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Control of *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae) by gamma radiation

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Abstract

The aim of the experiment was to determine the sterilizing dose from ionizing radiation by cobalt-60 to *Alphitobius diaperinus* (Panzer, 1797)(Coleoptera: Tenebrionidae) in peanuts. The study was conducted in the laboratory of Radiobiology and Environment of the Center for Nuclear Energy in Agriculture - CENA / USP at Piracicaba, SP - Brazil. Peanuts samples infested with adults of *A. diaperinus* with 8 treatments and 5 repetitions were used. Each repetition consisted of 20 adults, a total of 100 individuals per treatment. The samples were irradiated in doses of 0 (control), 25, 50, 75, 100, 125, 150 and 175 Gy, in a source of cobalt-60, Gammacell-220 type, with a rate dose of 381Gy / h. The experiment was conducted in a room with a relative of 25 ± 5 ° C temperatures and humidity of 70 ± 5%. After 50 days of irradiation process was evaluated of the number of emerged insects in each treatment. The results show that the sterilizing doses in F1 and F2 generations respectively were: 150 Gy and 125 Gy. The dose of 150 Gy of gamma radiation can be used in both generations as phytosanitary treatment to control of *A. diaperinus* infested peanuts.

Key words: Peanuts, *A. diaperinus*, Gamma radiation.

INTRODUCTION

Worldwide the loss of stored grain is a problem of economic order in importance, in view of the concern of the increased supply of food for a world population increasingly expanding. Associated with this fact, there is the problem of nutritional deficiency due to lack of protein, especially for the less privileged populations in the resources of a country. This lack could be met by adequate supply of grain produced, requiring for it, a system that provides optimum grain storage conditions in securing the quality until the time of consumption. The use of radiation in stored grain can solve the problem of the losses in these products, as not induce resistance of insects and leaves no toxic residue to the consumer, being considered an effective and safe method (Arthur *et al*., 2012; Arthur *et al.*, 2015).The first use of ionizing radiation on insects was performed by Hunter, (1912) when irradiated *Sitophilus oryzae* with X-ray, but did not get satisfactory results. Promising results were obtained only by Runner, (1916) that used X-rays to control *Lasioderma serricorne*, tobacco plague stored. From 1950 there was a major breakthrough in this type of research. Some factors such as the discovery of resistance to certain pests to chemicals, biological imbalance and toxicological problems caused by these products, contributed to this advance. Irradiation of the stored products can solve these types of problems, since it does not induce the emergence of resistance nor residues (Hossain, Brower and Tilton, 1972; Arthur, 2012, Arthur, 1997). Some control measures are adopted to solve the damage and losses caused by insects such as good storage practices, monitoring of pests and chemical treatment, this in turn end up causing some damage, besides the resistance of insects to the active ingredients used in composition of chemicals, and because of these problems, there is a need for more effective methods of control at low cost. Irradiation by numerous factors has been showed as the best solution to control pests (Arthur, 2012; Arthur, Machi and Mastrangelo, 2015; Follett *et al*., 2013, Hallman, 2013). Others authors studied the gamma radiation effects in various species and phases. In *Callosobruchus chinensis* (L.), in pre-larvae and pupae occur 100% of mortality in larvae at dose of 160 Gy, while pupae were fully killed under dose of 320 Gy (Huqueand Khan, 1969, Ruapongas, 1966). To other pests Banhan (1962) observed that doses of 80 to 100 Gy taken *Tribolium confusum* higher adult mortality. Nair and Subramanyan, (1963) founded in *Tribolium. castaneum*, doses ranging from 20 to 50 Gy that decreased the fertility adults. Research on this subject has been increasingly pronounced, thus, in order to clarify the advantages of this method and the benefits it provides in raising food for the population, being that recent years has been given greater attention by governments and private companies. The disinfestation of grain consists of a physical control method, inhibiting reproduction of insects or even killing him. However, for such control is of prime importance to know the lethal doses of ionizing radiation for the different stages of the life cycle of the pest, as the radiosensitivity varies according to several factors, including the stage of development (Arthur *et al*., 2012, Arthur *et al.*, 2015). The postharvest in phytosanitary irradiation is growing in commercial application and offers some advantages compared with other treatments for the control of quarantine pests on exported commodities. The Irradiation takes less time than fumigation and leaves no undesirable residues, while being at least as effective as any other existing method insect and mite control. Also, while the development of resistance to insecticides and acaricides is a growing problem, resistance to irradiation has never arisen in arthropods (Tilton and Burditt, 1983, Byrne, 1996). The use of methyl bromide as a fumigant to protect commodities is being eliminated; indeed the 1997 Montreal Protocol Agreement stipulated that methyl bromide usage would be completely phased out by 2005 in developed countries and by 2015 in developing countries (UNEP 2009). Nevertheless, certain uses of methyl bromide are exempt from phase-out, and these include the strictly regulated quarantine and pre shipment applications (Heather and Hallman 2008). Arthur et al., (1994) irradiated adults of *Sitophilus granarius* in wheat grains at doses of 50 to 150 Gy. The authors concluded that there was a significant reduction in the longevity of irradiated adults and absence of insects emerged from the dose of 60 Gy. Remember that a sterile population is an extinct population since there is no introduction of new fertile individuals, therefore mass of grain will be protected and this method of quarantine protection against *S. granarius* is feasible and recommendable. Arthur, (1974) also irradiated with increasing doses of gamma radiation, pupae of *Tribolium castaneum* aged from 1 to 5 days and concluded that the lethal dose was 46 krad and the sterilizing of 15 krad. The authors concluded that the dose of

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15 krad can be used to control this insect in wheat flour. Wiendl and Arthur (1974) studied the mortality of *Rhyzopertha dominica* in irradiated rice at doses up to 5.000 krad, the result was the increase in insect longevity that was proportional to the increase in radiation dose. Arthur *et al.* (1979) irradiated larvae and pupae of *Acanthoscelides obtectus* at doses of 3 to 12 krad and observed the emergence of adults, the dose of 11.82 krad was lethal for larvae, for pupae the dose was 13.86 krad. The sterilizing dose for irradiated adults in the pupae phase was 3 krad. Therefore a dose of 11.82 krad can be used to control this pest in beans. Arthur *et al.*, (1987), irradiated *Sitophilus granarius* with doses of 40 to 100 Gy of gamma radiation fractionated in 3 times and not fractionated. The results showed that the sterilize dose for the insects was 60 and 90 Gy respectively when irradiated with acute and fractionated doses. Arthur *et al.,* (1990), observed the longevity and adult reproduction of *Sitophilus oryzae* in rice irradiated at doses of 1 to 4 kGy. The results showed that the irradiation not affects the characteristics of the rice, so it not affects the longevity or the reproduction of the insects. Arthur *et al*., (1993) irradiated adults of *Sphenophorus levis* with a dose of 25 Gy and after crossing with normal insects, a total absence of offspring was observed without decreasing competitiveness among insects. The authors concluded that this methodology can serve as a basis for the programs of the sterile insect technique to control this pest in the field. Others authors are unanimous in affirm that the ionizing radiation can sterilize or kill insects of the order Coleoptera pests of stored grains (Arthur 1985, Arthur 1999, Arthur 1988, Arthur and Arthur 2001, Arthur and Franco 2000, Arthur and Wiendl 1993, Arthur, Berti-Filho and Arthur, 2003, Arthur *et al*., 1979, Arthur, Wiendl and Franco, 1994, Arthur, Wiendl and Henrique,1993).

Arthur *et al*., (1993), irradiated adults of *Rhyzopertha dominica* at doses of 50 to 250 Gy in a linear electron accelerator with energy of approximately 5 MeV, and concluded that doses of 150 and 250 Gy were the sterilizing and sub-sterilizing for this species of insects. Arthur and Silva, (2007), irradiated adults of *Trogoderma granarium* with doses of 25, 50, 75 and 100 Gy. They concluded that a dose of 100 Gy was sufficient for sterilizing adult insects. As we know a sterile population is an extinct population, so a dose of 100 Gy is sufficient to control this pest in stored products. But as safety margin a dose of 200 Gy was recommended by the authors. Arthur, (2012) irradiated adults *Lasioderma serricorne*, *Plodia interpuctella, Sitophilus zeamais and Sitophilus oryzae,* in four commercial brands of feed used to feed small animals. The samples were irradiated with doses of: 0 (control), 0.5, 1.0 and 2.0 kGy. The conclusion was that a dose of 0.5 kGy was enough to induce the sterilization and consequently the disinfestation of all rations studied.

The literature and current use of irradiation was show by various authors to control the pests of stored products and suggest new research to optimize its potential, according to Hallman (2013) food irradiation and pest commodity disinfestation is increasing in several countries. Doses to avoid pest reproduction of stored products range from 0.05 kGy to *Tenebrio molitor* and at 0.45 kGy to *Sitotroga cereallela*. Small and growing quantities of grain are being irradiated in the world today, especially in Asia. At least 33 countries allow the irradiation of some stored products, and that 14 countries allow irradiation for all stored products. Follett *et al*., (2013) irradiated *Sitophilus oryzae* in rice for quarantine treatment of this insect at doses of 30 to 120 Gy. The dose of 90 Gy was sufficient to control the egg, larvae and pupae phases, and 120 Gy for quarantine control of this insect in rice.

Follett (2014), affirm that USA approved the generic dose of 400 Gy for other insects, except adult pupae and Lepidoptera. Recent developments in phytosanitary irradiation are discussed. Current research is focused on: development of specific doses for quarantine treatment for Lepidoptera and not included by generic treatments; reducing dose levels for specific pests and commodities to reduce treatment time; development of generic doses lower than 400 Gy for groups of arthropods of quarantine importance other than fruit flies; and developing information about commodity tolerance and new methods to reduce injury and extend life. Therefore, several issues show barriers to wider use of irradiation as a phytosanitary treatment, including the 1 kGy limit, labeling requirements, restrictions on the use of modified atmosphere, and the limited number of country approvals for the use of phytosanitary irradiation. These issues should be directed to facilitate expansion. The small-scale development of cabinet model x-ray machines could provide farmers and food packaging markets with irradiation treatment capability and consequently accelerate the adoption of this technology. Mansur, (2016) irradiated all phases of the evolutionary cycle of *Trogoderma granarium* with doses of 50 to 200 Gy of gamma radiation. He concluded that a dose of 100 Gy was sufficient to control the insects and that this dose of radiation could be applied for quarantine treatment. Arthur *et al*., (2017) irradiated stages of the evolutionary cycle (eggs, larvae and pupae) of *Callosobruchus maculatus* at doses of: 0 (control), 25, 50, 75 and 100 Gy. They concluded that the dose of 50 Gy was lethal for eggs and larvae. A dose of 100 Gy was not sufficient to completely eliminate the pupae. Adult sterilization from irradiated pupae was obtained at the dose of 100 Gy. The authors recommend a dose of 150 Gy for the quarantine treatment for this insect in *Vigna sinensis* bean grains.

However study on the effects of gamma radiation with *A. diaperinus* not was fonded in the literature review. Therefore the aim of the experiment was determine the sterilizing dose to *A. diaperinus* by gamma radiation in peanuts at its control.

MATERIAL AND METHODS

The experiments were performed at Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture (CENA / USP), Piracicaba city, Sao Paulo, Brazil. The insect colony was obtained from cultures that had been maintained in the laboratory for more than 3 yr, the peanuts species *Arachis hypogaea* cv. IAC 8112 samples in flasks with capacity of 200 mL infected with adults of *Alphitobius diaperinus*. The study consisted of 8 treatments, 5 repetitions and each consisted of 20 individual adults, a total of 100 individuals per treatment. To irradiation were used doses of 0 (control), 25, 50, 75, 100, 125, 150 and 175 Gy, in a source of Cobalt-60, Gammacell-220 type, at a rate dose of 381 Gy/h. (Atomic Energy of Canada, Ottawa, Ontario, Canada) located in CENA / USP.

The intended doses for the irradiated samples were 25 - 175 Gy, gammachrome dosimeters with range dose of 0.1–3 kGy were used, and they were read with a Genesys 20 spectrophotometer. Dose certifications were made by the Institute for Energy and Nuclear Research – IPEN. The traceability of dose measurements was maintained by comparison with the international service assurance dose offered by the International Atomic Energy Agency, Vienna, Austria (Khoury *et al*., 2016). The 200 mL flasks were centralized inside the irradiator in order not to disrupt the uniformity of the radiation. Six dosimeters were positioned as follows: 1 on top of the flask, 1 at the bottom, and 4 equally-spaced at lateral positions. The uncertainty in each flask was ± 1.6%. The variation of measured doses was of ± 1.5% in the Gammacell-220 source.

The experiment was conducted in a room with a relative of 25 ± 5 ° C temperatures , humidity of 70 ± 5% and a 14:10 h L:D photoperiod. After 50 days of irradiation were performed evaluations using the method of counting of the number emergence insects in F_1 and F_2 generations. The experimental statistical design was completely randomized in an 8x5x1 scheme (8 treatments and 1 sampling time and 5 repetitions). The results of evaluations of the tests were subjected to variance analysis by F test, and the comparison of averages by 5% Tukey test, using the statistical system SAS (2002).

RESULTS AND DISCUSSION

Table 1 shows the number mean of adults emerged in the F_1 and F_2 generations from adults generation of *A. diaperinus* irradiated. From the results of this Table, we can observe that to all the treatments showed significant statistical differences being the gamma radiation effects in the adults were directly proportional to the increase of the doses of radiation. The doses of 50 Gy to 125 Gy caused a significant reduction in the development of the irradiated insects, above of 60% when compared to the control treatment, but these doses were not sufficient to cause total sterility in adults. The sterility of irradiated generation P adults was only obtained with the dose of 150 Gy where there was no emergence of adults in the F_1 generation. The sterilizing dose for F_1 generation adults from irradiated generation P was 125 Gy where there was no adult emergency in the F_2 generation. These results were in agreement with the results obtained by Wiendl, 1969, Arthur *et al*., 1980, Fontes and Arthur, 1994; Franco, Arthur and Franco, 2002, Follett *et al*., 2013, Mansur, 2016, Arthur *et al*., 2017, when irradiated insects of the species *Zabrotes subfasciatus*, *Acanthoscelides obtectus*, *Callosobruchus maculatus*, *Sitophilus oryzae* and *Trogoderma granarium*, and with the other results of the articles cited in the review of literature to insects of the same order as the mealworm.

According to international standards for phytosanitary measures 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 F_1 generation.

This standard identifies the minimum absorbed dose ranges for some pest groups based on research reported in the scientific literature. The minimum doses are obtained from many publications that are cited in the aforementioned regulations. Confirmation tests should be done prior to adopting the minimum dose for a specific pest treatment. For example, for Seed beetles (Bruchidae) to avoid adult reproduction were used doses of 70-300 Gy; Weevils (Curculionidae) to sterilize adult on active reproduction, doses of 80-165 Gy. Stored product beetles (Coleoptera) to sterilize active adult reproduction doses of 50-400 Gy. Borer (Lepidoptera) avoids adult development from the end of the larval stage doses of 100-280 Gy. Stored product moths (Lepidoptera) sterilize adult on active reproduction doses of 100 -1000 Gy.

For the phytosanitary treatment of *A. diaperinus* in peanuts, a dose of 150 Gy should be applied, consequently inhibiting the emergence of adults from paternal generation irradiated. This dose was sterilizing in adult insects, and as we know a sterile population is an extinct population. In accordance with the International Standards for Phytosanitary Measures ISPM No. 18, of (2003), which affirm that for beetles of stored products of Coleoptera order to sterilize adult in active reproduction requires doses of 50-400 Gy.

The radiosensitivity of insects increases of according to the development of the life cycle of insects (egg, larva, pupa and adult) and differs in various taxonomic groups, but the adult phase is considerable the more radioresistant.

According to Lee and Ducoff (1989) when are irradiated organisms they have ability to repair some of the damage caused in the DNA by irradiation. Gamma radiation effects also can cause more harmful to cells and consequently can take it from cellular inhibition to death. However, as explained by Muller (1950), La Chance (1967), Robinson (2005), the cause of cell death and the induction of sexual sterility by ionizing radiation is the induction of dominant lethal mutations most of which are chromosome breaks, chromosomal rearrangements and genetic imbalance created by the loss of chromosome fragments. In some species of the Curculionidae, such as *Anthonomus grandis*, the digestive enzymes of the gut are produced in apocrine cells that slough off the midgut epithelium and must be continuously replaced by cell division (Sakurai 2000, Riemann and Flint 1967). In any case, Hallman, (2000) including arthropods irradiated with doses that completely prevents reproduction can present result in equal or greater longevity compared with non-irradiated adults insect.

According to our results, irradiation of *A. diaperinus* the sterility of generation P adults was only obtained with the dose of 150 Gy, where there was no emergence of adults in the F_1 generation. The sterilizing dose for F_1 generation adults from irradiated generation P was 125 Gy where there was no adult emergency in the F_2 generation. Based on criterion of 100% of mortality P generation eggs the dose of 150 Gy can also be considered a viable candidate for phytosanitary irradiation of *A. diaperinus* infested peanuts.

Table 1: Mean number of adults (± SE) of *Alphitobius diaperinus* in the F_1 and F_2 generations emerged 50 d after irradiation with doses of 0 (control), 25, 50, 75, 100, 125, 150 and 175 Gy, when the P generation was irradiated.

Doses/Gy	Mean number of adults of the P generation irradiated	Mean number of the emerged adults in the F_1 generation	Mean number of the emerged adults in the F_2 generation
0	20	116.6±3.0a*	136.9±4.0a
25	20	102.5±2.0b	86.9±1.0b
50	20	79.1±1.0c	20.8±0.5c
75	20	36.1±0.7d	12.5±0.7d
100	20	22.1±1.2e	4.5±0.0e
125	20	10.5±0.7f	0.0±0.0f
150	20	0.0±0.0g	0.0±0.0f
175	20	0.0±0.0g	0.0±0.0f

*Means followed by the same letter do not differ by Tukey's test at 5%.

Conclusion:

The dose of 150 Gy of gamma radiation can be used as phytosanitary treatment to control of *A. diaperinus* infested peanuts.

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