IONIZING RADIATION AS A PHYTOSANITARY TREATMENT IN *Zea mays* L. VAR. *EVERTA*

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ABSTRACT

Quarantine treatment has been used to provide protection against pests by eliminating the stages evolutionary cycle that may be associated with the product. Popcorn is used in the food industry in the form of dry beans and is an economic option for producers and their sale generates foreign exchange worldwide. The aim of this work is to evaluate the effects of gamma radiation on seeds of popcorn (*Zea mays* L. var. everta). The seeds was be purchased in the retail trade of São Paulo city, later was be infested in the Laboratory of Food Irradiation at IPEN, then was be submitted to different doses of gamma radiation: 0, 25, 50, 75 and 100 Gy. A Gammacell-220 at ^{60}Co source at a dose rate of 1.4 kGy / hr at the Technology Center of Radiation (CTR) IPEN-CNEN / SP. Each treatment was consisting of 3 replicates with 100 grams of popcorn kernels. Samples was be packed and stored at a temperature of 25 ± 2 ° C and relative humidity of 70 ± 5%. By the results, it can conclude that the dose of 75 Gy can be indicated to control insects *Sitophilus zeamais* in popcorn.

Keywords: Disinfestations, quarantine treatment, stored grains, insect.

1. INTRODUCTION

The early history of food irradiation (1890s-1940s) is inseparably on linked to physics and to the development of the systems and sources to be used in food irradiation. This was followed by a period of intensive research and development (1940s 1970s) that overlapped with extensive studies on the wholesomeness of irradiated foods (1970s). Nowadays, food irradiation applications belong to either one of two basic types: those concerned with preventing food losses and those that result in microbial decontamination of food products or in inactivation of foodborne human parasites (Molins, 2001).

Irradiation is a versatile technology to disinfest fresh and durable agricultural commodities of quarantine pests. Quarantine or phytosanitary treatments eliminate, sterilize, or kill regulatory pests in exported commodities to prevent their introduction and establishment into new areas. The goal of irradiation as a phytosanitary treatment is to provide quarantine security for any regulated pests residing in or on commodity. This is most often accomplished by preventing development to the reproductive stage or sterilizing the reproductive stage of the insect (Follet & Griffins, 2006).

Primary pests are those that attack healthy grains and seeds and depending on what part of the grain they attack, can be termed primary external or internal pests. The inner primaries pierce the grains and seeds and penetrate them to complete their development. They feed on the entire interior of the grain or seed and enable the installation of other deteriorating agents. Examples of these pests are the *species Rhyzopertha dominica*, *Sitophilus oryzae* and *S. zeamais*. External primary pests destroy the outer part of the grain or seed (bark) and then feed on the inside without, however, developing inside them. There is destruction of grain or seed just for food purposes. (Lorini, 2008).

Insects, in general, are sensitive to radiation. As with other organisms, the effects of radiation on insects are closely related to the effects on constituent cells. For insect cells, radiation sensitivity is directly proportional to the reproductive activity of the cells and inversely proportional to their degree of differentiation. For each particular commodity, the insect species infesting it should be known. As the radiation sensitivity varies in insect orders, even in the development stages of the same insect species, a disinfestations dose should be established only after considering the species of insect, its development stages in the commodity, and the environmental conditions during the treatment (Ahmed, 2001).

Corn is one of the most cultivated cereals on all continents. There are approximately 150 species of corn today, with a great diversity of grain color and shape. It is an easy cereal to be planted and harvested, be it dent corn, flint corn or popcorn. Compared to corn crops, corn popcorn plantations are found to be fragile. Thus, in comparison to corn cultivars, popcorn plants are more susceptible to pests and diseases (Abimilho, 2015; Sawazaki, 2010).

The works involving the popcorn corn crop is scarce, especially those related to the incidence of pests. It is attributed that the pests that affect the common corn crop bring the same damages to the crops of the popcorn. Several insect species can infest corn crops, causing injury and damage from planting to harvesting. (Silva et. al, 2000)

The most important factor in grain of popcorn conservation concerns climate. Thus, considering the favorable climatic and ecological conditions of Brazil, the pests that occur in storage develop quite easily, compromising the preservation and quality of the grains. In this context, it highlight the caruncho of the corn *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae), due to their high reproductive potential, wide distribution in the national territory, existence of populations resistant to insecticides and mainly to the damages caused to stored rice, corn and popcorn and wheat (Potenza, 2004).

This insect are a weevils 2.0 mm to 3.5 mm in length, dark brown, with lighter patches on the elytra (anterior wings), visible shortly after emergence. The oviposition period is 104 days, and the average number of eggs per female is 282. The females' longevity is 140 days. The incubation period oscillates between 3 and 6 days, and the egg cycle until adult emergence is 34 days (Lorini, 2008).

Irradiated insects of the same order are unanimous in affirming the efficiency of ionizing radiation for the control of insect pests of stored grains Arthur, (1997); Arthur, (2012); Arthur; Berti-Filho; Arthur, (2003); Arthur; Machi; Mastrangelo, (2015); Arthur et., (2017); Arthur et al., (2000).

Considering the increasing demand for good quality foods and the increasing consumption of popcorn in the country, and of the scarcity of information about this product, the aim of this work is to evaluate the effects of gamma radiation on the evolutionary cycle of *Sitophillus zeamais* in corn popcorn seeds.

2. MATERIAL AND METHODS

2.1. *Sitophillus zeamais*

The insects used to perform the experiments were of the species *Sitophilus zeamais* Morts from a breeding kept in the laboratory of Entomology of the Center of Nuclear Energy in Agriculture - CENA /

USP, Piracicaba - SP. The experiment consisted of 3 replicates for each stage of the insect's life cycle, and each repetition consisted of 60 grams of popcorn per treatment, and the statistical design was completely randomized. The flasks used were glass with a capacity of 100 ml, with metal covers with screen to allow aeration.

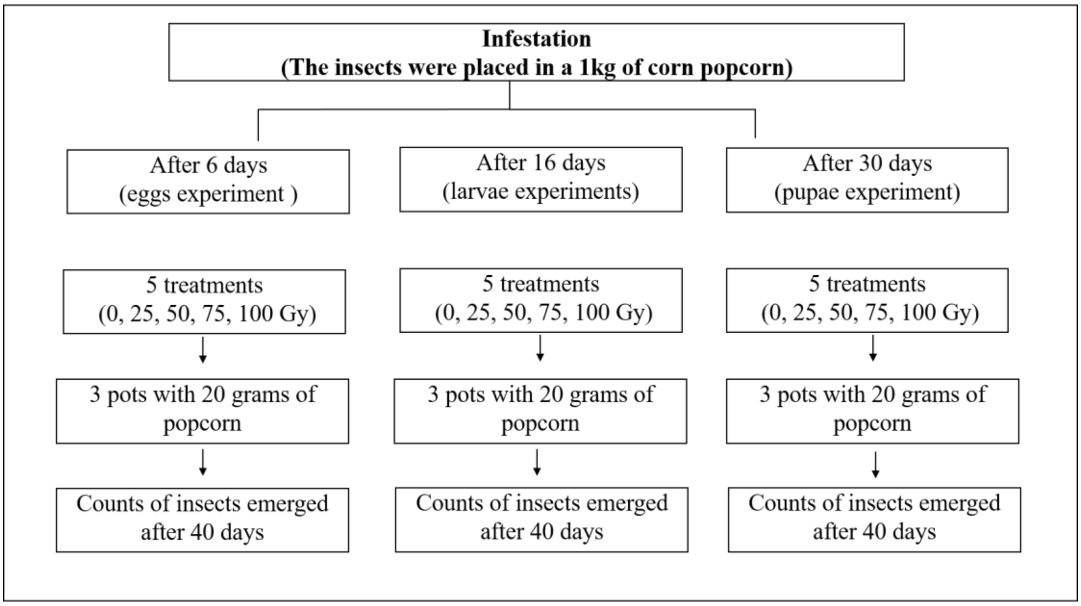
2.2 Irradiation of samples

The samples were irradiated at room temperature in a Gammacell-220 at ^{60}Co source at a dose rate of 1.4 kGy / hr at the Radiation Technology Center - CTR of the Institute of Energy and Nuclear Research - IPEN / CNEN / SP, in São Paulo city. Harwell Amber 3042 dosimeters were used to measure the radiation dose. After irradiation the flasks containing popcorn and insects were placed in an air-conditioned room with a temperature of 25 ± 2 ° C and relative humidity of 70 ± 5%.

2.3 Insect counting (Lethal Dose and Sterilization of adults)

Firstly, 1 kg of popcorn was placed in a 1000 ml pot glass, and then the insects were placed inside that glass for 6 days for infestation. Then a sample of 300 grams only of the popcorn kernels was removed, leaving the insects. These 300 grams were divided into 4 treatments (doses of: 0 (control), 25, 50, 75 and 100 Gy). After irradiation was expected the emergence of adult insects and was made counts the insect emerged within each repetition after 40 days (Test for lethal dose for eggs). The same methodology was performed after 16 days (lethal dose for larvae) and 30 days (Lethal dose test for pupae) as shown in Flowchart 1. Thus, lethal doses were obtained for the egg, larva and pupa phases, that is, the doses for quarantine treatment for these phases.

Flowchart 1 - Methodology used to determine the lethal doses for eggs, larvae and pupae stages of *Sitophilus zeamais.*



2.4 Statistical Analysis

The results were submitted to an analysis of variance and Tukey's test to compare the means, at the level of 5% of significance. The software used will be the SAS (Statistical Analytical System, SAS institute, N. C., USA, version 9.1).

3. RESULTS AND DISCUSSION

The Table 1 shows the average number of adults emerged in the F_1 generations from eggs of the generation paternal of *S. zeamais* irradiated with increasing doses of radiation gamma of Cobalto-60 of: 0 (control); 25; 50; 75 and 100 Gy in popcorn. From the results of this Table, we can observe that to all the treatments with radiation don't have significant statistical differences. Only the treatment control had statistical differences and the effects of the gamma radiation in the eggs were directly proportional to the increase of the doses of radiation. The dose of 25 Gy was sufficient to cause the lethality of the eggs.

Table 1. Mean and (± SE) of number insects adults emerged of eggs irradiated with doses increases gamma radiation after 40 days of process.

Dose (Gy)	Adults emerged
0	3,0±0,2a*
25	0,0±0,0b
50	0,0±0,0b
75	0,0±0,0b
100	0,0±0,0b

* Mean with same letters do not differ statistically by Tukey test at 5%

Table 2 shows the average number of adults emerged in the F_1 generations from larvae of the generation paternal of *S. zeamais* irradiated with increasing doses of gamma radiation of Cobalto-60 of: 0 (control); 25; 50; 75 and 100 Gy in popcorn. From the results of this Table, we can observe that to all the treatments with radiation don't have significant statistical differences. Only the treatment control had statistical differences. The effects of the gamma radiation in the larvae were directly proportional to the increase of the doses of radiation. The dose of 25 Gy was not sufficient to cause the lethality of the larvae because had the emergence of one insect in the F-1 generation then the lethal dose to larvae was of 50 Gy.

Table 2. Mean and (± SE) of number insects adults emerged of larvae irradiated with doses increases gamma radiation after 40 days of process.

Dose (Gy)	Adults emerged
0	6,0±0,1a*
25	1,0±0,0b
50	0,0±0,0b
75	0,0±0,0b
100	0,0±0,0b

* Mean with same letters do not differ statistically by Tukey test at 5%

Table 3 shows the average number of adults emerged in the F_1 generations from pupae of the generation paternal of *S. zeamais* irradiated with increasing doses of Cobalto-60 gamma radiation of: 0

(control); 25; 50; 75 and 100 Gy in popcorn. From the results of this Table, we can observe that to the treatments with radiation in the doses of 25 and 50 Gy don't have significant statistical differences with dose 0 (control). Only the treatments with doses of 75 and 100 Gy had statistical differences. The effects of the gamma radiation in the pupae were directly proportional to the increase of the doses of radiation. The dose of 75 Gy was sufficient to cause the lethality of the pupae because don't had the emergence of one insect in the F-1 generation.

Table 3. Mean and (± SE) of number insects adults emerged of pupae irradiated with doses increases gamma radiation after 40 days of process.

Dose (Gy)	Adults emerged
0	8,5±0,8a*
25	6,0±0,3a
50	5,3±0,1a
75	0,0±0,0b
100	0,0±0,0b

* Mean with same letters do not differ statistically by Tukey test at 5%

Table 4 shows the average number of adults emerged in the F_1 generations from adults of the generation paternal of *S. zeamais* irradiated with increasing doses of Cobalto-60 gamma radiation of: 0 (control); 25; 50; 75 and 100 Gy in popcorn. From the results of this Table, we can observe that to the treatments with radiation in the doses of 50, 75 and 100 Gy don't have significant statistical differences. The dose 0 (control) and 50 Gy don't have significant statistical differences. Only the treatments with doses of 75 and 100 Gy had statistical differences. The effects of the gamma radiation in the adults were directly proportional to the increase of the doses of radiation. The dose of 50 Gy was sufficient to cause the sterilization of the adults irradiated because don't had the emergence of insect in the F-1 generation.

Table 4. Mean and (± SE) of number insects adults emerged in generation F-1when the generation paternal was irradiated with doses increases gamma radiation after 40 days of process.

Dose (Gy)	Adults irradiated	Adults emerged F-1 generation
0	20	57,7±1,9a*
25	20	40,2±2,3b
50	20	0,0±0,0c
75	20	0,0±0,0c
100	20	0,0±0,0c

* Mean with same letters do not differ statistically by Tukey test at 5%

According to ISPM No. 18, (2003) the use of irradiation as a phytosanitary measure is to prevent the introduction or dissemination of regulated pests. This can be accomplished by obtaining certain responses in the target pest (s), such as: mortality; prevention of successful development (eg non-emergence of adults);

inability to reproduce (eg, sterility); or inactivation. A range of specific options can be determined when the necessary response is the inability to reproduce the pest. These may include: complete sterility; limited fertility of only one sex; egg laying and / or hatching without further development; altered behavior; and sterility of the F1 generation. For stored product beetles (Coleoptera) to sterilize active adult reproduction doses of 50-400 Gy. Then our results are according with those recommended by ISPM number 18 of 2003.

4. CONCLUSIONS

Treatment with ionizing radiation (irradiation) may be used for pest control of risk and Several researches proves efficacy of this treatment. All phases: egg, larva and pupa proved sensitive to gamma radiation. Dose of 50 Gy was sufficient to control the emergence of adults. Doses greater than 75Gy should be used for the quarantine treatment of Sitophilus zeamais in popcorn.

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5. REFERENCES

Anuário Brasileiro do Milho. (2015). *Brazilian Corn Yearbook*. Santa Cruz do Sul / Benno Bernardo Kist ... [et al.]: Editora Gazeta Santa Cruz, 2015.96 p.: il.ISSN 1808-3439.

Ahmed, M. (2001). *Disinfestation of stored grains pulses dried fruits and nuts and the dried foods.* In: Food Irradiation: Principles and application. In: MOLINS, R. A. Editora: Wiley Inter-Science. Cap.4. pág. 125-135

Arthur, P. B. Emprego da radiação gama do Cobalto-60 na desinfestação de alguns tipos de rações para alimentação de animais de pequeno porte. (Dissertação de Mestrado) IPEN, 68p. 2012.

Arthur, V. Controle de insetos pragas por radiações ionizantes. Arquivos do Instituto Biológico, v. 64, n.2, p. 77-79, 1997.

Arthur, V.; Machi, R. A.; Mastrangelo, T. Ionizing Radiation in Entomology: In: Topics in Ionizing Radiation Research, II Ed., In Tech. n. 1, p. 1-17, 2015.

Arthur, V.; Berti-Filho, E.; Arthur, P. B. Use of gamma radiation to control Sitophilus linearis (Herbst, 1785)(Coleoptera: Curculioniae) attacking Tamarindus indica (Leguminosae: Foboidae). Journal of Nuclear Agriculture and Biology, v. 29, n.3-4, p.217-219, 2003.

Arthur, V. ; Wiendl, F. M.; Arruda Neto, J. ; Tavares, M. Control of Rhyzopertha dominica (Fabricius) infesting wheat through accelerated electrons. Journal of Nuclear Agriculture and Biology, v. 29, n. 2, p. 117-119, 2000.

Arthur, V.; Fontes, L. S.; Arthur, P. B.; Machi, A. R.; Harder, M. N. C.; Rossi, R. S.; Franco, J. G.; Franco, S. S. H. Quarantine treatment by gamma radiation for different stages of Callosobruchus maculatus in bean Vigna sinensis. International Nuclear Atlantic Conference- INAC 2017 Belo Horizonte, MG, Brazil, October 22-27, 2017

Follet, Peter A. Griffin, Robert L. (2006). *Irradiation as a phytosanitary treatment for fresh horticultural commodities: Research and regulations*. In: Food irradiation research and technology: Sommers, C. H; Fan,X. Iowa: Blackwell,. 317p.

International Standards For Phytosanitary Measures. (2003). *Guidelines for the use of irradiation as a phytosanitary measure*. ISPM 18. Rome, IPPC, FAO.

Lorini, I. (2008). *Manejo integrado de pragas de grãos de cereais armazenados*. Passo Fundo: Embrapa Trigo. 72 p.

Molins, Ricardo A. (2001). *Food Irradiation: Principles and application*. Editora: Wiley Inter-Science.

Sawazaki, E. (2010). *Milho-pipoca*. XXVIII Congresso Nacional De Milho E Sorgo. IV Simpósio Brasileiro sobre a Lagarta do Cartucho. Goiânia, GO - 29 de agosto a 02 de setembro de 2010.

Silva, E. C. ; Galvão, J. C. C. ; Miranda, G. V. ; Picanço, M. C. ; Sawazaki, E. (2000). *Densidade populacional de Dalbulus maidis e do predador Doru luteipes em milho pipoca.*. In: XXIII Congresso Nacional de Milho e Sorgo, 2000, Uberlândia. A inovação tecnológica e a competitividade no contexto dos mercados globalizados, 2000.

Potenza, M. R. (2004). *Avaliação de produtos naturais irradiados para o controle de sitophilus zeamais mots (Coleoptera: Curculionidae) e Blatella Germanica (L.) (Dictyoptera: Blattellidae).* Dissertação Doutorado. Instituto de Pesquisa Energéticas e Nucleares.