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# Assessment of electron beam-induced abnormal development and DNA damage in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae)

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## HIGHLIGHTS

- Electron beam irradiation induced abnormal development of the cutworm.
- Electron beam irradiation induced the sterility of the cutworm.
- Electron beam irradiation increased levels of DNA damage.
- DNA damage by high irradiation exposure was not completely repaired.

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## ABSTRACT

The armyworm, *Spodoptera litura* (F.) is a polyphagous and important agricultural pest worldwide. In this study, we examined the effect of electron beam irradiation on developmental stages, reproduction, and DNA damage of *S. litura*. Eggs (0–24 h old), larvae (3rd instar), pupae (3 days old after pupation), and adults (24 h after emergence) were irradiated with electron beam irradiation of six levels between 30 and 250 Gy. When eggs were irradiated with 100 Gy, egg hatching was completely inhibited. When the larvae were irradiated, the larval period was significantly delayed, depending on the doses applied. At 150 Gy, the fecundity of adults that developed from irradiated pupae was entirely inhibited. However, electron beam irradiation did not induce the instantaneous death of *S. litura* adults. Reciprocal crosses between irradiated and unirradiated moths demonstrated that females were more radiosensitive than males. We also conducted the comet assay immediately after irradiation and over the following 5 days period. Severe DNA fragmentation in *S. litura* cells was observed just after irradiation and the damage was repaired during the post-irradiation period in a time-dependent manner. However, at more than 100 Gy, DNA damage was not fully recovered.



## 1. Introduction

*Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), also referred to as an 'armyworm' or 'cutworm', is an economically important and regular polyphagous pest, which seriously harms plants in the families of Cruciferae, Solanaceae, Cucurbitaceae, and Leguminosae. High resistance against a wide variety of insecticides has been reported from South Asia, and as a consequence, the management of this pest has become increasingly difficult. As international agricultural trade is increasing, the risk of introducing exotic insects into new areas where they may become plant pests is also on the rise. Therefore, importing countries only permit the entry of pest-free and secured commodities. In order to satisfy quarantine requirements, the following methods of disinfestation are used: fumigation with chemicals such as methyl bromide (MB), application of extreme temperatures (heat or cold), controlled atmospheres (level of $CO_2$ or $O_2$), irradiation, and combinations of these (Heather and Hallman, 2008). However, MB treatment is under regulation as a stratospheric ozone-depleting substance under the Montreal Protocol of substances that deplete the ozone layer (UNEP, 2009). Research is currently underway to develop alternatives to MB.

Irradiation is the ideal technology for developing generic treatments because it is effective against most insects at dose levels that do not affect the quality of most commodities. The electron-beams are generated by electrical energy, which is thought to be safe and effective for rapid surface treatment, with no consideration of remaining radioactivity. Moreover, it is an environmental friendly control technique, and does not produce harmful materials, being currently used widely throughout various areas, including medical, food, agricultural products, semiconductor (Taniguchi, 2005; Park et al., 2006; Moon et al., 2010). In our previous research (Koo et al., 2011; 2012), electron beam

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irradiation induced abnormal development, sterility, and DNA damage of *Plutella xylostella* and *Liriomyza trifolii*. Therefore, electron beam irradiation-induced changes in DNA could be used as the basis for detecting irradiation treatment in insects (Imamura et al., 2004). The microgel electrophoresis assay of single cells (DNA comet assay) is a sensitive technique for detecting DNA damage (Todoriki et al., 2006; Hasan et al., 2008). The comet assay has also been used to observe DNA repair in irradiated cells (Trzeciak et al., 2008; Kameya et al., 2012). The comet assay is quick, simple, sensitive, reliable, and inexpensive method for measuring DNA damage.

The objectives of the study are to examine the effects of electron beam irradiation on egg, larval, and pupal development, and on adult reproduction in *S. litura*, in order to identify a potential quarantine treatment dose. Also, the present study was designed to evaluate the levels of DNA damage and repair using the alkaline comet assay on *S. litura* adult after exposure to electron beam irradiation.

## 2. Materials and methods

### 2.1. Test insects

The cutworm, *S. litura*, larvae were collected from cucumber leaves in Gurye area (Republic of Korea) in 2010. The larvae were reared in the laboratory in plastic cages ($25 \times 19 \times 8$ cm$^3$) with artificial diet at $25 \pm 2$ °C, 50–60% RH and a 16 L:8D h photoperiod

### 2.2. Electron beam treatment

Electron beam irradiation was conducted in the EB-Tech Co., Ltd. (Daejeon, Republic of Korea) using a high energy linear accelerator (UEL V10-10S, 10 MeV). The different developmental stages *viz.* eggs, larvae, pupae and adults of *S. litura* were treated with doses of 0 (control), 30, 50, 100, 150, 200, and 250 Gy. The doses used were based on our previous study (Koo et al., 2012). Target doses were monitored by dosimetry with a radiochromic film dosimeter (GEX GAF3002DS, USA) (ISO/ASTM51275:2004(E)).

Thirty females and 60 males *S. litura* adults were placed in a parchment paper envelope ($27 \times 50$ cm) that contain cotton with sugar solution (10%). They were allowed to lay eggs for 24 h and then removed. Parchment paper piece with attached eggs (0–24 h old) was exposed to the electron beam. One hundred *S. litura* larvae (3rd instar) and pupae (3 days old after pupation) were placed in a plastic Petri dish with a net cover (10 cm diameter $\times$ 4 cm height) and exposed to the electron beam. Sixty adults (20 females and 40 males, 0–24 h old after emergence) were placed in a glass vials and exposed to the electron beam. To investigate lethal effects on various developmental stages, we observed the rate of egg hatch inhibition and rate of emergence inhibition. The numbers of adults (24 h after emergence) surviving after 2 days were also counted. In electron beam-irradiated eggs, the emergence rate of larvae and the longevity and fecundity of emerged adults were recorded. In electron beam-irradiated larvae, emergence rate, longevity and fecundity of emerged adults and hatchability of the eggs were recorded. In electron beam-irradiated adults (24 h after emergence), longevity and fecundity and hatchability of the eggs were recorded. Data on untreated controls were also recorded. Trials on the different stages of *S. litura* were done with three replicates.

### 2.3. Reciprocal crossing

Adult sterility tests were conducted using a reciprocal cross design. Pupae were isolated in individual plastic Petri dishes. After emergence, 20 unmated flies ($\leq 2$ days old) were irradiated with 30–200 Gy or left 20 untreated. Irradiated or untreated males and females in all combinations were then mated as pairs in individual Petri dishes. The number of eggs laid and the number of eggs hatched were recorded until all flies died. This test was done in three replicates.

### 2.4. DNA comet assays

DNA damage in *S. litura* adults was determined under alkaline conditions using the Comet Assay Kit from Trevigen (Gaithersburg, MD) with slight modifications. Another population of irradiated insects was sampled immediately after irradiation, and insects that would undergo a time course experiment of 1, 3, and 5 days after irradiation were instantaneously frozen with liquid nitrogen and subjected to the comet assay. Briefly, after irradiation, adults were frozen in liquid nitrogen and then gently homogenized into a powder. The powder was suspended in cold phosphate-buffered saline (PBS) and filtered through a 125 μm nylon mesh. The cells were combined with molten agarose and pipetted onto a comet slide. After solidification, the slides were immersed in lysis solution for 50 min at 4 °C. For alkaline single-cell electrophoresis which detects the combination of single- and double-strand DNA breaks, DNA cross-links, and base damage, the slides were placed in alkaline buffer and electrophoresed at low voltage for 30 min at 4 °C. Air-dried slides were stained with SYBR Green I and analyzed using a confocal laser scanning microscope (TCS SP2 AOBS; Leica). The cells with damaged DNA displayed migration of DNA fragments from the nucleus by forming a comet-like shape. The images were analyzed using CASP software (Comet Assay Software Project 1.2.2). At least 100 comets were analyzed for each sample. Comet assays were performed three times, each time in duplicate.

### 2.5. Data analysis

Data were compared by one-way analysis of variance (ANOVA) followed by Tukey's studentized range test (SAS Institute, 2003) when significant differences were found at $P < 0.05$. ED (effective dose) values were estimated by probit analysis. The regression analysis was carried out and the regression equation was obtained by iteration (standard probit analysis technique, Finney, 1964) by SPSS (1999).

## 3. Results

### 3.1. Effects of electron beam irradiation on developmental stage

At all stages, the rate of failure to develop increased with increasing doses of electron beam radiation from 30 to 250 Gy. Hatchability, pupation, emergence, longevity, and fecundity from irradiated eggs are shown in Table 1. When 1-day-old eggs were irradiated at more than 100 Gy, hatchability was completely inhibited. When third instar larvae were irradiated at 100 Gy and higher doses, pupation was significantly affected (Table 2) ($\chi^2 = 42.2$; df = 4; $P < 0.05$). The number of adults emerging from larvae was significantly reduced as the dose was increased ($\chi^2 = 4.5$; df = 2; $P < 0.05$). At 150 Gy and above, no adult emergence was observed. At 100 Gy, $F_1$ eggs did not hatch. Electron beam irradiation of larvae, for all doses, had no effect on adult longevity. Developmental period (larvae to pupae) of *S. litura* electron beam irradiated larvae is also given in Table 2. There was a dose-dependent delay in the developmental period. When third instar larvae were irradiated at 200 Gy, the developmental period was about 8 days longer than that of the untreated group. The results for electron beam-irradiated pupae are shown in Table 3. The mean number of adults that emerged from pupae irradiated with 30 and 50 Gy was not significantly different from that of the untreated control, but doses of