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Effect of gamma-ray irradiation to reproductive performance of *Aedes albopictus* and its offsprings

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Abstract
Aedes albopictus is an essential vector of dengue hemorrhagic fever (DHF) in the world. Currently, DHF vector control in Indonesia is still using chemical insecticide. However, chemical insecticide caused problems to human health, environment, and triggered the emergence of resistant *Ae. albopictus* colony. Sterile Insect Technique (SIT) has been developed for *Ae. albopictus* vector control. This study aimed to analyze the different effect doses of gamma-ray irradiation on *Ae. albopictus* and its offsprings. The research was held from October 2017 to January 2018 in the National Nuclear Energy Agency of Indonesia (BATAN), Jakarta. The design of this study was a true experiment. The treatment was irradiation of male *Ae. albopictus* pupae with five doses of gamma ray (30 Gy, 40 Gy, 50 Gy, 60 Gy, and 70 Gy). Irradiated *Ae. albopictus* male was immediately mass mated with the same aged normal females, and measured fecundity and eggs hatchability and its offspring. The results showed that irradiated of gamma-ray with doses of 50, 60, 70 Gy decreased fecundity in Parental and F1 of *Ae. albopictus*. Eggs produced by *Ae*. *albopictus* female which were mated with an irradiated male at doses of 60 and 70 Gy in Parental, was not hatched. Meanwhile, unhatched eggs occurred in F1 from an irradiated male at doses of 30, 40 and 50 Gy.

Keywords: *Aedes albopictus*, fecundity, hatchability, sterile insect technique

1. Introduction
Aedes albopictus is the mosquitoes which live outdoor and has wide distribution in all regions of Indonesia. These mosquitoes known as a vectors arboviruses (dengue virus, yellow fever virus, chikungunya virus), making these mosquitoes an important factor of infectious disease. The arboviruses can be transmitted to another vertebrate-host (horizontal transmission), also be passed from one arthropod generation to another by transovarial transmission (vertical transmission) [3, 9, 13].

Control of *Ae. albopictus* in Indonesia currently primarily based on chemical insecticides. Even though usage chemical insecticides more rapidly, but they also have potentially harmful effects on human health and the environment. As continuous use of an chemical insecticide cause resistant to vector. At present, several alternatives have been developed to control the chain of DHF vector transmission, including sterile insect techniques (SIT).

The SIT was among the first biological insect control methods (vector genetic) that targeting the same species (autocidal technique) [3, 4]. This technique would also present immense challenges in mass-rearing, sterilization, and release. The most commonly used gamma-ray irradiation in the form of radioisotopes are Cobalt-60 (Co-60) and Cesium-137 (Cs-137) which are exposed to male insects to produce sterile males. Sterile male insects are released to mate normal females so that they are expected to produce sterile offspring. In this case, the right radioisotope dose dramatically determines the success of the SIT application [2]. Maulidan (2017) [17] reported that *Ae. albopictus* which is irradiated by gamma-rays at a dose of 60 Gy, effectively sterilizes male mosquitoes up to 99%.

Irradiation causes cell damage resulting in genom mutations. This mutation has the potential to cause changes in the next generation [1]. Shetty *et al*. (2016) [22] reported that gamma-ray irradiation in *Ae. aegypti* mosquitoes in India for three generations affect reproductive performance. Study about the effects of gamma-irradiation on Ae. albopictus and its offspring is important because it does not conduct yet in Indonesia priorly. This report was aimed to analyze the effects of gamma-ray irradiation on the fecundity and hatchability of mosquitoes and its offspring on laboratory scale.

2. Materials and Methods

2.1 Research Design

The design of this study is a true experiment by measuring the effect of treatment, experiments were performed in batches for different doses and a control. The treatment in this study on a laboratory scale. Doses of 30, 40, 50, 60, and 70 Gy were chosen for the study in five replicates. Each batch consisted of 125 male pupae of *Ae. albopictus* receiving a specific dose of the radiation (gamma-ray).

2.2 Research Procedure

2.2.1 Mass Rearing of *Ae. albopictus*

Ae. albopictus used in this study was originally collected from around the National Nuclear Energy Agency of Indonesia neighborhood. Adults were maintained in 45 cm cubic cages of iron and fed on 10% sucrose solution in a jar with a cotton wick, Females were provided with the blood meal to produce eggs.

Furthermore, a plastic cup (ovitrap) containing clean water lined with filter paper was placed inside the cage for oviposition. Eggs from the ovitrap were hatched on a tray measuring 36x28x7 cm containing ± 1.5 L of water. The eggs reared into larvae as larval diet was provided biscuit until they developed into pupae. The pupae were transferred from the tray to the vial tube (231 mL) and placed inside the cage until they became adult mosquitoes. The mosquito colonization process was conducted until *Ae. albopictus* colonies were formed stable and ready to be tested. Mosquito colonies were maintained in laboratories with controlled climate parameters (temperature 26 ± 1 ^{0}C, humidity 70 ± 10%) and light:dark irradiation periods 12:12 hours [8].

2.2.2 Irradiation Procedure

Ae. albopictus pupae (less than 24 hours old) were separated between males and females used a plate separator (pupae separator). Male pupae were inserted into the vial tube (231 mL) containing water (± 10 mL). Furthermore, male pupae were irradiated by gamma-rays using Gamma Cell 220 irradiators with doses of 30, 40, 50, 60, and 70 Gy. Control variable of this study was non-irradiated male *Ae. albopictus* pupae which prepared in the laboratory of the same age as the irradiated male pupae.

2.3 Research Parameter

Ae. albopictus pupae were maintained in Bugdorm-1® until they develop into adulthood. The pupae were preserved in different Bugdorm-1®, namely normal female pupae, normal male pupae, and irradiated pupae at each dose level. Male and female mosquitoes were mated with a ratio of 1: 1 so that each Bugdorm-1® contains 25 males and 25 females. Adult male *Ae. albopictus* were irradiated by gamma-rays than mated with normal females at each dose as Parental in P30, P40, P50, P60, and P70. Parental descent was specified as F1 (F130, F140, F150, F160, and F170). Stages of *Ae. albopictus* offspring, starts female mosquitoes will do the oviposition. The oviposited eggs were hatched until they develop into adulthood. Mosquitoes that successfully develop into adults at each dose level, the male were separated and mated with normal females from the normal *Ae. albopictus* colony (not irradiated).

Fecundity was determined by counting the total number of eggs laid per female. Measurement of fecundity was done by transferred female mosquitoes that have been mated with male *Ae. albopictus* in Bugdorm-1® and blood-fed, individually into the vial tubes with moist filter paper for the oviposition process [25]. Calculation of fecundity was made based on the number of *Ae. albopictus* eggs oviposited per female in a gonotrophic cycle.

Eggs obtained from oviposition of *Ae. albopictus* females were hatched in a tray that has been filled with water and was observed until the seventh day. Hatchability is the percentage of the number of infertile eggs and the number of fertile eggs produced by female mosquitoes by observing the egg operculum under a microscope. An egg with an open operculum is a fertile egg, while a sterile egg is the egg whose operculum is not open [26].

2.4 Data Analysis

Data from the observations of fecundity and hatchability of each dose were presented in the form of tables and analyzed using One Way ANOVA to analyze the effect of differences in the dose of gamma irradiation on the parameters tested.

3. Results and Discussion

Table 1: Average of fecundity and hatchability of adult female *Ae. albopictus* mated with irradiated male mosquitoes

Reproduction	Dose (Gy)	Total Number of Eggs	Fecundity (eggs/adult female *Ae. albopictus*)	Hatchability (%)
P	0 (Control)	5457	43.66±1.14^a	94.58±0.34^a
	30	5241	41.93±1.03^a	0.23±0.07^b
	40	5349	42.79±1.89^a	0.21±0.67^b
	50	4123	32.98±1.99^b	0.21±0.11^b
	60	3772	30.18±2.50^{bc}	0.20±0.08^b
	70	3166	25.33±1.90^c	0.20±0.11^b
F1	0 (Control)	166	58.33±1.4^a	96.24±0.01^a
	30	202	40.40±2.94^b	0.00±0.00^b
	40	155	38.75±1.70^b	0.00±0.00^b
	50	77	38.50±2.50^b	0.00±0.00^b
	60	-	-	-
	70	-	-	-

Information: the same superscript word in the same column shows no difference (*p-value* >0.05)
(-) shows that there is no egg produced because there is no mosquito that develops into an adult

3.1 Fecundity

The average number of eggs *Ae. albopictus* produced at the higher parental at doses of 30 Gy (41.66 ± 1.03) and lowest at 70 Gy (25.33 ± 1.90) (Table 1). The average fecundity (of *Ae. albopictus* at P_{50}, P_{60}, and P_{70}) showed significant differences (P<0.05) with control, while P_{30} and P_{40} did not show significant differences (P> 0.05) with control, and P_{60} did not show significant differences (P> 0.05) with P50 and P_{70}. The average number of eggs *Ae. albopictus* produced at the higher F1 at doses of 30 Gy (40.40 ± 2.94) and lowest at 50 Gy (38.50 ± 2.50) (Table 1). The average fecundity at F130, F140, and F150 showed significant differences ($P<0.05$) with control, while F160 and F170 had no fecundity because hatched eggs are failed develop until the adult stage. The results showed that the average fecundity of *Ae. albopictus* decreases in a high doses when the oviposition per female conducted between 30 to 70 Gy.

Table 1 shows *Ae. albopictus* still produces eggs after normal female mosquitoes were mated with irradiated males. The result indicated that normal female *Ae. albopictus* successfully mated with irradiated male *Ae. albopictus*, and the oviposition by adult females of *Ae. albopictus* still occurs. Hassan *et al*. (2016) [10] and Ramadhani *et al*. (2017) [18] reported that if male *Culex pipiens* mosquitoes in Cairo and male *Cx. quinquefasciatus* mosquitoes in Indonesia irradiated by gamma-rays mated with normal female mosquitoes. A similar result was reported by Dandalo *et al*. (2017) [6] on *Anopheles arabiensis* that irradiation does not affect the survival of mosquitoes, such as mating.

Fecundity in this study decreased when compared with control. The decrease of the fecundity of mosquitoes can be affected by the inability of the female mosquitoes to lay eggs (infecundity), the inability of male mosquitoes to produce sperm (aspermia) or sperm do not function, and the inability to mate [15]. Dewi (2018) [7] reported that in *Aedes aegypti* mosquitoes irradiated by gamma rays of 60 and 70 Gy there was a decrease in fecundity caused by the inability of male mosquitoes to produce sperm (aspermia) or nonfunctional sperm produced. The previous study also showed a similar result that there was a decrease in fecundity of *Cx. pipiens* mosquitoes after exposure with gamma-ray irradiation at dosages 23, 41, 75 and 128 Gy [10]. Meanwhile, Light *et al*. (2015) [16] reported that adult male *Amyelois transitella* were irradiated using a laboratory scale x-ray irradiation dose 50-300 Gy, female fecundity was not affected.

Testicular histopathology due to gamma-ray irradiation will change the form of testis by rupture the testicular wall followed by necrosis and degeneration, abnormal distribution of spermatogonia and spermatocytes, causing a decrease in the rate of spermatogenesis, and changes in sperm shape that inhibit sperm movement and fertility, so that sperm are not successfully transferred [10]. The result of this research shows that gamma-ray irradiation has an impact on the testes and the sperm of insects resulting in a decrease in fecundity.

Gamma-ray irradiation can reduce mosquito fecundity and alter egg morphology. The irradiated male *Cx. quenquifasciatus* mosquitoes mated with normal female mosquitoes can cause infertile eggs. The morphology of a sterile egg has a shape that shrinks, curves, and shrinks. The morphology of sterile eggs at a dose of 50 Gy undergoes a change in shape to shrink, whereas at doses of 60 Gy and 70 Gy causes a small portion of the eggs produced change outer morphological forms. The 60 Gy gamma-ray irradiation tends to change the egg morphology to be slightly curved, while at 70 Gy the change in the morphology of the egg tends to shrink [21].

Fecundity of female mosquitoes in parental reproduction and F1 in this study showed significant differences compared to controls. The result of this study shows that damage to the testicles, sperm, and aspermia of male *Ae. albopictus* due to gamma-ray irradiation inherited to male offspring mosquitoes.

3.2 Hatchability

Hatchability of *Ae. albopictus* eggs at each dose level are presented in Table 1. Hatchability of eggs in Parental at each level dose showed significant differences ($P<0.05$) with control. The hatchability of eggs in Parental is obtained from reproductive eggs P30, P40, and P50 which succeed through the stages of the stage up to adults (males), the males are then mated with normal females. The mating results will produce F1, and those presented in Table 1 shows unhatched eggs. Hatchability *Ae. albopictus* in Parental and F1 between doses of 30 to 70 Gy showed significant differences ($P<0.05$) with control.

The hatchability of *Ae. albopictus* eggs in this study decreased when compared to control. The decrease of mosquito hatchability can be caused by the embryo in the formed eggs of *Ae. albopictus* mosquitoes cannot survive while the mating process continues (Table 1). The irradiation process does not affect the male mosquito to produce sperm. Spermatogenesis is the process of forming male gametes (sperm) that occur mainly at the end of the pupal stage. During the process of spermatogenesis sperm cells divide rapidly. If these cells are exposed to irradiation, chromatin in sperm will change and formed the abnormal sperm [11]. Observations at the cytology level, sterility or infertility are the results of chromosomal fragmentation (dominant lethal mutations, translocations, and chromosomal aberrations), which causes unbalanced gamete production, and subsequently, inhibits mitosis and the death of fertilized eggs or embryos do not develop [23].

Other researchers reported a decrease in hatchability when a normal female insects mated with irradiated male insects. Cai *et al.* (2018) [5] reported the similar result in *Bactocera dorsalis* (Diptera: Tephritidae) which was irradiated with Co-60 doses of 0 to 40 Gy; there were differences in hatchability after exposure to irradiation. The mating results of normal females with irradiated male *Cx. pipiens* at doses of 23 to 90 Gy experienced a decrease in hatchability of 72.6 to 0% when compared to control, i.e., 94 [10]. Another similar result was reported by Ramadhani *et al*. (2017) [18] which reported that a decrease in hatchability in *Cx. quinquefasciatus* in Pekalongan, Indonesia which was irradiated by Co-60 doses of 60 to 80 Gy derived from the mating of normal females with irradiated males, namely 4.81 to 1.38% and Rempoulakis *et al.* (2015) [19] reported, *Dacus ciliates* (Diptera: Tephritidae) was irradiated by Co-60 doses of 60 to 140 Gy, zero egg-hatching was only observed for eggs fathered by males that had been irradiated with 140 Gy. Below 140 Gy, some egg-hatching (<3.5%) was observed in tests including irradiated males. Light *et al*. (2015) [16] reported that Adult male *Amyelois transitella* were irradiated using a laboratory scale x-ray irradiation dose 50-300 Gy, embryonic development of eggs to the prehatch stage and egg eclosion did not occur at radiation doses ≥125 Gy, emergence of F1 adults was low with the result that no F2 eggs hatched for the test exposures of 50–100 Gy.

This study shows the percentage of hatchability of *Ae. albopictus* eggs which are sterile in Parental and F1 reproduction when compared to control. The result shows that exposure to gamma-ray irradiation in male mosquitoes resulting in the successful transfer of sterile sperm the female mosquitoes. Gamma-ray irradiation exposure causes damage to reproductive function, sperm cell death, and chromosomal aberration which results in a significant reduction in embryo production [14]. Robinson (2005) [20] explains that chromosome aberration in insects can occur due to exposure to high-dose gamma-ray irradiation. The broken chromosome can be rejoined with its own pieces or other pieces. The result is an individual chromosome mutation, a change in the structure of chromosomes, which means that the genetic material changes. Chromosomal aberrations have been reported after irradiated *Ephestia kuehniella* (Lepidoptera) males mated with normal females [24]. Hoa and Tien (2001) [12] prove that chromosomal aberrations are the main cause of infertility inherited from the moth of cabbage (*Plutella xylostella*). Sterility is expected to be effectively passed on to the next generation when sterile males mate with normal females in nature [23].

4. Conclusion
Results clearly demonstrate that, impact of radiation affect the offspring of *Ae. albopictus* mosquitoes which can be seen from their fecundity and hatchability. Compare to control, irradiation *Ae. albopictus* fecundity at dose 30 to 70 Gy not significant different. Whereas F1 egg producing only occurs at doses of 30 to 50 Gy. Meanwhile, parental egg hatchability occurs at doses of 30 to 50 Gy. This research is very important to figure the characteristic irradiated adult male of *Ae. albopictus* the inherited to its offspring.

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6. References
1. Abdalla AI, Kehail MAA, Ibrahim NA. Effect of X-Ray on the fertility, fecundity and sterility of female *Anopheles arabiensis* mosquito (Diptera: Culicidae) in Gezira State, Sudan. Int J Mosq Res. 2017; 4(3): 128-131.
2. Ayvaz A, Yilmaz S. Ionizing radiation disinfestation treatments against pest insect. Evolution of Ionizing Radiation Research. 2015. Doi: 10.5772/60923.
3. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Madon M, Kaiser A. Mosquitoes and their control. Second Edition. New York: Springer, 2010.
4. Bouyer J, Lefrancois T. Boosting the sterile insect technique to control mosquitoes. Trends in Parasitology. 2014; 30(6):271-273.
5. Cai P, Hong J, Wang C, Yang Y, Yi C, Chen J, Ji Q. Effect of Co-60 radiation on the activities of three main antioxidant enzymes in *Bactrodera dorsalis* (Hendel) (Diptera: Tephritidae). J Asia Pac Entomol. 2018; 21(1):345-351.
6. Dandalo LC, Kempc A, Lizette L, Koekemoera, Munhenga G. Effect of ionising (gamma) radiation on female *Anopheles arabiensis*. Trans R Soc Trop Med Hyg. 2017; 111: 38-40.
7. Dewi M. Produktivitas *Aedes aegypti* jantan (Diptera: Culicidae) yang diiradiasi sinar gamma dan keturunannya [tesis]. Institut Pertanian Bogor, Indonesia, 2018.
8. Ernawan B, Tambunan USF, Sugoro I, Sasmita HI. Effect of gamma irradiation dose-rate on sterile male *Aedes aegypti*. Proceeding of International Biology Conference, 2016, 2017.
9. Hadi UK, Koesharto F. Nyamuk. In: Hama Permukiman Indonesia: Pengenalan, Biologi, dan Pengendalian, (Sigit SH, Hadi UK. Eds.), Unit Kajian Pengendalian Hama Permukiman, Fakultas Kedokteran Hewan Institut Pertanian Bogor, IPB Press, Bogor, 2006, 23-50.
10. Hassan MI, Amer MS, Hammad KM, Gabarty A, Selim TA. Lattent effect of gamma irradiation on reproductive potential and ultrastructure of males tastes of *Culex pipiens* (Diptera: Culicidae). J Radiar Res. 2016; 3:1-9.
11. Helinski MEH, Parker AG, Knols BGJ. Radiation biology of mosquitoes. Malaria Journal. 2009; 8(2):1-13.
12. Hoa NTQ, Tien NTT. Radiation-induced F_1 sterility in *Plutella xylostella* (Lepidoptera: Plutellidae): potential for population suppression in the field. Florida Entomologist. 2001; 84(2):199-208.
13. Huang YJS, Higgs S, Home KM, Vanlandingham DL. Flavivirus-mosquito interaction. Viruses. 2014; 6(11):4703-4730.
14. Hurem S, Gomes T, Brede DA, Mayer I, Lobert VH, Mutoloki S *et al*. Gamma irradiation during gametogenesis in young adult zebrafish causes persistent genotoxicity and adverse reproductive effects. Ecotoxiology and Enviromental Safety. 2018; 154(15):19-26.
15. Lance DR, McInnis DO. Biological basis of the sterile insect technique. In: Sterile Insect Technique Principles and Practice in Area-Wide Integrated Pest Management, (Dyck VA, Robinson AS. Eds). Springer, Netherlands. 2005.
16. Light DM, Ovchinnikova I, Jackson ES, Haff RP. Effects of x-ray irradiation in male navel orangeworm moths (Lepidoptera: Pyralidae) on mating, fecundity, fertility, and inherited sterility. Journal of Economic Entomology. 2015; 108(5):2200-2212.
17. Maulidan D. Pengaruh radiasi sinar gamma terhadap daya saing kawin nyamuk jantan mandul *Aedes albopictus* sebagai pengendalian vektor dengan Teknik Serangga Mandul [skripsi]. Universitas Diponegoro, Indonesia, 2017
18. Ramadhani T, Hadi UK, Soviana S, Irawati Z, Sunaryo. Daya saing kawin nyamuk jantan steril (*Culex quinquefasciatus*) skala laboratorium: studi awal penggunaan Teknik Seragga Mandul dalam pengendalian vektor filariasis limfatik di Kota Pekalongan. Balaba. 2017; 13(1):65-72.
19. Rempoulakis P, Castro R, Nemny-Lavy E, Nestel D. Effects of radiation on the fertility of the Ethiopian fruit fly, *Dacus ciliatus.* Entomologia Experimentalis et Applicata. 2015; 155(2):117-112.
20. Robinson AS. Genetic basis of the sterile insect technique. In: Sterile Insect Technique Principles and Practice in Area-Wide Integrated Pest Management, (Dyck, VA, Robinson AS. Eds). Springer, Netherlands. 2005.
21. Setiyaningsih R, Widiarti, Heriyanto B. Pengaruh radiasi sinar gamma Co-60 terhadap sterilitas dan perkembangan emrio *Culex quinquefasciatus*. Media Litbangkes. 2015; 25(1):51-58.
22. Shetty V, Shetty NJ, Harini BP, Ananthanarayana SR, Jhac SK, Chaubeyd RC. Effect of gamma radiation on life history traits of *Aedes aegypti* (L.). Parasite Epidemiology and Control. 2016; 1:26-35.
23. Stringer LD, Harland DP, Grant JE, Laban J, Suckling DM. Effect of 40Gy irradiation on the ultrastructure, biochemistry, morphology and cytology during

spermatogenesis in the southern green stink bug *Nezara viridula* (Hemiptera: Pentatomidae). bioRxiv. 2017; 171991:1-20
24. Tothová A, Marec F. Chromosomal principle of radiation-induced F1 sterility in *Ephestia kuehniella* (Lepidoptera: Pyralidae). Genome. 2001; 44(2):172-184.
25. Zhang D, Lees RS, Xi Z, Bourtzis K, Gilles JRL. Combining the sterile insect technique with incompatible insect technique: III-robuts mating competitiveness of irradiated triple *Wolbachia*-infected *Aedes albopictus* male under semi-field conditions. Plos One. 2016; 11(3):1-15.
26. Zheng ML, Zhang DJ, Damiens DD, Lees RS, Gilles JRL. Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) – II – egg storage and hatching. Parasites and Vectors. 2015; 8:384.