Effect of Radiation Gamma Co-60 on Sterile Male Technique Competitiveness in Inhibiting Population of Rattus argentiventer

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Effect of Radiation Gamma Co-60 on Sterile Male Technique Competitiveness in Inhibiting Population of *Rattus argentiventer*

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Abstract---Increased rice field rat population in nature, due to birth (natality). One of the innovations that can inhibit the increase in wetland rat populations is the use of sterile male techniques (SMT) using Gamma Co-60 radiation as one of the components in integrated rat pest control systems (IRPCS). This study was designed in a randomized block design (RBD) with 7 radiation dose treatments. Start by mating radiation male rat (♂P) with fertile female rat (♀F) with treatments ♂(P0) × ♀(F), ♂(P1) × ♀(F), ♂(P2) × ♀(F), ♂(P3) × ♀(F), ♂(P4) × ♀(F), ♂(P5) × ♀(F) and ♂(P6) × ♀(F), each of which is repeated 5 times. The results of mating between radiation male rats with fertile female rats show that the greater the radiation dose received by radiation male rats, the fewer number of children born by fertile female rats. Statistical analysis shows that doses of 1 and 2Gy, the probability value of P <0.05 significantly decreases the number of pups in control. But at radiation doses 3, 4, 5 and 6Gy no longer significantly decreased the number of pups. Starting from 3Gy doses to 6Gy fertile female rats do not give birth to infants or male rats have experienced infertility. The infertility index was 33.33% at a dose of 1Gy, 77.78% at a dose of 2Gy and 100% for a dose of 3,4,5 and 6Gy. The minimum dose for spaying male rice field rats occurs at a dose of 3Gy. Control of the rat population can be achieved if the male infertile that is released has a 100% infertility index, but it is necessary to take into account the possibility that suppression of rice field rat in the release area can be achieved by releasing radiation male rat that is not 100% sterile but have good mating competitiveness. The radiation dose of 1Gy the marital competitiveness is 0.50, 2 Gy is 0.83 and at a dose of 3Gy, the mating competitiveness is only 0.04. The 2Gy dose with mating competitiveness of 0.83 is the right radiation dose to inhibit the population of rice field rat, although the infertility index is not 100% but has good mating competitiveness. The value of mating competitiveness or *C-Index* using the index to determine the number of infertile male rats to be released into the area. At a dose of 2Gy obtained a competitive C-Index of 0.83. This means the ratio of infertile male rats and fertile rats is 8 to 1 at the time of release in nature so that it can effectively reduce the population of the wet rat.
Keywords--- *SMT, population, rice field rat, radiation dose, infertility index, C-index.*

1 Introduction

Population dynamics aims to study changes in the size of a population's density at a particular time and place, and explain the mechanisms underlying these changes (Richards, 1982). In the population, some factors influence each other both internal and external factors, which affect the population density level over time. Factors that influence population density in nature include an increase in population due to birth (natality), population density due to the entry of similar individuals from other populations (immigration), a decrease in population due to death (mortality), and a decrease in population due to the release of several individuals from the population to other populations (emigration) (Krebs, 1994). Feed factors, shelter, natural enemies and competition affect the four main factors causing changes in the population.

Rice field rat, like pests in general, have the type of strategy r (r-strategist), which can breed in a short time, resulting in a rapid increase in population or often called a population explosion. The event can occur if the enabling environmental conditions such as the availability of abundant feed, there are adequate shelter and nesting grounds

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(Macdonald & Fenn, 1994). Increasing population abundance can affect the availability of food, shelter, and competition which ultimately affects the natural population decline (Murakami, 1992; Arnawa *et al.*, 2019).

Decreasing the paddy rat population can be done with SMT as one component of an integrated pest control system with Gamma Co-60 radiation (Yuliara *et al.*, 2018; Suryani *et al.*, 2018). In order to control the population of paddy rats with SMT, female paddy rat are not parthenogenetic, male rat should be easily bred in mass outside their natural habitat or laboratory, infertility (radiation) does not result in physiological and morphological abnormalities and decreased mating ability, female rat should can mate once and live shorter than male field rat (Praharsini *et al.*, 2018; Sutapa *et al.*, 2018). If female field rat only mate once then, normal females that have been married to infertile males, will not be married by normal males and will not produce offspring, male paddy rat should be able to mate more than once and live longer than females (Vallejo *et al.*, 2019; Delgado *et al.*, 2018). If male rats are long-lived and can mate more than once, sterile animals can marry several fertile females in the field, so the population decline will be greater (Knipling, 1955).

2 Materials and methods

This type of research is experimental, that is, the first experiment was designed to determine the effect of Co-60 gamma radiation doses on the number of children born from the marriage of radiation male rats with fertile female rats. From the number of children born by fertile field, rat can be determined the index of infertility of male paddy rats in the treatment of radiation doses (Hamid *et al.*, 2018; Citrawan *et al.*, 2018). The second experiment carried out a combination of mating fertile field rat with radiation field rat to determine the competitiveness of mating or *C-Index*. Both experiments used a randomized block design (RBD), which consisted of controls, six treatments of Co-60 gamma radiation doses and five replications.

Before the study was carried out, the grouping of rat was done based on the sex of the adult and was ready to be mated (female age 40 days and a male age 60 days). Irradiation conditions with 80 cm source surface distance (SSD) on a 20 x 20 cm field. As shown in Figure 1. below.

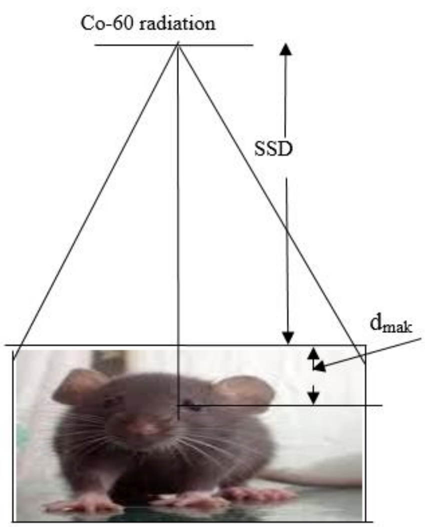

Figure 1. Irradiation conditions with a constant SSD (Dhanesar, 2008)

Radiation was carried out on male rats at the dose rate of the Co-60 FCC 8000F Teleterapi plane at 99.5631 cGy / min, then doses of 0, 1, 2, 3, 4, 5 and 6 Gy require irradiation times 0, 57, 115, 172, 229, 287 and 344 seconds. The radiation dose is calculated at a maximum depth of 0.5 cm. Where SSD is the distance between the radiation source and the surface of the radiation field, the impact, the maximum depth (gamma radiation Co-60 is 0.5 cm) and the equivalent field width is 20 x 20 cm^2.

2.1 Effect of dose on biological parameters

Determination of biological parameters mating between radiation male rats with fertile female rats according to the treatment dose of 0, 1, 2, 3, 4, 5 and 6Gy. Start by mating radiation male rat (♂ P) with fertile female rat (♀F) with treatments ♂(P0) × ♀(F), ♂(P1) × ♀(F), ♂(P2) × ♀(F), ♂(P3) × ♀(F), ♂(P4) × ♀(F), ♂(P5) × ♀(F), ♂(P6) × ♀(F), each of which is repeated 5 times. This mating process is left for approximately 5-7 days. After the mating process takes place, male and female rats have separated again and placed back in their respective cages. Then wait for an average pregnancy period of 21 days.

1) *Infertility index*

After giving birth each group from each treatment recorded the number of children born. From the percentage of children born between control and treatment, the infertility index is determined (the percentage of infertility or sterilization) by determining the level of fertilization of male rice rat with equation 1 (Hoper, 1976) as follows.

$$\text{Fertilization Rate} = \frac{\text{Number of treated children}}{\text{Number of control children}} \times 100\% \ldots\ldots\ldots\ldots(1)$$

With the level of fertilization, the infertility index can be determined as follows;
Infertility Index = 100% - Fertilization Rate ………………..(2)

From the infertility value for each radiation dose, the minimum dose that can cause permanent infertility in male rice rat can be determined.

2) *Mating competitiveness*

Determination of marital competitiveness in this study was designed in five groups consisting of 1 control group (P0) and 3 treatment groups (P1, P2, and P3) with five replications. The marriage comparison pattern for the three treatments is as follows: 0♂R×5♂F×5♀F, 5♂R×0♂F×5♀F dan 15♂R×5♂F×5♀F where R is the radiation rat and F is a fertile rat. The test was carried out in series one week intervals (Nahar, 2006). The value of the competitiveness of mating male infertile rats with fertile male rats, calculated by the formula Fried (1971) as follows;

$$\text{Mating competitiveness} = \frac{(Ha-E)}{(E-Hs)} \times \frac{R}{F} \ldots\ldots\ldots\ldots (3)$$

where ;
Ha = Number of cubs born with a mating pattern of 0♂R × 5♂F × 5♀F
Hs = Number of cubs born with mating patterns of 5♂R × 0♂F × 5♀F
E = Number of cubs born with mating patterns of 15♂R × 5♂F × 5♀F

The analysis used is the analysis of variance, and if the treatment is significantly different followed by the Mann-Whitney Test. To determine the minimum dose variance analysis was used using the SPSS (Statistical Product and Service Solutions) program, for Windows version 23 (Ridwan, 2019).

3 Results and Discussion

3.1 Biological parameters of field rat

Determining the effect of radiation doses on biological parameters is done by mating radiation male rat (♂P) with fertile female rat (♀F). Start by mating radiation male rat (♂P) with fertile female rat (♀F) with treatments ♂ (P0) × ♀ (F), ♂ (P1) × ♀ (F), ♂ (P2) × ♀ (F), ♂ (P3) × ♀ (F), ♂ (P4) × ♀ (F), ♂ (P5) × ♀ (F), ♂ (P6) × ♀ (F), each of which is repeated 5 times. This mating process is left for approximately 5-7 days. After the mating process takes place, the male and female rats have separated again and placed back in their respective cages. The result of mating in this study is the field rat shown in Table 1 as follows.

Table 1. Number of cubs produced by mating radiation male rat with a fertile female rat

Treatment	Number of rat cubs (tails)					Average rat (tails)
	1	2	3	4	5	
♂ (P0) × ♀(F)	8	10	10	8	9	9
♂ (P1) × ♀(F)	6	5	8	6	5	6
♂ (P2) × ♀(F)	3	1	3	2	1	2
♂ (P3) × ♀(F)	0	0	0	0	0	0
♂ (P4) × ♀(F)	0	0	0	0	0	0
♂ (P5) × ♀(F)	0	0	0	0	0	0

♂ (P6) × ♀(F)	0	0	0	0	0	0
Total	17	16	21	15	15	17

Data on the number of children from the mating between radiation wet rat and fertile rat can be represented in the graph as shown in Figure 2 below.

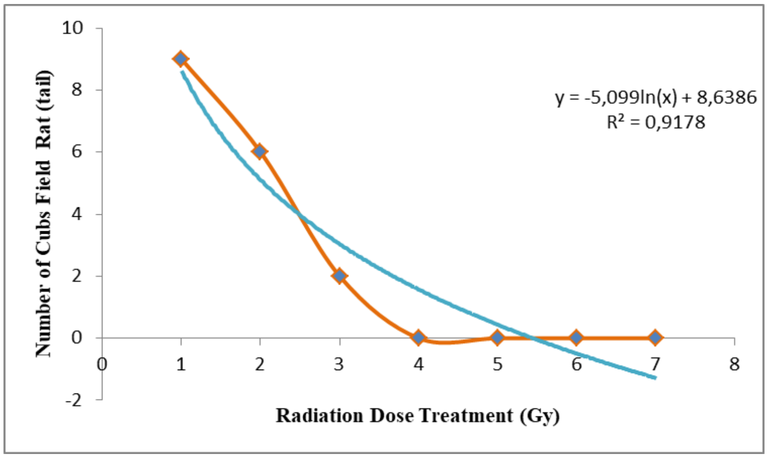

Figure 2. The number of cubs born to mating between radiation male rat and fertile female rat.
Note: Radiation dose 0Gy = Control, 1 Gy = P1, 2 Gy = P2, 3Gy = P3, 4Gy = P4, 5Gy = P5 and 6Gy = P6.

Figure 2. shows that the number of children born to rat between radiation male rat and fertile female rat, the greater the radiation dose received by male rat, the less number of children born by the fertile female rat. Statistical analysis shows that at doses 1 and 2Gy, the probability value $P < 0.05$ significantly decreases the number of pups in rats on the dick. But at radiation doses 3, 4, 5 and 6Gy no longer significantly decreased the number of pups. Likewise, the Mann-Whitney Test shows the same results. According to Hoper (1976) variations in radiation dose affect male rats, where the sperm produced becomes abnormal. Sperm will experience changes in shape and size. Abnormal sperm has a small head and short tail and low mobility (Mahmoud, 2011). Whereas normal sperm are larger and have higher mobility. Decreasing the number of rat children continues to occur until the 3Gy dose of fertile female rats does not give birth to more children. This condition can also be explained by the infertility index.

Infertility index From the number of pups produced after giving birth from each treatment as in Table 1. Infertility index (percentage of infertility or sterilization) can be determined by determining the level of fertilization of male paddy rats with equation 2. For example, calculations can be done for the treatment of 1Gy dose as following,

3.2 Infertility index

From the number of pups produced after giving birth from each treatment as in Table 1. Infertility index (percentage of infertility or sterilization) can be determined by determining the level of fertilization of male paddy rats with equation 2 (La Chance, 1967). For example, calculations can be done for the treatment of 1Gy dose as following,

$$\text{Fertilization Rate} = \frac{Number\ of\ treated\ children}{Number\ of\ control\ children} \times 100\%$$

$$= \frac{6}{9} \times 100\%$$

$$= 66{,}67\%$$

Then the index of infertility or sterilization = 100% - 66.67% = 33.33%. In the same way, the infertility index can be determined for 2Gy at 77.78%, whereas for doses of 3, 4, 5 and 6Gy the infertility index is 100%. The minimum dose for spaying male field rat at a dose of 3Gy. (Helinski, 2009; Fried, 1971) states that sterile doses are not standard doses, sterile doses can change by being influenced by many factors, one of which is the sexual maturity of rat. Control of rat populations can be achieved if the male infertile that is released has a sterility value of 100%, but it is necessary to take into account the possibility that suppression of rice field rat in the release area can be achieved by releasing radiation male rats that are not 100% sterile but have good mating competitiveness (Nahar, 2006).

3.3 Mating competitiveness

The competitiveness test of wetland rat is carried out in a room with a temperature of 24-31ºC and humidity of 77-82%. After the radiation process, each field rat is put in a cage with a pattern of male to female comparison, fertilized with radiation (Cunningham, 1983). The mating process is done naturally by placing males with females in a 1 x 1m cage for 5-7 days to maximize the marriage. Radiation males are coded R and females, fertile males are coded F. The mating pattern and number of cubs produced after giving birth from each treatment are shown in Table 2. as follows.

Table 2. Number of cubs produced by mating pattern between radiation male rat, fertile male rat and fertile females.

Radiation Dose (Gy)	Comparison of mating pattern (tail)	Average number of cubs born (tail)
1 (P1)	0♂R×5♂F×5♀F	9±1.22
	5♂R×0♂F×5♀F	4±1.00
	15♂R×5♂F×5♀F	6±1.00
2 (P2)	0♂R×5♂F×5♀F	9±1.00
	5♂R×0♂F×5♀F	2±0.71
	15♂R×5♂F×5♀F	4±1.00
3 (P3)	0♂R×5♂F×5♀F	9±1.00
	5♂R×0♂F×5♀F	0±0.00
	15♂R×5♂F×5♀F	8±0.71

Mating competitiveness is an important parameter that shows the quality of infertile male rat before the application in nature. The value of mating competitiveness or *C-Index* can be determined by equation 3. For example, the calculation of the value of mating competitiveness for treatment P1 as follows.

$$\text{Mating Competitiveness} = \frac{(Ha - E)/(E - Hs)}{R/F}$$

$$= \frac{(9-6)/(6-4)}{15/5}$$

$$= \frac{3/2}{3}$$

$$= 0{,}50$$

The complete competitiveness value of wet field rat at various doses of radiation with a comparison of mating patterns is shown in Table 3. as follows.

Table 3. The value of mating competitiveness of male field rat in various radiation doses with a comparison pattern of marriage

Treatment	Repetition	Number of cubs with mating ratio (tail)			R/F	*C-Indeks*
		0♂R×5♂F×5♀F	5♂R×0♂F×5♀F	15♂R×5♂F×5♀F		
P1	5	9	4	6	3	0,500
P2	5	9	2	4	3	0,833
P3	5	9	0	8	3	0,042

Note: P1 = Radiation dose 1 Gy, P2 = Radiation dose 2 Gy, P3 = Radiation dose 3 Gy, ♂R = Radiation male rat, ♂F = fertile male rat, ♀F = fertile female rat, R = Number of radiation rat, F = Number of fertile rat and C-Index = Value of mating competitiveness.

Mating competitiveness is an important parameter that shows the quality of infertile male techniques before implementing applications in the area. In this study, it was shown that at 1Gy radiation dose the marital competitiveness was 0.50, 2Gy was 0.83 and at the dose of 3Gy, the mating competitiveness was only 0.04. Infertile male rat must be able to compete with fertilized male rat in the wild at the time of release in the target area so they can mate in field females. The process of gamma radiation sterilization causes a decrease in competitiveness, due to the ionizing properties that can damage somatic cells, thereby reducing fitness in male rats. This condition will reduce the ability to mate with female rats (Ramadhani, 2017). In addition, Benni (2017), study showed the dose rate also affected the mating competitiveness of infertile male rat. The higher the dose can cause damage to somatic cells which can result in reduced physical ability to mate. Statistical analysis also supports the statement, where the probability value of $P<0.05$ significantly doses of radiation affect the mating competitiveness of infertile rats. Likewise, it can be seen from the results of the *Mann-Whitney Test*, the competitiveness of mating male infertility is significantly affected by radiation doses. In general, marital failure is caused by damage to genitalia due to gamma radiation (Nurhayati, 2006).

Supporting the statement of Helinski (2009) in this study, the 2Gy dose with mating competitiveness of 0.83 is the right radiation dose for suppression of rice field rat populations in the area. Population suppression can be achieved by releasing infertile male rats even though the infertility index is not 100% but has good mating competitiveness. The value of mating competitiveness or *C-Index* using the index to determine the number of infertile male rats to be released into the area. At a dose of 2Gy obtained a competitive *C-Index* of 0.83. This means that the ratio of infertile male rats and fertile rats is 8 to 1 at the time of release so that it can effectively reduce the population

4 Conclusion

1) The infertility index or percentage of sterilization at 1Gy dose is 33.33%, 2Gy is 77.78% and 3, 4, 5, 6Gy is 100%. So the minimum dose for spaying male rice field rat is 3Gy.
2) Mating competitiveness at 1Gy radiation dose reaches 0.50, 2 Gy is 0.83 and at dose 3Gy mating competitiveness is only 0.04.
3) 2Gy dose with mating competitiveness of 0.83 is the right radiation dose for suppression of rice field rat populations in the area. Population suppression can be achieved by releasing infertile male rats even though the infertility index is not 100% but has good mating competitiveness. The value of mating competitiveness or *C-Index* using the index to determine the number of infertile male rats to be released into the area. At a dose of 2Gy obtained a competitive *C-Index* of 0.83. This means that the ratio of infertile male rats and fertile rats is 8 to 1 at the time of release so that it can effectively reduce the population.

Suggestion

In research with rice field rats, it is necessary to do a smaller radiation dose interval range so that the C-index value obtained is more varied. With a variety of C-index can make it easier to get a comparison of infertile male rats with fertile rats released in the area of rice fields.

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