

## New Proposed Dose of Irradiation to Control Fruit Flies (Diptera:Tephritidae) and its Influence to Super Red Pittaya (*Hylocereus costaricensis*) Quality

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### ABSTRACT

As one of the important pests, fruit flies derived from Tephritidae family have caused the worst consequences in losing the economic value of fresh horticultural commodity including on the dragon fruits. As a prospective quarantine treatment, gamma irradiation has been thrived to eliminate totally the appearance of those adults' flies with less impact on the fruit quality degradation. In this study, a series of irradiation dosages were examined to both the third instar larvae (subsequently from *Bactrocera papayae*, *B. cucurbitae* and *B. carambolae*) infesting fruits and the non-infested ones afterwards at 0, 50, 100, 150, 200, 250, 300, 350 and 400 Gy. For a number of the infested fruits, a series of observations were persisted in at room temperature ( $27\pm 1^\circ\text{C}$ ) until two weeks of the post-treatment period. Whereas the non-infested fruits were stored at  $11\pm 1^\circ\text{C}$  for 17 days of the storage. Results showed that *B. papayae* required the highest recommended dosages (the effective dose = 248.4 Gy; the minimum dose = 225.1 Gy; the maximum dose = 279.6 Gy) for achieving  $\text{LD}_{0.99}$  rather than the other tested species. There were also no significant differences among each of the non-infested fruits in terms of altering of the observed physical attributes. This result has become a distinct indication that a higher dose value than the generic one is mostly recommended both for phytosanitary irradiation and stabilization of fruit quality.

## 1. Introduction

Pittaya also recognized as dragon fruit, is one kind of exotic horticultural plants firstly cultivated in several locations both in Central and South America (Mizrahi *et al.* 1997; Perween *et al.* 2018) and known as its magnificent ornamental value due to numerous spacious flowers blooming in late-night (Pushpakumara *et al.* 2006). One of the most attractive species of this fruit is recognized as *Hylocereus costaricensis* with distinctly distinguished through its red flesh with pink skin (Nerd *et al.* 1999). This fruit has also classified as the premium ones since it contains many antioxidant substances

(Mahattanatawee *et al.* 2006), a significant amount of bioactive compounds and a certain number of micro essential mineral substantially needed by human's body (Bellec *et al.* 2006).

However, several quarantine pests appearances have caused several serious consequences dealing with loss of economics potencies and trade values. McQuate (2010) reported certain patterns of *Bactrocera cucurbitae* and *B. dorsalis* infestation to white flesh dragon fruit on a local orchard in Hawaii island. Other important species are *B. papayae* and *B. carambolae* greatly impacting on several reported cases of severe tropical fruits deterioration (Salomon *et al.* 2018). Another previous study conducted by Drew *et al.* (2001) found several cases of fruit flies infestation on a number of the white flesh of dragon

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fruits traded in Vietnam. Several species of *Bactrocera* spp. such as *B. dorsalis*, *B. correcta* and *B. cucurbitae* have frequently infested on this commodity and therefore need further treatment to disinfest for fulfilling the requirement of the phyto-sanitary measures implemented on several trade partnership countries (Hoa *et al.* 2006).

At least, three reasons such as the appearance of converted sugars as the main source of energy and the protein source not only for achieving the optimum maturity but also together with lipids for determining the next generation, have previously proposed in supporting why several kinds of flesh fruits are preferably chosen as hosts (Fletcher 1987; Koswanudin *et al.* 2018). Learning from this case, an appropriate applying of the proper quarantine treatment shall be taken as a main priority in preventing the cross-contamination risks possibly occurred (Follet and Sanxter 2000). One of the technical schemes for minimalizing the fruit flies contamination on several kinds of horticultural commodities is through gamma irradiation.

Irradiation is one of the established quarantine treatments having auspicious prospects not only on eliminating the pests but also on allowing the minimum impacts of fruit degradation (Moy and Wong 2002; Follet and Sanxter 2003). Other benefits offered by this treatment are tangible to all process of fresh horticultural packaging and no remained residue (Hallman 2011). As a recognized treatment, irradiation has been conducted to prevent normal adults appearance and not subjected to total elimination of larvae of those tephritids (Hallman 2012). The first recommendations formulated by the International Consultative Group on Food Irradiation (ICGFI) firmly stated two generic doses in achieving those previous goals namely 150 Gy for fruit flies and 300 Gy for other insects (ICGFI 1991).

Unfortunately, previous studies describing the role of irradiation on risks mitigation of flies contamination to dragon fruits have been relatively rare to be published. This study has a crucial role since it would briefly describe whether the previous generic dose of fruit flies was sufficient or not and how its impacts on critically altering the attributes of the physical fruits.

## 2. Materials and Methods

### 2.1. Dose-response Tests

This study was conducted from January to December 2018. There were three kinds of tropical fruit flies species—*Bactrocera papayae*, and *Bactrocera carambolae* classified into *Bactrocera dorsalis* species complex (Schutze *et al.* 2015), and also *Bactrocera cucurbitae*—originated from Laboratory of Vapour Heat Treatment, Pest Forecasting Institute, Karawang Regency, West Java Province. While, red dragon fruits, mature index = 4, were supplied from a local market in Cibitung-Bekasi Regency, West Java Province. Those entire eggs were obtained in mid-day through an hour and half of nesting. Each of 150 pieces eggs were counted under stereo microscope (BOE 3500, Boeco, Germany) artificially inserted into a fruit through a square cut of windows located on the surface of peel sizing in 2 cm × 2 cm × 1 cm (expressed as length, width, depth), then those windows were tightly sealed. There were totally 45 eggs-infested fruits containing 6,750 pieces eggs, then a half of those eggs converting into mature larvae as the targeted life stage for this study.

All of infested fruits were stored at ambient temperature (27±1°C) for almost 5 days on giving sufficient period in growing the eggs into mature larvae (L-3) known as the most radio-tolerant stage (Balock *et al.* 1963; Follet *et al.* 2011; Follett and Armstrong 2004; Hallman 1999). Irradiation was then carried on Centre for Irradiation Application, National Atomic Agency, South Jakarta, Special Region of Jakarta Province using irradiation facility (Panoramic Gamma Irradiators, IAEA, Vienna, Austria). A series of irradiation treatment were conducted at a range of 50 (stated minimum and maximum doses, 50.40-58.93), 100 (100.79-117.87), 150 (159.17-187.50), 200 (212.22-249.99), 250 (256.94-272.22), 300 (308.13-326.67), 350 (357.37-443.95) and 400 (408.42-507.37) Gy to both infested and uninfested fruits. Un-irradiated ones (0 Gy) were used a control for observing natural response mortality of the insect.

Gamma irradiation was generated from a Co-60 energy source owning a counted activity until 100 kCi with a minimum dose rate at 2.5 kGy/h and a measured radiating spectrum was 1.33 MeV.

Each of dragon fruit was individually positioned on a cylindrical plastic container (diameter = 20 cm; height = 30 cm). Those filled containers were then placed on a-four of compass directions. At least two dosimeters (Gafchromic™ Dosimetry Media Type HD-V2, Bridgewater, NJ, USA) in each of compass positions were attached into a series of represented container for quantifying the absorbed doses on the four-different directions. The dose uniformity ratio for all of replicates was ≤1.24 (maximum/minimum ratio).

After irradiation, entire infested fruits were stored on ambient temperature (27±1°C) for almost 3 days on allowing those infested mature larvae in transforming into pupae. All of collected pupae gained from fruit’s dissections were moved into a number of cube-shaped confinements having 27 liter in volume for 14 days for further observation of adult’s appearances. On the other hand, overall non-infested fruits were then placed into a commercial showcase (POLYTRON SCP-18A, Japan) at 11±1°C until 17 days.

**2.2. Physical Fruit Quality Properties**

Color alteration occurred to post-irradiated and uninfested fruits was observed after a half-month storage period using a Konica Minolta Color Reader (CR-10 Plus, Osaka-Japan). Internal calibration was carried on before color’s testing to all of those fruits. The observed values (L,a and b ones) then subsequently converted into chroma values, hue angles and pericarb index using several kinds of mathematical formulas developed by Carreno *et al.* (1995).

In a different approach, the pulp’s strength was also measured utilizing a fruit hardness tester (Cat. No. 9300, Tokyo-Japan) according to a method described by Prasetia *et al.* (2018). While for analyzing of total dissolved solid to all juices fruits, a pocket refractometer (PAL-1, Atago, Tokyo-Japan) was employed during the entire tests. A proposed method developed by Rosman *et al.* (2018) was mainly selected for this analysis.

**2.3. Data analysis**

Data obtained from a series of dose-response tests were converted into certain proportioned values of no adult’s emergence through modified forms of Leibniz series giving a wide range of flexibility terms, expressed as follows:

$$A_{(n)} = \frac{\left(1 - \frac{n_{is}}{n_{ts}}\right) + \left(1 - \frac{n_{ps}}{n_{is}}\right)}{(n_{ts} + n_{is})} \times 100 \dots\dots\dots(1)$$

$$A_{t(n)} = 1 - \left(\frac{n_{is}}{n_{ps}} \times 100\right) \dots\dots\dots(2)$$

Where  $A_{(n)}$  and  $A_{t(n)}$  subsequently were proportion of no adults emerge from the entire inoculating stages and the final fraction of dead pupae. While  $n_{ts}$ ,  $n_{is}$ , and  $n_{ps}$  afterwards refer to total amount of inoculated eggs, a number of observed thirds instar larvae and the whole of noticed pupae. All of the previous proportioned values were then analyzed through a linier regression model.

Analysis of variance (ANOVA) has been conducted to evaluate whether slope and intercept values were significantly affected to a series of trial doses, means were compared by Duncan’s multiple range tests (DMRT). For obtaining a certain limit of trial dosages on achieving  $LD_{99}$  values, the dosage-mortality data were further analyzed using a probit model at confidence level of 95%. The statistical approaches using ANOVA and DMRT was also operated for analyzing physical fruit attributes.

**3. Results**

**3.1. Determination of the Most Tolerant Species of Fruit Flies**

All of three species fruit flies were well-developed until pupae stadium at each of tried dosages. However, there was a strong correlation in reducing populations of observed adult fruit flies appearance caused by the rising of gamma irradiation dosages observed during the entire trials (Figure 1). The entire three regression linear model described relations between those doses of irradiation and mortalities of observed pupae were significant ( $P < 0.05$ ).

There were similar flat-patterns in terms of the peak value of dead pupae percentage observed after treated with irradiation applied at a range of dose 250 to 400 Gy (data not shown). There were no significant differences among each of three species of tested fruit flies observed on entire of trial dosages ( $F = 3.6$ ;  $df = 2$ ;  $P = 0.09$ ). In contrast with the previous findings, *B. carambolae* had the least tolerant against gamma-irradiation exposures ( $F = 5.6$ ;  $df = 2$ ;  $P = 0.04$ ) than two others tested fruit flies (Table 1). The

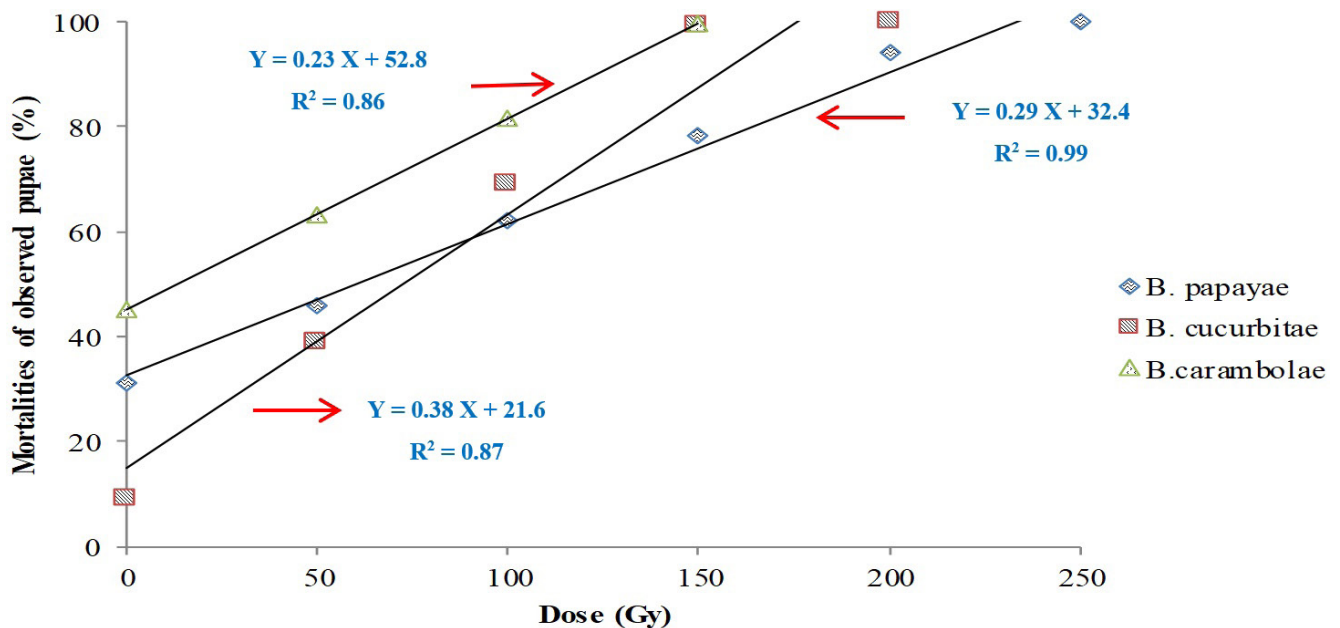


Figure 1. The response of raising dead pupae (preventing adults emergence) due to adding of irradiation dose tested on mature larvae instar artificially reared on dragon fruits. *B. papayae* was the most tolerant species among three species of tested fruit flies

altering of  $R^2$ -values was significant ( $P < 0.05$ ) in terms of describing intimate relations between each value of applied dosages and obtained relative percentages of dead pupae.

However, those predicted doses for total preventing adults appearance were different where adults papaya fly emergence was avoided by applying the biggest values of irradiation dosages. Consequently, *B. papayae* was needed higher values of applied dosages compared than those of two others kind of tested fruit flies (Table 1). Another a unique finding was *B. carambolae* had slight wider values than that of *B. cucurbitae* in terms of a range of needed dosages for avoiding adult fly appearance.

There was a significant difference observed on means value of the recommended dose for causing dead pupae on *B. papayae* than those of both *B. cucurbitae* and *B. carambolae* ( $P < 0.05$ ). Another that, there was no significant difference observed in means of recommended dose between *B. cucurbitae* and *B. carambolae*. Only *B. papayae* required the highest predicted value of dosage in dealing with achieving a total inhibition of their adults' appearance.

### 3.2. Effects of Irradiation to Physical Attributes Changes of Fruit

For non-infested fruits, several observations were carried on to study further about irradiation's effect

on physical fruit characteristics. The first observations were conducted to investigate the irradiation treatment to color attributes of fruit's pulp (Table 2). Those results indicated there were no significant differences among each of irradiated and non-irradiated fruits ( $p > 0.05$ ) in relating with observed chroma, hue angle, and pericarp color indexes.

At the end of storage periods, overall fruits color seemed not to be developed anymore. Most of the fruits were seemed into dark red indicating the saturated color conditions were reached at this time. This fact was also confirmed with the hue angle and pericarp color index showing the relatively same tendencies of color transitions into real red and strong intensities of red spectrum observed on the pulp.

Another of our findings confirmed there were relatively similar influences relating to irradiation effect to both of pulp hardness and sugar brix observed after the end of storage periods (Figure 2). These findings also strengthened the previous ones meaning all of irradiated dosages were not significantly impacted on lowering pulp hardness and sugar brix index in a drastic pattern.

In another word, irradiation treatment was not drastically altering the integrity of pulp structure. The same pattern was also strongly confirmed to the narrow changes of brix values indicating degradation of sugar was not completely occurred yet. Therefore,

Table 1. Linear regression of percentage dead pupae (inhibiting of adult emergence) against dose (Gy) practiced to third instar larvae of three fruit fly species artificially reared on dragon fruits

Species	N	Slope $\pm$ SE	Intercept $\pm$ SE	R <sup>2</sup>	Forecasted dose (95% CL) for 100 % mortality (Gy)
<i>B. papayae</i>	4468	0.29 $\pm$ 0.02 <sup>ab</sup>	32.4 $\pm$ 2.4 <sup>a</sup>	0.99	248.4 (225.1–279.6)
<i>B. cucurbitae</i>	4123	0.38 $\pm$ 0.07 <sup>b</sup>	21.6 $\pm$ 11.1 <sup>a</sup>	0.87	170.0 (156.0–188.1)
<i>B. carambolae</i>	4902	0.23 $\pm$ 0.05 <sup>a</sup>	52.8 $\pm$ 7 <sup>b</sup>	0.86	167.8 (145.8–200.3)

Linear regression expressed as  $y = ax + b$  where a is the slope, b is the intercept, x is the irradiation dose (Gy) and y is the percentage of mortality. Means within columns for each comparison followed by the same letters are not significantly different ( $P > 0.05$ ; DMRT)

Table 2. Color characteristics of post-irradiation fruits observed after 17 days of storage at 11 $\pm$ 1 $^{\circ}$ C

Dose (Gy)	Chroma	Hue angle	Pericarp color index
0	14.2 $\pm$ 2.9 <sup>a</sup>	23.3 $\pm$ 3.4 <sup>a</sup>	8.5 $\pm$ 1.2 <sup>a</sup>
50	13.8 $\pm$ 3.5 <sup>a</sup>	21.4 $\pm$ 1.5 <sup>a</sup>	10.1 $\pm$ 2.7 <sup>a</sup>
100	14.4 $\pm$ 3.9 <sup>a</sup>	23.1 $\pm$ 1.4 <sup>a</sup>	9.6 $\pm$ 1.8 <sup>a</sup>
150	11.4 $\pm$ 2.4 <sup>a</sup>	21.5 $\pm$ 5.8 <sup>a</sup>	10.7 $\pm$ 1.1 <sup>a</sup>
200	11.8 $\pm$ 3.6 <sup>a</sup>	22.3 $\pm$ 3.9 <sup>a</sup>	12.4 $\pm$ 3.0 <sup>a</sup>
250	14.2 $\pm$ 6.1 <sup>a</sup>	27.4 $\pm$ 9.6 <sup>a</sup>	13.3 $\pm$ 6.4 <sup>a</sup>
300	12.0 $\pm$ 3.3 <sup>a</sup>	21.9 $\pm$ 4.4 <sup>a</sup>	11.6 $\pm$ 2.6 <sup>a</sup>
350	13.2 $\pm$ 5.2 <sup>a</sup>	27.4 $\pm$ 12.2 <sup>a</sup>	12.7 $\pm$ 5.1 <sup>a</sup>
400	11.5 $\pm$ 5.0 <sup>a</sup>	30.2 $\pm$ 15.5 <sup>a</sup>	15.7 $\pm$ 7.1 <sup>a</sup>

Mean values followed by the same letter within a row indicate no significant differences ( $P > 0.05$ ; DMRT)

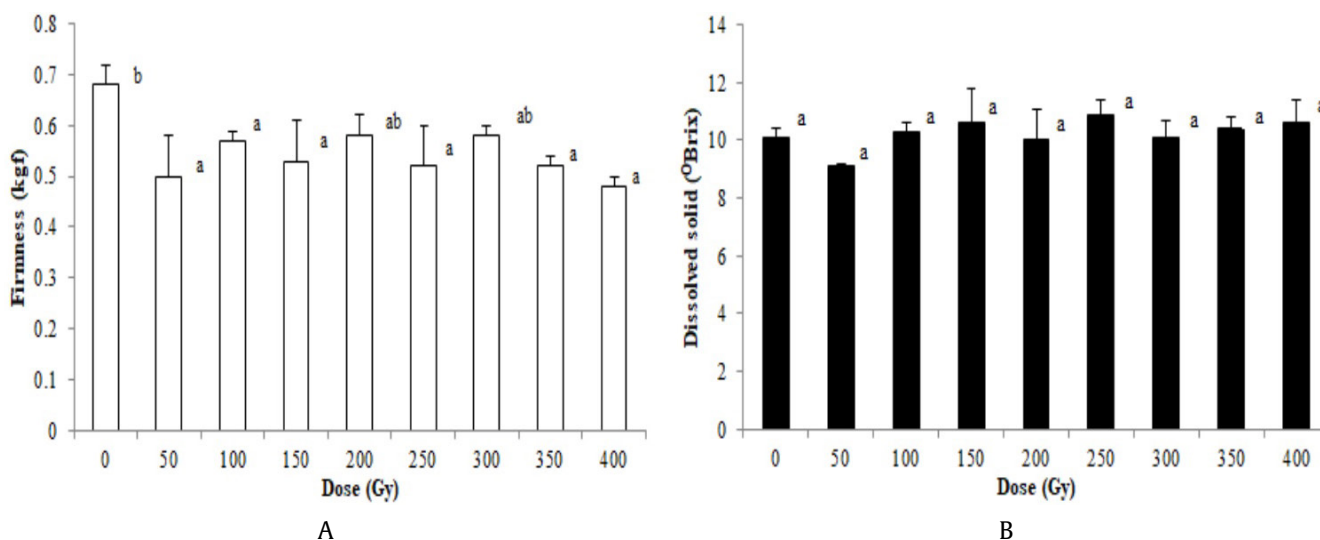


Figure 2. Alterations the two physical quality of fruits namely, (A) fruit firmness and (B) total dissolved solid, after 17 days of storage at 11 $\pm$ 1 $^{\circ}$ C. Values were expressed as means  $\pm$  standard errors. Values followed by the same letter indicated no significant differences ( $p \leq 0.05$ ; DMRT)

the fruits had still juicy characteristics during the storage periods.

#### 4. Discussion

Tropical fruit flies-*B. papayae*, and *B. carambolae* grouped into *Bactrocera dorsalis* species complex (Schutze *et al.* 2015), and also *B. cucurbitae* - have been considered as important pests infesting important horticultural commodities in South East ASIAN

countries (Indonesia, Malaysia, Cambodia, Myanmar, Brunei Darussalam, Laos and Thailand) (Hu *et al.* 2010; Prabhakar *et al.* 2009; Zhan *et al.* 2015). The minimum dose for sterilizing those flies has remained varies compared other Tephritid flies, as follows 30 Gy for *B. correcta* (Puanmee *et al.* 2010), 75 Gy for *B. invadens* (Ogaugwu *et al.* 2012), 70–90 Gy for *B. dorsalis* (Nasution *et al.* 2018), 90 Gy for *B. zonata* (Mahmoud and Barta 2011), and 200–250 Gy for *B. tau* (Cai *et al.* 2018). While for preventing adult flies appearance, the

third instar larvae have been previously irradiated on medium doses ranging from 85 Gy for *B. tau* (Zhan *et al.* 2015) to 125 Gy for *B. dorsalis* (Follett and Armstrong 2004). Our result confirmed that a series of regression linear trends have strongly correlated on achieving total sterility with gradual gamma irradiation doses, even though the final-values gained in this study were on higher ranges.

Previous studies demonstrated that performing low level of irradiating dose was not properly fit for insect sterilization as well as poor performance of mating after irradiated with the high dose (Parker and Mehta 2007). Therefore, in this case gamma-irradiation has formed a new character of fly-tolerant ability during their developmental stages (Zhan *et al.* 2015). A surprise result gained from another research showed irradiation at 400 Gy to the newly appeared male-imago did not modify their competitiveness on mating reflecting on producing their offspring (Cai *et al.* 2018). This phenomenon is quite interesting to be observed further, particularly in relating to the different variations of both of behaviors appeared from (Cai *et al.* 2018; Hansen *et al.* 2014; Kheirallah *et al.* 2017) and also determining which of developmental stages are essentially critical for managing the sterile insects populations (Bakri *et al.* 2005; Costa *et al.* 2020; Lopez-Guillen *et al.* 2008).

Meanwhile, difficulties on practicing the irradiating scheme often cause inaccuracies results leading some fundamental issues closely relating to determine the generic dose for the complete sterile ones. In this case, the rearing circumstances as well as their quality controls (Costa *et al.* 2020) should be assured through fulfilling basic requirements for implementation of the sterile insect technique regulated by FAO/IAEA (2014). Therefore, the gained result will be statistically satisfied and it is also well-understood to be reproduced for another case-experimental studies.

Improving life adaptation to the changes in solar radiation distribution, particularly on tropical area, is a key factor in understanding which approaches are suitable for fruit flies control management. As a part of the integrative post-harvest management system, irradiation aimed to prevent adults emergence of those flies has critically played on determining phytosanitary status of fresh horticultural commodities. Previous studies mentioned *B. dorsalis* have relatively higher dose until 250 Gy for fulfilling that purpose (Seo *et al.* 1973), while Follett and Armstrong (2004) reported at least needed three doses – 210, 225 and 250 Gy – subsequently for avoiding imago appearance of

Melon, Mediterranean and Oriental flies. Furthermore, Follett and Weinert (2009) and also Hallman *et al.* (2010) informed that the maximum dose applied on commercial facilities shall not exceed 1.5 until 2 times the minimum dose applied.

On many different studies observing irradiating effects in preserving the quality of fruits, it has revealed that there was not any significant differences on physics-chemistry and sensory attributes in between untreated control oranges and irradiated ones exposed at 200 to 400 Gy after cold-storing for almost a half month (Mc Donald *et al.* 2013). A slight difference finding was performed on mandarin's pulp becoming smoother after irradiated at 450 Gy (Miller *et al.* 2000). A distinct deviation observed on fruits physiological development was carried on soon after irradiated in a range of doses 600–800 Gy marked by darkening color and visual defect seen on the fruit surface (Mc Donald *et al.* 2012; O'Mahony *et al.* 1985) as well as off-flavor appearance as a result of coating combination treatment (Obenland *et al.* 2011, 2012). Our result confirmed that the overall irradiating doses did not drastically alter the fruit properties into more sensitive ones, therefore the whole fruits attributes were not degraded yet until the end of storage.

As previously informed, IPPC has allowed the usage of irradiation treatment under low-oxygen concentrations only for *Grapholita molesta* (IPPC 2010; Zhan *et al.* 2020). Therefore, the pest management studies through gamma-irradiation combined modified atmosphere storage should be further prioritized for decreasing flies resistant strongly related with severe hypoxia phenomenon (Dias *et al.* 2020; Zhan *et al.* 2020) as well as extending the shelf of life of fresh fruits directly related to slowing down of respiration rate (Barkai-Golan and Follett 2017; Lacroix and Follett 2015; Srimartpirom *et al.* 2018).

### Conflicts of Interest

The authors stated there is no conflicts of interest.

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