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STERILITY OF MALE *AEDES AEGYPTI* POST γ-RAY STERILIZATION

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ABSTRACT

Aedes aegypti (*Ae. aegypti*), a vector of dengue hemorrhagic fever (DHF), population control was developed using the sterile insect technique (SIT). Sterilization process was conducted using γ-ray sterilization. To support the SIT application, the sterility of male *Ae. aegypti* on the 1st-5th days post sterilization were studied. The first step of this research was the sterilization process of male *Ae. aegypti* with 70 Gy of γ-ray sterilization. Then, the sterile male Ae. aegypti mosquitoes on the 1st; 2nd; 3rd; 4th and 5th days after the sterilization process respectively were competed with un-irradiated males to mate with un-irradiated females. The number of eggs that were produced was incubated to determine the fertility of sterile male *Ae. aegypti*. The result showed that the average number of eggs produced from mating combination of sterile male *Ae. aegypti* mosquitoes on the 1st-5th days post sterilization, un-irradiated females and un-irradiated males was 1,135; 1,118; 1,243, 1,372 and 1,326. The average percentage number of eggs that did not hatch into larvae was 97.69%; 97.87%; 90.92%, 96.20%, and 86.91%. By analysis of variance (ANOVA), the number and percentage of the unhatched eggs were not significantly different at the level confident of 95%. The research showed that the mating ability and sterility of sterile male *Ae. aegypti* on the 1st-5th days post γ-ray sterilization show no significant difference.

Keywords: Sterile insect technique (SIT), sterility, *Aedes aegypti*, γ-ray sterilization.

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is one of major health problems in Indonesia. Epidemic of DHF is well-documented in Indonesia, and it was first recognized in 1968 on Java island, 15 years after its recognition in the Philippines (Nathin *et al*., 1988). Richards *et al*. (1997) reported outbreaks of DHF in Papua (formerly Irian Jaya) during 1993-1994, by the discovery of dengue virus (DENV) serotypes 1, 2, and 3 on the blood sample. Corwin *et al*. (2001) reported outbreaks of DHF in South Sumatra. DHF is transmitted by *Aedes aegypti* (*Ae. aegypti*) mosquitoes, which acted as the vectors/carriers of DENV. Now, there are 5 variants of DENV with the recent discovery of the new variant in Serawak Malaysia (Mustafa *et al*., 2015). DENV enters the human body through the bite of blood-sucking female *Ae. aegypti* mosquitoes, transmitting the virus from one person to another. Until the end of 2015, there had not been any drugs nor vaccines available for dengue disease (Tambunan *et al*., 2014; Fibriansah *et al*., 2015; Crunkhorn, 2015; Parikesit *et al*., 2013). However, the development of novel drugs and vaccine candidates are still in progress with the assistance of sophistication in silico, in vitro, and in vivo approach (Tambunan and Parikesit 2011; Tambunan and Parikesit 2010). In this respect, in line with the development in pharmaceutical technology, ecological measures to control *Ae. aegypti* as DENV vector are crucial. It should be noted that female *Ae. aegypti* mosquitoes need human blood as a source of protein for the egg maturation process (Harrington *et al*., 2001).

Various attempts have been made to control the population of *Ae. aegypti* as DENV vector, which among others are the use of pesticides and fumigation (fogging). The use of uncontrolled pesticides and other chemical compounds has negative impacts, such as environmental pollution that can kill non-target organisms and disrupt the balance of the ecosystem. Bhanu *et al*. (2011) reported that deltamethrin, which is a pyrethroid pesticide used to eradicate the mosquitoes, may cause chromosome damage in the root meristem tissues of onions (*Allium cepa*). In addition, pesticides also cause mosquitoes to become insecticide-resistant. Maestre-serrano *et al*. (2014) reported the *Ae. aegypti's* resistance to some insecticides in Colombia. Among the most appropriate strategies to control *Ae. aegypti* population is the integrated pest management (IPM), some methods of which are applied and evaluated together in order to produce more effective and efficient results. The Area Wide-Integrated Pest Management (AW-IPM) is increasingly accepted to control pest population, especially for mobile pests where large scale management is more effective and preferable to the uncoordinated field (Vreysen *et al*., 2007). One method that can be used in AW-IPM is the Sterile Insect

Technique (SIT) (Klassen and Curtis, 2005; Pimentel, 2007).

The basic principle of SIT is controlling the number of insects by themselves (autocidal) (Klassen and Curtis, 2005). These techniques include mass-rearing pest insects in laboratory, the sterilization process of male insects with ionization energy (irradiation), and release of sterile male insects into the target area in large numbers to get a high ratio between the released sterile insects and the native insects population (Knipling, 1955; Knipling, 1959; Knipling, 1970; Klassen and Curtis, 2005; Parker, 2005). The sterilization process of male insects uses gamma rays from Co-60 and Cs-137, X-rays and electron beam with a certain dose, in which the process affects gonad cells that cause sperm inactivation (Bakri *et al*., 2005). Sterile male *Ae. aegypti* mosquitoes irradiated with gamma rays would be released to the target area, and they would compete with wild male mosquitoes to mate with wild female. The mating between sterile male with wild female mosquitoes will produce no offspring. Therefore, by applying SIT, *Ae. aegypti* population in the target area will decrease significantly (Yakob *et al*., 2008). This technique has been applied before in different countries to control mosquito population in the area. Klassen (2009) reported the development of SIT to control African malaria vectors. Bellini *et al*. (2013) reported the application of SIT to control *Ae. albopictus* population in Rimini, Italy, where male *Ae. albopictus* were sterilized using gamma rays (Co-60) in the range 60-110 Gy, with the acceptable sterilizing dose of 80 Gy. To support the implementation of SIT in Indonesia, the researchers of the insect pest control group at the Center for Isotopes and Radiation Application-National Nuclear Energy Agency (CIRA-Batan) have successfully reared *Ae. aegypti* in laboratory and observed their life cycle. Starting with the hatching of *Ae. aegypti* eggs to the larvae stage, the larvae were then reared to the pupa stage. After the pupae turned into adult *Ae. Aegypti,* they were reared to produce eggs again. The sterilization process of males *Ae. aegypti* was conducted in the Irradiator and Electromechanical Division of CIRA-Batan, which have the facilities to support that process, such as gamma cell-220, panoramic irradiator, gamma chamber-4000A and natural rubber irradiator (source: Co-60) (http://www.batan.go.id/index.php/id/fasilitas-pair). Sterile male *Ae. aegypti* mosquitoes are available to be released periodically into the target area.

The SIT is a method to control the population of disease vector that utilizes radiation energy from a radioactive source (Knipling, 1955; Bakri *et al*., 2005). The use of radioactive sources requires permission from the Nuclear Energy Regulatory Agency of Indonesia (*Bapeten*) (http://www.bapeten.go.id/?page_id=1127). In addition, radioactive sources can only be found in certain places, such as CIRA-Batan in South Jakarta. This means that the sterile male *Ae. aegypti* mosquitoes have to spend a long trip into the target area. For example, while the sterilization process using gamma ray irradiation was conducted in CIRA-Batan, South Jakarta, the release site of the sterile male mosquitoes might be located in Banjarnegara district, Central Java, which was an endemic area of DHF in 2013 and 2014 (http://www.jatengprov.go.id/id/newsroom/waspada-demam-berdarah). Accordingly, transporting the sterile male *Ae. aegypti* mosquitoes by land can spend 18 hours to reach the release site. Transporting to other target areas with farther distance from Jakarta will spend more time. The length of time spent by sterile male *Ae. aegypti* mosquitoes on the trip to the target area is expected to decrease the sterility of the insect. The aim of this research is to investigate the sterility of male *Ae. aegypti* on the 1st-5th days post γ-ray sterilization. The authors propose a hypothesis that the sterility of sterile male *Ae. aegypti* mosquitoes will decrease on day-1 until day-5 post γ-ray sterilization.

MATERIALS AND METHODS

This research is designed using a completely randomized design (CRD) with a single factor level, sterile male *Ae. aegypti* sterility on the 1st-5th days post γ-ray sterilization. Each mating combination used 3 (three) replicates and was compared with control. The data were presented descriptively. The data obtained from this study, such as the number of eggs and hatchings, were tabulated using Microsoft Excel 2007 software and analyzed using the Statistical Analysis System (SAS) 9.1.3 portable to get the analysis of variance. Further tests were conducted with Duncan double interval test.

The study used *Ae. aegypti* colony reared in the Insect Pest Control Laboratory of CIRA-Batan, South Jakarta. These mosquitoes were collected from South Tangerang, Banten Province, Indonesia, and had undergone approximately ten generations from the field collections. This strain was reared in an environmental laboratory with the temperature range of 22-28^{o}C and 75% relative humidity. Adult *Ae. aegypti* mosquitoes are obtained by soaking about 3-month-old egg stock. Larvae from hatched eggs were maintained in a tray filled to a depth of 2 cm with water and fed until they transformed into the pupae stage, then male pupae and female pupae were separated based on pupal size with the visual technique. Male pupae are smaller than female pupae. Male pupae were put in a mosquito cage (Bugdorm®) sized 40x40x40 cm to obtain adult *Ae. aegypti* aged less than one day as an object of the study. The same procedure was performed to get the female *Ae. aegypti* aged less than one day, which were used in the treatment.

The sterilization process of male *Ae. aegypti* was conducted with Panoramic Irradiator that uses Co-60 as the source of gamma rays. The dosage of this process was 70 Gy. Then, the sterilized male *Ae. aegypti* mosquitoes

were combined with un-irradiated males to compete mating with un-irradiated females. Mating combinations of *Ae*. *aegypti* in this study were presented on Table 1.

Table 1. Mating combinations of *Aedes aegypti.*

No.	Mating combination
1	36 sterile ♂ 1 day post sterilization + 4 un-irradiated ♂ aged 1 day X 20 un-irradiated ♀ aged 1 day
2	36 sterile ♂ 2 days post sterilization + 4 un-irradiated ♂ aged 1 day X 20 un-irradiated ♀ aged 1 day
3	36 sterile ♂ 3 days post sterilization + 4 un-irradiated ♂ aged 1 day X 20 un-irradiated ♀ aged 1 day
4	36 sterile ♂ 4 days post sterilization + 4 un-irradiated ♂ aged 1 day X 20 un-irradiated ♀ aged 1 day
5	36 sterile ♂ 5 days post sterilization + 4 un-irradiated ♂ aged 1 day X 20 un-irradiated ♀ aged 1 day

Notes: ♂ indicates male(s) *Ae. aegypti*; ♀ indicates female(s) Ae. aegypti; + indicates combination; and X indicates mating

Sterile male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization were joined by un-irradiated males aged 1 day to compete mating with un-irradiated females aged 1 day. The mating combination was conducted for 2 days. After 2 days, both sterile male *Ae. aegypti* mosquitoes and un-irradiated males were removed from the cage. Each cage contained female *Ae*. *aegypti* mosquitoes fed with blood for the egg maturation process (oviposition) inside their body. After 2 days, the eggs were collected using an oviposition cup. Next, the eggs were soaked and the hatching process was observed. The data were obtained from the number of eggs and the number of larvae from the hatching process, and they were converted into sterility rates of male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization.

RESULTS AND DISCUSSION

The experiment pattern was designed with a ratio of 9:1 between sterile male *Ae. aegypti* mosquitoes and un-irradiated males. According to Knipling (1955), where the ratio between sterile males and un-irradiated males is 1:1, the breeding ability of insect populations will decrease by 50%; whereas if the ratio is 9:1, the ability of insect breeding will further decrease by 90%. Sterility refers to physiological inability of a living organism (which in this case is *Ae*. *aegypti*) that affects sexual reproduction, or the condition of *Ae*. *aegypti* being unable to bear offspring. *Ae*. *aegypti* sterility can be observed based on the number off eggs that do not hatch into larvae. The number and the percentage of the eggs that did not hatch into larvae from the mating combination of this study were presented in Table 2. Based on Table 2, the average percentage of unhatched eggs in mating combinations 1-5 stays higher than 90%, except in the mating combination number 5 (86.91%). It means that the sterility of *Ae. aegypti* resembles the pattern suggested by Knipling (1955). Double interval test with a level of confidence P≥0.05 on the number and the percentage of eggs that did not hatch showed no significant difference. In this study, the highest number of eggs (1,372 eggs) was produced by mating combination number 4. However, that number was not significantly different if compared with the control, mating combination number 1 (1,134 eggs). The number of eggs produced indicates there were the mating between sterile male *Ae. aegypti* mosquitoes and un-irradiated females and the mating between un-irradiated males and un-irradiated females.

The percentages of eggs that did not hatch in all mating combinations were not significantly different. The smallest percentage of eggs that did not hatch was generated by mating combination number 5 (86.91%). However, when these data were compared with the experimental controlled population, the mating combination number 1, the difference is not significantly different (97.69%). The percentage of eggs that did not hatch is closely related to the sterility and mating ability of sterile male *Ae*. *aegypti*. A high percentage of eggs that did not hatched (>90%) indicates that sterile male *Ae. aegypti* mosquitoes in this experiment were highly sterile.

Fertility means the ability of *Ae*. *aegypti* to produce offspring, which is observable from the percentage of eggs that hatched into larvae. Eggs viability, sterility, and mating ability are quality control parameters in sterile insect quality (Calkins and Parker, 2005). Fertility of this insect can also be observed from the data of the number of eggs that hatched into the larvae stage, and those eggs were probably produced from normal male *Ae. aegypti* mosquitoes mating with normal females. The highest percentage of the eggs hatched into larvae was found on the mating combination number 5, while the lowest percentage was found on the mating combination number 2. It is suspected that on the mating combination number 5, the ability of sterile male *Ae. aegypti* mosquitoes to compete with normal males to mate with females decreased due to the cellular damage caused by the exposure to gamma irradiation (Helinski *et al*., 2009). However, this result is not significantly different compared with the other combinations. In fact, the percentages of larvae in all mating combinations are not significantly different. Based on the data, the sterilized male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization have low fertility.

Table 2. The number and percentage of the eggs that did not hatch.

No. mating combination	No. eggs				Eggs not hatched (%)			
	Replication			Mean	Replication			Mean
	1	2	3		1	2	3	
1	1230	1040	1134	1135[a]	98.62	100.00	94.44	97.69[b]
2	1000	1117	1236	1118[a]	99.70	99.82	94.09	97.87[b]
3	708	1729	1292	1243[a]	100.00	91.50	81.27	90.92[b]
4	1417	1431	1268	1372[a]	91.81	97.41	99.37	96.20[b]
5	1321	1490	1166	1326[a]	94.25	91.34	75.13	86.91[b]

Notes: the numeral figure followed by the same letter means no significantly different at the level confidence of 95%

Sterilization process is one of main principles on the SIT. The sterilization process of male *Ae. aegypti* mosquitoes in this experiment was conducted using γ-ray sterilization. Gamma rays are electromagnetic energy with high penetrating power. When body tissues or cells are exposed to gamma radiation, the building blocks of the cell will be ionized, causing the formation of reactive free radicals that can cause damage to cells and tissues of living (Jagetia and Rajanikant, 2004).

Infertile state of insect's reproduction was generated by γ-ray sterilization that broke down the gonial cell chromosome. Thus, the fragmentation of germinal-cell chromosome causes dominant lethal mutations, translocations, and other chromosomal aberrations, and this could enhance the production of aberrant gametes. Mitosis inhibition and fertilized egg mortality could be guaranteed as well (Klassen and Curtis, 2005; Robinson, 2005). This experiment utilized a radiation dosage of 70 Gy (1 Gy = 100 rad; 1Gy = 1 J/kg) (Bakri *et al*., 2005). Gamma ray sterilization on *Ae. aegypti* is random, i.e. it was not only exposed on gonad cells, but also somatic cells (body). On one hand, sterilization dose can damage or dysfunction the gonad cells and cause inactivation of sperms, causing males to become sterile. On the other hand, radiation also causes disruption of somatic cells that damage the organs and can affect the sterility of male *Ae. aegypti*.

The hypothesis of this research is that the sterility of sterile male *Ae. aegypti* mosquitoes will gradually decrease on day-1 until day-5 post γ-ray sterilization. The hypothesis H_0 is tested by analyzing the sterile male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization. The results of analysis reject the H_0, which states that the longer day post γ-ray sterilization, the more sterility will decrease. The data on the number of eggs, hatching, and sterility do not show a significant difference among all mating combinations. Based on the experimental data and analysis, it is shown that the sterile male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization retained high ability to compete with un-irradiated males to mate with un-irradiated females. In other words, sterile male *Ae. aegypti* mosquitoes on the 1st-5th days post γ-ray sterilization have high sterility and low fertility. Data from this study can be used as a guideline to support the process of releasing sterile male *Ae. aegypti* mosquitoes into the target area of SIT.

Conclusion: Sterile insect technique (SIT) is one technique for controlling insect populations, especially *Ae. aegypti* as DHF vectors, which have good prospects in Indonesia. Sterility is one of important parameters in SIT. Results of this study show that the sterility of sterile male *Ae. aegypti* on the 1st-5th days post γ-ray sterilization was not significantly different from each other. In other words, there is no decrease on the sterility of sterile male *Ae. aegypti* on the 1st-5th days post γ-ray sterilization.

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