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Gamma irradiation as a disinfestations treatment against the greater wax moth, *Galleria mellonella* L., late pupae

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Abstract

The effects of gamma radiation on the *Galleria mellonella* L. late pupae were examined. About to emerge pupae were exposed to radiation dosages between 50 and 400 Gy and percentage adult emergence was recorded. Emerging moths were crossed to untreated insects of the opposite sex. Laid eggs were counted, incubated under a suitable temperature and examined one week later for egg hatch. Larvae resulting from the previous crosses (F_1) were given the chance to continue their development to pupae and adults and the number of formed pupae and emerging adults was recorded. Results showed that adult emergence was not significantly affected. Fecundity of females was negatively affected and fertility of both sexes decreased with increasing irradiation dose. Furthermore, irradiation decreased survival rate of the progeny of irradiated insects and at 400 Gy, all F_1 insects died before adult emergence. These results support a dose of 400 Gy as a disinfestations treatment against *G. mellonella* late pupae.

Keywords: Pupal stage; *Galleria mellonella*; gamma radiation; radiation disinfestations

1. Introduction

The greater wax moth, *Galleria mellonella* L., is a devastating pest of honeybee, *Apis mellifera* L. [1]. *G. mellonella* adult females deposit their eggs on unprotected honeybee combs and in the cracks of honeybee hives. Eggs hatch and young larvae crawl into the combs and start feeding on the wax. As they feed on the combs, they spin silk around their feeding promises to protect themselves from guarding bees causing bees to withdraw away from the feeding area. Eventually, larval feeding causes the destruction of the combs which may lead, in most cases, to the destruction of the whole colony [2]. Furthermore, honeybee combs in storage are also subject to moths attack [2-4] and can be completely destroyed.

Although this insect is very serious on beehives, strong colonies can protect themselves from *G. mellonella* attack. Stored honeybee combs, however, can be very susceptible and infestation brought with newly stored honeybee combs can lead to a total destruction of stored honeybee products [2-4].

Insecticides, specifically, paradichlorobenzene, phostoxin and methyl bromide (MB) are the most frequently used products to protect stored honeybee combs during storage against wax moth attack [3,5,6]. These products, however, are not suitable to protect combs with honey to be consumed by humans [7]. Insecticide residues in honey and wax can also be poisonous to bees [8]. Additionally, *G. mellonella* has developed resistance to these insecticides [9]. Consequently, developing more acceptable method for treating honeybee combs before storage can be very helpful.

Ionizing radiation is used for decades to disinfest agricultural products from insect pests [10,11]. In fact, ionizing radiation, as a disinfestations technique, has several advantages. Some of these are broad spectrum activity against target pests, fast application and resulted in residue free treated commodities [12]. In addition, ionizing radiation does not induce resistance in pest populations and, normally, does not cause any adverse effects on the quality of the treated products.

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The use of gamma irradiation as a disinfestations treatment against this pest requires good knowledge about the radiosensitivity of the targeted stages to gamma radiation. The effects of gamma radiation on wax moth eggs and larvae were reported before [13-15]. The effects of gamma radiation on *G. mellonella* late pupae, however, have not been reported yet.

This study was intended to examine the effects of gamma radiation on the greater wax moth late pupae as the most radiotolerant stage that may exist in the stored honeybee combs. In particular, it examines the effects of gamma radiation on adult emergence and fecundity and fertility of emerged adults resulting from irradiated pupae. In addition, it examines survival of F_1 insects resulting from crosses between irradiated and normal moths to adults. Furthermore, it discusses the possibility of using ionizing radiation as a disinfestations treatment for stored honeybee combs potentially infested with *G. mellonella* late pupae.

2. Materials and Method

2.1. Lab rearing of G. mellonella

The *G. mellonella* colony was maintained in a growth chamber under constant environmental conditions. Rearing conditions were set as stated by M. Mansour [13]. Specifically, they were set at 32±1 °C, 60 ± 5% RH and total darkness; under these conditions pupae start to emerge in day 8. For maintaining vigor, *G. mellonella* males from the wild populations were introduced into the colony several times every year. *G. mellonella* larvae were reared on a diet composed of their natural food (Pollen, honey and bee wax) in plastic dishes (19×14×5 cm^3) filled with the larval rearing medium (about 400 g). In such a diet, mature larvae pupate at the top of the medium. When close to maturation, folded paper strips ("larval holding strips") were placed at the top of the rearing media to provide pupation sites for mature larvae. "Larval holding strips" (with mature larvae inside) were collected daily and incubated under the same previously mentioned conditions for pupation and adult emergence. Emerging moths were transferred into polyethylene jars (15×10 cm×cm) for mating and oviposition (oviposition cages). Folded paper strips (accordion shaped) were placed in the jars for females to oviposit and the jar openings were covered with a fine mesh to prevent the escape of moths. The strips of folded paper were collected daily and replaced with new ones. Collected paper strips carrying *G. mellonella* eggs were incubated under the same conditions for the colony (32±1 °C and 60 ± 5% RH) and transferred into a rearing medium 4-5 days later.

2.2. Obtaining G. mellonella late pupae

When larvae were about to mature, strips of sterile white cardboard paper (25×2.5 cm×cm) folded at one cm distance (zigzag form) were distributed at the top of the rearing medium. Mature larvae searching for a place to pupate crawl into the folded paper strips and spun their cocoons in the paper folds. The cardboard paper strips holding the mature larvae were collected every 24 h and incubated under the same previously mentioned environmental conditions for pupation. Seven days later, the pupae (late pupae) were prepared for irradiation.

2.3. Preparing late pupae for irradiation

The paper strips containing the pupae were opened on day 7, pupae were carefully removed, sexed [16] and each sex (females or males) was placed in separate 9 cm Petri dishes (30 pupae in each dish). Care was taken not to include any underdeveloped or damaged insects.

2.4. Irradiation

G. mellonella late pupae were exposed to gamma radiation dosages in a gamma cell provided with a Co-60 source (Issledovatel Gamma Irradiator). The average dose rate at the time of irradiation was approximately 5.00 Gy/minute. The factor of homogeny (ratio of the maximum to the minimum received dose) was about 1.14 and we used Fricke solution to calibrate the absorbed dose. Four Petri dishes (4 replicates) were irradiated simultaneously at each dose level. The Petri dishes were irradiated with 50-400 Gy at 50 Gy increment.

2.5. Effects of gamma irradiation on adult emergence

Following irradiation, the Petri dishes containing the irradiated pupae were returned to the laboratory and incubated under the same environmental conditions for the colony (32±1 °C, 60±5% RH, total darkness) for adult emergence. The dishes were checked at about 24 h intervals and the number of emerging moth was noted. Percentage adult emergence was calculated by dividing the number of total emerging adults by the number of irradiated pupae.

2.6. Effects of gamma irradiation on fecundity and egg hatch

Emerging *G. mellonella* adults from the previous experiment were crossed to normal (untreated) insects of the opposite sex by confining them inside oviposition cages. Irradiated insects of one sex (10 insects) were placed in an oviposition cage and an equal number of untreated insects from the opposite sex were introduced. The cages were placed randomly on the shelves of the rearing room. Eggs were deposited on the "ovipositon paper strips" and the paper strips were replaced daily for 4 days. The ovipositon paper strips carrying deposited eggs were incubated under the same previously mentioned conditions for the colony and placed at the appropriate age (about 121-144 h old) on the surface of the larval rearing diet. The diet is placed in plastic dishes (19×14×5 cm^3) and the dishes were incubated under the same rearing conditions for the colony. Seven days later, the oviposition paper strips were removed and examined under a microscope for egg hatch. The number of deposited and hatched eggs in each replicate and treatment was recorded, and percentage egg hatch was calculated.

2.7. Effects of gamma irradiation on F_1 survival to adults

Following recording egg hatch, the dishes containing diet with larvae resulting from the different crosses were incubated under the same conditions (32±1 °C, 60±5% RH and total darkness) for larval and pupal development. As explained before, 4 weeks later, the larval holding strips were distributed at the top of the rearing medium to collect larvae trying to pupate. The dishes were covered with fine muslin mesh to prevent emerging insects from leaving and the dishes were examined daily. Emerging moths were removed, counted, their number was recorded and percentage survival to the adult stage was calculated.

2.8. Data analysis

Data from the various experiments were subjected to analysis of variance. Means (adult emergence, fecundity, fertility and survival of F_1 insects to the adult stage) were separated by Fisher's protected least significant difference test (PLSD, 5% level of probability). In addition, percentage egg hatch in the different crosses and survival of F_1 insects to pupae and adults (emergence of F_1 insects) data were subjected to regression analysis using the computer program SPSS Statistics for Windows (version 2008) [17].

3. Results and Discussion

Insect disinfestations of agricultural products refers to the process of getting rid of insect pests in these products. This is done using several techniques including insecticides, fumigants, temperature treatments and ionizing radiation [10]. Unfortunately, insecticides and fumigants are toxic to humans, leave unacceptable levels of residues in the treated commodities and may lead to the development of resistance in the insect populations. In addition, these products are not friendly to the environment and heat treatments may not be suitable for some products including honeybee combs. Radiation disinfestations possess several advantages over chemical treatments [18]. Consequently, this technique is increasingly used as a phytosanitary (quarantine and disinfestations) measure against insect pests in agricultural products [19].

The greater wax moth is a devastating pest of honeybee combs in storage [2-4]. Infestation brought by newly stored honeybee products can develop very fast, particularly when environmental conditions are suitable, and stored combs can be destroyed within in a short period of time [2-4]. Although insecticides, particularly fumigant, are still affective against this pest [3,5,6], they are not suitable to protect honeybee combs with honey intended for human consumption [7] and insecticide residues in bee products can be poisonous to bees [8]. Consequently, ionizing radiation, with all the advantages it posses, may be a suitable solution. Treatment with ionizing radiation requires good knowledge of the sensitivity of the most resistant stage of the pest potentially present in the stored commodity.

In reality, newly stored honeybee combs may contain greater wax moth eggs, larvae and, to less extent, pupae. Considering the fact that the radiosensitivity of insects decreases as they develop [11,20], late pupae should be the most radioresistant stage. Consequently, the irradiation treatment that proves to be affective against this stage, should also be sufficient to disinfest all other developmental stages potentially present in the stored combs.

Table 1. Effects of gamma radiation on adult emergence and female fecundity in irradiated *G. mellonella* late pupae

Dose (Gy)	**% Adult emergence ± SD**	**Female fecundity in the different crosses (Mean No. of eggs/female)**	
		IMxNF	***NMxIF***
0	95.0 ± 4.3[a]	291.0 ± 49.9[a]	291.0 ± 49.9[a]
50	93.3 ± 6.1[a]	298.3 ± 31.6[a]	299.8 ± 52.4[a]
100	95.0 ± 4.0[a]	287.8 ± 26.3[a]	286.7 ± 38.7[a]
150	95.8 ± 3.2[a]	301.2 ± 48.4[a]	263.6 ± 9.9[a]
200	90.0 ± 2.7[a]	288.8 ± 49.0[a]	210.6 ± 20.6[b]
250	91.7 ± 4.3[a]	270.0 ± 44.5[a]	179.5 ± 23.9[bc]
300	94.2 ± 3.2[a]	289.0 ± 36.1[a]	150.3 ± 9.6[cd]
350	91.7 ± 4.3[a]	279.9 ± 34.7[a]	130.7 ± 5.7[de]
400	92.5 ± 3.2[a]	276.5 ± 35.9[a]	102.0 ± 4.3[e]

Means followed by the same letter within each column are not significantly different (P<0.05, Fisher's LSD test).

Table 1 presents data on the effects of gamma radiation on adult emergence from irradiated wax moth late pupae and fecundity (mean No. of eggs/female) of emerged moths crossed with untreated insects of the opposite sex. At the examined dosages (50-400 Gy), the results do not show any significant negative effects ($P > 0.05$) on adult emergence. This suggests that preventing *G. mellonella* adult emergence from late pupae requires too high dose and, consequently, may not be a suitable criterion for measuring effectiveness [21]. These results are in general agreement with data reported previously for other Lepidoteran pupae [22,23].

Table 1 also shows that, within the used dosages, no significant effect on female fecundity was noticed when irradiated males were crossed to normal females ($P > 0.05$). However, when normal males were crossed to irradiated females, the effect was obvious, particularly at 200 Gy dose and higher where irradiation caused significant ($P < 0.0001$) and consistent decrease in the mean number of deposited eggs/female. For instance, while the mean number of produced eggs/female in the crosses between irradiated males and normal females (IM X NF) at 400 Gy was about 277 egg/female, this number decreased to about 37% in crosses where females were the irradiated sex (NM X IF).

The effects of gamma irradiation on fertility (% egg hatch) of irradiated insects (males or females) crossed to untreated individuals of the opposite sex are presented in (Figure 1). The results show that increasing irradiation dose caused consistent decrease in female fertility whether irradiated or crossed to irradiated male. The effect, however, was greater when females were the irradiated sex. For instance, fertility of irradiated males exposed to 200 Gy and crossed to untreated females was about 37% and at 400 Gy, fertility dropped to about 3%. In comparison, fertility of irradiated females exposed to 200 Gy and crossed with untreated males was less than 12% and no egg hatch was noted at 250 Gy dose and above. Regression analysis on the data show a significant inverse relationship between irradiation dose and percentage egg hatch, ($P < 0.001$); $R^2 = 0.84$ and 0.96 when females and males were irradiated, respectively (Figure 1).

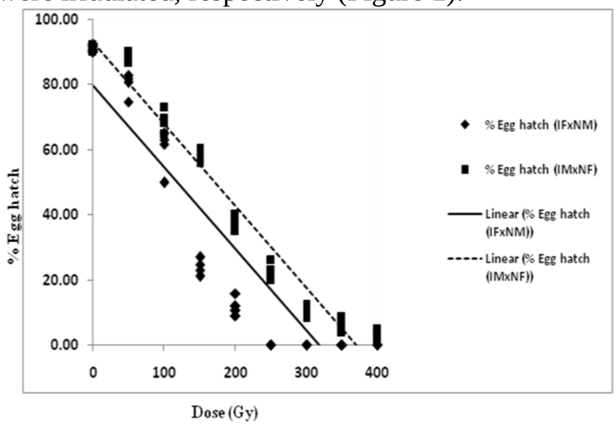

Figure 1. Effects of gamma irradiation on % egg hatch in *G. mellonella* adults resulting from irradiated late pupae. $y\ (IF) = -0.25x + 79.69$, $R^2 = 0.84$, $y\ (IM) = -0.250x + 93.02$, $R^2 = 0.96$

These results agree, in general, with data reported for other species in the order Lepidoptera [22-26]. The greater sensitivity of females to ionizing radiation has been explained before [27,28]. Basically, this may be due to the fact that Lepidopteran males possess a duplicate of the sex chromosome (homogametic) which makes them more radiotolerant than heterogametic females [27]. In addition, eggs are more complex than sperms providing higher possibilities for radiation-induced damage [28].

The effects of gamma irradiation on pupation and adult emergence of F_1 insects (insects resulting from crossings between irradiated and normal insects) are presented in Figures 2 and 3. The data clearly shows that increasing irradiation dose consistently decreased the percentage of insects that were able to reach the pupal (Figure 2) and adult stages (Figure 3). The effect, however, was much higher when females were the irradiated sex. For instance, while 250 Gy dose caused complete death of F_1 insects before pupation when females were irradiated (IF X NM), about 13 and 6% of F_1 insects were able to reach the pupal and adult stages (respectively) when males were the irradiate sex (IM X NF). These results are in general agreement with those reported for *L. botrana* adults [23,29].

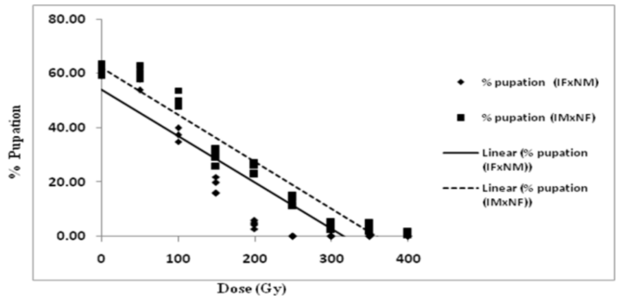

Figure 2. Effects of gamma irradiation on pupation of F_1 insects resulting from irradiated *G. mellonella* late pupae. y (IF) = -0.17x + 54.19, R^2 = 0.82, y (IM) = -0.173x + 62.20, R^2= 0.94

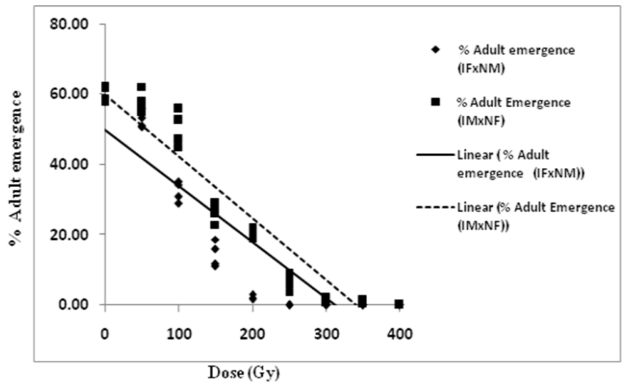

Figure 3. Effects of gamma irradiation on adult emergence of F_1 insects resulting from irradiated *G. mellonella* late pupae. y (IF) = -0.159x + 49.86, R^2 = 0.80, y (IM) = -0.176x + 60.08, R^2 = 0.91

Regression analysis on the data show a significant inverse relationship between irradiation dose and pupation and adult emergence ($P < 0.001$). When females were irradiated R^2 = 0.82 and 0.79 for pupation and adult emergence, respectively. When males were irradiated, however, R^2 value was about 0.94 and 0.91 for pupation and adult emergence in the same order (Figures 2 and 3).

4. Conclusion

The results of this study provide data on the radiosensitivity of *G. mellonella*, late pupae. They also show that the use of gamma radiation as a disinfestations treatment for honeybee combs infested with *G. mellonella* late pupae may be possible and requires a dose of about 400 Gy, provided that prevention of F_1 insects from reaching the adult stage is used as a criterion for measuring effectiveness. This dose (400 Gy) is much lower than the maximum allowed dose for food irradiation in the USA [30] and does not exceed the suggested generic irradiation disinfestations dose of 400 Gy for Lepidopteran pupae [21]. The small percentage of egg hatch at 400 Gy, particularly when males were the irradiated sex, should not pose much concern as all irradiated females stop laying eggs at a much lower dose (250 Gy).

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements and that they have no conflict of interest and all procedures involving human and/or animal subjects (if exist) respect the specific regulation and standards.

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