Larvicidal Effect of Kenikir Leaves Extract (Cosmos caudatus Kunth.) Against Aedes aegypti L. Larvae Vector of Dengue Hemorrhagic Fever

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Abstract—Background : Mosquito population is a well-known vector in transmitting many types of diseases causing serious public health problems (Gubler 1995; Lam, 1993; Nazri *et al.* 2013). Dengue Hemorrhagic Fever (DHF) is a disease caused by Dengue Virus that transmitted y mosquito bites of *Aedes aegypti*. Prevention of Dengue Hemorrhagic Fever can be done by controlling biological and chemical the vector of larvae stage. The vector has been resistance of chemical insecticides. The most accurate way to control propagation is by using the natural substance, for instance is the *Kenikir* leaves (*Cosmos caudatus* Kunth.). The Extract of *Kenikir* leaves contains of active substance *Alkaloid, Saponin, Flavonoid and Tannin* which are poisoning to *Aedes aegypti* L. larvae

Objective : The purpose this research to determine the larvacide effect of the Extract *Kenikir*'s leaves (*Cosmos caudatus* Kunth) against the *Aedes aegypti* L. as a Dengue Hemorrhagic Fever's vector.

Methods : This research used a Completely Randomized Design (CRD) design using 900 larvaes of *Aedes aegypti* L. instar III and divided into 6 treatments extract group (negative control ; 0,05%, 0,10% ; 0,15% ; 0,20%, positive control) which is in each treatment extracted with 4 replications.

Results : The Result of larvicide effect was analyzed by using Analysis of Variance (ANOVA) and Probit Analysis. Result the research showed that *Cosmos caudatus* Kunth's leaves extract affected the death of the third instar *Aedes aegypti* larvae with significant value (P <0,05). The Result of probit analysis showed that LC50 value of *Cosmos caudatus* Kunth's leaves extract was 0,19 % .

Conclusion : Larvicide effect of *Cosmos caudatus* Kunth's leaves extract cause from secondary metabolits such as *alkaloid, flavonoid, saponin, dan tannin*. The conclusion of this research is *Cosmos caudatus* Kunth's leaves extract have effectiveness as a larvicide the third instar of *Aedes aegypti*.

Keyword: Larvicidal, *Cosmos caudatus* Kunth's leaves extract , *Aedes aegypti*

1. INTRODUCTION

The mosquito Aedes aegypti (L.) is considered to be among the main vectors of the viral diseases dengue fever and dengue hemorrhagic fever (Gubler and Clark, 1995).

Aedes aegypti mosquito is a vector of Dengue Hemorrhagic Fever (DHF) disease which is an endemic disease in tropical countries, one of which is Indonesia. Dengue hemorrhagic fever is an infectious disease caused by dengue virus and transmitted to humans by the bite of the *Aedes aegypti* mosquito (Direktorat Jenderal, Pengendalian Penyakit dan Penyehatan Lingkungan, 2011). Dengue Hemorrhagic Fever (DHF), also known as dengue hemorrhagic fever (DHF), dengue fever (DF), dengue fever (DD), and

dengue shock syndrome (DSS) are diseases caused by dengue virus serotypes DEN-1, DEN -2, DEN-3, and DEN-4 (Soegijanto, 2012). The main vector of DHF is the *Aedes aegypti* mosquito.

Recently, Dengue Hemorrhagic Fever (DHF) is still getting serious attention from relevant agencies both at the national and regional levels due to the large number of cases due to Dengue Hemorrhagic Fever (DHF) which causes humans to suffer pain and even cause death. Based on the records of the Yogyakarta Special Region Health Office, in DIY dengue fever has increased in 2013 compared to 2012. This can be seen from the incidence of dengue cases in 2012 of 3.54 / 100,000 population and in 2013 amounted to 19.29 / 100,000 inhabitants. The death rate also increases. In 2012, there were no deaths from contracting the dengue virus, but in 2013 it was 0.6%. DHF cases continued to increase in 2014 with 882 dengue cases recorded and there were 7 deaths until May 2014 in Yogyakarta.

Various efforts have been made by the government to overcome the phenomenon of Dengue Hemorrhagic Fever (DHF) disease from prevention efforts to treatment. Prevention efforts are carried out through socialization in various media and eradication of mosquitoes in various ways. Vector-borne diseases, including those caused by Aedes aegypti should be handled with vector control. However, this did not go well as the use of insecticides exploded in the 1940s-1950s which caused all diseases transmitted by insect vectors as if they had been overcome properly. Lately, we can observe the use of insecticides regularly in our daily lives. However, disease outbreaks tend to re-emerge in far more severe conditions, accompanied by the fact that most types of vector insects are resistant to insecticides which are usually effective and easily accessible to the community (WHO, 2014).

The spread of DHF cases can be prevented by targeting the eradication of *Aedes aegypti* as the main vector of DHF. *Aedes aegypti* control is mainly achieved by cleaning container habitats using insecticides or biological control agents (WHO, 2009). Therefore, Recently, Dengue Hemorrhagic Fever (DHF) is still getting serious attention from relevant agencies both at the national and regional levels due to the large number of cases due to Dengue Hemorrhagic Fever (DHF) which causes humans to suffer pain and even cause death. Based on the records of the Yogyakarta Special Region Health Office, in DIY dengue fever has increased in 2013 compared to 2012. This can be seen from the incidence of dengue cases in 2012 of 3.54 / 100,000 population and in 2013 amounted to 19.29 / 100,000 inhabitants. The death rate also increases. In 2012, there were no deaths from contracting the dengue virus, but in 2013 it was 0.6%. DHF cases continued to increase in 2014 with 882 dengue cases recorded and there were 7 deaths until May 2014 in Yogyakarta.

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The key strategy for mosquito vector control is by eradicating larvae at nesting sites. The most common way used by the community is to use insecticides. However, the side effects caused by the use of chemical larvacides such as not degraded, environmental pollution, toxic to non-target populations and the development of mosquito resistance have continued to increase over the past five decades. Most insecticides are nonselective and can be harmful to other organisms and the environment. In addition, it increases the risk of residual pesticide contamination in water when used repeatedly.

2. MATERIALS AND METHOD

Plant collection and extraction

Kenikir leaves (*Cosmos caudatus Kunth*) collected from Yogyakarta, Indonesia. *Kenikir* leaves extract from LPPT, Faculty of Pharmacy, Gadjah Mada University. The plant parts were separated, dried and ground into fine powder using a laboratory grinding mill. Dry the *kenikir* leaves in a drying cabinet with a temperature of 45 ^{0} C for 48 hours. Powdered leaves with dried *kenikir* pollinator 1 mm filter hole diameter. Weight the *kenikir* leaf powder namely: 669 grams. Add ethanol 96%. Stir with ultraturaq for 30 minutes, let stand 24 hours, then strain (repeat 2 times). Apply filtate with Vacuum Rotary evaporator heater temperature 50 ^{0}C. Pour the thick extract in a porcelain dish. Heat it with a temperature of 70 ^{0}C while stirring occasionally. Weigh and pack the extract of the leaves of *kenikir*.

Larvicidal Activity Test

A larvicidal activity test was conducted using a bioassay according to the standards of the WHO (2005) with a slight modification. The third instar larvae of *A. aegypti* were acquired and allowed to develop at the Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University. A preliminary test was conducted to determine the range of concentrations of *Cosmos caudatus* extract that could be deadly to larva of A. aegypti from instar III. In further tests, temephos was used as a positive control at a concentration of 1%, whereas the negative control consisted of 100 mL of distilled water only. The selection of temephos dosage (1 %) was based on lethal damage consideration used in the field. Larvae of *A. aegypti* instar III were used and 25 larvae were used in each treatment medium and control, replicated four times. After 24 hours, the dead larvae of *A. aegypti* were counted. The temperature and pH of the media and humidity in the room were measured at the beginning and the end of the study.

Data analysis

The percentages of larval mortality were expressed as mean ± standard Error of the Mean (SEM). Statistical analysis was performed using one-way ANOVA to analyze the differences in the effect of the concentration of *kenikir* leaf extract (*Cosmos caudatus* Kunth) on the mortality of *Aedes aegypti* larvae. If the data were normally distributed and homogeneous; later LSD test was used. Statistically significant differences were indicated by $p<0.05$.

The ratio of LC_{50}/LC_{90} of concentration of *Cosmos caudatus* extract on *A. aegypti* larvae was calculated using probit regression analysis using SPSS version 24.

3. RESULTS AND DISCUSSION

Tabel 1. Percentage of Death of Larvae Aedes sp at Various Concentrations after Giving Extract of *kenikir* leaves (*Cosmos caudatus* Kunth) after 24 hours Treatment

Concentration (%)	No. of larvae	The number of death of Larvae (Replicate)				number	average	Percentage Death of Aedes Larvae (%)
		I	II	III	IV			
Kontrol (-)	25	0	0	0	0	0	0	0
0,05	25	18	14	13	8	53	13.25	53
0,1	25	25	24	11	4	64	16	64
0,15	25	23	16	9	10	58	14.5	58
0,2	25	18	23	19	21	81	20.25	81

Result

Larvicidal Activity of the environment considered in this study was the pH water, temperature and humidity. These were measured at the beginning and end of the study as pH 7, 25°C and 71%, respectively. The larvicidal activity test of *kenikir* leaf extract on *A.aegypti* larvae was performed in four replicate. The average percentage mortality of *A. aegypti* larvae after 24 h of observation is presented in Table 1.

Graphic 1. The Graph of mortality A.aegypti larvae in the group tread with *Cosmos caudatus* extract in a wide of concentration

Konsentrasi Ekstrak daun *Cosmos caudatus Kunth*	Mortality (%)	LC $_{50}$(%)	LC_{90}(%)
Konsentrasi 0,05%	53	0,19%	0,27%
Konsentrasi 0,1%	64		
Konsentrasi 1,5%	58		
Konsentrasi 2%	81		
Control	0		

Tabel 2. The average values of LC_{50} and LC_{90} of larvicidal effect from *Cosmos caudatus* leaf extract against larvae of *A. aegypti.*

The larvicidal effect from Cosmos caudatus leaf extract on *A.* aegypti larvae was reflected in the LC50 and LC90, which were determined by probit analysis using SPSS version 24. To measure the differences in the average number of deaths of A. aegypti larvae, ANOVA was used .The average values of LC50 and LC90 of larvicidal effect from *Cosmos caudatus* leaf extract against larvae of *A. aegypti* in the four larvicidal tests were o.19% and 0.27% (Table 2).

4. DISCUSION

Larvicidal Activity of *Cosmos caudatus Kunth* leaves extract againts *A. aegypti*

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes

to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides (Chndre et.al., 1998). Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. Mosquito control approaches based on synthetic insecticides have created many problems like insecticide resistance (Liu H, et al., 2005). Natural products of plant origin with insecticidal properties have been tried in the recent past in order to control a variety of insect pests and vectors. This has necessitated the need for a research and development of environmentally humidity of the environment at the time of the study were 7, 25°C and 71%. The levels of pH, temperature and room humidity were still within the optimal pH range (6.5-7), temperature (25-27°C) and air humidity (60-80%) for the development of *A. aegypti* larvae in bioassay research (WHO, 2005). Therefore, it can be concluded that the pH, temperature and humidity of the media at the time of the study did not interfere with the development of *A. aegypti* larvae. The death of *A. aegypti* larvae was not caused by these environmental factors. The growth and development of larvae were influenced by the adequacy of food sources, the temperature and the presence or absence of predators (Dharmagadda et al., 2005).

The plant's high larvicidal activity is supported by the presence of phytochemicals such as *alkaloids, saponins,flavonoids, steroids* and *tannins* which showed combination effects in terms of larvicidal action to mosquito larvae.

The death of *A. aegypti* larvae in the current study was due to the toxic leaves such as *saponins, polyphenol flavonoids,* and *essential oils* provide insecticidal effects that are accountable for their larvicidal efficacy potential. These compounds are chemical compounds that use metabolism that can be used in plant tissue, and can function as stomach and respiratory poisons. This is in line with what was done by Zuraida, et al. (2010) in Lestari (2012). Saponins that can cause destruction (damage) of channels by reducing the stress on the mucous membranes of the canal become corrosive.

Saponins are known to have various biological properties. They have *membrane-permeabilising, haemolytic, antioxidant, anti-inflammatory, immunostimulant and anticarcinogenic* activities, they affect feed intake, growth and reproduction in animals, and they can be used as fungicides, molluscicides and pesticides, as well as against some bacteria and viruses (Francis et al., 2002; Sparg et al., 2004; Avato et al., 2006; Tava and Avato 2006). Saponins give rise to increased mortality levels, lowered food intake, weight reduction, retardation in development, disturbances in development and decreased reproduction in pest insects. The mechanism underlying these actions is, however, still largely unknown, but it is likely that saponins have multiple activities.

The main hypotheses are that saponins could either make the food less attractive to eat (repellent / deterrent activity), bear digestive problems, because of moulting defects or have toxic effects on cells. (Ellen De Geyter et al., 2007). Moreover, saponins are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking with the cuticle membrane of the larvae, eventually disturbing the membrane, which is the main cause for larval death (Hostettmann and Marston 2005).

Flavonoid compounds contained in *kenikir* leaf extract (*Cosmos caudatus Kunth*) are also insecticides because they are respiratory poisons which cause larvae to be unable to breathe due to respiratory system damage and ultimately cause death of larvae. In addition, flavonoids are also CYP6Z2 inhibitors, family of cytochrome P450 which plays a role important for insecticide resistance in mosquitoes.

CONCLUSION

*J*oils have larvacidal effect against *Aedes aegypti* larvae .LC_{50} *Cosmos caudatus* Kunth Leaf Extract against *Aedes aegypti* larvae obtained at 0.19%

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