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The influence of transport and irradiation on the longevity of male *Aedes aegypti* (Diptera: Culicidae)

Authors
Tri Ramadhani[1], Upik Kesumawati Hadi[2,3], Susi Soviana[2], Zubaidah Irawati[2]
Hadian Imam[4]
[1]Post Graduate Student of Doctoral Program, Department of Parasitology and Medical Entomology, Bogor Agricultural University, Dramaga-Bogor, Jl Agatis Kampus IPB Darmaga, Bogor, 16680
Email: *3rdhani@gmail.com*
[2]Entomology Laboratory, Department of Parasitology and Medical Entomology, Faculty of Veterinary Medicine – Bogor Agricultural University, Dramaga-Bogor, Jl Agatis Kampus IPB Darmaga, Bogor, 16680
Email: *upikke@gmail.com*
[3]Corresponding author, Email: *upikke@gmail.com*, [4] Center for Isotope and Radiation Application (CIRA) - National Nuclear Enerry Agency (BATAN) Indonesia, Jl Jalan Lebak Bulus Raya No. 49,
North Jakarta 12440

ABSTRACT
Transport of sterile male mosquitoes from the location of irradiation to the release area possibly affects survival and mating competitiveness. The aim of the current research was to determine the influence of transportion the longevity of male irradiated Aedes aegypti (L.). This experiment compared the longevity of 180 irradiated male mosquitoes with 80 Gy dose in the pupal stadium to 100 virgin male mosquitoes (unirradiated control). The result is showed that irradiated male mosquito can survive in 13 days while fertile mosquito in 14 days. Based on the result of t-test, it show that there is no difference age mosquito on irradiated male mosquito and fertile male mosquito (control) with equal variance t-value not assumted of 0,000 with Sig. (2-tailed) is 1,000 or bigger than 0,05. There is a difference in the death rate in irradiated mosquitoes than control (t Equal Variance assumed value of 2.230 with Sig. (2-tailed) was 0.034). Suggested age irradiated mosquitoes no more than three days if it will be applied to the field.
Keywords: *irradiated, male mosquito, longevity, transportation, SIT.*

Introduction

Mosquito is known as main transmitter animal of various contagious vector disease in Indonesia. Malaria, dengue fever, JE, filariasis, is the example of contagious vector disease which until this day still become a healthy problem in the world especially in Indonesia. In addition, the effect of urbanization, globalization, and the change of climate are more accelerate the distributing and plague the contagious vector disease (mosquito). The control vector has long seen as the only available instrument to some main vector-borne disease, for example, dengue fever. The control vector with conventional way use insecticide is known less effective because it cause the death of non-target flora and fauna, also the emergence of environmental pollution and resistance to insecticide, even often occur cross resistance, which reduces its effectiveness control. Because the effort for control vector contagious disease not showing sufficient result, then it required another way to assist the control, include Insect Irradiated Technique (Sterile Insect Technique/SIT) (White R D,et al 1972, La-Change et al, 1967, Hoper, G.H.S. 1976)

SIT is one of the technique vector control genetically by sterilize or radiated the target insect then released to the nature so that happened marriage with insect in nature. It is expected the result of marriage obtained the sterile generation, so the release in stage can lower the population (Vloedt,2010). The application of SIT in mosquito control can be done by sterilize the male mosquito then it release to nature. The sterilizing process can be done by using gamma light Co-60 (Yodav, 2010, Esteva, 2006). In Indonesia, SIT application still in development process in laboratorium and in field. The test of SIT application has been done in two city, Banjarnegara District and Salatiga District with the aim is to reduce the population of *Ae. Aegypti* mosquito. The result of the release of irradiated male mosquito in Banjarnegara District show the average sterility value of 79.16% (Nurhayati S, 2013). While the result of release irradiated male mosquito in two location (sub-urbanized and urbanized) in Salatiga city show the sterility level as 96.06% and 94.05% in the last release (Setiyaningsih, R 2014). Even though the SIT concept is very simple but the implementation is not simple because it needs various research which cover biology base, field ecology, estimation of the insect amount in field for each season, the effectiveness population sampling method before the control is done and after it done, the orientation of dose radiation which is cause irradiation, the competiveness of marriage of irradiated insect, the economic mass rearing method, the methodology of release the irradiated insect, the transportation of long distance insect, the spreading and behavior marriage irradiated insect in field, implementing organization and personnel in field. The far distance of irradiation place with the location of the release of irradiation male mosquito is possible affect of the quality of irradiation male mosquito so that it will uncompetitive when it has been marriage with nature female mosquito. Based on this case is done a research the influence and the carriage of irradiation male mosquito towards longevity.

Method

Time, Place, and Material: The research of transportation test was done in two location, irradiation was done in BATAN while for releasing the mosquito was done in Banjarnegara. The distance of this two city if using four wheel transportation (bus) is needed around 8-12 hours. This research using pure experiment research with post test only control group design which is consist of treatment and control. The time of this research was done from April untulJuli 2016. The research material was pupa *Aedes aegypti* stadium ages 24 hours before it was done irradiation. The amount of the sample is used 180 irradiation pupa and 100 control pupa.

Irradiation Process: Irradiation was done using Gamma Chamber 2.0 Iradiation, PAIR, BATAN with used radioactive Cobalt-60 with 80 Gy doses. The using of *Gamma Chamber* based on the position of radiation source which is surround the materials, so the materials got more same absorption doses. Irradiation pupa in the petri cup by using wet cotton which is covered by filter paper.

Packing: For transportation pupa testing is used 100 ml plastic cup which is there is a little place in it for pupa that give a little water (±5 ml). The amount of pupa per plastic cup is 50 pupas. Then plastic cup was arranged in a box which is covered by banana midrib 30 cm x 15 cm before inside the box to keep the humidity in order to stable. Thus, it was done the measurement of humidity during in the journey by using hydrometer digital.

Design Experiment: When arrived in Banjarnegara, plastic cup of pupa was expelled from the box and placed in a transfer cage size (15 x 15 x 15 cm) to be maintained until become adult mosquito. Separate between irradiation pupa and control pupa. Take 20 mosquito (irradiation pupa) from transfer cage by using aspirator and placed to a cage size 12 cm x 12 cm x 12 cm which inside was equipped with 10% sugar water and honey as source energy. Do it in nine cages (repeated) for mosquito from irradiation pupa and mosquito from control pupa (five repeated). The observation of dead mosquito amount was done every day. The rest of dead mosquito was expelled from the cage to avoid double counting. Write the temperature and the humidity of the room by using thremohygrometer.

Analysis

Data of mosquito age was tabulated by using software *Microsoft Excell* and it was analyzed significantly by using SPSS version 15.0, before it was done normality data test using Kolmogorov-Smirnov Test and continued difference test with "t" test.

Result

Table 1. The amount of sterile and control dead male mosquito each day.

Cages	Male mosquitos	Dead of male mosquitoes														
		18/5	19/5	20/5	21/5	22/5	23/5	24/5	25/5	26/5	27/5	28/5	29/5	30/5	31/5	1/6
K 1	20	0	0	0	3	4	4	0	0	4	3	2	0	0	0	0
K 2	20	0	0	0	0	0	0	0	0	0	10	0	6	4	0	0
K 3	20	0	0	0	0	1	1	0	2	1	0	4	8	3	0	0
K 4	20	0	0	0	0	1	0	0	0	1	0	4	4	10	0	0
K 5	20	0	2	1	0	1	0	0	0	1	0	3	2	5	5	0
Jumlah	100	0	2	1	3	7	5	0	2	7	13	13	20	22	5	0
Mean Control		0,00	0,40	0,20	0,60	1,40	1,00	0,00	0,40	1,40	2,60	2,60	4,00	4,40	1,00	0,00
R 1	20	0	3	7	7	1	1	1	0	0	0	0	0	0	0	0
R 2	20	0	1	3	7	2	3	3	0	1	0	0	0	0	0	0
R 3	20	0	0	3	7	1	0	2	0	1	2	0	3	1	0	0
R 4	20	0	0	4	3	0	1	2	1	3	2	3	1	0	0	0
R 5	20	0	0	6	5	3	4	0	0	1	1	0	0	0	0	0
R 6	20	0	0	3	8	3	3	0	2	0	1	0	0	0	0	0
R 7	20	0	3	4	1	3	1	1	1	1	3	2	0	0	0	0
R 8	20	0	0	1	4	3	4	2	0	0	1	2	1	2	0	0
R 9	20	0	0	1	1	5	2	2	0	0	1	2	2	4	0	0
Jumlah	180	0	7	32	43	21	19	13	4	7	11	9	7	7	0	0
Mean Radiasi		0,00	0,78	3,56	4,78	2,33	2,11	1,44	0,44	0,78	1,22	1,00	0,78	0,78	0,00	0,00

Description:
K: Control Male Mosquito R: Irradiation Male Mosquito

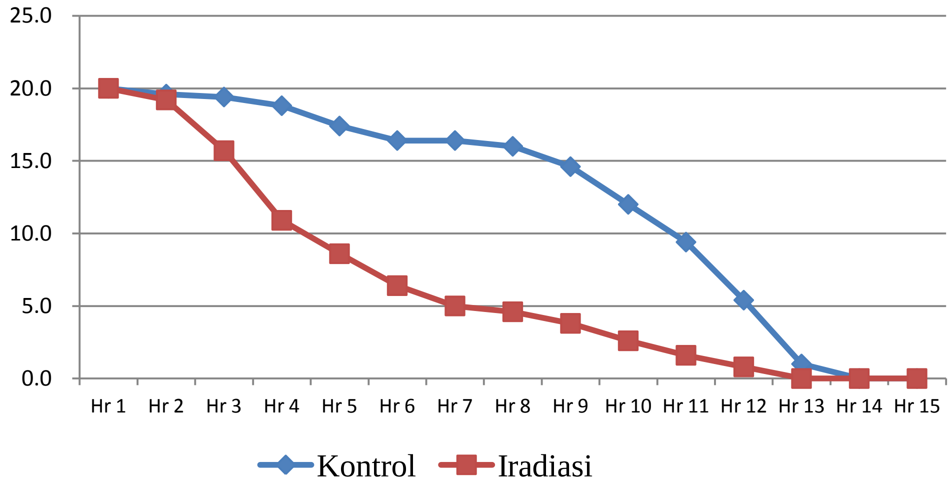

Figure 1 Longevity of male mosquitoes (irradiation and control) during observation

Figure above showed that irradiation male mosquito started dead in the second day of observation while the death of control male mosquito started in the third day. The death of irradiation male mosquito was more numerous if the sixth day, while the death of control mosquito was slow.

One-Sample Kolmogorov-Smirnov Test

		rata-rata kematian dikontrol	rata-rata kematian di radiasi
N		15	15
Normal Parameters[a,b]	Mean	1.333	1.333
	Std. Deviation	1.4356	1.3668
Most Extreme Differences	Absolute	.215	.214
	Positive	.215	.214
	Negative	-.177	-.165
Kolmogorov-Smirnov Z		.832	.828
Asymp. Sig. (2-tailed)		.493	.499

a. Test distribution is Normal.

b. Calculated from data.

The normality data test showed that the data distributed normally which is shown from Asymp Sig (2-tailed) value >0,05 value of irradiation mosquito data p= 0.493 and control 0,499 which is mean both of the data distributed normally so for the continue analysis using parametric test (t test).

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
rata-rata nyamuk hidup	Equal variances assumed	.000	1.000	.000	28	1.000	.0000	2.7931	-5.7214	5.7214
	Equal variances not assumed			.000	28.000	1.000	.0000	2.7931	-5.7214	5.7214

Output of the result of Independent-Sample t Test showed that there is two value t and the significant standard were Equal Variance assumed and Equal Variance not assumed. Equal Variance assumed mean that both of variant population was identic and Equal Variance not assumed was both of variant population was not identic. For decide was using F test. Standard sig. F is 1,000 means Ho is refused that both variant population was not identic (Equal Variance not assumed). Because sig. F have decision Equal Variance not assumed thus t test used Equal Variance not assumed. The value of t Equal Variance not assumed is 0,000 with Sig. (2-tailed) is 1,000 or bigger 0,05 thus it concluded that there is not differences between irradiation male mosquito and non-irradiation (control) mosquito. Although the age of mosquitoes no statistical difference but seen from irradiated mosquitoes percentage of deaths every day there is a difference. In figure 2 shows mosquitoes of irradiation on day 5th percentage mortality reached 60% while the control just happened on day 11th.

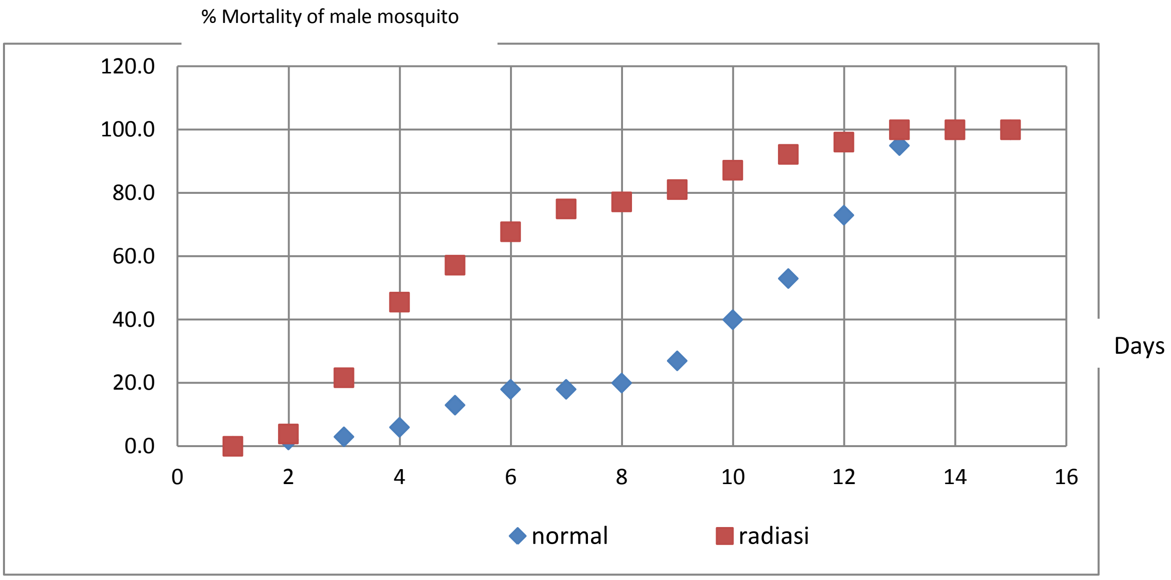

Figure 2 Percentage mortality of male mosquitoes to irradiation and control

Discussion

Vector control by using "Sterile Insect Technique" (SIT) now a days recently were back, this is due to the availability of new technology that has the potential to give improvement of the cost effectiveness significant to SIT, and many more research which is supported the lack of strategy vector control currently. Even though SIT has big potential to control the infectious vector disease but the result will be optimum if the implementation is integrated with other control effort. On the contrary, there is a case where SIT clearly not proper technic strategy for eliminate potential value of this approach in other cases. For that it required the result of research that support SIT so that in can be applied in the field.

Table 1 and figure 1 showed that the amount of dead mosquito or longevity irradiation male mosquito gamma Cobalt 60 with 80 Gy doses is not give the real differences ($p \leq 0.05$) if it compare with control mosquito. Mean while, it distinguish the dose of radiation that is used, besides it influence the sterility ovum produced also influence on mosquito age. The higher radiation doses which is given in pupa stadium can make mosquito have shorter life. It was caused by radiation besides it influence the occurrence of spermatogenesis process it can damage somatic cells. The higher doses is given the bigger damage cells that occur which is cause in shorter life of mosquito (Abdel-Malek, A.A. et al, 1967). The research result of KavitaYadav, et al 2010 showed that the influence of radiation on the longevity of mosquito *Anopheles stephensi Liston* in laboratory with 70 and 100 Gy radiation doses. Irradiation in mosquito with 100 Gy doses because the decrease of life chance (age) compared control mosquito (0 Gy) and radiation 70 Gy. The mosquito male barren must survive minimum a day after it release to compete with nature male mosquito to marry the female. Normal male mosquito has shorter life from the female less that 10 days (The American Mosquito Control Association). The success of application technic insect barren depends on the appropriate condition of irradiation male mosquito with nature female mosquito, also the time of application. The massive application of irradiation male mosquito is more effective done in abundance target low population and the ratio of irradiation male mosquito and nature male mosquito in maximum. The research result showed the longevity of normal male mosquito is longer that irradiation male mosquito, although in statistic there is no difference. This result same with Givemore Munhenga research result in *Anopheles arabiensis* mosquito, where male mosquito barren has longevity longer than nature mosquito.

The research result on *Anopheles stephensi* mosquito, the longevity of male mosquito with

100 Gy radiation written 11,8 ± 0.264, which is significantly different from control mosquito (13,2 days ± 0,35) and irradiation mosquito 70 Gy(11,8± 0,26) with value of p = 0,024, df = 2, F =7,447 (KavitaYaday, 2010) Helinskidkk (2006) reported that the occurrence and the survival of *An. Arabiensis* irradiation mosquito in pupa age 22-26 days has the same age or less that with the control mosquito in the same species. Most of the study which is done on the longevity mosquito revealed, there is age decrease of male mosquito after irradiation in 100-130 Gy doses (Abdel-Malek, A.A. et al, 1967) and 80 Gy (Sharma, V. P. 1978). Some of research results about radiation gamma effect on the longevity male mosquito barren around 10-12 days. This effect is not seen in female mosquito or the next generation. Doses 30, 35, and 40 Gy give stimulant effect in male mosquito from F1 generation, although in female this effect is observed just in F1 generation from 30 Gy in sample irradiation. The response of hormetic doses such as gamma radiation has recorded in many species of insects (Vinaya Shetty, 2016). In *An. Quadrimaculatus* in pupa stadium 1-4 hours age which is irradiated with 90 Gy doses will decrease the longevity of life (Davis, A.N, 1959)

Irradiation for each stadium in different species mosquito will show the increase or decrease effect in adult life range include in the next generation (Helinski et al, 2006; Abdel-Malek et al, 1996). Therefore, it was proven that life of adult mosquito very influenced by environmental changes or an earlier stage of mosquito life. However, irradiation cause the longevity of male mosquito increase that the control mosquito, as hermetic effect, that is induction radiation mutation main gen action in certain doses. Therefore, it was proven that the longevity of adult mosquito is very influenced by the environtmental changes or the earlier stage of mosquito life (Vinaya Shetty, 2016)

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