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Assessing the effects of gamma radiations on the protein concentration and mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract
The Red Flour Beetle, *Tribolium castaneum* (Herbst), is a key pest of stored products all over the world. The damages caused may result in reduced product quality and marketability. The use of insecticides and fumigants for the control of this pest has caused serious environmental problems and health hazards. Biologically based methods such as the use of botanicals and other means like the irradiation of insects, are gaining increasing role in the control of various storage pests. The aim of the present study was to determine the effects of gamma-irradiation on the mortality and protein content of *Tribolium castaneum* (Herbst). Doses ranging from 0.1kGy to 0.35kGy were applied. The mortality rates were higher in the irradiated insects than the control. The insect samples irradiated with 0.35kGy died within 6 Days after irradiation (DAI), while the samples irradiated with 0.1kGy died within 18 DAI. The protein content (estimated by Lowry's Method) was much also reduced in the irradiated insects than the unirradiated ones. These results are encouraging and indicate that irradiation is an effective measure in the control of *T.castaneum*.

Keywords: *T.castaneum*, gamma radiation, mortality, Lowry's method

1. Introduction
Stored Grains and processed products are subjected to attack by pests that feed on the products under a wide range of conditions. Most of them have become adapted to living in one or more links of the food storage ---processing---- distribution chain. Stored products pests, which enjoy a cosmopolitan distribution, are the prime concern to food security. *Tribolium castaneum, T.confusum, Sitophilus oryzae, S.granaries, Rhyzopertha dominica, Plodia interpunctella, Oryzaeplilus surinamensis, Lasioderma serricorne* are the major pests causing heavy damage and economic loss to a range of stored products in warehouses.
Among these storage pests, *Tribolium castaneum* Herbst (Coleoptera Tenebrionidae) is one of the most widespread and destructive pest. *Tribolium castaneum* is a polyphagous pest which feeds on different stored-grain and grain products, nuts and dried fruits [1, 2]. The adult is long lived. Females mate several times, and may lay 300-600 eggs. The egg is directly laid on the food material it feeds. Under favourable condition, about 90% of the eggs laid by young female are viable. The number of larval instars ranges from 5 to 11. The hatched larvae feed on the food for about 3-4 weeks and pupate. The adult is also observed to fly, but it is not a strong flier [3]. According to Howe, the optimum conditions for the rapid development of *T.castaneum* are between 35-37.5°C and a relative humidity over 70% [4].
The major damage caused by this pest is due to contamination of the stored product with large numbers of various life stages, dead bodies, cast skins and fecal pellets. They also produce many pungent substances which affect the quality of grain and result in reduced marketability and consumption by humans. Huge infestations of the pests cause buildup of heat in the containers and thereby result in mold growth. This causes a major reduction in the product quality and marketability of the products. The pest presence in food may cause also allergic responses, when product is consumed.
Management of this pest has proved to be a major obstacle in the food storage and processing industry. Control of *T. castaneum* populations is primarily dependent on repeated applications of conventional insecticides or fumigants [5, 6]. Although effective, their repeated use foster serious environmental and human health concerns [7, 8]. Synthetic insecticides have been widely developed and are extensively used because of their effectiveness, easy application and storage. The indiscriminate use of synthetic insecticides has caused environmental contamination and toxicity to living organisms [9]. Presently, one of the most common methods of controlling stored-products is the use of fumigant compounds like methylbromide and phosphine. These two pesticides are very toxic for humans and have very severe side effects and therefore, their use have become limited [10, 11].
These problems have highlighted the need for development of other pest control alternatives
One such alternative measure is the irradiation of the pests. Gamma radiations are applied so as to produce mortality or sterility in the insects. Irradiation of the insects with high doses can produce the desired effects. The present study focuses on the effect of gamma-rays on
T. castaneum and the results may be useful for commercial applications.

2. Materials and Methods
2.1 Culturing of Insect and Irradiation of the Samples
The beetle, *T. castaneum* was cultured in the laboratory and

was maintained at room temperature. Ten adult beetles, irrespective of sex, were collected from the stock. Ten each in five replications were kept in specimen tubes of uniform size. Samples were exposed to gamma radiation at the Gamma Irradiation facility in the Meat Technology Unit, Kerala Veterinary and Animal Science University, Thrissur. The specimen tubes were exposed to six doses of irradiation ranging from 0.1kGy to 0.35kGy. Treated beetles were transferred into another culture tubes along with the feed. Unirradiated beetles were kept as control. The development and mortality of insects were recorded daily and compared against the control.

2.2 Determination of total protein

Total proteins of *T. castaneum* were estimated according to Lowry *et al.*, [12]. For this purpose 50 mg of *T. castaneum* were homogenized in 0.4 ml of 10% TCA. The obtained homogenate was centrifuged at 10,000rpm for 10 minutes. The supernatant was collection and the amount of proteins was estimated.

3. Result and Discussion

3.1 Effect of Irradiation on mortality

The present study reveals that the irradiation of the *T.castaneum* has a major effect on its mortality. The samples exposed to higher doses achieved mortality within 6-9 days while in the samples treated with lower doses achieved mortality on a slower rate. (Table.1)

The mortality rate of the Control sample (unirradiated insect samples) was nil during the time of the experiment window.

Table 1: Mortality Rates of Samples of *T.castaneum* exposed to various doses of Gamma Radiation.

		MORTALITY (%)					
Sl.No	Dose	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
1	0.1kGy	2%	14.3%	23.8%	46.8%	58%	100%
2	0.15kGy	12%	18%	33.3%	75%	100%	-
3	0.20kGy	22%	33.3%	61.8%	100%	-	-
4	0.25kGy	28%	66.66%	100%	-	-	-
5	0.30kGy	32%	79.9%	100%	-	-	-
6	0.35Ky	48%	100%	-	-	-	-

Results of the present study are in accordance with previous research works. The present study reveals that 0.35 kGy can kill the pest in about3-6 Days after Irradiation and supports that low doses of irradiation can serve as disinfestation treatment and prevent further reproduction of adults. Also, it point out a close similarity in sensitivity of insects of other genus and species to the radiations. On irradiation at 0.1kGy from 1 to 5 days old *T. freemani*, complete sterilization was observed [13]. Another study indicated that doses higher than 0.20 kGy caused mortality of the *T. confusum* while, lower doses caused inhibition of development and sterility of surviving insects [14]. Lethal effect of gamma radiation on *Sitophilus granaries* when studied at different doses by Aldryhim and Adam [15], revealed that eggs and larvae were unable to develop adults at doses 0.30-0.30 kGy.

3.2 Estimation of protein content

Proteins are the major biomolecule in all living cells and are necessary for the proper functioning of an organism [16].The protein content estimated by Lowry method in the present study indicated that the concentration of protein was lower in the irradiated ones than the control (Table 2). The protein content was highly reduced in the samples irradiated with higher doses.

Table 2: The protein concentration of *T.castaneum* exposed to various doses of Gamma Radiation

Sl.No	Dose (kGy)	Protein Concentration (µg/mL)
1	Control	184.2
2	0.1	163.0
3	0.15	160.4
4	0.20	154.3
5	0.25	148.9
6	0.30	139.2
7	0.35	136.1

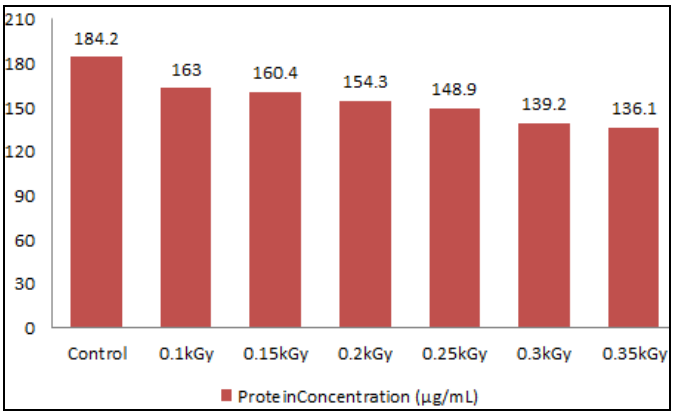

Fig 1: Graph indicating the protein content in *T.castaneum* exposed to Gamma Radiation

Similar work has been carried out in other insect species also. The protein content in the irradiated *Galleria mellonella* was drastically reduced than the control when exposed to radiations (iaea.org). The reduction in protein content is attributed to the effect of irradiation on the molecular mechanisms. When exposed to radiations, the protein synthesis may be blocked at cellular level which results into low availability of proteins [17].

It is proved by the results that the varying doses of gamma radiation cause high lethality in *T. castaneum* at significant doses and caused significant inhibition of metabolic constituents. Therefore, it is recommended that the application of Gamma radiations should be performed to control stored grain insects.

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