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MORTALITY TEST OF SPICE PLANTS AS BIOLARVICIDES AGAINST Aedes aegypti Larvae (Literature Review)

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ABSTRACT

Mortality Test Of Spice Plants As Biolarvicides Against Aedes Aegypti Larvae. *Dengue hemorrhagic fever (DHF) remains a global public health issue, transmitted through the bite of the Aedes aegypti mosquito. One method of disease prevention is the use of insecticides in the form of larvicides. Chemical larvicides have long been used but carry the risk of inducing resistance; therefore, research into the development of alternative larvicides is necessary. Indonesia's rich biodiversity of spices presents a promising opportunity for the development of spice-based biolarvicides. This study was conducted in the form of a literature review, aiming to provide an overview of mortality tests of spice plants as biolarvicides against Aedes aegypti larvae. The findings from the reviewed literature indicate that two spices—tobacco leaves (Nicotiana tabacum) and zodia leaves (Evodia suaveolens)—in ethanol extract form achieved 100% larval mortality at all tested concentrations. The review also identified a variety of other spices with potential as biolarvicides based on larvicidal tests, with 9 out of 20 spices exhibiting 100% larval mortality rates in the summarized studies. It can thus be concluded that various spice plants demonstrate significant mortality test results and hold potential as alternative biolarvicides against Aedes aegypti larvae. Continued efforts to conduct standardization tests on these spice plants are necessary to develop them into standardized biolarvicidal agents.*

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is a mosquito-borne disease, primarily transmitted by the Aedes aegypti species, whose incidence has continued to rise with each passing decade. To this day, DHF is classified as a serious global health issue, including in tropical countries such as Indonesia [1]. Over the past two decades, the incidence of DHF in Indonesia has increased significantly from year to year [2]. In 2017, a total of 68,407 cases were reported, which rose to more than 137,000 cases in 2019 [3], and further increased to 143,184 cases in 2022. This upward trend reflects the growing burden of DHF in Indonesia, emphasizing the urgent need for effective control and prevention strategies [4].

The use of insecticides, particularly larvicides, plays a crucial role in mosquito vector control programs, especially in disrupting the transmission cycle. The safe application of insecticides is essential to prevent the development of vector resistance [2,5].

The use of larvicides as a vector control method began in the 1980s, with the commercial application of chemical larvicides such as abate powder containing 1% temephos as the active ingredient [2,6]. Temephos, an organophosphate compound, is used to treat water bodies that serve as larval habitats for mosquito vectors, targeting the larval stage to prevent their development into adult mosquitoes [6].

Several studies have reported resistance to abate containing 1% temephos in Aedes aegypti larvae, including cases in Costa Rica (2013) and in several regions of Indonesia such as Demak, Banten, and Banjarnegara [7]. Therefore, the development of alternative plant-based biolarvicides is worth pursuing, particularly considering their advantages in terms of safety. Larvicidal compounds derived from plants are biodegradable and do not leave harmful residues in water, air, or soil [8].

As a tropical country with a population that largely depends on agriculture, Indonesia is renowned for its rich diversity of spices [9]. Spices are plant-derived substances primarily used to enhance the flavor and aroma of food [10]. Indonesia's wealth of spices is not only utilized as food additives but also as traditional medicines and fragrances [11].

The use of natural plant-based ingredients to control insects and pests has been widely practiced for a long time [12]. However, the use of spice-based larvicides, which are more environmentally friendly, remains limited when compared to chemical larvicides. This limited usage is partly due to a lack of public awareness and information regarding the potential of spices as larvicidal agents [13].

Based on the aforementioned background, it is essential to conduct a literature review on spices that exhibit biolarvicidal activity, with the aim of providing scientific information for future researchers and for communities unfamiliar with plant-derived biolarvicides. The objective of this study is to examine mortality tests of spice plants as biolarvicides against Aedes aegypti mosquitoes.

MATERIALS AND RESEARCH METHODS

This study was designed as a literature review, referring to various sources relevant to the topic. The references used were drawn from multiple sources, including scientific journals, books, and other literature published within the last ten years (2014–2024). The databases utilized for this review include Google Scholar and Scopus.

RESEARCH RESULTS AND DISCUSSION

Through a literature review of various references related to the topic, a set of spice plants with biolarvicidal activity was identified based on mortality tests. The reviewed literature was carefully filtered and standardized, resulting in the identification of several spice plants that exhibit larvicidal properties. From the analysis, a total of 20 spice plants were found to act as larvicides against *Aedes aegypti* larvae, as presented in Table 1.

Table 1. Results of the Literature Review on Selected Spice Plants with Larvicidal Potential Against the Larval Stage of *Aedes aegypti*.

No	Spice Name	Preparation and Solvent	Larvicidal Compounds	Concentration	Mean Larval Mortality Percentage within 24 Hours
1	Leaf Serai Dapur (Cymbopogon citratus) (14)	Juice of daun serai dapur crushed and mixed with water	Sitronelal, Sitronelol, Geraniol, Minyak Atsiri.	15% 30% 45%	67 82 100
2	Leaf Serai Dapur (Cymbopogon citratus) (15)	Minyak serai dalam bentuk granul dengan pengisian laktosa dan pengikat CMC-Na	Sitronelal, 3,4 Oktadiena, 7-metil-Z-Sitral, Trans-Geraniol, Sitral	36 ppm 39 ppm 42 ppm 45 ppm 49 ppm	41,67 53,33 60 76,67 88,33
3	Flower Kecombrang (Etlingera elatior) (16)	Ethanol extract bunga kecombrang	flavonoid, saponin, tanin, kuinon, monoterpen, steroid/triterpenoid, polifenolat	0,75% 1% 1,25% 1,5% 1,75% 2%	10 37 57,2 69,2 74 93,2
4	Lengkuas Putih (Alpinia galanga L. Willd) (17)	Filtrate of rimpang lengkuas putih	Saponin, flavonoida, polifenol, dan minyak atsiri	2% 2,5% 3,1% 4%	14 31,3 51,3 96,6
5	Serai Wangi/Rhizome (Andropogon nardus) (18)	Ethanol extract Serai wangi/ rimpang	Bahan aktif dominan berupa asam vetivetate	1,56% 3,12% 6,25% 12,50% 25,0% 50%	68 76 90,7 100 100 100
6	Leaf Tembakau (Nicotiana tabacum) (18)	Ethanol extract daun tembakau	Bahan aktif dominan berupa alkaloid	1,56% 3,12% 6,25% 12,50% 25,0% 50%	100 100 100 100 100 100
7	Leaf Zodia (Euvodia graveolens) (18)	Ethanol extract daun zodia	Bahan aktif dominan berupa evodiamine	1,56% 3,12% 6,25% 12,50% 25,0% 50%	100 100 100 100 100 100
8	Leaf Rosemary (Rosemarinus officinalis L) (18)	Ethanol extract daun rosemary	Bahan aktif dominan berupa alkaloida	1,56% 3,12% 6,25% 12,50% 25,0% 50%	78,7 86,6 96 100 100 100
9	Kayu putih (Melaleuca cajuputi) (19)	Essential Oil daun kayu putih	α-terpinena, terpinolena, dan γ-terpinena	62.5μg/mL 125 μg/mL 250 μg/mL 500 μg/mL 1000 μg/mL	33,75 76,25 100 100 100
10	Peel Jeruk Manis (Citrus sinensis) (20)	Air perasan kulit buah jeruk manis	Tanin, saponin, fitat oksalat, flavonoid, dan limonoid	0,05% 0,2% 0,4% 0,6% 0,8% 1% 1,2% 1,4%	0 57,32 82,64 94,64 97,32 100 98,64 100
11	Leaf Kemangi (Ocimum sanctum Linn) (21)	Ethanol extract daun kemangi	tannin, eugenol, flavonoid, tripenoid, minyak atsiri, saponin, pentose, xilosa, molludistin, dan beberapa jenis asam	500 ppm 1000 ppm 1500 ppm 2000 ppm 2500 ppm	18 25 48 73 90
12	Leaf Kerehau (Callicarpa longifolia Lam.) (22)	Ethanol extract daun kerehau	Saponin, tanin, dan flavonoid	2% 4% 6% 8%	24 36 48 88

13	Leaf Lemon (Citrus Limon) (23)	Formulasi Granul ekstrak etanol daun lemon	Bahan aktif dominan berupa Limonele	1g 2g 3g 4g	28 30 44 96
14	Lemon (Citrus Limon) (24)	Essential Oil buah lemon with solvent n-hexane	-	2 ppm 5 ppm 15 ppm 25 ppm 40 ppm	17 32 54 72 90
15	Leaf peppermint (Mentha piperita L.) (24)	Essential Oil daun peppermint with solvent n-hexane	-	10 ppm 20 ppm 30 ppm 40 ppm 50 ppm	14 46 66 80 94
16	Lavender (Lavandula angustifolia) (24)	Essential Oil lavender with solvent n-hexane	-	15 ppm 20 ppm 30 ppm 40 ppm 55 ppm	13 27 46 67 88
17	Leaf mimba/ neem (Azadirachta indica Juss) (24)	Essential Oil neem with solvent n-hexane		10 ppm 30 ppm 50 ppm 70 ppm 100 ppm	14 30 54 78 89
18	Leaf Marjoram (Origanum majorana, L.) (25)	Essential Oil daun marjoram	Pulegone, verbenone, trans-p-menthan-2-one, iso-menthone, piperitone, 3-octanol dan isopulegol	20 µg/mL 40 µg/mL 60 µg/mL 80 µg/mL 100 µg/mL	16 40 40 65,32 78,62
19	Bawang (Allium sativum) (26)	Essential Oil bawang	Bahan aktif dominan berupa diallyl disulfide	0.005 ppm 0.012 ppm 0.025 ppm 0.037 ppm 0.050 ppm	25 93 99 100 100
20	Adas manis (Pimpinella anisum) (26)	Essential Oil adas manis	Bahan aktif dominan berupa anethole	0.005 ppm 0.012 ppm 0.025 ppm 0.037 ppm 0.050 ppm	3 7 35 96 100

The World Health Organization (WHO) has issued laboratory guidelines for larvicide testing with standardized procedural mechanisms for evaluation [27]. In its guidelines, WHO states that the larvicidal activity of a substance can be measured by observing larval survival using mortality rate as the parameter following exposure.

In the testing procedures used to assess mosquito larval mortality after exposure to larvicidal substances, mortality is evaluated and calculated after a minimum of 24 hours, with the observation period potentially extended to 48 hours[27]. Accordingly, in the literature review on the use of spice-derived substances as larvicides, references were selected based on the availability of mortality data observed within a 24-hour period.

From the data presented in Table 1, 100% larval mortality was observed across all concentrations—from the lowest to the highest—for two types of spices: tobacco leaf (in ethanol extract form) and zodia leaf (in ethanol extract form). Both spices contain larvicidal active compounds classified as alkaloids. According to Veer & Gopalakrishnan [12], alkaloids are organic compounds predominantly containing nitrogen and are primarily sourced from plants. Alkaloids, particularly those in the pyrimidine group, are often utilized as herbal insecticides. This is consistent with Kumara's findings[28], which state that alkaloids act as larvicides by inhibiting larval feeding and acting as digestive toxins. Moreover, alkaloids inhibit cholinesterase enzymes that degrade neurotransmitters in the larval nervous system, thereby disrupting nerve signal transmission and inducing larval death.

The literature reviewed in Table 1 shows that out of 20 tested spice-based plants, 9 exhibited 100% larval mortality at the highest concentration observed within 24 hours. These included lemongrass leaf (juice extract), citronella rhizome (ethanol extract), tobacco leaf (ethanol extract), zodia leaf (ethanol extract), rosemary leaf (ethanol extract), cajuput (essential oil), sweet orange peel (juice extract), garlic (essential oil), and anise (essential oil).

Meanwhile, 11 spices did not reach 100% mortality even at their highest tested concentrations: lemongrass leaf (granulated lemongrass oil), torch ginger flower (ethanol extract), white galangal (filtrate), basil leaf (ethanol extract), kerehau leaf (ethanol extract), lemon leaf (granulated ethanol extract), lemon fruit (essential oil), peppermint leaf (essential oil), lavender (essential oil), neem leaf (essential oil), and marjoram leaf (essential oil).
Additionally, some spices showed less than 10% larval mortality at lower concentrations. For instance, anise essential oil resulted in 3% and 7% mortality at 0.003 ppm and 0.012 ppm, respectively, and sweet orange peel extract at 0.05% resulted in 0% mortality.
WHO also outlines in its guidelines that bioassay testing for larvicides should employ multiple concentration levels, with a maximum concentration of 1%, taking into account the solubility of test substances in aqueous media [27]. As shown in Table 1, nine studies tested spice-based substances at concentration levels below 1%, aligning with WHO guidelines. Of these, six spices were tested at concentrations measured in ppm (parts per million), where 1 ppm is equivalent to 1×10^{-4} %, thus conforming to WHO standards, although they did not achieve 100% larval mortality([29]. These included basil leaf (ethanol extract), lemon (essential oil), peppermint leaf (essential oil), lavender (essential oil), neem leaf (essential oil), and marjoram leaf (essential oil).
The literature review also identified three spices that achieved 100% larval mortality, namely cajuput (essential oil), garlic (essential oil), and anise (essential oil). These findings align with Manh et al. [30], who noted that essential oils contain complex mixtures of active compounds that act on various insect targets through multiple mechanisms, including neurotoxic effects on GABA receptors, octopamine receptors, and acetylcholinesterase enzymes in the nervous system. These combined actions synergistically enhance larvicidal activity and induce larval death.
According to WHO, a larvicidal effect is indicated when a substance causes 10–95% mortality in larval populations during testing[27]. Meanwhile, the Pesticide Commission of the Ministry of Agriculture [31] defines effective larvicidal agents as those achieving 90–100% mortality. Therefore, it can be concluded that all spice-derived substances reviewed in the literature possess effective biolarvicidal potential to induce Aedes aegypti larval mortality at various concentration levels and in various forms of preparation[32–37].

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the literature search and the reviewed and selected references, it was found that various spices exhibit biolarvicidal activity against *Aedes aegypti* larvae, as reflected by the larval mortality values obtained through larvicidal assays. These spices show potential as alternative biolarvicides to replace synthetic larvicides, offering a safer option for the environment and helping to prevent the development of resistance. Notably, essential oil preparations from cajuput leaf, garlic, and anise demonstrated 100% larval mortality within 24 hours of observation at concentrations below 1%. Further research is required to develop spice-based substances as biolarvicides by conducting quality control testing, toxicity evaluation, and efficacy testing in accordance with prevailing pesticide standards.

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