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Standardization of Gamma, X-ray and Electron Radiation against *Galleria mellonella* L., a Pest of Stored Honey Bee Combs

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ABSTRACT

The current study investigated the role of different types of ionizing radiation in causing damage to the eggs and larvae of *Galleria mellonella*. Moreover, the effect of varying dose rates on the sterilizing dose was investigated besides the sterilizing dose for male pupae of *G. mellonella* using gamma, x and electron beams. Egg hatchability was found to depend less on the energy of gamma and x-ray radiation in the MeV range and more on the rate at which a given dose was delivered, in addition to dosage amount. After 52.6 and 46.99 h of exposure to 0.662 MeV gamma rays and 6 MeV electrons, respectively, at a dose of 500 Gy, 50% larval death was noted. The male pupae of *G. mellonella* were sterilized with 350 Gy by all types of ionizing radiation in the MeV range of energy and the effect of dose rate in causing sterility was found to be minimal.

Key words: Greater wax moth, dose rate, sterility, electrons, energy

INTRODUCTION

Beekeeping is a significant source of revenue in many rural areas. In addition to making honey, honey bees are the world's most significant pollinator species for several crops (Hung *et al.*, 2018). Beekeeping creates numerous employment possibilities in rural regions and yields lucrative honey bee products, making it one of the most significant agricultural pursuits (Abou-Shaara and Staron, 2019). Despite increasing honey bee populations on a global scale, the growth rate is not keeping up with demand. Many pests attack the honey bee combs, and the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), is considered the deadliest pest of the honey bee combs. This pest is omnipresent worldwide and damages the stored wax combs, wherein the larvae feed on the wax and transform into adults. Given the fact that this insect can be discovered in bee hives or wax storage containers and causes a condition known as galleriosis, its occurrence is correlated with the beekeeping industry in many nations (Wojda *et al.*, 2020). Also, the wax moths spread infectious diseases, particularly the foulbrood (Goulson *et al.*, 2015). The management of *G. mellonella* relies mainly on the use of pesticides. Pesticide use leads to colony collapse disorder, which is responsible for substantial economic and ecological losses (Rucker *et al.*, 2019), besides leaving insecticide residues in the honey and honey products (Mansour, 2020). Pesticides also have an indirect impact by damaging the honeybees' food source. The resulting hive products are of poor quality and quantity (Fikadu, 2020). In recent studies, ionizing gamma radiation has been researched as a potential technique against *G. mellonella*. The irradiation method is quick to use, effective against a wide range of insect-pests and leaves no residue on the product. Ionizing radiation does not increase insect populations' resistance to it and rarely harms the treated goods' quality (Mansour, 2020). However, most studies have used only electromagnetic radiation (Cobalt-60 or Cesium-137 gamma radiation). The role of dose rate, energy and type of ionizing radiation has neither been investigated nor quantified. The present study investigated the effects of ionizing radiation's different types, dose rates and energies on egg hatch, pupation and adult

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emergence of *G. mellonella*, besides quantifying sterilizing doses of gamma, x-ray and electron beams for the male pupae of *G. mellonella.*

MATERIALS AND METHODS

G. mellonella culture was obtained from the Entomology Division of Sher-i-Kashmir University of Agricultural Sciences and Technology (SKUAST), Kashmir. The artificial diet to raise the culture was created using standard items along with slight alterations (Firacative *et al.*, 2020). The setting for the breeding circumstances was 30±2°C and 55±5% relative humidity. The eggs and larvae were collected according to standard practices (Mansour, 2020). Male pupae were identified and collected from the top of the diet medium using the Smith method and placed in paper cups before being exposed to radiation. The irradiation was carried out with multiple energy x-rays, gamma rays and electron beams at various dose rates using four radiation-generating machines: a Cesium-137 radioisotope-based Gamma irradiator, two Cobalt-60 radioisotope-based machines, and a Linear accelerator. The calibration of both photon and electron beams was carried out according to the international code of practice for dosimetry based on standards of absorbed dose to water issued by the international atomic energy agency. The *G. mellonella* eggs received radiation doses ranging from 50 to 450 Gy, with an incremental dose of 50 Gy. The irradiated eggs were observed for the next 10 days to count and record the number of hatching eggs. On the other hand, radiation was administered to a mixed population of *G. mellonella* larvae at doses ranging from 100 to 1500 Gy with a 100 Gy increment. Four samples and a sample size of 50 eggs were taken for each dose. The samples included eggs of all age groups (1-6 days) and all larval instars (first to seventh) to simulate an infected honeybee comb in the wild. The percentage mortality of the larvae to 500, 1000 and 1500 Gy was recorded after 24, 48, 72 and 96 h, respectively. The percentage of mortality was determined using Abbott's formula. The SPSS statistical software on the Finney technique was used to perform the Probit analysis for LD_{50} values. The pupae were irradiated with gamma, x-ray and electron beams with single doses ranging from 0 to 450 Gy with an incremental dose of 50 Gy. Four samples, each with a sample size of 10, were used for each dose. After irradiation, the irradiated pupae were kept in separate plastic bowls at a controlled temperature and humidity (30±2°C and 55±5% RH). These bowls were covered with muslin cloth and labelled with dose values. The pupae were watched over until they were fully grown into adult moths. The mature male moths that had emerged from the irradiated pupae were moved to various cubical plastic cages with dimensions of 1x1x1 m^3, where they were permitted to mate with regular female moths. The number of eggs laid by each female, the number of eggs hatched and the numbers of emerging larvae were all measured after the adult moths mated for each of the three forms of ionizing radiation at regular intervals.

RESULTS AND DISCUSSION

The hatchability of *G. mellonella* eggs and the survival of larvae showed a strong negative correlation with the dose of all types of radiation. The eggs of *G. mellonella* were found to be more radiosensitive than the larvae. It was observed that the egg hatchability depended more on the dose rate and less on the energy of the radiation for both electromagnetic and particulate radiation.

Larval mortality in response to Co-60 gamma radiation was 10% at doses of 290.91 Gy (R^2 = 0.986 at P=0.05) and 616.07 Gy (R^2 = 0.946 at P=0.05). The corresponding dose rates were 2.67 and 0.81 Gy/min. Larval mortality for Co-60 gamma radiation was 20% at doses of 523.47 Gy (R^2 = 0.986 at P=0.05) and 973.21 Gy (R^2 = 0.946 at P=0.05), respectively, for dose rates of 2.67 and 0.81 Gy/min (Fig. 1).

The doses needed to lower larval mortality by 10 and 20% were found for two types of radiation. For gamma rays (662 KeV, Cs-137), the doses were 269.55 and 507.64 Gy (R^2 = 0.988 at P=0.05), respectively. For X-rays (6 MeV), the doses were 323.81 and 499.25 Gy (R^2 = 0.984 at P=0.05), respectively (Fig. 2). For a variety of electron beams with energies ranging from 4 to 20 MeV and dose rates ranging from 100 to 888 MU/min. The relationship between larval mortality and dose rate and energy displayed a similar pattern.

These results were superior in terms of time

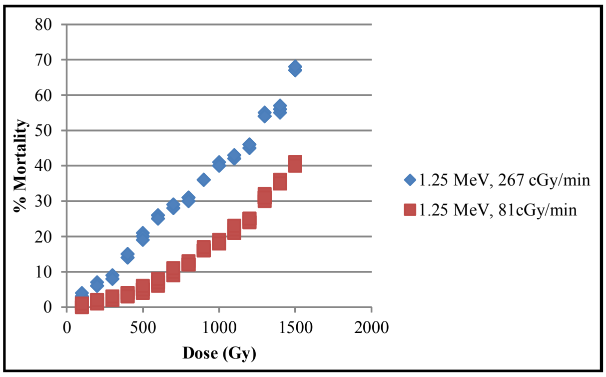

Fig. 1. Dependence of mortality of larvae on dose rate of ionizing radiation.

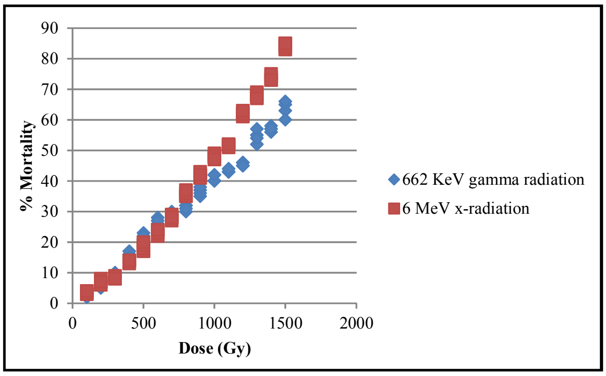

Fig. 2. Dependence of mortality of larvae on the energy of ionizing radiation.

taken to inflict larval mortality than those of Mahmoud and Abdel-Rahman (2021), who investigated the influence of some plant oils on the wax moths and observed that it took seven days for the clove oil to cause the highest mean percentage of larval mortality of *G. mellonella*, at 68.33±3.33%, followed by garlic oil at 51.66±1.66 and rosemary oil at 38.66±4.41% (Mahmoud and Abdel-Rahman, 2021).

Numerous laboratory investigations showed that entomopathogenic fungi were successful in controlling *G. mellonella* (Fergani and Yehia, 2020). According to another investigation, the biological control potential of the *B. bassiana* G-A and G-B isolates in *G. mellonella* larvae was high (Gençer and Bayramoglu, 2022). Greater wax moths may be managed using the fungus *Beauveria bassiana*, which naturally developed in soils all over the world and parasitizes several species of arthropods (Abou-Shaara, 2020). *Beauveria bassiana* is used to biologically manage agricultural pests; however, its effectiveness is mostly limited by environmental factors like high temperatures and low humidity. These restrictions, which

Table 1. Effect of different doses on the egg number, egg hatching and larval emergence of wax moth for gamma rays, x-rays and electrons

Dose (Gy)	Gamma (0.662 MeV)			Gamma (1.25 MeV)			X-ray (6 MV)			Electron (6 MeV)		
	Mean no. of eggs laid by each female	Mean no. of eggs hatched	Mean no. of larvae	Mean no. of eggs laid	Mean no. of eggs hatched	Mean no. of larvae	Mean no. of eggs laid	Mean no. of eggs hatched	Mean no. of larvea	Mean no. of eggs laid	Mean no. of eggs hatched	Mean no. of larvae
0	610.4±18	602.3±14	520.2±32	610.4±18	602.3±14	520.2±32	610.4±18	602.3±14	520.2±32	610.4±18	602.3±14	520.2±32
50	599.8±38	583.3±30	501.7±37	580.4±37	570.3±37	501.4±45	589.4±45	572.3±38	491.6±48	581.4±48	563.9±36	489.7±57
100	510.6±42	490.6±34	392.1±51	500.3±37	460.4±34	352.4±43	503.6±42	450.7±40	310.5±46	498.4±43	394.5±28	292.4±19
150	470.7±28	392.7±25	280.7±31	474.6±24	370.7±27	250.8±18	468.2±32	372.7±28	241.4±22	459.7±35	362.4±33	240.7±24
200	441.4±37	309.8±28	202.4±22	436.4±27	300.3±22	208.6±20	450.4±28	302.5±22	200.7±18	432.3±33	287.6±30	182.5±28
250	420.3±20	238.4±28	180.7±16	428.9±40	207.6±35	97.0±28	417. 3±32	201.9±31	88.3±26	396.7±24	200.8±26	120.4±19
300	383.7±14	203.3±12	30.5±4	380.6±12	192.8±12	42.8±6	369.7±13	173.8±14	44.5±6	351.8±17	152.1±15	12.3±7
350	331.4±15	20.7±1	0	321.2±12	05.7±2	0	335.5±12	10.4±2	0	251.4±14	02.1±0.8	0
400	17.3±8	0	0	28.7±3	0	0	20.4±2	0	0	12.1±3	0	0

would be magnified in a future of global warming, could render ineffective biological control methods based on this fungus. Also, the studies remain silent about the commercial viability of the G-A and G-B isolates because they haven't been tested in real-world settings. The findings on the effect of radiation dose on the sterility of male pupae demonstrated that male sterility was dose-dependent. The number of eggs laid and hatched by the female moths after mating with irradiated male moths significantly decreased as the dose increased (Table 1). All forms of ionizing radiation were found to significantly cause sterility in the exposed pupae at a dose of 350 Gy.

The findings revealed that all types of ionizing radiation had a detrimental effect on *G. mellonella's* larval mortality and egg hatchability. Numerous researchers recorded the gamma-ray dose values for a variety of insects (Mansour, 2015, 2020; Khan *et al.*, 2023). The results of the present study showed that the dose rate significantly impacted the larval mortality of *G. mellonella*. Since the amount of damage done by radiation was a combination of lethal and sub-lethal damage, one possible reason for this spike in mortality with an increase in dose rate may be the decline in the ability of the cell to repair its sub-lethal damage. Long-term exposures did not allow DNA repair. The electron beams in the MeV range of energy were slightly more effective against *G. mellonella*. A gamma radiation dose of over 200 Gy was sufficient to eliminate *G. mellonella* eggs from the stored honey bee combs. A dose of 500 Gy resulted in zero per cent survivability at 105.74 h for Cs-137 gamma rays and 89.04 h for 6 MeV electrons, respectively. Electromagnetic radiation (x-rays and gamma rays) lost energy and deposited dose in a medium by multiple modes depending on the energy of the incident radiation. The Compton effect is the predominant form of interaction of x-rays and gamma rays in the energy range of 30 KeV to 30 MeV, which helped to explain why the mortality of larvae was independent of the energy of the x-rays and gamma rays. The Compton effect was energy-independent in this range and only reliant on the medium's electron density, which was roughly the same for most elements.

Irradiated insects lost their ability to reproduce as a direct consequence of the harm to germ cells. As a result, the number of eggs laid declined as the dose given to the pupae increased. The pupal stage is chosen to determine the sterility dose because, at this stage, the somatic and gametic tissues differ most in sensitivity, the imaginal tissues have differentiated, and cell division is most active in the gonads.

Stored bee combs with the greater wax moth (*Galleria mellonella*) infestation can be treated with ionizing radiation. A dose of 350 Gy will significantly harm the *G. mellonella* eggs and larvae in addition to rendering the pupae sterile. This could boost agricultural production overall and honey production in particular without releasing any chemicals into the environment or into the food supply. Rural livelihoods might significantly improve as a result. High dose rate of gamma irradiation in the MeV range has advantages over electron and x-ray radiation in terms of cost and ease of usage.

CONCLUSION

In the treatment of *G. mellonella*, ionizing radiation had the potential to take the place of chemical pesticides. A dose of 350 Gy was sufficient to control *G. mellonella* in all of its developmental phases. Additionally, the *G. mellonella* can be sterilized with this dose. Ionizing radiation will make sure that no chemicals are released into the environment or the food supply. The ecosystem in general and human health specifically will benefit from this.

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