Low Doses Gamma Irradiation as Quarantine Treatment for Controlling *Bactrocera zonata* (Saund, 1841) and Its Impact on Guava Fruits Quality

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Abstract: The current laboratory study investigates the effect of low doses of gamma irradiation range of (5-50 Gy) cesium cell- 137 on the different developmental stages eggs, 1st, 2nd, 3rd instars larvae and pupae of peach fruit fly, *Bactrocera zonata*. In addition to that the evaluation of phytosanitary irradiation dose on 3rd instar larvae and their effects on the biochemical quality of guava fruits were proven. The findings indicate that irradiation of eggs with dose 25Gy prevents adult emergence, while the dose 45Gy prevents pupation and the dose 50Gy prevents the hatchability of eggs. Moreover, exposure of larvae with the dose 50 Gy resulted in 98.5, 91 and 86 % mortality for the 1st, 2nd and 3rd instars larvae, respectively. No adult emergence was reported in 1st, 2nd and 3rd instars at the dose level 30, 35 and 45 Gy, respectively. The 3rd instar larvae appeared to be the most tolerant stage to radiation treatment than the 2nd and 1st larval instars. When adult emergence was used as a criterion for determination of the effective irradiation dose, 50 Gy was adopted for phytosanitary irradiation dose for *B. zonata*. Large scale confirmatory tests were applied to 25,000 3rd instar larvae of *B. zonata* in guava fruits resulting in non-F1 adults’ production with a confidence level of 91.8%. In conclusion, the irradiation dose of 50 Gy induces the inhibition of adult emergence of 3rd instar larvae and is suggested as a possible minimum dose for phytosanitary treatment of *B. zonata* fruit fly without causing significant effect (p ≤ 0.05) on some biochemical characteristics of guava fruits.

Keywords: peach fruit fly, phytosanitary treatment

Introduction

The peach fruit fly, *B. zonata* (Saund, 1841) (Diptera: Tephritidae) is a serious devastating pest that causes great losses in fruit production in many parts of tropical and subtropical regions. It has been found especially, on peach. Hence it is called the peach fruit fly. It was recorded on more than 50 plant species, mainly those with fleshy fruits (Kapoor et al. 1993, Drew 1989). This pest was officially identified and recorded for the first time in Egypt in 1998 (El-Minshawy et al. 1999). The annual losses in Egypt due to infestation by the peach fruit fly are estimated at L.E 190 million (Eppo 2005).

Guava (*Psidium guajava* Linnaeus) is an important horticulture crop. Guava fruits are often consumed fresh but are also suitable for processing into jam, juice, nectar, wine and fruit leather among other products (Kumari et al. 2017). The fruit is highly nutritious and is rich in antioxidant activity, maybe due to its high vitamin C content (the concentration is ten times higher than in orange), vitamin A,
carbohydrates, proteins, minerals, pectin, calcium and phosphorus among other nutrients and can therefore help fight malnutrition (Youssef and Ibrahim 2016). However, guava transportation to fruit markets is regulated due to the risk of fruit fly infestations (Lin et al. 2020). The economic damage is due to larvae that feed the fruit, making it soft and causing punctures and blemished fruits. Also, the damage includes not only the direct loss of yield, but also the loss of exporting markets, and the cost of establishing and maintaining phytosanitary measures (Lysandrou 2009).

Phytosanitary is that type of treatment to control quarantine pests that have an economic importance but it is not distributed in a wide range and is official managed. Advantages over other treatments include tolerance by the vast majority of fresh commodities, the ability to treat in final packaging in pallet loads, and the lack of residues (Hallman 2011). Marples & Collis (2008) suggest that a low-radiation dose can encourage favorable effects on activity of insects. Ionizing radiation such as electron beams, gamma radiation, or X-rays, are increasingly used now as a phytosanitary treatment to disinfest fresh commodities of quarantine pests (Hallman 2012).

Chemical control has been the traditional control that uses insecticide to control harmful insects. But this insecticide should penetrate the fruit to kill larvae and cause ecosystem pollution, and harmful effects on humans. The use of irradiation technique as a physical control method is cheaper, and safer, does not significantly change the quality of the vast majority of fresh commodities and can be integrated with other control methods (Gabarty et al. 2020). Fresh fruits and vegetables tolerate phytosanitary irradiation better than any other broadly-used treatment. It has been commercially applied, as a phytosanitary treatment (Hallman et al. 2016). In Egypt, there is no sufficient information about the effect of specific irradiation doses on specific insect development stages and its life cycle. Therefore, the main objectives of this work to study the impact of gamma irradiation doses on the different development stages of B. zonata and confirming the phytosanitary irradiation dose using a large-scale confirmatory test and it’s on the biochemical quality of guava fruits.

Materials and methods

Rearing technique

The original colony of Peach fruit fly, B. zonata was obtained from the Natural Products Department, National Center for Radiation Research and Technology, Cairo, Egypt.

The insect was reared under laboratory conditions at 25±2 °C and 60 ± 5% relative humidity (Gabarty et al. 2020). Adults were reared in a cage (60 × 40 × 40 cm) with a wooden frame and metal screen sides. Caged adults were provided with food consisting of sugar and protein hydrolyzate (1:3) in Petri dishes. Water was put in a small plastic bottle provided with a piece of cotton for drinking. The cage was supplied with plastic fruits containing many small pores (as an ovioposition receptacle). These plastic fruits were filled with 3 ml of water to receive and prevent the dryness of the eggs.

Irradiation process

All specimens were exposed to gamma radiation at doses of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50Gy, received from a Cesium cell-137 installed at the National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. The dose rate of the radiation was 0.663 rad/sec.

Eggs collection

Eggs were collected from plastic fruits and transferred to a plastic container. Larvae are
reared in plastic trays that contain 250 g of the artificial diet of (330 g wheat bran, 84.5 g yeast, 84.5 g sugar, 3 g sodium benzoate, 3 g citric acid, and 500 ml tap water) for feeding. Then this container is transferred to a big plastic tray that contains sand for pupation.

Artificial infestation

Guava fruits were fresh, well- ripened, healthy and free from microbial infestation or insect infestation. The collected eggs were put in a small piece of paper by brush and put in the fruit by make small hole. Each guava fruit was infested with 50 eggs that were two days old.

Irradiation of eggs in guava fruit

Each guava fruit contains 50 eggs in a card of paper. guava fruits were irradiated by 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Gy. Four guava fruit were used for each dose, where each one fruit represents one replicate. After irradiation, the guava fruit is transferred to a plastic box (25 ± 2 °C and 60–70 RH%). All eggs were examined under a microscope on a daily to check the number of hatched eggs, pupation, and adult emergence.

Irradiation of larvae in guava fruit

Each guava fruit is infested with 1st, 2nd and 3rd instar larvae. Each guava fruit contains 50 larvae as one a replicate. These fruits were irradiated by 0, 5, 10, 15, 20, 15, 20, 25, 30, 35, 40, 45, and 50 Gy. Each dose is represented in 4 replicate. After that guava fruit is transferred to a plastic container and put in control conditions (25 ± 2 °C and 60–70 RH%). All fruits were examined daily to count larval mortality, pupation, and adult emergence.

Irradiation of pupae stage in the test tube

Three and seven days old pupae were placed in a 10 cm high test tube and pupae irradiated at doses of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50Gy. Each treatment was replicated four times with 50 pupae per each. After irradiation, the pupae were transferred to a cage (60 × 40 × 40 cm) with wooden frame and metal screen sides. The percentage of adult emergence was counted.

Large-scale confirmatory tests

Confirmatory tests were done by irradiated 250 guava fruits each one contains 100 3rd instar larvae that were replicated 100 times, and 10 guava fruit, each one contains 100 3rd instar larvae were represented as control. After irradiation, all fruits were stored in a big plastic container in the suitable control condition (25 ± 2°C and 60–70 RH%) and the adult emergence was checked.

Effect of phytosanitary irradiation dose on the chemical quality of guava fruits

The samples were prepared by purchasing the guava from a local market. The fruits were washed, weighed and cut into small pieces. The juice was prepared using a laboratory blender (each 10 g were blended in 20 ml distilled water), and strained using a muslin cloth.

Quantification of total phenolic

The total phenolics in fruits was determined by Folin – Ciocalteu method as modified by (Singelton and Rossi 1965). The Folin- Ciocalteu reagent was prepared by adding 100 gm sodium tungstate, 25 gm phosphomolybdic acid, 100 ml HCl, and 50 ml Phosphotungstic acid (85%) to 700 ml deionized water in a conical flask. The flask was refluxed for 10-hours, cooled and then 150 gm lithium sulfate was added. Few drops of liquid bromine were added to make the solution yellow in color, and then the final volume was completed by deionized water to 1 liter. Two hundred microliters of guava were introduced into test tubes. 1 ml of Folin-
Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 minutes. The absorbance by spectrophotometer at 760 nm was measured against blank, containing everything except the sample. Gallic acid standard (5 gm%) was used, and the total phenolic content was expressed as mg gallic acid per gm dry weight of the original sample.

**Acidity (%)**

The acidity was estimated using the standard AOAC method, 50 ml of distilled water was added to a 10 ml fruit juice sample. 3-4 drops of phenolphthalein indicator were added. It was titrated against 0.1N sodium hydroxide. The predominant acid in guava is citric acid. The percentage of acidity was calculated using the following formula (Ranganna 2001).

\[
\% \text{ acidity} = \frac{\text{Titres value} \times \text{Equivalent weight of acid} \times 100}{\text{Value of sample taken} \times 1000}
\]

**Carbohydrates**

The weight of one hundred milligrams of the guava sample were boiled tube. This was hydrolyzed by being keep in a boiling water bath for three hours after adding 10 ml of 2.5 N HCl, and cooled at room temperature. It was Neutralized it with sodium carbonate until effervescence. The supernatant was centrifuge and collected for analysis (Sadasivam and Manickam 1991). One milliliter of dinitrosalicylic acid aid reagent was added to 100 ul extract. The contents were heated in a boiling water bath for 10 minutes. After being cooled and the absorbance of the dark red color at 510 nm. A series of glucose standards were run to plot the standard curve.

**Vitamin C**

Vitamin C or ascorbic acid method (A.O.A.C. 1975) is based on measuring the extent to which, a 2,6-dichlorophenol-indophenol dye solution is decolorized by the presence of the ascorbic acid. To dry test tubes, pipette the requisite volume of sample or standard ascorbic acid solution and make up 5 ml with 2 % HPO3 (Metaphosphoric acid). Add 10 ml of the dye solution (0.02 %) with rapid delivery pipette, shake and take the reading within 15-20 second. The red color was measured at 518 nm against blank consisting of 5 ml 2% HPO3 and 10 ml of water.

**Statistical analysis**

One-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, version 17.0) was used in analyzing the experimental data and the significance among the samples was compared at P ≤ 0.05. Results were represented as mean ± SE.

**Results**

The obtained results in Table 1 indicate that the percentage of hatched eggs was 96% in the control group. Radiation treatment of eggs reduced hatchability and the reduction increased with the increasing radiation dose. The percentage of eggs hatching and pupation at the lowest dose (5 Gy) were 30 and 91.6%, respectively. At the radiation treatment of 10 Gy, eggs’ hatchability and pupation were decreased to 24.5 and 81.6%, respectively. Adult emergence was 87%. While, the dose of 15 Gy reduced eggs hatchability to 21.5% and pupation was decreased to 74.4%. Radiation treatment at 50 Gy completely prevented the egg hatching and consequently stopped pupation and adult emergence (Table 1).
### Table 1: Effect of gamma irradiation on egg hatching, pupation and adult emergence of *B. zonata* in guava fruit.

| Dose /Gy | No. hatched eggs Mean ±SE | Hatchability % | No. Pupae Mean ±SE | Pupation % | No. Emerging Adult Mean ±SD | Adult emergence % |
|----------|---------------------------|----------------|-------------------|------------|-----------------------------|------------------|
| C        | 48 ± 1.08*                | 96             | 47.25±0.853*      | 98.4       | 46±0.707*                   | 97               |
| 5        | 15 ± 1.58*                | 30             | 13.75±0.75*       | 91.6       | 12.25±0.85*                 | 89               |
| 10       | 12.25 ± 0.629*            | 24.5           | 10±0.408*         | 81.6       | 8.5±0.64*                   | 87               |
| 15       | 10.75 ± 0.478*            | 21.5           | 8±0.408*          | 74.4       | 5.75±0.62*                  | 65               |
| 20       | 6.75 ± 0.478*             | 13.5           | 4.75±0.478*       | 70.3       | 1.5±0.288*                  | 31.5             |
| 25       | 5 ± 0.408*                | 10             | 2.75±0.408*       | 60         | 0±0.00*                     | 0                |
| 30       | 3.5 ± 0.64*               | 7              | 2±0.408*          | 57         | 0±0.00*                     | 0                |
| 35       | 3 ± 0.577*                | 6              | 1.5±0.645*        | 50         | 0±0.00*                     | 0                |
| 40       | 1.75 ± 0.478*             | 3.5            | 0.5±0.288*        | 28         | 0±0.00*                     | 0                |
| 45       | 0.5 ± 0.288*              | 1              | 0±0.00*           | 0          | 0±0.00*                     | 0                |
| 50       | 0 ± 0.00*                 | 0              | 0±0.00*           | 0          | 0±0.00*                     | 0                |

*The mean difference is significant at the 0.05 level compared with control, The No. of eggs in each replicate were 50.

### Table 2: Effect of gamma irradiation on 1st larval instars of *B. zonata* in guava fruit.

| Dose /Gy | No. larvae dead Mean ±SE | Larval Mortality % | No. pupae Mean ±SE | Pupation % | No. Emerging adult Mean ±SE | Adult Emergence % |
|----------|---------------------------|--------------------|-------------------|------------|-----------------------------|------------------|
| C        | 2±0.408*                  | 4                  | 48±0.40*          | 96         | 46.75±0.85*                 | 97.39            |
| 5        | 22.25±1.31*               | 44.5               | 27.75±1.31*       | 55.5       | 22.5±1.65*                  | 81.08            |
| 10       | 25.25±0.62*               | 50.5               | 24.75±0.62*       | 49.5       | 18.25±0.85*                 | 73.7             |
| 15       | 27.25±0.478*              | 54.5               | 22.75±0.478*      | 45.5       | 16±0.70*                    | 70.32            |
| 20       | 33±0.816*                 | 66                 | 17±0.81*          | 34         | 10.75±0.47*                 | 63.2             |
| 25       | 34.5±1.32*                | 69                 | 15.5±1.32*        | 31         | 9±0.40*                     | 58               |
| 30       | 39.25±0.85*               | 78.5               | 10±1.04*          | 21         | 0±0.00*                     | 0                |
| 35       | 41±0.91*                  | 82                 | 9±0.91*           | 18         | 0±0.00*                     | 0                |
| 40       | 45.75±0.75*               | 90.5               | 4.25±0.75*        | 8.5        | 0±0.00*                     | 0                |
| 45       | 48.5±0.64*                | 97                 | 1.5±0.64*         | 3          | 0±0.00*                     | 0                |
| 50       | 49.25±0.47*               | 98.5               | 0.75±0.47*        | 1.5        | 0±0.00*                     | 0                |

*The mean difference is significant at the 0.05 level compared with control, The No. of larvae in each replicate were 50.

The mortality percentage of the 1st larval instar in the control group was 4%, pupation was 96% and adult emergence was 97.39%. The lowest irradiation dose 5 Gy resulted in 44.5% larval mortality and only 55.5% pupation. A adult emergence was 81.08%. The dose 10 Gy induced 50.5% larval mortality and reduced pupation to 49.5 and adult emergence to 73.7%. While at the dose level 30Gy, Larval mortality was 78.5%, pupation was 21% but it prevents adult emergence (Table 2). The data in Table 3 shows that the percentage of the 2nd larval instar mortality in the control was 2%, pupation was 98% and adult emergence was 98%. The irradiation dose 5 Gy resulted in 23% larval mortality, 77% pupation, and adult emergence was 87%. Treatment with the dose level 10 Gy resulted in 29% larval mortality, 71% pupation, and adult emergence reduced to 85.9%. At the dose 35 Gy larval mortality increased to 74 %, pupation was reduced to 25.5, and no adult emergence. The data in Table 4 indicates that the irradiation dose 5 Gy resulted in 19%, pupation 81.5%, and adult emergence 91%. While the dose 45 Gy resulted in 81% larval mortality and 19% pupation and prevented adult emergence. The 3rd instar appeared to be the most tolerant to radiation treatment than the 2nd instar, and the 3rd instar was more tolerant to radiation treatment. 45 Gy was required, when adult emergence was used as a criterion for measuring the effective irradiation dose.
Table 3: Effect of gamma irradiation on 2nd larval instars of *B. zonata* in the guava fruit.

| Dose /Gy | No. larvae dead Mean ±SD | Larval Mortality % | No. pupae Mean ±SD | Pupation % | No. Emerging adult Mean ±SD | Adult Emergence % |
|----------|--------------------------|--------------------|--------------------|------------|-----------------------------|-------------------|
| C        | 1±0.47*                  | 2                  | 49±0.408*          | 98         | 48.25±0.47*                 | 98                |
| 5        | 11.5±0.64*               | 23                 | 38.5±0.64*         | 77         | 33.5±0.64*                  | 87                |
| 10       | 14.5±0.64*               | 29                 | 35.5±0.645*        | 71         | 30.5±0.64*                  | 85.9              |
| 15       | 19.5±0.64*               | 39                 | 30.5±0.645*        | 61         | 25.5±0.64*                  | 83.6              |
| 20       | 23.5±0.64*               | 47                 | 26.5±0.645*        | 53         | 19.5±3.02*                  | 73.5              |
| 25       | 29.5±0.64*               | 59                 | 20.5±0.645*        | 41         | 13.5±0.64*                  | 65.8              |
| 30       | 32.75±1.108*             | 65.5               | 17.25±1.10*        | 34         | 8.5±0.64*                   | 49.2              |
| 35       | 37.25±0.853*             | 74                 | 12.75±0.85*        | 25.5       | 0±0.00*                     | 0                 |
| 40       | 40.5±0.64*               | 81                 | 9.5±0.64*          | 19         | 0±0.00*                     | 0                 |
| 45       | 43.75±0.47*              | 87.5               | 6.5±0.47*          | 12.5       | 0±0.00*                     | 0                 |
| 50       | 45.5±0.64*               | 91                 | 4.5±0.64*          | 9          | 0±0.00*                     | 0                 |

*The mean difference is significant at the 0.05 level compare with control, The No. of larvae in each replicate were 50.

Table 4: Effect of gamma irradiation on 3rd larval instars of *B. zonata* in guava fruit.

| Dose /Gy | No. larvae dead Mean ±SD | Larval Mortality % | No. pupae Mean ±SD | Pupation % | No. Emerging adult Mean ±SD | Adult Emergence % |
|----------|--------------------------|--------------------|--------------------|------------|-----------------------------|-------------------|
| C        | 0.25±0.25*               | 0.5                | 49.75±0.25         | 99.5       | 49.75±0.25                 | 100               |
| 5        | 9.5±0.64*                | 19                 | 40.5±0.64          | 81.5       | 37.25±0.85                 | 91                |
| 10       | 12.5±0.64*               | 25                 | 37.5±0.64          | 75         | 33.5±0.64                  | 89                |
| 15       | 16.5±0.64*               | 33                 | 33.5±0.64          | 67         | 28.5±0.64                  | 85                |
| 20       | 21.5±0.64*               | 43                 | 28.5±0.64          | 57         | 22.75±0.85                 | 79.8              |
| 25       | 25.5±0.64*               | 51                 | 24.5±0.64          | 49         | 14.5±0.64                  | 74                |
| 30       | 28.5±0.64*               | 57                 | 21.5±0.64          | 43         | 8.5±0.64                   | 61.8              |
| 35       | 31.75±0.85*              | 63.5               | 20.75±0.86         | 41.5       | 4.5±0.64                   | 42.8              |
| 40       | 37.5±0.64*               | 75                 | 12.5±0.64          | 25         | 1.5±0.28                   | 23                |
| 45       | 40.5±0.64*               | 81                 | 9.5±0.64           | 19         | 0±0.00                     | 0                 |
| 50       | 43±1.08*                 | 86                 | 7±1.08             | 14         | 0±0.00                     | 0                 |

*The mean difference is significant at the 0.05 level compare with control, The No. of larvae in each replicate were 50.

Table 5: Effect of gamma radiation on three and seven days old pupae of *B. zonata*.

| Dose /Gy | No. irradiated pupae Mean | 3 days old | 7 days old |
|----------|---------------------------|------------|------------|
|          |                           | No. Emerging Adult Mean ±SD | Adult Emergency % | No. Emerging Adult Mean ±SD | Adult Emergency % |
| C        | 50                        | 49.7±0.5* | 99.5       | 50±0.0* | 100 |
| 5        | 50                        | 47.5±1.29* | 95 | 49.75±0.50* | 99.5 |
| 10       | 50                        | 45.7±1.29* | 91.5 | 48±0.81* | 96 |
| 15       | 50                        | 42.2±0.95* | 84.5 | 47±0.81* | 94 |
| 20       | 50                        | 39.5±0.95* | 79 | 46±0.81* | 92 |
| 25       | 50                        | 37.5±1.29* | 75 | 43.5±1.29* | 87 |
| 30       | 50                        | 33.5±1.29* | 67 | 41.25±0.95* | 82.5 |
| 35       | 50                        | 30.7±1.29* | 61.5 | 38±0.81* | 76 |
| 40       | 50                        | 28.7±0.95* | 57.5 | 35±0.81* | 70 |
| 45       | 50                        | 25.5±0.95* | 51 | 32±0.81* | 64 |
| 50       | 50                        | 21.5±1.29* | 43 | 28.5±1.29* | 57 |

*The mean difference is significant at the 0.05 level compare with control, The No. of larvae in each replicate were 50.
The data in Table 5 shows that irradiation dose (5Gy) resulted in 95% adult emergence in 3-day old pupae, and 99.5% in 7-day old pupae. Dose 10Gy shows 91.5% adult emergence in 3-day old pupae, and 96% in 7-day old day pupae. At 20Gy adult emergence was 79% in 3-day old pupae and 92% in 7-day old pupae. At 40Gy adult emergence was 57.5% in 3-day old pupae and 70% in 7-day old pupae. This result refers to 7-day old pupae were more resistance to radiation than 3-day old pupae. The large-scale confirmatory experiment, resulting from the radiation treatment estimated 25,000 late 3rd instar at 50 Gy produced no F1 adults. (Table 6). The efficacy required was C=1-(1–0.0001)25,000 and the confidence level was 91.8% while the true survival of B. zonata was less than 0.0001.

Table 6: Large-scale confirmatory tests of irradiating 3rd larval instars in the host fruit of B. zonata.

| Dose/Gy | No/replicates | No/treated | No/F1 adult |
|---------|---------------|------------|-------------|
| 50Gy    | 250           | 25,000     | 0           |
| C       | 10            | 1000       | 975         |

Each one replicate contains 100 3rd instar larvae.

The results in Table 7 shows of guava fruits acidity, carbohydrate, glucose, reducing sugar, total phenol and vitamin C, the content of the juice extracted from the control group and irradiated guava fruits with 50 Gy. It is clear that the irradiation dose of 50 Gy almost did not effect on the quality attributes of guava as there was no change in different parameters, compared to the control group.

Table 7: Effect of phytosanitary irradiation dose (50 Gy) on chemical quality attributes of guava fruits.

| Parameters        | Unirradiated Control | Irradiated Guava by 50Gy |
|-------------------|----------------------|--------------------------|
| Acidity           | 0.67 ± 0.02          | 0.74 ± 0.02              |
| Carbohydrate      | 190.8 ± 7.05         | 189.5 ± 7.9              |
| Glucose           | 17.9 ± 0.753         | 23.7 ± 0.89              |
| Reducing sugar    | 75.7 ± 1.27          | 83.8 ± 2.19              |
| Total phenol      | 6.07 ± 0.23          | 4.9 ± 0.09               |
| Vitamin C         | 0.32 ± 0.006         | 0.302 ± 0.006            |

The mean difference is significant at the 0.05 level compare with control

Discussion

To investigate the efficacy gamma irradiation as phytosanitary treatment against Bactrocera zonata fruit flies, the relationship between dose and response for each stage of an insect is determined using a dose–response test (IPPC 2003). Irradiation of eggs with 25Gy prevents the adult emergence, while irradiation of eggs with 45 Gy prevents pupation and irradiation with 50 Gy prevents hatchability Table 1. This result is in agreement with Zhan et al. (2015) that found that a radiation dose of >35 Gy produced the lowest pupation rate and 100% prevention emergence from irradiated 1-d-old eggs of B. tau (Walker1849) indicates that it is more sensitive to radiation than other stages. On the other hand, Gabarty et al. (2020) found that irradiation of 1-d-old eggs of B. zonata with 100 Gy prevent the adult emergence. In general, the irradiation dose ≤ 100 Gy prevent adult emergence from irradiated 1-d-old eggs, with taking into consideration the difference in the dose/rate of the irradiated source used. Moreover, the percentage of the larval mortality in 1st, 2nd, 3rd instar larvae significantly increased by increasing the irradiation dose (Tables 2, 3, and 4), while pupation and adult emergence percentage significantly decreased. It is also clear that the 3rd instar larvae were more resistant. These results are in agreement with the previous finding, recorded by (Hallman et al. 2010; Gabarty et al. 2020).

This study shows that the irradiation dose 50Gy induces 98.5, 91, and 86 % larval
mortality for the 1st, 2nd and 3rd instars, respectively, and no 100 % larval mortality in any dose. But no adult emergence in the 1st, 2nd, and 3rd instars larvae at the dose level 30, 35, and 45 Gy, respectively. This is in agreement with the extensive review of the irradiation treatments literatures by Yusof et al. (2019) that 100 % larval mortality of B. dorsalis instars needs the radiation dose levels more than 250 Gy but prevention of adult emergence from irradiated larval instars required only 150 Gy. Zahran et al. (2013) finds out that tolerance to radiation increased with increasing age and due to the development of insects. The sequence of the tolerance to radiation in the stages of B. zonata is as: third instars> second instars> first instars> eggs. Therefore, the third instars were determined to be the most tolerant stage in guva fruits. Findings of this study shows that at a dose level, 45 Gy, all the pupae resulted from irradiated 3rd larvae instar were completely dead and failed to emerge as an adult. Therefore, this dose also meets the recommended generic dose, adopted for phytosanitary irradiation for 3rd instar larvae. This is in agreement with Hallman et al. (2010) that the most developed stage is invariably the most radio-tolerant when a common measure of efficacy is used. Fruit fly third instars are the most tolerant stage in their host fruits when conducting radiation tests (Hallman and Loaharanu 2002). Therefore, B. zonata third instars should be treated to validate the efficacy as the most resistant stage should be tested in the confirmatory tests even if it is not the most common one occurring in the commodity (IPPC 2003, 2007).

An irradiation treatment efficacy against tephritids is measured by the prevention of the emergence of adults capable of flight when irradiated as third instars inside fruit (Hallman and Loaharanu 2002, Follett and Armstrong 2004, Gabarty et al. 2020). In the confirmatory test, the target dose was ordinarily based on the statistical analysis of the dose–response data, where probit analysis was widely used for a number of fruit flies (Heather et al. 1991, Mansour and Franz 1996, Hallman and Thomas 2010, Bustos et al. 2004, Gabarty et al. 2020). Therefore, a radiation dose of 50 Gy was selected as target dose to conduct the confirmatory tests. In total, 25,000 third instars developed in guva fruits were irradiated in confirmatory tests, and as a result there was no adult emergence with a confidence level 91.8 % (Table 6).

The effect of gamma irradiation on three-d-old pupae and seven-d-old pupae were estimated in this study (Table 5). The adult emergence from irradiated pupae decreased with increasing dose, but 3-d-old pupae was more sensitive to irradiation than seven-d-old pupae. This results in agreement with. Mahmoud and Barta (2011), Zahran et al. (2013) and Gabarty et al. (2020) on B. zonata. It was important to test the phytosanitary dose 50 Gy to determine if it a harmful effect on the quality of the guava. In our research, it is shown that 50 Gy had no harmful effect on (acidity, carbohydrate, glucose, reducing sugar, total phenol, and vitamin C) compared to the control. Hossain et al. (2014) reports that 1.0 kGy radiation was most effective to the delay ripening, resulted in extending shelf life of guava. Sau et al. (2018) finds that exposed fresh guava to four doses of gamma-radiation (0, 100, 200, and 300 Gy) using Cobalt-60. They report that the irradiation of guava fruits with 200 Gy gamma radiations significantly increased the post-harvest life (93.8%) without any negative impacts on fruit quality (firmness, titratable acidity, soluble solids content, and vitamin C).

**Conclusion**

In comparison to the pervious study Gabarty et al. (2020) which used Indian Co-60 gamma Chamber, with the average dose rate 1.277 kGy /h (21.3 Gy needs 1 min.). the phytosanitary dose for the 3rd instar larvae of B.zonata was 150 Gy. In this study, a Cesium cell-137 gamma Chamber with the average
dose rate 0.663 rad/sec (1Gy needs 2.9 min) was used. So, using a lower dose rate, and longer irradiation time can be induced adverse effects on B.zonata. A dose of 50 Gy which gives the efficacy of inhibition of adult emergence of 3rd instar larvae is suggested as a possible minimum dose for phytosanitary treatment of B. zonata fruit fly. This dose was effective and sufficient to provide quarantine security for export/ import guava fruits without significant effects on guava fruits quality.

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