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#### SHORT COMMUNICATION

# PRELIMINARY STUDY OF THE TOXICITY OF *Toona sureni* LEAF HYDROSOL AND AQUEOUS EXTRACT AGAINST *Aedes aegypti* (DIPTERA: CULICIDAE)

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#### **ABSTRACT**

Dengue is a deadly disease transmitted by female Aedes mosquitoes, particularly Aedes aegypti. While insecticide-based control methods are widely used, they often lead to resistance and pose risks to human health and the environment. This has prompted interest in plant-based biopesticides, such as *Toona sureni* (Suren), which is traditionally placed around homes to repel mosquitoes. This preliminary study aims to evaluate the potential of Suren, not as a repellent as traditionally used, but as a larvicidal and pupicidal agent, particularly when formulated using simple and cost-effective methods. The study used a completely randomized design with 11 treatments and 3 replications, and data were analyzed using ANOVA and differences for each treatment were analyzed using DMRT at the 5% significance level. Treatment consists of hydrosol and aqueous extract of T. sureni leaves with concentration 10%; 5%; 2.5%; 1.25%; 0.625% and water as control. Each treatment was conducted on ten 3<sup>rd</sup> instar larvae and pupae which were placed in transparent plastic cups containing 150 ml of liquid treatment. The result indicated that Suren leaf aqueous extract is toxic to Ae. aegypti mosquito larvae with an LC<sub>50</sub> value of 13.6% and at concentrations of 10% and 5% causes larvae mortality of 36.7% and 26.7% respectively on 48 HAT (Hours After Treatment). These findings suggest that Suren leaf aqueous extract has the potential as a biopesticide for controlling Ae. aegypti larvae.

**Keywords**: Biopesticide; Suren; water solution; *Aedes aegypti; Toona sureni* 

## **ABSTRAK**

Denggi adalah penyakit yang membawa maut yang disebarkan oleh nyamuk *Aedes* betina, khususnya *Aedes aegypti*. Walaupun kaedah kawalan berasaskan racun serangga digunakan secara meluas, ia sering membawa kepada rintangan dan menimbulkan risiko kepada kesihatan manusia dan alam sekitar. Ini telah mendorong minat terhadap biopestisid berasaskan tumbuhan, seperti *Toona sureni* (Suren), yang secara tradisinya diletakkan di sekitar rumah untuk menghalau nyamuk. Kajian awal ini bertujuan untuk menilai potensi Suren bukan sebagai penghalau seperti yang digunakan secara tradisional, tetapi juga sebagai

agen larvisidal dan pupisidal, terutamanya apabila dirumus menggunakan kaedah yang mudah dan kos rendah. Kajian ini menggunakan reka bentuk rawak sepenuhnya dengan 11 rawatan dan 3 ulangan, dan data dianalisis menggunakan ANOVA dan perbezaan bagi setiap rawatan telah dianalisis menggunakan DMRT pada tahap 5%. Rawatan terdiri daripada hydrosol dan ekstrak akueus daun *T. sureni* dengan kepekatan 10%; 5%; 2.5%; 1.25%; 0.625% dan air sebagai kawalan. Setiap rawatan dijalankan ke atas sepuluh larva instar ke-3 dan pupa yang diletakkan di dalam cawan plastik lutsinar yang mengandungi 150 ml rawatan cecair. Hasil kajian menunjukkan bahawa ekstrak akueus daun Suren adalah toksik kepada larva nyamuk *Ae. aegypti* dengan nilai LC<sub>50</sub> sebanyak 13.6% dan pada kepekatan 10% dan 5% menyebabkan kematian larva masing-masing sebanyak 36.7% dan 26.7% pada 48 JSR (Jam Selepas Rawatan). Dapatan ini menunjukkan bahawa ekstrak akueus daun Suren berpotensi sebagai biopestisid untuk mengawal larva *Ae. aegypti*.

Kata kunci: Biopestisid; Suren; larutan air; Aedes aegypti; Toona sureni

Dengue virus is a life-threatening disease transmitted mainly by females Aedes mosquitos, particularly *Aedes aegypti*, the dominant vector in tropical region such as Indonesia. *Ae. aegypti* is highly adapted to human environment and undergoes a completed life cycle comprising egg, larvae, pupae, and adult stages. Eggs laid on damp areas at different heights above the waterline can remain dormant for months, up to nearly a year, before hatching upon contact with water. The larval stage consists of 4 instars, each lasting 1-2 days with development strongly influenced by temperature, where males typically develop faster than females. The pupal stage lasts 2-3 days before emerges as adults. Adult phase (imago) generally lives for 20-23 days, depending on environmental conditions where female can oviposit multiple times, at least 4-5 times per lifespan (Agustin et al 2017; Anoopkumar et al. 2017; Islam et al. 2021).

This mosquito is diurnal, meaning it is most active during the day. Peak biting activity occurs during the day and decreasing at night. It is frequently found in residential areas, and the presence of artificial lighting at night may further extend its biting behavior (Rund et al. 2020). Its abundance is strongly influenced by climatic condition. It increases under higher temperatures and reduced humidity and rainfall (Yusof et al 2018). Numerous efforts have been carried out to control *Ae. aegypti* due to its role as the primary dengue vector, including insecticide fogging, larvicidal applications and the removal of breeding sites around residential areas. However, the prolonged use of synthetic insecticides in domestic environment has led to the emergence of resistant (Lesmana et al. 2021). For example, *Ae. aegypti* populations have shown resistance to cypermethrin in Central Java, and to temephos in Riau, Indonesia (Lesmana et al. 2022; Sayono et al. 2023).

The growing resistance of mosquitoes to commercially available chemical insecticides, coupled with their detrimental effects on the environment and human health, underscores the urgent need for alternative control strategies mainly in plant-based biopesticides (due to their biodegradability, safety, and target specificity). Targeting the larval stage is particularly effective, as it prevents the development of adults capable of transmitting arboviral diseases. Numerous studies have explored environmentally friendly plant-derived compounds for larval control such as *Lavender angustifolia*, *Zingiber officinale*, *Artemisia herba-alba*, *Jatropha curcas*, *Mirabilis jalapa*, *Piper nigrum*, and *Toona sureni* (Alyahya 2023; Aziz et al 2021; Boesri et al. 2015; Boekoesoe & Ahmad 2022; Silvério et al. 2020). Suren leaves are traditionally recognized for their medicinal and insecticidal properties and are often placed around homes to repel mosquitoes (Latifah et al.

2019). This study evaluated the efficacy of Suren leaves extracts as a biopesticide against *Ae. aegypti* larvae and pupae, employing a simple and cost-effective formulation suitable for community level applications.

The preliminary assays was carried out in the Entomology Laboratory of Research Center for Estate Crops, Bogor in 2024. The procedure was a modified version of the WHO (2005) guidelines. *Ae. aegypti* larvae as the object in this test were sourced from eggs obtained through mass rearing at the School of Veterinary Medicine and Biomedicine, Bogor Agricultural University. These eggs were then reared to third-instar larvae and pupae in sufficient numbers, with fish pellets provided as feed. Larvae and pupae of uniform size and healthy condition were selected for experimentation. Suren leaves were collected from plants in the Cimanggu area, Bogor, West Java, Indonesia. Due to the slow action of biopesticides, observations were conducted for up to 48 hours. Mortality was recorded for larvae and pupae unresponsive to probing with a brush, including moribund larvae that lacked typical diving response upon water disturbance.

Hydrosol, a clear pale-yellow liquid with a strong Suren scent was obtained as a by-product of distilling Suren (*T. sureni*) leaves. The aqueous extract was made by blending 80 grams of Suren leaves with 500 ml of water, allowing the mixture macerated overnight and then filtering it. The filtered extract was allowed to separate into a sediment (bran) layer at the bottom and a clear liquid with a greenish yellow liquid at the top. A clear layer was used for this experiment. The 3<sup>rd</sup> instar larvae and pupae used as test objects in this experiment were obtained by hatching eggs in water.

The experiment followed a Completely Randomized Design (CRD) with 11 treatments and 3 replications. The treatment included varying concentrations (10%, 5%, 2.5%, 1.25%, 0.625%) of hydrosol and aqueous extract, along with control (water) as eleventh treatment. Each treatment was applied to 10 third instar larvae and pupae in transparent plastic cups containing 150 ml of treatment solution. Observations for mortality were made at 1, 3, 6, 24, & 48 HAT (Hours After Treatment). Data was analyzed using ANOVA and followed by Duncan's Multiple Range Test (DMRT) at the 5% significance level to identify significant differences among treatment means. The median lethal concentration (LC50) was estimated using probit analysis.

The hydrosol and aqueous extract of T. sureni leaves showed varying larvicidal effects on Ae. aegypti, with the highest mortality observed in the 10% aqueous extract at 48 HAT (Hours After Treatment) (Table 1). The hydrosol and aqueous extracts of T. sureni did not produce a significant effect on the mortality of Ae. aegypti pupae (Table 2). Probit analysis showed that the  $LC_{50}$  value of Suren leaf aqueous extract on  $3^{rd}$  instar Ae. aegypti larvae at 48 HAT was 13.6% (y = 6.5 + 1.7x) (Figure 1).

Table 1. Effect of *T. sureni* leaves on the mortality of *Ae. aegypti* larvae

Treatments	Mortality (%)						
	1 HAT	3 HAT	6 HAT	<b>24 HAT</b>	<b>48 HAT</b>		
Hydrosol 10%	0±0a	0±0a	0±0a	0±0a	3.3±0.6c		
Hydrosol 5%	0±0a	0±0a	0±0a	0±0a	$0\pm0c$		
Hydrosol 2.5%	0±0a	0±0a	0±0a	0±0a	$3.3 \pm 0.6c$		
Hydrosol 1,25%	0±0a	0±0a	0±0a	0±0a	$0\pm0c$		
Hydrosol 0.625%	0±0a	0±0a	0±0a	0±0a	0±0c		

Aqueous extract 10%	0±0a	0±0a	0±0a	6.7±1.5a	36.7±3.1a
Aqueous extract 5%	0±0a	0±0a	0±0a	3.3±0.6a	26.7±2.6ab
Aqueous extract 2,5%	0±0a	0±0a	0±0a	3.3±0.6a	$10.0 \pm 2.7 bc$
Aqueous extract 1,25%	0±0a	0±0a	0±0a	0±0a	$3.3 \pm 0.6c$
Aqueous extract 0.625%	0±0a	0±0a	0±0a	0±0a	$0\pm0c$
Control (Water)	0±0a	0±0a	0±0a	0±0a	$0\pm0c$

HAT = Hours After Treatment

Numbers followed by the same letter at the same column are not significantly different at 5% DMRT

Table 2. Effect of *T. sureni* leaves on the mortality of *Ae. aegypti* pupa

Treatments	Mortality (%)					
	1 HAT	<b>3 HAT</b>	6 HAT	<b>24 HAT</b>	<b>48 HAT</b>	
Hydrosol 10%	0±0a	0±0a	0±0a	0±0a	3.3±0.6a	
Hydrosol 5%	0±0a	0±0a	0±0a	0±0a	3.3±0.6a	
Hydrosol 2.5%	0±0a	0±0a	0±0a	0±0a	0±0a	
Hydrosol 1,25%	0±0a	0±0a	0±0a	0±0a	0±0a	
Hydrosol 0.625%	0±0a	0±0a	0±0a	0±0a	0±0a	
Aqueous extract 10%	0±0a	0±0a	0±0a	$3.3\pm0.6a$	$6.7 \pm 1.3a$	
Aqueous extract 5%	0±0a	0±0a	0±0a	0±0a	$3.3 \pm 0.6a$	
Aqueous extract 2,5%	0±0a	0±0a	0±0a	0±0a	$3.3 \pm 0.6a$	
Aqueous extract 1,25%	0±0a	0±0a	0±0a	0±0a	0±0a	
Aqueous extract 0.625%	0±0a	0±0a	0±0a	0±0a	0±0a	
Control (Water)	0±0a	0±0a	0±0a	0±0a	0±0a	

HAT = Hours After Treatment

Numbers followed by the same letter at the same column are not significantly different at 5% DMRT

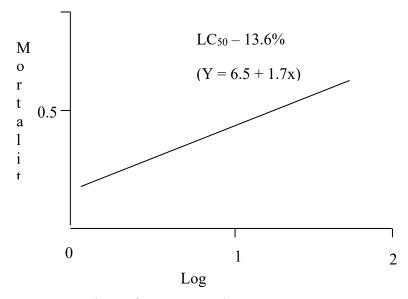


Figure 1. LC<sub>50</sub> Regression of *T. sureni* leaves aqueous extract on the larvae of *Ae.aegypti* 

At 1, 3 and 6 HAT, no larval mortality was observed, neither in the hydrosol nor in the aqueous extract treatment. Mortality was first recorded at 24 HAT in larvae exposed to 10% and 5% aqueous extract concentrations, but the rates were low and not significantly different from the control. At 48 HAT, a slight mortality was observed in the hydrosol treatment, which remained statistically similar to the control. In contrast, aqueous extract concentrations of 10% and 5% caused significantly higher mortality rates, namely 36.7 and 26.7% respectively. While the concentrations of 2.5% and below (1.25% and 0.625%) produced low and not significant rates of mortality levels.

Meanwhile the results of hydrosol and aqueous treatment on pupae showed no significant effects compared with the control. The highest mortality occurred at 10% aqueous extract concentration, both at 24 HAT and 48 HAT, although the values remained very low (Table 2). These findings indicate that both treatments had negligible effects on pupae. The minimal impact of hydrosol and Suren leaves aqueous extract on *Ae. aegypti* pupae suggests that these treatments lack sufficient potency to induce pupal mortality. This limited susceptibility may be attributed to pupal stage being a non-feeding stage (physiologically inactive phase) in *Ae. aegypti*, although pupae remain capable to limited movement in response to external disturbances (typically by performing downwards escape movements until the water surface stabilizes) (Anoopkumar et al. 2017; Zettel & Kaufman 2009).

Among the hydrosol and aqueous extract treatments tested on *Ae. aegypti* larvae and pupae, only the Suren leaf aqueous extract treatment showed a significant larvicidal. Probit analysis estimated a LC<sub>50</sub> value of 13.6%, indicating that a concentration of approximately 13,6% is required to achieve 50% mortality in 3<sup>rd</sup> instar *Ae. aegypti* larvae. The results indicate that Suren leaf aqueous extract has potential as biocontrol agents against *Ae. aegypti* larvae. The highest mortality rate (36.7%) observed at 48 HAT comparable to findings from other studies on biopesticides. Maris et al. (2025) reported 32.5% mortality on brown planthoppers 7 days post treatment. In *Callosobruchus chinensis*, the mortality rate at 48 HAT ranged from 13-30% (Javed 2014). Similarly, Pradinata et al. (2024) found that Tobacco and Babadotan (*Ageratum conyzoides*) leaf extract caused 5-30% mortality in Sp*odoptera exigua* at the same interval. Unlike chemical pesticides that act rapidly and produce high mortality, biopesticides typically exhibit slower action and lower efficacy.

Suren has been long known for its biopesticidal properties. Several studies on insects such as Doleschallia bisaltide (Widyastuti et al. 2020), red flour beetle (Tribolium castaneum) (Parvin et al. 2012), Hyposidra talaca (Nurawan & Haryati 2010), Spodoptera litura (Noviana et al. 2012), and mealybugs (Andini & Kuswandi 2022) have demonstrated its considerable effectiveness. A previous study combining Suren leaves with frangipani flowers revealed significant anti-mosquito activity in both materials (Nurseha & Asngad 2019). Other study has also reported that Suren is generally recognized as mosquito repellent (Boesri et al. 2015), Consistent with our findings, several studies have demonstrated that Suren exhibits sufficient toxicity to serve as an alternative biopesticide for mosquitoes' control (Nurhariyati et al. 1996; Nurseha & Asngad 2019). Suren leaves have demonstrated significant toxicity against various insects' pests. For instance, they caused over 85% mortality in Plutella xylostella (Hidayati et al. 2013), over 90% in mealybugs (Andini & Kuswandi 2022), and up to 86.7%, 88.3% and 85% mortality in T. castaneum adults, larvae, and pupae, respectively (Parvin et al. 2012). Their application against Hyposidra talaca in tea plantation also reduced infestation intensity, while improving yield and farmer income (Nurawan & Haryati 2010).

The aqueous extract of Suren leaves has been shown to effectively reduce both damage and reproductive capacity (fertility and fecundity) of S. litura (Noviana et al. 2012). Research conducted by Nofita & Nurlan (2020) recorded that phenolic content of aqueous extract is higher than in 70% ethanol extract, likely because the phenolic compounds possess polarity similar to water/aqueous, facilitating greater solubility. Among these, Methyl gallate (a phenolic compound isolated from Suren leaves) exhibits notable antioxidants and antibacterial properties (Ekaprasada et al. 2010; Ekaprasada et al. 2015). Other phenolic compounds, such as flavonoids, have also been frequently reported in Suren leaves (Ismanto et al. 2017; Prasetiadi et al. 2011; Sidabutar et al. 2021). They are known to affect insect pests by acting as repellents, feed deterrents (particularly during the larval stages), and inhibitors of detoxification systems (by diverting energy toward detoxification instead of digestion), nervous system, growth (causing malformations), development, and reproduction (reducing fecundity, oviposition, and egg hatching) (Pereira et al. 2024). Feeding deterrence during the larval phase particularly relevant for Ae. aegypti control. Starved larvae often fail to reach the second instar stage (Levi et al. 2014), and adults emerging from undernourished larvae tend to be smaller and require more blood meals for eggs production (Souza et al. 2019). They often possess smaller wings that limit their dispersal activity (Padmanabha et al. 2011). Reduced larval nutrition consequently decreases both fecundity and longevity, which significantly lowers mosquitoes' vectorial capacity and population density (Shapiro et al. 2016; Vantaux et al. 2016; Yan et al. 2021). In addition, Suren leaves contain triterpenoid compounds such as surenin, surenon, and surenolactone, which exhibit repellent properties and impart an astringent taste that deters insect feeding (Hidayati et al. 2013; Kraus & Krypke 1979).

The findings presented above suggest several possible explanations for the biopesticide activity of Suren leaf aqueous extract against *Ae. aegy*pti larvae. Further studies are required to elucidate its mode of action, chemical composition (via GC-MS), and speed of efficacy. Additional investigations should also examine its potential insect growth regulator (IGR) effects, including inhibition of larval-pupal and pupal-adult transitions via juvenile hormone analogues and chitin synthesis inhibitors. Despite its moderate toxicity, Suren leaf aqueous extract remains a promising candidate for environmentally friendly mosquito control due to its ease of preparation and application, particularly for early-stage control by treating potential breeding sites.

As a conclusion, the aqueous extract of Suren (*T. sureni*) leaves exhibited notable larvicidal activity against *Ae. aegypti* larvae at 48 HAT with an LC<sub>50</sub> value of 13.6%. These findings suggest that the extract possesses potential as an eco-friendly biopesticide due to its simplicity of preparation and cost effectiveness. It also supports prospective application in community-based or household mosquito control programs. Nevertheless, further studies are required to assess its mode of action, chemical composition (via GC-MS) and the speed of its efficacy. Moreover, comprehensive studies are recommended to evaluate its potential IGR (Insect Growth Regulatory) effects, particularly its ability to inhibit larval-pupal and pupal-adult transitions through juvenile hormone (JH) analogues and the chitin synthesis inhibitors.

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## **AUTHORS DECLARATION**

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

#### **Ethics Declarations**

No ethical issue is required for this research.

## **Data Availability Statement**

The authors confirm that the data supporting the findings of the study are available within the article. Raw data that support the findings of this study are available from corresponding author, upon reasonable request.

## **Authors' Contributions**

AK and PM: Designed study and statistical analysis. AK, NT, and PM: Carried out the preparation and observation, data interpretation, manuscript writing, and manuscript editing. All authors have read and approved the final manuscript.

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