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Research Article Phytosanitary Irradiation Against Cocoa Mealybug *Exallomochlus hispidus* (Morrison) (Hemiptera: Pseudococcidae) on Mangosteen Fruits

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Abstract

Background and Objective: The cocoa mealybug *Exallomochlus hispidus* (Morrison) is a quarantine pest on mangosteen. Gamma irradiation is a possible phytosanitary control treatment. This research investigated the lethal and sublethal gamma irradiation doses for controlling *Exallomochlus hispidus* (*E. hispidus*) and the impact of irradiation on mangosteen fruit quality. **Methodology:** Nymphs and adult females of *E. hispidus* were exposed to gamma irradiation (50-2000 Gy and a control) and newly emerged females were exposed to a sublethal dose of 50-400 Gy. Mangosteen fruit was irradiated at 250-1000 Gy. Reproductive development, longevity and fecundity of irradiated females were observed. The physical and chemical qualities of irradiated fruit were assessed. **Results:** The LD₉₉ for the first, second and third instar nymphs and adults were 423.7, 1026.0, 1276.0 and 1934.5 Gy, respectively. Irradiation doses of 50-400 Gy on adult females resulted in significantly longer pre and post birth periods but shorter birth period. These doses also increased the longevity and decreased the fecundity, observed as 99.7% unhatched eggs and 0.3% undeveloped nymphs. The quality of mangosteen fruit did not effect by irradiation unless the red color retain and vitamin C content of fruit was significantly decreased. **Conclusion:** The result indicates that irradiation is an effective phytosanitary treatment to control *E. hispidus* on mangosteen. Irradiation doses of 250 Gy are recommended for phytosanitary treatment without degradation of fruit quality.

Key words: Irradiation treatment, LD₉₉, mangosteen quality, ovoviviparous mealybug, quarantine pest

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cocoa mealybug Exallomochlus hispidus (Morrison) (Hemiptera: Pseudococcidae) is one of the main pests that attack the mangosteen Garcinia mangostana L. fruit¹. Exallomochlus hispidus is a polyphagous insect that can also be found on other hosts, such as soursop (Annona muricata), guava (Psidium guajava), rambutan (Nephelium lappaceum), durian (Durio zibethinus) and duku langsat (Lansium domesticum). This pest is often reported on postharvest products such as the mangosteen fruit, which violates export fruit trade provisions². Methyl bromide fumigant was commonly used to eradicate postharvest pests, but its application has been sharply decreased, if not banned, because it depletes the ozone layer³. An alternative method to control the cocoa mealybug is gamma ray irradiation of postharvest products, which is considered safer and more reliable than methyl bromide⁴. Gamma irradiation has several advantages, including effective killing of borer or hidden pests, relatively high penetration of rays in the target tissues, more practical treatment of packaged commodities, very low possibility of pest re-infestation after treatment and the absence of any residue that could endanger consumers⁵.

The use of gamma irradiation for phytosanitary control of mealybugs has been reported. For example, irradiation of females at a dose of 150 Gy inhibited egg hatching for the passion vine mealybug *Planococcus minor* (*P. minor*)⁶. Gamma irradiation at a dose of 400 Gy was able to kill 46% of an *E. hispidus* population⁷. The International Plant Protection Convention (IPPC) recommended 231 Gy as a suitable phytosanitary dose to control mealybugs *Dysmicoccus neobrevipes, Planococcus lilacinus* and *P. minor*⁸.

Although gamma irradiation can effectively eradicate pests, it may also damage the commodities affected by the pests. To avoid this problem, the Minister of Health of the Republic of Indonesia placed a limitation on the use of irradiation, according to regulation 701/MENKES/PER/VIII/ 2009, particularly on food⁹. This regulation indicates that 1 kGY is the maximum gamma irradiation dose permitted for fresh fruit and vegetables. This provision is also applied in both the United States and Australia⁵. Irradiation below 1 kGy on fresh fruits and vegetables is considered to be safe and to pose no hazard risk for consumers¹⁰. However, an exposure dose above 1 kGy has been found to cause physiological changes to plant tissues, for example, shrinkage of banana peel and grape skin and brownish discoloration of avocado flesh¹¹.

The purpose of the current study was to determine lethal and sublethal effects of gamma irradiation applied for

phytosanitary treatment of mangosteen fruit to control *E. hispidus*, which has not been investigated previously. The irradiation data are expected to provide valuable information in determining recommendations for controlling *E. hispidus* on postharvest mangosteen fruit.

MATERIALS AND METHODS

Mealybugs were collected from mangosteen fruit crops in the Bogor area, Indonesia. Irradiation of mealybugs and mangosteen fruit was conducted between June, 2015 and December, 2016 at the Center for the Isotope and Radiation Application, Indonesia National Nuclear Energy Agency.

Rearing of *E. hispidus* colony: Mealybugs were reared on an alternate host, kaboca (*Cucurbita moschata*) fruit, in the laboratory⁷. *Exallomochlus hispidus* has a deuterotokous ovoviviparous reproduction type; consequently, very few males are produced and all uncopulated females delivers nymphs¹². Five to ten gravid mealybugs were transferred from a reared colony to a kaboca fruit that was approximately 10 cm in diameter. After 1-2 days, females laid the first instar nymphs, the adult mealybugs were removed from fruit and the nymphs were allowed to develop to a uniform age for use in individual testing. These colonies were maintained in a rearing room at $27 \pm 1^{\circ}$ C with a relative humidity of $70\% \pm 5\%$.

Lethal dose testing of gamma irradiation ⁶⁰**Co against** *E. hispidus*: The irradiation treatment in this experiment was applied to the first, second and third instar nymphs and adults *E. hispidus* on kaboca fruit, with a radiation source of ⁶⁰Co delivering a dose rate of 1000 Gy h⁻¹ (Multipurpose Panoramamic Irradiator). Each test nymph was given five doses and the control treatment. First instar nymphs were irradiated with doses of 0, 50, 75, 100, 150 and 300 Gy; second instar nymphs received doses of 0, 75, 125, 200, 300 and 600 Gy; third instar nymphs were given doses of 0, 200, 300, 400, 550 and 1000 Gy; and adults received doses of 0, 600, 750, 900, 1100 and 2000 Gy. Fifty test insects were used in each treatment¹³, on replications of exposure. Mortality was observed daily until day 15 after irradiation.

Sublethal dose test of gamma irradiation 60Co against *E. hispidus*. Twenty adult *E. hispidus* were irradiated at doses of 50, 100, 200 and 400 Gy¹³ in the manner previously described. The irradiation treatment was repeated 3 times, with 20 insects each time. Two of the 20 irradiated mealybugs were separately transferred to a new kaboca fruit and then maintained in the rearing room. Adult developmental stages were observed in three periods: Prebirthing, which covers the life period from emergence until the first delivery of nymphs; birthing, which is period of productive birth; and postbirthing, which last from the cessation of progeny production until death⁷. The fecundity of mealybugs was observed by recording the number of nymphs laid during each female's life time. Longevity was the duration of life from emergence until death. Mealybug survival after irradiation was expressed by the percentage of surviving mealybugs.

Quality assessment of irradiated mangosteen: The mangosteen fruit was obtained from farmers in Purwakarta, Indonesia. The fruits used for the experimental test were mature fruits freshly picked from the field. They were index grade 4 according to export standard quality, with the following characteristic features: Red purple color, a fruit rind that appears gummy and the flesh can be easily separated from the rind and is a good quality for eating¹⁴.

Eight mangosteen fruits were each irradiated with a dose of 0 (control), 250, 500, 750 and 1000 Gy¹⁵. The experiments were conducted with 3 replications. The irradiated fruits were stored at $20\pm1^{\circ}$ C with a relative humidity of $70\pm5\%$. The physical qualities of the fruit included weight, hardness, color of rind and chemical quality of the fruit, including total soluble solid (TSS), titratable acidity (TA) and vitamin C. The qualities were observed at 3 days intervals during a 15 days period¹⁶.

Fruit weight was measured before and after irradiation using a Denver Instrument T-214 weighing balance at each interval observation and the weight loss during storage was calculated. Fruit hardness (expressed as kg. sec mm⁻¹) was assessed by measuring the depth to which penetrating needles could pass through the rind, using Fruit Hardness Tester FHR-5 (Tokyo) at a maximum load of 5 kg. Penetration points were measured in three locations, namely, at the base, the middle and the tip of each fruit. The color of the fruit was measured using a Konica Minolta CR-400 Chroma Meter, which captured the chromatic color and expressed it digitally as a number with the sign a (-) for green and a (+) for red, with b for the blue (-) to yellow (+) color range.

Total dissolved solids were evaluated by putting a drop of mangosteen fruit juice on a glass refractometer prism. The TSS value was measured with an Atago PR 201 refractometer and was expressed in °Brix. The TA content of flesh fruit was analyzed by a standard titration method using 0.1 N NaOH solution with a phenolphthalein indicator. The TA value was expressed as a percentage of titratable acidity. Similarly, a titration method was applied for determining vitamin C content, with 2, 6-Dichlorophenol indophenol used as an indicator. The vitamin C content was expressed in milligrams per 100 mg¹⁷.

Statistical analysis: Data on mealybug mortality after gamma irradiation were analyzed using the POLO-PC probit program¹⁸ and the mortality dose was expressed as the lethal dose for 50, 90 and 99% of the insects (LD_{50} , LD_{90} and LD_{99} , respectively). Effects of sublethal doses and mangosteen fruit quality data were calculated using analysis of variance in SAS version 9.1.3¹⁹. The differences of mean values were calculated based on the Tukey's test at a 95% confidence level.

RESULTS

Efficacy irradiation test against *E. hispidus*: Gamma irradiation for each specific application dose resulted in mortality for nymph and adult *E. hispidus*. Irradiation against the first instar nymph began to induce mortality at 2 days after treatment, whereas for second and third instar nymphs and for adults, mortality occurred at 3 and 4 days after treatment. Population mortality increased to 50% at 6, 7 and 8 days, respectively, in adults, first instar nymphs and second to third instar nymphs. At the end of the observation period, 100% mortality occurred for each of the highest dose treatments of 300, 600, 1000 and 2000 Gy, respectively, for first, second and third instar nymphs and adults on days 15, 13, 14 and 9 (Fig. 1).

The tolerance of *E. hispidus* to gamma irradiation was influenced by the dose. The tolerance that is indicated by the lowest LD_{50} , LD_{90} and LD_{99} in the first, second and third instar nymphs and adults are shown in Table 1. Based on that lethal dose, the irradiated tolerance of *E. hispidus* increased consecutively from the first, second and third instar nymphs, with adults having the greatest tolerance.

Sublethal irradiation test of *E. hispidus*. The observations showed that doses of 50-400 Gy generally lengthened the developmental period of the adult *E. hispidus* (Table 2). The prebirthing period of controls averaged 12.8 days, while mealybugs irradiated with doses of 50, 100 and 200 Gy had relatively longer prebirthing periods of 18.8, 19.0 and 19.4 days, respectively. An extended prebirthing period occurred with the dose of 400 Gy and averaged 21.7 days. The birthing period after irradiation at doses of 50 and 100 Gy was relatively longer than controls (8.5 and 11.4 vs. 8.2 days) and doses of 200 and 400 Gy induced significantly shorter birthing periods (3.3 and 2.9 days) than controls. Similarly,



Fig. 1(a-d): Mortality of *E. hispidus* after irradiation, (a) Mortality of first instar nymphs, (b) Mortality of second instar nymphs, (c) Mortality of third instar nymphs and (d) Mortality of adults

Table 1: Lethal dose of gamma i	rradiation [°°Co] against <i>E. hispidus</i> Lethal doses (Gy)			
Development phase	 LD ₅₀	LD ₉₀	 LD ₉₉	
First instar nymph	75.9 (60.8-89.9)	195.7 (153.7-302.7)	423.7 (280.5-951.5)	
Second instar nymph	126.9 (98.5-154.1)	547.0 (390.4-1007.0)	1026.0 (649.5-2357.6)	
Third instar nymph	339.0 (285.8-393.3)	703.6 (571.9-1018.7)	1276.0 (911.7-2444.3)	
Adult	837.5 (795.0-879.0)	1328.2 (1236.0-1459.1)	1934.5 (1719.2-2272.1)	
I D. Lethal dose confidence leve	1 95%			

ethal dose, confidence level 95%

Table 2: Development period and longevity of E. hispidus after irradiation treatment

Doses (Gy)	Development period (day	Development period (day) (Mean±SD)				
	Prebirthing	Birthing	Postbirthing	Longevity (days)		
0	12.8±1.0 ^b	8.2±2.1ª	1.0±0.9 ^b	22.0±2.8 ^b		
50	18.8±6.8 ^{ab}	8.5±7.0 ^{ab}	3.4±3.3 ^{ab}	31.8±9.9 ^{ab}		
100	19.0±8.6 ^{ab}	11.4±8.4 ^{ab}	4.0±3.6 ^{ab}	37.4±12.4 ^{ab}		
200	19.4±13.8 ^{ab}	3.3±2.7 ^b	3.2±3.0 ^{ab}	30.1±12.9 ^{ab}		
400	21.7±7.8ª	2.9±3.1 ^b	6.0±3.9ª	33.5±8.5ª		

Means within a column followed by the same letter do not differ significantly by Tukey's test ($\alpha = 0.05$)

compared to the control, irradiation at 50, 100 and 200 Gy doses was associated with a relatively longer postbirthing period (3.4, 4.0 and 3.2 days, respectively), while the highest dose of 400 Gy led to a significantly longer postbirthing period (6.0 days). The postbirthing period after irradiation at 50, 100 and 200 Gy was relatively longer at 31.8, 37.4 and 30.1 days, respectively, while at the highest dose of 400 Gy, it was significantly longer (33.5 days) compared to the control.

Gamma irradiation affects decreased the reproductive ability and fertility of E. hispidus. The percentages of reproductive adults at the doses of 50, 100, 200 and 400 Gy were 88.3, 81.3, 41.7 and 40%, respectively. Reproduction decreased significantly with increasing irradiation dose (Table 3). Gamma irradiation also has a significant effect on the fecundity of *E. hispidus*. The number of offspring laid by irradiated mealybugs decreased significantly as the irradiation dose increased (Table 3). Gamma irradiation also decreased the fecundity of *E. hispidus* significantly and the number of offspring laid by irradiated mealybugs decreased significantly as the irradiation dose increased (Table 3).

Quality assessment of irradiated mangosteen fruit: The result showed that the lowest fruit weight loss occurred in control, 8.88% and the highest at the dose of 1,000 Gy, 11.44%. The lowest firmness of the mangosteen of 250 Gy reached 2.06 and the highest at 750 Gy doses reached 2.23 kg. sec mm⁻¹ (Table 4). The weight loss and firmness of the fruit were relatively increased but not significantly than

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Doses (Gy)	Number of reproductive individual (%) (Mean \pm SD)	Fecundity (Nymphs/Parent) (Mean±SD)	Note
0	100.0±0.0ª	136.7±38.0ª	First instar nymphs
50	88.3±2.9 ^b	106.5±16.8 ^{ab}	First instar nymphs (0.3% died) Eggs unhatched (99.7%)
100	81.7±7.6 ^b	69.6±26.7 ^b	Eggs unhatched
200	41.7±5.8°	31.4±29.1 ^b	Eggs unhatched
400	40.0±5.0°	23.5±18.7°	Eggs unhatched

Table 3: Reproductive response of survival E. hispidus after irradiation treatment

Means within a column followed by the same letter do not differ significantly by Tukey's test ($\alpha = 0.05$)

Table 4: Physical quality assessment of irradiated mangosteen

		Firmness	Color (Mean±SD)		
Doses	Weight loss (%)	kg. sec mm⁻¹			
(Gy)	(Mean±SD)	(Mean±SD)	L	а	b
0	8.88±3.11	2.08±0.16	29.46±2.40	4.47±1.44ª	5.76±2.55ª
250	9.20±1.85	2.06±0.02	28.26±3.34	5.05±2.01ª	3.93±1.79ª
500	10.90±1.19	2.22±0.03	25.83±1.29	6.49±2.24ª	4.31±2.38ª
750	10.39±1.19	2.23±0.12	24.61±2.65	8.67±2.08 ^b	5.64±2.57ª
1000	11.44±0.01	2.12±0.11	25.83±3.49	12.32±2.80 ^b	8.14±3.44 ^b

Means within a column followed by the same letter do not differ significantly by Tukey's test ($\alpha = 0.05$). L: Brightness, a: Rind, b: Nilai

Table 5: Chemical quality assessment of irradiated mangosteen

	Chemical quality of mangosteen (Mean±SD)			
Doses (Gy)	 TSS (%)	TA (%)	Vitamin C (mg/100 g)	
0	19.67±0.58	0.59±0.07	6.35±0.39ª	
250	18.67±0.76	0.69 ± 0.06	5.86±0.18 ^{ab}	
500	19.00±1.73	0.65 ± 0.05	5.85 ± 0.05^{ab}	
750	17.50±2.18	0.65 ± 0.08	5.54±0.02 ^b	
1000	17.33±1.04	0.60 ± 0.06	5.53±0.16 ^b	

Means within a column followed by the same letter do not differ significantly by Tukey's test ($\alpha = 0.05$). TSS: Total soluble solids, TA: Titratable acidity

control. Irradiation of the mangosteen affected the rind color of the fruit. The L (brightness) value was relatively decreased but not significantly than control (Table 4). The 'a' value of the rind color increased significantly with increasing irradiation dose. The 'a' value after irradiation at doses of 250 and 500 Gy was relatively higher than controls, while the doses of 750 and 1000 Gy induced significantly higher than controls. The 'b' value Nilai b after irradiation at the dose of 250-750 Gy relatively higher than control but not significant, while at the dose of 1000 Gy induce significantly higher than control.

The results of TSS measurements were expressed in Brix sucrose. Higher the TSS value, high sweeter will be the fruit. The measured TSS and TA values showed that irradiation had no significant effect on TSS and TA in mangosteen fruit (Table 5). Irradiation affected the vitamin C content of the mangosteen fruit. The vitamin C content of mangosteen fruit after being irradiated at the doses of 250 and 500 Gy was relatively lower but not significantly different from controls, but irradiation at the doses of 750 and 1000 Gy was associated with significantly lower vitamin C content compared to controls (Table 5).

DISCUSSION

The effect of gamma irradiation on the mortality of *E. hispidus* depend on phases of the insect development and the doses applied. The 100% mortality of the first, second and third instar nymphs and adults occurred on the 15, 13, 14 and 9 days after they were treated with the irradiation doses of 300, 600, 1000 and 2000 Gy, respectively. The irradiation doses affected differently on the phases was showed in other insects. The doses applied for 100% mortality in the adult phase was higher than that applied for the younger phases. The mortality 100% of the first, second and third instar nymphs and adult of *D. neobrevipes* occurred on the 21st day treated with the doses of 224.6, 241.3, 330.9 and 581.5 Gy, respectively²⁰.

Based on that lethal dose, the irradiated tolerance of *E. hispidus* increased consecutively from the first, second and third instar nymphs, with adults having the greatest tolerance. The same pattern of tolerance to gamma irradiation also reported in *D. neobrevipes*. First instar nymph<second instar < third instar, with the greatest tolerance in adults⁸. The lethal dose of adult *E. hispidus* in the current experiment was relatively high at 1934.5 Gy, which is much higher than the dose permitted for fresh fruit irradiation (i.e., 1000 Gy). Likewise, it was reported that LD₉₉ values in adult mealybugs *Maconellicoccus hirsutus, Phenacoccus solenopsis* and *Paracoccus marginatus* of 2960, 3040 and 3020 Gy, respectively²¹.

The sensitivity of living organisms to gamma irradiation follows Bergonie and Tribondeau law²², which states that proliferating active cells are more sensitive to irradiation than

differentiated cells. In this case the immature insect cells proliferate during growth and maturation toward the next phase of development, so that the immature phases are more sensitive to irradiation. This is shown by the lethal irradiation doses of adult *E. hispidus* being higher than those for the nymphs.

The experimental results showed that the phytosanitary doses that killed the mealybugs, 423.7-1927.4 Gy, were higher than the specified generic dose range. According to the International Standard for Phytosanitary Measure¹³, the recommended generic dose for phytosanitary treatment is 400 Gy, while the irradiation dose permitted for fresh food safety is 1000 Gy. Therefore, treatment using sublethal doses was recommended as a phytosanitary treatment. Successful control of this pest is demonstrated by stalled development, inability to reproduce, or inactivation that ultimately leads to death.

Gamma irradiation affect differently decreased the insect productivity. Non-irradiated adults (controls) laid first instar nymphs at an average of 137.7 nymphs/parent (Table 3). Adult mealybugs irradiated at a dose of 50 Gy laid fewer offspring than controls, but their average of 106.5 nymphs/adult was not significantly different. However, adults irradiated at doses of 100, 200 and 400 Gy laid 69.6, 31.4 and 23.5 nymphs/adult and these averages were significantly lower than controls. Although the birthing periods at the doses of 50 and 100 were longer (Table 2), the number of eggs laid was fewer than controls. This result was due to the number of eggs per day also being lower than the controls. The birthing periods at the doses of 200 and 400 Gy were significantly shorter than the control and the number of eggs was significantly lower. This outcome indicated that the actual reproductive capacity decreased because of irradiation. Seth et al.23 reported that irradiation of adult mealybugs P. marginatus at doses of 56-165 Gy resulted in sterility.

Reproduction of *E. hispidus* is ovoviviparous; that is, eggs hatch in the body of adult and the first instar nymph is subsequently laid¹². Irradiation of *E. hispidus* at a dose of 50 Gy cause some eggs (0.3%) to hatch into first instar nymphs that died within 3 days (Table 3); 99.7% of eggs did not hatch. Normally, *E. hispidus* lay first instar nymphs, but irradiation at doses of 100-400 Gy led to the failure of eggs to hatch. Consequently, eggs rather than first instar nymphs were laid. The eggs laid by irradiated adults were darker than normal and eventually dried out. The irradiation treatment may have causes melanization of the eggs as reported by Supawan *et al.*²⁴, who found that *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) larvae were darker because of melanization after irradiation treatment.

The physical and chemical guality of mangosteen fruit did not influenced by the irradiation unless the rind color and vitamin C content. This finding means that irradiation decreased the brightness of the rind color of the mangosteen fruit. The value a showed the green to red. The result showed that at the dose of 0 (control), 250 and 500 Gy, the rind color changed from the green reddish to dark purple, while at the dose of 750-1000 Gy, the rind color still maintain red purple. The b values didn't affect by the irradiation unless the dose of 1000 Gy. The mangosteen fruit in this experiment had maturity index 4 and already had the red color of mature fruit that would quickly turn into a dark purple. The higher a and b values in fruit irradiated at 750 and 1000 Gy indicated that irradiation could cause mangosteen fruit to retain the red color because the controls were turn into dark purple. Typically, the color of mangosteen changes from green to red and then to purple as the chlorophyll degrades and anthocyanin forms²⁵.

The result showed that irradiation affects decreased the vitamin C content of the mangosteen fruit. The vitamin C content of the mangosteen fruit irradiated with 750 and 1000 Gy has lower vitamin C content compare to controls. Irradiation of dragon fruit (Hylocereus undatus) at the doses of 200-800 Gy was associated with significantly lower vitamin C compared to controls⁸. In contrast, Seth et al.²⁴ found that papaya irradiated at a dose of 200-400 Gy did not have decreased vitamin C content. Vitamin C is sensitive to irradiation, however and a decrease in vitamin C content depends on the type of commodity, cultivar, irradiation dose, duration and storage temperature¹¹. The doses of irradiation applied to the mangosteen fruits did not change the TTS and TA. Similar effect occurred on other fruits, the doses of 200 and 400 Gy applied on papaya (Carica papaya L.) did not significantly effect on the weight loss, rind firmness, TSS and TA²³.

The irradiation dose that is recommended for phytosanitary treatment of the mealybug *E. hispidus* on mangosteen fruits is 250 Gy. This dose is recommended by considering the results of this study. Irradiation at doses of 100-400 Gy in adult *E. hispidus* inhibited development and prevented eggs from hatching into first instar nymphs. Irradiation doses of 250-500 Gy did not degrade the physical and chemical qualities of the mangosteen fruit. Thus, irradiation for phytosanitary treatment of *E. hispidus* may be applied at a dose of 250 Gy. Hallman *et al.*⁴ proposed that a dose of 300 Gy (formerly 400 Gy) be used for all insect phytosanitary treatments (except against pupae and adult Lepidoptera) because it does not harm fresh fruit and vegetable commodities⁴.

CONCLUSION

The lethal doses (LD₉₉) of gamma irradiation against *E. hispidus* first, second and third instar nymphs and adults were 423.7, 1026.0, 1276.0 and 1934.5 Gy. Sublethal doses of 50-400 Gy may inhibited the reproduction, prolonged longevity and decreased the fecundity. Irradiation doses of 100-400 Gy effectively suppressed egg fertility. Gamma irradiation dose of 200-500 Gy on mangosteen fruit does not decreased fruit quality. Gamma irradiation doses of 750 and 1000 Gy were able to maintain the red color of fruit rind, but decreased the vitamin C content. A sublethal dose of 250 Gy can be recommended for phytosanitary treatment of *E. hispidus* on mangosteen.

SIGNIFICANT STATEMENT

This study found that gamma irradiation is effective for phytosanitary treatment of cocoa mealybug *E. hispidus* on mangosteen fruit. The irradiated doses obtained in this study can be used as recommendation material to the National Quarantine Agency as phytosanitary treatment to export mangosteen fruit.

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REFERENCES

- 1. Williams, D.J., 2004. Mealybugs of Southern Asia. United Selangor Press, Kuala Lumpur, Malaysia.
- 2. Ministry of Agriculture, 2012. Mangosteen profile in Indonesia. Directorate of Fruit Cultivation, Directorate General of Horticulture, Ministry of Agriculture, Jakarta, Indonesia. [In Indonesian].
- 3. EPA., 2017. Methyl bromide 2017. Environmental Protection Agency, USA. https://www.epa.gov/ods-phaseout/methylbromide.
- Hallman, G.J., Y.M. Henon, A.G. Parker and C.M. Blackburn, 2016. Phytosanitary irradiation: An overview. Florida Entomol., Vol. 99.
- Hallman, G.J., N.M. Levang-Brilz, J.L. Zettler and I.C. Winborne, 2010. Factors affecting ionizing radiation phytosanitary treatments and implications for research and generic treatments. J. Econ. Entomol., 103: 1950-1963.

- Ravuiwasa, K.T., K.H. Lu, T.C. Shen and S.Y. Hwang, 2009. Effects of irradiation on *Planococcus minor* (Hemiptera: Pseudococcidae). J. Econ. Entomol., 102: 1774-1780.
- Kuswadi, A.N., M. Indarwatmi, I.A. Nasution and H.I. Sasmita, 2016. Minimum gamma irradiation dose for phytosanitary treatment of *Exallomochlus hispidus* (Hemiptera: Pseudococcidae). Florida Entomol., 99: 69-75.
- Nguyen, T.K., T.K.L. Vo, T.L. Nguyen, T.T.A. Tran and H.H.T. Nguyen, 2016. Phytosanitary irradiation of the mealybugs, *Dysmicoccus neobrevipes, Planococcus lilacinus* and *Planococcus minor* (Hemiptera: Pseudococcidae), infesting dragon fruit in Viet Nam. Florida Entomol., 99: 159-165.
- 9. Ministry of Health, 2009. Regulation of the ministry of health: RI No. 701/MENKES/ PER/VIII/2009. Ministry of Health, Jakarta, Indonesia.
- FSANZ., 2014. Nutritional impact of phytosanitary irradiation of fruits and vegetables. Food Standards Australia New Zealand, February, 2014. http://www. foodstandards. gov. au/publications/Pages/Nutritionalimpact-of-phytosanitary-irradiation-of-fruits-andvegetables.aspx
- 11. Kader, A.A., 1986. Potential applications of ionizing radiation in postharvest handling of fresh fruits and vegetables. Food Technol., 40: 117-121.
- Indarwatmi, M., D. Dadang, S. Ridwani and E. Sri Ratna, 2017. The bionomics of the Cocoa Mealybug, *Exallomochlus hispidus* (Morrison)(Hemiptera: Pseudococcidae), on mangosteen fruit and three alternative hosts. Insects, Vol. 8. 10.3390/insects8030075.
- 13. IPPC., 2017. Guidelines for the use of irradiation as a phytosanitary measure. International Standard for Phytosanitary Measures (ISPM) No. 18. International Plant Protection Convention, FAO., Rome, Italy.
- 14. Ministry of Agriculture, 2007. Mangosteen profile in Indonesia. Ministry of Agriculture, Directorate of Fruit Cultivation, Directorate General of Horticulture, Jakarta, Indonesia, ID, (In Indonesian).
- 15. Ministry of Health, 2009. Guidance of ministry of health republic of Indonesia. No: 701. Ministry of Health, MENKES/PER/VIII/2009, Jakarta (ID), Indonesia.
- Fransiska, A., R. Hartanto, B. Lanya and Thamrin, 2013. Characteristics of mangosteen physiology (*Garcinia mangostana* L.) in modified atmospheric storage. J. Teknik Pertanian Lampung, 2: 1-6, (In Indonesian).
- 17. AOAC., 2012. Official Methods of Analysis. 19th Edn., Association of Official Analytical Chemists, Washington DC., USA.
- 18. LeOra Software, 1987. POLO-PC User's Guide. LeOra Software, Petaluma, CA.

- 19. SAS., 2010. SAS Users Guide. Version 9.13, SAS Institute Incorporation, Cary, North Carolina, USA.
- 20. The, D.T., N.T. Khanh, V.T.K. Lang, C. Van Chung, T.T.T. An and N.H.H. Thi, 2012. Effects of gamma irradiation on different stages of mealybug *Dysmicoccus neobrevipes* (Hemiptera: Pseudococcidae). Radiat. Phys. Chem., 81: 97-100.
- 21. Seth, R., M. Zarin, Zubeda and R.K. Seth, 2014. Bioefficacy of Ionizing Radiation as Phytosanitary Treatment against Mealybug Species of Quarantine Importance, Phenacoccus Solenopsis, *Maconelicoccus hirsutus* and *Paracoccus marginatus*. In: Development of Generic Irradiation Doses for Quarantine Treatments, Vienna, Austria, June 2-6, 2014, Hallman, G.J., Y.M. Henon, A.G. Parker and C.M. Blackburn (Eds.)., IAEA., Vienna, Austria, pp: 102-107.
- 22. Bergonie, J. and L. Tribondeau, 1959. [Interpretation of some results of radiotherapy and an attempt at determining a logical technique of treatment]. De quelques resultats de la radiotherapie et essai de fixation d'une technique rationnelle. Radiat. Res., 11: 587-588.
- 23. Seth, R., M. Zarin, Z. Khan and R.K. Seth, 2016. Towards phytosanitary irradiation of *Paracoccus marginatus* (Hemiptera: Pseudococcidae): Ascertaining the radiosensitivities of all life stages. Florida Entomol., 99: 88-101.
- 24. Supawan, J., P. Hormchan, M. Sutantawong and A. Wongpiyasatid, 2005. Effects of gamma radiation on azuki been weevil (*Callosobrunchus chinensis* L.). Kasetsart J. Nat. Sci., 38: 57-64.
- 25. Winarno, F.G., 2002. Post-Harvest Physiology of Horticultural Products. M-Brio Press, Bogor, Indonesia, (In Indonesian).