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The Level of Egg Sterility and Mosquitoes Age after the Release of Sterile Insect Technique (SIT) in Ngaliyan Semarang

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ABSTRACT

Dengue control efforts have not provided adequate results. Therefore we need other measures like sterile insect Technique (SIT). This study aimed to observe the level of egg sterility and age of *Aedes aegypti* mosquitoes after using SIT in Ngaliyan Semarang. The method of this research is a descriptive study with a cross-sectional design. A sample of the mosquitoes eggs are all the mosquitoes eggs on the paper trap in every house of RW2 population in Ngaliyan village. These samples are the result of the fertilization of male and female mosquitoes in nature. Data analysis using Wilcoxon sign test. The result showed that the average of egg sterility percentage in mosquitoes after using SIT indoors was 73.65% and the average of egg sterility percentage outdoors was 69.08%. Wilcoxon test showed significant differences in average age of mosquitoes egg ($p < 0.05$), males pupae ($p < 0.05$), females pupae ($p < 0.05$), and adult female mosquitoes ($p < 0.05$), before and after SIT. It can conclude from this research that the SIT release in Ngaliyan village Semarang city could increase the level of sterility mosquito's eggs and a decrease of the age of *Ae. aegypti* mosquitoes in this area.

Keywords: Sterile Insect Technique (SIT), egg sterility, mosquito's age, *Aedes aegypti*, Ngaliyan

INTRODUCTION

Dengue Fever is still a public health problem in Indonesia; it is likely to increase the number of cases and increasingly wide-spread. In Central Java, dengue fever cases in the period of May 2013, there were up to 10.000 cases, and 48 patients died, almost close to the total amount in 2012 that were up to 12.000 cases [1]. In Semarang, the number of dengue fever cases in May 2013 reached 1.226 cases, approaching the total cases during 2012 with 8 patients died, in 2012 the number of dengue cases was at 1.250 with 22 patients died. From the incidence of dengue in 16 districts in Semarang, Ngaliyan was ranked first with incident rate (IR) 207 per 100,000 population in November 2013. Although up to March 2014 Ngaliyan was the third, the number of cases remained high and endemic repeatedly [2]. Appropriate vector control methods are needed. Some vector control methods that can be used include the use of chemical,

biological, and environmental management of mechanic basis and by using radiation [3]. The use of insecticides is feared to give effect resistant (immune) against mosquitoes in the long term use and is dangerous for human health [4]. The sterile male technique is one alternative pest control, including potential disease vectors, effective and efficient, either individually or integrated with other technologies. The basic principle of the sterile male technique is very simple, which is to kill insects with the insect itself (autocidal technique) [5]. The use of sterile male technique had been done in some areas such as Salatiga, Banjarnegara, and West Bangka. From the results of the research in Salatiga, it can be concluded that the use of sterile male mosquitoes can decrease the population of *Aedes aegypti* vector [6]. This study aims to determine the level of sterility eggs and age of *Ae. aegypti* after the use of sterile male mosquitoes in the village Ngaliyan Semarang.

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MATERIALS AND METHODS

This research uses a descriptive survey method and cross-sectional study design. Data were collected by observation of *Ae. aegypti* in the field and laboratory. Samples of the mosquito eggs are all mosquito eggs contained in filter paper in every house in RW 2 village Ngaliyan, resulted from natural breeding between sterile male mosquitoes and normal female mosquitoes. Sample houses were 100 houses in RW 2 Ngaliyan Village when the new cases occurred at the same time with research. The data analyzed using univariate and bivariate analysis with Wilcoxon sign test.

RESULTS AND DISCUSSION

Gametophyte development

Population growth of *Ae. aegypti* mosquitoes in RW 2 Ngaliyan Village is done by the release of 3000 sterile male mosquitoes every week. From Table 1 and 2, it showed that the number of *Ae. aegypti* eggs in the house are found more than the outside. This is because of the *Ae. aegypti* mosquito prefers a dark, damp, and hidden places in the house or building, such as bedroom, closet, bathroom, and kitchen. These mosquitoes are rarely found outside the house, plants, or in another sheltered spot. Inside the room, *Ae. aegypti* resting under the house furniture, hanging objects such as clothes, curtains, as well as in walls [7]. *Ae. aegypti* mosquitoes usually lay its eggs in the clean water reservoirs located in the house, such as water reservoirs, bathtubs, water jar and sink in the bathroom [8] Additional factor that is rainfall also affects populations of *Ae. aegypti* [9] The temperature has an effect on the activities of eating and the development rate of eggs into larvae, larvae into pupae and pupae into imago [10]. The state of temperature causing stadium developmental disorders of mosquitoes so adult mosquitoes in the area may be more or less in order to produce eggs.

As seen on Tables 1 and 2, it is known that the average egg sterility of *Ae. aegypti* found indoor (73.65%) was higher than outdoor (69.08%). This shows that 73.65% of the total egg (100%) in ovi-traps in the house did not hatch. It is one of the consequences of radiation on insects, namely infertility. Infertility in a general sense is the inability of an organism to produce offspring or inability to give the genome on the further descent. The mechanism of infertility can be caused by infecundity, dominant lethal mutation, the failure to mate, inactivation of sperm, and aspermia [11]. The evidence from the studies has shown that the radioactive rays were able to decrease the number of fertile eggs, for ra-

dioactive rays can change the structure of chromosomes with the presence of chromosomal aberrations and resulting abnormal form and arrangement of chromosomes that it becomes sterile [12]. The study results from Purwanto and Sudaryadi [13] have proved that gamma-ray irradiation dose 100Gy (10 Krad) has an effect in suppressing the development of pest eggs *Bruchus* by 16.33% (control) to 3.50% (irradiated). Likewise, Handayani and Sudaryadi [14] research with treatment 100Gy of gamma ray irradiation (10 Krad) against the fruit flies *Drosophila melanogaster* male Meigen cause the mating results with normal females were able to suppress the first generation offspring (F1) by 89%.

The sterility of the eggs is one of the parameters of population decline [15]. Sterile eggs are eggs that do not contain an embryo [16]. The percentage of infertile eggs is calculated based on the eggs produced in each ovitrap. The increased egg sterility is caused by natural breeding of infertile males and normal females to produce infertile offspring [17]. The percentage of the sterility of mosquito eggs outdoors reached the lowest at the release I. It can occur due to the natural female mosquitoes failed to mate with sterile male mosquitoes to produce sterile offspring of 66.67%. Effects of irradiation are able to cause changes in some genetic aspects of reproduction

Table 1. The percentage of sterility of *Ae. aegypti* mosquito eggs found indoor in the houses in the Ngaliyan village after using SIT

No.	Number of		Sterility (%)
	Egg indoor	Mosquitoes indoor	
1	675	291	56.89
2	2208	213	90.35
3	548	151	72.45
4	1808	400	77.88
5	2092	613	70.69
Average			73.65

Table 2. The percentage of sterility of *Ae. aegypti* mosquito eggs found outdoor in the Ngaliyan Village after using SIT

No.	Number of		Sterility (%)
	Egg outdoor	Mosquitoes outdoor	
1	24	16	33.33
2	485	26	94.64
3	200	42	79.00
4	421	72	82.90
5	353	157	55.53
Average			69.08

Table 3. The average age of *Ae. aegypti* at every stadium before and after the release of sterile male mosquitoes in the Ngaliyan village

Stadium of Mosquito	Average age (days)			
	Before		After	
	Male	Female	Male	Female
Egg	4.71	4.71	4.51	4.51
Larvae	4.40	4.35	3.55	3.75
Pupae	2.20	2.47	2.40	2.70
Adult	14.14	16.71	12.95	14.50

Table 4. Wilcoxon test on the average age of *Ae. aegypti* after and before using SIT

	Z	Sig (2 tailed)
The age of eggs after using SIT – before using SIT	-3.508	0.000
The age of male larvae after using SIT – before using SIT	-1.890	0.059
The age of female larvae after using SIT – before using SIT	-0.577	0.564
The age of male pupae after using SIT – before using SIT	-2.828	0.005
The age of female pupae after using SIT – before using SIT	-3.276	0.001
	Z	Sig (2 tailed)
The age of adult male mosquitoes after – before	-2.405	0.016
The age of adult female mosquitoes after – before	-0.402	0.688

such as semi-sterile (decreased levels of fertility); decreasing capacity of mating and reproductive capacity. The conclusion derived from the effects of this irradiation is that the treatment with irradiation doses leads to acute doses. Carpenter stated that acute dose of reality has more effective influence than the chronic doses regarding: vitality, mating capacity, reproductive capacity and mating competition [18]. The effect of radiation on fertility cannot be immediately visible because the post-gonial cells are relatively resistant to the lethal implications of the influence of irradiation. If the sperm were irradiated at moderate to high doses, it will be found in the early rankings that remain fertile and then followed by a decline infertility or sterility. The length of time this infertility depends on the dose and the proportion of gonad cells, so temporary infertility does not result in significant changes in the hormonal balance, sexuality libido, or physical abilities. The post-gonial cells have a short lifespan; the gonad cells become necessary when

viewed in terms of fertility and genetically damage [19]. Therefore, it can be said that the probability or likelihood of permanent damage in a cell when the cell exposed by the radiation is determined by two main factors, namely: the amount of radiation dose interacts with cells and the degree of the sensitivity of cells to radiation that is generally associated with the ability to hold their own repairs from individuals of the related cells [20].

Based on Table 3, the lifespan of the eggs starting from the hatching into larvae before using SIT is 4.7 days in average, while the lifespan of the mosquito eggs after using SIT is 4.51 days in average. The lifespan from the larval stage into female pupae before using SIT has the average of 4.35 days, and the average for male larvae is 4.40 days, while the lifespan from the larval stage after using SIT for female larvae the average is 3.75 days and the average for male larvae is 3.55 days. It takes approximately 2.47 days from pupal stage to become female adult mosquitoes before using SIT, and for male pupae, the average is 2.2 days, while the lifespan from the female pupal stage after using SIT has the average of 2.7 days, and for male pupae, the average is 2.4 days. The average of lifespan before using SIT for adult female mosquitoes to death is 16.71 days, and for adult male mosquitoes the average is 14.14 days, while the average lifespan for adult female mosquitoes after using SIT is 14.5 days, and for adult male mosquitoes, the average is 12.95 days. Wilcoxon test showed no significant difference in the average age of the mosquito eggs ($p < 0.05$), male pupae ($p < 0.05$), female pupae ($p < 0.05$) and the adult female mosquito ($p < 0.05$) before and after using SIT (Table 4). Long-life differences may occur due to differences in survival for each of mosquitoes and the possibility of abnormal development of natural marriages on male and female mosquitoes sterile [21].

According to Sudaryadi [5] in his research, stated that the percentage of individual who managed to stay alive reached the adult stage showed that the increase in the number of male mosquito F1 irradiated in a mating ratio significantly suppressed the development of its population offspring to 65.6% (1: 1: 1), 46.4% (ratio of 5: 1: 1), 24.7% (10: 1: 1), and 19.6% (ratio 20: 1: 1). It proved that the eggs produced have the same quality so that means its ability to fertilize and mating of sterile male mosquitoes compared with normal males did not differ much so that the sterile males can compete with normal males. This is in line with the theory of Knippling [22] cites Carpenter [18], that the release of sterile male insects with an exceeded amount (at least 3-times) than

the number of natural population will ensure that the next generation of gradual population growth has been narrowed to zero. The results of this study showed that male mosquitoes as the outcome from gamma ray irradiation treatment are not completely sterile because they are still able to produce offspring population. So that it is known that gamma ray irradiation treatment Co-60 only cause *Ae. aegypti* males to become semi-sterile. The experimental results are consistent with the statement that the treatment radio-sterilized is intended to undermine the chromosomal DNA of the mosquitoes' sperm, and after mating, female mosquitoes will produce infertile eggs so it cannot successfully forward offspring or new generation [23].

CONCLUSION

The use of SIT in the Ngaliyan village can reduce the lifespan of mosquitoes and improve the eggs sterility of *Ae. aegypti*. The average percentage of the eggs sterility of *Ae. aegypti* found indoor (73.65%) was higher than outdoor (69.08%).

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