

Adverse effects of UV-C irradiation on the morphology of reproductive organs, fecundity, and fertility of the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera; Tenebrionidae)

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Abstract: The effects of UV-C irradiation on the size of male and female reproductive organs, reproductive performance and total protein amount in reproductive organs of *Tribolium castaneum* Herbst (1797) resulted from 0-d-old pupae exposed to 1-to-64 min were determined. UV-C irradiation from 4 to 64 min, resulted in a reduction in size of the gonads. The degree of atrophy increased as the increase of irradiation time where long duration of radiation resulted in decreasing on the size of testicular lobe, rod-shape accessory gland, tubular accessory gland and lacking of seminal vesicle in male beetle. Radiation for more than 4 min also reduced the size of germarium, lateral oviduct and lacking oocyte in ovarioles. No egg chamber formed in UV-C radiation groups for 8, 16, 32, and 64 min. Reciprocal crosses of female adults emerging from UV-C radiated pupae for 4 min and control or UV-C radiated male showed the decreased number of eggs laid and hatching rate compared to the control. Interestingly, the sterility index was 100% when UV-C radiated male was mated with UV-C radiated female. In addition, UV-C radiation clearly reduced the total protein amount in the reproductive organs of *T. castaneum* which correlate with the reduction on the size of reproductive organs.

Keywords: red flour beetle, reproductive organs, testis, ovary, fecundity, radiation

Introduction

Tribolium castaneum (Coleoptera; Tenebrionidae), is a stored product pest with many scientific research applications. Due to its adaptability, simplicity of feeding, and short life cycle, it is an important model organism for animal development, reproduction, and evolutionary studies (Weng *et al.* 2011).

The male reproductive organs of insects typically consist of a pair of testes connected to paired seminal vesicles and a median ejaculatory duct. *T. castaneum*'s testis consists of several testicular lobes and only two pairs of accessory glands, the rod-shaped accessory gland (RAG) and the tubular accessory gland (TAG), both of which function during the adult stage. The accessory glands are involved in spermatophore production and its transfer to the female reproductive tract, which may

induce behavioral and physiological changes in females (Chapman 1998). The testicular follicles contain the germ cells and are the sites of spermatogenesis. This process typically begins during the last larval instar or pupal stage and continues into the adult stage in some insect species (Romoser & Stoffolano 1994).

A pair of ovaries connect with a pair of lateral oviducts to form the female reproductive system. A median oviduct is formed by the oviduct's joint and opens into a genital chamber. Each ovary consists of several ovarioles, which are where oocytes develop. Each ovariole consists of a distal germarium where oocytes develop from oogonia and a more proximal vitellarium where yolk is deposited in the oocytes (Chapman 1998).

Several abiotic factors including stress, starvation, chemical stress, and radiation have

been shown to induce physiological and morphological changes in insect reproductive organs (Gruntenko *et al.* 2003, Blanckenhorn & Henseler 2005, Banu *et al.* 2006, Xu *et al.* 2009, Hassan *et al.* 2017). Irradiation as a phytosanitary treatment has gained widespread acceptance and is now used to control insects in fresh commodities, stored products, and ornamentals (IAEA 2002). Irradiation has been shown to influence male germ cells in Coleoptera insects such as the flour beetle, *Tribolium confusum* (Jacquelin du Val, 1863) (Cork 1957), the stored pulse beetle, *Callosobruchus chinensis* (Linnaeus, 1758) (Rahim & Norimah 1990), the hide beetle, *Dermestes maculatus* (De Geer, 1774) (Saha & Shahjahan 1998), the khapra beetle, *Trogoderma granarium* (Everts, 1899) (Abdel & El-Naggar 2000), the sal heart-wood borer, *Hoplocerambyx spinicornis* (Newman, 1842), the lesser meal-worm, *Alphitobius diaperinus* (Panzer, 1797), the red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas, 1849) (Khare & Khare 2015), and the cowpea weevil, *Callosobruchus maculatus* (Fabricius, 1775) (Ibrahim *et al.* 2017). Gamma-ray (15 Gy) treatments on *T. castaneum* pupae resulted in abnormalities of the testes and ovaries, resulting in sterility in males and females (Banu *et al.* 2006).

Ultraviolet (UV) radiation has been widely used to terminate insects at different stages (Baden *et al.* 1996). UV-C has the shortest wavelength (100–280 nm) and is the most damaging to biological systems (Pattison & Davies 2006). Previous research found that UV-C caused morphological abnormalities in *T. castaneum* to varying degrees. UV-C radiation inhibited adult emergence and reduced insect body mass in 0-day-old pupae. Radiation affected the size of the elytra, hindwings, and wing shape, and caused a deformity of the elytra surface, and decreased the number of hairs sensilla (Tungjitwitayakul *et al.* 2019). UV-C also caused antennal abnormalities in adults. The funicle and club lost segmentation, and the antennae were greatly reduced in size

overall. Radiated adults had abnormal antennae with wrinkled cuticles and few sensilla (Tungjitwitayakul *et al.* 2020). Aside from the effects on the wings, antennae, and legs, we preliminary found that adults emerged from UV-C radiated pupae produced fewer offspring. As a result, we hypothesized that UV-C radiation may have a significant impact on *T. castaneum*'s reproductive attribute organs.

In Coleoptera, spermatogenesis typically occurs during the pupal stage, often for most of the adult life (Tilton & Brower 1983). Germ cell proliferation occurs only in the germarium of *T. castaneum*'s telotrophic ovary during the larval and pupal periods (Trauner & Bünung 2007). Accordingly, we decided to expose the 0-day-old pupae to various UV-C radiation periods and assess the effects when the radiated pupae matured to 8–12-day old adults. This study assessed the effects of UV-C on reproductive organ size, fecundity, sterility, pupation rate, adult emergence rate, and total protein amount in male and female reproductive organs.

Material and Methods

The test insects and sexing *T. castaneum* pupae

Tribolium castaneum cultures were reared on whole wheat flour with 5% yeast (w/w). The insects were kept at 28±2°C, with a relative humidity of 65±5%, under a light:dark photoperiod of 16:8 hours.

Insect stages were checked for pupation daily, and pupae were sexed within 8 hours of pupation. Sexing was performed by separating pupae from the rearing medium and examining their external genitalia under a stereomicroscope, as described by Srivastava (1956).

Radiation treatments

The test cabinet had dimensions of 90×60×55 cm³, UV-C protective walls, and a UV-C lamp (17-watt UV germicidal lamp; TUV

F17T8, Philips, Amsterdam, The Netherlands) attached to its ceiling. The UV-C lamp measured $58 \times 2.5 \text{ cm}^2$ and emitted a 254 nm wavelength. The pupae were placed on an arena 35 cm away from the surface of the UV lamp. The 0-day-old pupae ($n=30$) were lined up on a glass slide and held in place with two-sided adhesive tape to expose their ventral sides to the UV-C radiation. UV-C exposure dosages of 0.2 W/cm^2 , 0.8 W/cm^2 , 3.2 W/cm^2 , 12.8 W/cm^2 , 51.2 W/cm^2 , 204.8 W/cm^2 , and 819.2 W/cm^2 were obtained by transferring the pupae to the test chamber and irradiating them for 0, 1, 2, 4, 8, 16, 32, and 64 minutes, respectively (Tungjitwitayakul *et al.* 2019). Three replicates were performed for each treatment. After irradiation, pupae were kept in a container of wheat flour until eclosion. Eight–twelve-day old adults were dissected under a stereomicroscope to look for gonads. Adults developed from unirradiated pupae were used as a control.

Dissection

Beetles were dissected under a stereomicroscope in a glass dissection with a drop of Ringer's solution. The reproductive organs were collected after the dorsal side of the abdomen was opened with dissecting forceps. The attached tracheoles and fat bodies were removed (Banu *et al.* 2006).

Documentation and image processing

The morphology of the *T. castaneum* gonads was observed under an Olympus SZ51 stereomicroscope (Olympus Corporation, Tokyo, Japan), and the images were digitally captured using an Optika microscope camera (4083 wifi; OPTIKA Srl, Ponteranica, Italy). Adobe Photoshop CS3 software was used to improve the quality of some images. This software is typically used to measure the length and width of the testicular lobes, the RAG, the TAG, and the seminal vesicle in male beetles, as well as the mature oocyte, germarium, and lateral oviduct in female beetles.

Reciprocal crosses

The resulting adults from pupae irradiated with UV-C radiation for 4 minutes, were then used for mating as follows: male (C) \times female (C), male (C) \times female (UV-C), male (UV-C) \times female (C), male (UV-C) \times female (UV-C). The experiments were done using five pairs of 12-day-old adults. Eggs were collected and counted at 48 hours after mating. The number of larvae, pupae and adults were counted on day 20, 40 and 48 after larvae hatched.

Percentage sterility

The sterility percentage was calculated according to Chamberlain's formula (Chamberlain 1962):

$$\% \text{ sterility} = 100 - \left(\frac{a \times b}{A \times B} \times 100 \right)$$

where:

a = number of eggs per female in the treatment,

b = percentage of hatched eggs in the treatment,

A = number of eggs per female in control,

B = percentage of hatched eggs in control.

Total protein amount

To determine the total protein amount in the testes and ovaries, samples were collected from the control, 4 min and 8 minutes of radiation. For each replicate, 20 pairs of testes or ovaries dissected from male and female beetles were gently homogenized in 1.5 ml microtubes containing 50 μl phosphate buffer saline, and protein concentration was then determined using Bradford reagent (Bio-Rad, California, USA) (Tatun *et al.* 2016).

Data analysis

A statistical analysis of the data was performed using a one-way analysis of variance (ANOVA; IBM SPSS Statistics 22), followed by a least-significance difference (LSD) multiple range test. The significance level was set at 0.05 ($p < 0.05$).

Results

Size of male reproductive organs after UV-C radiation

The internal reproductive organs of the adult male *T. castaneum* developed from UV-C treated pupae contain all the essential parts but are reduced in size (Fig. 1b–h). Examination of the irradiated testes revealed that the width of the TAGs and the length of the seminal vesicle in UV-C radiated groups for 2 minutes was wider than in the control and UV-C radiated groups for 1 minute. In contrast, the organ was very sensitive to UV-C radiation

from 4–64 minutes (Fig. 1d–h). Table 1 shows the reduction in the size of irradiated testes compared to unirradiated testes. The length of the testicular lobe, the width and length of RAGs, and the width of TAGs in adults developed from UV-C radiated pupae for 4–64 minutes were less than in the control and UV-C radiated groups for 1 and 2 minutes. Interestingly, the exact position of the seminal vesicle in adults that developed from UV-C irradiated pupae for 4–64 minutes could not be determined because the seminal vesicle appeared as a tiny tube and could not develop into a chamber.

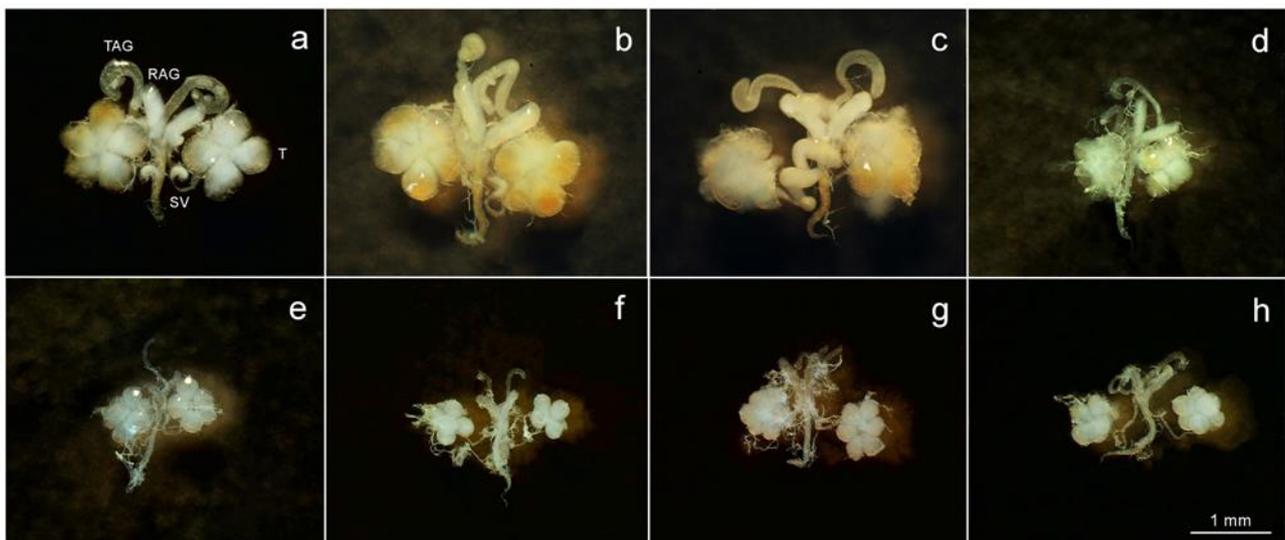


Fig. 1. Morphological changes in male reproductive organs of *T. castaneum* adult after UV-C radiation. Male reproductive organs of a) control and resulting adults of UV-C radiated groups for b) 1; c) 2; d) 4; e) 8; f) 16; g) 32; and h) 64 minutes were shown for comparison. RAG, rod-shaped accessory gland; SV, seminal vesicle; T, testis; TAG, tubular accessory gland.

Size of ovaries after UV-C radiation

The current study of the reproductive organs in female *T. castaneum* resulting from UV-C treated pupae exposed to UV-C showed that the size of ovaries is markedly reduced when compared to the control group (Fig. 2). After measurements were taken, the ovaries of irradiated insects from 4–64 minutes showed a significant difference to those of unirradiated insects, particularly in size (Table 2). The number of ovarioles in the ovary of a UV-C

radiated female for 32 minutes was significantly less than the control ($p < 0.05$). The width and length of the mature oocyte, germarium, and lateral oviduct were shorter in UV-C radiated females for 4–64 minutes than in control and UV-C radiated females for 1 and 2 minutes. In addition, the results showed that we could not detect a mature oocyte in a female adult developed from UV-C radiated pupae for 8–64 minutes (Table 2).

Table 1. Size of the male reproductive organs in adult developed from UV-C treated pupae for 1 to 64 min.

treatment	length of testicular lobe [μm]	rod-shape accessory gland		width of tubular accessory gland [μm]	seminal vesicle	
		width [μm]	length [μm]		width [μm]	length [μm]
Control	222.78 $\pm 5.05^a$	118.13 $\pm 8.44^a$	340.19 $\pm 17.23^a$	58.91 $\pm 2.72^a$	55.39 $\pm 5.46^a$	170.76 $\pm 29.24^a$
UV-C 1 min	221.12 $\pm 8.41^a$	121.71 $\pm 7.71^a$	373.97 $\pm 23.80^a$	63.49 $\pm 5.10^{ab}$	61.27 $\pm 9.88^a$	258.27 $\pm 45.21^b$
UV-C 2 min	232.71 $\pm 3.47^a$	110.16 $\pm 7.74^a$	356.73 $\pm 23.35^a$	68.03 $\pm 10.62^b$	80.24 $\pm 16.27^b$	362.82 $\pm 17.64^c$
UV-C 4 min	187.30 $\pm 5.01^b$	37.59 $\pm 9.14^b$	246.82 $\pm 25.48^{bc}$	19.39 $\pm 1.82^c$	*	*
UV-C 8 min	162.13 $\pm 26.00^c$	47.66 $\pm 12.68^{bc}$	216.97 $\pm 46.22^c$	22.91 $\pm 2.79^{cd}$	*	*
UV-C 16 min	162.86 $\pm 20.89^c$	51.93 $\pm 17.61^{bc}$	279.92 $\pm 34.35^b$	27.10 $\pm 4.44^d$	*	*
UV-C 32 min	138.78 $\pm 9.88^d$	59.73 $\pm 18.30^c$	273.29 $\pm 37.92^b$	22.58 $\pm 4.74^{cd}$	*	*
UV-C 64 min	153.62 $\pm 17.22^{cd}$	53.44 $\pm 7.86^c$	264.68 $\pm 24.30^b$	19.21 $\pm 1.17^c$	*	*

* Seminal vesicle appeared as a tiny tube and could not developed into a chamber.

Data are shown as the mean \pm standard deviation (SD) (n = 30) followed by the same letter in a column are not significantly different at $P < 0.05$ by LSD's test.

Table 2. Size of the female reproductive organs in adult developed from UV-C treated pupae for 1 to 64 min.

treatment	number of ovariole	mature oocyte		germarium		lateral oviduct	
		width [μm]	length [μm]	width [μm]	length [μm]	width [μm]	length [μm]
control	4.90 $\pm 0.22^a$	160.46 $\pm 32.63^a$	298.12 $\pm 40.48^a$	58.81 $\pm 5.76^a$	321.22 $\pm 37.11^a$	310.18 $\pm 44.29^a$	191.61 $\pm 17.88^{ab}$
UV-C 1 min	4.60 $\pm 0.42^{ab}$	171.15 $\pm 18.91^a$	296.38 $\pm 24.37^a$	65.20 $\pm 3.35^b$	439.18 $\pm 24.33^b$	281.17 $\pm 8.43^a$	196.88 $\pm 3.84^a$
UV-C 2 min	4.90 $\pm 0.42^a$	165.17 $\pm 28.32^a$	266.32 $\pm 41.94^a$	63.23 $\pm 7.32^{ab}$	351.20 $\pm 44.49^a$	240.62 $\pm 14.74^b$	173.06 $\pm 20.52^b$
UV-C 4 min	5.00 $\pm 0.35^a$	101.56 $\pm 26.29^b$	180.75 $\pm 8.18^b$	32.67 $\pm 3.25^c$	144.28 $\pm 24.60^c$	138.26 $\pm 16.67^c$	135.97 $\pm 13.48^c$
UV-C 8 min	4.7 $\pm 0.97^{ab}$	ND	ND	17.81 $\pm 4.65^d$	93.27 $\pm 7.03^d$	76.53 $\pm 18.21^d$	92.28 $\pm 16.13^d$
UV-C 16 min	4.3 $\pm 0.45^{ab}$	ND	ND	17.72 $\pm 4.98^d$	85.59 $\pm 10.33^d$	79.99 $\pm 14.86^d$	79.19 $\pm 14.55^d$
UV-C 32 min	4.1 $\pm 0.74^b$	ND	ND	14.70 $\pm 1.69^d$	93.42 $\pm 12.13^d$	83.13 $\pm 22.11^d$	80.29 $\pm 7.62^d$
UV-C 64 min	4.6 $\pm 0.42^{ab}$	ND	ND	20.47 $\pm 6.28^d$	118.06 $\pm 19.07^{cd}$	53.75 $\pm 3.83^d$	82.13 $\pm 5.29^d$

ND = non-detectable.

Data are shown as the mean \pm standard deviation (SD) (n = 30) followed by the same letter in a column are not significantly different at $P < 0.05$ by LSD's test.

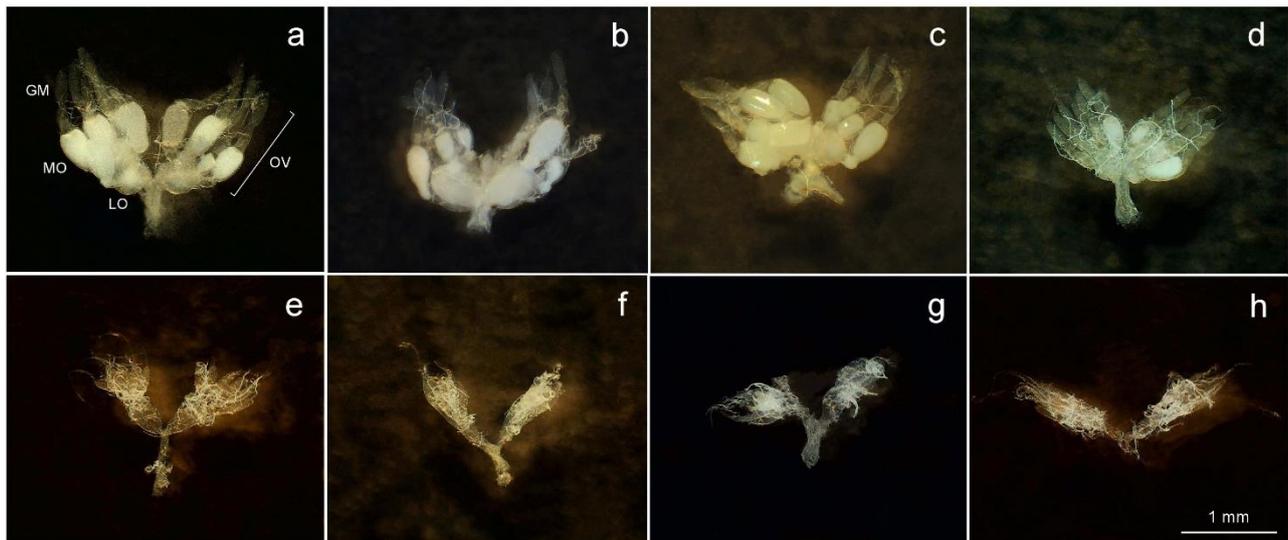


Fig. 2. Morphological changes in female reproductive organs of *T. castaneum* adult after UV-C radiation. Female reproductive organs of a) control, and resulting adults of UV-C radiated groups for b) 1; c) 2; d) 4; e) 8; f) 16; g) 32; and h) 64 minutes were shown for comparison. G, germarium; LO, lateral oviduct; MO, mature oocyte; O, ovariole.

Reproductive performance

UV-C radiation also had a remarkable effect on *T. castaneum*'s reproductive performance. Their fecundity, egg hatching rate, sterility index, pupation rate, and adult emergence rate all decreased after female pupae were irradiated with UV-C (Table 3). When comparing the number of eggs in the four tested groups, it was clear that the number of eggs produced by the male (C) × female (UV-C) and male (UV-C) × female (UV-C) was significantly lower than that produced by the male (C) × female (C) and male (UV-C) × female (C). Moreover, male (UV-C) × female (C) fecundity was significantly higher than the control. The overall hatchability rate differed significantly between the four tested groups.

The male (C) × female (C) hatchability percentage was significantly higher than in other groups. Interestingly, no larvae were produced by the male (UV-C) × female (UV-C) group. The sterility index was 100% in the male (UV-C) × female (UV-C) group and reduced to $84.12 \pm 2.85\%$ in the male (C) × female (UV-C). In contrast, there was no sterility effect in the male (UV-C) × female (C) group. However, the male (C) × female (UV-C) had the highest percentage of pupation, followed by male (C) × female (C), and male (UV-C) × female (C), respectively. In addition, the adult emergence rate in male (C) × female (UV-C) was recorded as 100%, followed by the male (UV-C) × female (C) and the male (C) × female (C), respectively.

Table 3. Reciprocal crosses of adults emerging from UV-C radiated pupae for 4 min.

treatment	number of eggs laid/ 5 females	hatching rate [%]	sterility index [%]	pupation rate [%]	adult emergent rate [%]
Male [C] x female [C]	49.67 ± 5.03^a	92.67 ± 6.43^a	-	87.86 ± 5.79^{ab}	91.33 ± 2.08^a
male [C] x female [UV-C]	21.63 ± 4.93^b	34.00 ± 4.36^b	84.12 ± 2.85^a	93.33 ± 5.77^a	100.00^b
male [UV-C] x female [C]	63.00 ± 9.54^c	83.33 ± 2.52^c	-15.60 ± 24.69^b	82.67 ± 5.03^b	96.83 ± 1.61^c
male [UV-C] x female [UV-C]	33.33 ± 6.66^b	0^d	100^a	0^c	0^d

Data are shown as the mean \pm standard deviation (SD) followed by the same letter in a column are not significantly different at $P < 0.05$ by LSD's test.

Total protein amount in male and female reproductive organs

UV-C radiation significantly reduced the total protein content of *T. castaneum* reproductive organs. When comparing the three groups, the protein amount in control testes was the highest at $0.89 \pm 0.03 \mu\text{g}/\mu\text{l}$, whereas the protein amount in adult testes developed from UV-C radiated pupae for 4

and 8 minutes was reduced to 0.62 ± 0.04 , and $0.17 \pm 0.02 \mu\text{g}/\mu\text{l}$, respectively (Fig. 3a). Similarly, the protein amount in the ovary of a control female was the highest at $1.81 \pm 0.19 \mu\text{g}/\mu\text{l}$, but the protein amount in the ovary of an adult developed from UV-C radiated pupae for 4 and 8 minutes was reduced to 0.53 ± 0.01 and $0.08 \pm 0.01 \mu\text{g}/\mu\text{l}$, respectively (Fig. 3b).

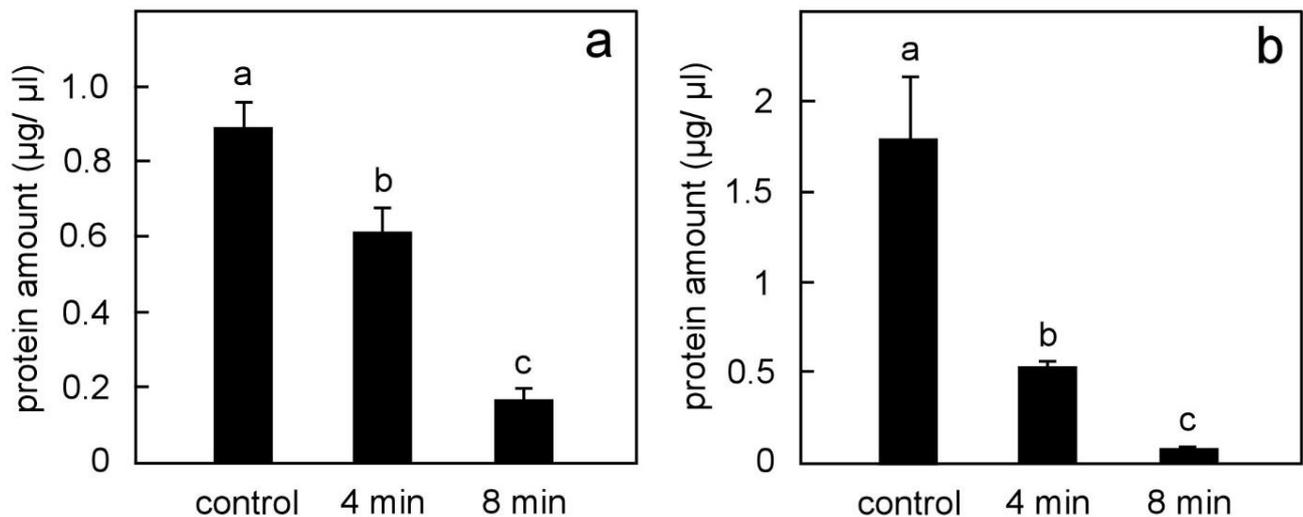


Fig. 3. Total protein amounts in a) testes, and b) ovaries of control and resulting adults of UV-C radiated groups for 4 and 8 minutes.

Discussion

Exposure of UV-C radiation to 0-day-old pupae resulted in poor development of *T. castaneum* adult male and female reproductive organs. Since *T. castaneum* ovaries, spermatheca, and testes primarily mature during the early pupal period (Sokoloff 1974, Mahroof *et al.* 2005), then resulting adults after UV-C treatment lacking functional testes and ovaries exhibit the ultimate states of sterility and infecundity.

Measurements of the male reproductive organs of adults developed from UV-C treated pupae showed that UV-C radiation for 4–64 minutes affected the size of testes, accessory glands, and seminal vesicles of the *T. castaneum*. Radiation for more than 4 minutes

reduced the length of the testicular lobe. Reduced growth of the testes after irradiation may account for decreases in or absence of spermatogenic activity as a possible consequence of radiation damage to the germ cells (Xu *et al.* 2015). UV-C radiation for more than 4 minutes caused a reduction in the size of RAG and TAG in the male beetles. Among the known functions of male accessory glands is the secretion of seminal fluid, which may contain chemicals involved in the activation of spermatozoa and the production of spermatophores. UV-C radiation may reduce the ability of *T. castaneum* seminal fluid production and secretion. In addition, accessory gland secretions may influence an inseminated female in various aspects, including stimulation of the oviposition,

acceleration of oocyte maturation, stimulation of contractions of the genital ducts that aid sperm movement, including vaginal plugs forming, which affects the female's behavior (Romoser & Stoffolano 1994). Hence, the reduced size of RAGs and TAGs in males may be related to the reproductive ability of female beetles.

UV-C radiation dramatically decreased the size of the ovary in *T. castaneum*. Radiation for 2 minutes resulted in a shorter and narrower lateral oviduct, while radiation for 4 minutes resulted in a smaller mature oocyte, germarium, and lateral oviduct. Female *T. castaneum* ovariole development is inhibited by UV-C treatment of pupae for 8, 16, 32, and 64 minutes. The degree of atrophy increased in line with longer irradiation periods where the long period resulted in ovariole lacking oocyte. It indicated that 8 minutes of treatment was sufficient to completely stop the process of oogenesis in *T. castaneum* adults. UV-C irradiation caused severe defects in the morphology of the testes and ovaries, similar to gamma irradiation. Many insects' male germ cells were severely damaged by gamma radiation. Radiation-induced aberrations in the spermatids and sperms of the progenies F1 and F2 generations of *C. maculatus* irradiated parental males have been observed (Ibrahim *et al.* 2017). These aberrations have also been recorded in other irradiated insects such as *Dermestes frischii* (Kugelann, 1792) (Hodges 1983), and include the specific damage that was caused to the red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Paoli *et al.* 2014), damage in the testes of the red pumpkin beetles, *R. foveicollis*, and a shrunken germinal epithelium of the testes in the lesser mealworm, *A. diaperinus* (Khare & Khare 2015). Pupal gamma radiation treatments for 15 Gy cause sterility in *T. castaneum* males and infecundity in females (Banu *et al.* 2006). Gamma radiation in screwworm fly, *Cochliomyia hominivorax* (Coquerel, 1858) decreased the rate of ovarian growth and caused cytopathological changes

in developing egg follicles (LaChance & Bruns 1963, LaChance & Leverich 1968). In addition, gamma radiation reduced ovarian growth in the peach fruit fly, *Bactrocera zonata* (Saunders, 1842) (Younes *et al.* 2007), reduced the size of ovaries in the fruit fly, *Drosophila melanogaster* (Meigen, 1830) (Cantwell & Henneberry 1963), the oriental fruit fly, melon fly and Mediterranean fruit fly (Keiser *et al.* 1965), and decreased egg production and egg hatchability in the medfly (Hafez & Shoukry 1972).

It has been reported that after 0-day-old pupae of *T. castaneum* were irradiated with UV-C for 4–64 min, the body mass of adults decreased as the exposure periods increased (Tungjitwitayakul *et al.* 2019). Accordingly, the reduction of the sizes of testes and ovaries in UV-C resulting adults were correlated with the decrease in insect body mass.

The width of the TAGs and the length of the seminal vesicle in UV-C radiated male for 2 minutes was wider than in the control and UV-C radiated groups for 1 minute. The width and length of germarium in UV-C radiated female for 1 minutes was also wider than in the control. This is in agreement with the study in *Ceratitis capitata* (Wiedemann, 1824) that gamma irradiation at low dose cause an enlarge of mesodermal accessory glands in male (Abraham *et al.* 2021).

When *T. castaneum* was reared in high temperature environment which is one kind of stress that induced female adults to produce larger gametes (Vasudeva *et al.* 2019). Besides, high temperature, it has been reported that the *Bicyclus anynana* (Butler, 1879) female butterflies lay larger eggs when they are exposed to low temperatures during oviposition (Fischer *et al.* 2003). Indicating that when insects exposed to various kind of stress including radiation, temperature changes may alter the morphology of reproductive organs.

UV-C radiation disrupts normal reproductive system functioning in both males and females, but *T. castaneum* females tend to be more vulnerable. When females were

radiated with UV-C, the adverse effects were greater than when males were exposed. Our oviposition studies suggest that male adults developed from UV-C radiated pupae showed no indication of sterility when mated with unirradiated (control) females. When mated to unirradiated (control) males, females from UV-C radiated pupae showed a considerable reduction in the number of eggs laid and egg-to-adult survival. It has been demonstrated that a decrease in the size of ovarioles may be related to a decrease in the size of all eggs, which might result in a smaller number of laying eggs, as reported in *D. melanogaster* (Gruntenko *et al.* 2003). Mating a UV-C radiated male with a UV-C radiated female impacted egg production and completely suppressed egg hatch. The findings were similar to those of Mahroof *et al.* (2005) who discovered that the magnitude of the adverse effects of high temperature (50°C) on reproduction was more severe in exposed female pupae than in male pupae. High temperatures are known to depress egg production more rapidly than sperm production in insects (Chapman 1998). Likewise, mating non-irradiated females of the peach fruit fly, *B. zonata* with radiated males did not affect egg production but severely reduced hatchability (Mahmoud & Barta 2011). The radiation time of 4 minutes was chosen for our study because it was the lowest dose capable of reducing the size of both male and female reproductive organs. UV-C did not affect male sterility during this radiation period, but it did reduce the hatching rate of the F1 generation. Since Farghaly *et al.* (2014) reported that the fecundity of irradiated insects was dose-dependent, UV-C radiation for 8–64 minutes may increase sterility in male *T. castaneum*.

The decrease in the total protein amount was related to a reduction in the size of reproductive organs. Protein levels in the testes and ovaries were dramatically decreased in the 4 and 8-minute UV-C radiated groups. The protein amount in the testes and

ovaries decreased 22.63 and 5.24 times respectively when exposed to UV-C radiated groups for 8 minutes compared to the control. According to the study in *Dysdercus koenigii* (Fabricius, 1775), UV-C radiation (254 nm) for 10, 15, and 20 minutes decreases protein, glycogen, and lipid positive granules in the ovaries through histochemistry. UV-C radiation successfully prevents oocyte development and yolk deposition in *D. koenigii* ovaries resulting in infertility in the irradiated insect (Mohan & Kumar 2016). Adult beetles' reproductive organs, such as the ovary and male accessory gland, increase in size in mature beetles soon after adult emergence when they begin feeding. Once the cell grows, most likely due to an increase in protein synthesis machinery (Xu *et al.* 2015), protein production decreases resulting in a reduction of ovary and male accessory gland size.

Several stresses can cause changes in the expression of certain proteins in insects. When spinning larvae were subjected to a 45°C heat shock treatment, the ovary showed a decrease in the expression of *Bombyx mori* (Linnaeus, 1758) vitellogenin mRNA (Paul & Kesham 2016). In addition, after treating the silkworm larvae with different concentrations of pyriproxyfen, the transcription levels of *Vg*, *Ovo*, *Out*, *Sxl*, and *Sxl-L* in the treatment group were lower than those in the control group (Qian *et al.* 2020). We expect that UV-C radiation may alter the synthesis of several important proteins in *T. castaneum*'s testes and ovaries, and therefore, further research is needed.

This study has demonstrated that UV-C radiation-induced abnormalities in reproductive organs result in a reduction in fecundity. As a result, the findings could provide important information for developing a sterile insect technique for this common stored product pest.

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References

- Abdel FK, El-Naggar SEM. 2000. Effect of gamma irradiation on male sterization and structure of male reproductive accessory glands of Khapra beetle *Trogoderma granarium* Everts. *Arab Journal of Nuclear Science and Application*, 33(2): 323-329.
- Abraham S, Díaz V, Moyano A, Castillo G, Rull J, Suárez L, Murúa AF, Pantano V, Molina D, Ovruski SM. 2021. Irradiation dose does not affect male reproductive organ size, sperm storage, and female remating propensity in *Ceratitidis capitata*. *Bulletin of Entomological Research*, 111(1): 82-90.
- Baden HP, Kollias N, Anderson RR, Hopkins T, Raftery L. 1996. *Drosophila melanogaster* larvae detect low doses of UVC radiation as manifested by a writhing response. *Archives of Insect Biochemistry and Physiology*, 32: 187-196.
- Banu PA, Ali IA, Salam MA. 2006. Effects of gamma radiation on the reproductive organs in the red flour beetle *Tribolium castaneum* (Herbst). *University Journal of Zoology, Rajshahi University*, 25: 11-14.
- Blanckenhorn WU, Henseler C. 2005. Temperature-dependent ovariole and testis maturation in the yellow dung fly. *Entomologia Experimentalis et Applicata*, 116: 159-165.
- Cantwell GE, Henneberry TJ. 1963. The effects of gamma radiation and apholate on the reproductive tissue of *Drosophila melanogaster* (Meigen). *Journal of Insect Pathology*, 5: 254-264.
- Chamberlain F. 1962. Chemical sterilization of the screw-worm. *Journal of Economic Entomology*, 55: 240-243.
- Chapman RF. 1998. *The insects: structure and functions*. 4th edition. Cambridge University Press, Cambridge, United Kingdom, 968 pp.
- Cork JM. 1957. Gamma radiation and longevity of the flour beetle. *Radiation Research*, 7: 551-557.
- Farghaly DS, El Sharkawy AZ, El Alfawy NA, Risk SA, Bader NF. 2014. Effect of gamma irradiation on some biological aspects of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), with ultrastructural studies on midgut. *Current Science International*, 3(4): 403-413.
- Fischer K, Eenhoorn E, Bot ANM, Brakefield PM, Zwaan BJ. 2003. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. *Proceedings of the Royal Society of London B*, 270: 2051-2056.
- Gruntenko NE, Bownes M, Terashima J, Sukhanova MZ, Raushenbach IY. 2003. Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. *Insect Molecular Biology*, 12(4): 393-404.
- Hafez M, Shoukry A. 1972. Effect of irradiation on adult fecundity and longevity of the Mediterranean fruit fly, *Ceratitidis capitata* (Wied.) in Egypt. *Zeitschrift für Angewandte Entomologie*, 72: 59-66.
- Hassan MI, Amer MS, Hammad KM, Gabarty A, and Seleam TA. 2017. Latent effect of gamma irradiation on reproductive potential and ultrastructure of males' testes of *Culex pipiens* (Diptera; Culicidae). *Journal of Radiation Research and Applied Sciences*, 10(1): 44-52.
- Hodges RJ. 1983. Morphology of the X-irradiated testes of *Dermastes frischii* Kugelann (Coleoptera: Dermestidae). II. Spermatids and sperm. *International Journal of Invertebrate Reproduction*, 6: 51-63.

- Ibrahim HA, Fawki S, Abd El-Bar MM, Abdou MA, Mahmoud DM, El-Gohary EE. 2017. Inherited influence of low dose gamma radiation on the reproductive potential and spermiogenesis of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae). *Journal of Radiation Research and Applied Sciences*, 10: 338-347.
- IAEA (Ed.). 2002. Irradiation as a phytosanitary treatment of food and agricultural commodities. Proceedings of a final research coordination meeting organized by the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency. Vienna. Australia.
- Keiser I, Steiner LF, Kamasaki H. 1965. Effect of chemosterilants against the oriental fruit , melon fly and Mediterranean fruit fly. *Journal of Economic Entomology*, 58: 682-685.
- Khare AK, Khare A. 2015. A method of controlling pest population using radiation techniques. *International Journal of Current Research in Biosciences and Plant Biology*, 2(6): 127-131.
- LaChance LE, Bruns SB. 1963. Oogenesis and radiosensitivity in *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Biology Bulletin*, 124: 65-83.
- LaChance LE, Leverich AP. 1968. Cytology of oogenesis in chemosterilized screwworm flies, *Cochliomyia hominivorax*, as related to endomitosis in nurse cells. *Annals of the Entomological Society of America*, 61: 1188-1197.
- Mahroof R, Subramanyam B, Flinn P. 2005. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. *Journal of Economic Entomology*, 98(2): 626-633.
- Mahmoud FM, Barta M. 2011. Effect of gamma radiation on the male sterility and other quality parameters of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Horticultural Science*, 38(2): 54-62.
- Mohan S, Kumar D. 2016. Effects of UVC (245 nm) irradiation on ovarian development of the red cotton bug, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae). *Biochemical and Cellular Archives*, 16(1): 33-39.
- Paoli F, Dallai R, Cristofaro M, Arnone S, Francardi V, Roversi PF. 2014. Morphology of the male reproductive system, sperm ultrastructure and irradiation of the red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae). *Tissue and Cell*, 46: 274-285.
- Paul S, Keshan B. 2016. Ovarian development and vitellogenin gene expression under heat stress in silkworm, *Bombyx mori*. *Psyche: A Journal of Entomology*, <https://doi.org/10.1155/2016/4242317>.
- Pattison D, Davies M. 2006. Actions of ultraviolet light on cellular structures. In: Bignold LP (Ed.). *Cancer: cell structures, carcinogens and genomic instability*. Switzerland, Birkhäuser Basel, pp.131-157.
- Qian HY, Zhang X, Zhao GD, Guo HM. 2020. Effects of pyriproxyfen exposure on reproduction and gene expressions in silkworm, *Bombyx mori*. *Insects*, 11(8): 467, DOI:10.3390/insects11080467
- Rahim MA, Norimah Y. 1990. Effects of gamma radiation on mortality and sterility of some stored product beetles. *MARDI (Malaysian Agricultural Research and Development Institute) Research Journal*, 18(1): 99-106.
- Romoser WS, Stoffolano JGJr. 1994. *The science of entomology*. 3rd edition. Wm. C. Brown publishers. United States of America, 532 pp.
- Saha AK, Shahjahan MR. 1998. Effect of neem and radiation on the larvae of hide beetle, *Dermestes maculatus* Degeer (Coleoptera: Dermestidae). *Bangladesh Journal of Zoology*, 26(1): 7-11.
- Sokoloff A. 1974. *The Biology of Tribolium: With Special Emphasis on Genetic Aspects*. Vol. 2. Clarendon Press, Oxford, 610 pp.

- Srivastava US. 1956. On the post-embryonic development of the female reproductive organs of *Tribolium castaneum* Herbst with a note on the homology of the reproductive organs in the two sexes (Coleoptera: Tenebrionidae). *Beitrage zur Entomologie, Band 6*, 274-284.
- Tatun N, Tungjitwitayakul J, Sakurai S. 2016. Developmental and lethal effects of trehalase inhibitor (validamycin) on the *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Annals of the Entomological Society of America*, 109(2), 224-231.
- Tilton EW, Brower JH. 1983. Radiation effect on arthropods. In: Josephson ES & Peterson MS. (Eds.). *Preservation of Food by Ionizing*. CRC Press, Florida, pp. 269-316.
- Trauner J, Bünung J. 2007. Germ-cell cluster formation in the telotrophic meriostic ovary of *Tribolium castaneum* (Coleoptera, Polyphaga, Tenebrionidae) and its implication on insect phylogeny. *Development Genes and Evolution*, 217: 13-27.
- Tungjitwitayakul J, Yasanga T, Tatun N. 2019. UV-C radiation during the pupal stage affects morphological changes of wings in *Tribolium castaneum* (Col; Tenebrionidae). *International Journal of Radiation Biology*, Doi:10.1080/09553002.2019.1625492.
- Tungjitwitayakul J, Yasanga T, Tatun N. 2020. Impact of UV-C radiation on morphology of the antenna and antennal sensilla in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Radiation Research and Applied Sciences*, 13(1): 648-656.
- Vasudeva R, Sutter A, Sales K, Dickinson ME, Lumley AJ, Gage M GJ. 2019. Adaptive thermal plasticity enhances sperm and egg performance in a model insect. *eLife*. DOI: 10.7554/eLife.49452.
- Weng Z, Huang C, Shu J, Zhang Q, Yu Y. 2011. Effects of heat shock on viability and fecundity in *Tribolium castaneum*. In: 5th International Conference on Bioinformatics and Biomedical Engineering (ICBBE). 10-12 May 2011.
- Xu J, Anciro AL, Palli SR. 2015. Nutritional regulation of male accessory gland growth and maturation in *Tribolium castaneum*. *Scientific Reports*, Doi:10.1038/srep10567.
- Xu J, Shu J, Qiu X, Wang Z, Zhao F, Zhang Z, Zhang Q. 2009. Effect of heat shock on ovary development and HSP83 expression in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Archives of Insect Biochemistry and Physiology*, 70(3): 204-216.
- Younes MWF, Shehata NF, Mahmoud YA. 2007. Anatomical effects of gamma rays on the peach fruit fly, *Bactrocera zonata* (Saund.) female gonads. *Research Journal of Agriculture and Biological Sciences*, 3: 481-484.

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