The effect of 200Gy ^{60}Co-γ radiation on the body stress responses sensitivity and reproductive reaction of *Plutella xylostella* (Linnaeus)

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Abstract: The diamondback moth, *Plutella xylostella* (Linnaeus), is one of the notorious pests causing substantial loses to many cruciferous vegetables across the nations. We have got the result that 200Gy dosage irradiated 6-day male pupae of *P. xylostella* inducing F1 generation sterile. First, in our research, we detected Oxidase system and stress response mechanism of irradiated pupae, the results displayed that 200Gy irradiation significantly alters the antioxidant enzyme regulation in 6-day male pupae of *P. xylostella*. The level of SOD, CAT

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were increased significantly in contrast the level of POD and GST were decreased in 12-24h post-treatment. The heat shock proteins (Hsps) gene expression level was significant increasing, maximum > 2 folds up-regulation of genes were observed in peak. But they also had a trend of gradual recovery with development. Second, in order to explore the irradiated sterility further, we detected the testis LDH and ACP activity found that in male adults testis they increased significantly than control during its development. Thus the present research investigation highlights that the ^{60}Co-γ radiation treatments alters the physiological development of diamondback moth. The results showed that 200Gy dosage resulted stress damage to the body and reproductive system of the diamondback moth.

Keywords: Diamondback moth; ^{60}Co-γ radiation; Antioxidant; Testis

1. Introduction

The diamondback moth (DBM), *P. xyllostella* (L.) is one of the most critical notorious pests of cruciferous vegetables across the nations[1]. The larvae mainly feed on leaves by opening hydraulic and makes holes results in serious loss of vegetable productions. Chemical pesticides have been used widely used to control this pest[2]. However, it has developed resistance against series of synthetic and biological based insecticides. So there is an urgent alarm in developing eco-friendly tool to reduce the global burden caused by the synthetic chemicals.

Sterile insect technique（SIT）have been widely recognized tool and displayed profound activity against lepidopteran insects including *Helicoverpa armigera* and *Episimus utilis Zimmerman*[3-4]. The SIT are environmentally friendly which can display increase efficiency and control target population density[5]. For instance, larval density of *Anopheles arabiensis*

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has been significantly reduced in Nuri area of Sudan by radiating more than 8100 males by 70 Gy ^{60}Co-γ radiation[6]. Sterility irradiation technology need to feed a large number of target insects and then treat different developmental stage with different radiation doses, according to mortality, hatching rate, emergence rate and other indicators to screen sub-sterile dosage and most suitable stage of insect. Then, the target insects irradiated by sub-sterile dosage is released to file[7] (Dyck et al., 2005).

During the physiological functioning of aerobic organisms, free radicals are generated and cleared; there is a balance between these two processes, and if the balance is disrupted, it will result in damage and bodily lesions. Insects are not immune to the ravages of reactive oxygen species (ROS), which are a by-product of oxidative metabolism in aerobic cells and are produced following exposure of cells and tissues to various stressors[8-10]. Low levels of ROS are generally considered to be harmless to cells and may even perform useful functions. The endogenous enzymatic antioxidant system is important for protecting the organism against high levels of ROS. This system is mainly composed of the enzymes superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) and glutathione S-transferase (GST)[11-14].

In general, radiation exposure can exhibit higher level of stress on the biological functions. Insects exposed to higher temperatures or various chemical and physical stresses will trigger a group of synthetic conserved peptides collectively referred as heat shock proteins (Hsps) [15]. The Hsp70 family is one of the most abundant Hsp families and is highly conserved. These proteins are characterized by the upsurge levels of transcription and are the most sensitive to various deleterious stimuli [16]. Hsp70 is expressed at low basal levels under non-stress conditions but can be quickly induced by heat shock and other

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environmental stresses [17, 18].

Based on the preliminary experiments, it is well known that the effect of irradiation on the reproductive system of insects is more obvious[19]. Lactate dehydrogenase (LDH) is the key enzyme in Embden-Meyerhof-Parnas pathway, and its activity changes directly affects the energy providing of cell. Acid phosphatase (ACP) is a hydrolase that catalyzes the hydrolysis of phosphoric acid to produce inorganic phosphoric acid under acid condition related to the absorption and transport of nutrients. LDH and ACP were used as a marker enzyme in the testis of *Bufo Gargarizans* intaking heavy metal[20]. LDH enzyme is a vital source of producing energy in glucose metabolism in spermatocyte[21]. And the ACP enzyme is mainly used as a biomarker enzymes to detect the incidence of spermatogenic disorders[22].

Based on the preliminary screening, 200 Gy was considered to be the suitable sub-sterilizing dose for *P. xylostella*. Thus the present investigation was aimed to detect the activity of oxidase and Hsp70s heat shock protein in the parental pupa of *P. xylostella*. In our lab, we have known there were many changes in reproductive indexes such as oviposition amount and hatchabilit. So, we detected the testis of pupae (after irradiated 24h) and adult males (after emegered 24h, 48h and 72h). Moreover, this was the primary study to determine the LDH and ACP activity in the testis of pupae and adult of irradiated *P. xylostella*. More research will carry out on the reproduction in the future.

2. Results and Discussion

2.1 Effect of ^{60}Co-γ radiation on the antioxidant enzymes of pupae irradiated with 200Gy

The SOD and CAT level was significantly higher from 12 h to 72 h in comparable to

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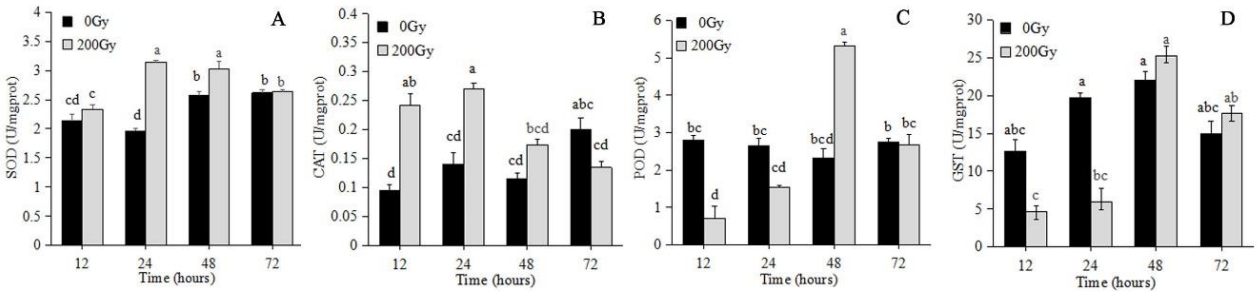
control, with the most significant difference being observed at 24 h. However, POD and GST level was prominent at 48 h after irradiation. Both enzymes have a same trend of enzyme regulation which showed a trend of increasing and then decreasing, reaching a peak at 24 h, followed by a gradual decrease.

SOD activity was significantly different at 24 h and 48 h compared to control however there was no significant difference between 72 h and control (Figure. 1A). Similarly, the change of CAT activity at 12h and 24 h was statistically different compared with control, while the significant difference was not prominent at 48 h and 72 h as compared to control (Figure. 1B). Correspondingly, the POD activity at 48 h displayed higher significant rate as compared to other treatments and control (Figure. 1C). In contrast the GST activity at 12h and 24 h post treatment was significantly lower than 48 h, 72 h and control (Figure. 1D).

Figure. 1. Effects of ^{60}Co-γ irradiation on SOD, CAT, POD, GST activity in the pupal stage of *P.* xylostella at different time points. Each bar represents the mean ± SD of three independent experiments. Different lowercase letters indicate significant differences (DMRT).



SOD is an antioxidant protein plays an important role in reducing high levels of intracellular superoxide radicals induced by extracellular stimuli such as ^{60}Co-γ irradiation. In the present study, the observed changes in SOD activity indicated that ^{60}Co-γ irradiation induced the production of superoxide free radicals in diamondback moth pupae. The antioxidant defense systems of two lepidopteran insect cell lines have been reported[23]. SOD

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activity gradually increased from 12 h to 48 h after the insects were exposed to irradiation, but no significant difference was observed compared with the control at 72 h, suggesting that SOD was stimulated by scavenging superoxide radicals to protect the pupae from radiation stress, and its activity subsequently returned to normal levels after 72 h. It has been found that high doses of UV irradiation suppress the activity of protective enzymes, such as SOD, in normal cells[24]. This finding is consistent with previous reports showing that CAT can protect against oxidative stress and extend the life of insects [25, 26]. This CAT activity was still sufficient to cope with the excess H_2O_2 induced by irradiation stress. Previous studies have shown that enzyme activity can be reduced via negative feedback from excessive substrate levels or damage by oxidative modification[27]. The significant increase in SOD and CAT activity observed in response to ^{60}Co-γ irradiation and the simultaneous decrease in POD activity suggested that CAT may play a more important role in scavenging H_2O_2 than POD[28]. GST effectively metabolizes lipid peroxides and can be considered the main antioxidant enzyme in insects[29]. These results suggest that 200Gy ^{60}Co-γ radiation destructed of the balance of the oxidase system in the stage of pupae, but this bad influence tend to recovery at 72h.

2.2 Effect of ^{60}Co-γ radiation on the expression of Hsp70s genes of pupae irradiated with 200Gy

The basal mRNA expression level of the six px-hsp70s was significantly altered at different time interval of treatment (Figure. 2). The basal relative mRNA expression levels of Px-hsp69-1, Px-hsp69-3 and Px-hsp69-4 showed up-regulation. The Px-hsp69-1 displayed higher expression rate from 12 h to 72 h, reaching a peak of approximately 3-fold at 48 h. However, the level of Px-hsp69-3 does not displayed considerable regulation at 12 h but the

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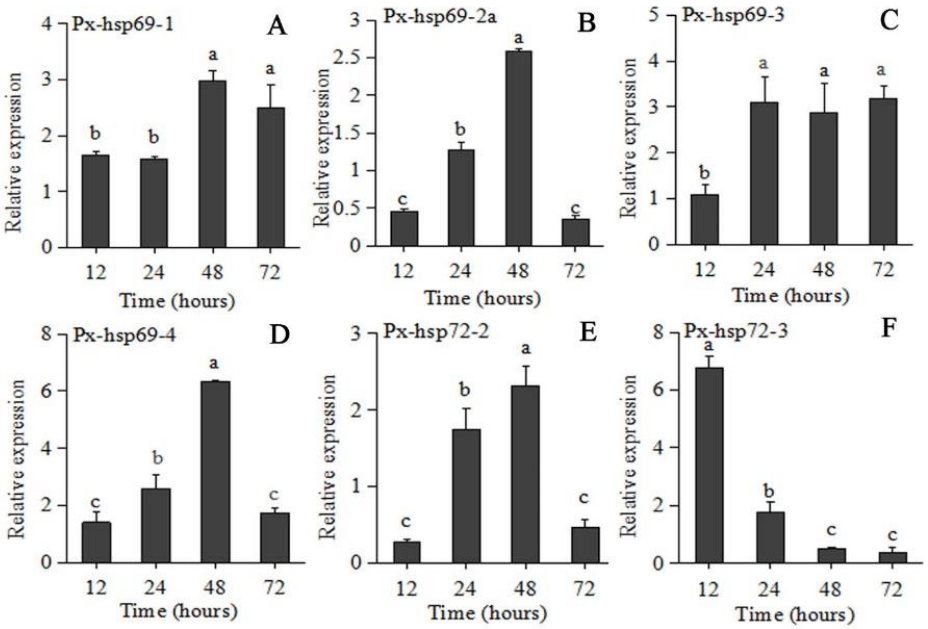
expression was up-regulated 3-fold higher from 24 h to 72 h. Correspondingly, Px-hsp69-4 displayed no significant up-regulation at 12 h and 72 h but displayed prominent expression level at 48 h. Similarly the expression levels of Px-hsp69-2a and Px-hsp72-2 were prominent at 48 h as compared to other treatments. The basal relative mRNA expression level of Px-hsp72-3 showed 7-fold higher expression rate at 12 h and it was significantly different to other treatments.

Figure. 2. Effect of ^{60}Co-γ radiation stress on the expression of Px-hsp70s (Px-hsp69-1, Px-hsp69-2a, Px-hsp69-3, Px-hsp69-4, Px-hsp72-2, Px-hsp69-4) in DBM pupae. Samples of total RNA were extracted from DBM pupae after the insects were irradiated with 200 Gy. Each bar represents the mean ± SD of three independent experiments. Different lowercase letters indicate significant differences (DMRT).



Previous research suggests that genes encoding Hsp70s displayed higher expression level when the insects were subjected to any external stress or stimuli. Insects can resist their external stress by increasing Hsps expression, however over expression may harm the development and reproduction rate of insects[30]. Hsp70 is mainly involved in protein folding,

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assembly, transport, cell protection, antigen presentation and tumor immunity. It can restore or accelerate the removal of denatured proteins and stabilize cell structure to achieve thermal tolerance, which plays an important role in the organism[31]. In this study, the expression of Hsp70 was shown to be altered under radiation stress. Over expression of heat shock protein may affect the development and reproductive state of the diamondback moth. The current results illustrates that the external stress through irradiation on pupae increased the Hsp70s expression pattern for initiating the endogenous protection mechanism[32-33].

2.3 Effects of ^{60}Co-γ radiation on LDH and ACP activity of testes irradiated with 200Gy

In present research, when pupae were exposed to 200 Gy irradiation the level of LDH and ACP gets increased first and then decreased and the activity of them was the strongest in 24h after adult emergence(Figure. 3). The level of LDH enzyme increased significantly in the pupal stage of 24h after irradiation and at 48h after emergence($P<0.01$). The ACP enzyme level was appeared to be higher than that of the control at different times, and the 48h increased significantly($P<0.01$), but increased significantly at 24h pupae after irradiation and 24h adult after emergence($P<0.05$).

Figure. 3. Effects of ^{60}Co-γ irradiation on LDH and ACP activity of *Plutella xylostella* testis. Values are the mean ± SD (n = 3). Asterisk designates statistically significant difference between control and irradiated males. (** $p < 0.01$; * $p<0.05$).

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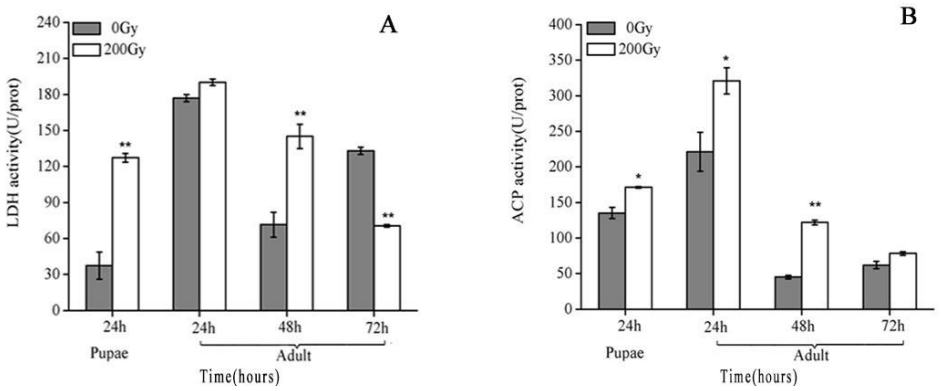

LDH and ACP are always as an common indicators indicating tissue damage produced by chemical stimulation in the testis of fish[34]. It was also detected in rat testis, in the experiment of selenium inducing reproductive damage of the male rat testis, the activity of LDH and ACP enzyme increased significantly[35]. The study found that 900MHz electromagnetic irradiation damage the reproductive system of rats, inducing the decrease of sperm count, deformity of sperm and the increase of LDH enzyme activity[36]. In our results, the activity of LDH and ACP was higher significantly than control and reach the peak at 24h after emergence. The mating trial of *P. xylostella* was considered to be the 0-day, and there was a higher mating rate at the one and two days after emergence, the mating rate being more than 70% [37]. It suggested that 200Gy ^{60}Co-γ radiation induced testis significant damage.

3.Experimental

3.1 Insects

The pupae of *P. xyllostella* were collected from a cabbage mustard field in Guangdong Province, China. Further, they were reared and maintained under laboratory conditions at 25±1℃ 60~70% RH, and 8:16h L:D. Further the emerged butterfly were confined in a cage (50 cm in length, 45cm in width, 45cm in height) with 10% honey for feeding and allowed to mate. Cabbage seedlings (three days) were placed in the tray for egg laying and also for larvae

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188 to feed (25±1°C 60~70% RH, and 8:16h L:D).

189 **3.2 Irradiation**

190 Irradiation was applied with ^{60}Co–γ rays from a Nordion (Ottawa, ON, Canada) ^{60}Co–γ
191 radiation was purchased from Furui High-energy Technology Co. Ltd. (Nansha District,
192 Guangzhou, Guangdong Province, China). The treatment dosage was 16.67 Gy/min and the
193 dosage rate was measured using a Fricke dosimeter adapted on ISO/ASTM E 1026-04. The
194 6-day old pupae were choose (due to radiation resistant and convenient for *in vitro*
195 experiments) and were transferred into a culture dish and exposed to ^{60}Co–γ radiation.

196 **3.3 Enzyme assay**

197 A total of 15 pupae per treatment were randomly selected and subjected to ^{60}Co–γ
198 treatment irradiation at regular intervals of 12, 24, 48 and 72 h. After treatment, the samples
199 were immediately frozen in to liquid nitrogen and stored at -80°C prior to analysis. The
200 controls were subjected to the same conditions but were not irradiated. For each treatment
201 three replicates were performed.

202 The activities of SOD, CAT, POD and GST were determined spectrophotometrically
203 according to the manufacturer's protocol, with a number of modifications, using commercially
204 available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). SOD
205 activity was measured at 550nm using the xanthine and xanthine oxidase systems. CAT
206 activity was determined by measuring the decrease in absorbance at 240 nm for H_2O_2
207 decomposition. Whereas, POD activity was determined at 420 nm via catalytic oxidation in
208 the presence of H_2O_2 and the substrate. GST activity was determined using
209 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The formation of the GSH-CDNB
210 conjugate was monitored by the change in absorbance at 412nm.

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3.4 Real-time quantitative PCR (qPCR) analysis of Hsp70s genes

Whole genomic RNA was extracted and first-strand cDNAs were synthesized, and real-time quantitative PCR (qPCR) analysis of Hsp70s was performed according to the manufacturer's instructions for the RNA Simple Total RNA Extraction Kit, the FastQuant RT Kit and the SuperReal PreMix Plus Kit, respectively (Tiangen Biotech Co., Ltd., Beijing, China). The primers for qPCR analysis of the Px-hsp70s were synthesized in the company (Sangon biological engineering Co., LtD., Shanghai, China) and primers for β-actin (housekeeping gene) were used as an endogenous control[38]. qPCR was performed with a SuperReal PreMix Plus (SYBR Green) Kit according to the following program: 95°C for 15 minutes, followed by 40 cycles of 95°C for 10 s and 55°C for 30 s, with plate reading for 32 s. Subsequently, the homogeneity of the PCR product was confirmed by melting curve analysis. The expression level of each gene was calculated according to the Cycle threshold (Ct) equation and the standard curve. Therefore, the normalized expression value for the target gene was calculated by comparing the expression value for the target gene with the expression value for β-actin [39, 40].

3.5 Dissection of pupae and males

A total of 200 testes was dissected and used for each treatment. The testis of irradiated pupae (24 h after irradiation) whose testis was more complete and males (24 h after irradiated pupae emergence) which was always 48 h after treatment. The pupae was dissected within 20 μL PBS on the glass slices directly under the stereoscope (Motic SMZ-171), and the adult males were killed in the alcohol for 10 s and then dissected under the stereoscope within 20 μL PBS. The testes were immediately placed in the liquid nitrogen or in the fixed liquid for the follow-up tests.

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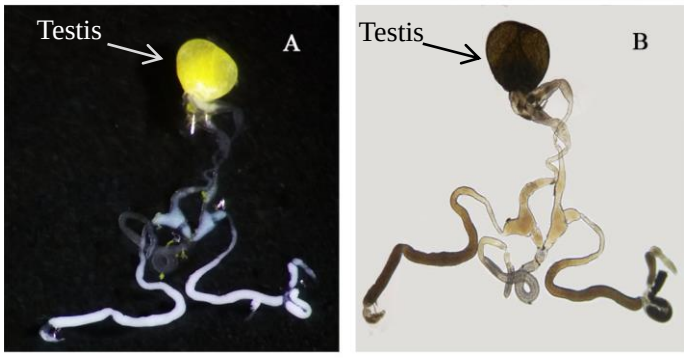
Fig. 4. The internal reproductive organs of male adults of *Plutella xylostella* A. photographed under the Light microscope. B. photographed under the Stereoscope.



3.6 LDH and ACP analysis

Fifty testes were dissected and treated with different dosage and immediately frozen in liquid nitrogen and stored at -80°C prior to analysis. Further the activities of lactate dehydrogenase (LDH) and acid phosphatase (ACP) were detected according to the instruction of matched test kit (Nan Jing Jian Cheng Bioengineering Institute). The absorbance of LDH and ACP was performed using microplate technique. The activity of LDH and ACP was detected at 450 nm and 520 nm respectively.

3.4 Statistical analysis

Statistical data from the experiments were subjected to analysis of variance (ANOVA) using SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA). The means were separated at the 5% significance level through Duncan Multiple Range Tests (DMRT).

4 Conclusion

Our research suggested that 200Gy ^{60}Co-γ radiation harmer the oxidase system during pupal stage, inducing the protection mechanism of Hsp70 family. However, there was a recovery with the development. The obvious damage to the male testis may be related to infertility.

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Author contribution

The listed authors contributed to this work as described in the following. X. L. was the leader of this experiment and prepared the manuscript. L. L., K. Z. and J. L. collected the test samples. Sengodan Karthi and Q. H. modified the article. Q. W. gave the concepts of work and modified the article. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no competing financial interest.

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