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Aedes Sp. Mosquito Resistance and the Effectiveness of Biolarvicides on Dengue Vector Mortality

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ABSTRACT

Until now, dengue fever is still a public health problem in Indonesia. To control mosquito vectors, various strategic approaches have been promoted such as chemical control, biological control, resource reduction and public education. The four main classes that are widely used for mosquito control are organochlorines, organophosphates, pyrethroids and carbamates. A literature study was conducted to determine the extent of *Aedes aegypti* resistance to several insecticides in several countries including Indonesia. This study also reviewed the effectiveness of biolarvicides on the mortality of *Aedes* sp. All research that has been done in the last 10 years is included in this topic. In Brazil, Sri Lanka, China and Peru, there have been reports of resistance to *Aedes* sp. against delthamethrin, pyrethroid and temephos insecticides. Meanwhile in Indonesia (Semarang, Surabaya, Banten, DKI Jakarta, North Sumatra, Jambi, Bandung, Bogor, Makassar and Palu), *Aedes* sp. resistance occurs to insecticides of the pyrethroid, temephos, malathion, cypermethrin, and permethrin groups. Continuous monitoring of mosquito vector resistance status is very important in the effectiveness of dengue fever control. In addition, research on plants that can be used as biolarvicides is very useful for alternative control and improving public health. This review also provides better insight into the effectiveness of laboratory-tested plants as larvicides and plans for further research to be applicable to the community.

Key words: Dengue fever, *Aedes* sp mosquito, Insecticide resistance, Biolarvicides.

INTRODUCTION

Zoonoses are diseases that are transmitted through intermediaries (vectors). Diseases that are transmitted through vectors include arbovirosis diseases such as dengue, malaria, chikungunya, inflammation of the brain (Japanese B. encephalitis), elephantiasis (lymphatic filariasis), bubonic plague (pestilence) and bush fever (scrub typhus). Dengue is an infectious disease transmitted by arthropods caused by dengue virus infection[1] and this disease is still a public health problem in Indonesia with quite high morbidity and mortality and can cause extraordinary events.[2] Several variables such as average temperature, relative humidity and mobility are factors that correlate with the incidence of DHF.[3]

Control of disease-carrying insects relies heavily on the use of insecticides. The worldwide evolution of insecticide resistance has been recognized as a major obstacle to effective vector control.[4] Various strategic approaches have been promoted to control mosquito vectors, including chemical control (indoor residue spraying, mass fumigation, use of household insecticides), biological control (use of mosquito predators, release of certain genetically modified mosquitoes), resource reduction and public education.[5]

Larvicidal chemicals, such as temephos Bacillus thuringiensis israelensis (Bti), and adult chemicals in very low volume sprays and mists are widely used to control the spread of disease. An estimated 2.5 million tonnes are used annually.[5] The four main classes of insecticides widely used for mosquito control are organochlorines, organophosphates, pyrethroids and carbamates.[6]

Although insecticides were once effective in controlling mosquito-borne diseases, the increasing trend of mosquito-borne diseases may indicate increased resistance or ineffectiveness of insecticides in controlling disease transmission.[6] Research related to insecticidal resistance in mosquitoes has been carried out by many researchers in the world such as resistance to Anopheles sinensis in four main classes of insecticides in Sichuan,[4] *Aedes* resistance to the pyrethroid deltamethrin in the adult generation in Mexico,[7] malaria vector resistance in Kenya,[8] high permethrin resistance in Dhaka City but susceptibility status to deltamethrin still exists in some populations,[9] phenotypic resistance in Anopheles arabiensis in Western Kenya,[10] *Aedes aegypti* resistance to deltamethrin in Rio de Janeiro Janeiro, Brazil,[11] Anopheles sp. resistance to four classes of insecticides in southeastern Senegal,[12] resistance to pyrethroid and temephos insecticides in the *Aedes aegypti* population in Sri Lanka,[13] resistance to Anopheles hyrcanus in Ubon Rachathani Province, Thailand,[14] in Brazil a study conducted in 2017-2018 reported that there had been resistance to malathion in most of the mosquito populations studied,[15] the occurrence of deltamethrin resistance to *Aedes albopictus* in Shandong, China,[16] the occurrence of resistance to Anopheles gambiae to three of the four classes of insecticides in Southeastern Nigeria,[17] the occurrence of *Aedes aegypti* resistance to several Insecticide welding in Peru[18] and in Sri Lanka, a study conducted by Fernando *et al* reported the occurrence of *Aedes aegypti* resistance to pyrethroid and temephos insecticides.

In Indonesia, various types of insecticides have been widely used in *Aedes* mosquito control programs, including malathion from the organophosphate

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group, which has been used since 1972, while synthetic pyrethroids, including permethrin and deltamethrin, have been used since the 1980s until now.[19] The continuous use of insecticides, the absence of rotation in the use of insecticides and errors in application (dose, technical, etc.) can cause resistance in *Aedes* mosquitoes.[20] According to WHO, resistance occurs if the mosquito vector is able to successfully avoid contact with insecticides through evolutionary phenomena or cannot be killed by standard doses of insecticides.[21] The use of insecticides continuously and repeatedly as an effort to control *Aedes aegypti*, not only causes resistance, but can also cause environmental pollution and kill other fauna.[22] From this, it is necessary to have an alternative effort as an effort to reduce vector density that does not cause resistance and environmental pollution.

Plants in Indonesia with essential oils produced, have great potential that can be used as an alternative to vegetable larvicides. Astrid *et al*, wrote that as many as twenty-five plant species were observed, there were 68% having high effectiveness as vegetable larvicides.[23] We will review the plants that have been studied effectively to kill the larvae of *Aedes* sp. and reviewing *Aedes* sp resistance in Indonesia.

METHOD

Resources

In this paper, we review the resistance of *Aedes* sp. 2015 until now. We also collected research on biolarvicides against *Aedes* sp. who have researched through two electronic databases (Pubmed and Google Scholar). In the first stage of searching the database, 369 studies were obtained. There are two criteria to include research in this review, the first is research related to resistance in *Aedes* sp. since 2015 until now. The second is a study related to the effectiveness of biolarvicides against dengue vectors published in 2010 until now. Review articles and unpublished research were not included in this review (figure 1). In the final search for resistance, 16 studies were included (table 1) and 26 studies related to biolarvicides (table 2).

Search term

The keywords for the search process consisted of two domains, "Resistance" (*Aedes* sp. resistance to insecticides) and "Biolarvicides" (biolarvicides to dengue vectors). This literature review limits the type or research design to experimental research.

Aedes sp. mosquito resistance

Chemical vector control using insecticides is one of the more popular control methods in the community compared to other control methods. Insecticide targets are mature and immature stages. Since insecticides are toxic so their use should reduce their impact on non-target organisms including the environment and mammals. In addition, the determination of the type, dose, and application of insecticides is an important prerequisite for understanding vector control policies. Chemical insecticides for controlling dengue fever include Organophosphates (Malathion, methylpyrimiphos) and Pyrethroids (Cypermethrine, Lambasihalotrin, Cypermethrin, Permethrin, S-Bioaletrin, etc.).[24]

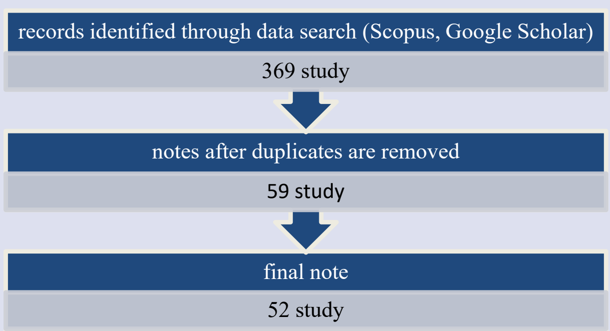

Figure 1: Study selection flowchart

The research collected consisted of research conducted on several islands in Indonesia (Table 1). For *Aedes aegypti* several insecticides commonly used in Indonesia. We reviewed for adult and immature *Aedes aegypti*. Table 1 shows the relevant studies on resistance in *Aedes* sp. in Indonesia in 2015 – 2020.

With the use of malathion in Indonesia and temephos as insecticides in the national dengue fever control program since the 1970s. One of the factors that determine the effectiveness of the use of these two insecticides is the level of susceptibility of vector mosquitoes both at the larval and adult stages.[25] The massive use of insecticide-based controls has contributed to the development of insecticide resistance, with increasing challenges in eliminating the *Aedes* mosquito and increasing the risk of dengue transmission.[26] Tolerance is the ability of insects (mosquitoes) to withstand the effects of insecticides which are usually lethal. *Aedes* sp. resistance. occurs against pyrethroid insecticides, temephos, malathion, cypermethrin, permethrin. Continuous monitoring of mosquito vector resistance status is very important in the effectiveness of dengue fever control.

Aedes Sp. resistance in Indonesia

Jawa Island: Java Island has six provinces. Several studies from 2015 until now on the island of Java have found resistance in several places. D.K.I. Jakarta, *Aedes aegypti* is resistant to organophosphate insecticides.[25,27,28] In West Java Province, *Aedes aegypti* larvae were indicated to be resistant to temefos.[29,30] In Central Java Province, *Aedes* aegypti is resistant to malathion[31] and the active ingredient of mosquito venom formulation for burns,[32] resistance has occurred in *Aedes aegypti* larvae resistance to pyrethroids was detected in *Aedes Aegypti*.[33]

Sumatera Island: On the island of Sumatra, we found three studies related to *Aedes* resistance. Bengkulu Province, *Aedes aegypti* 0.8n cypermethrin 0.05% against the insecticide malathion.[34] In Aceh, the susceptibility status of the larval stage DHF vector to 0.02 ppm temefos showed tolerance. Meanwhile, in North Sumatra and Jambi *Aedes aegypti* was resistant to malathion 0.8%, cypermethrin 0.05%, and lambda cyhalothrin alpha cypermethrin.[35]

Kalimantan Island: On the island of Kalimantan, in the Province of South Kalimantan, Kotan Banjarbaru, the results of the resistance test to the insecticides malathion, cypermethrin, lambdasihalothrin and deltamethrin by *Aedes aegypti* are already in the resistance stage.[36] Meanwhile in Banjarmasin City, it showed the development of *Aedes aegypti* resistance to the tested insecticides. *Aedes aegypti* mortality was less than 90% with the highest resistance observed to 0.75% permethrin.[37]

Sulawesi Island: On the island of Sulawesi, we found research in three provinces. In North Sulawesi Province, *Aedes aegypti* was highly resistant to malathion 0.8%,[30] while research by Yeslin Mantolu *et al*[30] *Aedes aegypti* larvae rejected the high position of four cities (Bandung, Bogor, Makassar, pal) to permethrin. While in Makassar, there is a relationship between the presence of *Aedes aegypti* larvae and humidity.[38]

Papua Island: We did not find any research related to Aedes sp. carried out in the Papua region since 2015.

Biolarvacides in Indonesia

Chemical insecticides such as malathion, DDT and pyrethroids which are commonly used in vector control, are known to cause problems such as contamination of mosquito species, residual effects and resistance.

Table 1: The relevant studies on resistance in *Aedes* sp. in Indonesia in 2015–2020.

No	Author	Sample (Location)	Finding
1	Heni Prasetyowati, *et al* (2016)	*Aedes aegypti* (West Jakarta, East Jakarta & South Jakarta, DKI Jakarta)	Vulnerability status Aedes aegypti is resistant to organophosphorus insecticides (temephos 0.02 ppm and malathion 0.8 %).
2	Dyah Widiastuti, *et al* (2016)	*Aedes aegypti* (Pekalongan Regency, Central Java)	Aedes aegypti is resistant to malathion
3	Hubullah Fuadzy (2015)	*Aedes aegypti* (Tasikmalaya City, West Java)	Aedes aegypti larvae are indicated to be resistant to temefos
4	Nur Handayani, *et al* (2016)	*Aedes aegypti* (Semarang City, Central Java)	Resistance has occurred in *Aedes aegypti* larvae in the buffer zone of Tanjung Emas Harbor
5	Steven Jacub Soenjono, *et al* (2017)	*Aedes aegypti* (Tomohon City, North Sulawesi)	*Aedes aegypti* is highly resistant to 0.8% malathion.
6	Miko Sudiharto, *et al* (2020)	*Aedes aegypti* (Bengkulu Province)	*Aedes aegypti* is resistant to the insecticide malathion 0.8% and cypermethrin 0.05%
7	Marlik, *et al* (2018)	*Aedes aegypti* (Kediri Regency, East Java)	*Aedes aegypti* is resistant to malathion 0.8%.
8	Mara Ipa, *et al* (2017)	*Aedes aegypti* (Aceh Besar District, Aceh)	The susceptibility status of the larval stage DHF vector to 0.02 ppm temefos showed tolerance.
9	Yerslin Mantolu, *et al* (2016)	*Aedes aegypti* (Bandung West Java, Bogor West Java, Makassar South Sulawesi and Palu Central Sulawesi)	*Aedes aegypti* larvae from four cities (Bandung, Bogor, Makassar, and Palu) were resistant with high status to permethrin.
10	Sunaryo dan Dyah Widiastuti (2018)	*Aedes aegypti* (North Sumatra Province and Jambi Province)	*Aedes aegypti* has been resistant to 0.8% malathion, 0.05% cypermethrin, and lambda cyhalothrin and is still tolerant to alpha cypermethrin.
11	M. Rasyid Ridha, *et al* (2018)	*Aedes aegypti* (Banjarbaru City, South Kalimantan)	*Aedes aegypti* are resistant to the insecticide's malathion, cypermethrin, lambdasihalothrin and deltamethrin.
12	Dwi Anggriani Wahyu Mukti (2016)	*Aedes aegypti* (Semarang City, Central Java)	*Aedes aegypti* are already resistant to Active Ingredients of Mosquito Poisons in Fuel Formulation
13	Penny Humaidah Hamid, et al (2017)	*Aedes aegypti* (Jakarta City, DKI Jakarta)	*Aedes aegypti* is already resistant to several insecticides that are often used.
14	Penny Humaidah Hamid, *et al* (2017)	*Aedes aegypti* (Denpasar City, Bali)	Development of *Aedes aegypti* resistance to insecticides. The highest resistance was observed to 0.75% permethrin.
15	P. H. Hamid, *et al* (2018)	*Aedes aegypti* (Banjarmasin City, South Kalimantan)	*Aedes aegypti* is already resistant to several insecticides that are often used. The highest resistance to 0.75% permethrin.
16	Tri Baskoro Tunggul Satoto, *et al* (2018)	*Aedes aegypti* (Magelang city, Central Java) Several KDR mutations associated with resistance to pyrethroids were detected in *Aedes Aegypti*.	Several KDR mutations associated with resistance to pyrethroids were detected in *Aedes Aegypti.*

Table 2: The relevant studies on biolarvicides in *Aedes* sp. in Indonesia in 2010–2020.

No	Author	Plant Type	Finding
1	Indriantoro Haditomo (2010)	Clove leaf (*Syzygium aroticum* L.)	The LC_{50} value is 0.040% or 400 ppm and LC_{99} 0.091% or 910 ppm.[40]
2	Nuning Irnawulan Ishak, *et al* (2013)	Lemon peel (*Citrus Amblycarpa*)	Utilization of lime peel extract has the potential to be an alternative natural larva for *Aedes aegypti* larvae.[41]
3	Wahyu Wira Utami, *et al* (2012)	Kepyar-Rizinusblatter *Ricinus communis* L.	The LC_{50} value was 138.995 ± 1.5µg/mL.[42]
4	Indri Ramayanti, *et al* (2016)	Papaya leaves (*Carica papaya* Linn)	The LC_{50} value is 3.73%.[43]
5	Sri Wahyuni Handayan, *et al* (2018)	Tobacco Leaves (*Nicotiana tabacum* L)	Tobacco extract from Temanggung has the strongest larvicidal activity against *Aedes Aegypti* at LC_{90} 212ppm, followed by Semarang tobacco at LC_{90} 241ppm and Kendal tobacco at LC_{90} 447ppm.[44]
6	Haqkiki Harfriani (2012)	Soursop (*Annona muricata* L.)	Leaf Extract sauerkraut leaf extract is effective in killing mosquito larvae.[45]
7	Khairun Nisa, *et al* (2015)	Noni Seed and Leaf Extract (*Morinda Citrifolia* L.).	Noni seed extract is known as *Aedes aegypti* azide larvae. Much more effective than noni leaf extract.[46]
8	Dyah Ayu Widyastuti, *et al* (2016)	Soursop (*Annona muricata*)	Extraction of bioactive compounds from A. muricata has been proven as a repellent for *Aedes albopictus* larvae and can be used as a safe natural insecticide with low LC_{50}. To be developed.[47]
9	Hebert Adrianto, *et al* (2017)	Grapefruit Leaves (*Citrus maxima*)	Non-polar extracts from C. maxima leaves are toxic and can cause *Aedes aegypti* mortality.[48]
10	Emi Minarni, *et al* (2013)	Ethyl Acetate of Kemuning Leaves (*Murraya paniculata* L)	Administration of ethyl acetate extract from yellow leaves can reduce the number of *Aedes Aegypti* larvae.[49]
11	Novi Ervina, *et al* (2014)	Cassava leaves (*Manihot utilissima* Pohl)	The LC_{90} value is 2.613%.[50]
12	Meidy Shadana, *et al* (2017)	Papaya Leaves (*Carica papaya*)	The LC_{50} value (24 hours) is 945,165ppm and the LC_{90} (24 hours) is 1495,219ppm.[51]
13	Kiki Rosmayanti (2014)	Soursop seeds (*Annona muricata* L.)	The LC_{50} value is 603ppm and the LC_{99} is 3713ppm.[52]
14	Dina Pratiwi, *et al* (2015)	Ethyl Acetate Antiting Herbs (*Alcalyphaindica*. L)	The LC_{50}value is 72.4435ppm.[53]
15	Sogandi & Fadhli Gunarto (2013).	Ethyl acetate leaves Bangun-bangun (*Plectranthus amboinicus*)	The LC_{50}value is 5.56%.[54]

16	Zulhar Riyadi (2018)	Rambutan seeds (*Nephelium lappaceum* L.)	The LC_{50} and LC_{90} values were 0.975% and 3.473%, respectively.[55]
17	Anna Yuliana, *et al* (2021)	Banana jackfruit leaf (*Musa x paradisiaca* L.)	Ethanol extract of jackfruit banana leaf is very effective as *Aedes aegypti* mosquito larvae.[56]
18	Wira Desy Kusumawati, *et al*	Soursop Leaf Extract and Lemongrass Stem Extract.	The mortality rate of *Aedes aegypti* larvae is influenced by the nature of the natural killer larvae extract.[57]
19	Susilawati dan Hermansyah (2015)	Bitter gourd (*Momordica charantia* L.)	The LC_{50} values are 0.13mg/mL (24 hours) and 0.11 mg/mL (48 hours).[58]
20	Apriangga (2014)	Lemongrass (*Cimbopogon citratus*)	The LC_{50} value is 973.7ppm or 0.973%.[59]
21	Roselina Panghiyangan, *et al* (2012)	Turmeric rhizome (*Curcumadomestica* val.)	Turmeric rhizome extract effectively kills *Aedes aegypti* larvae.[60]
22	Roni Koneri, *et al* (2016)	Mahogany seeds (S. *macrophylla* King)	The LC_{50} yield is 142.14ppm to 921.55 ppm.[61]
23	Maretta Rosabella Purnamasari, *et al* (2017)	Duftende Pandanblatter (*Pandanus amaryllifolius* Roxb)	The values obtained by LC_{50} and LC_{90} were 2.113% n 3.497%.[62]
24	Pranatasari Dyah Susanti (2013)	Bark Gemor (*Nothaphoebe coriacea* K.)	Bark extract as an effective biological larval agent for the death of *aedes aegypti* larvae.[63]
25	Ratna Sari Dewi (2020)	Aloe Vera Leaf (*Aloe vera* (L) Burm.f.)	The most effective concentration of aloe vera leaf extract as *Aedes aegypti* mosquitoes was 0.075%.[64]
26	Ratna Widyasari (2018)	Sweet Orange Peel (*Citrus x aurantium* L.)	The LC_{50} value is 0.20% and the LT_{50} value is 9.185 hours.[65]
27	Esy Maryanti, *et al* (2011)	Kaffir lime leaves (*Citrus hystrix* DC)	The LC_{50} value is 4015,880ppm and the LC_{90} is 6961,822ppm.[66]
28	Ratna Yuliawati, *et al* (2017)	Fruit Petals Sonneratia alba	Ethanol extract from Sonneratia alba is effective against the death of *Aedes aegypti* larvae.[67]
29	Ni Luh Komang Sumi Arcani, *et al* (2017)	Citronella (*Cymbopogon Nardus* L)	Ethanol extract of citronella from several concentrations was declared effective as a larvicide.[68]
30	Makkiah, *et al* (2019)	Fragrant Lemongrass (*Cimbopogon nardus* L.)	The LT_{50} value is 10.45 hours. this means that it takes 10.45 hours to kill 50% of the test larvae population.[69]
31	La Basri (2018)	Cinnamon (*Cinnamomum Burmani*)	The LC_{50} value is 0.10%.[70]
32	Evy Ratnasari Ekawati, *et al* (2017)	Skin of Lime Fruit (*Citrus aurantifolia*)	The LC_{50} of *Aedes aegypti* mosquito larvae is 3,419%.[71]
33	Fatma Sari Siharis, *et al* (2018)	Kirinyuh Leaves (*Chromolaena odorata*)	The LC_{50} result is 5.934%.[72]
34	Nazzirah A. Ammari, *et al* (2021)	Papaya Leaves (*Carica papaya* linn)	The LC_{50} value is 95.0%, estimated at 17.263 and LC_{90} is 95.0%, estimated at 38.900.[73]
35	Suhaimi, *et al* (2018)	Celery Stem (*Avium graveolens*)	The LC_{50} value is 0.221% The LC_{100} value is 0.839%.[74]
36	Yuneu Yuliasih, *et al* (2017)	Coastal Ironwood Seed (*Pongamia pinnata*)	The LC_{50} value for P. *pinnata* extracts with methanol solvent was 141.88ppm. $108._{19ppm}$ for *Aedes albopictus* against *Aedes aegypti*. At LC_{50} extract of P. *pinnata* using chloroform solvent was 346.06ppm against *Aedes aegypti* and 222.29ppm against *Aedes albopictus*.[75]

Therefore, we need to look for alternatives to insecticide abuse and look for alternatives that are safer and more environmentally friendly.[39] Research on plants that can be used as biolarvicides is very useful for alternative control and improving public health. Table 2 shows the relevant studies on biolarvicides in *Aedes* sp. in Indonesia in 2010–2020.

This review provides better insight into the effectiveness of laboratory-tested plants as larvicides and plans for further research for community application. In 2005, WHO published experimental guidelines for the field of larval killing testing by establishing standard procedures for larval killing testing mechanisms. There are three stages of testing the effectiveness of larvicides, namely the first phase (laboratory studies), the second phase (small field studies), and the third phase (large field studies).[76]

The study on biolarvicides that we collected in Indonesia is still in the first phase. Before being distributed to the public, these studies need further research in the second and third phases. The research in the second phase aims to determine the efficacy in ecological settings, determine the method and its application, determine the impact and residues, and to determine the effect on non-target organisms. While the research in the third phase aims to determine the operational and community acceptance.[76]

CONCLUSIONS AND FUTURE PERSPECTIVES

The use of insecticides in controlling *Aedes* as a vector of dengue is still being carried out in Indonesia. However, the occurrence of resistance will lead to the ineffectiveness of insecticides in controlling this disease. There needs to be a review of the concept of using this insecticide to cause the death of *Aedes* larvae and adult mosquitoes. Biolarvicides can be an alternative in dealing with chemical insecticide resistance. We found quite a lot of plants that can be used as an alternative. However, the research found is not enough to prove that the plant can actually be programmed by the government. Further research is needed in the small field phase and the large field phase to prove that these plants are effective against the mortality of *Aedes* larvae and are safe against the possibility of contamination. In short, resistance to the *Aedes* vector will worry the government's program to control DHF. A well-researched understanding of resistance is essential for developing effective methods of controlling *Aedes*.

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