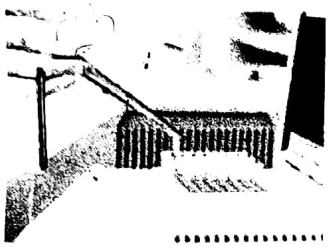
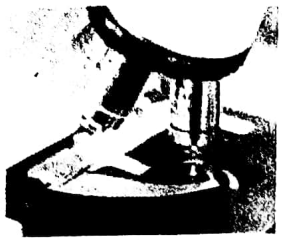
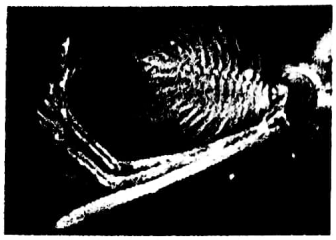

PROCEEDING

International Seminar

Health and Environmental Perspectives

Saturday, October 26 th 2013
at Patra Jasa Semarang Convention Hotel
Semarang, Indonesia

Editor
Praba Ginandjar
Dina Rahayuning Pangestuti
Lintang Dian Saraswati

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Editor
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PREFACE
CHAIR OF THE ORGANIZING COMMITTEE

In order to commemorate the 56th Anniversary of Diponegoro University as well as the mosquito day, School of Public Health held an international seminar.

Mosquito is a low-level organism including ancient invertebrates category. Characteristic of the low-level organisms are their ability that is very adaptable, so the mosquitoes still existed from the beginning until now. The ability of mosquitoes and insects is directly related to our efforts to control them. The organization th at control of vector and pest organisms currently is undertaken by the field of Health and Agriculture, and is mostly done using pesticides and insecticides.

Experience of WHO and USAID in using pesticides and insecticides from year to year in developed and underdeveloped countries in the world we can learned. This international seminar is our media to sit together and discuss their experiences in the field of health and agriculture to take a good benefit.

Will we continue to use pesticides? Is this time our farmers have been using pesticides safely? How to use pesticides safely, rational and produce minimum impact of health? Our homework on this case still a lot.

We hope that as an agricultural country, Indonesia will have a strong, healthy and fit farmers with agricultural products that are safe for the people consuming the product.

Organizations that associated with mosquito and vector control is Epidemiologist and Environmental Health. In this seminar occasion Entomology Society of Indonesia and Indonesia Environmental Health Spesialist Association opened a new member.

Besides seminars, other events that held are workshop and discussion of work. There are three workshops held to commemoration the mosquito day. Firstly Workshop about Molecular Entomology "Detection Sibling Vector and Dengue Virus" and the secondly Workshop about "Pesticides in Technology and Application", while the Thirdly Workshop about ISO 14001 Environmental Management System and EHSA.

We would like to say thank to the Committee who h ave worked hard to be able to organize this Workshop and Seminar. In particular, we also offered his congratulations and thank you to participants who interest to this event, members EHSA and ESI to present the results of research and thinking and at the s ame time we ask apologize for not all of the proposals can be presented. Our deep appreciation also goes to the keynote speakers and invited speakers.

Thank you also to all the people who have worked for the Workshop, Seminar and Work Dissucion thus lively and productive. Finally, we congratulate conduct workshops and seminars to commemorate the 2013 mosquitoes day. We hope that in the future we can continue to live in a healthy life, may Allah SWT bless our activities, Aamiin Yaa .. Robbal Alamin ....

Semarang, October 26, 2013

Committee

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PREFERENCES OF *Aedes aegypty*IN HACTCHING EGGS ON OVITRAPS WITH SEVERAL TYPES OF ATTRACTANT

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ABSTRACT

Background: A method to control *Aedes aegypti* in order to decrease the density of the vector is the use of the egg trap (the ovitrap). Attractant is usually used together with the ovitrap as attractant does not create a risk because of inhaling dangerous chemical contained in insecticide and fogging. The aim of this research was to identify the differences of the *Aedes aegypti's* preferences to hatch on the ovitrap using different types of attractants.

Method: This study was an experimental laboratory research with which variables measured were the number of *Aedes aegypti's* eggs trapped in the ovitrap and the attractants (yeast solution, hay infusion, and rain water.) Each unit of the experiment consisted of 25 blood fed *Aedes aegypti*. The number of eggs was calculated after five days. The number of replication of each unit was nine.

Result: ANOVA analysis showed that there were significant differences on the number of *Aedes aegypti's* eggs in the ovitrap using different attractants at 0.05. The average number of eggs in the ovitrap is, respectively, 669.1 eggs in the hay infusion, 297.3 eggs in the rain water, 114.4 eggs in the yeast solution, and control positive (+) 314.1. The highest number of *Aedes aegypti's* eggs in the ovitrap was the one uses hay infusion compare to yeast solution (p=0.000), rain water (p=0.000), and control (p=0.000).

Conclusion: Hay infusion was most effective than yeast solution and rain water attractant for oviposition of gravid *Aedes aegypti*.

Keywords: Ovitrap, hay infusion, attractant, *Aedes aegypti*

BACKGROUND

Aedes aegypti is a vector of several serious diseases exposed to human, such as, malaria, encephalitis, "yellow fever", dengue fever, filariasis, and arbovirus [1]. Among the most important vectors causing dengue fever; *Aedes aegypti, Aedes albopictus,* and *Aedes scutellaris; Aedes aegypti* is the major vector [2].The number of cases of dengue fever in Indonesia tends to increase from year to year. The increase of the dengue fever in many cities in Indonesia is caused by the difficulty to control the disease infected by *Aedes aegypti* [3,4].

Until today, there has no vaccine found to prevent dengue hemorrhagic fever [5,6]; so that, the management applied to these diseases is primarily aimed to cut off the infection spread out by controlling their vectors. The effort to control dengue fever's vector that has been carried out by all countries and endemic area proved to be not effective, unsustainable, and failing to break up the chain of infection [7].

The most effective precaution is by obliterating the mosquito's breeding sites through draining containers, to cover up tightly collecting and saving water, and burying unusable materials that might become the breeding place for *Aedes aegypti.* Moreover, the use of larvacide in wiping out the mosquito is also effective [8]. Some studies claimed that the knowledge of the society about the way to destroying *Aede saegypti* remains lacking [9]. As a result, restrain using chemical substances is popular in community and programs introduced to them although the use of insecticide to control dengue fever has both advantages and disadvantages [10]. Program to control vector implemented in many countries, including Indonesia, has not been successful because it depend on the application of fogging. This method costs 5 billion rupiahs per year, and it might develop resistance to the vector due to the inappropriate dosage use [11].

Recently, the use of alternative method to decrease the density of *Aedes aegypti* is considered significant in some countries, especially the use of the ovitrap. Unlike insecticide and fogging, attractant, mostly apply together with the ovitrap, does not contains dangerous chemical substances [12]. Mosquito can detect carbon dioxide (CO_2) from respiration of human, animal, and microorganism metabolism process. Mosquito approaching those sources of CO_2 will be trapped. The higher the volume of CO_2 and other attractants the easier the mosquito detects them. This caused chances of the mosquito to come closer increase [13].

The ovitrap modified using ten percent of hay infusion increases the number of eggs trapped into it eight times higher [14.15]. This attractant that produce CO_2, ammonia, and octane proves to be influencing the nerve system of mosquito's smell [16,17]. Meanwhile, fermented cassava (*Saccharomyces cereviceae*) produces methane gas, CO_2, small amount of other gases such as H_2S, and water [18]. Moreover, rain water is also suspected to be containing of more gases than soil water does, especially CO_2 and O_2 [19]. The objective of this study is to identify the different preferences of *Aedes aegypti* in hatching in the ovitrap using different type of attractants.

METHOD

Sample of this research was female blood fed *Aedes aegypti* collected from a survey of mosquito larvae and the ovitrap assembled in the field. At the rearing until maturity stage, they were fed by guinea pig blood, and the research was conducted in entomology laboratory of the Public Health Faculty of Diponegoro University. Research was carried out by conducting preliminary test to determine the concentration of yeast solution (0.5 grams, 1 gram, 2 grams, and 3 grams) dissolving in 200 milliliter of aqua distillation. Preliminary research showed that the concentration of 0.5 grams yeast dissolved in 200 milliliter (2.5 grams/l) was used to be an attractant. Other treatments were 10% hay infusion, and drinking water as positive control.

Variables of this research were number of eggs and larvae of *Aedes aegypti* trapped in ovitrap, and the type of attractants. This study was a true experimental research using pre-post test with control group design. The test to the type of attractants preferred by hatching *Aedes aegypti* was conducted by putting 25 blood fed *Aedes aegypti* into the 50 cm^2 cage with the ovitrap and the tested attractant for 5 days.

Number of eggs in the ovitrap with the attractants was calculated to be analyzed further. During the experiment, the room temperature was measured in the morning

(08:00-10:00 a.m.) and in the afternoon (01:00-03:00 p.m.). In addition, the temperature and pH of the attractant media was also noted. The experiment was conducted with 9 replications, and the data collected was analyzed using one way ANOVA.

RESULT

The highest average number of the pre-mature *Aedes aegypti* in the ovitrap using different type of attractants was 669.1 found in the ovitrap with 10% of hay infusion. The least attractant attracting mosquito to hatch was 114.4 pre-mature *Aedes aegypti* found in the ovitrap with yeast solution of 2.5 grams/lt. The following Table 1 shows the number of larvae found in the ovitrap after 5 days observation; the highest number of larvae was found in the ovitrap with 10% of hay infusion.

Table 1: Number of pre-mature *Aedesaegypti* (eggs and larvae) found in the ovitrap using different type of attractants.

Replication	The number of pre-mature *Aedesaegypti* based on the type of attractants							
	Yeast solution of 2.5 grams/ liter		Hay infusion of 10%		Rain water		Control	
	Eggs	Larvae	Eggs	Larvae	Eggs	Larvae	Eggs	Larvae
1	43	0	642	0	450	0	275	0
2	282	0	573	0	314	0	318	0
3	67	0	635	3	297	2	357	0
4	96	0	692	0	283	0	342	0
5	174	0	570	2	442	0	301	0
6	58	0	921	0	169	0	189	2
7	54	0	586	5	201	0	463	0
8	37	0	727	2	320	1	358	2
9	219	0	661	3	197	0	217	1
X	114.4 (a)		669.1 (c)		297.3 (b)		314.1 (b)	

Remark: the same letter following the average number of pre-mature (eggs and larvae) shows insignificant differences α=0.05 using Least Significant Different (LSD) Test

The test result using ANOVA (One Way Classification) shows that the value of $p<0.0001$; it means that there is a significant difference on the average number of the eggs of *Aedes aegypti* base on the type of attractants. Furthermore, LSD test shows that the hay infusion attractant attracts female *Aedes aegypti* to hatch compare to yeast solution (p=0.0001), rain water (p=0001), and control (p=0001); however, the result of the rain water attractant and control is p=0.720 (insignificant difference)

DISCUSSION

The significant difference of attractant using 10% of hay infusion to attract *Aedes aegypti* to hatch takes place; while, the attractant using 2.5 grams/liter of yeast solution seems to be not attracting *Aedes aegypti* to hatch because, physically, the feature of this

water was turbid. *Aedes aegypti* tend to hatch in the clean water and puddle water [20]. *Aedes aegypti* has a habit to breed in the puddle clean water; naturally or man-made one. The places that *Aedes aegypti* prefers are bathtub, abandoned used tire, used materials exposed to rain, and other places that rain water is stay in [21-25]. Research conducted by Phontas (2012) supported this finding. He claimed that the number of *Aedes aegyptis'* larvae is strongly affected by the degree of the turbid water and the light intensity. The higher the turbid water and the light intensity, the less the number of *Aedes aegyptis'* larvae is found [26].

Hay infusion is significantly the one that is preferred by *Aedes aegypti* to hatch. Hay infusion proved to produce CO_2, ammonia, and octenol that influence the nerve system of smell of the mosquito [14,15]. The ammonia and CO_2 in the hay infusion produce a specific smell that functions as an attractant to the mosquito. This specific smell is captured by mosquito's antenna that has a sensilla which has one or more bipolar smell nerve known as ORNs (Olfactory Receptor Neurons). ORNs are located in the tip of dendrite and of also that function to detect chemical substances. This sensory nerve transmits chemical impulse as electrical responds by carrying smelling information from perifer to lobus antenna as the first terminal in the brain. After entering the sendillum through cuticle pore, the smell molecule goes through lymph liquid to dendrite. Most of the smell molecule is easily evaporated and relatively hydrophobic. The smell tied up to OBPs (Odorant Binding Proteins) goes through lymph liquid. Not only is a carrying, OBPs also works to dissolve the smell molecule and function as smell information selector. When complex OBPs reach the membrane of dendrite, the smell will be tied up to trans-membrane receptor and it will be transferred to the surface of the intracellular membrane. Lastly, this electrical impulse will be delivered to the higher level of the center of the brain and it will be integrated to produce an appropriate behavior responds [27].

The number of *Aedes aegypti*'s larvae found in the ovitrap using hay infusion was bigger than the control. The number of *Aedes aegypti*'s eggs trapped in that ovitrap was higher than that of in the control; therefore, the chance of the eggs to hatch to be larvae stadium was bigger. The hatching reaction is caused by stimulation of the central nervous system of the larvae in the eggs. The hatching process is also probably caused by the crowded eggs [28].

The different level of acidity was found in the different attractants. The acidity of the attractant using 10% of hay infusion and the control was pH 7.0, the rain water' attractant was pH 6.5, and the pH of the yeast solution was 5.0. The mosquito larvae was mostly found in the neutral pH of 7.0 [15].The acidity (pH) of the cassava fermented water attractant changed to be lower (acid) of pH 5.0 because fermentation process was taken place in the ovitrap that caused the decrease of the pH in the ovitrap using yeast solution. This phenomenon does not support the *Aedes aegypti* preference that tends to hatch in the water with pH 7.0 (neutral) [29] or the development of the larvae to become mature ones [30].

The environment temperature during the experiment was 27.5-28.8^0 C (10:00 a.m.-03:00 p.m). Generally, the preferable temperature for *Aedes aegypti* to lay their eggs is 20-30^0 C. At the temperature of 30^0 C the eggs will be hatched in 1 day to 3 days, and at the temperature of 16^0 C the eggs will be hatched within 7 days [31]. The humidity measured was 73-75%; while, the preferable humidity for mosquito environment is 70-89% [32]. The fact shows that the temperature and the humidity in the laboratory during

the experiment did not affect the life cycle of the *Aedes aegypti* especially during the oviposition period.

CONCLUSSION

Attractant using hay infusion for preferable to *Aedes aegypti* to lay their eggs compare to other attractants (the using yeast solution and rain water). The average number of eggs in the ovitrap using hay infusion attractant is 667.4, rain water attractant is 297, and yeast solution attractant is 114.4.

Further study on the use of yeast solution with 0.5 grams concentration dissolved in 200 milliliter aqua distillation needs to done.

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