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LANSIUM DOMESTICUM CORR. LEAF EXTRACT SPRAY AS BIOINSECTICIDE FOR *AEDES AEGYPTI* MOSQUITO CONTROL

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ABSTRACT

Background: Dengue Haemorrhagic Fever vector eradication using chemical insecticides showed mosquitoes have become resistant and they cause environmental pollution. *Duku (Lansium domesticum corr.),* Indonesia's native plants, is a natural ingredient used as bio insecticide. The skin of fruit, seeds and stems contain alkaloids, flavonoids and saponins that have insecticide activities. This study aims to determine the efficacy of *duku* leaf extract as a bioinsecticide for *Aedes aegypti* mosquito control.

Materials and Methods: It used pre-test post-test only method. The samples are 500 mosquitoes for the experimental design of 5 treatment groups and 4 replications. Factors used as treatment were concentrations of extracts (35%, 40%, 45% and 50%) and controls (distilled water) were examined for 24 hours and measured the percentage of mosquitoes died. Anova was used followed by Least Significant Difference.

Result: The results showed the number of mosquitoes died at each concentration showed a significant difference ($p<0.001$). At a concentration of 35% the number of dead mosquitoes was 87.75%; 40% was 97.5%; 45% was 63.75%; 50% was 58.75% and in controls was 16.87%. LC50 calculation results of *duku* leaves were at a concentration of 35.97%.

Conclusion: It concluded that *duku* leaves can be used as a vegetable insecticide with an effective concentration of 40%.

Keywords: *Aedes aegypty*, Bio insecticide, *Lansium domesticum*, Extract, *Duku*

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1.0 Introduction

Data from all over the world shows that Asia ranks first in the number of dengue fever sufferers (DHF) every year. Meanwhile, starting from 1968 to 2009, the World Health Organization (WHO) recorded Indonesia as the country with the highest DHF cases in Southeast Asia and the second highest in the world after Thailand. The incidence of dengue has increased worldwide in decades. It is estimated, currently around the world there are around 50 million cases of dengue fever found every year, with 500,000 cases requiring treatment in hospitals. Of these cases, around 25,000 deaths occur annually (MOH RI, 2010 and Widyastuti, 2004).

Efforts to control and prevent DHF have been regulated in Regional Law No. 5 of 2010 concerning Disease Control of DHF, these efforts are more focused on controlling the vector, namely controlling *Aedes aegypti* mosquitoes (Cahyati, 2016). Mosquito control which is often used in the community is to use anti-mosquito spray, fuel, and anti-mosquito lotion made from chemicals. The chemicals used in anti-mosquitoes have negative impacts such as residues whose active ingredients are difficult to decompose in nature. The use of chemical insecticides if used on target, on the right dose, on time, and coverage will be able to control the vector and reduce the negative impact on the environment and non-target organisms, one example of the material used is alum as larva side against larvae of Anopheles mosquito (Cahyati & Sulastri, 2016). Although it can be used according to standards, but to prevent further effects from the effects of using insecticides, it is necessary to find alternatives to natural or plant-based insecticides.

Natural insecticide is an insecticide whose basic ingredients come from nature. This type of insecticide is biodegradable in nature, so it does not pollute the environment and is relatively safe for humans and livestock, because the residue will decompose and are easily lost (Sugiata in Mirnawaty, 2012). The use of insecticides at home has several advantages including not leaving residues in the environment so it is relatively safer than chemical insecticides, and when leaving residues, these residues do not cause resistance to the target because they break down faster than chemical insecticides (Qinahyu, 2016).

One effort that is being developed to reduce the negative impact is the search for insecticides from natural ingredients, especially products derived from plants that are selective, safe and environmentally friendly. Vegetable insecticides leave no residue in air, water and soil and have a higher level of security when compared to inorganic poisons. This is caused by the composition of plant-based insecticide molecules consisting mostly of carbohydrates, nitrogen, oxygen and hydrogen which are readily decomposed into compounds that are safe for the environment and also reduce the chance of animals that are not subject to residue exposure (Matsumura, 2008).

Several studies have investigated the potential of natural ingredients from plants as insecticides, although the results are still varied but are alternatives to replace synthetic materials because they are proven effective and do not cause disturbance to human, animal and environmental life (Chaitong et al., 2006). Plant families that are considered a potential source of plant-based insecticides are *Meliacea, Annonaceae, Astraceae, Piperaceae* and *Rutaceae.*

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One of the plants of the *Meliacea* family that has the potential to become an insecticide is *duku (Lansium domesticum corr).* Various groups of compounds that have activities as insecticides are alkaloids, flavonoids and saponins contained in *duku* plants (Oktavianti, 2009). These compounds are found in the skin of the fruit, seeds and bark of the *duku* plant (Astawan, 2008 and Octaviana et al., 2017). The study of Sampan et al. (2013) proved that the extract of *duku* fruit skin with a content of 35% is effective as an anti-electric mosquito against the killing power of *Aedes aegypti* mosquitoes. *Duku* fruit seeds, besides being effective as larvicides, are also effective for worm medication, fever medication and diarrhoea medicine (Astawan, 2009).

This encourages this research to be carried out, namely by giving extract *duku* leaf by trying out a sufficient concentration range to obtain LC50 information and total larval mortality in *Aedes aegypti*. By knowing the effect of *duku* leaf extract insecticide on *Aedes aegypti*, it is expected to provide information about the potential of *duku* as a vegetable insecticide.

2.0 Materials and Methods

This type of research used in this study is a true experiment using the post-test only control group design. The research subjects were Aedes aegypti mosquitoes which were divided into 2 groups: the experimental group was 25 Aedes aegypti mosquitoes in a glass chamber given *duku* leaf extract with concentrations of 35%, 40%, 45% and 50%; while the control group was 25 mosquitoes in the glass chamber given aquades. The sample was determined using a Completely Randomized Design with a total of 4 repetitions.

This research was conducted in two places. The making of *duku* leaf extract was carried out at the Faultas Biology Plant Physiology Laboratory. The effectiveness test was carried out at the Banjarnegara P2B2 Research and Development Laboratory.

Data obtained in this study were analysed using probit test and one way Anova test. Probit test is a test used to determine the killing power of *duku* leaf extract against the death of Aedes aegypti mosquitoes expressed in Lethal Concentration (LC), namely LC50 and KT90. One way Anova test is used to find differences in the number of mosquito deaths in the control group, and in the group treat 35%, 40%, 45%, and 50% of *duku* leaf extract.

3.0 Result

3.1 Making Duku Leaf Extract

Duku leaves were extracted using the 3 x 24 hour remaceration method. The extraction process was carried out at the Plant Physiology Laboratory at the Faculty of Biology Universitas Jenderal Soedirman. The extraction results from 2.5 kg of *duku* leaves produce 107 grams of *duku* leaf extract.

3.1.1 Phytochemical Test of Duku Leaf Extract

Phytochemical test was conducted to find out the effective content of insecticides contained in the extraction of *duku* leaves. The results can be seen in the following table.

Table 1 Phytochemical analysis results of *duku* leaf extract

No.	Compounds	Color	Description
1	Alkaloids	There is an orange-brown sediment	Positive
2	Flavonoids	There is dark green sediment	Positive
3	Saponins	Stable foam	Positive

Based on the table it is known that the positive *duku* leaf extract contains compounds of the alkaloids, flavonoids and saponins.

3.1.2 Temperature and Humidity Measurement

Temperature and Humidity in this study were carried out at any time before the insecticide was tested. This aims to ensure that there are no other factors that can influence mosquito mortality during the study. Analysis of the results of temperature and humidity measurements during the study can be seen in the following table.

Table 2 Analysis of room temperature and humidity measurement

Variable	N	Min	Max	Mean	SD
Temperature	27	25.43	29.86	27.37	1.44
Humidity	27	51.13	66.00	60.35	5.39

Based on the results of temperature measurements, it is known that the room temperature is around 27.37^0C and the humidity is around 60.35%. This temperature and humidity are normal conditions for mosquitoes to survive.

3.2 Death of the Aedes aegypti Mosquito

The number of Aedes aegypti mosquito deaths after being treated for 1 x 24 hours can be seen in the following table.

Table 3 Number of mosquito deaths in various concentrations of the extract

Replication	Number of mosquitoes in the glass chamber	Number of dead mosquitoes after 24 hours of treatment *duku* leaf extract concentration				
		Control	35%	40 %	45 %	50 %
1	25	3	20	20	9	9
2	25	2	10	13	16	13
3	25	6	14	21	13	15
4	25	4	23	24	13	10
Number		15	67	78	51	47
Mean		3.75	16.75	19.5	12.75	11.75
% Mosquito death		15 %	67 %	78 %	51 %	47 %

Based on table 3, the percentage of mosquito deaths in the control group was 15%. The lowest percentage of mosquito deaths was found at a concentration of 50% which is equal to 47%, and the highest was at a concentration of 40% by 78%. The difference in the number of mosquito deaths at each concentration can be seen from the results of the analysis using the ANOVA One Way test as follows.

Table 4 ANOVA one way test of the number of mortalities at 24 hours

	Sum of Squares	df	Mean Square	F	p
Between Groups	573.800	4	143.450	9.606	<0.001
Within Groups	224.000	15	14.933		
Total	797.800	19			

Based on the table, it is known that the results of p value (0.000 <0.05) then there is a significant difference in the death of mosquitoes using 4 *duku* leaf concentrates.

3.3 Lethal Concentration 50 (LC50)

The following is the result of LC50 analysis of *duku* leaf extract against Aedes aegypti mosquitoes using probit analysis.

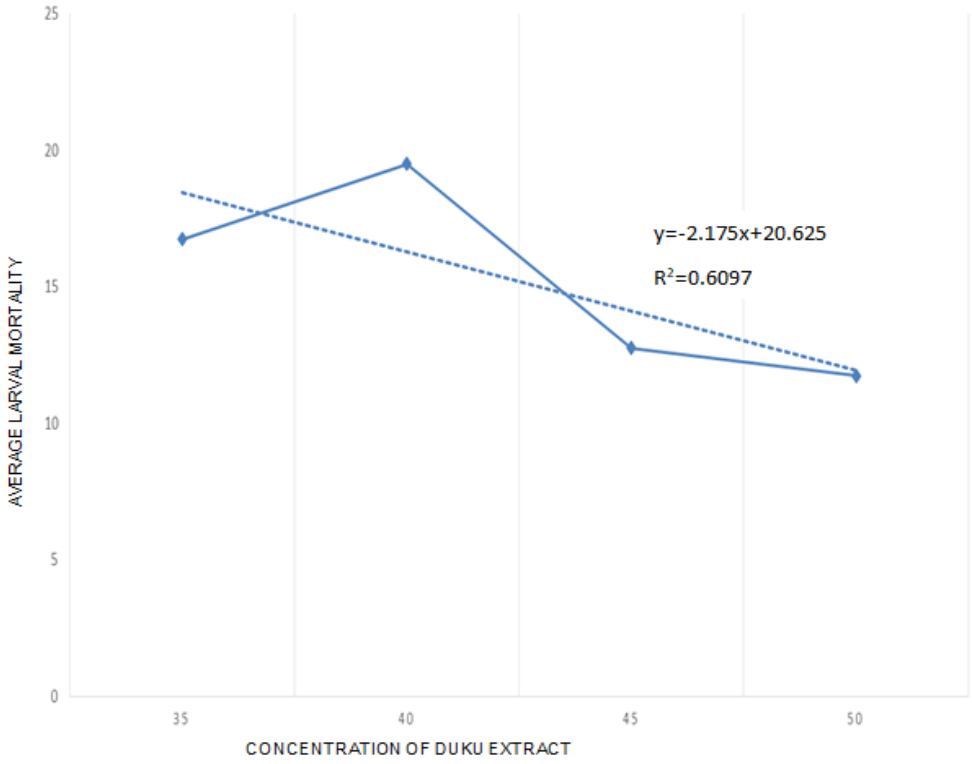

Figure 1 Probit analysis results of *duku* leaf extract

Based on probit analysis it is known that LC50 for *duku* leaf extract is at a concentration of 35.97%.

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4.0 Discussion

Phytochemical test results on *duku* leaf extract showed that the extract contained alkaloids, flavonoids and saponins. These results indicate that the content of the active insecticide contained in the leaves of *duku* is the same as that found in the skin of the fruit and seed skin of *duku* (Ni'mah et al., 2015). Among the three compounds the possibility of active substances that have insecticidal effects on Aedes aegypti mosquitoes are alkaloid and flavonoid compounds.

Alkaloid in *duku* leaves function as stomach poisons by weakening the nervous system in the digestive organs of mosquitoes. Mosquitoes will lose their appetite so mosquitoes will slowly die (Anizewski, 2007).

Flavonoid compounds in the leaves of *duku* function as a strong inhibitor of the respiratory system of adult insects. When mosquitoes do the process of breathing flavonoids will also be inhaled, then the compound will weaken the nerves and cause damage to the spiracle cells so that the respiratory system will run slowly and mosquitoes die due to lack of oxygen supply (Djojosumarto, 2008 and Hanum et al., 2013).

The results of research that have been done prove that leaf extract can affect the death of *Aedes aegypti* mosquitoes. In the administration of *duku* leaf extract the number of mosquito deaths is not directly proportional to the increase in concentration. The results showed that the number of mosquito deaths decreased with increasing concentrations. The concentration of *duku* leaf extract which was proven to be the most effective in killing mosquitoes was 40% and the least number of deaths was found at a concentration of 50%. This is not in accordance with Mulyana (2002) which states that the higher the concentration, the faster the insect dies, because the more active substances exposed to the insect.

A decrease in the percentage of mosquito deaths along with an increase in extract concentration may be influenced by the treatment process in the study. When the research was carried out the number of glass chambers used was very limited, so the implementation of the extract application was not carried out simultaneously but rotated for some time. In addition, there is also a considerable time difference between the time of extract and the application of extract. This is likely to affect the effectiveness of the active ingredient content of the contract. This is consistent with Prijono (1999) that plant-based insecticides that are stored and exposed in the field are very easily degraded so that their effectiveness decreases.

According to how it works, *duku* leaf extract can also be classified as an inhalation poison insecticide and contact poison, because based on observations made during the experiment, the Aedes aegypti mosquito undergoes changes before and after spraying *duku* leaf extract. Aedes aegypti mosquito from moving very actively trying to get out (due to the aroma released by *duku* leaf extract) until it eventually becomes sluggish and weak, and then paralyzed and then die. This shows that leaf extract can be used as an alternative ingredient for making plant-based insecticides. The use of *duku* leaves as natural insecticides is safer and more economical compared to synthetic insecticides (Elis, 2012 and Ni'mah, 2015).

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LC50 is a calculation to determine the toxicity of an extract or compound. Based on the results of probit analysis it is known that the concentration needed to kill 50% of test animals *(Aedes aegypti)* is 35% with the time required for 24 hours (1440 minutes).

This shows that the effectiveness of active insecticide spray made from *duku* leaf extract is still below the effectiveness of insecticide spray that is currently circulating in the market, all of which are active ingredients of toxic synthetic chemical (insecticide) 10 to 15%. However, despite losing effectiveness, mosquito spray insecticides derived from natural ingredients are superior in safety and health for users, because chemicals, which have been the main active ingredients of all insecticide products on the market, are poisonous and harmful to users especially children if their use is inappropriate. Likewise, in terms of the price of insecticide spray made from *duku* leaf extract, it is very possible to compete with insecticide spray mosquitoes with chemical active ingredients, because the raw materials do not need to be imported or can be met from within the country. For this reason, it needs to be explored and continuously researched so that the protective power of this natural material can be aligned and compete with insecticide spray insecticide made from synthetic chemical active ingredients. One way is to increase the protection and effectiveness of the spray, among others by adding fixative substances to retain the aroma so that its effectiveness can last longer.

5.0 Conclusion and recommendation

Duku leaf extract is proven to contain alkaloids, saponins and flavonoids that can be used as an alternative to control the Aedes aegypti mosquitoes which are safe for the environment and humans. The concentration of *duku* leaf extract which killed mosquitoes the fastest was 40% concentration at minute 59. LC50 of *duku* leaf extract by 35%. For further research, more tools and materials need to be provided to conduct insecticide effectiveness studies in adult mosquitoes. It is necessary to measure the content of the active ingredient of the insecticide contained in the *duku* leaf extract. The field uses of *duku* leaf extract also need to be examined.

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Declaration

Authors declare that there are no known conflicts of interest associated with this publication that could have influenced its outcome.

Authors' contribution

Author 1: Devi Octaviana, Author 2: Sri Nurlaela, Author 3: Dian Anandari, and Author 4: Firda Yanuar Pradani

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