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Research Article

Gamma Irradiation Treatment of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in Snake Fruit

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ABSTRACT

Snake fruit (*Salacca zalacca*) is a unique fruit and it has decadent prospects to be developed as an export commodity. Nevertheless, oriental fruit fly, *Bactrocera dorsalis* Hendel seems to decrease the quantity and quality of this fruit. On the other side, irradiation has been developed as a standard quarantine treatment to disinfest fruitfly on fruit. The objective of this research was to determine the impact of irradiation by Cobalt-60 gamma-ray on the development and survivorship of eggs and the third instar of fruitfly larvae using in-vitro and in-vivo approaches and minimum dose of Cobalt-60 gamma rays applied for snake fruit. Six doses of gamma-ray, i.e. 0 (control), 25, 50, 75, 100, 125 and 150 Gy were used in this experiment. The results showed that Cobalt-60 disturbed the development and survival rate of *B. dorsalis*. The development of eggs into pupa was failed when treated with Cobalt-60 at any doses, while the third instar larvae failed to become adult when irradiated with 75, 100 and 150 Gy of gamma-ray. The impacts were increased with the increment of dose. Furthermore, the impact of gamma–ray irradiation was greater on eggs compared to the third instar of larvae. The minimum dose of irradiation to prevent adults emerge was 118 Gy.

Keywords: fruit fly, irradiation, quarantine, snake fruit

INTRODUCTION

Snake fruit (*Salacca zalacca*) is one of the horticultural commodities originating from tropical countries that are prospective in supporting the economy and as one of the leading export commodities of horticultural products (Guntoro, 2008). The presence of fruit flies has always been an obstacle in exporting that fruit to other countries. In May 2013, the exporting of snake fruit from Indonesia to China was rejected due to fruit fly infestations suspected as *Bactrocera papayae* (synonym *B. dorsalis*) (Schutze *et al.,* 2014) and *B. carambolae* (Barantan, 2013). *B. dorsalis* (Hendel) is a type of fruit fly species mostly found attacks Indonesian horticultural commodities (Suputa *et al.,* 2010; Weems, 2016). *B. dorsalis* is one of the most important pests in Southeast Asia including Indonesia and polyphagous insect thus it has a broad host that allows fruit fly to find an alternative host. Damage caused by the attack of fruit fly can reduce the quality and quantity of fruit up to 100% (Adsavakulchai *et al.,* 1999; Follet *et al.,* 2009; Dohino *et al.,* 2016).

The current dynamic trade of agricultural commodities has resulted in an increased risk of entry and spread of quarantine pests to the free areas from fruit fly. Therefore, importing countries impose stringent import requirements including effective treatment in eradicating pests. The irradiation treatment is a quarantine treatment that has been approved internationally (IPPC, 2003). Some countries, i.e. Australia, the United States, Mexico, India, Vietnam, Thailand, and Pakistan, have reportedly adopted this method to prevent the pest infestation in fresh commodities (Hallman, 1999). The fast, practical, environmentally friendly, and because various types of fruit tolerant of irradiation are the advantages of using irradiation over other quarantine treatments (Heather, 2002).

The effectiveness of gamma rays irradiation on several species of fruit flies has been reported by several researchers. According to Mansour and Franz (1996), gamma rays irradiation can cause egg sterility, failure to form pupae and adults of *Ceratitis capitata* on mango. The minimum dose of gamma rays irradiation was reported to be able to inhibit the development of eggs and pre-adult instars, as well as the reproductive adult of *Anastrepha* spp., *B. jarvisi*, and *B. tryoni*; the development of *C. capitata* pupae in mango and guava (Hallman & Loaharanu, 2002; Torres-Rivera & Hallman, 2007; Kabbashi *et al.,* 2012). Odai *et al.* (2014) reported that gamma rays irradiation was effectively used to eradicate *B. invadens* on mango. Gamma rays irradiation has also been reported to be effective in inhibiting the development of *B. tau* in pumpkin (Guoping *et al.,* 2015). The application of gamma rays irradiation as a quarantine effort against *B. dorsalis* in snake fruit has never been reported in Indonesia, thus the efficacy of irradiation on the presence of these flies needs to be done. The purpose of this study was to obtain and evaluate the effectiveness of the minimum dose irradiation by Cobalt-60 gamma rays and determine its effect on *B. dorsalis* fruit fly for the purpose of eradication of *B. dorsalis* fruit fly on snake fruit.

MATERIALS AND METHODS

This research was conducted from January to April 2018 at the Laboratory of Phytosanitary, the Center of Radioactive Isotope Application–National Nuclear Energy Agency (PAIR BATAN), Jakarta.

Mass Rearing of Tested Insect and its Diet (Natural and Artificial)

The insect colony used in this study was *B. dorsalis* obtained from the Laboratory of Phytosanitary, PAIR–BATAN. *B. dorsalis* are then reared in the same laboratory. The technique modified by Kuswadi *et al.* (1999) was used to rearing *B. dorsalis*. The composition of the artificial diet is 232 g of wheat bran, 28 g of yeast bread, 1000 g of sugar, 0.79 g of sodium benzoate, 0.79 g of nipagin, and 0.75 ml of HCl. Pondoh snake fruit was used as a natural diet with a maturity level of 70–80% and weight ranged from 100–105 g. Snake fruit was harvested from plantations in Sleman Regency, Yogyakarta. Snake fruit was wrapped in the white paper that easily absorbs water condensation from the fruit to prevent the pest re-infestation. Then fruits were stored one day before being used as eggs laying media for in-vivo tests.

In–vitro test: Irradiation of Eggs and Third Instar Larvae Using Artificial Diet

B. dorsalis eggs were inoculated in an artificial diet. The diet was placed in a gauze-covered container with a size of 8 × 8 × 5 cm. Each treatment container was inoculated with 50 eggs. The gamma irradiation treatment was conducted on an artificial diet inoculated with eggs, each container was treated on the first day after inoculation. For the third instar larvae, eggs that have been inoculated on the diet were treated with gamma irradiation on the 5th day after inoculation (Ratna *et al.,* 2015). Each diet container containing eggs and third instar larvae was put into the irradiator and treated with the irradiation doses of 0 (control), 40, 50, 75, 100, and 150 Gy. The irradiator used was Gamma Cell 220 Upgrade owned by PAIR-Batan. The irradiator has a cylindrical sample area of 150 mm in diameter, 200 mm in height and a capacity of 3.7 l with Cobalt-60 gamma rays (Ratna *et al.,* 2015). Each irradiation treatment was repeated 5 times. After irradiation, eggs and larvae of *B. dorsalis* in an artificial diet were moved to different containers containing sawdust as pupation material. After *B. dorsalis* become pupae, those pupae were separated from sawdust by sieving and then moved to the oviposition cage.

In–vivo test: Irradiation of Eggs and Third Instar Larvae on Snakefruit

Irradiation test on snake fruit was performed by inoculating the natural diet, Pondoh snake fruit. Inoculation was carried out by two methods: natural and artificial (Hallman & Thomas, 2010). The natural inoculation was conducted by exposing the snake fruit to a fruitfly cage containing 5 pairs of adults over 3 hours to get the expected number of 50 eggs. The pair of *B. dorsalis* adults used was 14 days old because they are mature sexually (Rattanapun *et al.,* 2009). Before the exposure, snake fruit was wounded with a sterile needle at the end of the fruit to ease *B. dorsalis* laying eggs (Odai *et al.,* 2014). Afterward, snake fruit was taken and irradiated. Irradiation of eggs and third instar larvae was carried out at the eggs aged 24 hours and 5 days after fruit exposure, respectively. Artificial inoculation was done by laying eggs on snake fruit that has been injured on the fruit tissue with a size of 1 × 1 cm, inoculated by 50 eggs, then covered with a plaster to prevent the larvae

crawl out from fruit tissue. The irradiation treatment was performed 24 hours after the egg inoculation on fruit, while the treatment of third instar larvae was carried out 5 days after inoculation according to the method by Hallman & Thomas (2010). The source and irradiation dose used is the same as the treatment of an artificial diet with 10 replications. After irradiation, snake fruit was moved into pupal and adult rearing media as in the treatment using an artificial diet.

Data Analysis

Mortality data were analyzed by the R program. The growth of pupae and adults were transformed using arcsin to normalize the distribution in ANOVA, further analysis by Tukey test ($\alpha = 0.05$). The lethal doses of 50% (LD50) and 99% (LD99) were calculated using probit analysis by R program. Adult mortality was corrected using Abbott's formula (Follet & Armstrong, 2004).

RESULTS AND DISCUSSION

Effect of Irradiation on Eggs and the Third Instar Larvae towards the Growth and Development of B. dorsalis

The results of irradiation doses of *B. dorsalis* eggs by in-vitro and in-vivo (natural and artificial) affect the development of the *B. dorsalis* (Figure 1). The greater the irradiation doses, the smaller the pupal survivorship. In in-vitro with the lowest doses (40 Gy), the failure of larvae become pupae were 44.4%, higher than control (3.4%). The failure of larvae become pupae, in natural and artificial in-vivo was 25% and 57%, respectively. At the highest dose (150 Gy) revealed that 100% of larvae failed to be pupae. The results of irradiation treatment on *B. dorsalis* eggs proved that irradiation with a dose of 40–150 Gy inhibit the development of egg up to 44.4–100%. The irradiation dose which caused the failure of *B. carambolae* pupa formation of 93.4% was at 50 Gy (Ratna *et al.*, 2015). Guoping *et al.,* (2015) also reported that irradiation on eggs and third instar larvae at a dose of 49 Gy caused the failure of the formation of *B. tau* pupa up to 85–95.7%.

The percentage of larvae become pupae in the in-vivo egg irradiation showed that all treatment doses were significantly different than control. Artificial in-vivo egg irradiation treatment at a dose of 40–150 Gy was 43–97.2%, while natural was 75–98.6%. This result showed that the egg irradiation treatment with a dose of 40–150 Gy by in-vivo, both natural and artificial, can suppress the growth of *B. dorsalis*. The irradiation test on the third instar larvae by in-vivo (artificial and natural) at doses of 40 and 50 Gy were unable to suppress the growth of *B. dorsalis* pupae. At a dose of 75–150 Gy showed the survival rate of pupae showed a significantly different with control (76–83.2%). The effective irradiation dose to inhibit the survival rate of the third instar larvae of *B. dorsalis* was 75–150 Gy (Figure 2).

Inhibition of growth of *B. dorsalis* in the irradiation treatment of third instar larvae was lower than in egg irradiation. Hallman and Blackburn (2016) suggested that the effect of radiation on insects depends

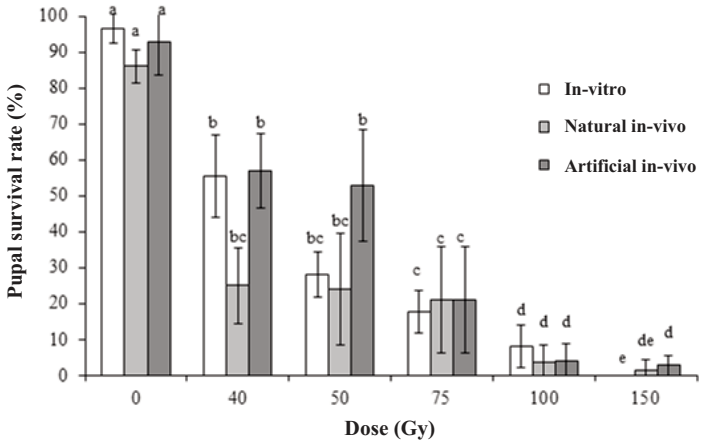


Figure 1. The pupal survival rate at six irradiation doses on *Bactrocera dorsalis* eggs; the same letter on the bar was not significantly different between the different treatment methods (in-vitro, natural in-vivo and artificial in-vivo) according to HSD Tukey ($\alpha = 0.05$)

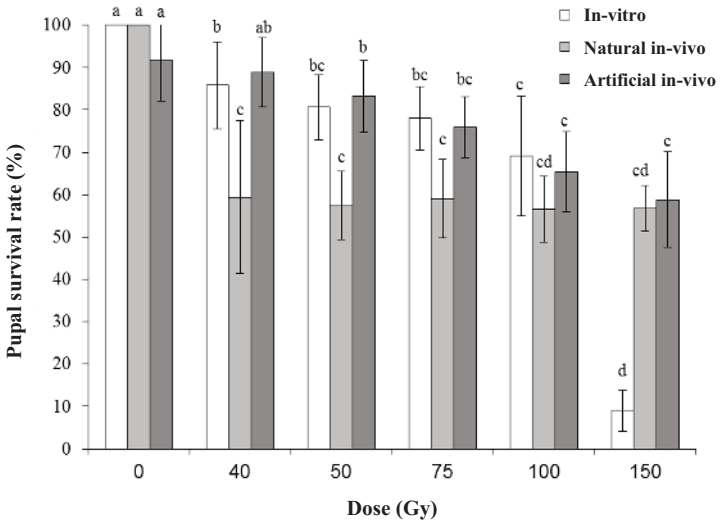


Figure 2. The pupal survival rate at six irradiation doses on third instar larvae of *Bactrocera dorsalis*; the same letter on the bar was not significantly different between the different treatment methods (in-vitro, natural in-vivo and artificial in-vivo) according to HSD Tukey (α = 0.05)

Table 1. The unemerged adults of *Bactrocera dorsalis* due to the irradiation treatment on third instar larvae by in-vivo natural and artificial inoculation

Dose (Gy)	Unemerged adult (%)			
	Natural		Artificial	
	Egg	Third instar larva	Egg	Third instar larva
0	8.2 ± 9.4a	5.6 ± 6.2a	8.0 ± 9.4a	13.8 ± 8.0a
40	100 ± 0.0b	96.2 ± 5.1b	100 ± 0.0b	94.6 ± 3.2b
50	100 ± 0.0b	97.4 ± 2.3b	99.6 ±1.3b	98.6 ± 2.1b
75	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b
100	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b
150	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b

Remarks: Values ± SD followed by the same letters in the same column was not significantly different according to HSD Tukey (α = 0.05)

on which stadia of the insect when the irradiation takes place and the sensitivity of cells to radiation is in line with the activity of insect reproduction and reverse to the differentiation level of insect. Irradiation on the third instar larvae both in vitro and in-vivo (natural and artificial) showed the success of adult formation only occurred at the lowest dose of 40 and 50 Gy (5.4% and 3.8%, respectively) and at a dose of 50 Gy (2.6% and 1.4%, respectively) (Table 1). A similar result was reported by Kuswadi *et al.* (2011) on *B. carambolae* that a dose of 80 Gy and 120 Gy reduced the percentage of pupae become adult to be 4% and 2%, respectively. This finding showed that gamma rays treatment affected the percentage of pupae become adults on *B. dorsalis*.

The Effect of Larva and Egg Irradiation on B. dorsalis *Mortality*

Gamma rays irradiation at various doses by in-vitro and in-vivo (natural and artificial) on eggs and third instar larvae of *B. dorsalis* caused the mortality of *B. dorsalis* (Table 2). Egg mortality by in-vitro in control was 4.2%; while mortality in the doses of 40–150 Gy was 60.2–100%. The lowest dose of irradiation in suppressing the hatching of *B. carambolae* eggs on guava fruit was 50 Gy by in-vitro and in-vivo (Ratna *et al.*, 2015). The irradiation treatment with the lowest dose of 30 Gy can also suppress the hatching of *C. capitata* eggs on mango up to 95% (Mansour & Franz, 1996).

Gamma rays irradiation on third instar larvae showed a higher level of resistance compared to eggs.

Table 2. The effect of gamma rays irradiation on the mortality of eggs and third instar larva of *Bactrocera dorsalis* by in-vitro and in-vivo (natural and artificial)

Dose (Gy)	Egg Mortality (%)	Larval Mortality (%)		
	In-vitro	In-vitro	In-vivo	
			Natural	Artificial
0	4.2 ± 4.4c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
40	60.2 ± 13.2b	11.8 ± 11.3bc	5.6 ± 4.4bc	7.2 ± 7.3bc
50	71.0 ± 6.1b	12.8 ± 7.6bc	10.6 ± 9.5bc	12.8 ± 8.5bc
75	82.2 ± 5.5a	16.0 ± 8.3ab	14.4 ± 7.8bc	14.2 ± 7.0bc
100	91.8 ± 8.2a	20.2 ± 16.2ab	23.4 ± 7.8b	24.6 ± 9.5b
150	100 ± 8.8a	28.4 ± 13.2a	32.4 ± 6.6a	34.2 ± 11.2a

Remarks: Values ± SD followed by the same letters in the same column was not significantly different according to HSD Tukey (α = 0.05)

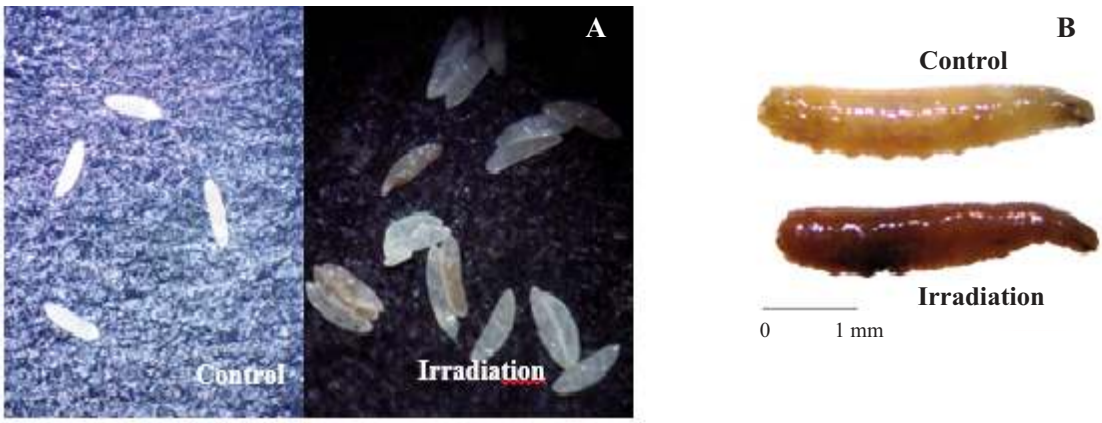


Figure 3. Melanization due to irradiation treatment on eggs (40× magnification) (A) and larvae (B) of *Bactrocera dorsalis*

The 75 Gy gamma rays irradiation has an impact on the mortality of third instar larvae (14.4%). The highest mortality by in-vitro and in-vivo (both natural and artificial) was at 150 Gy (28.4%, 32.4%, and 34.2%). The irradiation treatment at several doses causes death and discoloration of the eggs, larvae and pupae (Figure 3). Nation *et al.,* (1995) stated that irradiation treatment can reduce the activity of the phenoloxidase enzyme in *Anastrepha suspensa* larvae. This brown formation is triggered by an oxidation reaction catalyzed by the phenoloxidase enzyme. This enzyme can catalyze the oxidation of phenol compounds into quinone and then polarized into a brown melanoidin pigment (Nation, 1995).

Morphological changes also occur in the pupae irradiated with the highest doses of 150 Gy (Figure 4). The pupal size is longer control and undergoes melanization. Abnormalities in pupa size treated using irradiation cause inhibition of larval muscle development during the process of metamorphosis (Thomas & Hallman, 2011). *B. dorsalis* pupae that succeeded to be adults would have two forms of abnormality (Figure 5). The adult that succeeded in molting has undeveloped wings (Figure 5A), whereas the pupa did not succeed in molting would have the damaged abdomen (Figure 5B). The effect of irradiation in tissues can cause DNA breakdown, thus causing the failure of cell replication or division (Ferrier, 2010). Thomas & Hallman (2011) also reported that irradiation of third instar larvae of *A. ludens* in grapes at a dose of 40 Gy caused the failure to be adults. Gamma rays irradiation caused the apoptosis of *B. dorsalis* adults. The same thing was reported by Nirmala *et al.,* (2015) that gamma rays irradiation on *A. suspensa* caused pro-apoptotic genes (Asrpr and Ashid) to be active and develop.

Eradication of pests in export commodities requires a high level of security for quarantine treatment, commonly called probit 9. Probit 9 response (LD Probit 9) results in an efficacy rate of 99.9968% (Heather, 2002; Hallman, 2012). The expected result is to prevent the adult development of fruitfly

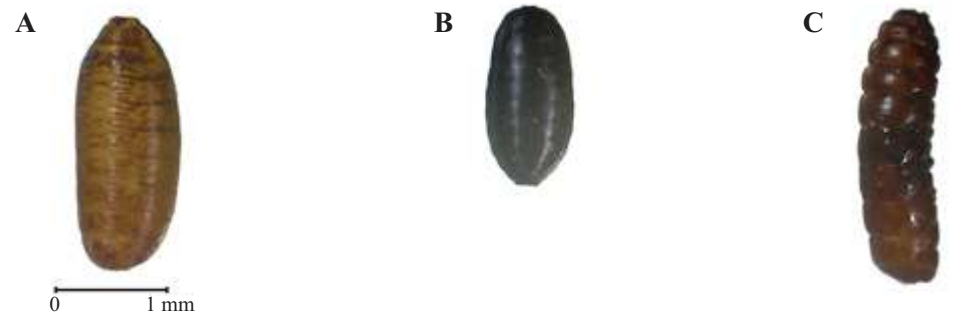


Figure 4. The abnormality of *Bactrocera dorsalis* pupae due to irradiation treatment on third instar larvae by in-vivo: (A) control, the size of pupa is 2.96 × 1.44 mm; (B) irradiation at doses of 40–100 Gy undergoes melanization and pupae shrink to be 2.50 × 1.30 mm; and (C) irradiation at a dose of 150 Gy undergoes melanization and pupae shrink to be 2.90 × 1.00 mm

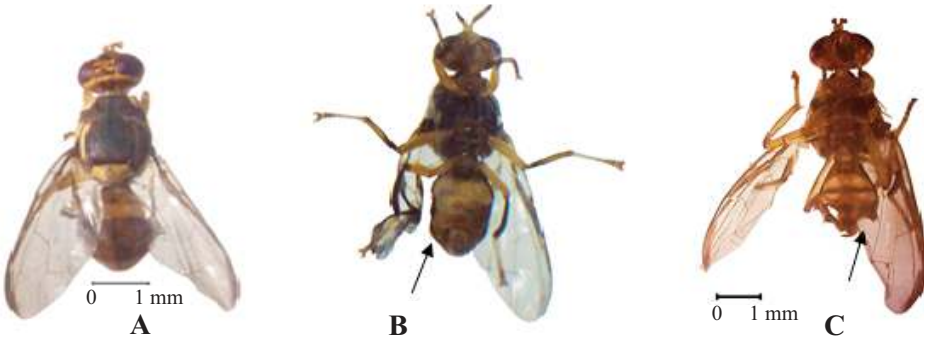


Figure 5. (A) Normal adult of *Bactrocera dorsalis*; the abnormal adults of *B. dorsalis* due to irradiation treatment by in-vivo on third instar larvae: (B) the damaged wing, (C) incomplete molting in the abdomen

Table 3. Three lethal doses of gamma rays irradiation on third instar larvae of *Bactrocera dorsalis* by in-vivo (natural and artificial) on snake fruit based on the adult emergence

Treatment	LD_{50}	LD_{95}	LD_{99}
Natural In-vivo	20.23 (12.35–26.72)	41.91 (36.25–43.18)	118.8 (93.85–198.92)
Artificial In-vivo	19.92 (11.25–25.23)	39.42 (35.56–41.91)	104.8 (80.41–187.17)

(Hallman & Loaharanu, 2002; Follett & Armstrong, 2004). The minimum dose of irradiation on snake fruit naturally and artificially that can inhibit the survivorship of adults is 118 Gy (Table 3). That dose is considered able to provide quarantine safety in preventing the emergence of *B. dorsalis* adults. The minimum dose obtained in this test is lower than the recommended dose of IPPC to prevent the emergence of the Tephritidae family on 150 Gy (IPPC, 2003). The application of the minimum doses of irradiation aims to ensure that the treatment will disinfect the fruit fly. Lower dose application is expected to be able to reduce the control costs, speed up the treatment time, and reduce damage to commodities, especially for the marketing purposes of important commodities. Follet *et al.* (2008) and Hallman (1999) stated that the application of irradiation is attempted to use the lowest possible dose with a level of efficacy sufficient for quarantine safety, hence the application becomes faster, reduces damage to commodities, and reduces costs.

CONCLUSION

In-vitro and in-vivo (natural and artificial) exposure by Cobalt-60 gamma rays has an impact on the inhibition of *B. dorsalis* development. Higher the radiation dose, longer duration of the third instar

larvae and failed to be pupae. Gamma rays irradiation causes morphological abnormalities and mortality of *B. dorsalis*. The minimum dose of gamma rays irradiation treatment that can inhibit the formation of *B. dorsalis* adults of snake fruit is 118 Gy.

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