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Effectiveness of Biolarvicides of *Imperata cylindrica*, *Saccharum spotaneum* and *Andropogon aciculatus* on *Aedes aegypti* larval Mortality and Egg-laying Ability in Adults

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ABSTRACT

Vector-borne disease such as Dengue Hemorrghagic (DHF), transmitted by *Aedes aegypti* and *Aedes albopictus*, remain a significant public health concern in Indonesia. Controlling these disease often involves insectides; however, the negative impact of chemical insecticides have prompted interest in organic alternatives derived from plants. Certain weeds, including cogon grass (*Imperata cylindrica*), wild sugarcane (*Saccharum spontaneoum*), and needle grass (*Andropogon aciculatus*), have shown potential as botanical insecticides. Research findings showed that weed root extracts significantly affect larval mortality rate of *Ae. Aegypti*. At 1000 ppm, larval mortality was significantly higher compared to 100 ppm and the control, while treatments of 1 ppm and 10 ppm showed similar results to the control. Probit analysis revealed that *I. cylindrica* root extract achieved an LC50 of 974.99 ppm within 24 hours, indicating it could kill 50% of *Ae. Aegypti* larvae. Within 48 hours, the LC50 dropped to 889.20 ppm. Toxicity tests further revealed significant differences in *Ae. Aegypti* egg-laying abilities when treated with extracts. Analysis of variance yielded p-values of 0.000 for egg hatching within 72 and 96 hours, highlighting significant differences across samples. These findings suggest the extracts influence mosquito reproduction, warranting further studies to assess the quality of egg hatched from larvae exposed to these treatments. The potential of botanical insecticides derived from weeds represents a promising step toward sustainable mosquito control in the fight against vector-borne diseases.

1. Introduction

Dengue, a mosquito-borne virus, is the leading cause of arthropod-borne viral disease globally, posing a significant health concern. Since early 2023, continuous transmission and an unexpected surge in dengue cases have led to nearly record levels, with over five million cases and more than 5,000 dengue-related deaths reported in over 80 countries and territories across five WHO regions: Africa, the Americas, Southeast Asia, the Western Pacific, and the Eastern Mediterranean (World Health Organization 2023).

DHF is a disease transmitted by mosquitoes as a vector. The two invasive mosquito species in Indonesia are *Aedes aegypti* and *Ae. Albopictus* is not just a significant nuisance to humans but also the main carrier of various foreign pathogens, including the viruses responsible for dengue, zika, and chikungunya. It's crucial to control these diseases effectively. Are a significant nuisance to humans and serve as the main carriers of various foreign pathogens, including the viruses responsible for dengue, Zika, and chikungunya.

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(Rhida *et al*. 2023). Dengue Hemorrhagic Fever (DHF) has been increasing and spreading throughout Indonesia since its discovery in 1968 (Giroth *et al*. 2019).

Mosquito control is a worldwide public health practice worldwide in tropical and sub-tropical areas (Listiono *et al*. 2023: Duval *et al*. 2023). Mosquitoes are the most dangerous disease vector belonging to the arthropod family. They are significant for public health because of their blood-feeding habit (Siam *et al*. 2022; Nebbak *et al*. 2022; Attaullah *et al*. 2023). Mosquitoes exhibit high species diversity, with approximately 3,490 officially recorded (Kirik *et al*. 2021). Despite ongoing control efforts, the mosquito population and transmitted diseases rapidly expand globally, posing significant health problems (Lim *et al*. 2023). Disease management strategies to reduce the burden of mosquito-borne diseases depend on vector or mosquito control (Ramayanti *et al*. 2023). The unregulated and excessive use of insecticides and pesticides to control pests has led to mosquito resistance to these larvicides (Raul *et al*. 2021).

Breaking the vector transmission chain can be achieved during the larval phase. One way to control DHF during the larval stage is using chemicals known as insecticides. However, the use of chemical insecticides has several side effects. The long-term use of synthetic insecticides has made mosquitoes resistant, necessitating new vector control methods. The synthetic chemical insecticides currently in use have some disadvantages. Factors such as vector resistance and toxicity to humans and non-target organisms increase the interest in exploring new control alternatives (Pavela 2016; Benelli 2018).

There has been an increase in the use of plants as an alternative to synthetic insecticides due to their broad insecticidal properties, biodegradability, and adaptability to ecological conditions (Demirak and Canpolak 2022; Lim *et al*. 2023). One effort to mitigate these negative impacts is the utilization of organic insecticides derived from natural compounds found in plants (Giroth *et al*. 2019; Pereira *et al*. 2024). Several studies regarding the potential of plant extract in controlling mosquitoes, including the fern Actiniopteris radiata, were tested with novel mosquitocidal activity against larvae of *Ae. aegypti* and *Anopheles stephensi* (Kamaraj *et al*. 2018). Seed oil extract of *Acacia nilotica* possessed robust larvacidal action against major mosquito vectors (Vivekanandhan *et al*. 2018). Plant essential oils were also reported to have mosquitocidal potential. The crude volatile oil (CVO) from Piper betle leaves possessed significant larvacidal, ovipositional and repellency effects against *Ae. aegypti* (Vasantha-Srinivasan *et al*. 2018; Vivekanandhan *et al*. 2018; Chellappandian *et al*. 2019).

Weed plants contain various active substances, including tannins, phenolics, terpenoids, alkaloids, saponins, and flavonoids. These phytochemical compounds can trigger various reactions in the mosquito larvae, disrupting their growth and development and ultimately leading to larval death. Flavonoids function as potent respiratory inhibitors, causing larval death; saponin compounds can corrode the mucous membrane of the larval digestive tract; tannin compounds act as stomach poisons for mosquito larvae, disrupting their nutrition; and steroid compounds can inhibit the molting process in larvae, causing them to become unconscious (Fitriyani *et al*. 2023). The presence of these bioactive compounds suggests that cogon grass (*Imperata cylindrica*), wild sugarcane (*Saccharum spontaneum*), and needle grass (*Andropogon aciculatus*) can be used as natural larvicides.

Imperata cylindrica is among the ten worst weeds in tropical and subtropical regions. Despite having many benefits, the issues related to its invasive nature outweigh the positive aspects (Rusdy *et al*. 2020). *Imperata cylindrica* contains organic phenolic compounds that are toxic to *Aedes aegypti* mosquito larvae, but the concentration used in experiments is not high enough to kill the larvae. *Imperata cylindrica* can paralyze larvae within the first twenty-four hours, with larval movement observed a few hours later (Calago *et al*. 2015). Furthermore, chemical component analysis of wild sugarcane reveals that the resulting bio-oil is dominated by acetic acid, phenol, and 1-hydroxy-2-propanone (Wibowo *et al*. 2015).

The objective of this research was to determine the effectiveness of bio larvicides derived from weed plants, specifically cogon grass (*I. cylindrica*), wild sugarcane (*S. spontaneum*), and needle grass (*A. aciculatus*) on the mortality of *Ae. aegypti* mosquito larvae.

2. Materials and Methods

This study was pure experimental research with a post-test-only control group design. The purpose of this study was to investigate the effectiveness of secondary

metabolites from weed plants, namely cogon grass (*I. cylindrica*), wild sugarcane (*S. spontaneum*), and needle grass (*A. aciculatus*) on the mortality of *Aedes aegypti* mosquito larvae. The research was conducted at the Health Research and Development Center, Litbangkes Entomology Laboratory Baturaja, South Sumatra, from January to August 2023.

Preparation of larvae. Two thousand mosquito eggs of *Ae. aegypti* were obtained from the Health Research and Development Center in Baturaja, South Sumatra, and colonized until they became larvae. After the larvae hatched and reached the third instar, 500 third-instar larvae were taken to be treated with weed extract.

Preparation of weed extract. Weeds of *I. cylindrica*l, *S. spontaneum*, and *A. aciculatus* collected from agricultural and plantation areas were cut into small pieces and dried in the sun. Once completely dry, the weed plants were ground into a fine powder using a blender and then extracted using the maceration method with 96% this was ethanol, followed by evaporation using a rotary evaporator. The weed extract obtained is then diluted according to the treatment, namely one ppm, ten ppm, 100 ppm, and 1000 ppm. The weed extraction method is carried out separately between the three weeds. This concentration is based on the Guidelines for Laboratory and Field Testing of Mosquito Larvicides (WHO/CDS/WHOPES/GCDPP/2005).

Aplication of weed extract. This study involved five treatment groups, including one control and four experimental groups, with four repetitions based on the Federer formula.

The negative control group was treated with water without the addition of weed extract. In contrast, the experimental groups were exposed to four different concentrations of weed extract, one ppm, ten ppm, 100 ppm, and 1000 ppm. Six different plant parts were used as samples, including leaves and roots of cogon grass (*I. cylindrica*), wild sugarcane (*S. spontaneum*), and needle grass (*A. aciculatus*).

They are testing the effectiveness of weed extract on *Ae. aegypti* mosquito larvae was carried out using a 100 ml plastic cup, where the extract solution was dropped into the plastic cup according to the respective concentration. One plastic cup contains 25 larvae x 5 concentrations and four repetitions = 500 intra three larvae, which have been selected, and healthy larvae are taken after the larvae are put into the plastic cup. Next, the number of dead larvae was counted during the 24-hour and 48-hour test periods.

To test the *Ae. aegypti* mosquito's ability based on the number of hatched eggs, third-instar larvae that had been exposed to the extract treatment and survived were bred to become adult mosquitoes. The research was done by transferring them into mosquito cages. After the mosquitoes had matured in each sample type, specifically for the 1000 ppm treatment, two healthy male and female mosquitoes were taken and bred in one mosquito cage for every kind of sample. They received a blood meal from a shaved-back male guinea pig during breeding. After the mosquitoes had mated, fed on blood, and laid eggs, the number of eggs was counted from 72 hours to 96 hours.

One-way ANOVA or Kruskal-Wellis analysis was used to analyze the data obtained in the research. If the One-Way ANOVA or Kruskal-Welis test results were significant, a post-hoc analysis would be conducted to determine which groups were significant. For One-Way ANOVA, the post-hoc analysis used was Bonferroni, and for the Kruskal-Wallis test was Mann-Whitney (Hastono 2018). The LC50 was determined by using the probit test (Ekawati *et al*. 2017).

Analysis of secondary metabolite content from the three types of weeds was carried out using GS-MS. Weed extracts of *I. cylindrica*, *S. spontaneum*, and *A. aciculatus* using the maceration or soaking method. Then, it was put in a micro tube containing 0.5 g of powder and 1.5 ml of ethanol solvent, vortexed for 1 minute, and centrifuged for 3 minutes at a speed of 90 rpm. The supernatant formed was continued for GC-MS testing. The time was 60 minutes with an injector temperature of 260°C, detector 250°C, and column 325°C. The carrier gas used is a helium gas carrier with a constant flow rate of 1 ml per minute. The identification process using the GC-MS tool produces several bioactive which can be seen from the peaks of the chromatogram as identification data from chromatography and mass spectrometry (MS) results seen from the mass spectrum with each molecular weight of the bioactive compound.

3. Results

3.1. Effect of Extract on The Death of *Ae. aegypti* Larvae

The average larval mortality in the thatch root treatment significantly differs in each concentration. The results show that that of 1000 ppm significantly differs from the concentration of 100 ppm and the control. However, the one ppm and ten ppm treatments

were not significantly different from the control (Table 1).

Based on the results of this research, it is clear that the *I. cylindrica*l root extract has quite a practical effect on killing *Ae. Aegypti*. The leaf extract of *I. cylindrica*, *S. spontaneum*, and *A. aciculatus* was compared with the roots extract of *S. spontaneum* and *A. aciculatus*.

Based on the probit analysis in Table 1, it can be seen that extracts from *I. cylindrica* leaves, *S. Spontaneum*, and *A. aciculatus* were not toxic to *Ae. Aegypti* larvae within 24 hours, as well as root extracts of *S. spontaneum* and *A. aciculatus* were not toxic to *Ae aegypti* within 24 hours after application. In contrast, *I. cylindrica* root extract was toxic to *Ae. aegypti* larvae within 24 hours and were able to kill 50% of the *Ae. aegypti* larvae are equivalent to 50 out of 100 larvae.

The average larval mortality after 48 hours of applying the extract significantly differs at each concentration. The results show that the concentration of 1000 ppm significantly differs from that of 100 ppm and the control. However, the one-ppm and ten-ppm treatments were not significantly different from the control (Table 2).

Based on the probit analysis, *A. aciculatus* leaf extract and *S. spontaneum* leaf extract were non-toxic to *Ae aegypti* larvae at 48 hours. In contrast, *I. cylindrica*l roots extract was toxic to *Ae. aegypti* larvae within 48 hours and could kill 50% of *Ae. aegypti* larvae., which is equivalent to 50 larvae out of 100. For *S. spontaneum* roots, the result showed LC 50>1000 ppm (141579.4), and for *A. aciculatus* roots, the result indicated LC 50>1000 ppm (141579.4), suggesting that both root extracts were non-toxic to *Ae. aegypti* larvae at 48 hours. Symptoms of the effect of the three grass extracts on *Ae aegypti* larvae were not different from each other (Figure 1)

Table 1. Effect of extract on the death of *Ae. aegypti* larvae in the 24 hour after aplication

Concentration	Differences in average larval mortality					
	Leaf *I. cylindrica*	Leaf *S. spontaneum*	Leaf *A. aciculatus*	Root *I. cylindrica*	Root *S. spontaneum*	Root *A. aciculatus*
Control	0.00	0.00	0.00	0.00c	0.00	0.00
1 ppm	0.00	0.00	0.00	0.00c	0.00	0.00
10 ppm	0.00	0.00	0.00	0.00c	0.00	0.00
100 ppm	0.25	0.00	0.00	2.00b	0.00	0.00
1000 ppm	6.50	1.25	0.50	13.25a	0.75	1.25
F Value	1.26ns	2.09ns	3.00ns	63.72*	2.65ns	2.88ns
P Value	0.33	0.13	0.06	3.12 × 10-9	0.07	0.06
BNJ 5%	-	-	-	0.71	-	-
LC 50	3681.29 ppm	141579.4 ppm	141579.4 ppm	974,989 ppm	141579.4 ppm	141579.4 ppm

* significantly different; values in columns followed by the same letter are not significantly different at P < 0.05 according to the BNJ test. Original data were transformed using square root transformation

Table 2. Effect of extract on the death of *Ae. aegypti* larvae in the 48 hour after aplication

Concentration	Differences in average larval mortality					
	Leaf *I. cylindrica*	Leaf *S. spontaneum*	Leaf *A. aciculatus*	Root *I. cylindrica*	Root *S. spontaneum*	Root *A. aciculatus*
Control	0.00	0.00b	0.00	0.00c	0.00	0.00
1 ppm	0.00	0.00b	0.00	0.00c	0.00	0.00
10 ppm	0.00	0.00b	0.00	0.00c	0.00	0.00
100 ppm	0.25	0.00b	0.00	2.50b	0.00	0.00
1000 ppm	7.50	1.50a	0.75	15.00a	0.75	1.25
F Value	2.68ns	4.34*	2.65ns	129.20*	2.65ns	2.88ns
P Value	0.07	0.01	0.07	1.97 × 10-11	0.07	0.06
BNJ 5%	-	0.57	-	0.54	-	-
LC 50	3169.567 ppm	141579.4 ppm	141579.4 ppm	889,201 ppm	141579.4ppm	141579.4 ppm

* significantly different; values in columns followed by the same letter are not significantly different at P < 0.05 according to the BNJ test. Original data were transformed using square root transformation

3.2. Identification of Molecular Formulas using The Gas Chromatography-Mass Spectrometry (GC-MS) Method

The result of the analysis of secondary metabolite compounds present in the three types of grass used can be seen in Table 3.

Table 3 shows that the chemical compounds in weed leaves that are thought to be used as bioinsecticides are campesterol, sitosterol, -Phenylalanyl-glycine, and sigmasterol. In *I. cylindrica* leaves, campesterol appeared at 21.64 minutes with an area size of 1.22%, 21.79 minutes with an area size of 2.87%, 21.95 minutes with an area size of 1.93% and sitosterol appeared at 26.40 minutes with an area size of 28.18%. In *S. spontaneum* leaves, there is 1-Phenylalanyl-glycine appearing at 9.98 minutes with an area size of 0.85%, and in *A. aciculatus* leaves, there is

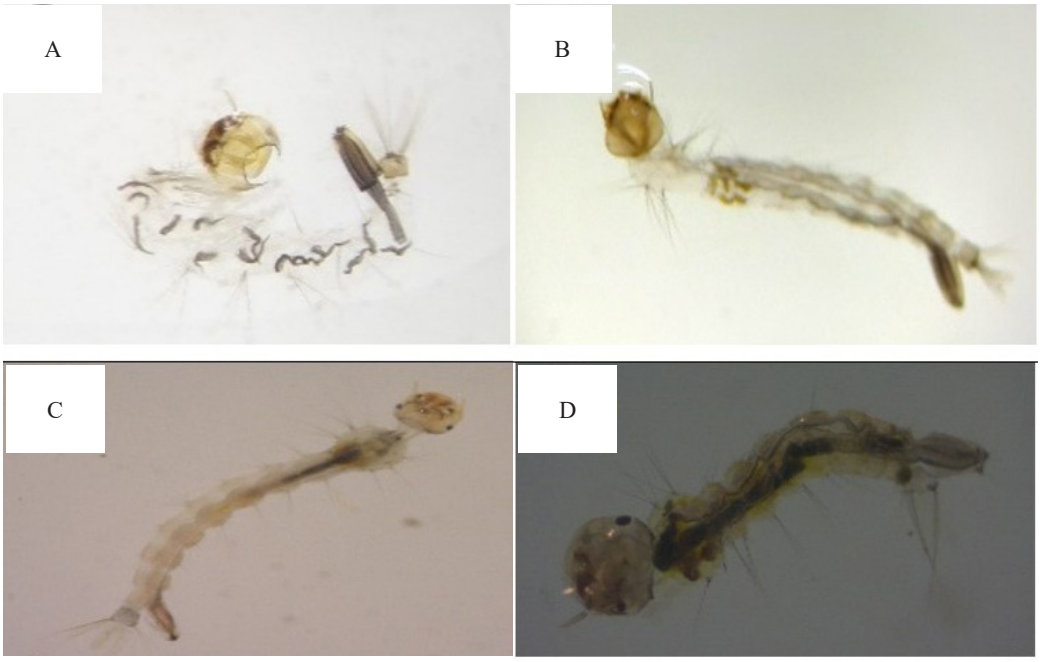
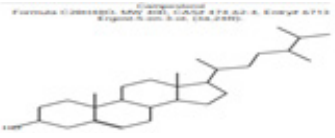
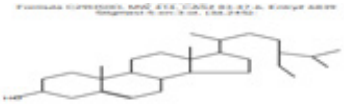
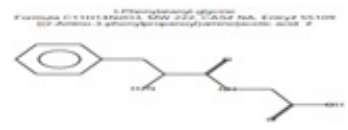
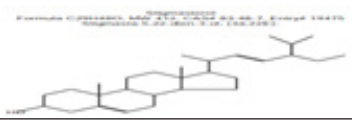


Figure 1. Effect of extracts of *I. cylindrica*, *S. spontaneum*, *A. aciculatus* on *Ae. Aegypti* larvae. (A) Larvae from the extract test whose body shape is incomplete. (B) Larvae from the extract test whose body parts are broken down. (C) Larvae as a result of the extract test whose insides are broken down. (D) Healthy larvae

Table 3. GCMS test results on *I. cylindrica*, *S. spontaneum*, *A. aciculatus* leaves

Weed leaves	Compound name	Chemical compound formulasi	Information
Imperata cylindrical	Campestrol		Campesterol appeared at 21.64 minutes with an area size of 1.22%, 21.79 minutes with an area size of 2.87%, 21.95 minutes with an area size of 1.93%
	Sitosterol		Sitosterol appeared at 26.40 minutes with an area size of 28.18%
Saccarum spontaneum	1-Phenylalanyl-glycine		1-Phenylalanyl-glycine appears at 9.98 minutes with an area of 0.85%
Andropogon aciculatus	Stigmasterol		Stigmasterol appeared at 23.86 minutes with an area size of 2.44%

Stigmasterol appearing at 23.86 minutes with an area size of 2.44%.

It can be seen in Table 4 that chemical compounds in weed roots that are thought to be able to be used as bioinsecticides are Phenol,2,4-bis, Phenol,2,6-bis, Phenol,3,5-bis, and Sitosterol. In the roots of *I. cylindrica* Phenol, 2,4-bis, appears at 13.74 minutes with an area size of 0.58%. In the roots of *S. spontaneum*, there is Phenol, 2,6-bis, appearing at 13.74 minutes with an area size of 0.58% and Sitosterol appearing at 26.40 minutes with an area size of 1.56% as well as in the roots of *A. aciculatus* There is Phenol,3,5-bis appearing at 13.74 minutes with an area size of 1.28%.

Effect of weed extract toxicity test on mosquito larvae in their ability to lay eggs.

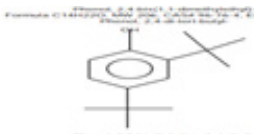
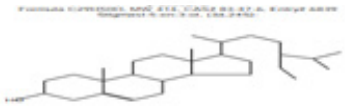
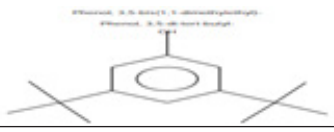
The results of observations of the effect of plant extracts on the egg-laying ability of *Ae aegypti* showed that the longer the application period, the more eggs were hatched. The number of eggs that hatched at a concentration of 1000 ppm in 72 hours showed the average number of eggs that hatched: 75 *I. cylindrica* leaves, 77.6 *S. spontaneum* leaves, 80.3 *A. aciculatus* leaves. *I. cylindrica* roots 9.6, *S. spontaneum* roots 27.3, and *A. aciculatus* roots 13.3. For 96 hours, the average number of eggs hatched was as follows: *I. cylindrica* leaves 76.33, *S. spontaneum* leaves 90, *A. aciculatus* leaves 83. *I. cylindrica* roots 14, *S. spontaneum* roots 19.3, *A. aciculatus* roots 17.6 (Figure 2).

Analysis of the effect of weed extract on *Ae aegypti* mosquito larvae at 72 hours and 96 showed a very significant effect on the egg-laying ability of adult mosquitoes-the results of further tests on the effect of weed extract on the ability to lay of *Ae. Aegytpti* egg can be seen in Table 5 and 6.

Table 5 shows significant differences in the number of eggs produced by mosquitoes subjected to various treatments. Notably, differences were observed between mosquitoes treated with *I. cylindrica* leaf extract and those treated with *I. cylindrica* root extract ($p = 0.009$), *I. cylindrica* leaf extract and *A. aciculatus* root extract ($p = 0.015$), and *I. cylindrica* leaf extract and *S. spontaneum* root extract ($p = 0.014$). Additionally, there were differences between mosquitoes treated with *S. spontaneum* leaf extract and *I. cylindrica* root extract ($p = 0.007$), *S. spontaneum* leaf extract and *S. spontaneum* root extract ($p = 0.011$), *S. spontaneum* leaf extract and *A. aciculatus* root extract ($p = 0.010$), *A. aciculatus* leaf extract and *I. cylindrica* root extract ($p = 0.005$), *A. aciculatus* leaf extract and *S. spontaneum* root extract ($p = 0.008$), and *A. aciculatus* leaf extract and *A. aciculatus* root extract ($p = 0.007$).

Table 6 presents Bonferroni's post-hoc test result, revealing significant differences in the number of eggs mosquitoes produce under various treatments. There were notable differences between mosquitoes treated with *I. cylindrica* leaf extract and those treated with *I. cylindrica* root extract ($p = 0.001$), *I. cylindrica* leaf extract and *A. aciculatus* root extract ($p = 0.003$), *I. cylindrica* leaf extract and *S. spontaneum* root extract ($p = 0.002$), *S. spontaneum* leaf extract and *I. cylindrica* root extract ($p = 0.000$), *S. spontaneum* leaf extract and *S. spontaneum* root extract ($p = 0.000$),

Table 4. GCMS test results on roots of *Imperata cylindrica*, *Saccarum spontaneum*, *Andropogon aciculatus*

Weed leaves	Compound name	Chemical compound formulasi	Information
Imperata cylindrical	Phenol,2,4-bis		Phenol,2,4-bis, appears at 13.74 minutes with an area size of 0.58%
Saccarum spontaneum	Phenol,2,6-bis		Phenol,2,6-bis, appears at 13.74 minutes with an area size of 1.17%
	Sitosterol		Sitosterol appears at 26.40 minutes with an area size of 1.56%
Andropogon aciculatus	Phenol,3,5-bis		Phenol,3,5- appears at minute 13.74 with an area size of 1.28%

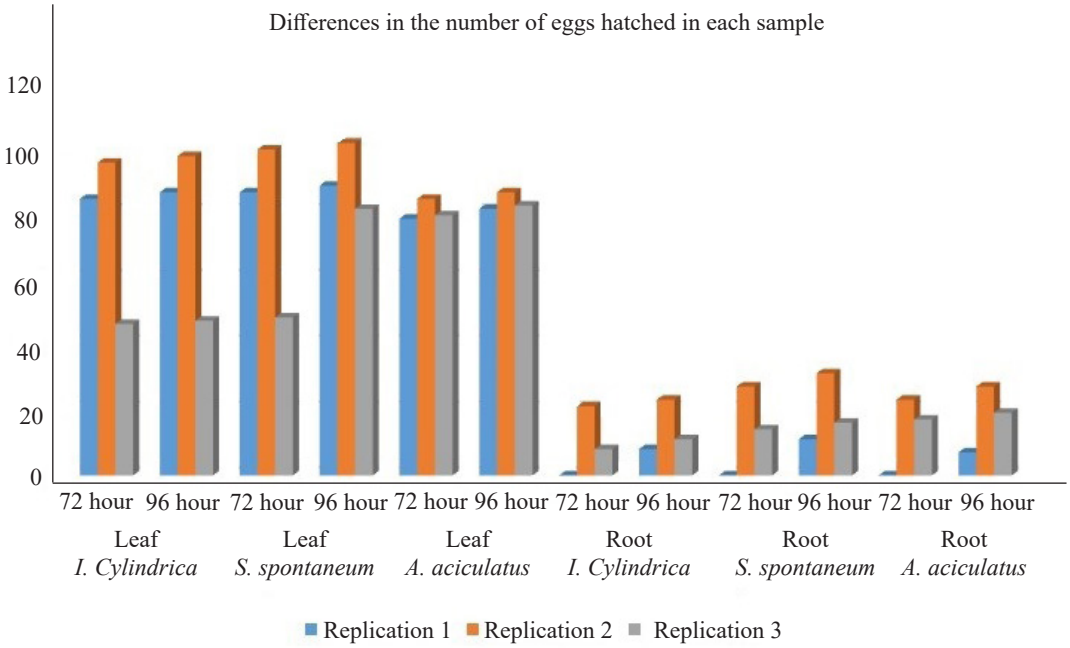


Figure 2. Effect of weed extract on *Ae aegypti* eggs hatching

Table 5 . Effect of weed extract on *Ae. aegypti* larvae and the ability of their adult mosquitoes to lay eggs at 72 hours after application

(I) Jenis sample	(J) Jenis sample	Mean difference (I-J)	Std. error	Sig.	95 % Confidence interval	
					lower bound	upper bound
Leaf	Leaf *S. spontaneum*	-2,667	14,184	1.000	-54.42	49.09
I. cylindrica	Leaf *A. aciculatus*	-5,333	14,184	1.000	-57.09	46.42
	Root *I. cylindrica*	65,000*	14,184	0.009	13.58	117.09
	Root *S. spontaneum*	61,000*	14,184	0.015	9.58	113.09
	Root *A. aciculatus*	61,667*	14,184	0.014	9.91	113.42
Leaf	Leaf *I. cylindrica*	2,667	14,184	1.000	-49.09	54.42
S. spontaneum	Leaf *A. aciculatus*	-2,667	14,184	1.000	-54.42	49.09
	Root *I. cylindrica*	68,000*	14,184	0.007	16.24	119.76
	Root *S. spontaneum*	64,000*	14,184	0.011	12.24	115.76
	Root *A. aciculatus*	64,333*	14,184	0.010	12.58	116.09
Leaf	Leaf *I. cylindrica*	5,333	14,184	1.000	-46.42	57.09
A. aciculatus	Leaf *S. spontaneum*	2,667	14,184	1.000	-49.09	54.42
	Root *I. cylindrica*	70,667*	14,184	0.005	18.91	122.42
	Root *S. spontaneum*	66,667*	14,184	0.008	14.91	118.42
	Root *A. aciculatus*	67,000*	14,184	0.007	15.24	118.76
Root	Leaf *I. cylindrica*	-65,333*	14,184	0.009	-113.09	-9.58
I. cylindrica	Leaf *S. spontaneum*	-68,000*	14,184	0.007	-115.76	-12.24
	Leaf *A. aciculatus*	-70,667*	14,184	0.005	-118.42	-14.91
	Root *S. spontaneum*	-4,000	14,184	1.000	-47.76	55.76
	Root *A. Aciculatus*	-3,667	14,184	1.000	-51.42	52.09
Root	Leaf *I. cylindrica*	-61,333*	14,184	0.015	-113.09	-9.58
S. spontaneum	Leaf *S. spontaneum*	-64,000*	14,184	0.11	-115.76	-12.24
	Leaf *A. aciculatus*	-66,667*	14,184	0.008	-118.42	-14.91
	Root *I. cylindrica*	4,000	14,184	1.000	-47.76	55.76
	Root *A. aciculatus*	0,333	14,184	1.000	-51.42	52.09
Root	Leaf *I. cylindrica*	-61,667*	14,184	0.014	-113.42	-9.91
A. aciculatus	Leaf *S. spontaneum*	-64,333*	14,184	0.010	-116.09	-12.58
	Leaf *A. aciculatus*	-67,000*	14,184	0.007	-118.76	-15.24
	Root *I. cylindrica*	3,667	14,184	1.000	-48.09	55.42
	Root *S. spontaneum*	-0,333	14,184	1.000	-52.09	51.42

Table 6. Effect of weed extract on *Ae. aegypti* larvae and the ability of their adult mosquitoes to lay eggs at 72 hours after application

(I) Jenis sample	(J) Jenis sample	Mean difference (I-J)	Std. error	Sig.	95 % Confidence interval	
					lower bound	upper bound
Leaf	Leaf *S. spontaneum*	-13,333	10,919	1.000	-53.18	26.51
I. cylindrica	Leaf *A. aciculatus*	-6,333	10,919	1.000	-46.18	33.51
	Root *I. cylindrica*	60,000*	10,919	0.001	22.82	102.51
	Root *S. spontaneum*	57,333*	10,919	0.003	17.49	97.18
	Root *A. aciculatus*	59,000*	10,919	0.002	19.16	98.84
Leaf	Leaf *I. cylindrica*	13,333	10,919	1.000	-26.51	53.18
S. spontaneum	Leaf *A. aciculatus*	7,000	10,919	1.000	-32.84	46.84
	Root *I. cylindrica*	76,000*	10,919	0.000	36.16	115.84
	Root *S. spontaneum*	70,667*	10,919	0.000	30.82	110.51
	Root *A. Aciculatus*	72,333*	10,919	0.000	32.49	112.18
Leaf	Leaf *I. cylindrica*	6,333	10,919	1.000	-33.51	46.18
A. aciculatus	Leaf *S. spontaneum*	-7,000	10,919	1.000	-46.84	32.84
	Root *I. cylindrica*	69,000*	10,919	0.001	29.16	108.84
	Root *S. spontaneum*	63,667*	10,919	0.001	23.52	103.51
	Root *A. aciculatus*	65,333*	10,919	0.001	25.49	105.18
Root	Leaf *I. cylindrica*	-62,667*	10,919	0.001	-102.51	-22.82
I. cylindrica	Leaf *S. spontaneum*	-76,000*	10,919	0.000	-115.84	-36.16
	Leaf *A. aciculatus*	-69,000*	10,919	0.001	-108.84	-29.16
	Root *S. spontaneum*	-5,333	10,919	1.000	-45.18	34.51
	Root *A. aciculatus*	-3,667	10,919	1.000	-43.51	36.18
Root	Leaf *I. cylindrica*	-57,333*	10,919	0.003	-97.18	-17.49
S. spontaneum	Leaf *S. spontaneum*	-70,667*	10,919	0.000	-110.51	-30.82
	Leaf *A. aciculatus*	-63,667*	10,919	0.001	-103.51	-23.82
	Root *I. cylindrica*	5,333	10,919	1.000	-34.51	45.18
	Root *A. aciculatus*	1,667	10,919	1.000	-38.18	41.51
Root	Leaf *I. cylindrica*	-59,000*	10,919	0.002	-98.84	-19.16
A. aciculatus	Leaf *S. spontaneum*	-72,333*	10,919	0.000	-112.18	-32.49
	Leaf *A. aciculatus*	-65,333*	10,919	0.001	-105.18	-25.49
	Root *I. cylindrica*	3,667	10,919	1.000	-36.18	43.51
	Root *S. spontaneum*	-1,667	10,919	1.000	-41.51	38.18

S. spontaneum leaf extract and *A. aciculatus* root extract (p = 0.000), *A. aciculatus* leaf extract and *A. aciculatus* root extract (p = 0.001), *A. aciculatus* leaf extract and *S. spontaneum* root extract (p = 0.001), and *A. aciculatus* leaf extract and *A. aciculatus* root extract (p = 0.001).

4. Discussion

4.1. Effect of Extract on The Death of *Ae. aegypti* Larvae

The average larval mortality in the thatch root treatment significantly differs in each concentration. The results show that the concentration of 1000 ppm significantly differs from the concentration of 100 ppm and the control. However, the 1 ppm and 10 ppm treatments were not significantly different from the control. These results showed two sample types with significant differences in the average among the 5 concentration series, namely the *I. cylindrica* root and *S. spontaneum* root extracts. Furthermore, based on the probit analysis, for the *I. cylindrica* root extract, LC50 < 1000 ppm (974.989), indicating that *I. cylindrica* root extract was toxic to *Ae, aegypti* larvae within 24 hours. At a concentration of 974.989 ppm, the *I. cylindrica* root extract could kill 50% of *Ae. aegypti* larvae, which is equivalent to 50 eggs out of 100 larvae.

The potency of various concentrations of *I. cylindrica* root extract in killing *Aedes* sp. dengue mosquito larvae. There was an increase in the average mortality. The higher the concentration and treatment time applied, the higher the average mortality of *Aedes* sp. dengue mosquito larvae. (Antou *et al*. 2023). In Figure 1, it can be seen that an increase in concentration indicates a toxic compound that enters the body of the test larvae, resulting in disrupted larval life and causing mortality. When treated with concentrations of stolon extract from weeds, the death of the larvae caused a

change in activity, where when the larvae were about to die, there was movement up and down on the surface. The death of the larvae if the larvae do not move and do not respond, and float above the surface indicates that the larvae are dead.

Plants are rich in biologically active substances with the potential to control *Ae. aegypti* are considered attractive alternatives to traditional chemical insecticides (Muangmoon *et al*. 2018; Azevedo *et al*. 2019). The phytochemical analysis of the *I. cylindrica* root extract revealed the presence of flavonoid compounds, known for their phenolic content (Tungmunnithum *et al*. 2018). Flavonoids have been shown to act as potent respiratory inhibitors that can lead to larval mortality (Fitriyani *et al*. 2022). Several chemical factors can influence the extraction results, including internal factors like the type of active compounds in the material, qualitative composition of active compounds, quantitative composition of active compounds, and average total content of active compounds. External factors, on the other hand, include the extraction method, the type of extraction equipment, the characteristics of the material, and the solvents used in the extraction process (Zhang *et al*. 2023). Another factor is believed to affect the LC50 of the *I. cylindrica* root extract on *Ae. aegypti* larval mortality is the difference in flavonoid content between *I. cylindrica* root, *S. spontaneum* root, and *A. aciculatus* root. *I. cylindrica* root is thought to contain more flavonoids than wild sugarcane and *A. aciculatus* roots. *I. cylindrica* roots are obtained from terrestrial plantation areas, while *S. spontaneum* and *I. cylindrica* roots are collected from aquatic areas. Environmental factors, such as temperature stress (Utomo *et al*. 2020), can also affect the production of secondary metabolites. In this context, terrestrial plantation areas have higher temperatures than aquatic areas.

Based on the observations of *Ae. aegypti* mosquito larvae, signs of mortality were evident, with the larvae displaying disrupted body integrity and visible deterioration in their internal structure. The researchers continued this observation until the fifth day, indicating that most larvae had not yet transformed into pupae or adult mosquitoes. Additionally, the larvae exhibited reduced activity. These observations were believed to result from inhibiting larval growth by secondary metabolites found in the *I. cylindrica* root extract. The mortality of *Ae. aegypti* larvae, particularly at a concentration of 1000 ppm of *I. cylindrica* root extract, was suspected to be caused by the active compounds coming into direct contact with the larvae. In the ethanol extract of *I. cylindrica* roots, active compounds such as flavonoids, alkaloids, and tannins are present (Maharani *et al*. 2018). Flavonoids, secondary metabolites derived from plants and other microorganisms, influence insect development and physiology. These compounds can act as larvicides against *Ae. aegypti*. For instance, several plants' flavonoid extracts or purified flavonoids have shown larvicidal activity against *Ae. aegypti* and other vector mosquitoes (Pessoa *et al*. 2018; Pavela *et al*. 2019; Al-Massarani *et al*. 2019; Swargiary *et al*. 2019).

4.2. Effects of Weed Extract Toxicity on The Ability of *Ae. Aegypti* Larvae to Lay Eggs

The results of the *Ae. aegypti* larva breeding test regarding their ability to lay eggs at a concentration of 1000 ppm within 72 hours showed variations in the number of eggs hatched among the different weed extract samples. The average number of eggs hatched for each sample was as follows: *I. cylindrica* leaves 75, *S. spontaneum* leaves 77.6, *A. aciculatus* leaves 80.3, *I. cylindrica* roots 9.6, *S. spontaneum* roots 27.3, *A. aciculatus* roots 13.3. At 96 hours, the average number of eggs hatched for each sample was as follows: *I. cylindrica* leaves 77.3, wild sugarcane leaves 70.3, needle grass leaves 66, *I. cylindrica* roots 14, *S. spontaneum* roots 19.3, *A. aciculatus* roots 17.6.

Based on the Analysis of Variance for the Effects of Weed Extract Toxicity Testing on *Ae. aegypti* mosquito larvae's egg-laying ability at 72 hours, the average number of eggs hatched in the larval test treatment at a concentration of 1000 ppm was as follows: for cogon grass leaves (75.00), wild sugarcane leaves (77.67), needle grass leaves (80.33), *I. cylindrica* roots (9.67), *S. spontaneum* roots (13.67), and *A. aciculatus* roots (13.33). The obtained p-value was 0.000, indicating a significant difference in the average number of eggs hatched among the six samples.

It is similarly based on the variance analysis of weed extract toxicity testing effects on *Ae. aegypti* mosquito larvae's egg-laying ability at 96 hours, th e average number of eggs hatched in the larval test treatment at a concentration of 1000 ppm was as follows: for *I. cylindrica* leaves (76.67), *S. spontaneum* leaves (90.00), *A. aciculatus* leaves (83.00), *I. cylindrica* roots (14.00), *S. spontaneum* roots (19.33), and *A. aciculatus* roots (17.67). The obtained p-value was 0.000, indicating a significant difference in the average number of eggs hatched among the six samples.

Based on the analyses above, it was evident that there was a significant difference in the average number of

eggs hatched among the six testing samples, both at the 72-hour and 96-hour time intervals. From these results, it could be concluded that in the testing of *I. cylindrica* root extracts, the number of hatched eggs was lower than the number of eggs in the testing of *I. cylindrica* leaf extracts. Based on phytochemical test results, the *I. cylindrica* root extracts contain flavonoid compounds with phenolic content (Maharani *et al*. 2018).

The crude extracts of leaves, flowers, fruits, rhizomes, and endosperms of plants exhibited significant larvicidal activity against *Ae. aegypti* was measured by lethal concentration (LC) and larval mortality (Priya *et al*. 2023). The ethanolic seed extract of *Annona mucosa* (Annonaceae) demonstrated an LC50 value of 2.6 mg/ml against *Ae. aegypti* larvae within 24 hours (Rodrigues *et al*. 2021). The dichloromethane extract from *Ateleia glazioviana* (Fabaceae) leaves showed significant larvicidal activity at a concentration of 500 mg/ml. Annona glabra, the aqueous extract, exhibited LC50 values of 2.43 mg/L and 1.17 mg/L against *Ae. aegypti* larvae at 24 and 48 hours, respectively (Almadiy 2020; Amarasinghe *et al*. 2020). The steroidal alkaloid solasodine, extracted from the fruit of Solanum paludosum, resulted in a 63% mortality rate of fourth instar larvae at a concentration of 150 µg/ml (Cruz *et al*. 2019).

Based on the research by Alivianti (2021), during a 15-day observation period, mosquito eggs in ovitraps in the control group (aquades) had a small portion (21%) of mosquito eggs with a count of 46 eggs. In contrast, the intervention group with water soaked in *I. cylindrica* extracts showed a smaller portion (16%) of mosquito eggs, with a count of 191 eggs, indicating the influence of using soaked water from *I. cylindrica* on the number of mosquito eggs trapped in the ovitraps.

These findings align with the research conducted by Ridha *et al*. (2019), which found that using *I. cylindrica*-soaked water resulted in the highest oviposition compared to other soaking methods. For hatching results, rice-soaked water, water from egg residues, and aquades all produced high hatching rates, while soaking in lemon grass and cogon grass resulted in lower hatching rates (Ridha *et al*. 2019).

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