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Research Article

Effectivity Test of Tobacco Leaf (*nicotiana tabacum* l.) 96% Ethanol Extract on *Culex Quinquefasciatus* Larvae Mortality

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ABSTRACT

Culex quinquefasciatus acts as a vector for Filariasis disease. The transmission of this disease can be prevented by cutting the life cycle of these mosquitoes at the larvae phase. Tobacco leaf contains an alkaloid, flavonoid, essential oil, saponin, and tannin which can kill Culex quinquefasciatus larvae. This study is a laboratory experiment with a post-test only controlled group design. This study used the larvae of Culex quinquefasciatus third-instar larvae. Six hundreds larvae were divided into 6 groups and 4 repetitions (each consisted of 25 larvae), i.e. negative control (distilled water + CMC), tobacco leaf ethanol extract (EEDT) with 0.025%; 0.05%; 0.075% and 0.1% doses and positive control (temephos). The number of dead larvae was counted at 6, 12, 18, and 24 hours after treatment. The results of the Kruskal-Wallis test showed p-value = 0.000, followed by the Mann-Whitney Post-hoc test, which resulted in all four doses of EEDT significantly differed compared to the negative control. However, 0.1% concentration was insignificantly different than the positive control. This showed that the ethanol extract of Nicotiana tabacum L. was effective in killing Culex quinquefasciatus larvae and EEDT with 0.1% concentration had the same potential as temephos as a larvicide.

Keywords : Tobacco leaf extract, *Culex quinquefasciatus*, Larvicide, Filariasis.

INTRODUCTION

Culex quinquefasciatus is a vector for *Wuchereria bancrofti*, a nematode that causes filariasis in tropical and subtropical countries. The habitat of *C. quinquefasciatus* includes rainwater, obstructed drainage, and places with dirty puddles. This mosquito is active during the evening. At night, the microfilaria of *W. bancrofti* is active around the peripheral blood of the body of the affected. When *C. quinquefasciatus* bites, the microfilaria is transferred from the human body to the mosquito (Arimurti, 2018).

Filariasis, also known as elephantiasis, is a chronic infectious disease caused by the infection of filaria worms, whereas these worms attack lymph tracts and nodes, causing the destruction of the lymph system, which leads to swelling on the arms, legs, mammary gland, and the scrotum (Masrizal, 2018). Filariasis can cause lifetime disability and social stigma for its sufferers and families (Pan, 2015). The indirect impacts of this disease include decreased work productivity, family burden, and substantial economic loss for the country (Taylor, Hoerauf, and Bockarie, 2015).

To date, it is estimated that almost 1.4 billion people in 73 countries throughout the world are threatened by filariasis. The larvae of the worm had infected more than 120 million people in the world, where 40 million of them experienced disability and paralysis due to this disease (WHO, 2016). Around 65% of those infected lived in the South East Asia region. In Southeast Asia, there are 11 countries that are filariasis endemic, and one of which is Indonesia (Otsuji, 2016).

Indonesia implements two main strategies in eliminating filariasis, i.e. cutting the transmission chain and the effort in the clinical management of filariasis. The termination of the transmission chain is divided into two main programs, i.e. mass treatment and vector control. The administration of preventive mass medication (*Pemberian Obat Massal Pencegahan*; POMP) for filariasis was conducted annually, for at least 5 consecutive years. Vector control was conducted by cutting the life cycle of *Culex* mosquitoes, from the egg to the adult stage. During the larva stage, its development can be cut by larvicide, either using synthetic or vegetable larvicide (Ahdiyah and Purwani, 2015).

There are several widely used synthetic larvicides, including temephos (Abate®), Dichlorodiphenyltrichloroethane (DDT), Diethyltoluamide (DEET), and Propoxur. Excessive and continuous use of synthetic larvicide can lead to new problems, which are environmental pollution and health problems of other organisms in contact with the environment (Fuadzy and Hendri, 2015). Therefore, the use of vegetable larvicide is more recommended because it has a similar ability to kill larvae as a synthetic larvicide, albeit more biodegradable. Therefore, it is environmentally-friendly and safe for health (Astriani and Widawati, 2016).

One of the plants that can be used as natural larvicide is tobacco leaf (*Nicotiana tabacum* L.). According to Astriani & Widawati (2017), there are 8 plants that have larvicide concentration with LC_{50} < 50 ppm, i.e. lemongrass, zodiac, jasmine, patchouli, tobacco, galangal, citronella grass, and teak wood. These plants have active larvicide content. Currently in Indonesia, especially in Central Java, tobacco (*Nicotiana tabacum* L.) is a widely cultivated plant. Although tobacco is widely used for cigarettes, it can also be used as a larvicide due to its flavonoid, alkaloid, saponin, essential oil, and tannin contents. Previous studies conducted by Rizki Khalalia (2016) and Handayani (2018) stated that tobacco (*Nicotiana tabacum* L.) leaf extract was effective in exterminating the larvae of *Aedes aegypti*.

Wijayanti (2015) investigated tobacco leaf extract with tween 80 solution using 0.005%, 0.011%, 0.018%, 0.031%, 0.066%, and 0.095% doses and showed that the highest dose (0.095%) could kill 90% of *Culex quinquefasciatus* larvae. According to these backgrounds, the author suspected that tobacco leaf can be used as an effective larvicide for *Culex quinquefasciatus*. Therefore, the author initiated to conduct a study to determine whether tobacco leaf ethanol extract is effective on the mortality of *Culex quinquefasciatus* larvae. This study aimed to determine the effect of 96% ethanol extract of tobacco (*Nicotiana tabacum* L.) and to determine the optimal dose of this extract as a larvicide for *Culex quinquefasciatus*.

This study also performed Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) probit analyses, which were used to determine the concentration and time needed to kill *Culex quinquefasciatus* larvae. The results of this study can be used for the utilization of tobacco leaf as a natural larvicide for *Culex quinquefasciatus* to reduce the incidence of filariasis in Indonesia. The expected hypothesis was the ethanol extract of tobacco (*Nicotiana tabacum* L.) leaf had a mortality effect on *Culexquinquefasciatus* larvae.

METHODS

This study is a laboratory experiment with a post-test only with controlled group design. The tobacco leaves (*Nicotiana tabacum* L.) used in this study were obtained from Trucuk District, Klaten Regency, Central Java, with an average rainfall of 3287 mm per year. Afterward, the tobacco leaves were measured to 5000 gram, cleansed, minced, and dried for around 1-2 weeks, and blended (*simplisia*). The extraction method used in this study was maceration. The extract was made in the Pharmacology Laboratory, Faculty of Medicine, Muhammadiyah Surakarta University and larvae treatment were conducted in *Balai Besar Penelitian dan Pengembangan Vektor dan Reservoir Penyakit* (B2P2VRP), Salatiga, Central Java. The sample size in this study was 600 larvae, consisted of 2 control groups and 4 treatment groups with 4 repetitions and 25 third-instar larvae for each treatment group placed in treatment glass. The samples were taken using a purposive sampling technique.

Data analysis was conducted using computer software, i.e. normality test (Shapiro-Wilk) homogeneity variance (Levene test), and the differences between each treatment variables onmosquito mortality (Kruskal Wallis test). The analysis was continued with Post-hoc test (Mann Whitney), and lethal concentration and lethal time probit analyses.

RESULTS AND DISCUSSION

Table 1. The mean results of mortality.

Treatment	Number of Larvae	Mean Mortality of Larvae after 6-24 hours ± SD				Mortality percentage after 24 hours
		6	12	18	24	
K- (negative)	25	0±0	0±0	0±0	0±0	0%
K+ (positive)	25	25±0	25±0	25±0	25±0	100%
EEDT (0.025%)	25	0.25±0.5	3.25±0.95	5.25±0.95	7±0.81	28%
EEDT (0.050%)	25	1.25±0.95	6±0.81	9.25±0.95	13.25±0.57	54%
EEDT (0.075%)	25	2.5±0.57	9±0.81	13.5±0.57	17.75±0.95	71%
EEDT (0.1%)	25	4.5±0.57	14.75±0.95	20±1.41	24.25±0.57	98%

(Source: Primary Data, 2019)

The table above demonstrated that the highest mortality rate was in the EEDT (0.1%) group, which caused 98% mortality of *Culex quinquefasciatus*. The higher the concentration of tobacco leaf extract, the higher the mortality rate of the larvae (Wijayanti, 2015). Tobacco leaf ethanol extract was suspected to contain flavonoid, alkaloid, saponin, essential oil, and tannin, which are toxic to larvae. Flavonoid that enters the body of the larva through the respiratory system will cause weakness of the nerve and damage respiratory system, which lead to the inability to breath and death. Alkaloid works by inhibiting the acetylcholinesterase enzyme, which also acts as a stomach toxin. If the substance enters the body of the larva, it will cause damage, which leads to death. Saponin works by denaturing protein and enzyme in cells, creating leakage (Taufiq, 2015). Saponin can also cause delayed growth of larvae (Chaieb, 2010). Furthermore, it can also reduce the surface tension of the digestive tract mucous membrane of the larvae, which corroded the digestive tract and cause death (Khalalia, 2016). Tannin works by reducing the activity of protease and amylase, thus inhibiting the activity of intestines, which will cause death. Tannin also has a bitter taste, which causes antifeedant to the larvae (Fajriani *et al*., 2019). Essential oil is a nerve toxin that causes nerve weakness, which then leads to death (Khalalia, 2016).

Normality and homogeneity tests showed $p < 0.05$, which means that the data were not normally distributed and not homogenous. Therefore, the Kruskal-Wallis non-parametric test was

conducted. The results of the Kruskal-Wallis test showed sig (0.000) P < 0.05, in which there were significant differences between treatments. To determine which group differed significantly, Post-hoc test of Mann-Whitney was conducted, comparing between control group (distilled water + CMC) and EEDT group (0.025%), EEDT (0.05%), EEDT (0.075%), EEDT (0.1%), which demonstrated p-value < 0.05, which means that there was significant difference. Therefore, it can be concluded that tobacco leaf extract (*Nicotiana tabacum* L.) had an effect on the mortality of *Culex quinquefasciatus* larvae. Comparison between control + abate with all treatment groups (EEDT 0.025%, 0.05%, 0.075%) showed significant differences, thus 0.025%, 0.05%, and 0.075% were less effective than abate as larvicide. Comparison between control + abate and EEDT (0.1%) showed p-value > 0.05, which was insignificantly different, thus EEDT (0.1%) had similar potential as abate as larvicide. The result of lethal concentration probit analysis showed that the LC_{50} value (the concentration of tobacco leaf extract that can cause 50% larvae death within 24 hours) was 0.062%. Based on the above, the higher the concentration means the higher the mortality of *Culex quinquefasciatus*, thus the higher the content of substances within the larvicide that can kill larvae. (Minarni *et al*., 2013).

The results of lethal time probit analysis showed that the LT_{50} of EEDT 0.1% was 9.792 hours, which did not exceed the observation time limit. Therefore, this concentration was effective if used as a larvicide for *Culex quinquefasciatus*third-instar larvae. The higher the concentration is given to the larvae, the higher the chemical substances exposed to the larvae, thus the faster the time required to kill the larvae.

A previous study conducted by Wijayanti (2015) stated that tobacco (*Nicotiana tabacum* L.) had larvicide effect on *Culex quinquefasciatus* larvae with 0.005%, 0.011%, 0.018%, 0.031%, 0.066%, and 0.095% concentrations. The highest dose (0.095%) had a 90% *Culex quinquefasciatus* mortality percentage using tween 80 solvent. The result of lethal concentration probit analysis was 0.058%. Khalalia (2016) investigated the killing power of tobacco extract granules on *Aedes aegypti* using 10%, 15%, and 20% doses. The highest dose (20%) could kill 31.25% larvae. Meanwhile, in this study, tobacco leaf extract with a 0.1% dose can kill 98% of *Culex quinquefasciatus* larvae using the CMC solvent. The LC_{50} analysis was 0.062%. Therefore, the increased concentration will increase the mortality rate of *Culex quinquefasciatus*. The results showed that 96% ethanol extract of tobacco leaf (*Nicotiana tabacum* L.) had significant ability in killing *Culex quinquefasciatus* larvae.

CONCLUSION

Tobacco leaf (*Nicotiana tabacum* L.) extract had a larvicide effect on *Culex quinquefasciatus* third-instar larvae. The 0.1% dose of tobacco leaf extract was effective in killing 98% of *Culex quinquefasciatus* larvae. The LC_{50} of tobacco leaf extract on *C.* quinquefasciatus larvae mortality

within 24 hours was 0.062%. The LT_{50} of tobacco leaf extract on the mortality of *C. quinquefasciatus* larvae with 0.1% was 9.792 hours.

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