

Activity of Activated Charcoal *Cymbopogon nardus* as a Larvicide in Controlling the Larvae of *Aedes aegypti*

Abstract

Dengue Hemorrhagic Fever (DHF) is a disease caused by ectoparasites. The ectoparasite in this disease is the *Aedes aegypti* mosquito which acts as a vector. *Aedes aegypti* is a type of mosquito that can carry the dengue virus that causes dengue fever. The symptoms of this disease are rarely noticed and can spread quickly. In addition, this disease also attacks all ages and can cause death. *Cymbopogon nardus* (L.) Rendle is an aromatic grass from the Poaceae family that produces essential oil mainly in the leaves. The essential oil from *C. nardus* is known as citronella oil has repellent activity. To determine the effect of citronella powder (*Cymbopogon nardus*) as activated charcoal packaged in bags for controlling *Aedes aegypti* in bathroom tubs. This study is an experimental posttest-only control group design consisting of a control group and a treatment group using activated charcoal from lemongrass at a concentration of 1%, 2%, and 2.5%, which was treated on *Aedes aegypti* mosquito larvae instar III five times test. Data analysis used the One-way Anova Statistical Product and Solutions (SPSS) Version 26 test. The results showed the effect of citronella powder (*Cymbopogon nardus*) as activated charcoal on the mortality of the third instar *Aedes aegypti* mosquito larvae. This is indicated by the p-value of 0.197. The high concentration of activated charcoal from lemongrass powder determines the high mortality of third instar *Aedes aegypti* larvae. This is indicated by the significant difference between the concentration treatments. For further research, an increase in the concentration of activated charcoal is needed to increase larval mortality.

Keywords: Activated charcoal; Lemongrass; Mortality; Third instar *Aedes aegypti* larvae, *Cymbopogon nardus*, Dengue Hemorrhagic Fever

INTRODUCTION

Mosquitoes are insects that significantly interfere with human life through their bites which cause itching and red spots on the skin. Mosquitoes act as vectors that can transmit diseases dangerous to humans, especially Dengue Hemorrhagic Fever (DHF). Dengue Hemorrhagic Fever (DHF) is one of the diseases caused by ectoparasites (Suwandono, 2019). Ectoparasites, as well as the primary vector of the Dengue virus, are the *Aedes aegypti* mosquito (Noshirma et al., 2020). Symptoms caused by DHF are rarely noticed and can be transmitted quickly. The disease affects all ages and can lead to death. Until now, no cure or vaccine has been found, so prevention is carried out by

breaking the chain of transmission through vector control (Kementrian Kesehatan Republik Indonesia, 2017; Suwandono, 2019).

Dengue cases in Indonesia in 2022 have been reported as many as 36,596 cases with 377 deaths. In April 2022, it was reported that there were an additional 2093 cases, and 27 deaths had been reported from 444 regencies/cities in Indonesia (Kementrian Kesehatan Republik Indonesia, n.d.).

Given the dangers and severe impacts, there is an effective method to prevent or minimize the transmission of dengue hemorrhagic fever, namely by breaking the life cycle of dengue vector mosquitoes through the destruction of their larvae (Sumi Arcani et al., 2017). The public often uses eradication with chemical insecticides because it is considered adequate and practical. Temefos is one of the most commonly used organophosphate group insecticides in the form of granules that can control mosquito populations in their breeding sites directly (Ipa et al., 2017). However, some studies have reported that the use of temefos impacts the resistance of an *Ae. aegypti* mosquito larva. The countries reporting this are Costa Rica, Brazil, Martinique Island, Colombia, Thailand, and Malaysia (Grisales et al., 2013; Poupardin et al., 2014). Mulyatno, K. C., Yamanaka, A., & Konishi, (2012) reported that in some areas of Surabaya Indonesia *Ae. aegypti* is also resistant to temefos. Prasetyowati et al., (2016) stated that the DKI Jakarta area *Ae. aegypti* is resistant to temefos. This needs to be watched out for because chemical insecticides are bioactive. Their residues can pollute the environment because they contain chemicals that are difficult to degrade in nature, are toxic to humans and pets, and cause resistance to disturb animals or larvae (Swacita, 2017).

Based on the losses caused by these chemical insecticides, alternative insecticides that are environmentally friendly, safe for humans, easy to obtain, and effective in controlling pest attack populations are needed, namely by using natural materials from plants as insecticides.

Based on the results of literature studies, it is said that the citronella plant (*Cymbopogon nardus*) contains compounds that can potentially be larvicides (Giroth et al., 2021). Larvicide is the best method of controlling the *Aedes aegypti* mosquito population. The action of larvicides as ectoparasiticides is by inhibiting the action of the acetylcholinesterase enzyme, which can later cause larval death (Kusumawati et al., 2018). Essential oils (ester) produced by lemongrass plants contain compounds such as citral, geraniol, citronellal, eugenol-methyl ether, dipentene, eugenol, kadinen, kadinol, and limonene. The most abundant chemical content contained in lemongrass is citronellal (34.6%), geraniol (23.17%), and citronellol (12.09%) (Bota et al., 2015). The Citronella contained in lemongrass essential oil has a characteristic and robust aroma that insects, including mosquitoes, are not like or avoid. In addition, it is a toxin that causes larval death because the larvae are continuously dehydrated (Aulung et al., 2014).

Using natural larvicides from lemongrass plants is expected to become later an alternative in breaking the chain of the spread of the *Aedes aegypti* mosquito. This is because using conventional larvicides has a harmful effect on the body, affecting the

work of the central nervous system through the inhibition of cholinesterase. The natural larvicides made will later be formed as activated charcoal. Activated charcoal is a carbon that is used as an adsorbent because it can absorb anions, cations, and molecules in the form of organic and inorganic compounds, both in the form of solutions and gases. Activated charcoal in health often is used as a therapy for poisoning caused by microorganisms. According to Lempang, (2014), external poisoning and secretory diarrhea therapy caused by bacterial toxins such as cholera, Salmonella, and Shigella, as well as the Coli pathogen group, can be given adsorbents to avoid absorption of toxins in the form of activated charcoal. According to Millenia, (2022), activated charcoal can also prevent insect bites. Based on the preceding, this study aimed to analyze the activity of activated charcoal as a larvicide of Citronella (*Cymbopogon nardus*) to control the larvae of *Aedes aegypti*.

MATERIALS AND METHODS

Research Place and Time

The development, rearing, and treatment of *Aedes aegypti* test larvae were conducted at the Tropical Disease Center (TDC) laboratory, Universitas Airlangga Surabaya. Meanwhile, Citronella activated charcoal (*Cymbopogon nardus*) is manufactured at the Health Chemistry Laboratory, Faculty of Health Sciences, University of Muhammadiyah Surabaya. This research was conducted from September 2021 to February 2022.

Materials and Instruments

The instrument used include binocular microscopes, ovens, sieves with sizes of 106 μm and 212 μm , larval test containers, porcelain cups, and whatman filter paper number 12. The material used is larva mosquito *A. aegypti* instar III with the same larval size criteria; tail does not allow bending, agile movement, and large. Activated charcoal is made from lemongrass plants (*Cymbopogon nardus*), temefos 1% use the ABATE brands 1GR, NaOH, EtOH 1%, and aquadest.

Data Collection Techniques

This research is experimental with Post Test Control Group Design, which uses the Larva test *A. Aegypti* instar III as the object of study. There were five treatments used in this study, namely the first treatment by giving a concentration of activated charcoal from lemongrass plants at 1% (S1), the second treatment by giving a concentration of activated charcoal from lemongrass plants at 2% (S2), the third treatment with the provision of serai plant activated charcoal concentration of 2.5% (S3), the fourth treatment was negative control using EtOH 1% and aqueous (K-), and the fifth treatment was positive control using temefos 1% brand ABATE 1GR (K+). Each of the perpetrators was repeated five times. The number of larvae of *A. aegypti* in each treatment was 25 heads. Testing is carried out for 24 hours.

- a. Observations of larval mortality of *Aedes aegypti*
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The results of the observations were obtained from the number of larvae that died in each treatment by looking at the absence of movement in the larvae.

A.aegypti larval mortality data were analyzed using the One Way ANOVA Statistical Product and Service Solutions Version 26 test. The results of the study are considered significant if the p-value < 0.05.

RESULTS AND DISCUSSION

Activated charcoal in this study is a porous solid containing 85-95% carbon produced from heating at high temperatures. This charcoal is obtained from lemongrass plants which are processed through the activation stage with NaOH. Activated charcoal is shown in Figure 1.



Figure 1. Activated charcoal from the citronella plant (*Cymbopogon nardus*)

Data from observations of the mortality of *A.aegypti* larvae treated with each treatment, namely S1, S2, S3, K (-), and K (+), with five repetitions, there were differences (Table 1). The highest average death of *A. aegypti* larvae was in the K(+) treatment of 25 heads, followed by the S3 treatment of 20 heads, followed by the S2 treatment of 15 heads, the S1 treatment of 11 heads, and the K(-) treatment the dead larvae were zero.

Table 1. *Aedes aegypti* Mosquito Larva Mortality Data

CONCENTRATION	NUMBER OF LARVAE	AVERAGE MORTALITY OF LARVAE <i>A. aegypti</i>	STANDARD DEVIATION
1%	25	11	1.14
2%	25	15	1.10
2.5%	25	20	1.00
NEGATIVE CONTROL (EtOH 1% and Aquades)	25	0	0.00
POSITIVE CONTROL	25	25	0.00

For data on the percentage of larval mortality in each treatment is presented in Figure 2.

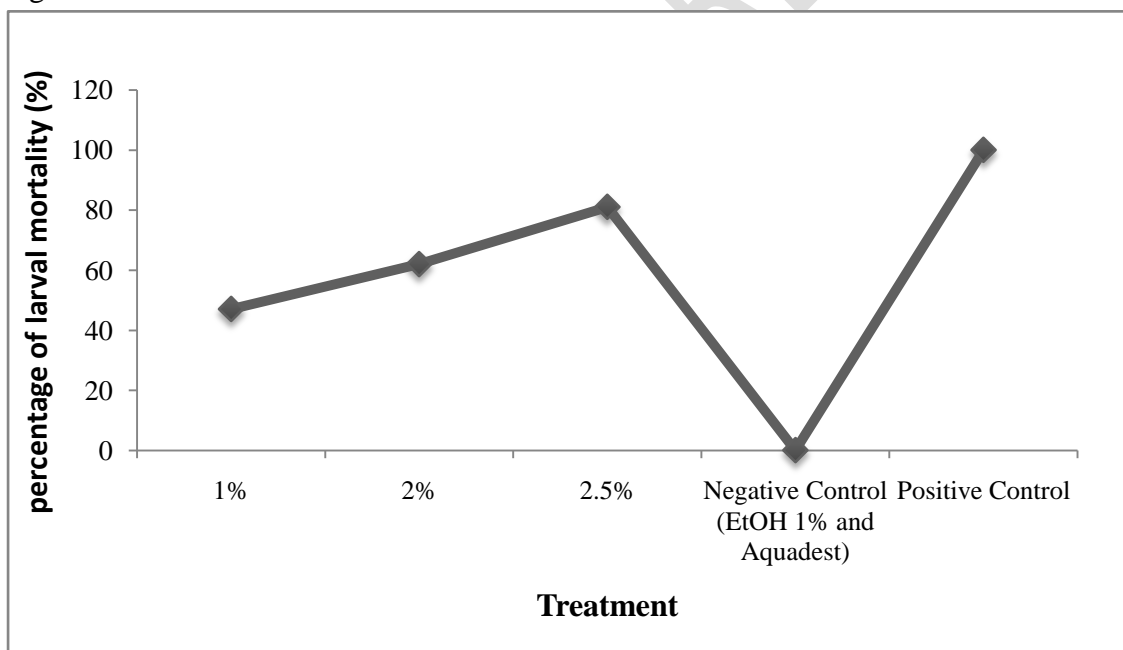


Figure 2. Percentage of Larval Mortality of *Aedes aegypti* in each of the activated charcoal and Control treatments

Statistical tests with one-way ANOVA show a meaningful difference in **pada** concentration due to the $p < 0.05$. Based on these data, it can be seen that the treatment that provides the most significant larvicide effect is on group concentration of activated charcoal of 2.5 %, with larvicide activity of 81% compared to the 1% group and 2%. It

can also be seen that from the post hoc test, the three **constituencies** significantly had a more negligible larvicide effect compared to the positive control group.

The results of this study showed that activated charcoal with a concentration of 1% could cause average larval mortality of 11 heads with a percentage of 46%, at a concentration of 2% causing average larval mortality of 15 heads with a percentage of 62%, at a concentration of 2.5% larval mortality an average of 20 heads with a percentage of 81% compared to negative controls and positive controls whose larval mortality averaged 0 and 25 respectively with percentages of 0 and 100%. This proves that the higher the concentration of activated charcoal in the lemongrass plant (*Cymbopogon nodus* L), the more it will influence a larvicide on *Aedes. aegypti*. Yatuu et al., (2020) stated that the concentration of larvicides is considered adequate according to WHO if the ability to kill larvae ranges from 10% - 95%. The high mortality rate due to the content in the lemongrass stem can shed the chitin layer that makes up the insect's cuticle. Active ingredients that affect mortality caused by toxic substances present in the material

The difference in the results in this study was also due to differences in the concentration of NaOH activators for activated charcoal activation. From the data obtained, the number of larvae mortalities shows that the greater the concentration of NaOH, the more larvae die. This is because activated charcoal activated using NaOH with a concentration of 2.5% has low levels of volatile substances, so organic compounds contained in the lemongrass plant are still present. The higher the concentration of NaOH used for activated carbon activation, the lower the level of volatile substances (Sahara et al., 2017). These lagging organic compounds are the toxic substances that kill larvae (Makkiah et al., 2019).

Ae.aegypti larvae exposed to toxic compounds when in contact with the activated charcoal of the lemongrass plant are slow in their movement and hanging position and then die. This means that the larvae experience toxic effects that impact energy depletion. This state is in line with the chemical mechanisms of the largest lemongrass plants, such as citronellal and geraniol Toxic compounds contained in *Citronella* (*Cymbopogon nardus* L) are an active substance that is harmful to **iron fat** when in contact with the respiratory system of larvae (Kusumawati et al., 2018). Acute poisoning such as seizures, disorders of the central nervous system, and respiratory paralysis is a function of **Citronella Citronella** in *Citronella* which works by inhibiting the enzyme acetylcholinesterase, so that acetylcholine accumulation occurs, which results in death in insects (Aulung et al., 2014; Giroth et al., 2021; Kusumawati et al., 2018).

Contact poison insecticides are insecticides that enter the body of the target insect through the skin (cuticle), natural cracks/holes in the body (trachea), or directly hit the insect's mouth and are translocated to the part of the insect body where the insecticide actively works (Moustafa et al., 2021). Insects will die when they come into direct contact (contact) with the insecticide. Most contact poisons also act as stomach poisons. Symptoms of insect poisoning arise due to acetylcholine hoarding leading to the

impaired motor nervous system, seizures, respiratory paralysis, and death (Angelica et al., 2020).

CONCLUSION

The most effective treatment of Activated Charcoal *Cymbopogon nardus* in controlling *Aedes aegypti* larvae was at a concentration of 2.5% of 81% of dead larvae. This proves that *Cymbopogon nardus* as activated charcoal can be used as a larvicide.

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