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Research Article

Neem leaves (*Azadirachta Indica*) and Jasmine flowers (*Jasminum sambac*): A toxic combination of *Aedes aegypti*

Putri Ayu Irodah [a,1,*], N. Nurwidodo [a,2], Moh. Mirza Nuryady [a,3]
[a] Department of Biology Education, Faculty of Teacher and Training Education, Universitas Muhammadiyah Malang, Jl. Tlogomas No. 246Malang, East Java, 65144, Indonesia
[1] putri.ayuirodah@gmail.com; [2] nurwidodo@umm.ac.id; [3] mirzanuryady@umm.ac.id *
* Corresponding author

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ABSTRACT

Background: The high number of cases of dengue hemorrhagic fever (DHF) in Indonesia indicates the lack of diseases prevention caused by mosquito vectors. Chemical control efforts can cause resistance to mosquitoes, so it is necessary to have natural insecticides derived from plants, one of which is neem and jasmine which has active ingredients as larvicides.
Objectives: This research aims to analyze the effect of different concentrations of extracts from neem (*Azadirachta indica*) and jasmine (*Jasminum sambac*) leaves on the mortality of Aedes aegypti larvae.
Methods: This research is true experimental research using 7 treatments, there are negative control group using distilled water and a positive control group using 1% abate and 5 treatment groups using combination of extracts from neem leaves (*Azadirachta indica*) and jasmine flower (*Jasminum sambac*) with concentration ratio 0%:100%, 25%:75%, 50%:50%, 75%:25%, dan 100%:0%. In this study, it is used *Aedes aegypti* larvae taken by landing collection in Nguling, Pasuruan.
Results: The results of identification of larvae showed that the larvae taken were Aedes aegypti larvae in terms of morphological characteristics. The results showed that the combination of 50%: 50% concentration caused a lot of mortality in larvae and obtained LC50 from the extract of neem (*Azadirachta indica*) and jasmine flower (Jasminum sambac) was 253,57 µg / mL.
Conclusion: Extracts from neem leaves (*Azadirachta indica*) and jasmine flowers (*Jasminum sambac*) can be used as an candidate alternative to *Aedes aegypti* larvicide.

Keywords: *Aedes aegypti, Azadirachta indica, Jasminum sambac*, larvacide, toxic

SDGs Relevance: This research is aligned with SDG 3: Good Health and Well-being. This research contributes to the development of natural larvicides to control dengue vectors that pose a significant public health challenge.
Laboratory Affiliation: The in-vitro experimentation and analysis were conducted at the Biology Laboratory, University of Muhammadiyah Malang, where the extraction and testing of neem and jasmine plant compounds were performed.

INTRODUCTION

Indonesia is a country that has high cases of dengue fever among other ASEAN countries (Kemenkes, 2016). Early 2019, dengue fever (DHF) has become a threat in several areas. In January 2019, there were 15,132 dengue patients with death rate of 145 people who occurred in 34 provinces. This number is two times higher than the end of January 2018 which recorded 6,167 dengue fever sufferers with 43 people died (Yuningsih, 2019). The high case of dengue fever is strongly influenced by the density of disease vectors. This disease is transmitted through the bite of *Aedes aegypti* mosquito (Agustin et al., 2017). The Ministry of Health has given many efforts to solve the problem of dengue fever. Those efforts are being made to control mosquitoes by spraying synthetic pesticides. The use of synthetic pesticides can be negative impact for environment (Indrayani & Sudarmaja, 2018), therefore it is necessary to look for vegetable insecticides that are environmentally friendly and effectively kill the mosquitoes that are vectors of dengue disease. One of the plants that can be used as larvicide is neem (*Azadirachta indica*) and jasmine (*Jasminum sambac* L).

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Neem is a plant that can be used as natural insecticide. Neem leave has been studied to contain many active compounds that can be used as insecticides, anti-bacteria, mosquito larvicides, and antimalarials (Dewi et al., 2017). All parts of the neem plant contain chemical compounds that can be used as larvicides, the leaves and seeds are the parts that contain lots of chemical compounds. Containing azadirachtin, meliantriol, salanin, nimbin and nimbidin are limonoid compounds contained in neem leaves (Nur et al., 2019). Apart from neem plants, jasmine flowers also have potential as a larvicide. Jasmine flowers can also produce essential oils that can repel mosquitoes so that essential oils are widely used as a bacteria killer, insect repellent and insecticide (Hidayat et al., 2016). Jasmine flower plants (*Jasminum sambac*) contain chemical compounds, namely flavonoids, tannins, alkaloids, and saponins which can be used as natural larvicides (Husna et al., 2020)

The use of natural larvicides provides several advantages, namely natural larvicides that are used leave little residue in the environment and decompose quickly in nature, so they are safe to use. Research conducted by Utomo & Budiarti, (2010) on the effectiveness of neem leaves, which are 80% effective as larvicides for the *Aedes aegypti* mosquito. Husna et al., (2020) conducted research on the effectiveness of jasmine flower extract against Aedes aegypti larvae which can cause 100% mortality. Based on existing research it is estimated that neem leaves and jasmine flowers have effective larval killing power. Research on the effectiveness of the combination of neem leaves and jasmine flowers as a larvicide that is friendly to the environment has never been done so it is important to do research. This research aims to analyze the effect of different concentrations of extracts from neem (*Azadirachta indica*) and jasmine (*Jasminum sambac*) leaves on the mortality of Aedes aegypti larvae.

METHODS

This research started from the hatching of Aedes aegypti mosquito eggs obtained from landing collections in Mlaten and Nguling Villages, Nguling District, Pasuruan Regency. Larvae were taken from 5 houses, namely 2 houses in Nguling Village at the coordinate point area Nguling (7˚4252.5''S 113˚04'37.3''E) and Nguling (7˚43'01.6''S 113˚04'39.9''E), while 3 houses in Mlaten Village are at point Mlaten (7˚4210.2''S 113˚05'09.0''E), Mlaten (7˚4210.2''S 113˚05'11.6''E), and Mlaten (7˚4210.2''S 113˚05'13.0''E). The following is a map of Nguling District.

Figure 1. Points of *Aedes aegypti* larvae collection location

The larvae obtained from the landing collection will be filtered at the Chemistry Laboratory of UMM under light conditions for 12 hours. Aedes aegypti eggs, hatched in a plastic tray filled with ± 1000cc of clean water. The hatched larvae were fed with poultry pellets every day. The larvae are maintained until stage III, for approximately 2-3 days. There were 560 larvae used for the reseach. Larvae that have been instar III will be identified to prove that the larvae taken is Aedes aegypti larvae. The larvae were observed using a digital microscope.

The materials used in the research were the third instar Aedes aegypti mosquito and extracts from neem leaves (*Azadirachta indica*) and jasmine flowers (*Jasminum sambac*), 1% abate, and aquades. The extraction process was carried out by means of 1000 grams of neem leaf simplicia powder and 1000 grams of jasmine flower simplicia powder, each immersed in ± 1L 96% ethanol solvent for 24 hours with 2x stirring. Filtering is done with a filter cloth, then macerated for 24 hours. The next step is evaporated at a temperature of 40˚C-60˚C. The extracts of neem leaves and jasmine flowers will be diluted to obtain a concentration ratio of 0%: 100%, 25%: 75%, 50%: 50%, 75%: 25%, and 100%: 0%.

This research is true experimental research with plans posttest only controlled group design. This research used 7 treatments where there was a negative control group using distilled water and a positive control group using 1% abate and 5 treatment groups using extracts from neem leaves (*Azadirachta indica*) and jasmine flowers (*Jasminum sambac*) with concentrations of 0%: 100%, 25%: 75%, 50%: 50%, 75%: 25%, and 100%: 0%. The treatment stage was to prepare 7 containers, 2 for the control group and 5 for the treatment group of neem leaves and jasmine flower extracts with a concentration ratio of 0%: 100%, 25%: 75%, 50%: 50%, 75%: 25%, and 100%: 0% with 4 repetitions. Each treatment contained 20 larvae in the container. Larvicidal activity was observed for 1x24 hours. The next treatment was calculating the mortality number of third instar Aedes aegypti larvae.

The extraction result was tested by secondary metabolic compound, they were: flavonoid, tannin, saponin, terpenoid tests. Flavonoid test, if the sample shaped has orange color, so it contains flavonoid compound (Mihra et al., 2018). Tannin test, when the sample formed white sediment and turbidity, it contains tannin compound (Masitoh, 2011). Saponin test, if the sample tested by boiling foam, then added by one drop of HCl 2 N and the foam was stable after 10 minutes, it means that the sample contains saponin compound (Syafitri et al., 2014). Terpenoid test, the sample was added with chloroform and stirred. After that it was added by concentrated H_2SO_4, if there was red color it shows terpenoid compound (Widiyati, 2006).

The obtained data will be processed using normality test, homogeneity test, ANOVA test and further test. Before that, there will be normality and homogeneity test in the probability level of 5%. in order to find out whether the population variants were normally and homogeneous distributed. Next, ANOVA test (Analysis of Variants). if there were some significant differences, further test will be conducted using the smallest real difference test (BNT). followed by a probit regression test to determine $LC_{50.}$

RESULTS

Mosquitoes experience a metamorphosis from eggs, larvae, pupa, and adult mosquitoes. The research result can be seen on Figure 2. Figure 2.a. shows the shape of Aedes Aegypti larva is instar III in size 4-115 mm, locomotor on the chest is clear, while in Figure 2.b shows that the siphon and comb teeth is clear (Sudibyo, 2012) (Figure 3).

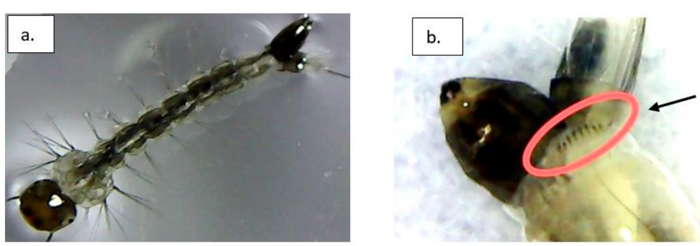

Figure 2. The Identification result microscopic morphology of Aedes aegypti larva: a. Aedes aegypti larva and b. Siphon part

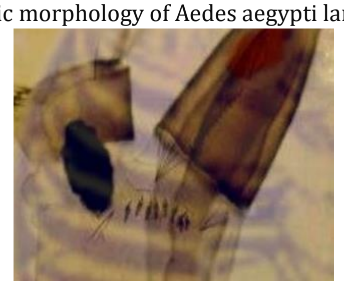

Figure 3. The Identification result microscopic morphology of Aedes aegypti larva: a. Aedes aegypti larva and b. Siphon part

The secondary metabolic compound contained in neem leaves and jasmine flower were identified in Table 1.

Table 1. Qualitative Test Result

Sampel	Identification	
Neem leaf extract	(+) Flavonoid	(+) Tanin
Jasmine flower extract	(+) Saponin	(+) Triterpenoid

Based on the conducted research by giving a treatment such as negative control using aquades, positive control using abate, and neem leaf extract (*Azadirachta indica*) and jasmine flower (*Jasminum sambac*) with the concentration of 0%:100%, 25%:75%, 50%:50%, 75%:25%, and 100%:0% gained the different result on the mortality Aedes aegypti larva. Those result are presented by the following Figure 4.

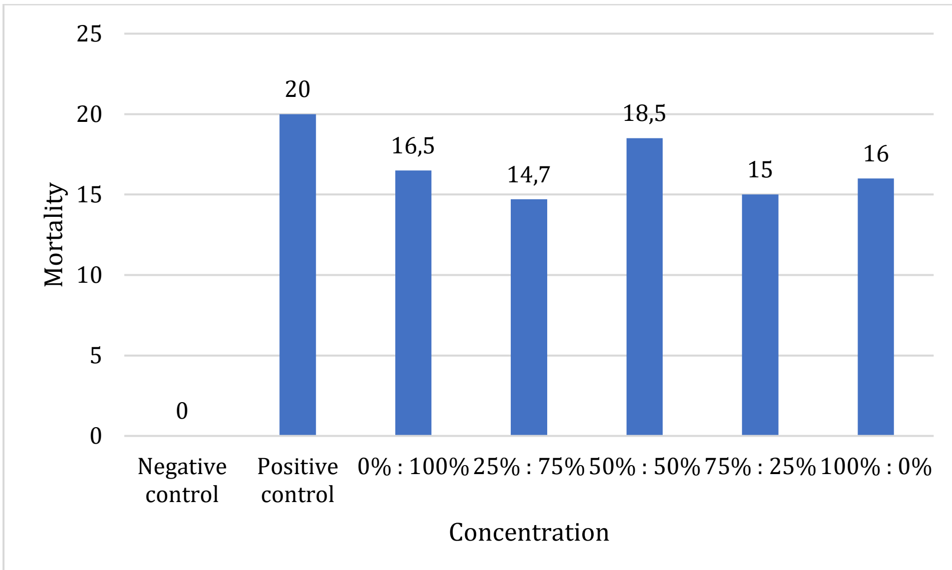

Figure 4. Graphics result of mortality *Aedes aegypti*

Based on the calculation of normality test, it was gained a skewnes value that is 1.311 and kurtosis value that is 1.211, means that the effect of neem leaf extract (*Azadirachta indica*) and jasmine flower (*Jasminum sambac*) with some concentration toward the mortality of Aedes aegypti larvae distributed normal. Homogeneity shows that Sig 0.116 > 0.05 means that the distributed data was homogeneous. The calculation result of one way ANOVA gained the calculated f value 32.446with the Sig = 0.001, hence Sig value <0.05, so H0 was refused. It can be concluded that there was an effect of neem leaf extract (*Azadirachta indica*) and jasmine flower (*Jasminum sambac*) with some concentration toward the mortality of Aedes aegypti larvae. The effect found in this research needs to be conducted the smallest real difference test to find out the difference in each treatment, whether it was real or not. Based on the result of the smallest real difference test shows that the average of treatment mortality 50%:50% was the real different toward negative control, positive control, concentration 0%:100%, 25%:75%, 75%:25%, and 100%:0%.
LC_{50} test value is the concentration used to kill 50% of the population of larvae tested. LC_{50} is used to assess the toxicity of larvacides. LC_{50} value determined based on the total of tested larvae death wich was gained in each concentration. Based on the regression test result were obtained that LC_{50} value cause the death of tested larvae from total treatment 253,57 µg/Ml that identifies the compund that is toxic.

DISCUSSION

The Aedes aegypti mosquito is the main vector that carries the dengue virus, zika virus, yellow fever, and chikungunya (Sukesi, 2012). The Aedes aegypti mosquito is an intermediary that transmits the dengue virus and usually these mosquitoes like to suck human blood during the day (Astriani & Widawati, 2016). Mosquitoes contain the dengue hemorrhagic fever virus when it sucks the sufferer's blood, then the virus replicates in the hemocoelum and eventually enters the salivary glands and transmits the dengue virus to sufferers (Palgunadi & Rahayu, 2011). The Aedes sp. Mosquito is very easy to infect humans, because this mosquito lives around the house, in contrast to the Aedes albopictus mosquito which lives in plantation areas (Yudhastuti & Vidiyani, 2005). The Aedes aegypti mosquito undergoes a complete metamorphosis, starting from eggs, larvae, pupa, and adult mosquitoes.

The development of Aedes sp from egg to adult takes about 10-12 days. Aedes sp mosquito larvae go through four stages, namely instars I, II, III, and IV. First instar larvae have a length of 1-2 mm, body size is very small and it is not clear that the chest and siphon have not turned black. Second instar larvae are 2.5-3.9 mm long, their bodies have begun to grow bigger, third instar larvae 4-115 mm, the chest is clear and the respirator starts to look black. Fourth instar larvae have a clear anatomical structure and can be distinguished on the head, chest and abdomen (Haditomo, 2010). The Ministry of Health has made many efforts to overcome the problem of dengue fever by eradicating mosquito larvae by spraying chemical insecticides. The use of chemical insecticides can cause environmental problems; therefore, it is necessary to have natural insecticides as environmentally friendly larvicides.

The difference in the number of larvae deaths can be seen in the table of observation results, if seen from the concentration ratio, the higher concentration level, the higher level of poison which can cause many larvae to die. Jamal et al., (2016) states that an increased concentration can increase toxicity levels, so that the killing power is higher. It can be seen in Graph 4.1 the number of mortalities of Aedes aegypti mosquito larvae that died in the test larvae due to the concentration and content of secondary metabolite compounds. Secondary metabolite compounds in plants consist of alkaloids, flavonoids, saponins, tannins, phenolics, and steroids (Hikma & Ardiansyah, 2018). These compounds can be used as larvicides. Neem leaves and jasmine flowers can be used as mosquito larvicides because of their secondary metabolite compounds. Neem leaves contain flavonoids, alkaloids, tannins, and limonoid groups namely azadirachtin, nimbidin, nimbin, meliantriol, and salanin (Indrayani & Sudarmaja, 2018) as well as neem leaves in jasmine which contain flavonoids, saponins, and essential oils (Husna et al., 2020). The tannin content in neem leaves when extracted in water is 0.55% (Mihra et al., 2018). The phytochemical content of the combination of neem leaf larvicides and jasmine flowers causes a synergistic effect so that if formulated it can increase the potential for insecticides (Kemenkes, 2016).

Limonoids are compounds that act as inhibitors of larval growth. Flavonoids are toxins that can interfere with the respiratory system so that larvae have difficulty breathing and experiencing death (Indrayani & Sudarmaja, 2018). In insects, saponins also act as metamorphosis inhibitors and triterpenoids causing loss of appetite in larvae, causing death (Wahyuni & Loren, 2015). Tannins have the ability to precipitate protein for larval growth (Husna et al., 2020). The compounds above act as toxic substances that can cause death in the test larvae.

Thus, the difference in the mortality of Aedes aegypti mosquito larvae was influenced by the combination of insecticide from neem leaf extract (*Azadirachta indica*) and jasmine flower (*Jasminum sambac*). The observations showed that the highest number of mortality was at a concentration of 50%: 50%. This is because the combination of insecticide contains more secondary metabolite compounds so that it can inhibit Aedes aegypti mosquito larvae in the metabolic process. The amount of solvent in the extract can affect the content of secondary metabolite compounds. The greater the volume of the solvent, the more it reduces the levels of extracted metabolites (Yulianingtyas & Kusmartono, 2016). Aedes aegypti larvae that do not move when touched at the bottom of the water and do not appear on the surface of the water are said to be dead. The dead larvae appear pale white.

Based on the results of the regression test, it was obtained that the LC_{50} value of neem (*Azadirachta indica*) leaf extract and jasmine flower (*Jasminum sambac*) which could cause the death of 50% of the test larvae, the total treatment was 253.57 μg / mL $\pm$ 1.5 μg / mL. Compounds from plants if the value of $LC_{50} \leq 30$ μg / mL are very toxic, if the value is $31 \leq LC50 \leq 1000$ then they are toxic (Ningdyah et al., 2015). This shows that the extracts of neem leaves (*Azadirachta indica*) and jasmine flowers (*Jasminum sambac*) are effective as larvicides against third instar *Aedes aegypti* larvae because they are toxic.

CONCLUSION

Based on the research results, the effectiveness of neem (*Azadirachta indica*) leaf extract and jasmine flower (*Jasminum sambac*) is effective in killing the third instar Aedes aegypti larvae and can be used as an alternative. LC50 values can cause high toxicity.

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Conflicts of Interest: The authors declare no conflicts of interest.

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