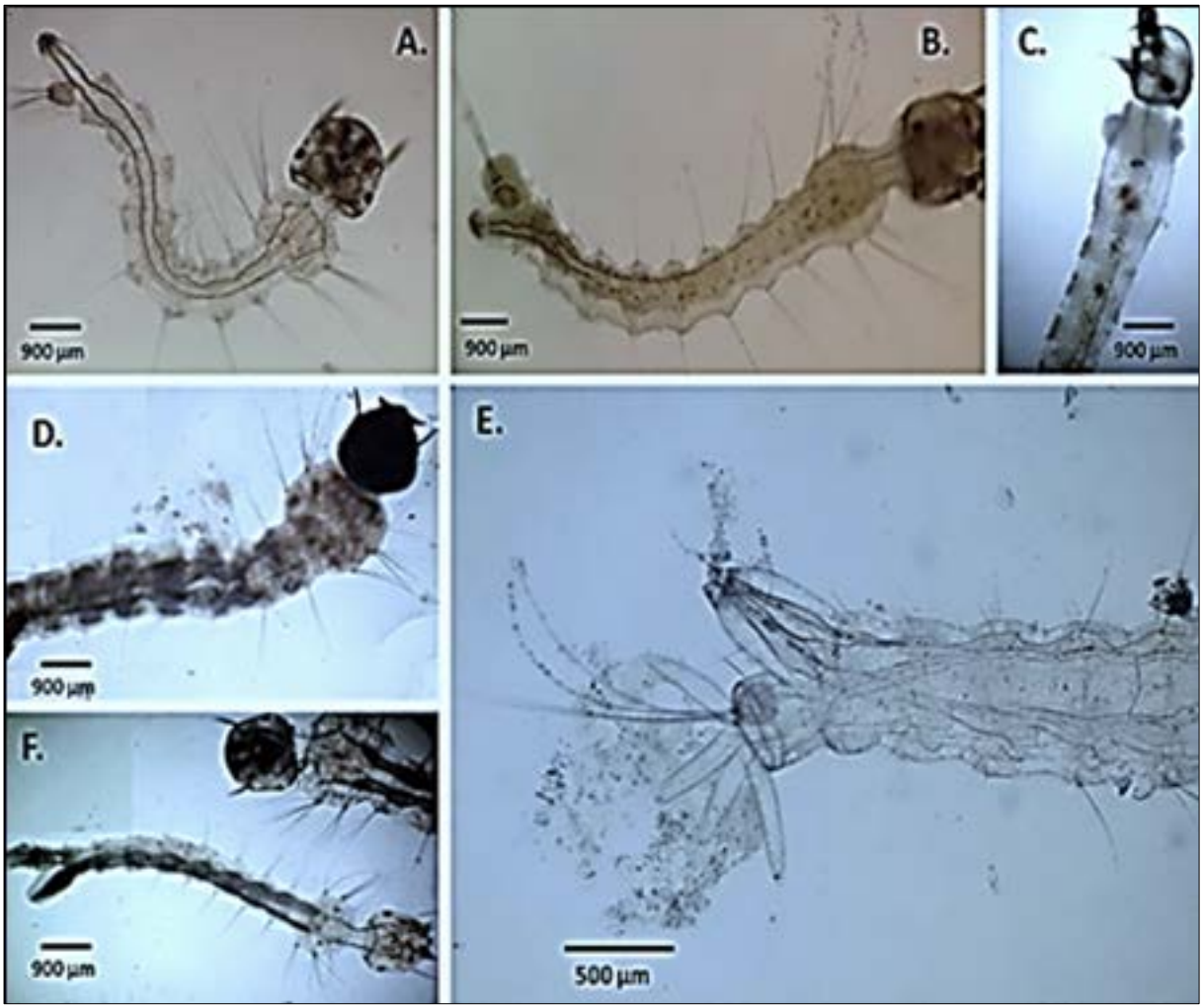
Data on the percentage of larval death at various concentrations of *E. indica* leaf ethanol extract were then analysed using Probit to obtain LC10 at a concentration of 320.92 g/L and LC_{90} at a concentration of 1282.276g/L. Data on the number and percentage of deaths of *Aedes aegypti* larvae in the final test are in table 2. It shows 100% death at a concentration of 1.198 g/L and 25% mortality at 0.2998g/L.

Data on larval mortality in the final test were analysed using Probit so that it was found that LC_{50} and LC_{90} were located at a concentration of 466.907 g/L and LC_{90} was located at a concentration of 868.42g/L.

Table 2: Number and percentage of *Aedes aegypti* larvae that died at various concentrations of *E. indica* leaf ethanol extract in the final test

		Repetition				
Concentration (g/L)	**Number of test larvae (*Aedes aegypti*)**	**1**	**2**	**3**	**Average**	**Mortality (%)**
0	30	0	0	0	0	0
0.2996	30	3	14	6	8	25.56
0.3995	30	8	21	18	16	52.22
0.5993	30	9	24	29	21	68.89
0.799	30	27	26	29	27	91.11
0.9988	30	30	27	24	27	90
1.1986	30	30	30	30	30	100
1.3984	30	30	30	30	30	100

3.1.1 Morphological changes caused in *A. aegypti* larvae by *E. indica* ethanolic extract: In this study, the control group, containing water and DMSO, presented active larvae with a defined vermiform appearance, quite characteristic of the larva. The head, thorax and abdomen regions were well represented, with lateral spicules, aculei, intact respiratory siphon, transparent body and visible segments (Figure 2: A). In the test groups in which the larvae were in contact with the dissolved leaf extract, it was possible to observe morphological changes such as the displacement of the head (Figure 2:B, F), darkening of the body (Figure 2:D), exit from the periotrophic matrix (MP) through the anus (Figure 2: E), darkening of the respiratory siphon (Figure 2: H), loss of needles or bristles (Figure 2: C, H), parts imposing on breathing. Some showed thickening or swelling in the larval body (Figure 2: G, E), and others led a thinner or stretched aspect of the larval body (Figure 2: F).



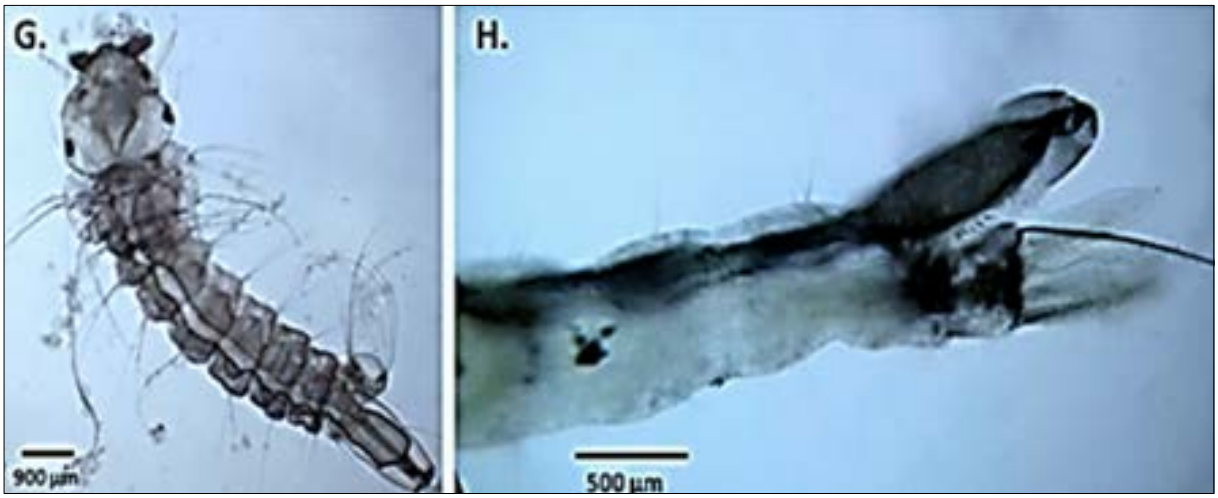


Fig 2: Morphology of *Aedes aegypti* larvae after 24 hours of treatments with different concentrations of *E. indica* leaf extract. A: Negative Control Larvae; B: Larva with poorly defined segmentation, internal spreading of the PM, thickening of the anus; C: Loss of segmentation, loss of bristles, deformity; D: deformity and darkening of the head; E: Larvae showing PM exit through the anus; F: Larvae showing elongation or stretching of the body; G: Larva showing swelling of the body; H: Respiratory siphon thickened and blackened

3.2 Larvicidal Effect of *E. indica* ethanolic Extract on *Culex vishnui* Larvae

3.2.1 Preliminary Test

This preliminary research at each repeated dose used 20 larvae, pH 7, temperature 250 C. A dose of 0.2 gr/100 ml is the minimum dose that can kill 100% of *Culex vishnui* larvae, whereas, from 0.2 gr/100 ml, the dose will be reduced gradually. Step by step to get LD_{50} and LD_{90} (Figure 3).

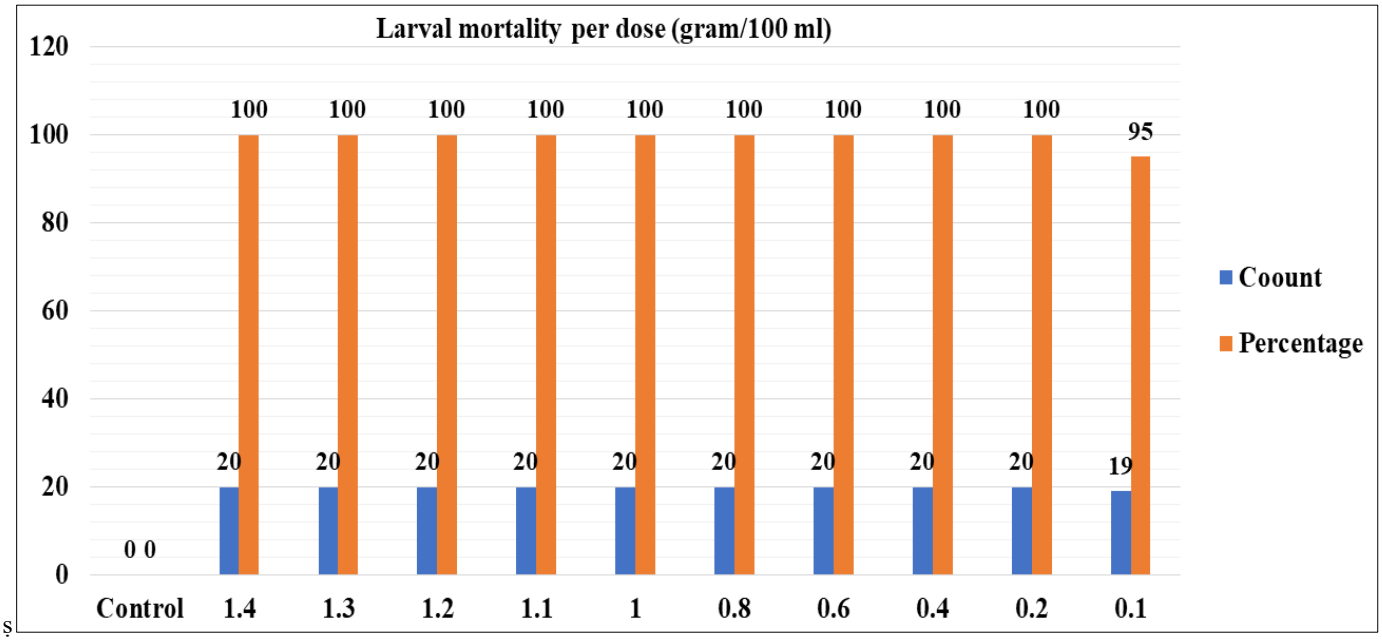


Fig 3: Preliminary test of *E. indica* leaf extract against *Culex vishnui* after 24 hours

3.2.2 Larvicide Test

This test uses 20 larvae/100ml, pH 7, temperature 250 C, using a dose of *E. indica* leaf *extract* of 0.2 g/; 0.1 g/100 ml; 0.09 g/100 ml; 0.08 g/100 ml; 0.07 g/100 ml; 0.06 g/100 ml; 0.05 g/100 ml; 0.05 g/100 ml; 0.04 g/100 ml; 0.03 g/100 ml; 0.02 g/100 ml, 0.01 g/100 ml. Table 3 shows that *E. indica* leaf extract at a dose of 0.2 g/10s0 ml can kill *Culex vishnui* larvae with an average number of larvae deaths of 20 and a percentage of larval deaths of 100%. Meanwhile, the control could not kill *Culex vishnui* larvae, with an average number of deaths of 0 individuals and a percentage of larval deaths of 0%.

Table 3: larvicide test against *Culex vishnui* after 24 hours

			Larval mortality per dose (gram/100ml)										
Test	**Control**	**0.01**	**0.02**	**0.03**	**0.04**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**0.1**	**0.2**
1	0	14	15	16	17	17	18	19	19	20	20	20
2	0	14	14	15	19	17	18	18	18	19	19	20
3	0	13	15	16	14	18	19	17	19	19	20	20

∑	0	41	44	47	50	52	55	54	56	58	59	60
X	0	13.7	14.7	15.7	16.7	17.3	18.3	18	18.7	19.3	19.7	20
s%	0	68.3	73.3	78.3	83.3	86.7	91.7	90	93.3	96.7	98.3	100

3.2.3 Normality test
The results of the normality test using Shapiro-Wilk show that not all groups have a p-value> 0.05 so the data distribution is abnormal. The analysis was continued with the Kruskall-Wallis test.

3.2.4 Kruskall-Wallis test
The results of the Kruskall-Wallis test obtained a value of p=0.001 (p<0.05), which can be interpreted as a difference in the average number of larval deaths in each treatment group (table 4).

Table 4: Kruskall-Wallis Test Results

E. indica **dosage (g/100 ml)**	**Median (min-max)**	**P**
Control	0 (0 – 0)	0.001
0.01	14 (13 – 14)	
0.02	15 (14 – 15)	
0.03	16 (15 – 16)	
0.04	17 (14 – 19)	
0.05	17 (17 – 18)	
0.06	18 (18 – 19)	
0.07	18 (17 – 19)	
0.08	19 (18 – 19)	
0.09	19 (19 – 20)	
0.10	20 (19 – 20)	
0.20	20 (20 – 20)	

Number repetitions: 3(N)

In the probit test (table 5), it is known that the dose of *E. indica* leaf extract, which can kill 50% of *Culex vishnui* larvae (LD_{50}), is 0.01 g/100 ml of distilled water, while the dose of *E. indica* leaf *extract* which can kill 90% of *Culex vishnui* larvae (LD_{90}) is 0.06 grams/ 100 ml distilled water.

Table 5: Probit Test Results

	CI 95%		
Probability	**Estimate**	**Lowest**	**Highest**
0.5	0.01	0.01	0.02
0.9	0.06	0.05	0.07

4. Discussion
Aedes aegypti larvae
Research data shows that ethanol extract of *E. indica* leaves has a larvicidal effect on *Aedes aegypti* larvae. Larval mortality increased at higher extract concentrations, whereas in the negative control, there was no larval mortality because it did not contain ethanol extract of *E. indica* leaves. In this study, LC_{50} and LC_{90} were obtained at concentrations of 466.907 g/L and 868.42 g/L, different from research conducted [20] that at a concentration of 0.9093 g/L, *E. indica* leaf extract had the effect of killing 50% of the *Aedes aegypti* larvae population in 24 hours. Differences in outcomes *E. indica* leaf larvicide can be influenced by the location where the *E. indica* plant grows.

The difference in the larvicidal effect of *E. indica* leaves can be influenced by the dosage form of the test material. In this study, researchers used 96% ethanol because ethanol is a polar solvent, so it extracts polar compounds such as alkaloids, flavonoids and chlorophyll but cannot extract nonpolar compounds such as secondary metabolites. The inability to extract nonpolar compounds makes the sample viscosity relatively moderate compared to the semipolar solvent ethyl acetate. This is different from the research conducted by Betriyon and Yahya- using *betel* leaf extract, which was dissolved using distilled water. Based on its polarity, water is a very polar solvent with low temperature. The optimal for extraction using water is 100 ºC. According to Yuliantari. (2017) [22], if the extraction temperature is more than 50 ºC, it will easily damage the polar compounds contained, resulting in lower extract obtained. Saponin enters through the larva's mouth to the digestive tract. The target organ for this compound is the peritrophic membrane in the middle intestine, which produces digestive enzymes. This compound is known to have the ability to damage cell membranes, but the exact mechanism has yet to be discovered. Experts believe that the binding of saponin to the cell membrane causes changes in the structure of the membrane so that the osmosis process takes place. This can change the surface tension of the cell as water enters the cell. The cells will burst so that the larvae fail to digest food as an energy source [23]. Safrole is one of the essential oils contained in plant leaves. The effect caused by this compound is a neurotoxic mechanism. Experts say that the death mechanism in insects can be caused by inhibiting acetylcholinesterase and positive allosteric modulation of GABA. Activation of GABA receptors by neurotransmitters causes Cl- channels to open and trigger hyperpolarisation, inhibiting action potentials. Activation of GABA receptors causes depression of the central nervous system through convulsions [24].

In this study, researchers did not use isolation techniques to separate the active compounds, so it cannot be ascertained which compounds have more potential as larvicides. It is possible that the active compound components work synergistically to cause the death of the larvae.

Culex vishnui larvae
In this study, third and fourth instar larvae of *Aedes aegypti* were used because, at this stage, the mosquito larvae are already large in body size, have a large tolerance for the toxic power of the extract given, and do not die easily if given

mechanical action so that the results obtained later will not be false positive mechanics so that the results obtained later are not false positives.

In this study, preliminary tests were carried out, and it was found that at the lowest dose, namely 0.2 grams, larval mortality reached 100%. For the control group, there was no death of *Culex vishnui* larvae; this proves that the death of *Culex vishnui* larvae in the treatment group was caused by *E. indica* leaf extract, not by distilled water or disturbing variables. After that, a core test was carried out with repetition 3 times and the results were that *E. indica* leaf extract at the lowest dose, namely 0.2 grams, could kill all *Culex vishnui* larvae with a percentage of 100%.

This is different from previous research conducted by Reuben (1994) [25] regarding the larvicidal power of using neem leaf extract on third-instar *Culex vishnui* larvae, which proved that a dose of 0.04 grams was the minimum dose that could kill the larvae and 1.1 gram is the minimum dose that can kill all larvae, 3. In contrast, in this study it is known that the dose that can kill all larvae is 0.2 gram. From the results of this research, it is known that *E. indica* leaf extract with a lower concentration kills third-instar *Culex vishnui* larvae than neem leaf extract.

The differences in the results of this study may be caused by various factors, namely the type of larvae such as *Aedes aegypti* larvae, *Cule*x larvae, *psorophora* larvae; types of larvicide preparations such as granules, extracts, solutions; and the type of plant such as garlic, sour sop, sugar apple used for treatment, these three things are factors that can influence the strength or larvicidal power [26].

5. Conclusion

The present study reported the larvicidal activity of *E. indica* leaves extract, which was active from the third and the fourth *Aedes aegypti* larval stage, demonstrating that the higher the larval stage, the higher the concentrations necessary for the lethality of the larvae. Based on the results of this research, it can be concluded that the LC_{50} of ethanol extract of *E. indica* leaves against *Aedes aegypti* larvae is 466.907 g/L and the LC_{90} of ethanol extract of leaves of *Aedes aegypti* larvae population is 868.42 g/L. The dose of *E. indica* leaf extract, which can function as a larvicide in *Culex vishnui* larvae, LD_{90}, is 0.06 g/100 ml of distilled water, while the LD_{50} is 0.01 grams/ 100 ml of distilled water.

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7. Conflict of Interest

The authors declare no conflicts of interest with respect to this work.

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