

## Dengue vector surveillance (*Aedes albopictus*) with ovitrap and attractants from imperata immersion (*Imperata cylindrica*)

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### ABSTRACT

Efforts to control the vector in reducing the population of *Aedes albopictus* can be done by using attractants with ovitrap. This study aims to determine the variation of *Imperata cylindrica raeusch's* immersion straw immersion dose on the number of eggs and hatchability of *Aedes albopictus* in the laboratory. Research type is a true experiment with a completely randomized design (CRD). The material used is *Imperata cylindrica raeusch* soaking with 5%, 10%, 15%, 20% concentration and aquadest as a control with 5 replications. The results showed that Imperata grass soaking was proven to be effective as an attractant in *Aedes albopictus* mosquitoes and was able to inhibit the number of eggs that hatch. Utilization of Imperata straw soaking can be used as an additive in the ovitrap in dengue vector surveillance.

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## 1. INTRODUCTION

Vector borne disease is a public health problem. One vector that needs to be watched out is *Aedes albopictus*. Arbovirus (arthropod borne virus) disease such as La Crosse encephalitis [1] West Nile virus, Zika, Dengue, chikungunya, yellow fever, and rift valley fever (RVF), [2] but it can also be an intermediary for tapeworm disease in dogs *Dirofilaria immitis* (Leidy), (Spirurida: *Onchocercidae*) in Southeast Asia and *D. immitis* and *D. repens* (Raillet and Henry) in Italy are diseases transmitted by *Aedes Albopictus* [3]. Arbovirus is increasingly becoming a global health problem because of its rapid geographical spread and high disease burden. Especially over the past 30 years, the distribution and impact of public health have increased dramatically, because the widespread distribution of these vectors correlates with increased trade and travel [4]. A large number of studies have mapped the global or regional distribution of *Aedes albopictus* and determined the ecological and habitat requirements to see the latest distribution and development. Recent developments have been mapped regarding the global distribution of *Aedes albopictus* together with *Aedes aegypti* and found that the range of habitat suitability for this species is the widest, occurring on all continents including North America and Europe [5]. *Aedes albopictus* originally from Southeast Asia, islands in the Western Pacific and Indian Ocean, but currently its distribution has expanded to have spread to Africa, the Middle East, Europe and America (north and south) and the Pacific islands [6].

Arbovirus transmission in *Aedes albopictus* is proven to be transovarial, especially dengue and zika [7]. Transovarial transmission of the virus is vertical transmission from female mosquitoes that are infective to the offspring of the virus. If conditions in nature do not allow it to reproduce, one of the ways the dengue

virus to maintain their existence is through transovarial transmission. Dengue virus maintains its life in an optimal amount through transovarial transmission to mosquito populations that have a genetic advantage, where such populations are very important in the dynamics of dengue transmission [8].

Vector control efforts in reducing the population of *Aedes albopictus* can be done in the adult and pre-adult phases. One method that can be done is to use attractants to attract mosquitoes by using ovitrap [9]. Ovitrap is a mosquito laying trap that is used to break the life cycle of mosquitoes before the larvae or pupa turns into mosquitoes [10]. The addition of attractants in the ovitrap proved effective in various research results, including isolation of rhizoma fungi [11], rice straw (*Oryza sativa*) with field application [12] and others. The use of Imperata grass immersion was done at a concentration of 20% and proved effective in mosquito oviposition, [13] but experiments on the variation of the dose percentage have never been done either on the number of eggs and hatchability. Therefore, it is necessary to further research whether there is a more effective dose as an attractant. The purpose of this study was to determine the variation of *Imperata cylindrica* Raueuschs immersion doses to the number of eggs and hatchability of *Aedes albopictus* in the laboratory.

## 2. RESEARCH METHOD

Type of the research is a true experiment with a completely randomized design (CRD). The research activity was carried out in the Entomology Laboratory of the Health Research and Development, Tanah Bumbu District in January-July 2019. *Aedes albopictus* used in the test were phenotype (F) 27, Tanah Bumbu strains. Methods for Mass Rearing of *Aedes albopictus* is modified from the *Aedes aegypti* Mass Rearing method [14].

### 2.1. Hatching eggs into larvae

The eggs from the pottery containers are moved in a tray measuring 27cm x35 cm x 5 cm. Each tray contains 4-5 filter paper that already contains eggs. The eggs will hatch after 1-2 days. The water used for the life of pre-adult mosquitoes are aquades water with a volume of 1.5 liters. Observation of eggs that hatch into first instar larvae is done every 24 hours. Egg hatches calculations are performed on five groups.

### 2.2. Rearing *Aedes albopictus* larvae

Instar larvae I and II were fed using mashed cat food (Whiskes®) which amount was adjusted to the size of the instar hatched in a container made of earth (pottery). Feeding is done once a day. In instar larvae III and IV feed (not pounded) was given twice, namely morning and evening. Larvae that have become to pupae from the breeding place are taken with a pipette and then placed in a small plastic container and put into a mosquito cage.

### 2.3. Rearing adult *Aedes albopictus*

The larvae that have become pupae are then moved using a pipette into a plastic tray with a diameter of 6cm below the top, 8cm, height 6.5cm and water+50ml. Containers containing pupae are placed in bugdorm cages of 30cm x 30cm x 30cm size. In general, after two days the pupa will turn into a mosquito. Female and male mosquito food is given a solution of 10% sugar water which is placed in cotton and put in a bottle. Mosquitoes will copulate with a cage for 2-3 days. Female mosquitoes will suck the blood of guinea pigs in the process of egg maturation. While male mosquitoes will suck 10% sugar liquid. After 2-3 days of sucking blood the female mosquito will lay eggs on the pottery.

### 2.4. Making imperata grass immersion

Imperata that is found in nature is collected, then dried in the sun for about 3-6 days until it is completely dry. The attractant stock solution is made by soaking 125 grams of Imperata straw with 15 liters of water in a closed bucket for 7 days. The attractant stock solution was diluted with distilled water to obtain a treatment concentration of 5%, 10%, 15% and 20% in a 1 liter volume. Aquadest are used as controls. The treatment and control solution is put into an ovitrap in the form of a 150 ml plastic glass that has been affixed with filter paper on the inside, then put into a 45x45x45 cm mosquito cage.

### 2.5. Testing mechanism

An Immersion that is done by immersion is inserted into the ovitrap with the laying design of each forming a rectangle shown on Figure 1. A total of 25 female full of blood *Aedes albopictus* mosquitoes were put into a cage that already contained an ovitrap. Each treatment and control was repeated 5 times. The duration of treatment was carried out for 7 days, after which the ovitrap was removed and its filter paper was taken to observe and count the mosquito eggs attached with the aid of a microscope and a counter.

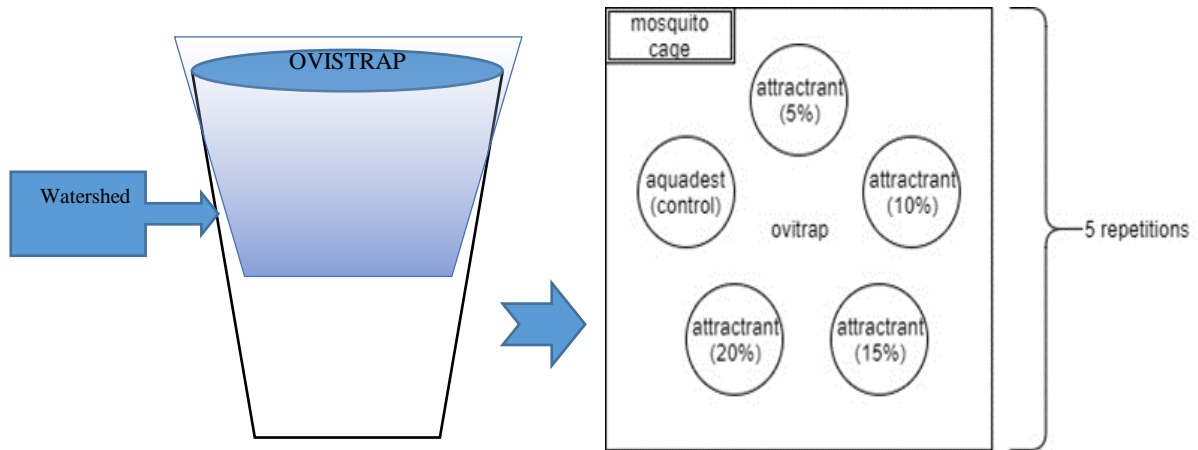


Figure 1. Ovitrap form and test design scheme

**2.6. Data analyze**

The results of the calculation of the number of eggs entered into the table for analysis. Data were analyzed by Anova test, normality test and LSD follow-up test to find out significant differences.

**3. RESULTS AND DISCUSSION**

The number of eggs laid in the ovitrap with the highest concentration is 20% with the number of 811 eggs almost 2 times the total concentrations of 15%, 415 eggs, while the least is the aquadest with the number of 39 eggs shown on Figure 2a. The highest number of hatching eggs is 20% with 458 larvae, while the least is aquadest, which is 20 larvae shown on Figure 2b.

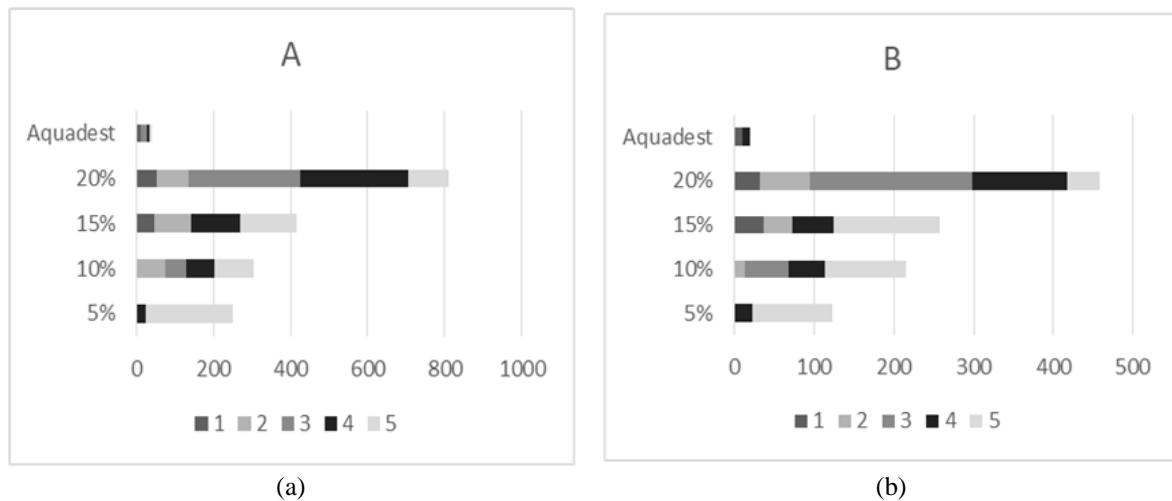


Figure 2. (a) Number of eggs *Aedes albopictus*, (b) Number of eggs *Aedes albopictus* that hatch into larvae at various concentrations

In percentage, 44.56% of the concentration of 20% with the number of hatches compared to the number of eggs is 42.74%, but in proportion to the concentration of 10% can hatch up to 70%. Based on the Anova test, there was an effect of concentration on egg plumes in *Aedes albopictus* mosquitoes shown on Table 1. The effect of the concentration is then continued with further LSD testing. There are 3 concentrations that have a real influence, namely a concentration of 5%, 10% and aquadest to a concentration of 20% shown on Table 2.

Table 1. Percentage of number of eggs and hatching of *Aedes Albopictus* and Anova testing influence concentration on number of eggs

Treatment	n	SE	Average (min-max)	Percentage of number of eggs	Percentage of number of hatches	The proportion of hatches	F	Sign
5%	5	44.47	50 (0-227)	13.74	11.36	48.80	2.899	0.048
10%	5	17.09	61(0-103)	16.76	20.11	70.82		
15%	5	26.71	83 (0-146)	22.80	23.93	61.93		
20%	5	51,07	162.2 (52-291)	44.56	42.74	56.60		
Aquadest	5	2.44	7.8 (1-15)	2.14	1.86	51.28		

Table 2. LSD test analysis results on average the number of eggs trapped in the ovitrap based on the type of treatment

Treatment	SE	Sign	
5%	10%	47.316	0.819
	15%	47.316	0.494
	20%	47.316	0.028*
	Aquadest	47.316	0.383
10%	5%	47.316	0.819
	15%	47.316	0.647
	20%	47.316	0.045*
	Aquadest	47.316	0.274
15%	5%	47.316	0.494
	10%	47.316	0.647
	20%	47.316	0.110
	Aquadest	47.316	0.128
20%	5%	47.316	0.028*
	10%	47.316	0.045*
	15%	47.316	0.110
	Aquadest	47.316	0.004*
Aquadest	5%	47.316	0.383
	10%	47.316	0.274
	15%	47.316	0.128
	20%	47.316	0.004*

Efforts to control vectors by utilizing natural materials that are widely available in nature are economical and environmentally friendly. These efforts can use of fruit, flowers, leaves, stems, roots and other available materials. Many of these natural materials used as larvicides and also as attractants. The use of natural materials as biolarvicides which has been scientifically proven to be able to kill mosquito larvae, among others are extracts of grapefruit [15], frangipani leaves [16], tobacco leaves [17] and others, while attractants that are proven capable of attracting mosquitoes to lay eggs (oviposition) are agnasma leaves with nano particle extraction [18], rice straw (*Oryza sativa*) soaking water, Goose grass (*Eleusine indica*) [13] with a 15% soaking water content.

The use of attractants is usually combined with ovitrap/egg traps in the field. Attractants are chemicals that can cause insects to move towards the source of these substances [19]. Attractant aims to centralize insects and be able to ignore the presence, disturbance, and danger of the area around attractants both other insects and other predators [20]. Based on the results of this study, Imperata grass soaking was proven to be effective as an attractant for *Aedes albopictus* mosquitoes and other effects were also able to inhibit egg hatching. The ability of Imperata straw immersion as an attractant may be due to the presence of fatty acid content [21]. Fatty acids can stimulate in influencing the attractiveness of oviposition of *Aedes* sp. [22, 23] Variability of the manifestation of the immersion resulted in the isolation and identification of specific chemicals present in the immersion that affected the oviposition response of the *Aedes* (*Stegomyia*) spp [23, 24].

Acidic metabolism (RCOOH) is the end result of digestion of fatty acids and is subsequently modified through the beta oxidation cycle to produce acetyl-CoA [25]. The acetyl-CoA formed will be converted into CO<sub>2</sub> + H<sub>2</sub>O + Adenosine triphosphate (ATP) energy through the Krebs cycle [26]. CO<sub>2</sub> is a gas produced by human or animal respiration which is a marker of the natural "signal" of mosquitoes to search for food sources. These markers are issued by Imperata grass soaking as a preference for oviposition because mosquitoes can smell CO<sub>2</sub> from 10-50 meters distance [27]. The organ to detect these markers in mosquitoes is located in the dendritic receptors of the olfactory receptor neurons (ORN) which protrude into the empty spaces formed by sensory fibers that called sensilla. Sensilla is found in the palpi maxilla and antenna. Different mosquito species can respond differently to certain odors (attractants). Action mechanism of the olfactory neuron response to attractants takes place at the molecular level so that it is able to distinguish attractant odors from other odors. The receptor will deliver to the olfactory neurons and then give an order to approach the attractant odor. One of the receptors that plays a role is the family gene Or83b, which is expressed in almost all olfactory neurons [28, 29].

Imperata grass in agriculture are considered as weeds or plant pests because of their massive nature and disrupt the growth of other plants, but Imperata straw that contains cellulose can be used as raw material for bioethanol production [30]. The content of Imperata grass is mannitol, glucose, saccharose, malic acid, citric acid, coixol, arundoin, cylindrin, fernenol, simiarenol, anemonin, grit acid, resin and alkali metals, alkaloids by 1.07% and flavonoids by 4.8 % [31]. The area of Imperata grasslands in Indonesia is around 10 million ha (4.5% of the land area). The most widespread Imperata grass area is found in Sumatra and Kalimantan, which are 2.13 and 2.19 million ha, [32] this is a great potential for Imperata can be used easily and cheaply as an attractant in ovitrap.

The use of ovitrap in entomological surveillance can be used if the Bretau index (BI) is low or less than 5. The use of ovitrap can determine the ovitrap index (OI), estimated mosquito density and what types of mosquitoes are in the area [10]. The ovitrap index is used to detect *Aedes aegypti* gravid female, *Aedes albopictus* gravid female and other genera of *Aedes*. However, ovitrap will not be effective anymore if placed more than one week because it can become a new breeding place for mosquitoes [33].

Through this research, the use of attractant straw soaking water in the ovitrap can be a new alternative in controlling dengue vector. The advantage of ovitrap is the low cost of manufacture, minimal maintenance and can be used in a long time. In the future it is necessary to conduct field-scale research and the combination of attractants and bioinsecticides is expected to strengthen this research.

#### 4. CONCLUSION

Imperata grass (*Imperata cylindrica*) straw soaking water has proven to be effective as an attractant for *Aedes albopictus* oviposition at a dose of 20%. The other effect is also able to reduce the number of eggs that hatch.

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