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Determination of Oviposition, pH, and Salinity of *Aedes aegypti's* Breeding Places in Semarang Regency

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Abstract

Dengue Hemorrhagic Fever (DHF) is still a health problem in Indonesia. This is because DHF can cause decease, and the incidence is always increasing. Vector control efforts in Semarang Regency can be done by knowing mosquito bionomics, so one of the actions can be taken is to modify the environment, so that the Ae. aegypti mosquito feels uncomfortable about our environment. This type of research is pure experimental. In this research, mosquito larva will be the subject to be breeded using water with various degrees of acidity (pH) and salinity, then the ability to reproduce in various water with the pH and salinity levels is observed. The sample used was F1 larva originating from breeding places using ovitrap in Semarang Regency. Based on the results of the study showed that the most preferred container of the Ae. Aegypti mosquito for oviposition is a container made of plastic and can. The most optimal water pH for instar 2 larva breeding is water with a pH of 9, followed by water with a pH of 8 and 7. The most optimal water salinity for instar 2 larva breeding is water with a 0-6gr / l salinity.

Introduction

Dengue Hemorrhagic Fever (DHF) is still a health problem in Indonesia. This is because DHF can cause decease, and the incidence is always increasing. DHF is one of the diseases caused by dengue virus. This virus can be transmitted from patient to others through mosquito bites from the genus Aedes. The main vector of DHF is Ae. Aegypti mosquito, while Ae. Albopictus is a secondary vector. According to the Republic of Indonesia Health Office, starting in 2014, the number of DHF sufferers in Indonesia had increased. This can be seen from the Indonesian Health Profile which stated that in 2015 there were 129,650 dengue cases in Indonesia, with a total of 1,071 deceases. The increase in dengue cases cannot be separated from the effect of increasing population mobility, which is also accompanied by the spread of mosquitoes which become dengue vectors (infectious vectors) that are increasingly spread in Indonesia (Depkes RI, 2005).

Prevention of DHF can be done by increasing public knowledge about DHF, for example through simple counseling activities or by avoiding contact with the dengue vector, namely Ae. Aegypti mosquito, including by mosquito nets application, closing house vents with wire mesh, and using mosquito repellent (Sayono et al., 2011). Vector control efforts in Semarang Regency can be done by knowing mosquito bionomics, so actions can be done by modifying the environment, so that the mosquito feels uncomfortable about our environment. Thus, gradually it will leave our environment, and we will be free from the risk of several diseases that can be transmitted by Ae. Aegypti mosquito. Some research also informs that insecticide resistance has occurred in several countries.

One of the efforts of the Semarang

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Regency Health Office in controlling DHF is to use fogging. The active ingredient used in the DHF control fogging in Semarang Regency is sipermetrin 19: 1 since 2013 until now. The use of insecticides with the same type in a long time continuously will run the risk of resistance. This encourages the promotion of environmental modification measures that are considered safest in controlling mosquito population (Yotopranoto et al., 1998). But the weakness in this activity is that it requires quite high community participation to carry it out. It is expected that the results of this research can be used as references in environmental modification activities to control Ae. aegypti mosquito population.

Method

This research type was pure experimental. In this research, mosquito larva would be the subject of treatment using water with various degrees of acidity (pH) and salinity, then the ability to reproduce is observed. The sample used was F1 larva originating from breeding places taken by larva capture using ovitrap in Semarang Regency. During the treatment, the room temperature was controlled by recording the temperature (morning, afternoon, evening), and keeping it in optimal condition for mosquito.

The stages of this research began by preparing the Aedes aegypti mosquito. Researchers installed ovitrap at the study site for a week. Next, the mosquito eggs that had been attached to the filter paper werr then taken. Eggs taken from the location to the laboratory for rearing. The eggs would turn into larva and then turned into pupae in about 7 days. The larva were fed liquid sugar, pet food or fish food, and occasionally blood. Eggs that hatched into larva were transferred to a tray that contained 2 liters of water. Larva density in trays was estimated to range from 0.5 to 1 larva / cm3 with 2.5 cm water depth. Living larva were fed 0.5 gram of chicken liver from day 1 to 5 and the next day larva were fed as much as 1 gram. The pupa would turn into an adult mosquito for about 3 days. After becaming an adult mosquito, the male and female mosquito will mate, so the female mosquito would lay eggs. The eggs from the mating would then be hatched, the mosquitoes that hatch were F1 offspring and would be tested for their ability to breed under various conditions of pH and water salinity. This research useed Aedes aegypti mosquitoes from Semarang Regency. The sample in this study was a number of Aedes aegypti eggs taken randomly from the Aedes aegypti mosquito population from 18 villages / sub-districts in Semarang Regency.

Sampling was done by installing an egg trap (ovitrap) and larva examination because the number of Aedes aegypti mosquitoes in each village cannot be known certainty. The number of ovitraps placed were 100 ovitraps and done randomly. Ovitrap installation distance was 100-600 meters to avoid eggs from the same mosquito and placed in every randomly chosen house. The collection of Aedes aegypti mosquito eggs used ovitraps installed in every home, inside or outside the house. After the sample was bred to F1, and the age of the mosquito reaches 2-3 days stepping on the adult stage can fly, then the sample was taken using an aspirator. The data collected were used as primary data and obtained from the calculation of the number of Aedes aegypti mosquito deaths during the research. Data analysis was performed descriptively to see how the ability of 2nd instar larva to become mosquitoes under various conditions of pH and salinity of water.

Result and Discussion

The sample in this study used Ae. aegypti larva instar 3, each as many as 25 larvas for each treatment. Previously, researchers conducted mosquito rearing starting from capturing larva in nature using ovitrap, then bred in a laboratory. After the larva develop into adult mosquitoes, then both female and male Ae. aegypti who have almost the same age were

Table 1. Room Temperature During Experiment of Mosquito Breedstock Place Tendency

Measurement Time	Temperatur on Experiment Day (^{0}C)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morning	26	26	26	25	25	26	26	26	26	26	25	26	26	26
Noon	28	28	28	28	27	28	28	28	28	28	28	28	28	28
Afternoon	27	27	27	27	26	27	27	27	27	27	27	27	27	28

Source : Primary Data, 2019

Table 2. Distribution of *Ae. Aegypti* Breeding Place Tendency

Breeding Container	Data taking	Number of Pupa found on Day-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Plastic	1	-	-	5	43	21	5	4	-	-	-	-	-	-	-
	2	-	-	3	38	60	9	8	1	-	-	-	-	-	-
	3	-	-	4	52	43	14	4	1	1	-	-	-	-	-
	4	-	-	-	34	57	15	8	-	1	-	-	-	-	-
	Avg	-	-	3	41,8	45,3	10,8	6	0,5	0,5	-	-	-	-	-
Tin	1	-	-	3	35	15	5	5	-	-	-	-	-	-	-
	2	-	-	-	42	17	9	2	1	-	-	-	-	-	-
	3	-	-	2	21	23	7	12	-	2	-	-	-	-	-
	4	-	-	-	26	24	11	5	-	1	-	-	-	-	-
	Avg	-	-	1,3	31	19,8	8	6	0,3	0,8					
Soil	1	-	-	-	-	-	1	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Avg	-	-	-	-	-	0,5	0,5	-	-	-	-	-	-	-
Plastic with soil bed	1	-	-	5	20	11	2	3	-	-	-	-	-	-	-
	2	-	-	-	13	8	4	-	2	1	-	-	-	-	-
	3	-	-	-	16	13	9	4	-	2	-	-	-	-	-
	4	-	-	-	7	11	12	3	1	-	-	-	-	-	-
	Avg	-	-	1,3	14	10,8	6,8	2,5	0,8	0,8	-	-	-	-	-
Tin with soil bed	1	-	-	-	24	9	2	-	-	-	-	-	-	-	-
	2	-	-	-	12	8	-	2	-	-	-	-	-	-	-
	3	-	-	-	9	11	2	-	-	-	-	-	-	-	-
	4	-	-	-	16	5	7	-	-	-	-	-	-	-	-
	Avg	-	-	-	15,3	8,3	2,8	0,5	-	-	-	-	-	-	-

Source : Primary Data, 2019

put in a mosquito cage, and then mated. One to two days after copulation, female mosquitoes and male mosquitoes were separated. An easy way to distinguish Ae. aegypti female and male mosquitoes was by sticking a clean hand (without smearing anything, and does not smell anything) to the side of the cage. If a mosquito approached the hand, it was most likely that it was female, because it was trying to find blood to ripen its eggs. Another way is to look at the special characteristics of the mosquito's body. Generally female is bigger than male. You can also see the mosquito palpus, which is the female is shorter than male. The tip of the female mosquito's abdomen also looks darker colored and pointed edges.

Female Ae. aegypti was taken using an aspirator, and then separated into other cages.

Table 3. Development of *Ae. aegypti* Mosquito Larva in various pH condition

Water pH	Data taking	Number of Larva becoming Mosquito	Percentage (%)
3	1	0	0
	2	0	0
	3	0	0
	4	0	0
	Average	0	0
4	1	11	44
	2	9	36
	3	9	36
	4	10	40
	Average	9,75	39
5	1	18	72
	2	15	60
	3	16	64
	4	17	68
	Average	16,5	66
6	1	21	84
	2	18	72
	3	17	68
	4	20	80
	Average	19	76
7	1	21	84
	2	19	76
	3	22	88
	4	19	76
	Average	20,3	81
8	1	25	100
	2	25	100
	3	22	88
	4	21	84
	Average	23,3	93
9	1	24	96
	2	24	96
	3	23	92
	4	25	100
	Average	24	96
10	1	20	80
	2	16	64
	3	18	72
	4	20	80
	Average	18,5	74

511	1	0	0
	2	1	4
	3	1	4
	4	0	0
	Average	0,5	2
12	1	0	0
	2	0	0
	3	0	0
	4	0	0
	Average	0	0

Source: Primary Data, 2019

The female mosquito separated from the male was then fed with guinea pig blood. Guinea pig that would be put into mosquito cage was slightly scraped off their feathers first, so that mosquito can easily suck blood. After 2 hours, the guinea pig was removed from the mosquito cage, and the Female Ae. aegypti had now become gravid 1, by looking at a mosquito's stomach which becomes bigger and forms like a blood sac. It was then taken to be put into a cage that had been prepared for oviposition.

In research, the tendency of Ae. Aegypti mosquito eggs placement was carried out for 14 days, this is because after 14 days all mosquitos have died. The number of Ae. aegypti female mosquito samples in this experiment were 25 animals. During the experiment, the room temperature was measured and managed in accordance with the optimal temperature for mosquitoes.

From table 1, it can be seen that the temperature in the laboratory was very optimal for mosquito breeding, where Ae. aegypti can develop optimally at a temperature of 25-27^{0}C, but Ae mosquitoes. aegypti can live normally at temperatures of 20-30^{0}C. Thus, the temperature factor did not affect the tendency of mosquito breeding places.

To see the tendency of laying Ae. aegypti mosquito eggs on various types of container materials / water containers, then an experiment was conducted by placing 5 kinds of containers in a mosquito cage containing 25 gravid female mosquitoes. The container was filled with well water. The tendency of laying eggs was seen from the number of larva contained in the container. The larva found were then exterminated and thrown away, so as not to mix with ones that hatch later, so as not to confuse the calculation. Based on table 2. it can be seen that the tendency of Ae. aegypti mosquito for laying eggs is in a plastic container. It can be seen that the average total number of larva found in plastic containers was 108, followed by cans of 67. For containers made of soil, on average only one larva was found.

To see the development of mosquito in water with various pH conditions, an experiment was carried out using instar 2 larva. The instar 2 larva were obtained from Ae. aegypti mosquito female gravid 1 which had been separated from male mosquito. Then the hatched eggs were observed until they became instar 2 larva. These instar 2 larva were put into containers containing water with a different pH, and each container was put 25 instar 2 larva mosquito. The reason of Instar 2 chosen for this experiment was because Instar 1 was still too weak, so there was a higher risk of dying. instar 3 and 4 were not chosen because in this research we wanted to know the maximum breeding of mosquito. So by choosing instar 2, the observation phase could be longer. The results of this larva breeding can be seen in table 3. below.

From table 3, it could be seen that largest pecentage of instar 2 larva that successfully hatch into mosquito were larva placed in water with a pH of 9, which was as much as 96%, followed by larva placed in water with a pH of 8, which is as much as 93%, and larva placed in water with a pH of 7, which is as much as 81%. Instar 2 larva were placed in water with a pH of 3 and a pH of 12 no larva have hatched into mosquito.

To see the development of mosquitoes in water with various salinity conditions, an experiment was carried out using instar 2 larva. The larva were obtained from gravid 1 ae. aegypti female mosquito which had been separated from male mosquito. Then the hatched eggs were observed until they become instar 2 larva. These larva were put into containers containing water with different salinity, and each container was put 25 larva. The reason Instar 2 was chosen for this experiment was because Instar 1 is still too weak, so there is a higher risk of dying. As for instar 3 and 4 were not chosen because in this study we wanted to know the maximum breeding of mosquito, so by choosing instar 2, the observation phase could be longer. The results of this breeding larva can be seen in table 4 following.

Based on table 4. it can be seen that the largest percentage of instar 2 larva that successfully hatched into mosquito were larva placed in water with a salinity of 0, which was as much as 90%, followed by larva placed in water with a pH of 8, which was as much as 93%, and larva placed in water with salinity 4, which was as much as 81%. Instar 2 larva placed in water with salinity 7 and salinity 8, none successfully hatched into mosquito, although in salinity 7 there were 3 larva that successfully become pupae, but died before becoming mosquitoes.

From the results it can be seen that the most preferred container by the Ae. for oviposition was one made of plastic and can. In this research, the tendency of Ae. aegypti mosquito for laying eggs was in a plastic container. It can be seen that the average total number of larva found in plastic containers was 108, followed by cans with 67. For container made of soil, on average only one larva was found. In theory, Ae. aegypti mosquito will lay the eggs slightly above the surface of the clear water. In addition, it will avoid water where the wall is made of soil (Budiyanto, 2012; Schneider, 2011). But in certain circumstances, as in this research, Ae. aegypti mosquito wants to lay their eggs in a container made of soil or a plastic container that has soil bed, although the percentage is very small when compared to the number of eggs laid in a plastic container or can (Baserra et al., 2010).

Mature Ae. aegypti who have just come out of the pupa shell will remain for a while in the pupa shell to dry their wings. After the wings are felt dry, then Ae. aegypti immediately flies. To ripen the eggs, mature female Ae. aegypti must suck blood as food, while the male only eats fruit and flower fluids. After copulation, female Aedes aegypti will sucks blood and three days later it will lay about 125 eggs and an average of 100 eggs, then it will suck blood again.

The results of this research are in accordance with the research of Hendri et al., (2010) which states that of the 39 positive larva of containers, the highest number is found in plastic-based buckets. The attractiveness of female mosquito to lay their eggs is affected by the color of the container, temperature, humidity, light, and environmental conditions. Other factors that also affect the tendency of female mosquitoes to lay their eggs include the ability to absorb water, texture, and color of the material. In Buenos Aires, Argentina, it was found that containers of black and black plastic materials contained a lot of Ae. aegypti larva (82.1%). Based on the results of these studies it can be seen that mosquito in each particular region have tendency in different water reservoirs, in the type, basic material, and color used. These factors are estimated to affect the percentage of larval acquisition in various containers in each region (CDC, 2016).

In this research, Ae. aegypti mosquito larva was placed in regulated water, so that it had a pH between 3 - 12. The pH range was chosen because we wanted to know whether the larva can adapt to pH conditions that are rather extreme and capable of breeding. In previous studies, no results had yet been obtained that Ae. mosquito larva was able to breed at pH less than 4 or more than 10. According to the research of Sayono et al., (2011), eggs from oviposition of gravid I female Ae. aegypti can be found in water with a pH range of 4-10 in the attached ovitrap, and have different results in each treatment. Research conducted by Yulidar (2015) stated that the hatchability of Ae. Aegypti eggs at the laboratory scale reached 80.09%. Eggs produced by Ae. Aegypti mosquito were then collected to be hatched. In this research, gravid female mosquito can lay their eggs in

Table 4. Development of *Ae. aegypti* Mosquito Larva in Various Salinity Condition

Water Salinity (gr/l)	Data taking	Number of Larva becoming Mosquito	Percentage (%)
0	1	23	92
	2	23	92
	3	23	92
	4	21	84
	Average	22,5	90
4	1	21	84
	2	19	76
	3	21	84
	4	20	80
	Average	20,3	81
5	1	18	72
	2	18	72
	3	16	64
	4	19	76
	Average	17,8	71
6	1	12	48
	2	8	32
	3	7	28
	4	8	32
	Average	8,8	35
7	1	0	0
	2	0	0
	3	0	0
	4	0	0
	Average	0	0
8	1	0	0
	2	0	0
	3	0	0
	4	0	0
	Average	0	0

Source: Primary Data, 2019

acidic pH 3 conditions. But after being hatched, only a small number that managed to hatch into larva. The hatched larva were only able to survive less than 24 hours, then died (mosquito larva cannot develop). This might be caused by environmental conditions that were too acidic. Dead larva were white. Water pH is one of the factors that affect larval development. In research conducted by Clark (2004), it stated that mosquito larva will die at pH <3 or >12. Based on the result of research conducted by Sayono et al., (2011), instar III larva cannot develop into pupa at pH 4. But the results of the research are different from this one, because in this research, the development of larva into adult mosquito can occur in water with pH 4.

Based on the results of the research it can be seen that the most optimal water pH for breeding instar 2 larva into Ae. aegypti mosquitoes is water with a pH of 9, followed by water with a pH of 8 and 7. One important factors that can affect the percentage of hatching of Ae. Aegypti mosquito egg is temperature and pH. In addition, the process of developing an egg to the next stage (becoming an adult stage) is also affected by temperature and pH. If the temperature and pH factors are at optimal conditions, the process of mosquito breeding will also be higher. Vice versa, if the temperature is too hot or cold, it will interfere with mosquito growth, even stop or kill the mosquito (Agustin et al., 2017; Wahyuningsih, 2009).

Based on the results of Jacob's research (2014), Ae. aegypti cannot develop in alkaline water, and can develop optimally in neutral water. However, this research shows that the biggest percentage is Ae. aegypti can develop into adult mosquito at pH 9. That is, based on this research, the most optimal mosquito larva develop in more alkaline water (pH 9) than in neutral water (pH 7). The ability of Ae. aegypti larva to develop in water conditions with an alkaline pH due to it's adaptability in various environmental conditions (CDC, 2016).

The chemical condition of water that is acidic or alkaline does not affect gravid female mosquito to lay their eggs (Agustina, 2013). That is, if there is a pool of water that is considered safe by the mosquito to lay its eggs, then that's where the gravid female mosquito will lay her eggs. This is consistent with the results of research Tilak et al., (2005) which states that the pH of water does not affect the oviposition response of female mosquito. However, the pH of water will affect the ability of larva to develop into adult mosquito. The results of this study are in line with research of Sayono (2011) which states that the Ae. aegypti mosquito can grow and develop well in water drains that are still. This is because the pH of the sewage water itself is not all neutral, because the water comes from various sources of waste water.

In this research, the largest percentage of instar 2 larva that successfully hatched into mosquitoes were larva placed in water with a pH of 9, which was as much as 96%, followed by one placed in water with a pH of 8, which was as much as 93%, and larva placed in water with a pH of 7, which is as much as 81%. Instar 2 larva placed in water with a pH of 3 and a pH of 12 could not hatch into mosquito.

In the condition of water salinity 0 gr / l up to 6 gr / l, Ae. aegypti larva is able to develop into an adult mosquito. When the salinity of water is 6 g / l, the mosquito larva developed some appear to be larger than usual size, where the larval instar IV size is 5 mm. This can occur because the liquid absorbed the body of the larva, so the size of the larva appear larger than normal size (CDC, 2016). In Clark's research (2004) stated that Ae. aegypti develops in water salinity with a concentration of 3.5 gr/l.

Mortality of Ae. aegypti larva in various water salinity in the laboratory in the first 24 hours occurred in the treatment with salinity of 18 gr / l - 22 gr / l. High concentration of NaCl resulted in an imbalance between the body fluids of larva and brood media. This difference in osmotic pressure results in the death of Ae. aegypti larva. Osmosis plays an important role in the body of a living creature, because the fluid in the body of the larva is pulled out so that the body of the dead larva shrinks and is damaged called crenation (CDC, 2011). This is in accordance with research by Badrah & Nurul (2011) which stated that the content of Cl in water can interfere with the process of development and hatching of mosquito eggs. Ae. Aegyptil larva can survive on salt levels at concentrations of 10.0 mg - 59.5 mg. When viewed from the length of the development of the larva to adult mosquito, at a concentration of 6 gr / l some mosquito developed abnormally. The metamorphosis cycle of the mosquito exceeds the normally from egg to adult + 10 days (Ahmad et al., 2009; MOH RI, 2005). This has an impact on the size of larva and pupa in normal conditions. Larva and pupa that do not develop, will not survive and died.

In addition to temperature and pH, the presence of organic matter also affects the development process of mosquito, ranging from larva (pre-mature stage) to mature mosquito (Olayemi et al., 2010; Curto & Schweigmann., 2006). The growth of mosquito larva is divided into several instars, which then become pupa. The process is affected by the presence of detritus or organic matter as food. The media used in this research was clean water from well, which is used as a breeding ground for Ae. aegypti mosquito.. Usually well water has a sufficient amount of organic matter. The content of organic matter also affects the penetration of light and oxygen content in a medium. If the content of organic matter is too much, it can interfere with the penetration of light into the media so that it can interfere the development of pre-mature mosquito. So, too much organic matter can also inhibit the breeding of Ae. aegypti larva (Agustin et al., 2017).

The longevity of Ae.aegypti also depends on the temperatures, humidity of the air, water supply, food and predators (Wong et al., 2011). Aedes aegypti can live at 20^{0}C with a humidity of 70%, but can only last for about 100 days. Ae.aegypti will die if it is at 6^{0}C for 24 hours (Yulidar & Wilya, 2015)

Based on the results of the research it can be seen that the most optimal water salinity for instar 2 larva breeding into Ae. aegypti mosquito is water with a 0-6gr / l salinity.

Conclussion

The results of this research showed that the most preferred container of the Ae. Aegypti mosquito for oviposition is a container made of plastic and can. The most optimal water pH for breeding instar 2 larva into Ae. aegypti mosquito is 9, followed by pH of 8 and 7. The optimal water salinity for breeding instar 2 larva into Ae. Aegypti mosquito is water with a 0-6gr / l salinity.

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