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RESEARCH ARTICLE

Control of rice weevil (*Sitophilus oryzae* L.) infestation during storage using X-ray treatment

Uma Sankar Pal[1], Raghabananda Nayak[2*], Nihar Ranjan Sahoo[1] & Minati Mohapatra[1]

[1]All India Coordinated Research Project on Post-Harvest Engineering and Technology, Department of Agricultural Processing and Food Engineering, College of Agricultural Engineering and Technology, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India

[2]Department of Soil Science and Agricultural Chemistry, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India

*Correspondence email - rnnayak.soil@ouat.ac.in

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Abstract

The present experiment was undertaken to study the effect of X-ray radiation treatment on mortality of rice weevil (*Sitophilus oryzae* L.). About 500 g of clean rice inoculated with 14 adult rice weevil (T_1) and 500 g of infested rice with eggs and larvae (T_2) were packed in cloth bags and then placed inside polyethylene zip pouch. The samples were then exposed to X-ray radiation at 4 different doses of 50, 100, 150 and 200 gray (Gy). Dead and live insects were counted at 7, 14 and 21 days after the treatment for calculation of mortality percentage. Insect re-emergence was recorded at regular interval of one month during storage. Complete (100 %) mortality was achieved at 150 and 200 Gy treatment after 7 days of the treatment, whereas samples treated with 50 and 100 Gy showed total mortality after 21 and 14 days of X-ray treatment, respectively. Insect infestation observed after 30 days of storage and population of rice weevil increased significantly with time in control sample. Progeny was not detected up to 90 days in all X-ray treated T_2 sample and insect infestation was noticed in control sample after 30 days. From the above study, it was concluded that 150 Gy X-ray treatments could control rice weevil infestation during storage. X-ray dose of 50 Gy is effective for controlling eggs and larvae of *S. oryzae*. Scanning electron microscopy (SEM) of X-ray treated dead insects showed that the genital organ of rice weevil was deformed as compared to naturally dead insects.

Keywords: mortality; rice weevil; *Sitophilus oryzae*; X-ray radiation

Introduction

After milling, rice is generally stored in bags in godowns and warehouses. During storage, 5–10 % cereals are lost due to insect pests (1). Damaged grain has reduced nutritional value, reduced weight and lowered market value. Rice weevil (*Sitophilus oryzae* L.) is one of the most common cosmopolitan pests of stored rice causing considerable qualitative and quantitative loss during storage. Hot and humid climate favors its development. Adult rice weevil is about 2.5 mm long and dark brown in color and female weevil starts laying eggs after 4–5 days of the emergence and 300–400 eggs are laid by everyone (2). Insecticides such as phosphine (Aluminium phosphide), formaldehyde etc. are widely used to control insects in stored grain but residues development and development of resistance in certain species have been of some concern (3). Chemical methods for insect disinfestations can cause serious damage to environment and human health. So, application of radiation for safe storage of food grains may be used as an alternative environment friendly, cheap and effective treatment to overcome the drawbacks of chemical method of disinfestation (4). Radiation treatment can cause mortality at various stages of these insects or sterilise the adults without any toxic residues on the treated products (5). The residue free character is an advantage of irradiation disinfestation over chemical fumigation (6). Mostly ionising radiation (e.g. γ-rays and X-rays) has been used to manage storage pests, but there were also attempts with a variety of other irradiation types, such as radio waves, radar, infrared, microwaves and ultraviolet. Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed.

Electron beams are produced from particle accelerators capable of accelerating electrons to near the speed of light (~190000 miles/sec). This electron beam generator uses commercial electricity as an energy source and can be simply switched on or off. However, widespread application of irradiation for food preservation was not commercially implemented earlier mainly due to the consumer skepticism regarding the wholesomeness of the foods that are irradiated with gamma rays from radioactive isotopes such as ^{137}Cs or ^{60}Co. The application of electron beams accelerated mechanically to foods without using any radioactive source is changing the attitudes of the consumers.

X-rays are a form of ionising electromagnetic radiation that travels at a speed of light, 3×10^8 m/s. Its wavelengths ranging from about 10^{-8} to 10^{-12} m and corresponding frequencies from about 10^{16} to 10^{19} Hz. X-ray radiation lies between ultraviolet radiation and gamma radiation in the electromagnetic spectrum. It is ionising radiation in the form of packet of energy or photon which has no charge. X-ray is produced by the bombardment of high energy

stream electron beam at a thin plate of gold or other metal in an X-ray tube. X-ray can pass through thick food and require heavy shielding for safety. The unit of radiation absorbed by the food is expressed in gray which is absorption of 1 Joule of radiation energy per kg of matter. The typical dose range used is up to 200 Gy for food disinfestation.

The physiological cause of this phenomenon is the release of free-radicals and consequent molecular membrane damage (7). When X-rays interacts with insect, they are energetic enough to cause neutral atoms to eject electrons. When passing through living tissue, X-rays can cause harmful biochemical changes in genes, chromosomes and other cell components viz. inhibiting DNA synthesis, cell membrane alteration, denaturation of enzymes, alterations in RNA synthesis, effects on phosphorylation and DNA compositional changes. The biological effects of X-ray radiation are complex and highly dependent on the length and intensity of exposure.

It is reported in a study of X-ray on the life cycle of rice weevil, that in 12 out of the 14 X-ray irradiated groups, the females lived longer than the males and there was a highly significant shortening of the mean duration of life (8). In a study of the lethal and sterile effects of X-ray irradiation on cigarette beetle, it was suggested that X-ray irradiation at 0.1 k Gy completely broke the reproductive cycle of the cigarette beetle, even though the action was not immediate for sterility, but it would be applicable for the disinfestations of stored tobacco (9). In a similar study scientists have compared X ray irradiators for insect sterilisation and reported that traditional radiation from ^{60}Co for insect sterilisation and they confirmed the suitability of this new generation of X-ray irradiators for pest control programs (10). Mortality and sterility consequences of 10, 50, 70, 100 and 200 gray (Gy) X-ray radiation on maize grain against granary weevil (*Sitophilus granaries)* confirmed complete sterilising effect of the doses of 70 Gy and above. At doses of 50 and 70 Gy, 95 % insect mortality was recorded only on 21 day and high mortality values of 83.5 and 97.5 % were observed on 14 days at 100 and 200 Gy doses (11). In an experiment conducted for quarantine control of coffee berry borer (*Hypothenemus hampei)* using 50, 75, 100 or 125 Gy of X-ray radiation and it was concluded that adults irradiated at 100 and 125 Gy were completely sterilised (1). Though many works have been done on control of pests by X-ray irradiation, study on control of rice weevil is meagre in the literature. Therefore, the effect of different X-ray dose on mortality and re-emergence of rice weevil during storage is studied in the present work.

Materials and methods

Rearing of rice weevil

Sitophilus oryzae were reared on whole raw rice grain (Lalat) inside a glass jar covered with well-ventilated textiles placed in incubator maintained at 30 ± 1 °C, 75 ± 5 % R.H. Adults of 7–14 days old were used for the experiments.

Sample preparation

Untreated, clean and infestation-free raw rice (Lalat) purchased from local market of Bhubaneswar, India was used for the experiment. Rice sample of 500 g was packed in each cloth bag for the study. The sample was divided into two groups. In one group of samples, fourteen healthy *S. oryzae* adults of mixed sex were added to rice samples in cloth bags and sealed (T_1). Rice already infested by *S. oryzae* was collected from a farmer and cleaned by air screen to remove the live adult insects. Sample of 500 g of this infested rice was packed in cloth bag and sealed (T_2). Ten packs were prepared from each group for the radiation treatment (0, 50, 100, 150 and 200 Gy) with 2 replications. The packs are labelled based on treatment and replications.

Agricultural radiation processing facility description

The X-ray treatment was given at 'Agricultural Radiation Processing Facility of Raja Ramanna Centre for Advanced Technology, Indore. The e-beam radiation facility is based on the indigenously developed 10 MeV (beam energy) 5 kW (beam power) travelling wave electron linear accelerator (linacs). The facility has three major parts viz., electron accelerator and its system, product handling system and radiation shielding and safety systems. Dose delivery to a product is primarily determined by the the electron beam energy, beam current and conveyor speed. The beam energy required for processing a product is selected based on its areal density (thickness and density). Dose uniformity is achieved by varying scanning-magnet current and controlling the conveyor speed.

X-ray treatment

X-ray doses of 0 (control), 50, 100, 150 and 200 Gy were chosen for sample treatment. The rice sample cloth packs were placed inside low-density polyethylene (LDPE) zip packs for uniform thickness of sample inside the pouch i.e. 4 cm. The pouches required to be treated at same dose were mounted on the side of a corrugated fibreboard (CFB) box with adhesive tape. The boxes were placed on the roller conveyor which automatically took the product to the process chamber past X-ray at specific speed and brought it back. The system was set for 50 Gy X-ray treatments per pass. For doses of 50, 100, 150 and 200 Gy, the sample was passed through the process chamber past X-ray for 1, 2, 3 and 4 times, respectively. After passing through the radiation field for the required number of times as per the treatment dose by moving the sample on the conveyor in forward and backward direction, the product was collected at the exit end.

Mortality percent (%)

The X-ray treated rice samples with insect inoculation were opened after 7, 14 and 21 days and the number of dead rice weevils were counted to find out the mortality percentage.

Mortality percent was calculated by the following formula:

$$\text{Mortality percent} = \frac{\text{Number of dead rice weevil}}{\text{Total number of rice weevil inoculated}} \times 100 \quad \text{(Eq. 1)}$$

Mortality was evaluated as a function of both time after exposure and dosage rate. The data were transformed into arcsine value and analysed using two-way ANOVA in MS-Excel software, with weevil mortality as the response variable and time after exposure and dose rate as the main factors.

Insect re-emergence during storage

The re-emergence of insects in control as well as X-ray treated samples for treatments T_1 and T_2 during storage was studied for four months. The adult insects were counted every month. The data were analysed after square root transformation using two-way ANOVA in MS-Excel software to find out the effect of X-ray dose and storage time on rice weevil re-emergence.

Scanning electron microscopy for external morphology of Sitophilus oryzae and X-ray treated rice

The external morphology of rice weevil treated with different doses of X-ray and naturally dead insects was studied in scanning electron microscope (12). The morphology of X-ray treated rice samples was also observed in scanning electron microscopy (SEM). The insect specimens were analysed using a scanning electron microscope (S-3400N) without carbon or gold coating to avoid damage to delicate body structures.

Results and Discussions

Effect of X-ray radiation on mortality of rice weevil inoculated in clean raw rice

The effect of X-ray treatment dose on cumulative mortality of rice weevil is shown in Fig. 1. The mortality of insects increased to 57 % in the control samples with progress of time. Total mortality (100 %) was observed in samples treated with 150 and 200 Gy after 7 days of treatment, whereas samples treated with 50 and 100 Gy showed total mortality after 21 and 14 days of X-ray radiation treatment, respectively. Cumulative mortality varied significantly with X-ray treatment dose and time after exposure ($p < 0.05$). Higher irradiation doses induced highest mortality. There was a significant difference between the mortality of insects in control sample and x-ray treated sample. However, there was no difference among X-ray treated samples at higher dose (150 and 200 Gy) and days after treatment.

Re-emergence of rice weevil during storage in X-ray treated rice sample with insect inoculation

No adult progeny was observed in samples treated with X-ray dose above 100 Gy after 60 days of exposure (Fig. 2). The rice weevil population increased significantly with time in control sample and significantly different from X-ray treated samples. Progeny was detected in all X-ray treated samples after 90 days. So, 150 Gy treatments may be used for safe storage of rice up to 90 days with no progeny. Both X-ray treatment dose and time after exposure had significant effect on rice weevil re-emergence ($p < 0.05$). There was no significant difference in rice weevil re-emergence in all samples after 120 days of exposure.

Re-emergence of rice weevil during storage in X-ray treated infested rice

Already infested rice was treated with different doses of X-ray and insect re-emergence during storage was studied to find out the effect on insect eggs and larvae (Fig. 3). Progeny was not found up to 90 days in rice samples treated even with lower dose of X-ray i.e. 50 Gy. Insect infestation was noticed in control sample after 30 days. Insect re-emergence varied significantly with X-ray radiation dose ($p < 0.05$), whereas storage period had no significant effect.

Scanning electron microscopy of X-ray treated rice

Scanning electron microscopy of rice samples treated with different doses of X-ray showed gradual changes in the starch structure (Fig. 4). The starch granules of control sample were polygonal with distinct boundary whereas fusion of starch molecules was observed in rice samples treated at higher X-ray dose of 200 Gy. In contrast, rice samples treated with 150 Gy exhibited less starch granule fusion. The figure shows that realignment of starch molecule has occurred by application of X-ray treatment. Similar finding is reported by scientists in a study of gamma ray radiation on brown rice (13). Additionally, the fusion of starch granules indicates less entanglement of amylase molecules within amylopectin chains giving rise to higher swelling and less hard structure during cooking of rice grain.

Effect of X-ray treatment on external morphology of *Sitophilus oryzae*

The effect of X-ray was found on external morphology of rice weevil. The X-ray-treated specimens exhibited several morphological abnormalities. The body hairs were reduced in length, adhered to the body or were malformed (Fig. 5). The genital organ of X-ray treated rice weevil was deformed as compared to naturally dead weevils (weevils dead without exposure to X-ray radiation). So, the fecundity of weevil may be controlled by X-ray treatment.

It was observed that in the natural death facing specimens all the body parts were as usual including antennae and the hairs are straight and normal. Cloaca is normally closed with elongated hairs surrounding. In X-ray treated rice weevil, the cloaca remained simply opened, the body hairs at the tip of the snout were also reduced much and hairs were contracted in size.

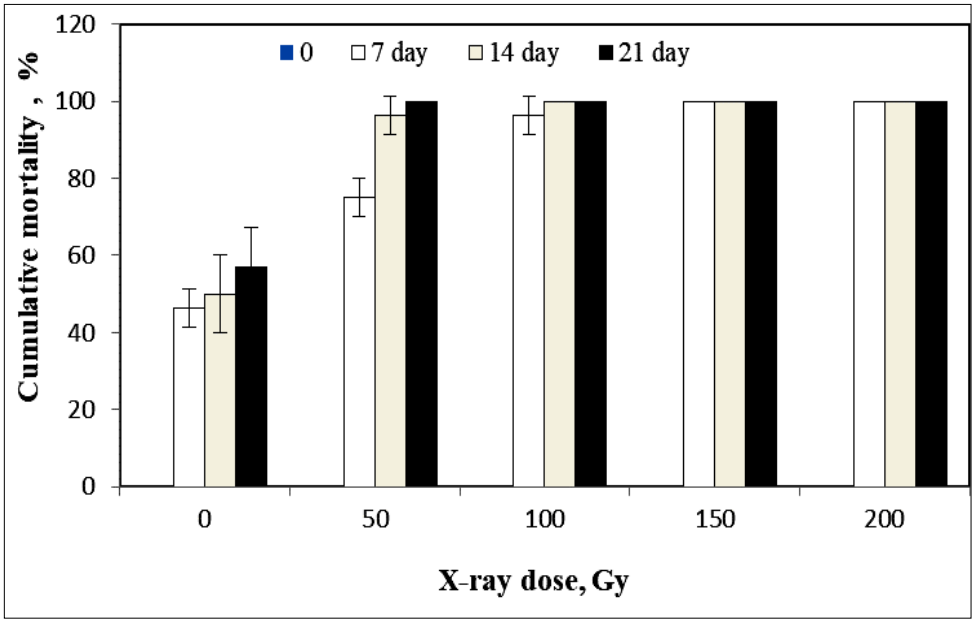


Fig. 1. Effect of X-ray treatment dose on cumulative mortality of rice weevil.

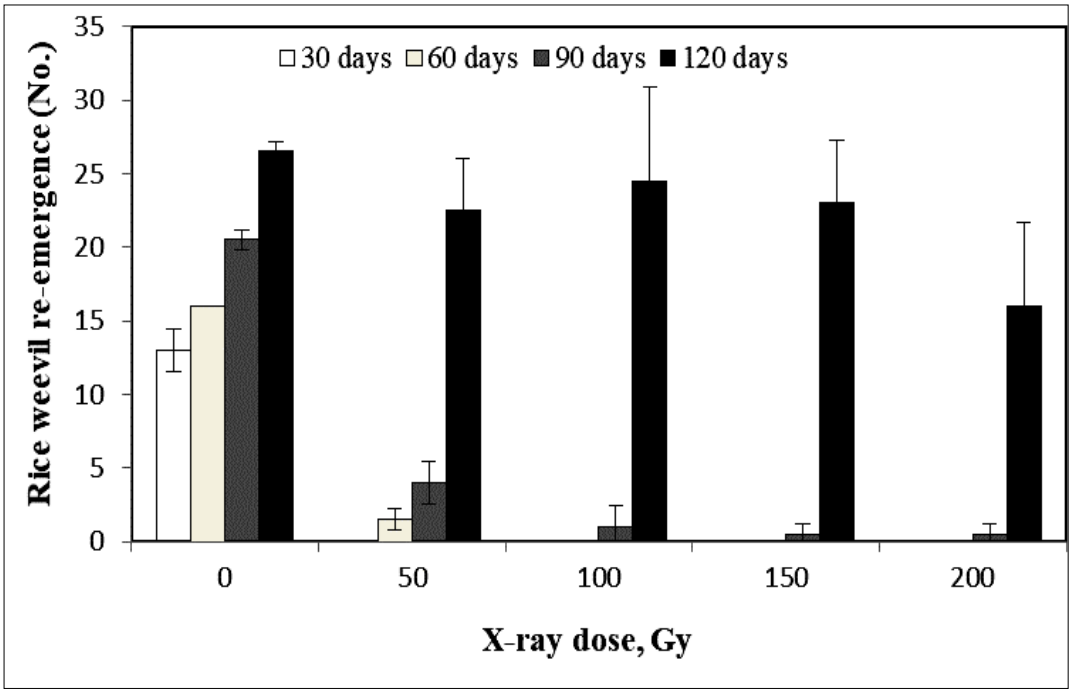


Fig. 2. Effect of X-ray dose on re-emergence of rice weevil in insect inoculated sample.

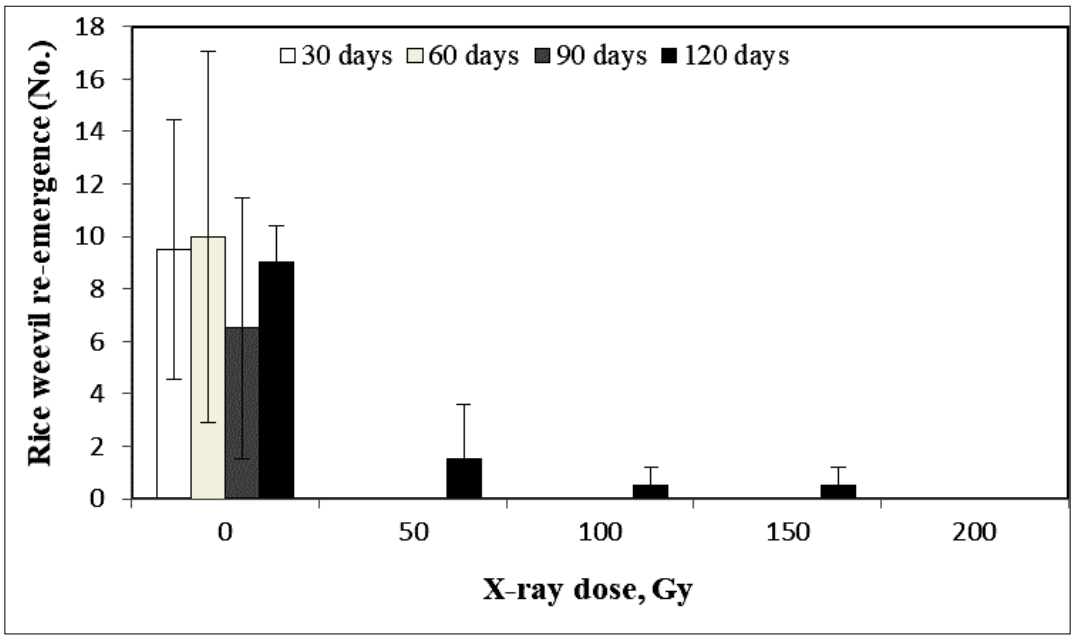
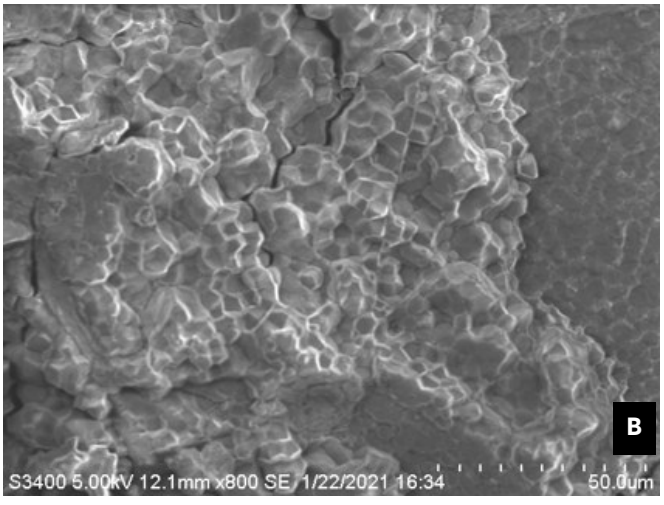


Fig. 3. Effect of X-ray dose on re-emergence of rice weevil in infested rice sample.





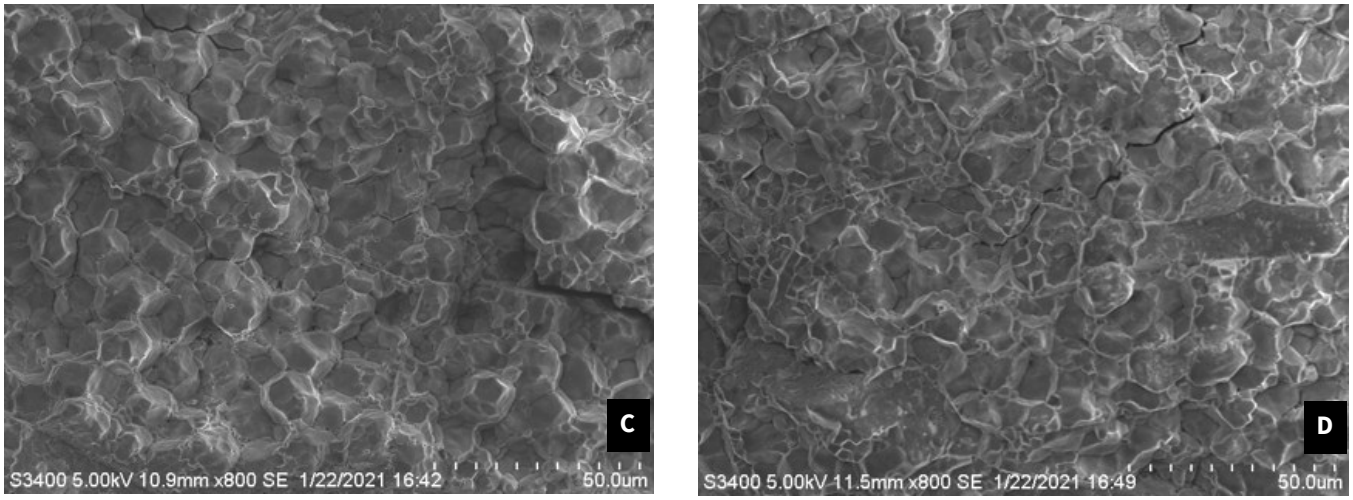


Fig. 4. Scanning electron micrographs of rice treated with different doses of X-ray radiation: (**A**) Control; (**B**) 100 Gy; (**C**) 150 Gy; (D) 200 Gy.

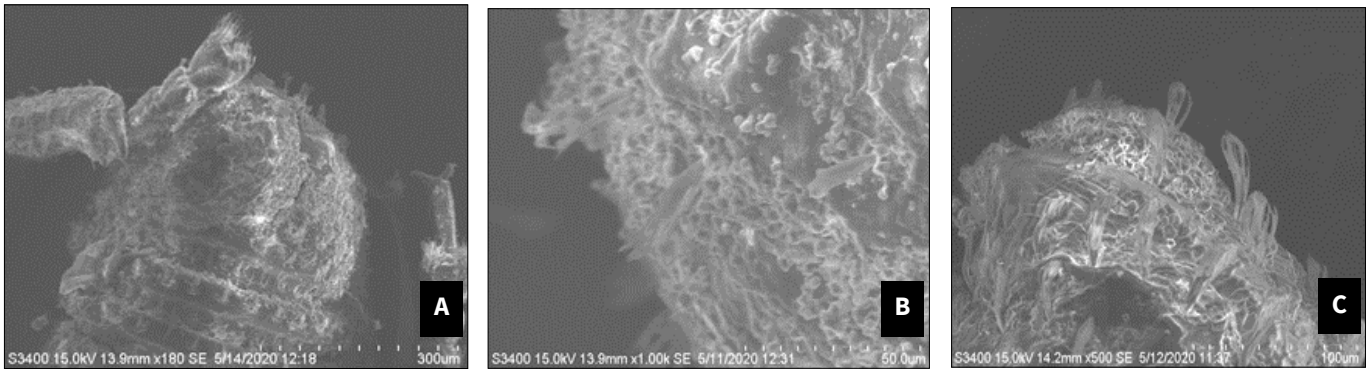


Fig. 5. External morphology of X-ray-treated *Sitophilus oryzae*: (**A**) X-ray-treated cloaca and hind leg; (**B**) X-ray-treated genitalia; (**C**) cloaca of a naturally dead insect.

Similar results of radiation treatment are also reported on the effect of different doses of gamma radiation on morphology and distribution of the sensory receptors of lesser grain borer, *Rhyzopertha dominica* (*Coleoptera: Bostrichidae*). Irradiation at a dose of 75 Gy had no effect on the general distribution of different types of sensilla as compared to the control, whereas doses of 100, 150 and 200 Gy showed malformation which increased with increased doses (14).

Conclusion

Cumulative mortality varied significantly with X-ray treatment dose and time after exposure ($p < 0.05$). Total mortality was observed in samples treated with 150 and 200 Gy after 7 days of treatment, whereas samples treated with 50 and 100 Gy showed total mortality after 21 and 14 days of X-ray radiation treatment, respectively. The rice weevil population increased significantly with time in the control sample and was significantly different from X-ray treated samples. No adult progeny was observed in samples treated with X-ray doses of 150 Gy and 200 Gy after 90 days of exposure. X-ray treatment dose of 150 Gy may be used for safe storage of rice for up to 90 days. Progeny was not found up to 90 days in infested rice samples treated with 50 Gy X-ray dose. Insect infestation was noticed in the control infested sample after 30 days and increased steadily during storage. The starch granules of control sample were polygonal with distinct boundary and fusion of starch molecules were observed in rice samples treated at higher X-ray dose of 200 Gy. Scanning electron microscopy (SEM) of X-ray treated dead insects showed that the genital organ of rice weevil was deformed as compared to naturally died insects indicating sudden death of the insect. This research finding can be a torch bearer for study of X-ray treatment to control other store grain pests of rice and can be a cutting edge technology with eco-friendly approach in future for handling large scale go downs for a better export prospective.

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Authors' contributions

USP, RN and NRS conceptualised the study. RN and NRS conducted the laboratory analyses. USP and MM wrote the manuscript. MM and RN participated in data analysis. RN, USP and NRS edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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