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Botanical Larvicide from Jeruk Limau (*Citrus amblycarpa*) and Serai (*Cymbopogon citratus*) for Killing *Aedes aegypti* Larvae

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Abstract

BACKGROUND: Dengue hemorrhagic fever is caused by the dengue virus which is transmitted through the bite of the Aedes aegypti mosquito which breeds in densely populated areas with humid and warm climates. The use of anti-mosquito drugs in the form of burns, sprays, lotions, or other forms has been widely used, but these drugs are not completely profitable. Anticipatory efforts need to be made by utilizing natural materials so that they can be used effectively and safely for both humans and the environment.

AIM: Our study tries to find the effectivity of the infusion from Jeruk Limau (Citrus amblycarpa) and Serai for botanical larvicide against Aedes aegypti larvae.

METHODS: This research is a pure experimental study with a Post-test Only Control Group Design by making larvicide products from lime and lemongrass waste infusions, the results of which are analyzed descriptively and analytically. The research used two factors, namely treatment (P) using infusion products whereas control (K) using distilled water.

RESULTS: From the results of effectiveness tests on larvae, it was found that there was a reduction in live larvae until all of them died. Lime and lemongrass waste contain flavonoid compounds which are respiratory toxins in larvae. The combination of saponin and tannin as a stomach poison has the power to kill mosquito larvae. The abundant availability of materials, enriching research treasures on local potential, as well as a good level of public acceptance are the advantages of the product.

CONCLUSION: Apart from that, it is recommended to optimize the dosage and durability of the product so that it can be further patented as a concrete step in realizing the sustainable development agenda in the health sector.

Introduction

The UN's Sustainable Development Goals (SDGs) 2030 present a comprehensive vision striving for a better world by 2030. These goals follow the Millennium Development Goals (MDGs) conclusion in 2015 and encompass 17 major objectives with 169 specific targets. The third goal emphasizes universal access to quality health care and well-being across all ages, targeting the eradication of diseases such as AIDS, tuberculosis, malaria, hepatitis, and water-borne and communicable diseases, including neglected tropical diseases such as dengue hemorrhagic fever (DHF) transmitted primarily by mosquitoes [1].

DHF, or severe dengue, exhibits high fever, accompanied by symptoms such as headaches, joint and muscle pain, bone pain, and ocular discomfort [2]. *Aedes aegypti* mosquitoes, thriving in warm, densely populated areas, transmit this disease in Indonesia, a tropical and humid country, experiencing a high prevalence of dengue fever [3].

Indonesia ranks second globally in reported dengue fever cases after Brazil with an estimated 500,000 DHF patients needing annual hospitalization. It is a leading cause of hospitalization and fatalities in several tropical Asian countries, marking Indonesia as a significant hub for dengue fever in Southeast Asia [4]. To tackle dengue fever, the Indonesian Ministry of Health introduced the 3M program – closing, draining, and burying potential *Aedes aegypti* mosquito breeding grounds, and using Abate powder in stagnant water. Despite these efforts, dengue fever rates have not significantly decreased, prompting the exploration of natural products from traditional plants to disrupt the mosquito lifecycle, particularly *Aedes aegypti*, as a preventive measure [5], [6].

Numerous anti-mosquito products, such as coils, sprays, and lotions, carry risks due to toxic substances. Chemicals such as carbamates (e.g. propoxur), pyrethroids (e.g. permethrin), organophosphates (e.g. DDVP and DEET), and organochlorines can harm health and the environment. They may cause skin irritation, muscle spasms, and respiratory issues and are linked to asthma and respiratory cancer [7], [8]. To mitigate these risks, using natural materials effective

and safe for humans and the environment is advocated. Utilizing citrus and lemongrass waste infusions as larvicides is a promising preventive measure. Although traditional plant-based therapies are cost-effective and have fewer perceived side effects, their improper use emphasizes the importance of dosage, administration, timing, and ingredient selection aligned with medical guidance [9], [10]. Jeruk Limau (*Citrus amblycarpa*) generates significant waste and its compounds act as larvicides, hindering insect growth. Lemongrass or serai containing citronella and geraniol, dehydrates larvae, contributing to their demise [11], [12]. Then, our study tries to find the effectivity of the infusion from Jeruk Limau (*Citrus amblycarpa)* and Serai for botanical larvicide against *Aedes aegypti* larvae.

Methods

This pure experimental study employed a Post-test Only Control Group Design, creating a larvicide product from citrus and lemongrass waste infusions. Descriptive and analytical analysis assessed causal relationships between dependent and manipulated independent variables in a controlled setting. Employing a completely randomized design (CRD), the study analyzed effectiveness using two factors: A treatment group using larvicide and a control group using aquades. The experiment observed larval mortality in buckets and evaluated acceptance levels through descriptive analysis with ten panelists. Interviews assessed acute reactions during product use. SPSS analyzed data descriptively to depict public acceptance levels, presented graphically. Brief observations evaluated initial hypersensitivity reactions and conducted from August 5, 2020, to September 30, 2020, at Bukit Tambun Tulang, Kenagarian IV Koto Hilie, Batang Kapas.

Results

From the infusion, 300 ml of larvicide was obtained and tested in mosquito larva-filled buckets. Testing for larval death duration in both groups showed significant efficacy in the treatment group (P); all larvae died. In the control group (K) using aquades, no larval deaths occurred. Interviews and observations found no acute hypersensitivity reactions among panelists within the WHO-recommended 2-h monitoring post-product usage.

Discussion

The death of *Aedes aegypti* larvae in this study is likely due to secondary metabolites found in the Jeruk Limau *(Citrus amblycarpa)* extract. A study of phytochemical analysis confirmed alkaloids, tannins, saponins, and flavonoids in citrus peel extract, whereas fruit extract contained saponins, tannins, alkaloids, and lacked flavonoids [13]. These compounds are presumed to be lethal to *Aedes aegypti* larvae. Plant chemical defenses, such as saponins, terpenoids, alkaloids, and flavonoids, are toxic to insects, hindering their food digestion capabilities [14].

Alkaloids act as toxic plant defenses, impeding insect feeding and damaging their cells. They degrade cell membranes and affect the nervous system by hindering acetylcholinesterase, making larval bodies transparent, slowing their movement, and causing a curved posture. Observing motionless larvae floating in containers indicates their death [15], [16].

Saponins in citrus peel extracts are stomach poisons for larvae, hindering their metabolism by disrupting cell membranes. These compounds disturb larval digestion through the digestive system, entering through the gastrointestinal tract, and interfering with the larval digestion system [17], [18]. Saponins denature proteins and enzymes, diffusing through fragile outer cell membranes, binding to cytoplasmic membranes, causing cell leakage and death [19].

In addition, tannins act as stomach toxins, hindering digestive enzymes in the insect's system from binding proteins, affecting *Aedes aegypti* larval digestion. Tannins disrupt enzymes by forming complexes with proteins in enzymes and substrates, disturbing digestion and damaging cell walls. These compounds are primarily absorbed in the digestive system, affecting enzymatic activity and causing uncontrolled insect metabolism [20], [21].

Saponins, having soap-like properties, are water-soluble insect repellents. They function as bitter, foamy steroid glycosides or triterpenes, exhibiting various biological properties such as hemolytic abilities, antibacterial, molluscicidal, antiviral, cytotoxic or anticancer effects, cholesterol-lowering effects, and antiprotozoal activities [19], [22]. Tannins, found in woody plants, act as plant defenses by hindering insect digestion, resulting in reduced food intake and growth [23], [24].

Flavonoids, the largest natural phenolic group in plants, enter larvae through respiration, causing nerve atrophy and respiratory damage, leading to larval asphyxiation and death. These compounds, combined in citrus peel extracts, including alkaloids, saponins, tannins, and flavonoids, exhibit toxic effects on larvae, resulting in their death due to the inability to detoxify the ingested toxins [18], [25].

Contact with active compounds in lemongrass (*Cymbopogon nardus* L) caused *Aedes aegypti* larvae death. Lemongrass active components are toxic upon respiratory contact with larvae, inhibiting acetylcholinesterase, causing acute poisoning such as seizures, central nervous system disease (CNS), and respiratory paralysis, leading to insect death [12], [26].

Utilizing citrus peel and lemongrass waste extracts potentially serves as a bio-larvicide due to their secondary metabolites – alkaloids, saponins, flavonoids, and tannins – disturbing and killing *Aedes aegypti* larvae. The larvicidal mechanism involves contact poisoning, penetrating larval bodies, and disrupting membranes, sensory glands, tracheae, or other cuticle-related systems. The large toxic amount absorbed by insect bodies acts as a respiratory or fumigant poison and stomach poisoning through ingestion [27], [28], [29].

This research demonstrated that citrus peel waste extracts caused 100% mortality in *Aedes aegypti* larvae in the treatment group. Conversely, aquades lacked larvicidal effects, allowing larvae survival, as water serves as the mosquito habitat without toxic content. In addition, aquades with a pH of 7 provide optimal conditions for *Aedes aegypti* larval development [30].

Conclusion

There is a difference in *Aedes aegypti* mosquito larvae mortality rates with the addition of citrus peel and lemongrass waste infusion compared across the two groups. Observations and literature review confirm this solution as a natural larvicide for the primary DHF vector. No acute reactions were observed among respondents. Further research should optimize dosage and expand control groups across various concentrations. Purification of secondary metabolites in lemongrass extracts is necessary to identify their role as *Aedes aegypti* larvicides. Extensive testing for acute and chronic effects of the product's administration is vital to ensure safety.

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