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Original Article

The Longevity of *Aedes aegypti* Larvae in Several Water Sources in Surabaya

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ABSTRACT

Aedes aegypti transmits the dengue virus that causes Dengue. The high number of DVI cases is the existing breeding places of *Ae. aegypti*. The water sources used by the community and the surrounding environment are essential media for living *Ae. aegypti* larvae. This recent study aimed to detect the longevity of *Ae. aegypti* larvae in different water sources in Surabaya and the killing effect of temephos. An analytical observational and experimental study was conducted in August–September 2021. Twenty-instar III *Ae. aegypti* larvae were put in each 100 ml beaker glass containing different water sources, such as rain, well, mineral, new and used bath water, and antiseptic soapy water. Fungi in water sources were examined. Two groups were set with and without temephos, the final temephos concentration was of 0.00001 ppm. Live *Ae. aegypti* larvae, pupae, mosquitoes were observed every 24 hours for seven days without feeding. Living larvae were still found on Day 7 in all water sources with and without temephos. There were more larvae live in soapy water without temephos, particularly on Day 2 to Day 6, compared to other water sources either without or with temephos. In contrast, many larvae died in mineral water with temephos. Some larvae turned into pupae, started on Day 1. Pupae and mosquitoes were mostly found in rain water with temephos. *Ae. aegypti* larvae survived better in soapy water either with or without temephos. Temephos seemed to be effective to kill *Ae. aegypti* larvae in mineral water, and might induce larvae in turning to pupae and mosquitoes quickly at low concentration.

Keywords: *Ae. Aegypti*, larvae, water sources, Surabaya

ABSTRAK

Aedes aegypti menularkan virus dengue penyebab Infeksi Virus Dengue. Penyakit ini terjadi tertinggi di Asia dan menempati urutan pertama setiap tahun, termasuk Surabaya, Indonesia. Faktor penyebab tingginya angka kasus IVD adalah keberadaan tempat perkembangbiakan larva *Ae. aegypti*. Sumber air yang dimanfaatkan oleh masyarakat dan lingkungan sekitar merupakan media yang penting bagi kehidupan larva *Ae. aegypti*. Penelitian terbaru ini bertujuan untuk mendeteksi keberlangsungan hidup *Ae. aegypti* di berbagai sumber air di Surabaya dan efek membunuh temefos. Studi observasional analitik dan eksperimental dilakukan pada bulan Agustus–September 2021. Dua puluh instar III *Ae. Larva aegypti* dimasukkan ke dalam masing-masing gelas beker 100 ml yang berisi sumber air yang berbeda, seperti air hujan, sumur, mineral, air mandi baru dan bekas, dan air sabun antiseptik 0,5 ppm. Jamur dalam sumber air diperiksa. Dua kelompok ditetapkan dengan dan tanpa temefos, dengan konsentrasi temefos akhir 0,00001 ppm. Larva *Ae. aegypti* yang hidup, pupa, nyamuk diamati setiap 24 jam selama 7 hari tanpa diberi makan. Banyak Larva yang hidup

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13 dalam air sabun tanpa temephos, terutama pada hari ke-2 hingga hari ke-6, dibandingkan dengan sumber air lain baik tanpa maupun dengan temephos. Sebaliknya, banyak larva mati dalam air mineral dengan temephos. Beberapa larva berubah menjadi pupa dimulai pada hari 1. Pupa dan nyamuk banyak ditemukan di air hujan dengan temephos. Larva *Ae. aegypti* bertahan lebih baik dalam air sabun baik tanpa atau dengan temephos. Temephos efektif untuk membunuh larva *Ae. aegypti* dalam air mineral, dan dapat menginduksi larva berubah menjadi pupa dan nyamuk dengan cepat pada konsentrasi rendah.

Kata kunci: *Ae. Aegypti*, larva, sumber air, Surabaya

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INTRODUCTION

Aedes aegypti mosquito is a global vector of human diseases, such as yellow fever, dengue, and Zika through the bite of the adult female mosquito. The size and the success for being a mosquito are determined by environmental conditions during the larval growth phase to pupation.¹ The geographic expansion of *Ae. aegypti* has a significant value that has been causing epidemics in different countries of Africa, the Indian Ocean, Asia, Pacific, Europe, and America despite all the considerable efforts made for their control.² Almost all tropical countries are not free from the spread of these viruses' diseases by these mosquito carriers. Especially, as a carrier of the dengue virus, *Ae. aegypti* is the primary vector.³

In the Southeast Asia and Western Pacific region, about 1.8 billion people are at risk of contracting the dengue virus. Dengue Fever (DF)/Dengue Hemorrhagic Fever (DHF) epidemics have been reported in Bhutan, India, Maldives, Bangladesh, and Pakistan, and due to the porous borders with India, Nepal is at high risk of DF/DHF outbreaks.⁴ Dengue Virus Infection (DVI) is a public health problem in Indonesia with a fairly high morbidity and mortality rate, and has the potential to cause Extraordinary Events and can also have an impact on community economic losses.⁵

In 2015, cases of DVI in Surabaya experienced many changes, where there was an increase and decrease in different cases every month.^{6,7} In 2019, there were 138,127 DVI cases with an Incidence Rate (IR) of 51.48 per 100,000

populations. This number increased compared to 2018 of 65,602 cases. Deaths due to DVI in 2019 also increased compared to 2018 from 467 to 919 deaths.⁸

The development of the *Ae. aegypti* mosquito is based on its ability to adapt to the environment so that it is possible to overcome disturbances caused by natural phenomena. The ability mentioned is about surviving dry conditions and living without water for several months on the sides of the container walls or to adapt to human intervention, such as eradicating mosquito nests.⁹ Reproduction sites of *Ae. aegypti* are defined as any water retention container in which the immature stages of *Ae. aegypti* are found. Usually *Ae. aegypti* oviposition sites are found in artificial containers, such as flower pots, stems or water storage tanks, discarded plastic or metal containers, buckets and tires.^{10–12}

Clean water used for daily needs produces domestic liquid waste, like waste water from bathrooms that contains soap (NaOH and KOH/alkali).¹³ In a study, it showed that *Ae. aegypti* eggs grow more quickly in water with soap than clean water. This defines bath soap and waste water as the most chosen and better site in the development of *Ae. aegypti* larvae into adult.¹³ Another study reveals that *Ae. aegypti* larvae are able to survive in sewer water that has been remained in a single site till it is clear, which means the *Ae. aegypti* eggs which become mosquitoes are more able to breed in clear water than dirty water.¹⁴ Another previous study stated that the most preferred water reservoir properties for the reproduction of *Ae. aegypti* mosquitoes are

well water sources with the complements such as dark in color, without a lid, unexposed to direct sunlight and without draining during more than a week.¹⁵

In addition, *Ae. aegypti* larvae are able to live together alongside other microorganisms, such as fungi. Fungi usually can be found growing in the same water site as *Ae. aegypti* larvae¹³ and could be served as food for the larvae¹⁴. However, fungi could also be as a lethal pathogen to these larvae and they have been used to control mosquito vectors.^{14,15}

Surabaya is a DVI endemic area, and has various water sources in various circles of society. Therefore, research is needed on some of these water sources in order to pay attention to their effect on the growth of *Ae. aegypti* larvae. Moreover, the effectiveness of water sources as a breeding ground for *Ae. aegypti* larvae have not yet been fully studied. The purpose of this study was to detect the longevity of *Ae. aegypti* larvae in several types of water sources in Surabaya, as well as the effect of using temephos on both types of water sources.

MATERIALS AND METHODS

Sample Collection

An analytical observational and experimental study was conducted in Institute of Tropical Disease (ITD) Universitas Airlangga, Surabaya, Indonesia from August-September 2021. The sample in this study is *Ae. aegypti* instar III larvae that were collected from the breeding at the Entomology Laboratory, ITD Universitas Airlangga. These larvae were selected using simple random sampling with a total of 20 individuals for each 100 mL beaker glass (Herma, Germany).

Type of water sources

Variables in this study were rain water, antiseptic soapy water (Dettol¹⁶ with concentration of 0.5 ppm (mg/L)), well water, mineral water, new and used bath water.

Temephos Preparation

Evaluation of the positive control in this study on the longevity of *Ae. aegypti* instar III larvae used temephos with a concentration of 0.00001 ppm (mg/L). The usage application of temephos was in accordance with the WHO recommendation using the commercial product Abate® 1G (BASF, Indonesia).¹⁶

Fungi examination

Fungi examination of each water source was only carried out once on the first day at the Laboratory of Medical Microbiology Faculty of Medicine Universitas Airlangga. The water sources were homogenized by vortex mixer for 30 seconds. One milliliter of each homogenized water source was put in the Saboroud Dextrose Agar (SDA) medium, and kept at room temperature for seven days. Then, fungi were identified from sample film stained with Lactophenol Cotton Blue under light microscope (Olympus© CX22, Japan) with 400 and 1000 magnifications.

Bioassay

The bioassay for the longevity of *Ae. aegypti* was performed in 14 of 100 ml beaker glasses, divided into two groups of water type. Each group contained six beaker glasses Each beaker glass was filled with each water type. First group was without temephos, second group was treated with 0.00001 ppm of temephos. Each beaker glass was filled with 20 larvae. The other two glass beakers were used as controls, filled with tap water from the laboratory either with or without temephos.

There were 14 beaker glasses, and the total sample was 280 *Ae. aegypti* instar III larvae. The variables were divided into two groups, then the first group was not mixed with temephos, while the second group was mixed with temephos with a concentration of 0.00001 ppm. Then these water sources were filled one by one in 100 mL beaker glass.

These *Ae. aegypti* larvae were observed every 24 hours for seven days without feeding until one had turned into a pupae or mosquito.

Statistical Analyzes

The data collected are in the form of numbers and percentages, and will be carried out in an average figure completed with its mean value.

The data variables were also analyzed using the *Chi-square* test with a significant comparison or difference determined by $p < 0.05$ value.

Ethical Clearance

This study has been approved with the license from Medical Research Ethics Commission, Faculty of Medicine, Universitas Airlangga Number 242/EC/KEPK/FKUA/2021.

RESULTS AND DISCUSSION

This study is the first to be conducted using several different water sources in Surabaya. In addition, this study also used temephos which was mixed in several water sources. Drastically, the average live larvae in mineral water without temephos was reduced significantly on Day 3 (D3) compared to Day 1 (D1), 6/20 vs 16/20 (p -value = < 0.00001 , $p < 0.05$, *Chi-square* test). Therefore, the average live larvae in mineral water without temephos seemed to be equal compared to used bath water without temephos since D4 until D7. Interestingly, the average live larvae in soapy water without temephos were decreased little by little per day so that in soapy water without temephos many larvae could still survive (Figure 1a).

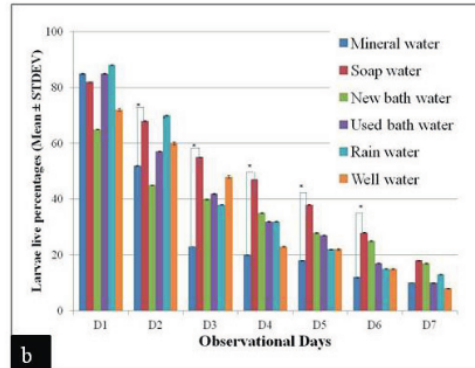
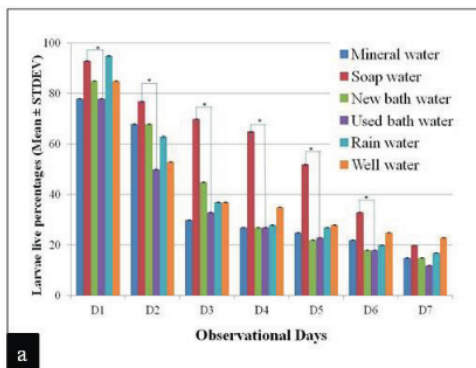


Figure 1. Live *Ae. aegypti* larvae inside **a)** water sources without temephos, and **b)** water sources with temephos during seven days of observation. Dark blue bar is mineral water, red bar is soapy water, green bar is new bath water, purple bar is used bath water, blue bar is rain water, and orange bar is well water. *means $p < 0.05$, *Chi-square* test Y Axis: Percentage of live larvae \pm SD

In mineral water with temephos it was also decreased significantly on D3 compared to D1, 5/20 vs 17/20 (p -value = < 0.00001 , $p < 0.05$, *Chi-square* test). Rain water with temephos was the highest among others until D2; however, soapy water with temephos took first place and remained on top until the last day (D7) of the observation (Figure 1b).

In control water, in which the water was taken from the laboratory, it did not demonstrate a significant decrease in the number of live larvae. The results are shown in Figure 2, where on D1 to D4, control water with temephos was higher than without temephos.

However, on D7, the number of live larvae in control water with temephos was lower compared to that of water without temephos, and they were not significantly different (4/20 vs 6/20, p -value = < 0.24305 , *Chi-square* test).

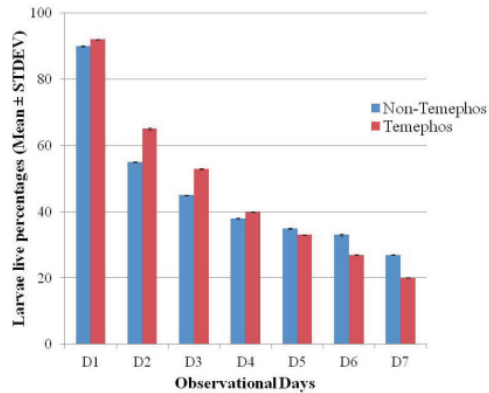


Figure 2. Average living *Ae. aegypti* larvae in control water during seven days of observation. Blue bar is control water without temephos, and red bar is control water with temephos

Table 1 shows a calculation of significant differences using *Chi-square* test on the number of live larvae in six different water sources with and without temephos on D1 to D7 of observation. Apparently, only the number of live larvae on D6 and D7 in all water sources without temephos were insignificantly different. Thus, only the number of live larvae in all water sources with temephos on D7 was insignificantly different. The calculation in the control waters was all insignificantly different.

Apart from the larvae that managed to survive, there were also several larvae that achieved in turning to pupae and mosquitoes during the seven days of observation of this study. Afterwards, the results of each water sources demonstrated, even on D1, that there were still some live pupae from mineral water, used bath water and well water without temephos. More pupae were also found in rain water, new bath water, soapy water and mineral water with temephos.

During the seven days of observation, rain water with temephos resulted in the highest number of pupae. Pupae transformation to adult mosquito took at least two days at average.

However, in temephos water sources, the total number of mosquitoes and pupae were unmatched due to the other two pupae that died during the study. The fact showed that some water sources, such as new bath water and rain water without temephos, as well as used bath water and well water with temephos, did not produce pupae at all (Table 2).

Examinations of fungi on the culture of water samples used in study were also performed. However, no fungi were found in either samples of water, but only the blue color of results was seen on the surface of water culture. This was the absorption of LPCB (Lactophenol Cotton Blue) into the water sample (Figure 3).

Table 1. *P*-Value of the Comparison Among the Average of Live *Ae. aegypti* Larvae Inside Water Sources Either with or without Temephos During 7 Days of Observation

Water Sources	D1	D2	D3	D4	D5	D6	D7
Non-temephos	0.0008	0.0118	0.0000	0	0.0000	0.0970	0.3549
Temephos	0.0014	0.0023	0.0002	0.0007	0.0255	0.0211	0.1839
Control	0.6213	0.1489	0.2577	0.7719	0.7655	0.3546	0.2430

p<0.05 is significant, *Chi-square* test

Table 2. Development of pupae and mosquito during 7 days of observation

Water sources	n	D0		D1		D2		D3		D4		D5		D6		D7		Total	
		p	m	p	m	p	m	p	m	p	m	p	m	p	m	p	m	p	m
a	60	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1
b	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c	60	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	2	2
d	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e	60	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1
f	60	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1
a+	60	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	2	2
b+	60	0	0	6	0	1	0	6	0	1	0	0	0	0	0	0	0	7	7
c+	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d+	60	0	0	4	0	1	1	2	3	2	2	0	1	0	0	0	0	9	7
e+	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
f+	60	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	2	1

Abbreviations: n = larvae total of three times repetition, p = pupae, m = mosquito, a = mineral water, b = new bath water, c = used bath water, d = rain water, e = well water, f = soap water, a+ = mineral water with temephos, b+ = new bath water with temephos, c+ = used bath water with temephos, d+ = rain water with temephos, e+ = well water with temephos, f+ = soap water with temephos

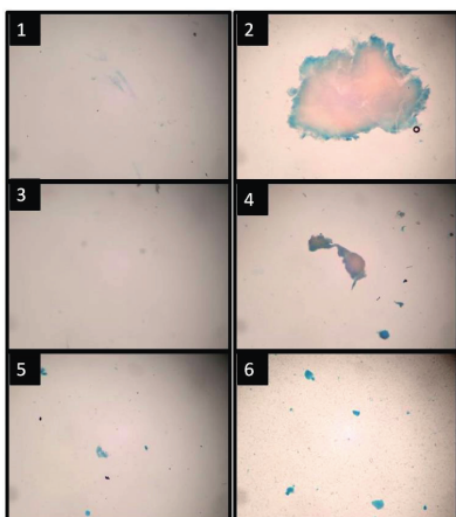


Figure 3. No fungi were detected in the water sources; 1) Mineral water, 2) Rain water, 3) Soapy water, 4) Well water, 5) New bath water, and 6) Used bath water

The interesting results of this study showed that larvae were not able to survive in mineral water either with or without temephos. This might explain that the mineral water as a clean water could inhibit the process of larval development to survive and to become pupae. In fact, mineral

water identified as microbiologically healthy water had a guarantee of the absence of the most important contamination indicators¹⁷ and categorized its division into macronutrients like calcium, phosphorus, magnesium, sodium and potassium, and micronutrients like cobalt, iron, iodine and copper.¹⁷ In addition, the experiment was conducted in a glass container, which was not the usual breeding place of *Ae. Aegypti*. The natural breeding places of this mosquito are flower pots, stems or water storage tanks, discarded plastic or metal containers, buckets and tires.¹⁰⁻¹² Moreover, Baharuddin and Rahman²² found that *Ae. aegypti* larvae were mostly obtained in plastic containers such as plastic barrels and used rubber tires¹⁸. It suggested that *Ae. aegypti* larvae could not live long inside a clean glass containing mineral water.

On the other hand, soapy water either with or without temephos was very prominent with a high percentage of live larvae. This means that *Ae. aegypti* larvae could still survive better in water mixed with antiseptic soap 0.5 ppm, rather than other types of water. This might be because the soapy water contains sodium palmate, talc, sodium palm kernelate and paraffin liquidum¹⁹ that could provide food for these larvae to survive. Another study stated that soapy water with an equivalent concentration on water pollution in

nature also could become a good breeding place for *Ae. aegypti* larva to survive; however, it only works if the pH of the soapy water is less than 12.8.²⁰ It is suggested that temephos with a little concentration of 0.00001 ppm did not work effectively in soapy water. Therefore, the water for bathing and water reservoir should be drained.²¹

In control waters, no significant difference was found between water with or without temephos. It seemed that the control waters as media for living larvae were similar condition and the concentration of 0.00001 ppm temephos showed a low efficacy of larvicide.

There was no discovery of fungi in water sources used in this study. This happened because it was possible that the water sites where the water was taken had no prospect to grow fungi. Fungi usually grow in environments that have soil debris, insect remains, or dead leaves and plants.²²

A study revealed that fungi are used as food and provide nutrients for larval development. Therefore, fungi-mosquitoes associations are able to form a more commensal period in the gut of mosquito with slight or no effect on host survival.²³ An example is the yeast, *Saccharomyces cerevisiae*, which is commonly used to feed the larvae during its developmental phase.²³ The feeding behavior of adult mosquitoes also leads to the formation of adhesions of the fungi in the mosquitoes' hindgut. At least there are four fungi species of the genus *Smittium*, of the order *Harpelellas* that can attach to and increase on various mosquito species' hindgut without affecting larval development or survival.²³

On the other hand, there have been studies demonstrated the potential usage of fungi as a successful and ecologically safe strategy to control mosquito vectors.²⁴

Since few studies reported that the number and diversity of fungi are greater found on the surface water than in groundwater and tap water^{25–27}, the fungi are possibly contact with adult mosquitoes, some of which fungi are already infused together with chemical insecticides.^{28,29} Besides chemical materials, some of the fungi itself are pathogens to mosquitoes and larvae. Fungi species such

as *Entomophthora* sp. and *Coelomomyces* sp. are known as obligate pathogens, while other fungi order such as *Eurotiales*, *Hypocreales* and *Mucorales* are opportunistic pathogens that unfortunately cannot actively invade the mosquito body, but can set up an infection if ingoing through breaches in the cuticle.²³ Other fungal pathogens from water molds such as those in the genera *Lagenidium*, *Leptolegnia* and *Saprolegnia* are identified as facultative pathogens of mosquitoes, and obviously there are no commensals between these fungi and mosquitoes nor larvae.²³ Thus, there were no fungi in water sources in our study, which showed no fungi effecting into the larva life of *Ae. aegypti* in our study.

The use of temephos with concentration of 0.00001 ppm was applied in this study in order to find out its effect on the larval longevity. Temephos worked very well on killing *Ae. aegypti* larvae in mineral water, and water sources with temephos showed *Ae. aegypti* larvae turned to pupae and adult mosquitoes rapidly. The temephos' concentration of 0.00001 ppm seemed to effectively induce larva development into pupa.

Several studies have shown that the use of temephos could kill *Ae. aegypti* larvae very quickly, because the toxicity of temephos is absorbed into the body of the larvae.^{30–32} The absorbed toxin attacks the larvae's central nervous system, causing symptoms such as restlessness, hyperexcitability, tremors, convulsions, and paralysis.³²

Temephos inhibits cholinesterase enzyme, which causes a disorder in the larva nervous system due to the accumulation of acetylcholine in nerve endings, and this will lead to the larval mortality.^{30–33} A study in South Kalimantan showed that the lowest concentration of temephos was 0.005 ppm resulted in 39% of larvae mortality. The highest concentration of 0.030 ppm resulted in 100% of larvae mortality.³⁴ Comparing to other study, the *Ae. aegypti* larvae were continuously exposed with larvicide such as temephos, over a particular time at the larvicide would make a modification in the larvae genetics and brings resistance to temephos and other larvicides.^{35,36}

In this study, the use of temephos was at a concentration of 0.00001 ppm, where this concentration was very small and probably the concentration lacked the scale of larval killing when compared to the study in South Kalimantan. However, if observed from the overall point of view, this very small concentration of temephos could still kill *Ae. aegypti* larvae, particularly in mineral water, and showed the induction of larva development into pupa. Regarding this point, the use of temephos for larvicide should be adequate and in appropriate dose, based on the instruction written on the package and guidelines by Kemenkes RI and WHO.^{21,37}

CONCLUSIONS

Ae. aegypti larvae endured better in antiseptic soapy water with concentration of 0.5 ppm either with or without temephos compared to other water sources. Temephos with concentration of 0.00001 ppm was effective to kill *Ae. aegypti* larvae in mineral water, and might induce larval development into pupae and mosquitoes more quickly.

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CONFLICT OF INTEREST

There are no conflicts of interest between authors in this study.

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PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9
