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Effects of UV-Radiation on the Larvae of the Lesser Mealworm, *Alphitobius diaperinus* (Panzer)(Coleoptera: Tenebrionidae) and Their Progeny

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Abstract: The effect of UV-radiation on the larvae of the lesser mealworm, *Alphitobius diaperinus* (Panzer) was investigated for different exposure periods. Larval mortality, formation of pupae, adult emergence and deformities; growth of larvae, pupae and adults and reproductive potential of the developing progeny were recorded. The early instar larvae were more susceptible to UV-rays by increasing their mortality. UV-rays significantly inhibited development to pupae and adults, growth of larvae, pupae and adults. It was observed that irradiation produced deformities in different developmental stages. Fecundity and egg-fertility were also notably reduced following by irradiation.

Key words: UV-irradiation, *Alphitobius diaperinus*, mortality, pupal and adult recoveries, deformities, growth, fecundity, egg-fertility

INTRODUCTION

Fumigants and other chemical insecticides are widely used to protect stored commodities from insect infestation and contamination but their use leads to problem of undesirable residues^[1] and development of resistance in certain insect species^[2-4] demonstrated the acceptability of irradiation technology as an alternative treatment for food protection^[7]. Moreover, the improper use of insecticides especially in developing countries and its concomitant impact on environment, has necessitated exploration of alternative non-toxic pest control methods. Irradiation becomes an established technique for controlling stored grain insects because of residue free advantages over chemical fumigation^[8]. The feasibility of Ultra Violet (UV) rays as pest control agent was investigated for this purpose.

The Ultra Violet (UV) portion of the spectrum is widely used as germicide and as an attractant for insects^[9], in embryological-physiological studies^[10] and for the surface disinfection of insect eggs from pathogens^[11]. Islam *et al.*^[12] stated that UV-rays are generally less harmful to living organisms than the ionizing radiations as they penetrate only the surface layer of cells. However, a number of investigators have considered the possibility of using UV-rays to control or at least suppress development of various species of stored product insects^[13-16].

Guerra *et al.*^[11] reported the dose-mortality response of eggs of the tobacco budworm, *Heliothis virescens* (F.) and the bollworm, *H. zea* (Boddie) to short wave lengths

of UV. Dipterous eggs are sensitive to UV irradiation and Beard^[17] showed the variability among strains of the house fly, *Musca domestica* L. Wharton^[18] reported that UV irradiation (254 nm) killed nymphs of the American cockroach, *Periplaneta americana*.

UV radiation has not been exploited extensively as an agent for controlling stored products insects population. Keeping in view the importance of the problem and feasibility of this technique we decided to conduct the present investigation on the control of lesser mealworm, *Alphitobius diaperinus* using UV rays.

MATERIALS AND METHODS

Throughout the experiment *A. diaperinus* was maintained on a food medium: a mixture of whole wheat flour, corn flour and brewer's yeast blended thoroughly at the ratio of 10:10:1.5, respectively. A 15 W germicidal lamp, GE15T8 measuring 20x4 cm was the source of UV radiation, emitting at a wavelength of 254 nm. For irradiation the test insects were kept in 15 cm diameter petri dishes placed on the surface apart 12 cm from the lamp. Second and 3rd instar larvae were collected from the food medium, kept in separate petri dishes (15 cm diameter) and irradiated with UV-rays. The experiment was set up with two batches: the first batch was for determining the larval mortality, progeny formation and their deformities, the exposure period was 2, 4, 8 and 16 min and the second batch was for recording growth and reproductive potential, the exposure period was 1, 2 and 4 min. After irradiation the same amount of standard food

was added in each petridish containing larvae. The food was renewed after every 7-days to avoid conditioning. For the first batch each treatment was replicated thrice with 50 larvae each. The same number of non-irradiated insects of each instar were raised simultaneously on the same food medium as controls. Larval mortality was assessed at 3- and 10-day post-irradiation and up to pupation. After pupation, insects were collected and pupal recovery (%) was recorded. Pupae were then kept in petri dishes for adult eclosion. The number of adults that emerged was counted and their recovery (%) was also noted. Any deformities at larval, pupal and adult stages were carefully observed.

Experiments for determining growth and reproductive potential of *A. diaperinus* all the exposure periods i.e. 1, 2 and 4 min including controls (for each instar) were replicated five times with 100 larvae each. Surviving healthy larvae of 45- and 60-day old were individually weighed on an electronic balance (OSK, fx 300) in milligram. Freshly formed pupae and adults were weighed individually according to sex on the second day of their formation. Fifty observations were taken randomly from each exposure period for determining larval growth and forty for pupal and adult growth. Twenty freshly enclosed beetles of opposite sexes from each exposure period were allowed to mate and the females were observed for oviposition. Each pair of beetles was kept in a glass vial (25x50 mm) containing standard food covered with cotton. Eggs from each pair were collected every 3-days of intervals for a period of 30-days and their number counted and kept in petri dishes for observing fertility. The food was also changed during the time of egg collection.

All the experiments were conducted in an incubator at a temperature of 30±1°C without light or humidity control.

Mortality data were corrected by Abbott's^[19] formula and then subjected to probit analysis following the methods in Busvine^[20]. The data on pupal and adult recoveries (%); growth of larvae, pupae and adults and fecundity and egg-fertility (%) were subjected to analysis of variance. The Percentages of Reproduction Control (PRC) were calculated using the formula of Rizvi *et al.*^[21] as: $PRC = (V_1 - V_2) / V_1 \times 100$, where, V_1 = mean fecundity and egg-fertility of non-irradiated group and V_2 = mean fecundity and egg-fertility of irradiated group. A comparison test was done between control and doses by Duncan's^[22] Multiple Range Test and Tukey's Multiple Comparison procedure^[23].

RESULTS

Effect on larval mortality and their progenies: It has been found that the time required for 50% kill (LT_{50}) was gradually increased as the larval age increased and the

duration of exposure decreased (Table 1). From the χ^2 -test it was observed that early stage larvae (2nd instar) were more sensitive to UV-rays at any level of mortality count than 3rd instar larvae. UV-irradiation significantly reduced pupation in both the instars ($F=16.47$ and $F=50.50$, $p<0.01$ for exposure periods and instars, respectively). A similar result was recorded in adult emergence ($F=16.24$ and $F=40.06$, $p<0.01$ respectively for exposure periods and instars). It has been found that the rate of pupation and adult emergence was appreciably reduced for all exposure periods in both instars in comparison to non-irradiated controls (Table 2). This reduction increased as the exposure period increased. It has also been noted that no adults enclosed at 16 min UV-rays in 2nd instar. In the present experiment deformity was observed at different developmental stages and recorded (Table 2). A significant number of larval and adult deformities were recorded at 8 and 16 min in both the instars of *A. diaperinus*. However, deformity was observed at controls.

Effect on growth and reproductive potential: UV radiation produced deleterious effects on larval weight ($F=17.41$ and $F=25.25$, $p<0.05$ for 45- and 60-day old larvae respectively) but no significant differences between the instars (Table 3). It has been observed that in both the age groups of each instar the weight was almost halved at 4 min as compared to controls. Analysis of variance showed that UV rays caused decreased weight of male pupae ($F=84.10$, $p<0.01$) and female adults ($F=11.15$, $p<0.05$) (Table 3) whereas no significant effect on female

Table 1: Lethal time for UV-radiation against larvae of *A. diaperinus*

Level of mortality count	Larval instars	LT_{50} (min)	Regression equation	χ^2 -value (df)
72 h	2nd	23.95	$3.07+1.40x$	8.57 (2)
	3rd	215.34	$2.22+1.19x$	2.55 (2)
10-day	2nd	12.58	$2.90+1.91x$	24.03 (2)
	3rd	29.29	$2.51+1.70x$	0.96 (2)
Up to pupation	2nd	3.60	$3.63+2.45x$	9.05 (2)
	3rd	9.74	$3.05+1.98x$	20.17 (2)

Table 2: Formation of various developmental stages and their deformities in *A. diaperinus* obtaining from UV-irradiated larvae (N=150)

Larval instars	Exposure time (min)	% recoveries		% deformities		
		Pupa	Adult	Larva	Pupa	Adult
2nd	0 (Control)	64.67	64.00	00a	00a	00a
	2	16.67	15.33	0.67a	0.67b	0.67a
	4	13.33	13.33	2.67ab	0.67b	1.33a
	8	4.67	4.00	5.33cd	00a	3.33b
	16	0.67	0.00	00a	00a	00a
3rd	0 (Control)	68.67	67.33	00a	00a	00a
	2	33.33	32.67	00a	00a	00a
	4	31.33	29.33	0.67a	0.67b	1.33a
	8	26.67	24.00	3.33bc	00a	3.33b
	16	9.33	6.67	7.33d	1.33c	5.33c

Figures in each column followed by the same letter(s) are not significantly different at $p<0.01$ (DMRT)

Table 3: Mean weight (mg±SE) of various developmental stages of *A. diaperinus* followed by UV-irradiation

Larval instars	Exposure time (min)	Larval weight (N = 50)		Pupal weight (N = 40)		Adult weight (N = 40)	
		45-day-old	60-day-old	Male	Female	Male	Female
2nd	0 (Control)	1.60±0.07a	5.02±0.20a	14.25±0.35a	16.53±0.45a	10.95±0.34ab	15.30±0.55a
	1	1.50±0.05ab	3.18±0.14cd	12.55±0.37b	14.85±0.39ab	10.83±0.36ab	13.35±0.41ab
	2	1.48±0.05ab	3.24±0.14cd	12.43±0.38bc	12.98±0.44b	10.83±0.33ab	12.07±0.50b
	4	0.72±0.03c	2.62±0.13d	11.98±0.37c	12.55±0.42b	9.95±0.34b	11.98±0.33b
3rd	0 (Control)	1.68±0.05a	5.32±0.15a	13.95±0.31a	15.73±0.56a	13.30±0.49a	15.32±0.74a
	1	1.48±0.04ab	4.04±0.14bc	12.43±0.38bc	15.50±0.54a	11.05±0.27ab	15.22±0.64a
	2	1.16±0.03bc	3.18±0.13cd	12.23±0.32bc	17.78±0.40a	10.64±0.39ab	13.53±0.43ab
	4	0.80±0.03c	2.52±0.12d	12.17±0.34bc	16.65±0.38a	9.96±0.43b	12.94±0.55b

Means in each column followed by the same letter(s) are not significantly different ($p < 0.05$) by Tukey's multiple comparison procedure

Table 4: Mean±SE of reproductive potential of *A. diaperinus* females obtaining from UV-irradiated larvae

Larval instars	Exposure time (min)	Fecundity	PRC	Egg-fertility (%)	PRC
2nd	0 (Control)	74.65±4.44a	-	90.98±0.97a	-
	1	56.00±3.51b	24.98	82.04±1.25b	9.83
	2	52.41±5.06b	29.79	81.91±1.25b	9.97
	4	32.88±3.05cd	55.95	70.40±2.20d	22.62
3rd	0 (Control)	78.05±5.16a	-	90.60±0.42a	-
	1	46.06±3.08bc	40.99	79.71±1.29bc	12.02
	2	42.35±3.88c	45.74	76.75±1.66b-d	15.29
	4	23.50±2.19e	69.89	72.60±1.60cd	19.87

PRC = Percent Reproduction Control; Means in each column followed by the same letter(s) are not significantly different ($p < 0.05$) by Tukey's Multiple Comparison Procedure

pupae and male adults. This peculiar effect of UV rays on pupae and adults of *A. diaperinus* needs further investigation. In the present experiment it has been observed that female pupae developing from 3rd instar were comparatively heavier at 2 and 4 min than control group.

For all exposure periods UV-irradiation significantly reduced the production of eggs ($F = 35.98$, $p < 0.01$) and egg-fertility ($F = 27.01$, $p < 0.05$) (Table 4). It was observed that fecundity and fertility gradually reduced with increased exposure period. It was also observed that the subsequent egg production in female progeny was halved at 4 min in 2nd instars and $\frac{1}{3}$ in 3rd instars in comparison to controls.

DISCUSSION

The present findings showed that the early larval stages were more sensitive to UV-radiation than older ones which corroborate with the findings of Qureshi *et al.*^[24] who reported that larval sensitivity depended on age at treatment i.e. older larvae were less sensitive than the younger ones. In the present test, the mortality of the irradiated larvae was directly proportional to the dose of radiation i.e. mortality gradually increased with the increasing of doses. This findings agreed with the results of Faruki and Khan^[15] working with *Cadra cautella* using UV-rays mentioned that larval mortality was positively correlated with radiation doses. UV-rays significantly lowered the production of progeny in

A. diaperinus which correlates with the findings of Beard^[25] who reported adult emergence inhibited progressively by higher dosages when late stage larvae of the Indian meal moth, *Plodia interpunctella* were irradiated with UV rays. Kumar *et al.*^[26] and Hasan *et al.*^[27] also reported a significant reduction in adult emergence from gamma- and UV-irradiated pupae of *Exorista sorbillans*, respectively. Besides, UV rays produced higher numbers of larval and adult deformities at long exposure periods. It was found that some of the larvae failed to shed their exuviae during molting results lower number of progeny. Larval-pupal and pupal-adult intermediate forms were common at each exposure period showing various morphological deformed characters. The larval-pupal intermediate forms had pupal head with larval body and pupa with larval skin. The pupal-adult intermediate forms had adult head with pupal abdomen and small pupa that unable to emerge as adult. Some of the adults that emerged from treated larvae had incomplete elytra, widely spreaded and crumpled wings, small winged and short abdomen. Burges and Bennett^[28,29] observed when 4th instar larvae and pupae of the alfalfa weevils irradiated with gamma rays emerged adults had abnormal antennae, legs and elytra. In this present investigation, it was observed that UV-treatment did not influence the sex ratio of the test insect despite sex related effects.

From the present investigation it is evident that the production of fewer progeny by irradiation as compared to non-irradiated progeny shows potential for using UV rays in controlling pests of stored commodities. Furthermore, a significant reduction in reproductive abilities in females of *A. diaperinus* from different larval instars irradiated with UV rays was observed. The lower fecundity in females developed from early age larvae treated at higher exposure period as well as lower proportion of fertile eggs than in females developed from late age and lower exposure period indicates that younger stage is more sensitive to radiation than older stage and the effect is dose-dependent. These results support the findings of Hasan *et al.*^[27] who observed reduced fecundity and fertility in adults of *E. sorbillans*

developing from UV irradiated pupae. The reduced fecundity and fertility of adults indicates the disturbances in oogenesis and spermatogenesis. To discuss the reduced fecundity in *E. sorbillans*, Hasan *et al.*^[27] stated that reduction may probably occur due to both reduced transfer of active sperms by irradiated males to females and limited production of oocytes in the irradiated females. Knipling^[30] suggested that reduced fertility resulting from chromosomal translocations and other effects of ionizing radiation could be used to control insects of economic importance.

However, the present investigation reveals that UV-radiation is promising and effective for the control of the lesser mealworm, *A. diaperinus* either in grain storage or poultry farms. Thus, this radiation can be used with no toxic residue created for suppressing the population of certain insect pests in grains or grain products has special appeal where avoidance of the use of toxic insecticides is advisable.

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