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Research Article

Investigating the effect of gamma irradiation on the control of cigarette beetle *Lasioderma serricorne* F. (Coleoptera: Anobiidae) for development of Phytosanitary irradiation (PI) treatments

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Abstract

World trade of agriculture commodities continue to grow. This will also result in introduction and establishment of exotic pests into new areas where the pests are absent. The phytosanitary irradiation (PI) treatment of food commodity cause uniform penetration into the commodity, does not have any risks but helpful in killing and inactivating the contaminating microorganisms, food borne pathogens and insect pests. The technology is worldwide accepted and useful in trade promotion of agriculture commodities for better returns. In the present study, gamma irradiation doses were examined on the susceptibility of different developmental stages of *Lasioderma serricorne* F. The results showed that there was no larval emergence when 24 hours eggs were treated at 10Gy dose of gamma irradiation. A 100 Gy dose reduced adult emergence in 7 days old irradiated larvae and F_1 adults were sterile. The 7 days old treated pupae and adults when exposed to a series of irradiation doses, exhibited radio-tolerance and F_1♂and ♀adults were sterile at 80Gy. The radiation effect on longevity of each stage also exhibited a dose dependent increase in developmental periods. Adult's growth index decreased with increasing doses of gamma radiation against larvae and pupae. It is concluded that a maximum of 100Gy dose of gamma irradiation may effectively control all developmental stages of *L. serricorne* and therefore, recommended as PI treatment for *L. serricorne* control. Being environment friendly, the PI technology will ease farmers to export and sell their products at higher prices in the world market, save their money and spare them from unnecessary use of hazardous pesticides.

Keywords: Cigarette beetle; Developmental stages; Export; Gamma irradiation; Longevity; Growth index; Sterility

Introduction

Cigarette beetle *Lasioderma serricorne* is the most serious pest of high-value commodities and stored products, widespread in temperate regions worldwide. It attacks stored commodities of wide ranges such as processed products, stored cereals, oilseeds, nuts and dried fruits [1]. It causes serious quantity losses and deteriorate the quality of stored products via feeding, contaminating, leaving feces and casting the skin [2, 3]. The control of this pest is being achieved by fumigation and contact use of insecticides. For this purpose hydrogen phosphide and methyl bromide are common fumigants which are most often used in controlling insect pests of stored products. Methyl bromide causes depletion of ozone and is banned in most of countries [4-6]. Extensive use of synthetic fumigants also causes resistance in insects. Environment friendly alternative techniques are required to fill this gape and use of gamma irradiation in controlling insect pests and pathogens is accepted worldwide as an alternative method to control insect pests of stored and fresh agriculture commodities [7-9]. Sensitivity of *L. serricorne* to gamma radiation is noticed in recent past. There exist sufficient body of research on the use irradiation for the control of in almost all type of store commodities [10, 11] or fresh fruits and vegetables [12, 13] and irradiation is approved as direct control method [14] and advantageous in a sense that it prevents development of insect pest resistance with minimal changes in the properties of treated products without any residual effects. Current research work was carried out to assess minimal doses effects of gamma irradiations against developmental life stages of *Lasioderma serricorne*.

Materials and Methods

Insect culture

For all experiments cigarette beetle immature stages were derived from established culture of adults which were collected from local markets. Rearing medium composition was (95:5 by weight) of wheat flour and yeast [15]. 1 kg volume of plastic jar was used for insect rearing and covered with muslin cloth to prevent any escape of beetles over several generations. Humidity at temperature was set at R.H 50%±10 and 30°C±2 for rearing insects within the medium. All experiments were done in plant protection laboratory at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar.

Gamma radiation treatments

Radiation applications were done at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. The source was Co60 research irradiator (ISOGAMMA LLCo from Hungary). The dose administration was done by placing samples inside a 15×27cm chamber that was lowered into the radiation field through an electric hoist for a predetermined length of time based on the dose rate 4.10 kGy/hr and current activity, 12000 Ci during experiment. For the doses range and accuracy verification a dose mapping was conducted where samples were placed within the chamber. Fricke dosimeters was used for absorbed dose measurement at 3 irradiation positions.

Effect of gamma radiation on eggs

Eggs were obtained by confining pair of mature adults in glass tubes for 24 hours and allowed them to mate and lay eggs over on the substrate. Freshly laid eggs were collected via sieving from 50mm mesh size sieve with camel hair brush. Collected eggs (n=150/ treatment) were put into plastic vials for gamma radiation treatments of 0, 10, 20, 30 and 40 Gy. There were three replications for each dose treatment under completely randomized design (CRD). Post irradiated eggs were observed regularly. Eggs hatch data was recorded under stereomicroscope.

Effect of gamma radiation on larvae

Seven days old larvae were collected via sieving from the rearing medium. During sieving larvae remained on sieve meanwhile rearing diet dropped down and camel hair brush was used for larval separation and collected in plastic vials. Larvae (n=150/ treatment) were treated with gamma irradiations doses in a range 0, 40, 60, 80 and 100 Gy. Post treatment, larvae were shifted into plastic jar having rearing medium and covered with muslin cloth and kept at 30°C

±2 and R.H 50%±10 rearing conditions. Mortality was recorded as larvae color changed to dark brown, prolongation to pupae formation and adult emergence were recorded in every 2 days after irradiation treatment and sterility test in newly emerged adults was done. Data was replicated thrice under completely randomized design (CRD) [16].

Effect of gamma radiation on pupae

Seven (7) days old pupae were collected by sieving through rearing medium. Pupae (n=100/ treatment) were kept in plastic vials for sample preparation with camel hair brush and exposed to gamma radiation doses from 0, 40, 60, 80 and 100 Gy. Irradiated pupae were placed at rearing conditions of 30°C ±2 and R.H 50%±10. Data on pupal mortality and time prolongation to adult stage was recorded after every 2 days. Sterility test was also done in newly emerged adults. There were three replication for each treatment under completely randomized design (CRD).

Effect of gamma radiation on longevity of developmental stages

The longevity in developmental stages after irradiation treatments of larvae and pupae to adults were recorded. The growth index was calculated by formulae: percent adult emergence /total developmental period and Koplan-Meier chart was prepared using formula: total/survived*100).

F_1 sterility in emerged adults from gamma radiated larvae and pupae of 7 days age

Similar procedures were used for sterility test of newly emerged virgin adults of cigarette beetle emerged from irradiated larvae or pupae of cigarette beetles were observed. The sterility tests were performed by crossing virgin adults emerged from treated larvae and pupae crossed with opposite virgin sex reciprocally such as treated female crossed with untreated males and treated male crossed with untreated female with a control for comparison and allowed them to lay eggs on medium. Any eggs hatching from crossed pairs was recorded with the help of stereomicroscope. There were three replications for each treatment with completely randomized design (CRD).

Effect of gamma radiation on adults

Cigarette beetle adults of homogenous age were collected from rearing medium. For virgin pair of adults, male and female pupae of similar age were identified microscopically, separated in jars having rearing medium and allowed them to convert into adults. After adult emergence, virgin pair of adults were collected in plastic vials for gamma irradiation treatments. Adults were exposed under gamma radiation doses of 0, 40, 60, 80 and 100 Gy. The adults crosses were made as treated both virgin male and female and crossed reciprocally with untreated male and females. After treatment, adults were placed at R.H 50%±10 and 30°C ±2 in vials having rearing medium and allowed them to lay eggs. The data about eggs laid and hatched was recorded in numbers with the help of stereomicroscope. There were 7 replications for each treatment under completely randomized design (CRD)

Statistical analysis

Statistical analysis was done through Statistix 8.1 by using least significant difference (LSD) test and Fisher's Analysis of Variance (ANOVA) technique at probability level of 5% to compare the treatment means [16].

Results and Discussion

Effect of gamma radiation on eggs

The data in (Table 1) revealed significant difference between control and gamma irradiated eggs. Being sensitive to irradiation, there was no eggs hatching noticed even in 10 Gy treated eggs while 0 Gy showed 92.2% eggs hatch.

Table 1. Eggs hatching inhibition of 24 hrs. old eggs of *Lasioderma serricorne* exposed to gamma irradiation

Treatments (Gy)	Hatch Eggs (%)
0	92.2±0.33[a]
10	0.0±0.0[b]
20	0.0±0.0[b]
30	0.0±0.0[b]
40	0.0±0.0[b]

Values are means of three replicates ±S.E

Effect of gamma radiation on larvae

Data in (Table 2) revealed significant differences in mortality, pupae formation and adult emergence of gamma irradiated larvae. Mean percentage values unveiled that the highest mortality was recorded from 100 Gy (34.43±0.33%) and the lowest mortality was recorded from control 0 Gy (4.43±0.33%) while mortality in other treatments as recorded was27.8±0.33%, 26.7±0.00% and 20.0±0.00% from 80 Gy, 60Gy and 40 Gy respectively. In terms of pupae formation from gamma irradiated larvae, the highest pupae formed in 0 Gy (94.4±0.33%) and the lowest pupae was recorded from 100 Gy (65.6±0.33%).The highest percentage of adult emergence was recorded as 88.90±0.33% in control 0 Gy and the lowest (23.3±0.00%) from 100 Gy. Other treatments followed were as 40.0±0.58% (40 Gy), 32.2±0.33% (60 Gy) and 25.6±0.33% (80 Gy).

Table 2. Post Irradiation Mortality[a], pupae formation[b] and adult emergence[c] of gamma irradiated 7 days old larvae of cigarette beetle

Treatments (Gy)	Mortality (%)	Pupae formation (%)	Adult emergence (%)
0	4.43±0.33[d]	94.4±0.33[a]	88.9±0.33[a]
40	20.0±0.00[c]	80.0±0.00[b]	40.0±0.58[b]
60	26.7±0.00[b]	74.4±0.33[c]	32.2±0.33[c]
80	27.8±0.33[b]	72.2±0.33[c]	25.6±0.33[d]
100	34.4±0.33[a]	65.6±0.33[d]	23.3±0.00[d]

Values are means of three replicates ±S.E, Means carrying different letters in a column varied significantly (P ≤0.05). Larval mortality[a], pupae formation[b] and adult emergence[c] were statistically analyzed separately

F_1 sterility in emerged adults from gamma radiated 7 days old larvae of cigerrate beetle

The data in (Table 3) revealed that F_1 adults of irradiated larvae showed the highest number of eggs laying in 0 Gy (43.67±1.76) and the lowest in 100 Gy (6.00±0.58). Other treatments as followed, were 40 Gy (19.00±1.15), 60 Gy (12.33±0.33) and 80 Gy (9.33±0.33). Eggs hatching followed the same pattern. The highest percentage of eggs hatching (92.44±1.33%) was recorded in control (0Gy) and lowest was (0.0%) in 100 Gy treatment while other treatments followed, were 40 Gy (87.85±1.20%), 60 Gy (64.80±0.58) and 80 Gy (42.59±0.58) of eggs hatching.

Table 3. F_1 sterility in adults emerged from 7 days old irradiated larvae of cigarette beetles

Treatments (Gy)	Gamma radiated larvae	
	F_1 adults eggs	
	Laid(n)	Hatched (%)
0	43.67 ± 1.76^a	92.44 ± 1.33^a
40	19.00 ± 1.15^b	87.85 ± 1.20^a
60	12.33 ± 0.67^c	64.80 ± 0.58^b
80	9.33 ± 0.33^c	42.59 ± 0.58^c
100	6.00 ± 0.58^d	0.0^d

Values are means of three replicates ±S.E, In a column, means carrying different letters varied significantly, n (number)

Effect of gamma radiation on pupae

Data in (Table 4) revealed significant differences in pupae mortality and adult emergence due to gamma irradiation of pupae. Mean values in Table 4 exhibited highest percentage of pupae mortality (51.7±0.33%) in 100 Gy and the lowest 6.67±0.33% in (0 Gy). Mortality in other treatments was 80 Gy (43.3±0.33%), 60 Gy (28.3±0.33%) and 40 Gy (25.0±0.58%) respectively. Adult emergence followed the same with highest percent value of (93.3±0.58%) in control 0 Gy and the lowest (48.3±0.58%) in 100 Gy was recorded. Similar pattern for radiotolerance was recorded for other treatments such as 40 Gy (75.0±1.00%), 60 Gy (71.7±0.58%) and 80 Gy (56.7±0.58%) respectively.

Table 4. Mortality[a] and adult emergence[b] of 7 days old pupae of cigarette beetle exposed to gamma irradiation treatments

Treatments (Gy)	Mortality (%)	Adult emergence (%)
0	6.67 ± 0.33^d	93.3 ± 0.58^a
40	25.0 ± 0.58^c	75.0 ± 1.00^b
60	28.3 ± 0.33^c	71.7 ± 0.58^b
80	43.3 ± 0.33^b	56.7 ± 0.58^c
100	51.7 ± 0.33^a	48.3 ± 0.58^d

Values are three replicates means±S.E, In a column, means carrying different letters varied significantly, pupal mortality[a] and adults emergence[b] were statistically analyzed separately

F_1 sterility in emerged adults from 7 days old irradiated pupae of cigarette beetle

The data in (Table 5) as recorded, showed that F_1 adults of gamma irradiated pupae exhibited the highest numbers of eggs laying from females in 0 Gy (51.67±2.19) followed by 22.33±1.76, 11.33±0.67 and 8.67±0.67 from 40, 60 and 80 Gy doses respectively while no egg laying was recorded from females of 100 Gy treated pupae. Eggs hatching data from F_1 male irradiated pupa showed maximum hatching of 92.47±0.67% in control (0 Gy) and no hatching 0.0% in 80 Gy dose. Other eggs hatching values for irradiation with their doses were 45.0±0.58% and 41.0±0.58% from 40 Gy and 60 Gy doses respectively. While in F_1 females, the highest numbers of eggs laying was noted in 0 Gy (51.67±2.19) and lowest were in 80 and 100 Gy (0.0%). The eggs hatching as recorded was 9.00±1.53% and 6.67±0.33% from 40Gy and 60 Gy respectively. Similarly, eggs hatching values, the highest was in 0 Gy (92.47±0.67%) and no hatching was noticed in 80 and 100 Gy. However other values shadowed as (42.07±0.33%) 40 Gy and (34.17±0.67%) 60 Gy.

Table 5. F_1 sterility in adults emerged from 7 days old irradiated pupae of cigarette beetle

Treatments (Gy)	Radiated pupae			
	F_1♂ irradiated adults		F_1♀ irradiated adults	
	eggs Laid (n)	Hatched (%)	eggs Laid (n)	Hatched (%)
0	51.67±2.19[a]	92.47±0.67[a]	51.67±2.19[a]	92.47±0.67[a]
40	22.33±1.76[b]	45.0±0.58[b]	9.00±1.53[b]	42.07±0.33[b]
60	11.33±0.67[c]	41.0±0.58[b]	6.67±0.33[b]	34.17±0.67[b]
80	8.67±0.67[c]	0.0[d]	0.0[c]	0.0[c]
100	0.0[d]	0.0[d]	0.0[c]	0.0[c]

Values are means of three replicates ±S. E, In a column, means carrying different letters varied significantly, n (number), ♂ (male), ♀ (female)

Effect of gamma radiations on longevity and developmental stages of cigarette beetle

The gamma radiation treatments caused significant effects on developmental time of treated larvae and pupae to adult stages. Developmental period increased by dose range increment which are shown as mean values in (Table 6). The highest period taken by larvae to adult was (36.3±0.67 days) at100 Gy followed by 80 Gy (34.3±0.67), 60 Gy (30.3±0.67), 40 Gy (28.3±0.67) and the lowest by control 0 Gy (25.0±0.00). Meanwhile, growth index was decreased by increasing dose range as the highest growth index showed by 0 Gy (3.57±0.04) and the lowest (0.63±0.01) by 100 Gy while others indices were 40 Gy (1.43±0.04), 60 Gy (1.06±0.05) and 80 Gy (0.77±0.02). Similarly, developmental period taken from pupae to adult was the highest as 25.0±0.00 by 100 Gy and the lowest as 5.33±0.67 by 0 Gy while other treatments followed were as 21.3±0.67 (80 Gy), 17.3±0.67 (60 Gy) and 15.3±0.67 (40 Gy). Growth index from pupae to adult unveiled the same pattern, highest by 0 Gy (18.20±2.79) and the lowest by 100 Gy (1.83±0.04) and other treatments shadowed as 40 Gy (4.83±0.29), 60 Gy (3.57±0.12) and 80 Gy (2.57±0.08). Longevity and survival from treated larvae and treated pupae is also shown in (Fig. 1 & 2) where 100 Gy showed more mortality and less survival while 0 Gy showed less mortality and more survival of radiated larvae and radiated pupae.

Table 6. **Developmental period of cigarette beetle larvae and pupae to adults[a] after gamma irradiation treatments**

Treatments (Gy)	Developmental DAT to adults[a]		Index[b]	
	Treated larvae	Treated pupae	Treated larvae	Treated pupae
0	25.0±0.00[e]	5.33±0.67[d]	3.57±0.04[a]	18.20±2.79[a]
40	28.3±0.67[d]	15.3±0.67[c]	1.43±0.04[b]	4.83±0.29[b]
60	30.3±0.67[c]	17.3±0.67[c]	1.06±0.05[c]	3.57±0.12[b]
80	34.3±0.67[b]	21.3±0.67[b]	0.77±0.02[d]	2.57±0.08[b]
100	36.3±0.67[a]	25.0±0.00[a]	0.63±0.01[e]	1.83±0.04[b]

Values are means of three replicates ±S.E, In a column, means carrying different letters varied significantly, adults emergence[a] and growth index[b] were analyzed statistically separately, DAT (days after treatment)

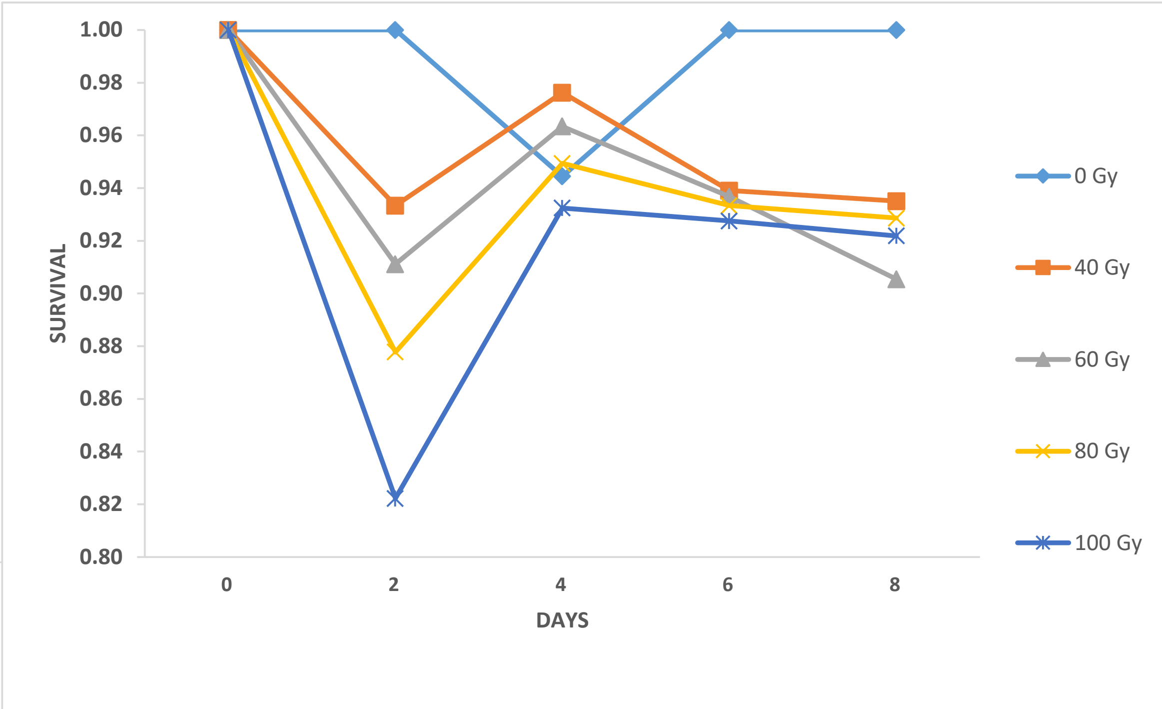

Figure 1. K. M. graph survival days of *Lasioderma serricorne* 7 D larvae after radiation treatment (DAT) (Koplan-Meier formula: total/survived*100)

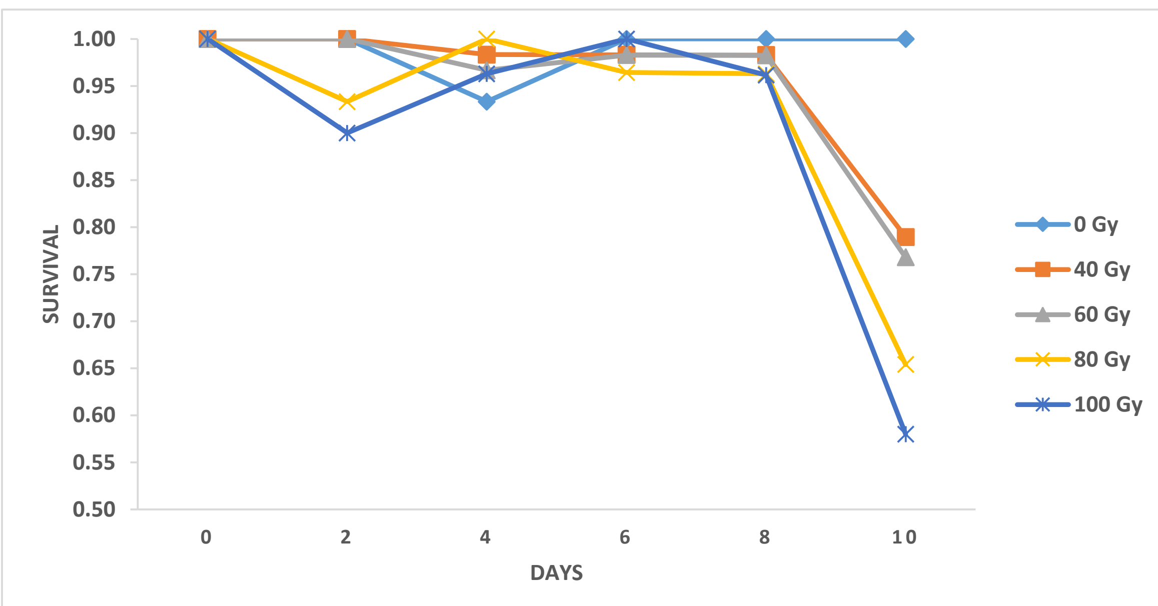

Figure 2. K. M. survival days graph of *Lasioderma serricorne* 7 D pupae after radiation treatment (DAT) (Koplan-Meier formula: total/survived*100)

Effect of gamma radiations on adults

The data summarized in (Table 7), exhibited significant differences in eggs laying (n) and hatching (%) from gamma irradiated male, female and both male×female. Mean values of irradiated exposed males revealed the highest eggs laid by 0 Gy (39.86±1.5) and the lowest in 100 Gy (3.00±1.43) and other treatments showed as 40 Gy (23.29±1.21) and 60 Gy (18.57±0.65) and 80 Gy (10.71±0.61) and the highest hatching was recorded in 0 Gy (92.67±1.83%) and lowest in 100 Gy (0.0%) and other values followed as 40 Gy (62.14±0.74%), 60 Gy (50.30±0.64%) and 80 Gy (34.32±0.52%) respectively.

Similarly, radiation exposed female uncovered the highest eggs laying in 0 Gy (39.86±1.75) and the lowest (2.00±0.44) by 100 Gy exposed females followed by 40 (18.29±1.08), 60 (12.71±0.81) and 80 Gy (3.86±0.51). While, maximum eggs hatch was noticed from 0 Gy (92.67±1.83%) minimum (0.0%) from 100 Gy and the rest of doses revealed 61.62±0.87%, 44.62±0.48% and 13.10±0.30% from 40, 60 and 80 Gy respectively.

When both sexes were irradiated, again highest eggs laying was recorded from control 0 Gy (39.86±1.75) and the lowest (0.0) from 100 Gy. Other values were (12.14±0.83) 40 Gy, (4.29±0.61) 60 Gy and (2.57±0.65) 80 Gy. However, in control 0 Gy highest (92.67±1.83%) eggs hatching and in 100 and 80 Gy no eggs hatching (0.0%) was recorded. A 40 Gy exposure caused (60.95±0.78%) and 60 Gy (30.99±0.30%) eggs hatching.

Table 7. Eggs deposition and eggs hatch data from irradiated reciprocal crosses of cigarette beetle

Treatments (Gy)	Irradiated (♂)		Irradiated (♀)		Irradiated (♂×♀)	
	Eggs Laid (n)	Hatched (%)	Eggs Laid (n)	Hatched (%)	Eggs Laid (n)	Hatched (%)
0	39.86±1.75^{a}	92.67±1.83^{a}	39.86±1.75^{a}	92.67±1.83^{a}	39.86±1.75^{a}	92.67±1.83^{a}
40	23.29±1.21^{b}	62.14±0.74^{b}	18.29±1.08^{b}	61.62±0.87^{b}	12.14±0.83^{b}	60.95±0.78^{b}
60	18.57±0.65^{c}	50.30±0.64^{c}	12.71±0.81^{c}	44.62±0.48^{c}	4.29±0.61^{c}	30.99±0.30^{c}
80	10.71±0.61^{d}	34.32±0.52^{d}	3.86±0.51^{d}	13.10±0.30^{d}	2.57±0.65^{cd}	0.0^{d}
100	3.00±1.43^{e}	0.0^{e}	2.00±0.44^{d}	0.0^{d}	0.0^{d}	0.0^{d}

Values are means of three replicates ±S. E, In a column, means carrying different letters varied significantly, n (number), ♂ (male), ♀ (female)

Discussion

The control of cigarette beetle (*Lasioderma serricorne*) at different stages by gamma irradiation treatments uncovered that gamma radiation treatments can effectively control the beetle. Eggs were the most radio-sensitive stage to gamma irradiations and resulted in no emergence at 10 Gy. Similarly, the 100 Gy dose was effective for causing maximum but gradual mortality of larvae, pupae formation and adults' emergence. When larvae were exposed to gamma irradiation. prolongation was observed in larval stage as compared with control. Similarly, pupal exposure to gamma irradiation also showed mortality and a delay in pupae to adult emergence was also noted. Similar prolongation in the larval and pupal stages has also been reported for *L. sericona* **[17]** and prolongation for months followed by death of larva **[18]**. The 100 Gy dose showed more delay in adult emergence among all treatments. Furthermore, adults treated with gamma irradiations for inducing sterility at different doses, showed different eggs laying and hatchability by irradiated males, irradiated females or male and female both irradiated. Males showed some margin of resistance while female and pair of both sexes showed infertility of eggs at 80 to 100 Gy. The results are in accordance with **[18]** who reported compete sterility of females at 100 Gy dose of X-ray exposure. Additionally, we observed eggs laying ability of females and eggs hatching was reduced with increase in gamma irradiation from 80-100 Gy dose. Similarly, F_1 adults of irradiated larvae and pupae had less ability to lay eggs and most of them were infertile. Dose of 80 to 100 Gy showed good results in in terms of sterility from adults obtained from irradiated larvae pupae and adults.

Similar studies with insects from other genera and species have reported radio-tolerance of eggs of store pests **[19]** Our results are in agreement with the study on *Tribolium confusum* **[20]** where 40 and 50 Gy gamma irradiation on eggs and larvae were enough to prevent adult's emergence and 100 Gy dose prevented emergence of 15 day old larvae *T. castaneum*. **[21]** Other studies on eggs of *Oryzeaphilus surinamensis* **[22, 23]** have reported 8 Gy of irradiation prevented eggs hatch of *O. surinamensis* and resulted sterility Similar studies on eggs of *Sitophilus granaries* exposed to 30 Gy dose **[24]** prevented adult's emergence and 100 Gy dose caused adult's sterility. A delay in developmental stages due to increase in radiation doses has been supported by other studies on phytosanitary irradiation of fresh horticultural commodities **[26]** and store grain pests **[27-36]**.

Conclusion and Implications

The use of radiation technology is worldwide accepted and encouraged by the food and agriculture organization (FAO) and international atomic energy agency (IAEA) for trade promotion of agriculture and other commodities. This technology is residue free and environment friendly with no health hazards. The present research has investigated gamma irradiation doses as phytosanitary treatment for the control of *L. sericona*, a commonly found insect pest of store grains and dry fruits. The main focus of the current research was on the application of gamma irradiation at lower doses to prevent eggs hatching, development of the insect to the next stage and sterility without any focus on acute mortality. A synthesis of the data successfully demonstrated that 100 Gy dose of gamma radiation can deter the *L. sericona* to develop further and therefore, the 100Gy dose may be used as Phytosanitary treatment for the control of all stages of *L. sericona*. Further large scale studies for ~ 30000 insect are warranted to confirm 100 Gy dose as PI treatment for *L. sericona.*

Authors' contributions

Conceived and designed the experiments: I Khan, Performed the experiments: NI Jajja, Analyzed the data: NI Jajja & A TAmkin, Contributed materials/ analysis/ tools: I Khan & NI Jajja, Wrote the paper: NI Jajja &I Khan.

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