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Effects of gamma radiation on adults of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) collected in the retail trade in São Paulo/SP city

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ABSTRACT

Brazil is one of the world's largest grain producers, and inadequate storage favors the appearance of pests. Among the most important insect species, the genus *Sitophilus* and *Tribolium* stand out. The constant use of chemical pesticides leads to the selection of resistant populations and the need to study different methods for integrated control. Considering these aspects, the present study aimed to evaluate the mortality of adult individuals of *S. zeamais* in brown rice and *T. castaneum* in wheat flour, collected on substrates sold in the retail trade in São Paulo/SP city, exposed to increasing doses of gamma radiation. The experiment was carried out at the Radiation Technology Center at the Institute for Energetic and Nuclear Research – IPEN/USP, the substrates with the specimens were irradiated with doses of 0 (control), 25, 50, 75, 100, and 200 Gy in a Cobalt-60 source, type Gammacell 220, at a dose rate of 0.903 kGy/hour. The experiment consisted of 4 replicates per treatment and each replicate 10 insects, in a total of 40 insects per treatment, and the statistical design was completely randomized. The dose of 75 Gy was sufficient to obtain 100% efficiency for *S. zeamais*, but the dose of 200 Gy did not reach satisfactory efficiency for controlling *T. castaneum*. Therefore, a dose of 400 Gy should be recommended for the control of these two insect species in their respective substrates.

Keywords: **gamma radiation, stored grain pests, control, insects.**

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1. INTRODUCTION

Worldwide post-harvest losses can reach 30% of agricultural production, in Brazil, losses between harvest and storage reach 20% [1]. Approximately, 45.53% of the losses occurred in the storage logistics activity; 21.67% in road transport from the farm to the warehouse; 13.31% in road transport; 1.62% in waterway multimodal transport; 8.24% in multimodal rail transport; finally, 9.04% at the port [2]. Inadequate storage of grains, and the deficiency in temperature and humidity control, favors the appearance of pests. Insects of genus *Sitophilus* (Curculionidae) and *Tribolium* (Tenebrionidae) can cause quantitative and qualitative losses [3, 4].

S. zeamais Motschulsky (Curculionidae) is considered an internal primary pest, as it attacks seeds and whole and healthy grains, piercing to feed and complete its development. *T. castaneum* Herbst (Tenebrionidae) is a secondary pest that feeds on processed grains (farinaceous) or previously damaged by primary insects, accidentally broken or cracked [5]. The Technical Regulation of Identity and Quality of Wheat Flour recommends that the grains should not exceed 1.5% of damage [6], so to reach this percentage Da Silva *et al*., observed that it would be necessary to have a population of *S. zeamais* composed of 180 insects kg-1, associated with 0.13% wetting, 0.4 kg hL-1 reduction in hectoliter weight and 0.7% dry matter loss [7].

The use of chemical products is one of the most used methods to control insects, however they present several drawbacks, including the possibility of causing intoxication to the consumer by leaving residues in the treated foods and promoting the selection of individuals resistant to the product. Therefore, as a residue-free method of pest control, radiation treatment is a viable substitute for chemical control to satisfy the quarantine regulations of many countries [8].

The treatment of food with ionizing radiation, whether particles or electromagnetic waves, have enough energy to change the structure of matter, in order to obtain the desired purpose, such as inhibition of sprouting, delay in the maturation process, disinfection of insects, disinfection of parasites, reduction of microbial load and reduction of fungi [9].

The mode of action of ionizing radiation in organisms consists of a chain of oxidative reactions that occur during irradiation, forming highly reactive metabolites such as free radicals, causing irreversible damage to DNA, such as chromosome breaks and lethal mutations [10]. These effects may be somatic (irradiated individual) or hereditary (offspring of the individual) as a result of damage to cells in the reproductive organs. Chromosomal breaks do not interfere with the ability of gametes to participate in fertilization, so the affected sperm fertilizes the egg in the normal way, and major lethal mutations kill most embryos during the first cleavage divisions [11].

In the review Bakri *et al*. on radiation doses for disinfestation and reproductive sterilization of arthropods, among the 78 coleopteran species, the Curculionidae and Tenebrionidae families, represent the main groups tested for radiation sterilization, with 26 and 11 species, respectively, requiring an average dose of approximately 76 Gy [12]. For disinfection of insects in food, the doses used are considered low, up to 1kGy [9]. Considering these aspects, the experiment evaluated the mortality of adult individuals of *S. zeamais* in brown rice and *T. castaneum* in wheat flour collected on substrates sold in the retail trade in São Paulo/SP city exposed to increasing doses of gamma radiation.

2. MATERIALS AND METHODS

The experiment was carried out at the Radiation Technology Center at the Institute for Energy and Nuclear Research – IPEN/USP, São Paulo. The substrates (Brown rice with *S. zeamais* and wheat flour with *T. castaneum*) were irradiated with doses of: 0 (control), 25, 50, 75, 100 and 200 Gy in a source of Cobalt-60, type Gammacell 220, under a dose rate of 0.903 kGy/hour. The insect populations (One of *S. zeamais* and one of *T. castaneum*) were collected on substrates sold in the retail trade in São Paulo/SP city and were kept in a room conditioned at 27 ± 2°C with relative humidity of 60 ± 10 % and photoperiod equal to 12h.

Newly emerged adults aged 3 to 7 days, unsexed, were used in the experiment. The experiment consisted of 4 replicates per treatment and each replicate 10 insects, in a total of 40 insects per treatment, and the statistical design was completely randomized. The cylindrical flasks used were acrylic with a capacity of 30mL, and plastic lids under pressure, with small perforations to allow aeration. The amount of substrate per vial was approximately 20g. For the evaluation of the experiment, weekly counts were carried out, removing and recording the number of dead insects, for a period of 35 days, respecting the development cycle for the first generation. Data were submitted to Probit analysis [13], using the POLO PLUS program [14] and variance analysis compared by Tukey's test at a 5% probability level.

3. RESULTS AND DISCUSSION

Evaluating the accumulated mortality (336h), the doses of 75, 100 and 200 Gy reached 100% of efficiency for *S. zeamais* (Tab. 1). Negrão verified in popcorn seeds that the lethal dose for eggs was 25 Gy, for larvae - 50 Gy and pupae - 75 Gy, promoting sterility in adults of *S. zeamais* [15]. Follett *et al*. when irradiating *S. oryzae* in rice, achieved control with doses of 90 Gy for the egg, larvae and pupae stages, and a dose of 120 Gy for the adult [16]. Although, Ramos and Arthur studied the effect of gamma radiation in diets for domestic animals infested by this pest and concluded that doses from 500 Gy were sufficient to cause lethality of eggs and larvae and induce sterility [17, 18]. The irradiation process can be influenced by temperature, humidity, irradiation atmosphere (presence of oxygen), as well as the physical state of the material to be irradiated [19].

Table 1 - Application of increasing doses of gamma radiation (Gy) on adults of *S. zeamais* and *T. castaneum*. Mean of mortality (Mean *) and efficiency (% Ef **) accumulated with 336h.

Dose (Gy)	*S. zeamais*		*T. castaneum*	
	Mean*	%Ef**	Mean*	%Ef**
25	4,00 b	10,00	0,25 bc	0,62
50	9,00 a	22,50	-	-
75	10,00 a	100,00	1,00 bc	2,50
100	10,00 a	100,00	2,50 b	6,25
200	10,00 a	100,00	5,50 a	13,75
C.V.	4,40		10,43	

Means followed by the same letter did not differ significantly from each other by the Tukey test ($P \leq 0.05$). C.V.: Coefficient of Variation.

Another important factor to stand out in the face of this difference in values is related to the dose rate, because the higher the dose rate, the faster the irradiation process and the greater the effect induced by radiation on biological systems [20], decreasing the probability of recovery of somatic cells. Fontes *et al*. when studying different dose rates to control *Callosobruchus maculatus* (Fabr.) observed that when applying a high dose rate of 1418 Gy/hour the letal dose was less than 25 Gy, while on a low dose rate of 55 Gy/hour the lethal dose had a fourfold increase, corresponding to 100 Gy [21].

To *T. castaneum*, a statistically significant difference was observed starting 100 Gy. At the dose of 50 Gy, there was no more emergence of adult insects, possibly suggesting sterility in adults. In eggs, Fontes and Arthur concluded that the lethal and sterilizing doses were respectively 30 and 20 Gy [22]. In pupae, the lethal dose found was 460 Gy and the sterilant dose was 150 Gy [23] and in adults Fontes *et al*. reached a sterilizing dose of 75Gy in wheat flour [24].

In an Egyptian population of *T. castaneum* submitted to an irradiation rate of 0.27 Gy/s, Tabikha concluded that a dose of 90 Gy would be ideal to inhibit the development of immature stages and cause complete sterility of the mature stage [25], in turn, Santos *et al*., observed that for the control in dehydrated mushroom the lethal dose would be 2 kGy [26], which explains the low mortality in this study. The 200 Gy dose caused 100% mortality in irradiated adults 14 days after treatment. There is a dose-dependent increase in the periods of different stages of development age and strain used [27]. In the bioassays performed by Farias, the dose of gamma radiation that proved to be efficient for the control of *T. castaneum* adults 4 hours after irradiation was equal to 2.91 kGy [28].

Currently, an alternative to reduce the radiation dose used to control stored grain pests would be the combination with essential oil. This technique was used by Hossain *et al*. to control *S. oryzae*, the synergism observed between radiation and gases from eucalyptus essential oil resulted in a 3- to 6-fold increase in mortality compared to treatment by radiation alone, remembering that the dose rate must be maintained high for better use [29]. El-Gizawy *et al*. combining 162.56 µl/l (50% lethal concentration) of rosemary essential oils with 350 Gy obtained total mortality of *T. castaneum* adults in one week, and to obtain 50% of mortality it took only 1.12 days [30].

As in the research carried out by Da Silva and Arthur, has been verified that the insects of the species *S. zeamais* were more radiosensitive [31].

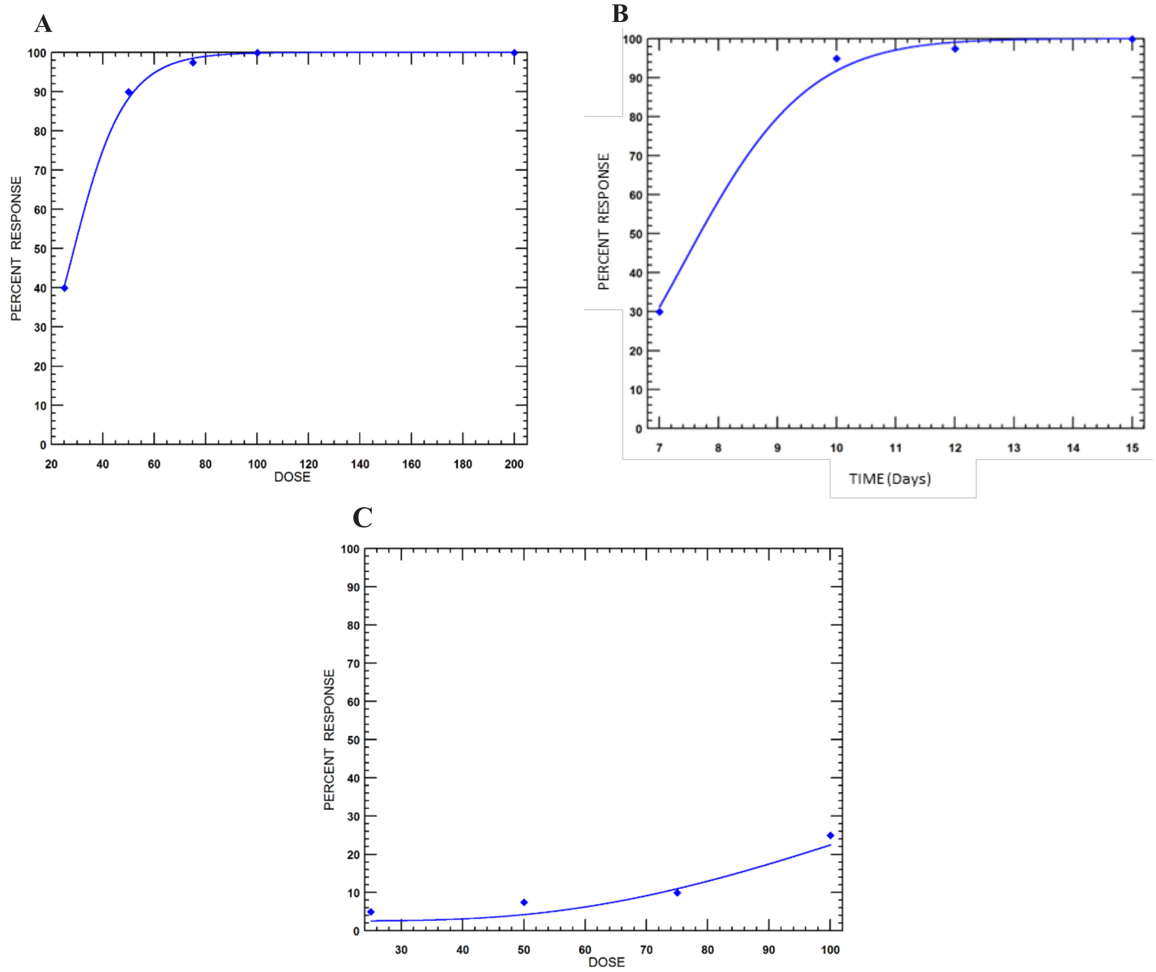
The estimated adult lethality of *S. zeamais* (LD_{50}) was 33.09 Gy and the doses needed to control 90% of the study population (LD_{90}) were 83 Gy (Tab. 2). Considering the 200 Gy dose, the mean lethal time to reach 90% control (LT_{90}) was approximately 12 days (Fig. 1). The dose-response for *T. castaneum* was below 30% with the dose of 100 Gy. These results demonstrate superior sensitivity of the population of *S. zeamais* in relation to the population of *T. castaneum*. Therefore, *T. castaneum* presented the dose response percentage value at the dose of 200 Gy 70% lower, the result similar to that of Arthur *et al*. [23].

Table 2. Estimate of Lethal dose (LD - Gy) and Lethal time (LT - days) of *S. zeamais*.

	n	**(95% C.I.)**	**Slope ± SE**	**χ^2**	**D.F.**
LD_{50} (Gy)	240	33.09 (25.16 - 39.41)	5.825 ±1.11	0.46	3
LD_{90} (Gy)	240	83.00 (66.50 - 98.23)			
LT_{50} (200 Gy)	40	7.678 (7.140 - 8.154)	3.158 ±1.857	1.667	2
LT_{90} (200 Gy)	40	11.928 (10.769 - 14.231)			

Confidence Interval – C.I.; Slope and e Standard Error - S.E.; Chi-square - $\overline{\chi^2}$; Degrees of Freedom - D.F.

Figure 1: Percent Response (A – Letal Dose (Gy) and B – Letal Time (Days) – 200Gy) of *S. zeamais* and C – Letal Dose (Gy) of *T. castaneum*.



According to the International Standards for Phytosanitary Measures - ISPM nº 18, the minimum absorbed dose to sterilize stored product beetles is in the range of 50 to 400Gy [32]. Regarding the substrate, Zanão *et al*. analyzed the effect of gamma radiation on the physicochemical and sensory characteristics of rice, considering the sterilizing dose of 1 kGy for *S. oryzae*, there was no change in the percentage of grain breakage during processing, changes in the proximate composition and in the amylose content present, did not influence the cooking time, had good acceptance in the tasting without promoting changes in color, and this dose is recommended for food conservation [33].

Sileen *et al*. observed in *T. castaneum* that the dose of 1 kGy provides total mortality of individuals from the seventh day after treatment in wheat flour, in addition, they found that radiation eliminates fungi and bacteria from the surface of the food and inhibits the effect of quinones, secreted by the insect, which can cause cancerous changes in human and animal tissue cells after consumption [34].

According to World Health Organization, Food and Agriculture Organization and International Atomic Energy Agency, foods irradiated with an adequate dose to achieve the technical objective are safe for consumption and nutritionally adequate. Thus, irradiation applied for radurization (0.4-10 kGy) or radicidation (40-45 kGy) does not reduce the nutritional quality of the food [35, 36].

Here, we demonstrate the difference in the use of gamma radiation to control 2 populations of weevil species and thus establish a single and satisfactory dose to optimize the irradiation process. In this case, we considered the results cited in the literature for the species *T. castaneum*, whose minimum absorbed dose to sterilize beetles from the stored product is in the range of 50 to 400Gy [32].

4. CONCLUSION

Gamma radiation is an important strategy for the control of stored grain pests. The dose of 75 Gy was enough to obtain 100% efficiency for *S. zeamais* but the dose of 200 Gy did not reach satisfactory efficiency to obtain 100% of control for *T. castaneum*. Therefore, a dose of 400 Gy should be recommended for the control of these two insect species in their respective substrates.

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