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Effect of electron beam irradiation on developmental stages of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

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HIGHLIGHTS

- Electron beam irradiation induced abnormal development of *Helicoverpa armigera.*
- ED_{99} value for inhibition of adult emergence was estimated at 197.8 Gy for egg.
- ED_{99} value for inhibition of adult emergence was estimated at 189.6 Gy for larva.

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ABSTRACT

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), is an economically important and polyphagous pest, which harms various kinds of food crops and important agricultural plants, such as cotton and paprika. Effects of electron beam irradiation at six dose levels between 50 and 350 Gy on the egg (24–48 h old), the larval (4–5th instar), and the pupal (7-d old for female, 5-d old for male) development, and on the adult (1-d old) reproduction were tested to identify a potential quarantine treatment dose. Increased doses of irradiation on eggs decreased egg hatchability, pupation and adult emergence and increased larval period. ED_{99} values for inhibition of hatching, pupation and emergence were 460.6, 236.9 and 197.8 Gy, respectively. When larvae were irradiated with more than 280 Gy, no larvae could develop into pupae. ED_{99} values for inhibition of pupation and adult emergence were 265.6 and 189.6 Gy, respectively. Even though the irradiation on pupa did not completely inhibit adult emergence, most of the pupae emerged to deformed adults. When adults were irradiated, fecundity was not affected. However, F_1 egg hatching was completely inhibited at the dose of 350 Gy. ED_{99} value for inhibition of adult emergence was estimated at 366.5 Gy. Our results suggest that electron beam irradiation could be recommendable as an alternative to MB and as a phytosanitary treatment for quarantine. A treatment dose of less than or equal to 220 Gy is suggested as a potential quarantine treatment to *H. armigera* egg for prevention of pupation and to larva for prevention of adult emerge.

1. Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), is a serious polyphagous pest, which inflicts losses to various kinds of food crops and important agricultural plants, such as cotton, tobacco, tomatoes, paprika, and *Prunus* in addition to ornamental plants and flowers (EPPO, 2007). The *H. armigera* infected produces are particularly hard to detect and hard to control, because the larvae bore into plants with few visible external symptoms. Though *H. armigera* is widely distributed in Old World including Oceania, it has not been recorded in the American continents until 2013 (Czepak et al., 2013; Tay et al., 2013). Therefore, it had been considered a quarantine pest in Brazil and Chile (APQA, 2013; Czepak et al., 2013).

Rapid growth in agricultural products trade has increased the risk of introduction of exotic pest into new area. This trade-induced introduction of pests may lead to significant negative economic and environmental impact. Therefore, importing countries only permit the entry of quarantine pest-free and secured agricultural commodities. In order to satisfy quarantine requirements, several treatments are used for disinfestation: chemical

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treatments including fumigants such as methyl bromide (MB), physical treatment including the application of extreme temperature (heat and cold), controlled atmospheres, and irradiation, and combinations of them (Sharp and Hallman, 1994; Follett and Neven, 2006; Jung et al., 2014). Among the disinfestation methods, MB has been widely used for controlling insect pests in quarantine treatments. However, after regulation of ozone depleting substance, MB is limited in use (UNEP, 2006).

Phytosanitary irradiation has been suggested as a useful alternative to MB with the following advantages: non-development of insect resistance, absence of pesticide residue, and applicability to packed commodities. Among the phytosanitary irradiation treatments, electron beam radiation is composed of a narrow stream of electrons produced from machine sources. It is widely used in the semiconductor and medical industries, food processing and agricultural quarantine (Migdal et al., 1993; Sadat et al., 1993; Calvo et al., 2012; Koo et al., 2012). However, we are not aware of studies reporting the effect of electron beam radiation to control of *H. armigera*.

The objective of this study was to examine the effects of electron beam irradiation on egg, larval, and pupal development, and on adult reproduction in *H. armigera* to identify a potential quarantine treatment dose.

2. Materials and methods

2.1. Test insect

H. armigera larvae were initially obtained from National Institute of Horticultural and Herbal Science (Suwon, Korea) in 2011 and have been maintained in the insectarium at Insect Chemical Ecology Laboratory, Gyeongsang National University (GNU). *H. armigera* was successively reared through several generations in plastic cages ($35\times35\times30$ cm^3) with artificial diet (General purpose Lepidoptera, Bio-serv; Flemington, NJ) under 25 ± 2 °C 60 ± 10% RH and a 16 L:8D h photoregime. A larva was individually reared after the 3rd instar in a plastic cup (1 Oz, Bio-serv; Flemington, NJ) on the artificial diet until adult emergence. We have not felt any decrease in vitality of the insectarium colony until use in the experiments.

2.2. Electron beam treatment

Eggs, larvae, pupae, and adults of different developmental stages of *H. armigera* were exposed directly to radiation dose of 0 Gy (control), 50 Gy (10 MeV, 0.25 mA, 7.63 m/min), 100 Gy (10 MeV, 0.2 mA, 7.63 m/min), 220 Gy (10 MeV, 0.14 mA, 8.25 m/min), 280 Gy (10 MeV, 0.05 mA, 8.25 m/min), and 350 Gy (10 MeV, 0.02 mA, 7.13 m/min) in air using a high energy linear accelerator (UEL V10-10S) at EB-Tech Co., Ltd. (Daejeon, Korea). Absorbed doses were monitored by a Bruker EMS 104 EPR analyzer (Bruker Instruments, Rheinstetten, Germany) with an alanine pellets dosimeter (Bruker Instruments, Rheinstetten, Germany) at Advance Technology Radiation Institute (Jeongup, Korea). From regression analysis, the absorbed doses were calculated from the dose recorded by the dosimeter suing the following formula: absorbed doses $=1.11$ $(\pm0.08)\times$ dosimeter reading (Gy)–27.27 (±18.70) ($R^2=0.94$, $F_{1,13}=200.78$, $p<0.0001$). The absorbed dose ranges were 90–97 Gy for 100 Gy, 93–171 Gy for 150 Gy, 162–241 Gy for 220 Gy, 297–311 Gy for 280 Gy and 347–350 Gy for 350 Gy.

Two-day-old (24–48 h) eggs laid on paper towel (Kimtowel, Yuhan-Kimberly, Seoul, Korea) were put into a meshed-lid Petridish (10×4 cm^2, SPL Life Science, Pocheon, Korea) in which artificial diet were provided, and irradiated. After irradiation, the development was observed until adult stage. The treatment was replicated three times.

Larvae of *H. armigera* (4–5th instar, $n=60$) with artificial diet were placed in the plastic cup (1 Oz, Bio-Serv) individually and exposed to the electron beam. After irradiation, the development of *H. armigera* was observed until adult stage. This treatment was repeated three times.

Pupae (20 females: 7-D-old, 20 males: 5-D-old, $n=40$) were put individually into the plastic cup (1 Oz, Bio-Serv) and exposed to the electron beam. After adult emergence, the emerged adults at male to female ratio of 2:1 were moved into a plastic cage ($2\times20\times20$ cm^3) where paper towel was attached as an oviposition place. Sucrose solution (10%) was provided as food. The eggs laid by adults were counted every two day for 10 days. For investigating hatchability, hatched larvae were observed every day. This procedure was performed four times.

A one-day old adult was put individually into a plastic cup (1 Oz) and then 5 females and 10 males exposed to the electron beam. After irradiation, the adults were moved into the plastic cage ($20\times20\times20$ cm^3). In the plastic cage, paper towel was attached inside for oviposition and 10% sucrose solution was provided. The eggs laid by adults were counted every two day for 10 days. For investigating hatchability, hatched larvae were observed every day. This treatment was repeated four times.

After irradiations, the following biological reactions on each developmental stage of *H. armigera* were investigated:

(1) For eggs: Rates of eggs hatched, pupation, adult emergence and deformed adult emergence, and periods of larval and pupal development.
(2) For larvae: Rates of pupation and adult emergence and deformed adult emergence, and pupal period.
(3) For pupae: Pupal period, rate of adult emergence, deformed adult emergence, and F_1 eggs hatched, and the fecundity of emerged adults.
(4) For adults: Rate of F_1 eggs hatched and fecundity of the emerged adults.
Based on the above investigation, the effects of electron beam irradiation on various developmental stages–the inhibitory rates of egg hatching, pupation inhibition, adult emergence and hatchability of F_1 eggs were calculated.

2.3. Statistical analysis

Mean values for treatment data were compared by one-way analysis of variance (ANOVA) or general linear mode (GLM) and separated by Tukey's test. Percentage data and the number (χ) of eggs laid by adults were transformed to arcsine and log($\chi+1$) respectively to normalize the distribution (Sokal and Rohlf, 1995). ED_{99} (effective dose) values for inhibition of developmental stage were estimated by probit analysis. Before subjected to probit analysis, each inhibition rate values were adjusted using Abbott's formula (Abbott, 1925). All statistical analyses were conducted using JMP, ver. 9.0.2 (SAS Institute, 2010).

3. Results

3.1. Effects of electron beam irradiation on eggs

Biological reactions of electron-beam irradiated *H. armigera* eggs are given in Table 1. When eggs were irradiated with dose above 100 Gy, hatchability rates significantly decreased ($F_{6,14}=178.38$, $p<0.0001$). At 350 Gy, no larva hatched from the eggs irradiated. Increased doses of irradiation significantly increased the period of larvae ($F_{5,12}=97.63$, $p<0.0001$). Pupation