Effects of \( \gamma \)-Irradiation on Larval and Adult Stages of \textit{Tribolium castaneum} (Red Flour Beetle)

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\textit{Tribolium castaneum} の幼虫と成虫へのガンマ線の影響

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Summary

Food irradiation using \( \gamma \)-rays is one of powerful tools to reduce insect infestation in crops and spices. We examined the effects of \( \gamma \)-irradiation on larvae and adults of \textit{Tribolium castaneum}. Eclosion for larvae was completely inhibited by irradiation at 500 Gy. A survival rate for adults was markedly reduced by irradiation at more than 500 Gy. The neutral comet assay presented that irradiation irreversibly increased DNA damage at 500 and 1000 Gy and transiently increased DNA damage at 100 Gy, suggesting possible DNA repair in larvae and adults stages. These results indicate that \( \gamma \)-irradiation at 500 Gy is available to eradicate the beetle at larval and adults stages.

Key words: gamma ray irradiation, \textit{Tribolium castaneum}, comet assay, DNA damage

Introduction

Loss in the quality and quantity of stored grain due to insects is a serious problem worldwide and it is estimated that more than 10% of stored grain is lost due to insect pests each year. \textit{Tribolium castaneum} is a major secondary pest which can be of major impor-

tance on grains previously damaged either mechanically or by other insects\(^1\). Under optimum conditions a single pair of red flour beetles can multiply its progeny to one million in 150 days\(^2\). Chemicals including methyl bromide have been widely used for disinfestations of the beetles in storage of foods all over the world, accounting for environmental deterioration\(^3\). Therefore, usage of non-chemical methods, natural components and biological control against pests are

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increasing\textsuperscript{46}.

Irradiation is an approved method for the direct control of stored product insects in wheat and flour in many countries, and probably would be approved for all grain, grain products, and other dry food commodities\textsuperscript{88}. The advantages of irradiation as a pest control measure include no undesirable residues in the irradiated food products, few changes in their physiochemical properties or nutritive value, and no development of resistance in pest insects\textsuperscript{88}. Susceptibility to $\gamma$-irradiation is known to be different between and within the different stages of the same species of insects\textsuperscript{88}. As both adults and larvae feed on grain dust and broken grain, but not the undamaged whole grains and spends its life cycle outside grain kernels\textsuperscript{88}, it is important to know the irradiation susceptibility of each stage of insects and this information can be used to determine the most tolerant stage and effective irradiation dose for control of pests.

The most important target molecule for cell killing by ionizing radiation is DNA\textsuperscript{89}. Gamma irradiation induced significant damage at DNA level in insect cells as manifested by increased strand breaks compare to non-irradiated ones\textsuperscript{88}. DNA damage detecting single cell gel electrophoresis (SCGE) or comet assay showed less DNA strand breaks in both cells (insect and mammal cells) induced by X-rays or $\gamma$-rays\textsuperscript{11} and higher efficiency of DNA repair in insect cells were responsible for its radio resistance. However, to our knowledge, $\gamma$-ray-induced DNA damage of $T$. castaneum has not been reported previously.

In order to clarify susceptibility at different stages, we investigated $\gamma$-ray-induced death and DNA damage at larval and adult stages of $T$. castaneum.

Materials and Methods

1. Test insects

$T$. castaneum used in this study was a laboratory strain of Evolutionary Ecology, Graduate School of Environmental Science, Okayama University, Japan\textsuperscript{12}. The beetles were reared with a mixture of wholemeal (Yoshikura-shokai, Tokyo) enriched with brewer’s yeast (Asahi beer, Tokyo) as the rearing medium and kept in a chamber (Sanyo, Tokyo) maintained at 25°C and 60% relative humidity under a photoperiod of 16:8 (Light : Dark) (lights on at 7.00, lights off at 23.00).

2. Irradiation

The insect samples were irradiated with $\gamma$-rays from a $^{137}$Cs source (CS-500C, Yoshizawa Namari Kogyo Co., Osaka, Japan) at Okayama University, Japan. The absorbed dose rate was measured at 0.1 kGy/h with a Fricke dosimeter. Larvae and adults of $T$. castaneum were irradiated at dose of 10, 100, 500, and 1000 Gy. After irradiation, insects were reared and were observed at indicated time. The non-irradiated samples were used as the Control.

To analysis the comet assay, 5 larvae and 5 adults were subjected to irradiation at indicated dosages.

3. Assessment of DNA damage

Low melting agarose gel, lysis solution, and SYBR green were purchased from Trevigen Inc. (Gaithersburg, MD, USA). 1,4-phenylenediamine dihydrochloride was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Irradiated larvae and adults were ground with a mortar and pestle in 20 mM EDTA in phosphate buffered saline (PBS) and centrifuged separately. Then 75 $\mu$L of cell suspensions was collected and mixed with 250 $\mu$L of low melting agarose gel. Seventy five microliters of these cell-gel mixtures were dropped on the comet slide and incubated at 4°C for 10 min. The slides were immersed in a prechilled lysis solution and incubated at 4°C for 30 min. After lysis, the slides were then placed in a horizontal gel electrophoresis tank containing freshly prepared neutral electrophoresis solution for 20 min to allow the DNA in the nuclei to unwind, followed by electrophoresis at 300 mA, 1 Vcm\textsuperscript{-1}. After electrophoresis, slides were soaked in ethanol for 5 min and then stained with 30 $\mu$L of dilute SYBR green and 1 drop of 1,4-phenylenediamine dihydrochloride solution was added. Comet images were captured using a fluorescent microscope (Biozero BZ-8000, Keyence, Osaka, Japan) with filter: OP-66835 BZ filter GFP (excitation wavelength, 480/30 nm; absorption
wavelength 510 nm; and dichroic mirror wavelength, 505 nm) and then analyzed by using image analysis software (CASP; CaspLab.com). Measure of tail moment (defined as the product of the fraction of DNA in the comet tail) was used as an indicator of DNA damage. Distance between the means of the head and tail distributions was determined following the method of Olive et al. More than 100 comets were analyzed for evaluation of DNA damage in each stage.

4. Statistical Analysis

The significance of differences between mean values was assessed using one way analysis of variance (ANOVA) with Tukey’s test. Differences were considered significant for p values of < 0.05.

Results and Discussion

The effect of γ-irradiation on larval eclosion rate of larvae of T. castaneum was examined (Fig. 1A). When larvae were irradiated at 10 Gy, 100% of the larvae were eclosed. Eclosion rate of larvae was 2% when irradiated at 100 Gy. Irradiation at 500 Gy completely inhibited the eclosion of larvae.

The DNA damage of T. castaneum at larval stage exposed to γ-irradiation at 10 Gy to 1000 Gy was examined using the neutral comet assay (Fig. 1B). Irradiation induced DNA fragmentation at 10 Gy to 1000 Gy. The DNA fragmentation induced by irradiation at 100 Gy was significantly decreased 3 h after irradiation but the fragmentation by 500 Gy and 1000 Gy irradiation was not changed.

The survival rate of T. castaneum at adult stage irradiated with γ-rays was examined (Fig. 2A). When irradiated at 100 Gy, 50% of the adults survived for 60 days. No adults irradiated at 500 Gy survived for 40 days and at 1000 Gy for 26 days. Gamma irradiation reduced the survival rate of adults in a dose-dependent manner.

Figure 2B shows DNA fragmentation in γ-irradiated T. castaneum at adult stage. DNA damage in the adult exposed to γ-irradiation at 10 Gy to 1000 Gy was examined using the neutral comet assay. Irradiation induced DNA fragmentation at 10 Gy to 1000 Gy. The DNA fragmentation induced by irradiation at 100

![Fig. 1](image)

Fig. 1 Larval eclosion rate and DNA damage level of γ-irradiated T. castaneum. A) Larvae were treated with γ-irradiation at 10 Gy, 100 Gy, and 500 Gy; B) Larvae were treated with γ-irradiation at 10 Gy, 100 Gy, 500 Gy, and 1000 Gy for comet assay. Averages for three independent experiments (more than 100 comets per bar) are shown. Error bars represent standard deviations. Values indicated by the same letter do not differ significantly at the 5% level, as determined by ANOVA with Tukey’s test.
Gy was significantly decreased 3 h after irradiation but the fragmentation by 500 Gy and 1000 Gy irradiation was not changed.

In this study, larvae irradiated at 500 Gy did not eclose at all (Fig. 1A) and adults irradiated at 10 Gy and 100 Gy did not have a mortality of up to 100% but adults irradiated at 500 Gy and 1000 Gy have a mortality of up to 100% (Fig. 2A). It has been reported that irradiation at 150 Gy completely prevented larval development and pupation and that irradiation at higher than 200 Gy killed T. castaneum adults 28 days after irradiation\textsuperscript{16}. There are some differences in susceptibility to  \( \gamma \)-irradiation among development stages. The differences may be attributed to differences in the age of the experimental larvae and adults at the time of irradiation or differences in the strain of T. castaneum used.

Insect cells are more resistant to irradiation because of less susceptibility to irradiation and more efficient DNA repair\textsuperscript{15}. Our results show that DNA damage of T. castaneum at larval and adult stages was restored 3 h after irradiation at 100 Gy but not 3 h after irradiation at 500 Gy and 1000 Gy (Fig.1B and 2B). Hence, the restoration of DNA damage may be due to the efficient DNA repair and irradiation at 500 Gy and 1000 Gy may temporarily hamper repair of the DNA fragmentation.

In Conclusion, larvae and adults of the T. castaneum can be successfully controlled by  \( \gamma \)-irradiation at 500 Gy.

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**和文要旨**

ガムマ線を用いた食品照射は、穀物やスパイスの害虫の侵入を抑制する手段の一つである。我々は、*Tribolium castaneum*の幼虫と成虫へのガンマ線照射の影響を調べた。羽化は、500Gyの照射で完全に阻害された。成虫の生存率は、500Gy以上の照射で著しく減少した。中性条件でのコメトアッセイの結果より、500Gyまたは1000Gy照射によるDNA損傷は、不可逆的に増加したが、100Gy照射によるDNA損傷は一過的に増加した。100Gy照射ではDNAの修復が行われていることが観察された。以上の結果より500Gy以上のガンマ線照射は、*T. castaneum*の幼虫と成虫の駆除に十分であることが示された。

**キーワード：ガムマ線照射, *Tribolium castaneum*, コメトアッセイ, DNA損傷**

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