Effectivity Of Benalu Coffe Extract (*Loranthus Ferrugineus-Jack*) As Insecticide Against *Culex Sp.*

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Abstract.

Benalu coffe (Loranthus ferrugineus Jack) contains phytochemical compounds such as alkaloid, flavonoid, saponin and tannin. The compounds possess natural insecticide properties against mosquito larva. The aim of this study was to obtain the effectivity of Benalu coffe extract as larvicidal agent against Culex sp. The study was designed as a post test only control group with a total of 80 tested larvae and 24 hr of observation. The concentration of administered extract was 0% (K0), 0.7% (P1), 0.9 % (P2) and 2% (P3). Based on ANOVA test, there was a significant difference between control group and treatment group ($p=0.001 < \alpha(0.05)$). Based on a post hoc analysis result, the larvicidal percentage was significant between K0 and P2 ($p=0.013$), K0 and P3 ($p=0.001$), P1 and P2 ($p=0.045$), P1 and P3 ($p=0.002$), and between P2 and P3 ($p=0.023$). In this case, there was no significant difference between K0 and P1 group ($p=0.697$). The Benalu coffe extracts were then proved to exhibit larvicidal activities against Culex sp. larva.

Keywords: *Benalu coffe, Culex sp, Natural larvicide*

I. INTRODUCTION

Mosquitoes are insects in the order of Diptera, importantly known as the primary vector of human parasites which cause serious disease. These vector insects are generally neglected and may spread viruses and parasites such as malaria, dengue hemorrhagic fever (DHF), filariasis, chikungunya fever and encephalitis [4]. *Culex* spp. are mosquito members in Culicidae as one of the main transmission vectors for filariasis [8]. This parasite lives in the mosquito can cause filariasis after the mosquito bites person to person who suffer from lymphatic filariasis in the blood circulation. Filariasis has some clinical manifestations that can cause disability in chronic patients [11]. Preventive measures and termination of the life cycle of *Culex* sp. has been promoted through mosquito nest eradication (MNE) and other efforts by killing the larvae and mosquitoes. Recently, the existing methods for general eradication or physical methods carried out by the community through the implementation of the 3M program, namely draining, burying and removing all possible places for mosquito breeding have not been optimal. Furthermore, there is a chemical approach which is the application of chemical larvicides such as Abate® in the household which is still less effective as the main alternative larvicide in vector control efforts [6].

The prolonged use of Abate® can lead to larval resistance in the future leading to the emergence of larvicide-resistant population. In addition, there is a possibility that the chemical compounds will also impact seriously to the environment in the form of ecosystem imbalances, therefore it is necessary to explore other natural alternative larvicides that are environmentally friendly, for example those originating from vegetable [5]. A good candidate of plants are those containing flavonoid compounds, alkaloids, steroids, terpenoids, tannins and saponins which have toxic effects on insects, but do not cause side effects on humans and the environment [2]. Previous research conducted by Santi et al on the larvicidal activity by the extract of the Mangkokan leaf (*Nothopanax scutellarium*) was reported to be effective in killing *Culex* sp. larvae while the extracts also contained bioactive phytochemicals such as saponins, tannins, alkaloids and flavonoids [7]. One of the underexplored plant species that contain these promising compounds as larvicide is Benalu coffe. Yulian and Safrijal reported that the extract of Benalu coffe contain a significant proportion of tannins [10]. There is still a limited information regarding the study of Benalu coffe extract as a larvicidal agent against mosquito. In this study, we have evaluated the larvicidal potential of Benalu coffe extract against *Culex* sp. larvae. The crude extracts have proven to exhibit some toxicity against mosquito larvae.

II. METHODS

This study used a post test only with control group design with 4 treatment groups, namely a control group (K0), a group exposed with 0.7% of Benalu coffe extract (P1), a group exposed with 0.9% of Benalu coffe extract (P2) and a group exposed with 2.0% of Benalu coffe extract. The experimental procedure was described as follows:

Specimen collection

A collection of 80 *Culex* sp. larvae was used as the object in our study. The sampling was conducted randomly and the larvae were identified morphologically under dissected microscope and stored in a container for further experimentation.

Extraction of Benalu coffe Leaves

Benalu coffe leaves (*Loranthus ferrugineus* Jack) were cleaned and air-dried without direct sunlight exposure. The dried leaves were crushed into powder while 200 g of it were dissolved into 800 mL EtOH (menstruum) and macerated for 24 hr in a closed bottle. The macerate was filtered using a Whatman filter paper while the residue was macerated twice using a new EtOH with a ratio of 1 : 2. The macerates were pooled and evaporated from the remaining solvent in a laminar air flow at 50°C for $\pm$ 24 hr. The plant extract was further concentrated in a water bath at 40 - 70°C to obtain a crude extract [10].

Larvicidal test

The crude extract of Benalu coffe was prepared in a serial of concentrations namely 0%, 0.7% (P1), 0.9% (P2) and 2% (P3). Ten larvae of *Culex* sp. were placed by

hand using a plastic pipette into a reaction tube containing 5 mL of each extract. The larvicidal activity was measured as the number of dead larvae after 24 hr of exposure to plant extract [1].

III. RESULT AND DISCUSSION

Results

The effectivity of Benalu coffe extracts as larvicidal compounds against *Culex* sp. larvae is presented in Table 1.

Table 1. Larvicidal test results of Benalu coffe (*L. ferrugineus*) against *Culex* sp. larvae

No	Concentration	Duration	Dead larvae	Percentage	Aver.	Category
1	K0	24 Jam	0	0%	0%	I
			0	0%		
2	P1	24 Jam	1	10%	10%	E
			1	10%		
3	P2	24 Jam	3	30%	35%	E
			4	40%		
4	P3	24 Jam	6	60%	65%	I
			7	70%		

Notes :

K0 : Control (without exposure to Benalu coffe extract)

P1 : Benalu coffe concentration at 0.7%

P2 : Benalu coffe concentration at 0.9%

P3 : Benalu coffe concentration at 2%

E : Effective (Average between 10% and 95%)

I : Ineffective (Average value <10%)

Based on the results presented in **Table 1**, the average number of dead larvae in K0 was 0 or no mosquito larvae were killed during the incubation period. Meanwhile in P1, P2 and P3, the percentage of larvicidal activity against *Culex* sp. larvae was 10%, 35% and 65%, respectively. The utilization of Benalu coffe extract (*Loranthus ferrugineus* Jack) was toxic to *Culex* sp. larvae based on WHO (2005), which categorized the effectivity of a larvicide lies between 10% to 95% [9].

Data analysis

Based on the normality test of acquired data using Kolmogorov-Smirnov, it was revealed that the data may proceed to one way ANOVA since it displayed an equal distribution of data

*One way ANOVA test results***Table 2.** Statistical significance between treatment groups using One way ANOVA

Group	Average $\pm$ S.D	P
K0	00. $\pm$ 0.00	0.001
P1	1.00 $\pm$ 0.00	
P2	3.50 $\pm$ 0.707	
P3	6.50 $\pm$ 0.707	

Notes :

K0= Control, P1 = Benalu coffe concentration at 0.7%, P2 = Benalu coffe concentration at 0.9%, and P3 = Benalu coffe concentration at 2%

Based on the results presented in **Table 2**, there was a significant difference among groups in terms of the number of dead larvae as revealed from the value $p=0.001$ ($p<0.05$). The results were further analyzed using a post hoc test to obtain the multiple comparison among significant groups.

*Post hoc Bonferroni's test results***Table 3.** Multiple comparisons of treatment groups using post hoc Bonferroni's test

Group	P			
	K0	P1	P2	P3
K0	-	0.697	0.013	0.001
P1	0.697	-	0.045	0.002
P2	0.013	0.045	-	0.023
P3	0.001	0.002	0.023	-

Notes:

K0= Control, P1 = Benalu coffe concentration at 0.7%, P2 = Benalu coffe concentration at 0.9%, and P3 = Benalu coffe concentration at 2%

Based on the results presented in **Table 3**, There were significant groups in terms of in terms of the number of dead larvae between K0 and P2 ($p = 0.013$), K0 and P3 ($p = 0.001$), P1 and P2 ($p = 0.045$), P1 and P3 ($p = 0.002$), and between P2 and P3 ($p = 0.023$). In this case, there was no significant difference between K0 and P1 group ($p=0.697$).

Discussion

In this study, the effectiveness of the Benalu coffe extract (*Loranthus ferrugineus* Jack) was tested based on the larvicidal activity against *Culex* sp. larvae under various concentrations. The purpose of preparing the Benalu coffe extract was to utilize any available natural larvicides or known as vegetable larvicides, which are types of larvicides derived from plants and contain active substances that are toxic to larvae. In this study, Benalu coffe plants were used and reported to harbor some toxic phytochemicals although never been tested against mosquito larvae. This study used 4

treatment groups with different concentrations, each group containing 10 larvae in a 5 ml of solution. The concentration of the Benalu coffe extract (*Loranthus ferrugineus* Jack) used in this study was 0% (K0), 0.7% (P1), 0.9% (P2) and 2% (P3). Based on the results, we noticed that all tested concentrations (0.7%, 0.9% and 2%) were effective to kill *Culex* sp. larvae and was found to be directly proportional to the concentration of used extracts. We reported here that the higher the concentration of Benalu coffe extract, the higher the percentage of the number of dead larvae which also indicated the higher amount of toxic phytochemicals contained in the extracts.

The content of bioactive compounds such as alkaloids, terpenoids, flavonoids and tannins are chemical defense compounds commonly found in the Benalu coffe to suppress herbivory by herbivorous insects by killing the sensitive larvae. Our results were also supported by Arti et al (2018) on the effectiveness test of Pandan Wangi (*Pandanus ammaryllifolius*) leaf extract which also exhibit larvicidal activity against *Culex* sp. with a high mortality result through the use of higher concentration [1]. Some negligible factors are thought to improve the larvicidal activity against *Culex* sp. by the Benalu coffe extracts namely pH and temperature. Prior test, the pH of Benalu coffe extract incorporated into the treatment solution was 7 and after treatment, the pH was dropped until 6 in each treatment group. However, the larvae was reported to grow in the pH range of 5.8-7.6. Furthermore, the resulting temperature of treatment group was 28°C, hence the temperature was still tolerable by the larvae as they show growth in the temperature range of 27°C-31°C [3]. This study still has several limitations, for example the addition of Benalu coffe extract may alter the color of the water and the aroma which are not applicable in a larvicidal product. The crude extract was also hard to dissolve since it appear as a paste thereby rendering its solubility in the aqueous environment and can not be used consistently in further experimentation.

IV. CONCLUSION

Based on the results, all tested concentration of Benalu coffe extract (0.7% , 0.9% dan 2%) exhibit larvicidal activities against *Culex* sp. larvae and may be used as an alternative to synthetic larvicides.

V. ACKNOWLEDGMENTS

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