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EFFECT OF IONIZING (GAMMA) AND NON-IONIZING (UV) RADIATION ON THE DEVELOPMENT OF *TRICHOGRAMMA EUPROCTIDIS* (HYMENOPTERA: TRICHOGRAMMATIDAE)

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Abstract - The potential of using gamma and ultraviolet radiation as an alternative treatment to increase the efficiency of *Trichogramma euproctidis* (Girault 1911) (Hymenoptera: Trichogrammatidae) was investigated in the laboratory. The developmental and adult stages of *T. euproctidis* were exposed to gamma radiation of different doses (0-30 Gy) and ultraviolet radiation of 254 nm wavelengths (UV-C) for different durations (0-10 min) to assess their effect on each of the instars and their potential in breaking the developmental cycle of the egg parasitoid. The LD_{50} values for eggs, prepupae, pupae and adults were 8.1, 10.0, 22.7 and 9.5 Gy for gamma radiation and 9.5, 0.12, 2.0 and 11.9 min for UV radiation, respectively. The pupa and adult stages were more radioresistant to both gamma and UV radiation. The most interesting and unexpected result obtained for the prepupal stage was that UV radiation has a greater effect on prepupal stages than gamma radiation.

Key words: Parasitoid, *Trichogramma euproctidis*, longevity, gamma radiation, UV radiation, LD_{50} and LD_{99}

INTRODUCTION

Nuclear techniques could play a vital role in enabling the cost-effective mass production of beneficial insects for use in augmentative biological control. There are significant opportunities for increasing the use of classical and augmentative biological control through nuclear techniques for the production and release of biological control agents (Brower, 1982; Greany, 1999).

The Ultraviolet (UV) portion of the spectrum has been widely used as a germicide and as an attractant for insects (Bruce, 1975) in embryological-physiological studies for the surface disinfection of insect eggs from pathogens (Bodenstein, 1953; Guerra et al., 1968) and for the suppression of insects and different stages of the life cycle (Beard, 1972; Baden et al., 1996). Among these, UV-B/C radiation is very important in influencing biological systems (Kovacs and Keresztes, 2002). Egg parasitoids in the genus T*richogramma* are one of the most important biological control agents of pest insects in different crops. Augmentative releases of the *Trichogramma* species require mass rearing. The eggs of *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) are used as an alternative host for rearing *Trichogramma*. *E. kuehniella* larvae that hatch from unparasitized eggs and can attack parasitized ones. To prevent host larvae hatch, ultra violet light (UV) can be used to kill host eggs. Gamma radiation is an ionizing radiation type and has a very high penetrating ability (Ayvaz and Tunçbilek, 2006). Nevertheless, host eggs exposed to gamma irradiation can be used as a food for *Trichogramma* rearing (Mansour, 2010). In Turkey, some studies have been conducted on the effect of gamma rays on *Trichogramma* (Tuncbilek and Canpolat, 2003; Tuncbilek and Ayvaz, 2001). Unfortunately, no research has been conducted on the effect of ultraviolet radiation on the *Trichogramma*. Keeping in mind the importance and feasibility of the use of UV-rays, the present investigation was undertaken to evaluate the effect of gamma and UV-irradiation on the commercially relevant aspects of *Trichogramma* rearing. It is, however, difficult to make direct comparisons between gamma and UV radiation studies as the level of gamma and UV dose achieved is not always stated, and radiation intensities vary with sources. The ability of UV to penetrate surfaces is very limited, and thus its effect is limited to surfaces, whereas gamma rays can penetrate deep into tissues. In principle, ultraviolet C (UVC) radiation may provide an effective means of combating pest infestations associated with the structure of a building and may serve as a potential new hygiene measure. Although the use of gamma radiation as a method of pest control has been extensively investigated (Tuncbilek, 1995; Tuncbilek and Kansu; 1996; Tuncbilek, 1997; Carpenter et al., 2001; Makee and Saour, 2003; Bloem et al., 2006), the use of UV-C radiation has not been widely studied due to the lack of penetration through substrates (Bruce and Lum, 1978).

These results led us to explore the idea that radiation is very important in enhancing parasitoid effectiveness in biological systems. The research described herein was conducted to examine the possible effects of UV and gamma radiation use in *T. euproctidis*.

MATERIALS AND METHODS

Rearing of Ephestia kuehniella

All insects were taken from an *E. kuehniella* stock culture kept in the laboratory in 3 l plastic jars covered with mesh. The larvae were reared on a pesticide-free mixture consisting of 1 kg wheat flour, 5% yeast and 30 g of wheat germ. Throughout the rearing, cultures were kept in a rearing room equipped with a controlling system, at 27±1°C and 70±5% R.H., 16:8 h L:D. Light was controlled using a 24 h time switch and fluorescent tubes.

Rearing of Trichogramma euproctidis

The wasp *Trichogramma euproctidis* (Girault 1911) was reared on the eggs of the flour moth and kept in test tubes at 27±1°C, 70–80% RH, 16:8 h L:D. Strips of lightweight cardboard (2.5 by 4 cm) were brushed with gum arabica. The eggs were sprinkled onto these cards and placed in tubes along with adult *T. euproctidis*. Individual *T. euproctidis* females (24 h old) were prepared for the tests by isolating them in small tubes. To do this, the adults were scattered from the rearing tubes onto white paper and captured by placing test tubes (180 mm long and 18 mm in diameter) over them. When the parasites walked up into the tube towards the source of light, their sex could be easily determined under a binocular microscope and then the egg card was placed into the tube (Wührer and Hassan, 1993). After 24 h, the egg cards were removed from the tubes and incubated in controlled conditions.

Gamma and UV radiation sources

T. euproctidis eggs, prepupae, pupae and adults were placed in glass tubes and irradiated in a calibrated ^{137}Cs irradiator (IRACEL OK-GK17) with a source strength of ca 111 TBq and a dose rate of ca 3.20 Gy/min. Dose rate was verified with Fricke dosimetry. They were exposed to 7 dose levels ranging from 0, 5, 10, 15, 20, 25 and 30 Gy. An unirradiated control population (0 dose) was similarly started.

All the developmental stages of the wasp were irradiated with UV-rays of 254 nm wavelength at different duration: 0, 2, 4, 6, 8 and 10 min (Mineralight Lamp, shortwave UV, 254 nm 215-250 V, 56-60 Hz, 0.12A). For irradiation, the test insects were kept in 10 cm quartz tubes placed on the surface 5 cm away from the lamp. During irradiation, the room was illuminated with two 40W fluorescent bulbs and the temperature of the laboratory was 27±1°C.

Experiments

One-day-old *T. euproctidis* adults were obtained from the stock culture. Male and female adults were allowed to mate by pairing them inside glass tubes in a growth chamber at 27±1°C, 70± 5% r.h, at a photoperiod of 16:8 h light:dark, respectively. Individual *T. euproctidis* females (24 h old) were prepared for the tests by isolating them in small tubes for laying. Parasitized eggs were isolated into different groups, one for the control, six and five for the different doses of gamma and UV radiation, respectively. The 1-day-old parasitized eggs in each replicate were exposed to gamma radiation by placing the eggs in glass tubes, or UV doses by placing the eggs in quartz tubes for egg stage irradiation of *T. euproctidis*. Each group consisted of five replicates, with 50 eggs/replicate. Control eggs were placed in the room for the designated time without being exposed to radiation. Eggs parasitized by *T. euproctidis* were treated at target gamma radiation doses of 0, 5, 10, 15, 20, 25 and 30 Gy. In the UV radiation test, the eggs were treated at 0, 2, 4, 6, 8 and 10 min exposure. After irradiation, the eggs were returned to their corresponding glass tubes and placed into rearing room.

The prepupae chosen for irradiation were 4-day-old parasitized eggs. This means that the parasitized eggs were irradiated 4 days after laying. The appearance of black deposits on the surface was an indication of the onset of the prepupal stage (Dahlan and Gordh, 1997). Pupae were irradiated 8 days after laying and one-day-old *T. euproctidis* adults were irradiated with the doses mentioned above. After exposure to radiation, irradiated stages and controls were kept in the rearing rooms under the conditions mentioned above. The numbers of parasitized eggs, adult emergence and sex ratio for each stage were scored.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) for the determination of differences between means, and when significant differences occurred, a Tukey-HSD test was applied for mean separation. The data were transformed to square roots before statistical analysis was performed; back-transformed data are presented in the tables and figures. LD_{50} and LD_{99} values were estimated by using probit analysis with doses of radiation as sources of variation (SPSS, 1999).

RESULTS

Egg

The effects of applying different doses of gamma and UV irradiation on the subsequent development of eggs are shown in Fig. 1. It was found that mean parasitization and adult emergence were significantly reduced with both increased gamma radiation doses but not significantly by UV radiation exposure time ($F=22.911$, df=6, $P<0.001$; $F=1.568$, df=5, $P=0.207$ for gamma and UV radiation, respectively). The effect of gamma radiation was more pronounced than UV radiation at the studied doses. An increase in gamma radiation dose and in time of exposure to UV-rays caused a gradual decrease in the number of hatching eggs. Although very few adults (2.8) emerged from gamma irradiated *T. euproctidis* eggs, more adults (8.6) emerged from UV irradiated eggs after 25 Gy and 10 min, respectively. One-way ANOVA showed that gamma and UV-radiation had significant effects on adult emergence in comparison to controls ($F=35.445$, df=6, $P<0.001$; $F=4.879$, df=5, $P=0.003$ for gamma and UV radiation, respectively). Gamma radiation was more effective than UV radiation in the egg stage in the studied doses.

Although female emergence was influenced by both gamma and UV radiation ($F=25.666$, df=6, $P<0.001$; $F=6.096$, df=5, $P=0.001$ for gamma and UV radiation, respectively), male emergence was not changed by the radiation treatments ($F=4.803$, df=6, $P=0.002$; $F=1.041$, df=5, $P=0.417$ for gamma and UV radiation, respectively).

Prepupae

The results of the effect of gamma and UV radiation on prepupae are shown in Fig. 2. There was a significant reduction in the adult emergence numbers produced by UV-treated females compared to gamma radiation-treated females ($F=60.620$, df=6, $P<0.001$; $F=206.324$, df=5, $P<0.001$ for gamma and UV radiation, respectively). The effect of UV radiation on adult emergence was more evident than gamma radiation. Although very few adults emerged from both gamma

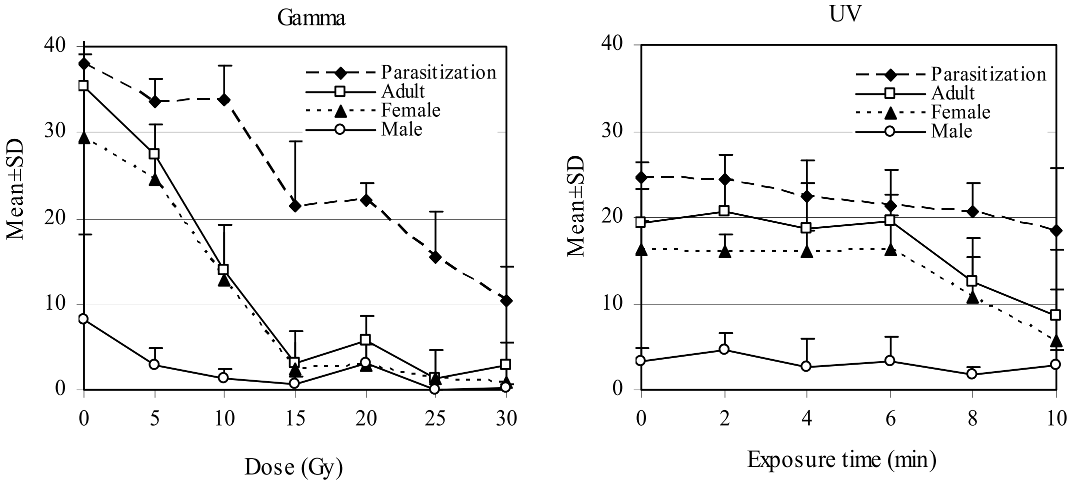

Fig. 1. Mean (±SD) parasitization and adult emergence from gamma and UV irradiated eggs of *T. euproctidis*.

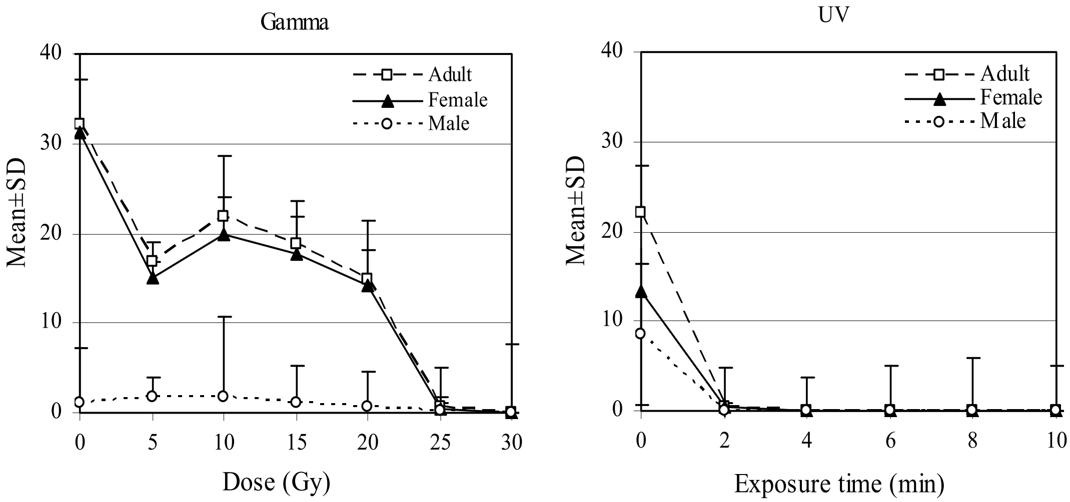

Fig. 2. Mean (±SD) adult emergence from gamma and UV irradiated prepupae of *T. euproctidis*.

and UV irradiated *T. euproctidis* prepupae after 25 Gy and 2 min, respectively, no adults developed from prepupae irradiated with higher doses or exposure times. *T. euproctidis* prepupae were unexpectedly more vulnerable to damage from UV radiation than from gamma radiation. To our knowledge, this is the first report of UV radiation being more efficient than gamma radiation on the prepupal stage.

Female and male emergences were influenced by radiation treatments (Female: F=52.571, df=6, P<0.001; F =14.378, df=5, P<0.001 for gamma and UV radiation, respectively: Male: F=3.683, df=6, P=0.008; F =7.833, df=5, P<0.001 for gamma and UV radiation, respectively).

Pupae

Fig. 3 shows the effect of exposing *T. euproctidis* pupae to gamma and UVC radiation on their subsequent development. It was found that adult emergence decreased with both increased gamma and

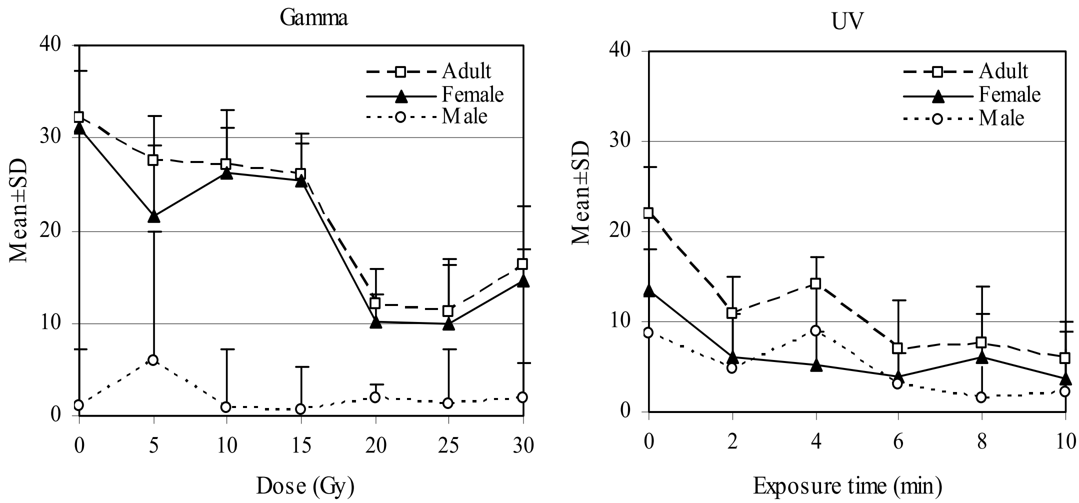

Fig. 3. Mean (±SD) adult emergence from gamma and UV irradiated pupae of *T. euproctidis*.

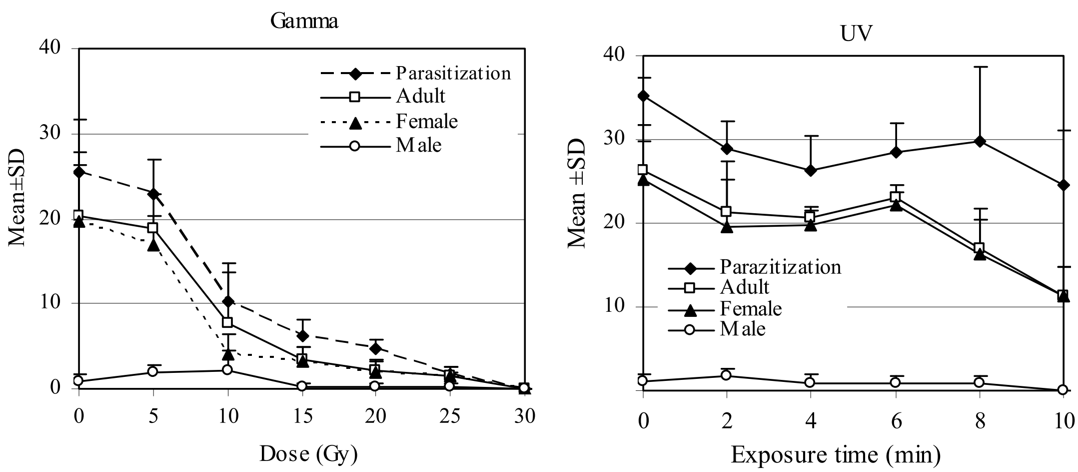

Fig. 4. Mean (±SD) parasitization and adult emergence from gamma and UV irradiated adults of *T. euproctidis*.

UV radiation doses (F=11.300, df=6, P<0.001; F =6.793, df=5, P<0.001 for gamma and UV radiation, respectively). Adult eclosion from irradiated *T. euproctidis* pupae gradually decreased as the gamma radiation doses increased, but adult eclosion from UV irradiated *T. euproctidis* pupae drastically decreased with duration of exposure to radiation. Although *T. euproctidis* pupae were more tolerant to both UV and gamma radiation, UV radiation was more effective than gamma radiation in the pupal stage.

Gamma and UV radiation did not influence either female emergence (F=5.190, df=6, P=0.001; F =1.357, df=5, P=0.275 for gamma and UV radiation, respectively) or male emergence (F=1.605, df=6, P=0.183; F=2.479, df=5, P=0.060 for gamma and UV radiation, respectively) when irradiated in the pupal stage.

Adult

The results of the effect of gamma and UV exposure

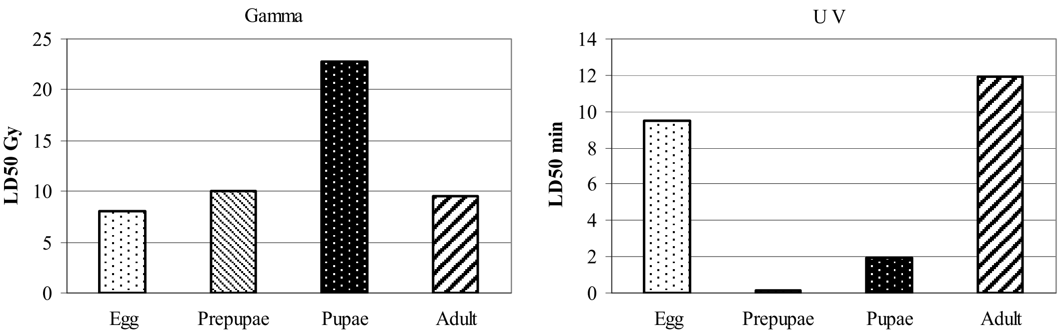

Fig. 5. LD_{50} values of gamma and UV radiated development stages of *T. euproctidis*.

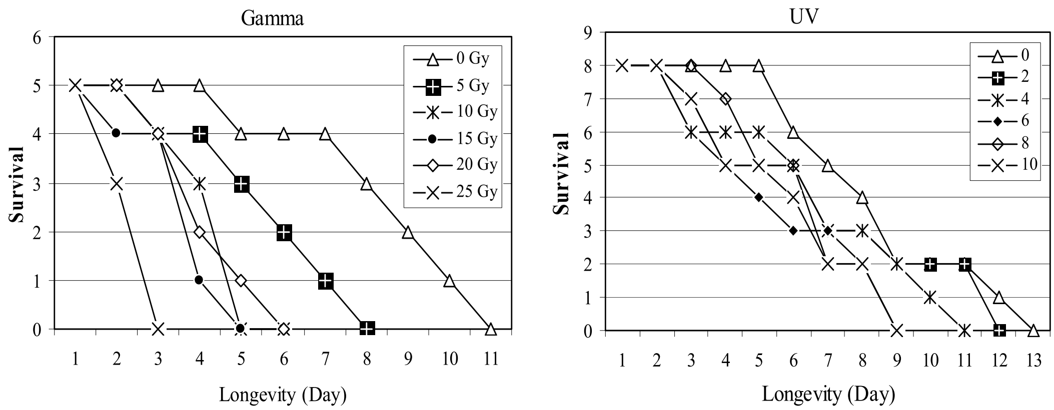

Fig. 6. Longevity of irradiated adult of *T. euproctidis* with gamma and UV radiation.

on adults are shown in Fig. 4. The mean parasitization (F=66.586, df=6, P<0.001; F =4.342, df=5, P=0.060 for gamma and UV radiation, respectively) and adult emergence (F=33.970, df=6, P<0.001; F =6.262, df=5, P=0.001 for gamma and UV radiation, respectively) decreased with both increased gamma and UV radiation doses. Although *T. euproctidis* adults were more tolerant to both UV and gamma radiation, the effect of gamma radiation on parasitization and adult emergence was more pronounced than UV radiation with the studied doses.

Gamma and UV radiation did not influence either female (F=53.551, df=6, P<0.001; F=1.616, df=5, P=0.194 for gamma and UV radiation, respectively) or male emergence (F=5.459, df=6, P=0.001; F =2.023, df=5, P=0.111 for gamma and UV radiation, respectively) when UV-treated females were compared to gamma radiation-treated females.

T. euproctidis adults were more resistant to UV radiation than gamma radiation. Only a few adults emerged from irradiated adults after 25 Gy and no adults emerged after 30 Gy of gamma radiation, but adult emergence was observed for all durations of exposure to UV. LD_{50} values for eggs, prepupae, pupae and adults exposed to gamma radiation were 8.1, 10.0, 22.7 and 9.5 Gy, respectively, and LD_{50} values for eggs, prepupae, pupae and adults exposed to UV radiation were 9.5, 0.12, 2.0 and 11.9 min, respectively. The comparison of LD_{50} doses of gamma and UV radiation for the developmental stages and adults of *T. euproctidis* showed that these values differed statistically. The pupae and adult stages were more radioresistant to gamma radiation and to UV radiation, respectively. The egg and prepupal stages were more sensitive to gamma radiation and to UV radiation, respectively.

Longevity

The longevity of *T. euproctidis* adults irradiated with gamma and UV radiation are shown in Fig. 5. Although the longevity of adults irradiated with UV radiation gradually decreased from 13 days to 9 days with increasing doses, it significantly decreased from 11 days to 3 days with increased doses of gamma radiation.

DISCUSSION

In the present study, gamma and ultra-violet radiation (UV) were used for *T. euproctidis* to enhance its parasitization and longevity. A search of databases for research on gamma and UV radiation and *T. euproctidis* did not yield any information on the minimum radiation doses for suppressing/stimulation. Therefore, we chose radiation doses arbitrarily to evaluate the effect of UV doses on the developmental stages of *T. euproctidis*. However, the striking difference in the observed effect could be attributed in part to the difference in the nature of the two types of radiation. The ability of UV to penetrate surfaces is very limited, and thus its effect is limited to surfaces, whereas gamma rays can penetrate deep into tissues. It is difficult to compare the effect of UV irradiation with the effect of gamma irradiation on *T. euproctidis* because of the difficulties involved in relating UV doses to gamma ray doses.

Our study showed that the effect of gamma radiation was more evident than UV radiation in the studied doses. An increase in exposure time to UV-rays caused a gradual decrease in the percentage of hatching eggs. The results obtained in our study may be correlated with the findings of Tilton and Brower (1983), who observed that the embryonic stage of an animal is a time of extreme radiosensitivity and insects are no exception. It was reported that an increase in exposure time to UV-rays caused a gradual decrease in the percentage of hatching eggs and no hatching occurred after 24 min of exposure in 2- and 3-day-old eggs of *T. confusum* (Faruki et al., 2007). Studies have been made on unlaid eggs (Hussain et al., 1994), on embryos produced parthenogenetically (Ahmed et al., 1976) and on embryos produced by fertilization (Ghomomu, 1989).

It was observed that the prepupae of *T. euproctidis* were more sensitive to UV rays than the eggs. The prepupae were unexpectedly most vulnerable to damage from UV radiation in comparison to gamma radiation. To our knowledge, this is the first report of UV radiation being more efficient than gamma radiation on the prepupal stage. The results obtained in our study may be correlated with the findings of Daimona et al. (2010) who observed that silkworm prepupae were highly sensitive to UV-B irradiation and their pupation rates dramatically decreased with the increase of radiation dose. Early research into the effects of irradiation on larvae was made using different storage pests. Larvae are more resistant to radiation than eggs and there is a variation in susceptibility between larval stages (Nair and Subramanyam, 1963; Yang and Ducoff, 1971; Allotey, 1985; Johnson and Patrick, 1988; Hasan et al., 1989).

Adult eclosion from irradiated *T. euproctidis* pupae gradually decreased as gamma radiation doses increased, however, adult eclosion from UV irradiated pupae drastically decreased with duration of exposure to radiation. *T. euproctidis* pupae were more tolerant to both UV and gamma radiation. In many ways, the pupal stage is analogous to the embryonic stage when intense cell division and differentiation occur. Therefore, it is not surprising that the overall pattern of pupal radiosensitivity is similar to the pattern exhibited by developing eggs (Tilton and Brower, 1983). Most studies of pupal radiosensitivity have not been precise enough, or at least not correlated with the morphogenetic changes occurring to reveal the fine details in the pattern of radiosensitivity. Irradiation can have several distinct effects on pupae, among which are pupal mortality, adult malformations and delayed adult mortality. The time sequence of mortality in pupae is very difficult to determine and their failure to emerge is usually used to assess overall pupal mortality. Working with wheat infested by the granary beetle, *Sitophilus granarius* (L.), Cornwell (1966) demonstrated a marked increase in the radiation resistance of the pupae compared with larvae.

Mean parasitization and adult emergence from irradiated females decreased with both increased gamma and UV radiation doses. Also, the effect of gamma radiation on parasitization and adult emergence was more pronounced than UV radiation. Adult insects are more radioresistant than the other stages in the developmental sequence (Tilton and Brower, 1983). In spite of many studies on the effects of radiation on insects, the specific mechanisms whereby the damage is produced and later manifested are still not well understood. Bhuiya et al. (1991) showed a dose-dependent reduction in adult emergence from the irradiated eggs, larvae and pupae of *Sitotroga cerealella* and *Oryzaephilus surinamensis*. Although it is difficult to make direct comparisons between gamma and UV radiation on their biological effects, further research will more precisely identify the wavelengths responsible for prepupal death or the stimulating effect and relationships between gamma and UV radiation in events.

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