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Gamma radiation as a phytosanitary treatment against larvae and pupae of *Bactrocera dorsalis* (Diptera: Tephritidae) in guava fruits

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ABSTRACT

A low-dose gamma radiation phytosanitary treatment against the oriental fruit fly, *Bactrocera dorsalis* Hendel, was developed for guava fruits. The measure for efficacy of the treatment is preventing adult emergence from late third instars that were reared in the fruit of guava, *Psidium guajava* L. The dose–response tests with 1-, 2-, 3-, 7-d-old larvae in guava were initiated to determine the most tolerant stages, the late-aged third instars. No adult emerged from a total of 100,684 late-aged third instars irradiated at the dose of 97–116 Gy, resulting in an efficacy of 99.9970% at the 95% confidence level. The minimum dose for 100% preventing adult emergence from 2-, 5-, 7-d-old pupae (1800 pupae in each dose) reared in artificial diets was 100, 500, and 1750 Gy, respectively. Quality determinations on 'Taiwan' guavas were conducted at 1, 3 and 7 days after gamma radiation at doses of 200, 400, 600, 800, 1,200, 2000 and 6000 Gy. The guavas could tolerate radiation dose up to 600–800 Gy as there were no significant changes in organoleptic characteristics (≤800 Gy), the chemical and nutritional contents (sugar, sucrose, total sugar, titratable acid, vitamin C, and soluble solid) (≤600 Gy). Therefore, a dose of 116 Gy, which give the disinfestations efficacy of 99.9968% for the late-aged larvae in guavas and 100% mortality of 2-d-old pupae, is suggested as the minimum absorbed dose for phytosanitary irradiation treatment of *B. dorsalis* in fruits.

1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is one of the most destructive quarantine insects of tropical and subtropical fruits and vegetables (Shi and Ye, 2004; Chang & Mclnnis 2010). In particular, it has caused severe losses to Guava, Carambola and Mango in the southern and southwestern provinces and Guangxi autonomous regions of China (Shi and Ye, 2004; Zhao Liang, Liang, Hu 2006). Guava (*Psidium guajava* L.) is an exceptionally common host plant for more than 20 species of fruit flies in the genus *Bactrocera* (Allwood et al. 1999; Hancock, Hamacek, Lloyd, Elson-Harris, 2000). Postharvest phytosanitary treatments are used to disinfest imported hosts of fruit flies to prevent the pests from being transported across quarantine barriers (Hallman 2002).

Application of ionizing radiation as a phytosanitary treatment is increasing in commercial uses because it possesses some advantages over other treatments, such as applicability to packed commodities and broad tolerance by fresh fruit (Heather & Hallman, 2008; Hallman, 2011). Phytosanitary irradiation treatments are promising measures to overcome quarantine barriers to trade and are currently used in several countries (Hallman, Levang-Brilz et al., 2010). The U.S. Animal and Plant Health Inspection Service (APHIS 2008, 2010a,b) has approved several phytosanitary irradiation treatments. The minimum dose for phytosanitary irradiation treatment of 7 species of fruit flies and a generic dose of 150 Gy for tephritid fruit flies have been built by the International Plant Protection Convention, but the specific dose for the oriental fruit fly was absent (IPPC 2009a, 2011). The United States and the IPPC has approved a generic dose of 150 Gy for all tephritid fruit flies,

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reduction of dose levels for specific pests and commodities to shorten treatment time and minimize any deleterious effects of radiation treatment on commodity quality (Follett 2009; IPPC 2009b). Thus, the minimum dose for phytosanitary treatment of oriental fruit fly is needed to be established.

When applied on a commercial scale, many irradiated fruit in each load can be expected to receive two-three times the minimum required dose for treatment efficacy (Follett and Weinert 2009; Hallman, Levang-Brilz et al., 2010). Acceptance of irradiation by fruit growers, packers, and consumers requires knowledge of its effect on nutritional, chemical, and sensory characteristics of the irradiated fruits (Terri, Reitmeier, Moy, Mosher, & Taladriz, 2002). Therefore, the destination of this study is to develop the minimum dose for phytosanitary irradiation treatment of oriental fruit fly in guavas, to determination the irradiation effects on the quality of "Taiwan" guavas for the practical use of phytosanitary irradiation treatments in shipped fruits.

2. Materials and methods

2.1. Insect rearing

Insects used in this study were obtained from a guava orchard in the outskirt of Guangzhou, China. Late third instars of *B. dorsalis* that emerged out of guava fruits were transferred to moist sand for pupariation in laboratory. One day before adult eclosion, the pupae were placed in adult rearing cages (57 by 40 by 40), and the adults were fed with fresh orange slice and a solid mixture of sucrose and hydrolyzed yeast (3:1) as Zhan, Liang, Liang, Hu (2015) done. Eggs were collected by placing perforated plastic cups in the adult cages and washed out. Larvae were reared on a wheat mill feed diet modified form Vargas, Miyashita, Nishida (1984) for the development to late-aged third instars emerge. All the stages were reared under 25 ± 2 °C, 70 ± 5% relative humidity (RH) with a photoperiod of 12:12 (D:L) h. The insects are replaced every 9–12 months.

2.2. Irradiation treatments

2.2.1. Irradiator and dosimetry

All the irradiation treatments were conducted in Guangdong Radiation Technology Development Center with a Colbalt-60 source of gamma radiation (2.22 PBq). Reference standard and routine dosimetry were done with the Fricke system (ASTM E1026-13, 2002). This dosimetry system was calibrated in accordance with the international standard ISO/ASTM 51261 (2002) and ASTM E1026-13 (2002), and the uncertainty of the measured value was calculated according to ISO/ASTM 51707 (2002).

2.2.2. Tolerance comparison tests

Every 30 guavas (~95 g) were placed in one adult rearing cage for *B. dorsalis* female lay eggs for one hour. The infested guavas were then held for 1, 2, 3, and 7 days for the eggs develop to late-aged eggs (mostly), first, second, and late-aged third instars, respectively. To compare the radio-tolerance between developmental stages, infesting guavas were exposed to gamma radiation at the same time; the target dose, replications, number of guavas used in the treatment were shown in Table 1. The dose rate detected was 2–4 Gy/min with the dose uniformity of 1.15.

2.2.3. Dose–response test

To estimate the minimum absorbed dose for preventing adult emergence from late-aged third instars, the most radiotolerant stages in fruits, the dose–response test was conducted on 7-d old larvae in guava (Table 1). The dose rate detected was 0.8–4.0 Gy/min with the dose uniformity of 1.09.

2.2.4. Confirmatory tests

In the two confirmatory tests, every 14 guavas infested with 7-d-old larvae were placed in one plastic box (20 × 33 × 23 cm) for radiation. On each of the treatment dates (July and September 2012), nine box was selected as control and the other eighty-four or forty-three boxes were exposed to gamma radiation simultaneously on a platform surrounding the source, where the boxes were placed within 150 cm from the source. The irradiator was stopped at half exposure time and the boxes were rotated 180° to give a more uniform exposure. In each confirmatory test, five Fricke dosimeters (2 in the center and 3 on the surface of guavas) were included in every twentieth box to measure dose variation (Zhan et al. 2014, 2015). The dose rate detected was 2.7 Gy/min.

2.2.5. Irradiation of pupae

2-, 5-, 7-d-old pupae, that developed from artificial rearing larvae were placed in plastics cups (5 cm inside diameter, 5 cm high, with moist sands), was placed in the different distance from the source for gamma radiation at the dose of 50–1750 Gy (Table 1); the dose rate detected was 4–35 Gy/min. The eclosion rate was checked to investigate the minimum dose for preventing adult emergence.

After irradiation, the guava (irradiated as well as controls) were stored at about 25 ± 2 °C in plastics basin (covered with 40 mesh gauze) until the fruits were fully desiccated, all the emerging late-aged third instars were transferred to moist sand for pupation and adult eclosion. The number of late-aged third instars, pupae, and adults were finally checked 3 weeks after the late 3rd instars emerged.

2.3. Quality evaluation of irradiated guava

To determine the effect of irradiation and storage time on fruit quality, fresh guava fruits (cv. Taiwan) that were growing in the outskirt of Guangzhou were irradiated at the target dose of 0 (control), 200, 400, 600, 800, 1,200, 2000 and 6000 Gy, respectively. Eighteen guavas were used in each dose.

2.3.1. Instrumental and chemical analysis

After irradiation, the fruit were placed at indoor room of 25 ± 2 °C, 70 ± 5% RH for 7days. Nutrition and chemical contents including soluble solids, reducing sugar, sucrose, total sugar, titratable acids and Vitamin C were measured at about 1, 3, 7 days after irradiation with 200, 400, 600Gy by using standard practices.

Table 1
Design for the irradiation treatment of oriental fruit fly larvae and pupae.

Stages	No. insects per dose	Replications	Target dose (Gy)
1-,2-,3-,7-d larvae in guava	3 guavas	3	0, 24, 32, 50, 70
Late-aged 3rd instars in guava	12 guavas	1	0, 9, 16, 25, 34, 43, 54, 74, 92
2-d pupae	600	3	0, 50, 100, 150
5-d pupae	600	3	0, 100, 200, 300, 400, 500
7-d pupae	600	3	0, 150, 200, 400, 500, 600, 800, 1000, 1400, 1750

2.3.2. Organoleptic testing

A 12-member panel evaluated the heuristic taste comparisons 7 days after irradiation. The sensory evaluation included firmness, taste and appearance, each sensory scale for ratings were: 3 = normal, 2 = acceptable, 1 = unacceptable, 0 = highly distasteful. Panelists evaluated samples under white light in individual booths. Distilled water was provided for oral rinsing between samples.

2.4. Data analysis

Radiotolerance comparing data on the percentage mortality to adult from late-aged third instars in each dose and control were subjected to one-way analysis of variance (ANOVA), means were separated by Tukey's multiple comparison tests (DPS, 2010). The results were presented in Table 2. To determine the target dose for conducting confirmatory tests on oriental fruit fly late third instars, the dose-mortality data on percentage mortality to adult from emerged third instars were analyzed with the probit model and logit model by using computer program PoloPlus (LeOra Software 2002). Data used in the analysis included any radiation doses causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. For the confirmatory tests, mortality proportion (1-P_u) associated with treating a number of Asian fruit fly with zero survivors is calculated by the equation,

$$1 - P_u = (1 - C)^{1/n} \quad (1)$$

Where C is the confidence level and n is the number of test insects (Couey and Chew 1986).

For objective analysis of soluble solids, titratable acids, reducing sugar, sucrose, total sugar and Vitamin C in guava quality evaluation, two-way analysis of variance (2-way ANOVA) was used to analyze the effects of irradiation treatment (control and irradiated) and storage time (1, 3 and 7 days) and interaction of irradiation treatment and storage time in a randomized complete block design. Means separation were done using Tukey model at $P \leq 0.05$ (DPS 2010).

3. Results

3.1. Radio-tolerance comparing

The number of late-aged third instars of *B. dorsalis* developing out of irradiated larvae in guavas at all doses did not significantly decreased as compared with their controls ($P > 0.05$), indicating that the development to late-aged third instars was not strongly affected by radiation up to 70 Gy (Table 2). However, the percentage of adult eclosion was decreased significantly as increasing dose for 24Gy ($F = 139.44$; df = 3.8; $P < 0.0001$), 32Gy ($F = 35.32$; df = 3.8; $P = 0.0001$), respectively. No adult emerged from 1-, 2-, 3-, or 7-d-old larvae at the dose above 24 Gy, 50 Gy, 50 Gy, and 70 Gy, respectively (Table 2), indicate that the minimum dose to 100% prevention of adult eclosion increase with increasing age and developmental stage. In addition, the percentage rate of adult ecolsoion for irradiated 7-d-old larvae was significantly higher than the other stages at all doses (Table 2). Therefore, the late-aged third instars likely to be found in fruits were determined to be the most tolerant stage in guavas, and they should be used to conduct the following dose–response tests and large-scale confirmatory tests.

As the pupae irradiated, the percentage rate of adult eclosion was decreased as the dose increased for all irradiated 2-, 5-, and 7-d-old pupae (Fig.1). There was no adult developed from irradiated 2-d-old pupae at 100 Gy, or 5-d-old pupae at 500 Gy, or 7-d-old pupae at 1750 Gy, indicating that the radiotolerence increased with increasing age and pupae were more tolerant than 7-d-old larvae.

3.2. Dose-response test

In the dose–response test on *B. dorsalis* late third instars, mortality to adult was 8.2%, 10.7%, 32.7%, 76.9%, 93.5%, 99.1%, 99.2%, 100%, and 100% at the dose of 0 (control), 9, 16, 25, 34, 43, 54, 74, and 92 Gy, respectively. All the data were subject to probit analysis (probit and logit model) and the results were presented in Table 3. The small value of heterogeneity (chi-square divided by degrees of freedom) deviated from probit model (logit transformation of dose, 2.26) and logit model (logit transformation of dose, 1.78) indicated that the estimations had good fit to the data. While the estimated ED_{99} and $ED_{99.9968}$ analyzed by the three models was similar except for the large value of $ED_{99.9968}$ (160.5 Gy vs. 84.1 or 70.6 Gy) from the logit model with the dose transformation. Then, the estimation of $ED_{99.9968}$ value of 84.1 Gy (95%CI: 73.6, 99.3) that derived from probit model is suggested as the target dose for conducting confirmatory test.

3.3. Confirmatory tests

In the confirmatory tests, the dose of 90 Gy was selected as target dose as the 84.1 Gy (95% CI: 74.5, 101.1) was estimated to 99.9968% preventing adult eclosion from late 3rd instars (the most resistant stage in harvested fruits) of *B. dorsalis*, and the dose of 70 or 92 Gy lead 100% mortarlity of 7-d-old larvae in guavas (Tables 2 and 3). No adults emerged from a total of 100,684 late 3rd instars developed in the guava fruits, whereas the percentage of adult eclosion in the control was >80% (Table 4). Actual absorbed doses measured by dosimetry ranged from 97 to 116 Gy in the first and 100–112 Gy in the second confirmatory test (Table 4), resulting in the dose uniformity ratio (maximum/minimum) of 1.20 and 1.12, respectively.

3.4. Quality evaluation of guava

When measuring the interaction effects of irradiation doses and storage time on the chemical and nutritional contents in guava fruits irradiated at 0, 200, 400, and 600 Gy, no significant difference was found for the content of soluble solids ($F = 1.9$; df = 6.24; $P = 0.12$), titratable acids ($F = 1.5$; df = 6.24; $P = 0.1201$), reducing

Table 2
Number and eclosion of *Bactrocera dorsalis* third instars emerged from irradiated guava infesting with 1-, 2-, 3-, and 7-d larvae.

Dose (Gy)	No. late-third instars emerged from				Eclosion rate (%)			
	1-d	2-d	3-d	7-d	1-d	2-d	3-d	7-d
0	149.0 ± 16.0	165.0 ± 20.2	170.3 ± 24.2	201.7 ± 36.0	89.4 ± 1.6a	91.9 ± 2.3a	89.6 ± 2.5a	90.0 ± 3.4a
24	208.7 ± 34.9	202.0 ± 18.1	184.3 ± 15.5	163.3 ± 21.4	0 ± 0c	1.8 ± 0.4bc	5.4 ± 1.0b	28.2 ± 2.0a
32	171 ± 25.2	179.0 ± 4.2	150.0 ± 14.6	238.0 ± 29.1	0 ± 0c	0.9 ± 0.4bc	3.4 ± 0.6b	9.0 ± 1.2a
50	177.7 ± 19.9	17.01 ± 11.6	215.7 ± 62.5	158.7 ± 12.4	0 ± 0	0 ± 0	0 ± 0	0.54 ± 0.54
70	237.3 ± 9.5	192.7 ± 17.8	176.7 ± 20.0	161.3 ± 17.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0

*Mean value followed by the same letter within a row are not significantly difference ($P > 0.05$; Tukey's multiple comparison test).

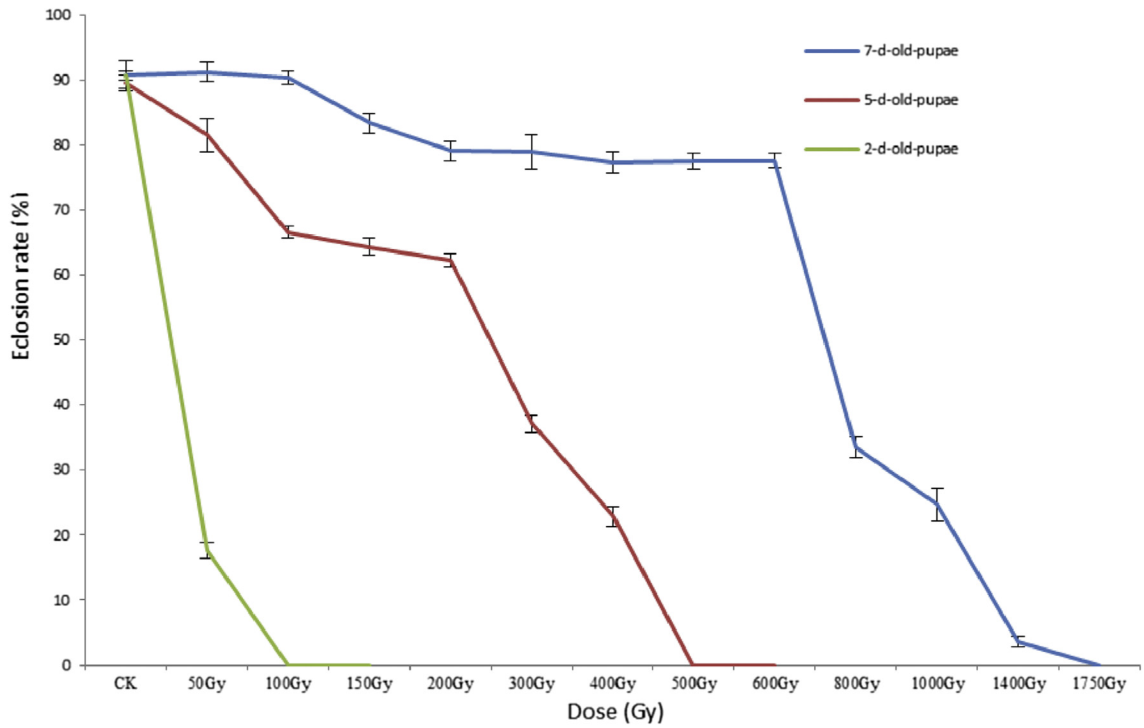

Fig. 1. Eclosion rate of irradiated *Bactrocera dorsalis* pupae.

Table 3
Probit analysis on preventing emergence of adults from *Bactrocera dorsalis* late-aged third instars in guavas.

No. 3rd instars	Analyzing model		Slope±SE[a]	Estimated minimum dose (Gy)		Hetero- geneity
	Model	Dose transformation		ED_{99}(95%CI)	$ED_{99.9968}$(95%CI)	
6341	Probit	logit transformed	6.395 ± 0.195	46.0 (42.7, 50.4)	84.1 (73.6.99.3)	2.26
	Logit	logit transformed	11.428 ± 0.36	50.2 (46.4, 55.3)	160.5 (133.2, 202.2)	1.78
	Logit	none	0.207 ± 0.006	42.7 (38.2, 50.1)	70.6 (60.5, 87.8)	11.35

[a] Mean±SE; heterogeneity means chi-square divided by degrees of freedom.

Table 4
Large-scale irradiation tests for third instars of oriental fruit fly in guava.

Date of radiation	No. guava	Dose monitored (Gy)		No. late-aged 3rd instars[a]	No. adults emergenced
		Min.	Max.		
Jul. 2012	1173	97	116	64143	0
(Control)	102	0	0	5706	4595
Sept. 2012	602	100	112	36541	0
Control	120	0	0	5712	4659

[a] The late third instars were collected from guava fruits within 1d after irradiation.

sugar ($F = 2.3$; $df = 6.24$; $P = 0.0618$), sucrose ($F = 1$; $df = 6.24$; $P = 0.4734$), total sugar ($F = 2.1$; $df = 6.24$; $P = 0.0946$), Vitamin C($F = 0.6$; $df = 6.24$; $P = 0.7321$), respectively. Therefore, the results will discuss the effects of irradiation treatment and storage time on the objective attributes. Data will be presented for the individual irradiation treatment and storage time effects when the interactions were significant.

3.4.1. Effects of radiation

Comparisons of the effects of irradiation at 0, 200, 400, 600 on the objective attributes of guava were presented the Figs.2–7. There was no significant difference between treatments and control in, reducing sugar ($F = 0.9$; $df = 3.24$; $P = 0.5046$) (Fig.2), sucrose ($F = 2.5$; $df = 3.24$; $P = 0.1588$) (Fig.3), total sugar ($F = 2.6$; $df = 3.24$; $P = 0.0773$) (Fig.4), soluble solids ($F = 0.4$; $df = 3.24$; $P = 0.7562$) (Fig.5), Vitamin C ($F = 2.3$; $df = 3.24$; $P = 0.1756$) (Fig.6) and titratable acids ($F = 0.6$; $df = 3.24$; $P = 0.6258$) (Fig.7), indicating that the irradiation treatment had no significant effects on the objective attributes.

3.4.2. Effects of storage time

The storage time (1, 3, 7) had significant effects on titratable acids ($F = 84.1$; $df = 2.24$; $P < 0.0001$) (Fig.7), reducing sugar ($F = 38.9$; $df = 2.24$; $P = 0.0004$) (Fig.2), sucrose ($F = 221.1$; $df = 2.24$; $P < 0.0001$) (Fig.3), total sugar ($F = 28.8$; $df = 2.24$; $P < 0.0001$) (Fig.4), respectively; while there was no significant changes in soluble solids ($F = 0.3$; $df = 2.24$; $P = 0.7308$) (Fig.5) of Vitamin C ($F = 0.8$; $df = 2.24$; $P = 0.5007$) (Fig.6). The sucrose

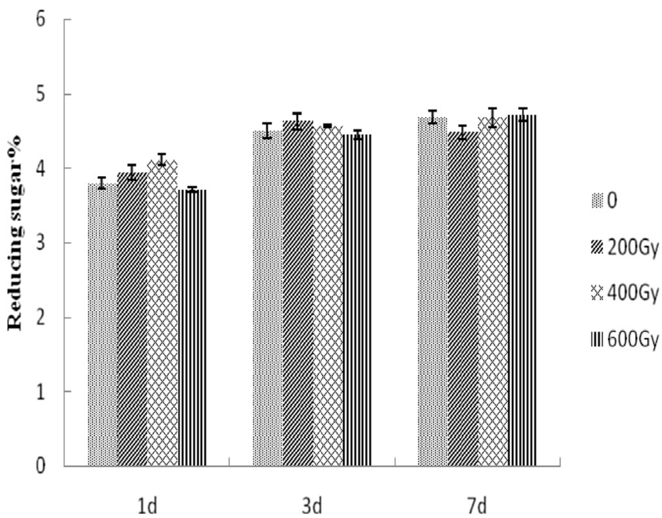

Fig. 2. Changes in reducing sugar of guava through time after irradiation at 0, 200, 400 and 600 Gy.

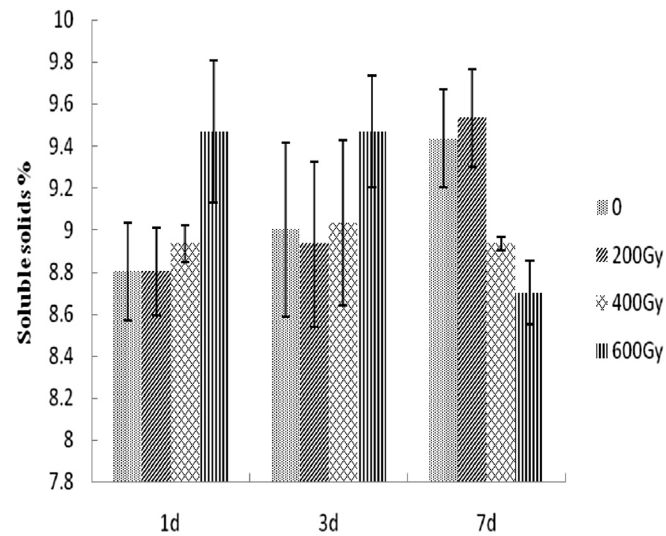

Fig. 5. Changes in soluble solids of guava through time after irradiation at 0, 200, 400 and 600 Gy.

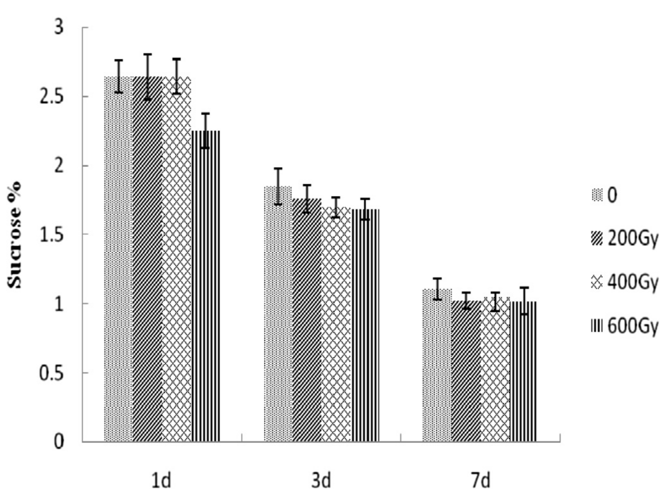

Fig. 3. Changes in sucrose of guava through time after irradiation at 0, 200, 400 and 600 Gy.

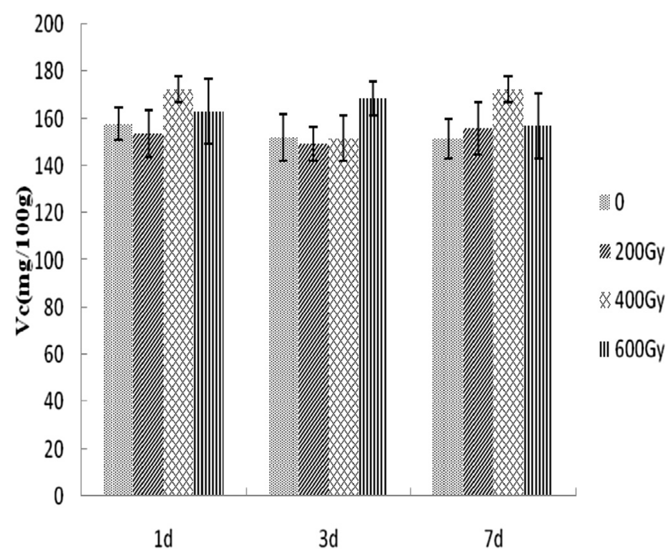

Fig. 6. Changes in Vitamin C of guava through time after irradiation at 0, 200, 400 and 600 Gy.

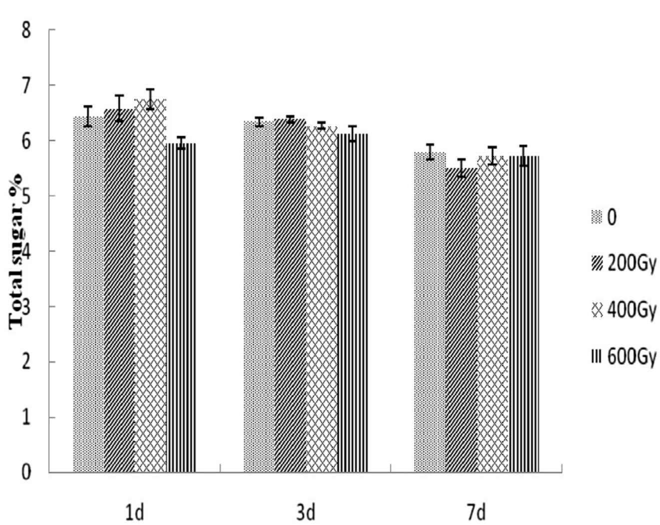

Fig. 4. Changes in total sugar of guava through time after irradiation at 0, 200, 400 and 600 Gy.

decreased significantly from 1d to 7d (Fig.3), but reducing sugar and titratable acids increased from 1d to 3d (Figs. 2 and 7), so total sugar only decreased a little.

3.4.3. *Organoleptic testing*

The results from the 12-menber panel evaluated the organoleptic characteristics of guavas were presented in Table 5. The results showed that irradiation at 1200, or 2000, or 6000Gy produced significant decreased in the scale vales for firmness ($F = 60.92$; $df = 7.72$; $P < 0.0001$), taste ($F = 51.77$; $df = 7.72$; $P < 0.0001$), and appearance ($F = 137.01$; $df = 7.72$; $P < 0.0001$), respectively. There was no significant changes in organoleptic characteristic when irradiated below 800 Gy, except the guavas turned yellow and shrinkle at 800Gy. Furthermore, the chemical and nutritional contents were not affected by the irradiation dose below 600 Gy, thus, guavas could tolerate irradiation doses up to 600–800Gy.

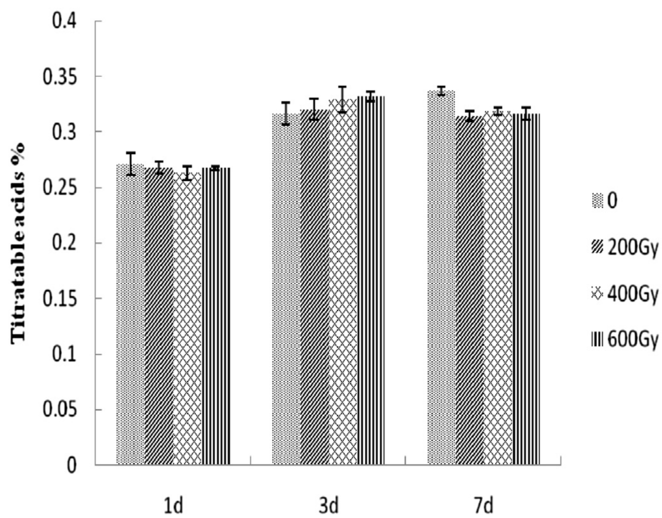

Fig. 7. Changes in titratable acids of guava through time after irradiation at 0, 200, 400 and 600 Gy.

Table 5
Organoleptic testing of guavas 7 days after irradiation.

Dose(Gy)	Organoleptic testing		
	Taste	Firmness	Apperance
0	2.96 ± 0.02a	2.92 ± 0.04a	2.95 ± 0.04a
200	2.95 ± 0.09a	2.84 ± 0.04ab	2.94 ± 0.02a
400	2.92 ± 0.10a	2.88 ± 0.04a	2.89 ± 0.04a
600	2.84 ± 0.10a	2.85 ± 0.05ab	2.72 ±0.10a
800	2.75 ± 0.09a	2.77 ± 0.07ab	2.20 ± 0.12b
1200	2.03 ± 0.18b	2.46 ± 0.14b	1.26 ± 0.13c
2000	1.67 ± 0.14c	1.79 ± 0.17c	1.07 ± 0.13c
6000	0.67 ± 0.11d	0.94 ± 0.06d	0.25 ± 0.05d

*Scale for ratings was: 3 = normal, 2 = acceptable, 1 = unacceptable, 0 = highly distasteful.
Mean vale followed by the same letters within a column are not significantly difference (P > 0.05; Tukey's multiple comparison test).

4. Discussion

To identify the most resistant stage, the relationship between dose and response for each stage of an insect is determined using a dose–response test (IPPC, 2003). In general, tolerance in *B. dorsalis* to radiation is increased with increasing age and developmental stage when comparing the efficacy on preventing eclosion. The fact that 24 Gy produce 100% preventing eclosion from irradiated 1-d-old larvae indicates that it is more sensitive to radiation than other stages, while a minimum dose of 70 Gy was required to produce 100% preventing eclosion from 7-d-od larvae present it is the most tolerant stages that likely to be presented in fruits (Table 2). The minimum dose for 100% mortality of pupae reared in artificial diets also increased with the developmental time (Fig 1). This is in agreement with the extensive review of the irradiation treatments literatures by Hallman, Levang-Brilz et al., (2010) that the most developed stage is invariably the most radiotolerant when a common measure of efficacy is used. Efficacy of an irradiation treatment against tephritids is measured by the prevention of the eclosion of adults capable of flight from third instars, the most tolerant stage in their host fruits (Hallman and Loaharanu 2002; Follett and Armstrong 2004).

Young pupae are involved principally because of extremely rare possibility that larvae might mature and pupate in packing material after fruit has been packed for shipment but before treatment (Balock, Burditt, Christiansonet al. 1963). A minimum dose of 116 Gy is sufficient for disinfestations of young pupae as a minimum dose of 100 Gy produce 100% mortality of 2-d-old pupae (Fig 1) in this study. In the previous study, a minimum dose of 95 Gy resulted in completely sterilization (failed in reproduction) of *B. dorsalis* 7-d-old pupae (Liang, Liang, Wu, Li et al., 2003).

The minimum dose for phytosanitary irradiation treatment of oriental fruit fly is decreased as the development of study, especially in the confirmatory tests. A minimum dose of 250 Gy, which was suggested by Seo, Kobayashi, Dollar, and Hanaoka (1973), was used by APHIS for the phytosanitary irradiation treatment of *B. dorsalis* (APHIS, 2000). The 250 Gy dose for *B. dorsalis* is substantially higher than that indicated by any other studies with any *Bactrocera* (Hallman and Loaharanu, 2002). Melon fly was the most tolerant of the three species to irradiation, and oriental fruit fly was more tolerant than Mediterranean fruit fly. An irradiation dose of 150 Gy (measured doses of 129–144 Gy) applied to 93,666 Melon fly late third instars in papayas resulted in no survival to the adult stage. Irradiation doses of 100 and 125 Gy was applied to 55,743 oriental fruit fly late third instars, that were reared in diet and transferred to papaya 24 h before irradiation, resulted in no survival to the adult stage (Follett and Armstrong, 2004). In this experiment, larvae were reared in guava by using natural oviposition and irradiated directly at late third instars, the methodology is closer to nature and superior.

To develop a phytosanitary irradiation treatment, late-aged third instars of *Bactrocera dordalis* should be subjected to confirmatory tests to validate the efficacy as the most tolerant stage(s) should be tested in the confirmatory tests even if it is not the most common one occurring in the commodity (IPPC, 2003, 2007). In the confirmatory tests, the target dose of 100 Gy (measured dosed of 97–116Gy) was applied to 100,684 oriental fruit fly late third instars in guavas resulted in no survival to the adult stage (Table 4), the mortality proportion (1-*Pu*) in *B. dorsalis* late 3rd instars calculated by formula (1) was 99.9970% at the 95% confidence level when counting all the irradiated late 3rd instars in the two confirmatory tests. Then, this dose is sufficient to provide quarantine security at the probit-9 level (99.9968% mortality, minimum 93,613 individuals treated with no survivors) (Couey and Chew 1986; Robertson Priestler, Frampton, 1994). The highest absorbed dose in the confirmatory tests should be used as the recommended minimum absorbed dose for a phytosanitary treatment (IPPC, 2003, Hallman, Levang-Brilz et al., 2010). Therefore, a dose of 116 Gy (Table 4) is suggested as the minimum dose for treatment of *B. dorsalis* in fruits and vegetables. These results accordingly support the assertion that relatively low doses of radiation can serve as phytosanitary treatment s against many tephritids (Hallman, 1999), and also support the proposal that a generic dose of 150 Gy for all eggs and larvae of the family of Tephritidae on all host commodities (IPPC, 2009b).

When the treatment is applied on a commercial scale, most fruit will receive 2–3 times the recommended minimum dose. Guava subjected to 3 times the minimum recommended dose found in this study will receive a maximum of about 348Gy. We did not observe detrimental effects to guava fruits at up to 600 Gy by analyzing of the nutritional contents (sugar, sucrose, total sugar, titratable acid, vitamin C, soluble solid concentrations) in irradiated and untreated guava (Figs. 2–7). There were no significant changes in organoleptic characteristic when irradiated at 200, 400, and 600 Gy (Table 5). Furthermore, Kabbashi, Nasr, Musa, Rshdi (2012) also analysis of irradiated and untreated guava for vitamin C reflected no significant difference at 2000 Gy. To facility the application of phytosanitary irradiation treatment for guavas, the effect on shelf life shall be investigated in the future. After all, a phytosanitary irradiation treatment with the minimum absorbed dose of

116 Gy would be practical as a phytosanitary treatment schedule for controlling *B. dorsalis* on all commodities in international trade.

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