Impact of Electron Beam Irradiation on the Developmental Stages of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and *Bactrocera zonata* (Diptera: Tephritidae)[1]

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Abstract An eco-friendly electron beam irradiation (EBI) technology was used to assess the effect of EBI on the different development stages of *Callosobruchus maculatus* (F.) and *Bactrocera zonata* (Saunders). No adults emerged after 3-d-old eggs of *C. maculatus* were irradiated with 304.8 Gy of EBI, and no adults emerged from the F_1 generation of larvae and pupae irradiated with 103.6 Gy. The adult stage of *C. maculatus* was the most tolerant of EBI, with a high dose (414.3 Gy) irradiation of the adult stage resulting in complete prevention of the F_1 generation adult emergence. Large-scale tests confirmed that 414.3 Gy was an effective dose for the phytosanitary and security treatment for *C. maculatus*. An EBI dose of 414.3 Gy prevented *B. zonata* egg hatching. EBI doses of 304.8, 414.3, and 653.5 Gy prevented the development of first, second, and third larval instars to pupation, respectively. Using the criterion of adult emergence from the F_1 generation, targeting the third larval stage, considered the most tolerant stage to EBI, a dose of 304.8 Gy was effective for quarantine and security treatment of *B. zonata*.

Key Words electron beam irradiation, *B. zonata, C. maculatus,* phytosanitary treatment

Most food and agriculture products irradiated in the world are treated in facilities using gamma radiation from ^{60}Co as a source of ionizing radiation. The recent development of high-power and high-energy accelerators and X-rays has made electron beams and X-ray machines available as an alternative to gamma radiation for treating food. Electron beam irradiation (EBI) technology has many advantages; it is a continuous process, environmentally friendly, leaves no residues, lacks development of resistance, and causes few changes in the quality attributes of the irradiated products. In addition, the production of ionizing radiation by accelerators uses electricity instead of radioactive isotopes, allowing it to be turned on and off as needed (Ahmed 2001, Hallman 2011, Stewart 2001).

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Among the different species of insects infesting various pulses (i.e., edible seeds of leguminous plant) are the pulse beetles, *Calosobruchs* spp., which are major insects in pulse storage. Generally, infestations start in the field, but the population increases in storage as the insects feed inside the seeds and emerge as adults resulting in secondary infestations and heavy losses (Bhalla et al. 2008). The cowpea weevil, *Callosobruchus maculatus* (F.), is a cosmopolitan field-to-store pest ranked as the principal pest of several pulses including chickpea (*Cicer arietinum* L.) and cowpea (*Vigna unguiculata* [F.]).

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious polyphagous insect pest in several countries which attacks a wide range of host plants, including chickpea and cowpea. This pest was officially identified and recorded for the first time in Egypt in 1998 (El-Minshawy et al. 1999) and has since become widespread in most Egyptian provinces causing severe damage to host plants.

EBI is an alternative technique that uses ionizing radiation in the treatment of agricultural commodities and food products to eliminate microbial contamination and insect pests. Generally, too little research has been conducted on the effect of EBI against insect pests infesting agricultural products. Imamura et al. (2004) studied the effect of EBI on the control of cut flower pests and found that an electron beam with an energy of 2.5 MeV at 400 Gy inactivates all pests of cut flowers that were tested. Soft-electron beam at 60 KeV effectively inactivated eggs, larvae, pupae of red flour beetle (*Tribolium castaneum* [Herbst]) and the Indian meal moth (*Polodia interpunetella* [Hübner]) and eggs of the adzuki bean weevil (*Callosbruchus chinensis* [L.]) at a dose of 1 kGy. Al-Farisi et al. (2013) reported that EBI at 0.5, 1.0, and 2.0 kGy eliminated all stages of *Oryzaephilus surinamensis* (L.) in dates. Also, Hosseinzadeh et al. (2010) recommended EBI between 600 and 700 Gy for controlling populations of *O. surinamensis*.

Follett et al. (2013) found that treatment of *Sitophilus oryzae* (L.) in rice with X-ray at 120 Gy halted the development of rice weevil and sterilized its adult, thus providing quarantine security. Sang et al. (2016) found that the early and intermediate stages of *C. maculatus* eggs never hatch after exposure to an EBI dose of 30 Gy, whereas a substantial portion of black-headed (i.e., late-stage) eggs survived EBI. Chizh et al. (2018) reported that *O. surinamensis* infesting grains were completely inhibited after exposure to low-energy EBI at a doses ranging from 200 to 1,000 Gy.

To our knowledge, very few (if any) studies have been conducted on the effect of EBI on all development stages of *C. maculatus* and *B. zonata*; thus, the main objectives of our study were to (1) evaluate the effect of different EBI doses on the developmental stages of *C. maculatus* and *B. zonata* and (2) determine the most radio-tolerant stage and establish the EBI phytosanitary irradiation efficacy through large-scale confirmatory tests.

Materials and Methods

EBI was performed at an electron beam accelerator (ICT, Vivirad Co., Handschuheim, France) located at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The details of general electron

Table 1. General operating parameters and operating parameters during irradiation treatment.

Operating Parameters	General Operating Parameters	Operating Parameters During Irradiation Treatment
Beam energy, MeV	Up to 3	2.50
Beam current, mA	Up to 30	0.50
Beam Power, kW	90	1.25
Scan width, cm	90	90
Distance between scanner and conveyor system, cm	53	53
Scan speed, m/h	Up to 16	6–16

beam parameters and operating parameters during irradiation treatment are given in Table 1. A single layer of infested cowpea samples or artificial feeding medium (5 g) were spread on the bottom in small plastic boxes (5 × 5 × 3 cm). Small holes were punched in the sides for air movement. These small boxes were then arranged in a large plastic box (36 × 36 × 15 cm) covered with a plate (8 mm) of polymethyl methacrylate to attenuate the radiation field to reach the desired doses of 100, 125, 300, 400, 500, and 650 Gy. Gafchromic™ HD-V2 film dosimetry (dynamic dose range 10 to 1,000 Gy; lot 01091801) was used. HD-V2 films were calibrated at the ^{60}Co-Gamma Cell 220 Excel irradiation unit (MDS Nordion, Ottawa, Canada) at NCRRT. The absorbed dose rate of water at the center of the gamma cell was calibrated by the National Physical Laboratory in England using a transfer alanine dosimeter. The HD-V2 was calibrated in this gamma cell at the center in the dose range of 10 to 1,000 Gy and measured at 660 and 605 nm using a ultraviolet-visible (UV-V) spectrophotometer (UVICON 860, Kontron Co. Ltd., Rotkreuz, Switzerland). The obtained calibration curve was then used to measure the absorbed dose imparted to samples of cowpea seeds or artificial feeding medium during EBI. To establish the calibration curve, the absorbance of 10 nonirradiated film dosimeters was measured at 660- and 605-nm wavelengths (to determine the background absorbance), and then several HD-V2 films were irradiated at different doses ranging from 10 Gy to 1 kGy (five dosimeters at each dose). The absorbance was measured at the same wavelengths of 660 and 605 nm. The thin-film alanine was used to compare the absorbed dose measured by HD-V2 films in an electron beam for the doses higher than 500 Gy. For every treatment, HD-V2 films were placed with the infested cowpea or artificial feeding medium during irradiation and read by the UV-V spectrophotometer, which was used to measure the actual absorbed dose.

Laboratory rearing of the *C. maculatus* colony followed techniques of the Entomology Laboratory at Plant Protection Research Institute, Dokki, Giza, Egypt, and those according to Hammad et al. (2020). For the EBI of the *C. maculatus* egg stage, 5 g of cowpea seeds were infested by five pairs of tested insects. After 3 d, the adults were removed, then the cowpea seeds, which were infested with 3-d-old eggs, were exposed to doses of 0.0, 100, 125, 300, 400, 500, and 650 Gy, which corresponded to the measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy of the electron beam. The treatments were replicated five times. The irradiated 3-d-old eggs in each replicate were counted. Moreover, the hatch and adult emergence percentages were estimated. For the EBI of *C. maculatus* larval and pupal stages, 400 g of cowpea seeds were infested with 200 insects. Three days later, the cowpea seeds were sieved and the weevils were discarded. Five grams of infested cowpea were separated for each treatment. After 13 d of infestation, insects were treated at the doses of 0.0, 100, 125, 300, 400, 500, and 650 Gy as larvae. After 17 days of infestation, insects were treated by 0.0, 100, 125, 300, 400, 500, and 650 Gy, which corresponded to the measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy as pupae. Adult emergence and reduction percentage from irradiated larvae and pupae were estimated. Five pairs (males and females) resulted from each irradiated dose; these were separated and placed on 3 g of cowpea. The infested cowpea was stored in a controlled environment cabinet (28 ± 2°C and 75 ± 5% relative humidity [RH]). The adult weevils that emerged (F_1 progeny) were counted, and the percentage of insect reduction was estimated. For the EBI of the *C. maculatus* adult stage, 5 g of cowpea seeds were infested by 25 pairs of *C. maculatus*. Adults (1 d old) were exposed to 0.0, 100, 125, 300, 400, 500, and 650 Gy, corresponding to the measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy of the electron beam (five replicates per treatment). The mortality percentage of irradiated adults was estimated after 24 h. Five living pairs resulting from each irradiated dose were separated and placed on 3 g cowpea seeds. Three days later, the cowpea was sieved in each treatment, and the adult weevils were discarded. The eggs were counted in each treatment, and the number of emerging adults was estimated. Large-scale confirmatory tests were conducted by treating a large number of cowpea weevil adults with an EBI dose of 414.3 Gy. Approximately 700 adults (1 –d old) were counted and placed on 5 g cowpea seeds (40 replicates). In controls, 500 adults were counted and placed on 5 g of cowpea seeds (10 replicates). Adults were removed from the containers 7 d later, and the treated and untreated cowpea seeds were held for an additional 60 d for the emergence of F_1 adults.

The peach fruits fly used in the study were obtained from a continuously reared strain (under conditions of 25 ± 2°C and 60–70% RH) and maintained in the Entomology Laboratory at Plant Protection Research Institute, Dokki, Giza, Egypt. The rearing protocol was that of Gabarty et al. (2020). For the EBI of *B. zonata* egg stage, 5 g of artificial medium in plastic vials was infested with 1-d-old eggs. The artificial medium infested with eggs was irradiated at doses of 0.0, 100, 125, 300, 400, 500, and 650 Gy, corresponding to the measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy. Each treatment had five replicates with 100 eggs each. After irradiation, the plastic vials were stored in a controlled environmental cabinet (25 ± 2°C and 60–70% RH). All eggs were examined under a microscope daily to check the number of hatched eggs, pupation, and adult emergence. For the EBI of *B. zonata* larvae stage, 5 g of the artificial medium in plastic vials was infested with first, second, and third instars. The artificial medium infested with larvae was irradiated at the doses of 0.0, 100, 125, 300, 400, 500, and

650 Gy, corresponding to the measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy. Each treatment had five replicates with 100 larvae each. After irradiation, the plastic vials were stored in a controlled-environment cabinet (25 ± 2°C and 60–70% RH). All infested artificial medium was examined daily to check larval mortality and the percentage of pupation, and adult emergence was estimated. For the EBI of the pupal stage of *B. zonata*, 3- and 7-d-old pupae were placed in a plastic vial. The pupae were irradiated at the doses of 0.0, 100, 125, 300, 400, 500, and 650 Gy, corresponding to measured doses of 0.0, 103.6, 123.3, 304.8, 414.3, 488.3, and 653.5 Gy. Each treatment had five replicates with 50 pupae each. After irradiation, the pupae were transferred to a cage ($6 \times 40 \times 40$ cm) with a wooden frame and metal screen sides. The percentage of adult emergence was estimated. Large-scale confirmatory tests were conducted by treating a large number of third instar larvae with an EBI dose of 304.8 Gy. Approximately 250 larvae were counted and placed on 5 g of artificial medium in plastic vials (85 replicates). In the controls, 250 larvae were counted and placed on 5 g of artificial medium in plastic vials (five replicates). All irradiated and nonirradiated infested artificial media were stored in a controlled-environment cabinet at 25°C ± 2 and 70 ± 5% RH and observed daily to monitor adult emergence of the F_1generation.

Statistical analysis. One-way analysis of variance using SPSS (ver.17.0) was used to analyze the experimental data, and significance among the samples were compared at $P \leq 0.05$. Results are presented as mean ± SD ($n = 5$). In case of the large-scale confirmatory tests, the level of confidence associated with treating some insects with zero survivors is given by the equation of Couey and Chew (1986). Confidence levels were calculated as 1 - (1 - pu)n, where pu is the acceptable level of survivorship (0.0001) and n is the number of treated insects.

Results

In general, the mean percentage of 3-d-old egg hatching and adult emergence of *C. maculatus* in cowpea seed samples significantly decreased with increasing EBI dose in comparison with nonirradiated (0.0 Gy) control samples (Table 2). The irradiation dose of 103.6 Gy decreased egg hatching from 90% in the nonirradiated control to 51%, whereas adult emergence decreased from 88% in control samples to 57.1%. The irradiation dose of 304.8 Gy decreased egg hatching to 39.8%, while completely preventing adult emergence. The highest irradiation dose (653.5 Gy) prevented egg hatching.

The number of emerging adults from irradiated larvae significantly decreased as the irradiation dose increased (Table 3). The irradiation dose of 103.6 Gy reduced adult emergence by 89.2%, and completely halted adult emergence of the F_1 generation. An irradiation dose of 304.8 Gy was required to prevent adult emergence of P_1 generation when the larval stage was irradiated.

As the irradiation dose applied to the pupal stage increased, the number of emerging adults significantly decreased (Table 4). The lowest irradiation dose of 103.6 Gy completely prevented adult emergence of the F_1 generation. No emerging adults of the P_1 generation were observed when pupae had been irradiated at 414.3 Gy. As irradiation dose increased, the percentage of adult mortality significantly

Table 2. Effect of electron beam irradiation on 3-d-old eggs of *C. maculatus*.

Dose, Gy	No. of Eggs Irradiated, Mean ± SD	No. of Hatched Eggs, Mean ±SD	Hatchability, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	25.8 ± 5.2	23.2 ± 4.5	90	20.4 ± 3.8	88
103.6	22 ± 2.5	11.2 ± 2.6*	51	6.4 ± 2.3*	57.1
123.3	22.4 ± 3.9	10.2 ± 2.9*	45.5	5.2 ± 2.7*	51
304.8	20.6 ± 2.7	8.2 ± 2.4*	39.8	0 ± 0	0.0
414.3	26.2 ± 3	6.6 ± 2.2*	25.2	0 ± 0	0.0
488.3	25.8 ± 5.3	3.4 ± 1.1*	13.2	0 ± 0	0.0
653.5	23.8 ± 7.7	0.0 ± 0.0*	0.0	0 ± 0	0.0

* The mean difference is significant at the 0.05 level compared with control.

increased (Table 5). The irradiation dose of 414.3 Gy completely prevented eggs from being laid and adult emergence of the F_1 generation. The highest irradiation dose (488.3 Gy) increased the adult mortality to 44.4%, but the EBI dose of 414.3 Gy prevent the adult emergence so this indicating that the adult stage was the most tolerant to EBI. Accordingly, the adult stage is the most radio-tolerant stage, and prevention of an F_1 generation is used for measuring the effective EBI dose of 414.3 Gy required as phytosanitary irradiation dose. This irradiation dose could be used for quarantine treatment to control *C. maculatus* in cowpea seeds. The irradiation dose of 414.3 Gy, which prevented the adult emergence, was applied to 28,000 *C.*

Table 3. Effect of electron beam irradiation on the larval stage of *C. maculatus*.

Dose, Gy	No. of Emerging Adults From Irradiated Larvae, Mean ± SD	Reduction, %	No. of Emerging Adult Progeny (F_1 Generation), Mean ± SD	Reduction (F_1 Generation), %
0 (Control)	31.4 ± 9.1	0.0	52.2 ± 6.2	0.0
103.6	3.4 ± 2.5*	89.2	0 ± 0	100
123.3	1.4 ± 1.3*	95.5	0 ± 0	100
304.8	0 ± 0	100	0 ± 0	100
414.3	0 ± 0	100	0 ± 0	100
488.3	0 ± 0	100	0 ± 0	100

* The mean difference is significant at the 0.05 level compared with control.

Table 4. Effect of electron beam irradiation on the pupal stage of *C. maculatus.*

Dose, Gy	No. of Emerging Adults From Irradiated Pupae, Mean ± SD	Reduction, %	No. of Emerging Adult Progeny, Mean ± SD	Reduction, %
			F_1 Generation	
0 (Control)	36 ± 3.5	0.0	42.8 ± 8.7	0.0
103.6	15.4 ± 5.5*	57.2	0 ± 0	100
123.3	14 ± 3.4*	61.1	0 ± 0	100
304.8	3.4 ± 2.9*	90.5	0 ± 0	100
414.3	0 ± 0	100	0 ± 0	100
488.3	0 ± 0	100	0 ± 0	100
653.5	0 ± 0	100	0 ± 0	100

* The mean difference is significant at the 0.05 level compared with control.

maculatus adults in cowpea seed, resulting in no adult emergence of the F_1 generation. This shows that this dose is sufficient to control this insect and to provide quarantine security. The calculated confidence (C) level (C = 1 − [1 − 0.0001]^{28,000}) was equal to 93.9198%. Nonirradiated control samples of infected cowpea with 500 *C. maculatus* adults produced an average of 4,097 adults (Table 6).

The percentage of egg hatching, pupation, and adult emergence of *B. zonata* significantly decreased as the irradiation dose increased (Table 7). The lowest

Table 5. Effect of electron beam irradiation on the adult stage of *C. maculatus.*

Dose, Gy	No. of Adults Irradiated, Mean	No. of Adult Mortalities, Mean ± SD	Adult Mortality, %	No. of Eggs, Mean ± SD	No. of Emerging Adults (F_1 Generation,) Mean ± SD
0 (Control)	50	0.0	0.0	56.8 ± 11.8	46.2 ± 1.3
103.6	50	3.4 ± 2.6*	6.8	47.2 ± 9*	30.4 ± 2.3*
123.3	50	5.6 ± 1.1*	11.2	43.4 ± 4.7*	19.4 ± 5*
304.8	50	12.4 ± 2.9*	24.8	9.4 ± 3*	2.4 ± 1.14*
414.3	50	15.8 ± 3*	31.6	0 ± 0	0 ± 0
488.3	50	22.2 ± 6.6*	44.4	0 ± 0	0 ± 0

* The mean difference is significant at the 0.05 level compared with control.

Table 6. Large-scale confirmatory tests of irradiation of adult stage of *C. maculatus* with electron beam in cowpea seeds.

Measured Dose	No. of Replicates	No. Treated	No. of F_1 Adults
414.3 Gy	40	28,000	0
Control	10	5,000	4,097

irradiation dose (103.6 Gy) applied to 1-d-old eggs decreased the percentage of egg hatching, pupation, and adult emergence from 99.4, 98.6, and 98.8% in nonirradiated (0.0 Gy) control samples to 24.6, 15.4, and 47.4%, respectively. The EBI dose of 304.8 Gy reduced egg hatching and pupation to 4.8 and 2.5%, respectively, while it completely prevented adult emergence. The irradiation dose of 414.3 Gy completely prevented egg hatching and pupation. The EBI dose of 123.3 Gy resulted in 94.8% mortality of the first instars, and only 5.2% pupation, while completely preventing adult emergence. The irradiation dose of 304.8 Gy caused 100% larval mortality and no pupation (Table 8). The irradiation dose of 123.3 Gy caused 97.6% mortality of the second instars and 2.4% pupation, while completely preventing adult emergence. However, the irradiation dose of 414.3 Gy caused 100% larval mortality and no pupation (Table 9). The irradiation dose of 304.8 Gy caused 92.2% third instar mortality, 7.8% pupation, and prevented adult emergence. The irradiation dose of 653.5 Gy resulted in 100% third larval instars mortality and zero pupation. These results indicate that different larval instars exhibit different radiosensitivity. The older larval instars were more radio-tolerant than the younger ones (Table 10). The EBI dose of 103.6 Gy completely prevented adult emergence of 1-d-old pupae. The EBI dose of 123.3 Gy completely prevented adult emergence of 3-d-old pupae. However, the irradiation dose of 653.5 Gy completely prevented the adult emergence of 7-d-old pupae. The radio-resistance of pupae increased as their age increased. The younger pupae were more radiosensitive than the older pupae. It is obvious from our results that the 7-d-old pupae were the most tolerant stages to EBI (Table 11). The irradiation dose of 304.8 Gy, which prevented the adult emergence of the third instars, applied to 21,250 *B. zonata* adults in an artificial medium, resulting in no survival to the adult stage. This shows that this dose is sufficient to control *B. zonata* and to provide quarantine security. The calculated confidence level was equal to 88.058%. The nonirradiated control samples of infected cowpea with 1,250 *B. zonata* adults produced an average of 1,210 adults (Table 12).

Discussion

Use of ^{60}Co facilities as the source of ionizing radiation is approved on a commercial basis for a quarantine treatment of fresh fruits and stored products. Gamma irradiation sources available worldwide are limited, and it is difficult to deliver large amounts of radioisotopes due to the logistical complexities associated with transboundary shipments and security concerns. Using the electron beam as alternative modes of irradiation that employ electricity to generate ionizing radiation

Table 7. Effect of electron beam irradiation on the maturation of 1-d-old eggs of *B. zonata* in artificial medium.

Dose, Gy	No. of Eggs Irradiated, Mean	No. of Hatched Eggs, Mean ± SD	Hatchability, %	No. of Pupae, Mean ± SD	Pupation, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	100	99.4 ± 0.9	99.4	98 ± 1.1	98.6	96.8 ± 1.6	98.8
103.6	100	24.6 ± 2.1*	24.6	3.8 ± 0.8*	15.4	1.8 ± 1.1*	47.4
123.3	100	13.6 ± 2.7*	13.6	2 ± 1*	14.7	0.8 ± 0.8*	40
304.8	100	4.8 ± 1.8*	4.8	1.2 ± 0.8*	2.5	0 ± 0	0.0
414.3	100	0 ± 0	0.0	0 ± 0	0.0	0 ± 0	0.0
488.3	100	0 ± 0	0.0	0 ± 0	0.0	0 ± 0	0.0
653.5	100	0 ± 0	0.0	0 ± 0	0.0	0 ± 0	0.0

* The mean difference is significant at the 0.05 level compared with control.

Table 8. Effect of electron beam irradiation on first larval instars of *B. zonata* in artificial medium.

Dose, Gy	No. of Irradiated Larvae, Mean	No. of Dead Larvae, Mean ± SD	Larval Mortality, %	No. of Pupae, Mean ± SD	Pupation, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	100	3.4 ± 2.1	3.4	96.6 ± 2.1	96.6	92.2 ± 2.7	95.4
103.6	100	82.8 ± 2.3*	82.8	17.2 ± 2.3*	17.2	8.2 ± 1.5*	47.7
123.3	100	94.8 ± 2.3*	94.8	5.2 ± 2.3*	5.2	0 ± 0	0.0
304.8	100	100 ± 0*	100	0 ± 0	0.0	0 ± 0	0.0
414.3	100	100 ± 0*	100	0 ± 0	0.0	0 ± 0	0.0
488.3	100	100 ± 0*	100	0 ± 0	0.0	0 ± 0	0.0
653.5	100	100 ± 0*	100	0 ± 0	0.0	0 ± 0	0.0

* The mean difference is significant at the 0.05 level compared with control.

Table 9. Effect of electron beam irradiation on second larval instars of *B. zonata* in artificial medium.

Dose, Gy	No. of Irradiated Larvae, Mean	No. of Dead Larvae, Mean ± SD	Larval Mortality, %	No. of Pupae, Mean ± SD	Pupation, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	100	3.6 ± 1.1	3.6	96.4 ± 1.1	96.4	94 ± 3.1	97.5
103.6	100	95.6 ± 1.1*	95.6	4.4 ± 1.1*	4.4	2.6 ± 1.1*	59.1
123.3	100	97.6 ± 1.5*	97.6	2.4 ± 1.5*	2.4	0 ± 0	0.0
304.8	100	98.8 ± 1.3*	98.8	1.2 ± 1.3*	1.2	0 ± 0	0.0
414.3	100	100 ± 0.0*	100	0 ± 0	0.0	0 ± 0	0.0
488.3	100	100 ± 0.0*	100	0 ± 0	0.0	0 ± 0	0.0
653.5	100	100 ± 0.0*	100	0 ± 0	0.0	0 ± 0	0.0

* The mean difference is significant at the 0.05 level compared with control.

Table 10. Effect of electron beam irradiation on third larval instars of *B. zonata* in artificial medium.

Dose, Gy	No. of Irradiated Larvae, Mean	No. of Dead Larvae, Mean ± SD	Larval Mortality, %	No. of Pupae, Mean ± SD	Pupation, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	100	1.2 ± 1.8	1.2	98.8 ± 1.8	98.8	96.8 ± 1.6	98
103.6	100	75.8 ± 5.5*	75.8	24.2 ± 5.5*	24.2	11.4 ± 1.1*	47
123.3	100	90.4 ± 3*	90.4	9.6 ± 3*	9.6	1.2 ± 0.8*	12.5
304.8	100	92.2 ± 2.5*	92.2	7.8 ± 2.5*	7.8	0 ± 0	0.0
414.3	100	96.2 ± 1.9*	96.2	3.8 ± 1.9*	3.8	0 ± 0	0.0
488.3	100	97.8 ± 2.8*	97.8	1.4 ± 1.1*	1.4	0 ± 0	0.0
653.5	100	100 ± 0.0*	100	0.0 ± 0.0*	0.0	0 ± 0	0.0

* The mean difference is significant at the 0.05 level compared with control.

Table 11. Effect of of electron beam irradiation on 1-, 3-, and 7-d-old pupae of *B. zonata*.

		1 d old		3 d old		7 d old	
Dose, Gy	No. of Irradiated Pupae	No. of Emerging Adults, Mean ± SD	Adult Emergence, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %	No. of Emerging Adults, Mean ± SD	Adult Emergence, %
0 (Control)	50	48 ± 2.3	96	48.8 ± 1.8	97.6	49.6 ± 0.5	99.2
103.6	50	0 ± 0	0.0	0.8 ± 0.8*	1.6	47.8 ± 2.7	95.6
123.3	50	0 ± 0	0.0	0 ± 0	0.0	44.8 ± 3.4*	89.6
304.8	50	0 ± 0	0.0	0 ± 0	0.0	41.6 ± 2.1*	83.2
414.3	50	0 ± 0	0.0	0 ± 0	0.0	30.2 ± 2.4*	60.4
488.3	50	0 ± 0	0.0	0 ± 0	0.0	10.8 ± 5.2*	21.6
653.5	50	0 ± 0	0.0	0 ± 0	0.0	0.0 ± 0.0*	0.0

* The mean difference is significant at the 0.05 level compared with control.

Table 12. Large-scale confirmatory tests of irradiation of adult stage of *B. zonata* with electron beam in artificial medium.

Measured Dose	No. of Replicates	No. Treated	No. of F_1 adults
304.8 Gy	85	21,250	0
Control	5	1,250	1,210

would enable food irradiation to be used more widely without increasing the demand for radioisotope sources (^{60}Co).

Application of EBI for insect pest control in agricultural product quarantine has effectively prevented development and/or reproduction of insects representing different taxonomic orders, including *C. maculatus* and *C. chinensis* (Shashi et al. 2016), the lepidopteran *Spodoptera litura* (F.), the leafminer *Liriomyza trifolii* (Burgess) (Yun et al. 2015), *Myzus persicae* (Sulzer), *Planococcus citri* (Risso), and *Bactericera cockerelli* (Šulc) (Lee et al. 2014, Lei et al. 2020). Irradiation technology is effective for all developmental stages of insects. However, acute mortality is not necessary for pest control efficacy, and studies are needed to evaluate the impact of specific irradiation doses on specific stages of the life cycle of an insect. That will help to identify the optimal treatment strategy for each species. In the present study, we performed dose-response analyses of EBI in all developmental stages of *C. maculatus* and *B. zonata* to determine which dose prevents the adult emergence of P_1 generation. Also, we established the EBI phytosanitary irradiation dose using large-scale confirmatory tests that are potentially useful for elimination of infestations of these pests.

EBI effects on *C. maculatus*, as illustrated in Tables 2–6, include the following: (1) the EBI dose of 304.8 Gy completely prevented adult emergence of the P1 generation when 3-d-old eggs were irradiated; (2) when larval and pupae stages were irradiated by EBI, the dose of only 103.6 Gy completely prevented adult emergence of the F_1 generation; and (3) when the adult stage was irradiated, the EBI dose of 414.3 Gy completely prevented adult emergence of the F_1 generation. These results indicate that the adult stage was the most tolerant to EBI. Based on the prevention of F_1 generation, when the most radio-tolerant stage (adult) is irradiated, 414.3 Gy is required as a phytosanitary irradiation control dose for quarantine and security treatment.

In the present study, irradiated *C. maculatus* at an EBI dose of 488.3 Gy resulted in 24.8% adult morality (Table 5). These results agree with those obtained by Sang et al. (2016) who did not observe immediate death of *C. maculatus* of male and female adults until doses of 150 and 500 Gy from the electron beam were applied. They added that EBI at a dose of 2 kGy resulted in approximately 50% acute mortality, indicating that the adult stage was the most tolerant to EBI. Also, our results revealed that the adult stage of *C. maculatus* was the most tolerant. Compared to gamma radiation, our previous results indicated that 1.0 and 1.3 kGy resulted in 75 and 100% acute mortality of *C. maculatus* (Hammad et al. 2020). Hence, a gamma irradiation dose of 650 Gy was required for quarantine treatment of cowpea seed, when prevention of F1 generation was used for measuring the effective irradiation dose.

In the present study with EBI, the dose level 414.3 Gy was needed for the prevention of the F_1 generation, indicating that EBI was more effective in the control of *C. maculatus* than gamma irradiation. Darfour et al. (2012) irradiated different varieties of cowpea seeds ('Asontem', 'Nhyira', 'Nigeria', 'Togo') infested with *C. maculatus* adults at gamma irradiation doses of 0.25, 0.5, 0.75, 1.0, and 1.5 kGy. They found that the irradiation dose of 0.75 kGy caused 100% mortality in the Asontem variety, whereas irradiation doses of 1.0 and 1.5 kGy did not achieve 100% mortality in other cowpea seed varieties after 6 d. Our results are similar to those of Sutantawong (1991) who reported that a gamma irradiation dose of 500 Gy resulted in 100% mortality of *C. maculatus* larval and pupal stages. Nonetheless, Follett et al. (2013) treated rice weevil, *Sitophilus oryzae* (L.), with X-rays and found that only 120 Gy arrested the development of rice weevil and sterilized its adults, thus providing quarantine security.

The effects of EBI on *B. zonata* (Tables 7–12) were as follows: (1) when 1-d-old eggs were irradiated, the irradiation dose of 304.8 Gy prevented adult emergence, whereas the irradiation dose of 414.3 Gy prevented egg hatching and pupation; (2) when the first instars were irradiated, the irradiation dose of 123.3 Gy prevented adult emergence, while the irradiation dose of 304.3 Gy caused 100% larval mortality and no pupation; (3) when the second instars were irradiated, an irradiation dose of 123.3 Gy prevented adult emergence, whereas 414.3 Gy resulted in 100% larval mortality and no pupation; (4) when the third instars were irradiated, an irradiation dose of 304.8 Gy prevented adult emergence, whereas 100% larval mortality and no pupation required 653.5 Gy; and (5) when 1-, 3-, and 7-d–old pupae were irradiated, the adult emergence was prevented at 103.3, 123.3, and 653.5 Gy, respectively.

The sensitivity of larvae and pupae to EBI decreased as their age increased. The third instars and 7-d-old pupae were the radio-tolerant stages. Apart from the 7-d-old pupal stage, which usually does not exist in fresh fruit hosts (peach, mango, guava, pomegranate, etc.), the third larval stage is considered the most tolerant stage. When this most radio-tolerant stage is considered and adult emergence was used as a criterion for measuring the effective phytosanitary irradiation dose, 304.8 Gy was required, which was higher than that reported by the other researchers with gamma radiation. Follett and Armstrong (2004) reported that currently approved irradiation doses for quarantine treatment of exported fruits and vegetables from Hawaii to the continental United States infested with *Bactrocera cucurbita* (Coquillett), *Ceratitis capitate* (Wiedemann), and *Bactrocera dorsalis* (Hendel) are 210, 220, and 225 Gy, respectively. Their results on these fruit flies support a proposed generic irradiation quarantine treatment dose of 150 Gy for all tephritid fruit flies. Hallman (2004) reported that a dose of 232 Gy was recommended to disinfect any fruits from oriental fruit moths under ambient and hypoxic atmosphere. To the contrary, lower irradiation doses were reported by Zhan et al. (2015) indicating that an irradiation dose of only 85 Gy prevented adult emergence of *Bactrocera tau*. Our previous results with gamma radiation (Gabarty et al. 2020) on *B. zonata* revealed that an irradiation dose of 150 Gy applied to 17,000 late third instars of *B. zonata* resulted in no adult emergence of the F_1 generation. This indicated that this dose is sufficient for providing quarantine security of export and import of pomegranate. The results of the present study indicated that EBI of 1- and 3-day-old pupae of *B. zonata* with 103.6 Gy resulted in 0 and 2% adult emergence, respectively. Zahran et al. (2013) found that a gamma irradiation dose of 90 Gy applied to *B. zonata* pupae (5 d old) decreased adult emergence to 22.66%. Naveed et al. (2015) found that gamma radiation of 1-day-old pupae of *B. zonata* and *B. cucurbitae* at 80 Gy reduced adult emergence from 83.25 and 87.5% (nonirradiated pupae) to 53.75 and 57%, respectively. This indicated that the adult emergence decreased when the irradiation doses decreased.

The overall objective of the present study is the development of practical techniques to irradiate food and agricultural products using an electron beam in the control of stored and fresh-product insects such as C. *maculatus* and *B. zonata*. Adult stage and third larval instars of *C. maculatus* and *B. zonata*, respectively, were the most tolerant stages. The EBI dose levels of 414.3 and 304.8 Gy, respectively, can be used as safe phytosanitary treatments to control *C. maculatus* and *B. zonata*.

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