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ADULT TIME-MORTALITY RESPONSE AND CHANGES IN REPRODUCTIVE ATTRIBUTES IN *CALLOSOBRUCHUS MACULATUS* (FAB.) FOLLOWING UV IRRADIATION

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Abstract: Using 254nm UV radiations of exposure periods from 2 to 16 min against adults. Time mortality response of the cowpea weevil, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae), adults and alterations in vital reproductive attributes as fecundity, immature duration, adult emergence and adult longevity of the beetles from parental through F_1 generation have been estimated. Time mortality response of the adults of the cowpea weevil *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae), and alternations in vital reproductive attributes is fecundity, immature duration, adult emergence and longevity from parental through F_1 generation using 254nm UV radiations of response periods from 2-16min have been estimated. Results show that an exposure time to kill 50% of the adults is around 20 min (LT_{50} = 19.99min), and irradiation significantly reduced egg-laying ($P<0.001$), lengthened immature durations (*i.e.* larval and pupal developmental periods; $P<0.05$), decreased adult emergences as well as longevity in both sexes ($P<0.001$) in the parental generation. The effects of the UV-rays on F_1 progenies, however, were less pronounced than that expressed in the parental generation, but the immature duration was significantly increased ($P<0.01$) but the longevity in both sexes was significantly reduced ($P<0.001$). Relevance of this study in relation to prospective phytosanitary treatments of the stored pulses with UV-rays has been discussed.

Key words: UV irradiation, *Callosobruchus maculatus*, time-mortality response, reproductive attributes, stored pulses

mvivsk: Wv‡ji †cvKv, *Callosobruchus maculatus*-Gi cYeq¯‹ `k‡K 254 b¨v‡bwgUvi Zi½‰`‡N¨i AwZ‡e¸bx i‡k‡i 2 †_‡K 16 wgwbU chš c‡qvM K‡i Zv‡`i mgq gZ¨nvi mvov Ges ¸i“ZcY cRbb ^e‡kó¨ †hgb, wWgcvov, Acvßeq¯‹‡`i (jvfv I wcDcv `kvi) ¯vqxZ, cYeq‡¯‹i Awefe Ges Avq®vj wcZv-gvZvi esk †_‡K $F_1$ eskai chš wbaviY Kiv nq| †`Lv hvq, AwZ‡e¸bx i‡k‡i cvq 20 wgwbU (LT_{50} = 19.99min) c‡qv‡Mi d‡j 50 kZvsk cYeq¯‹ †cvKvi gZ¨ N‡U Ges AwZ‡e¸bx i‡k‡i cfv‡e Zv‡`i wWgcvov ZvrchcYfv‡e K‡g hvq ($P<0.001$)| ZvQvov Acvßeq¯‹‡`i ciùU‡bi ¯vqxZ `xNwqZ nq ($P<0.05$), cYeq¯‹‡`i Awefve I Zv‡`i Avq®vjI Zvrchfv‡e K‡g hvq ($P<0.001$)| wcZv-gvZvi esk A‡c¶v $F_1$ eskai‡`i g‡a¨ AwZ‡e¸bx i‡k‡i cfve Dc‡iv³ ^e‡kó¨¸wji †¶‡Î Zjbvgjkfv‡e Kg j¶¨ Kiv †M‡jI Acvßeq¯‹‡`i ¯vqxZ I cYeq¯‹‡`i Avq®vj ZvrchcYfv‡e K‡g ($P<0.001$)| GB wbe‡Ü AwZ‡e¸bx i‡k‡i e¨envi‡i gva¨‡g ¸`vgRvZ Wv‡ji msi¶Y wel‡q eZgvb M‡elYvi cvmw½KZv Av‡jvKcvZ Kiv n‡q‡Q|

Introduction

Owing to an alarming threat to the environment and its biota, accompanied by increasing treatment costs and development of resistant strains among the pest population, researchers now-a-days are emphasizing on such alternative approaches as irradiation, phytochemicals, biopesticides, insect hormones and natural enemies instead of traditional and synthetic chemical pesticides (Follett *et al*. 2007; Begum *et al*. 2009). These approaches are destined to be non-hazardous to human health, eco-friendly, less expensive, and more importantly, are more specific to the target pest population. Ionizing radiations like x-rays and gamma rays (Islam and Laz 2001; Follett 2006, Follett *et al*. 2007; Tandon *et al*. 2009), and non-ionizing radiations like ultraviolet (UV) rays (Islam *et al*. 1992; Faruki *et al*. 2005, 2007; Begum *et al*. 2007, 2009) and microwave radiation (Zhao *et al*. 2007; Gasemzadeh *et al*. 2010) have been employed to limit reproduction and survival of a variety of insect pest species. Interest in the use of irradiation as a phytosanitary treatment for agricultural commodities is growing worldwide, particularly since publication of the International Plant Protection Convention (IPPC) standard that endorses and facilitates trade based on this disinfestation method (Follett *et al*. 2007; Gasemzadeh *et al*. 2010).

UV-rays are electromagnetic radiation with wavelengths shorter than that of visible light, but longer than x-rays or gamma rays, in the range of 10 to 400nm (Harm 1980) that include three main sub-types (Diffey 1991): UV-A or black light (315-400nm), UV-B or medium wave (280-315nm), and UV-C or germicidal or short wave (100-280nm). The uses of UV irradiation, however, as attractant for the pest insects (Bruce 1975), as germicide (Allen 2001), and as suppressor of immature development and adult emergence in Coleoptera (Calderon *et al*. 1985; Sharma and Dwevedi 1997; Faruki *et al*. 2005, 2007; Begum *et al*. 2007, 2009), in Diptera (Beard 1970; Krishna and Srivastava 1991; Hasan *et al*. 1998; Khan *et al*. 2006) and in Lepidoptera (Calderon and Navarro 1971; Faruki and Khan 1993; Faruki *et al*. 2007) are quite encouraging from pest management point of view.

Being a major pest of cowpeas (*Vigna unguiculata* Walp.), black grams (*V. mungo* Hepper) and other grain legumes, the spotted cowpea weevil, *Callosobruchus maculatus* (Fabricius) is an important pest of pulses in Africa and tropical Asia both in field crops and in stores (Gorham 1987; Hill 1990). The pest causes damage only at immature stages because the adults normally do not feed in the granaries (Fox *et al*. 2004). In an elaborate study with *C. maculatus* and *C. chinensis*, Mustari (2007) compared efficacies of two wavelengths (254 and 366nm) of UV-rays on pupae and adults, where 254nm were found to be much detrimental on adults in both species of the beetle. In the present report, effects of 254nm UV-rays for 2 to 16 min on newly emerged adults, and their consequences on adult mortality, fecundity, immature duration and adult longevity in parents and in the next generation offspring of *C. maculatus* have been assessed.

Materials and Methods

Mass rearing of the experimental insects: C. maculatus infested black gram seeds (*V. mungo*) were procured from wholesale markets of Katakhali, Lashmipur and Shaheb Bazar, Rajshahi, and brought to laboratory for mass rearing. The seeds were kept in 500ml beakers, mouths were covered with coarse cloth and tied with rubber bands. The beakers were housed in an incubator at 28±0.5ºC without light and relative humidity control. After every 10 days fresh seeds, disinfected and sterilized at 60ºC in an incubator for 24h to destroy any previously laid eggs or immature stages, were added to the beakers. In order to eliminate natural and/or deleterious mutations that might have accumulated in their genome over time, the beetles were inbred for two successive generations prior to setting up the experiments.

Estimation of UV-induced adult time-mortality response: A 15W germicidal lamp (GE1578) that emitted a wavelength of 254nm (1nm=1×10^{-9}m) and installed at the Genetics and Molecular Biology Laboratory, Department of Zoology, Rajshahi University, was used as a source of UV radiation. Time-mortality response tests were conducted with newly emerged adults at a series of irradiation exposure periods *viz*., 2, 4, 8, 12 and 16min. For irradiation, 100 anaesthetized or chilled 1-3 day-old adults (1:1 sex-ratio) were kept in 15cm diameter Petri dishes, placed on table surface 12cm below the lamp and were exposed to UV-rays for estimating their respective mortalities at 24h interval up to 72h post-irradiation. The same number of non-irradiated insects was maintained as controls. The experiments were conducted in the laboratory at an ambient temperature (25±2°C) and normal daylight between 10:00 and 13:00hrs.

Estimation of reproductive attributes: Four reproductive attributes that constitute vital parts in the life-history of an insect *viz*., fecundity (48h egg-laying), immature duration total number of adults emerged, and longevity of both male and female adults, were considered for assessing UV-induced changes in the experimental beetles. In parental generation, 20 replicates for each treated line (T♀♀×T♂♂) and 10 replicates for the control (C♀♀×C♂♂) were maintained. *C. maculatus* that emerged from the parental generation were not further subjected to UV-rays, but they were continued for the F_1 generation as follows: 5 replicates for each of the treated and control lines were maintained; and apart from the control and treated groups, two reciprocal crosses *viz*., T♀♀×C♂♂ and C♀♀×T♂♂ were also used for evaluating the reproductive attributes in the progenies. All the experiments were conducted in an incubator at 28±0.5º C without light and relative humidity control.

Statistical analyses: UV-induced time-mortality response data were subjected to Probit analysis (Finney 1964) using a *GWBASIC* software for estimating an LT_{50} value i.e the median lethal time to kill 50% of the treated adults. Then a regression line was plotted based on log time and Probit mortality. Data on reproductive attributes recorded for the parental and F_1 generations were subjected to one-way analysis of variance (ANOVA), followed by least significant difference (LSD) tests for comparisons between the control and/or treatment groups. A statistical package (SPSS version 11.5 for Windows) was used for analyzing the data.

Results and Discussion

UV-induced time-mortality response in adults: Compared to the control, 72h post irradiation adult mortality of *C. maculates* increased from 10% at 2min exposure period to 50% at 16min, thus yielding an estimated LT_{50} value of 19.99min (Fig. 1) and it was positively significant (r^2=0.93, y=1.48x+3.04). This result suggests that adult mortality in *C. maculatus* due to UV irradiation, perhaps coupled with inherited sterility, might contribute substantially to the suppression of the pest under laboratory as well as storage conditions.

Effects on fecundity: A significant reduction in egg-laying occurred ($F_{5,104}$ = 4.270; P<0.001) in the parental generation (Table 1) following UV-treatment from 2min (76.20±19.46) to 16min (67.60±6.61) in comparison with the control (80.00±11.81). In the F_1 generation (Table 2), however, decrease in fecundity was not significant ($F_{3,76}$ = 0.437; P>0.05), but oviposition in C♀♀×T♂♂ lines was always less than that in corresponding T♀♀×C♂♂ and C♀♀×T♂♂ lines, indicating that males of *C. maculatus* are more sensitive to UV radiations than their female counterparts.

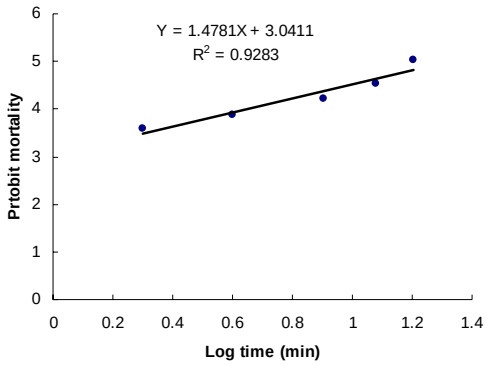

Fig. 1. Adult time-mortality response in *C. maculatus* following UV irradiation treatments.

Effects on immature duration: The duration of the immature stages (larval and pupal periods) in *C. maculatus* was found to increase significantly both in the parental ($F_{5,104}$ = 2.485; P<0.05) and F_1 generation ($F_{3,76}$ = 4.204; P<0.01), showing that UV rays lengthened the immature developmental period up to 4 days (Tables 1 and 2).

Effects on adult emergence: The number of *C. maculatus* adults emerged from the treated lines in parental generation (Table 1) was gradually and significantly reduced than that in the control lines ($F_{5,104}$ = 10.152; P<0.001). But this trend was not maintained in the F_1 generation ($F_{3,76}$ = 1.957; P>0.05). While the control had an adult emergence of 57.20±10.26, UV irradiation reduced the trait to 28.20±3.59 at 16min exposure in the parental generation (Table 1), and to 34.18±5.98 in F_1 generation (Table 2).

Effects on adult longevity: Longevities in both the males ($F_{5,104}$ = 11.197; P<0.001) and females ($F_{5,104}$ = 10.34; P<0.001) in UV-treated lines of *C. maculatus* were significantly reduced from about 12 days in the controls to around 8 days in the irradiated female and male beetles in both the parental and F_1 generations (Tables 1 and 2).

Treatment effects: Results presented in Tables 1 and 2 clearly demonstrate that 254nm UV-rays reduced egg-laying, lengthened immature developmental period, decreased adult emergence as well as their longevity in both sexes of *C. maculatus* in an exposure time-dependent manner.

Crossing effects: Compared to the controls (C♀♀×C♂♂), treated (T♀♀×T♂♂) and reciprocal crosses (T♀♀×C♂♂ and C♀♀×T♂♂) among the F_1 progenies showed only significant lengthening of immature stages and reduction in adult longevity in both the sexes (Table 2), but unlike parental generation, fecundity and adult emergence were not adversely affected.

Generation effects: Comparison of reproductive attributes between the parental and F_1 generations revealed that UV irradiation in the experimental beetles induced pronounced changes in all life-history parameters in parental generation than those recorded in F_1. This is suggestive of a diminishing effect of the UV rays on the reproductive attributes of the offspring of the treated grandparents.

Recent uses of gamma irradiation against a number of stored product and other pests (Lester *et al.* 2003; Hallman 2004; Follett and Armstrong 2004; Follett 2006; Follett and Neven 2006; Follett *et al.* 2007; Tandon *et al.* 2009)

demonstrate that unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live but sterile and/or not viable insects may occur with the exported commodities. Experiments with irradiation other than gamma, for example, microwave radiation and UV rays, for the management of coleopteran pests have shown to be very promising. Zhao *et al*. (2007) and Gasemzadeh *et al*. (2010) used microwave irradiation to induce higher mortality and increased developmental stages in the flour beetle *Tribolium castaneum* and the rice weevil *Sitophilus oryzae*. Calderon *et al*. (1985) reported that the egg-hatching in *T. castaneum* was negatively affected by UV radiation, whereas Sharma and Dwevedi (1997) observed adverse influences of UV-rays on the egg-to-adult development of the pulse beetle *C. chinensis*. Hoque and Islam (1999) and Islam and Kabir (2000) integrated UV radiation with cytoplasmic incompatibility to suppress population growth in *T. castaneum*, while Faruki *et al*. (2007) noted that UV treatment in the same insect decreased egg-hatching and reduced adult emergence. The growth and development in the lesser mealworm *Alphitobius diaperinus* has been shown to be manipulated by UV treatments on eggs, larvae, pupae and adults by a number of workers (Parween *et al.* 2004; Faruki *et al.* 2005; Begum *et al.* 2007). These results nicely corroborate with the findings of the present study.

The present study thus clearly demonstrated the UV irradiated adult time-mortality response, followed by UV radiation-induced alterations in various reproductive parameters in parental through F_1 generation in *C. maculatus*.

Table 1. Effects of adult irradiation with 254nm UV-rays on some reproductive attributes in *C. maculatus* (Parental generation)

Exposure period[1] (♀♀×♂♂)	Fecundity[2]	Immature duration (days)	No. adult emergence	Adult longevity (days)	
				Females	Males
0 (C×C)	80.00±11.81[a]	27.98±2.61[a]	57.20±10.66[a]	12.60±1.14[a]	12.00±1.58[a]
2 (T×T)	76.20±19.46[a]	30.32±0.44[a]	55.20±12.35[a]	7.80±1.30[b]	6.80±0.84[b]
4 (T×T)	78.80±6.46[a]	30.66±0.51[b]	46.80±9.04[b]	9.20±0.84[c]	8.40±1.14[c]
8 (T×T)	67.80±12.15[b]	30.60±0.82[b]	37.60±11.44[b]	8.60±1.14[c]	9.00±1.58[c]
12 (T×T)	73.20±3.42[a]	30.76±0.56[b]	34.40±5.94[b]	8.40±1.14[c]	8.00±0.71[c]
16 (T×T)	67.60±6.61[b]	32.16±1.39[b]	28.20±3.59[c]	8.20±1.30[c]	8.20±0.84[c]
F-ratios	4.28***	2.48*	10.05***	11.20***	10.34***

[1]in min; [2]48-h oviposition; C=control; T-treated; Values are mean±SD of 10 replicates for control and 20 replicates for each treatment (N=110); Dissimilar superscripts indicate significant difference by LSD tests at P<0.05; *=P<0.05; ***=P<0.001.

Table 2. Effects of adult irradiation with 254nm UV-rays on some reproductive attributes in *C. maculatus* (F_1 generation)

Exposure period[1] (♀♀×♂♂)	Fecundity[2]	Immature duration (days)	No. adult emergence	Adult longevity (days)	
				Females	Males
0 (C×C)	76.40±9.56	28.74±0.56	52.80±10.64	12.80±1.30	12.80±1.48
2 (T×T)	74.40±17.38[a]	29.96±1.80[a]	48.87±19.50[a]	10.07±1.39[a]	10.13±1.55[a]
2 (T×C)	75.11±10.96[a]	28.98±1.24[a]	48.18±11.01[a]	10.98±0.86[b]	9.00±1.87[b]
2 (C×T)	70.09±7.18[b]	29.13±0.78[a]	47.93±8.12[a]	10.01±0.12[a]	9.00±1.87[b]
4 (T×T)	66.53±10.50[a]	30.35±0.45[a]	38.87±8.68[a]	9.13±1.25[a]	8.67±1.54[a]
4 (T×C)	64.37±8.11[a]	28.15±1.33[b]	39.17±9.12[a]	9.54±0.98[a]	9.00±1.12[b]
4 (C×T)	60.18±5.19[b]	29.84±0.96[a]	40.01±6.18[b]	9.76±2.01[b]	9.12±0.86[b]
8 (T×T)	68.07±11.57[a]	30.85±0.66[a]	37.07±7.20[a]	9.80±1.08[a]	9.93±1.28[a]
8 (T×C)	66.13±12.59[a]	28.98±1.72[b]	36.84±8.17[a]	9.52±2.11[b]	9.37±1.14[b]
8 (C×T)	62.14±8.12[b]	29.94±0.55[a]	36.11±9.12[a]	8.98±1.11[c]	9.18±1.10[c]
12 (T×T)	78.40±7.05[a]	32.15±1.55[a]	33.87±11.29[a]	8.73±1.49[a]	9.93±1.53[a]
12 (T×C)	76.98±8.11[a]	30.12±0.88[b]	31.36±8.12[b]	9.08±1.55[b]	9.11±0.78[b]
12 (C×T)	60.88±5.13[b]	32.08±0.97[a]	32.57±10.11[a]	8.22±0.98[a]	8.98±2.01[c]
16 (T×T)	64.27±10.88[a]	33.23±0.94[a]	36.13±10.26[a]	8.93±1.91[a]	9.13±1.77[a]
16 (T×C)	63.15±7.85[a]	31.92±0.68[b]	35.42±8.17[a]	9.01±0.87[a]	9.18±1.33[a]
16 (C×T)	62.14±8.37[a]	32.18±1.68[a]	34.18±5.98[b]	8.00±2.12[b]	8.88±2.42[b]
F-ratios	0.44[ns]	4.20**	1.96[ns]	10.41***	15.58***

[1]in min; [2]48-h oviposition; C=control; T-treated; Values are mean±SD of 5 replicates for control and each treatment (N=80). Dissimilar superscripts within exposure periods indicate significant difference by LSD tests at P<0.05; ns= not significant; **=P<0.01; ***=P<0.001.

The present estimated LT_{50} value suggests that half of the adult beetles in the infested pulse seeds could be killed with 254nm UV treatment for 20min, and reduced longevity of the remaining half, perhaps coupled with inherited sterility, might contribute to reduction in the number of the next generation offspring as shown by the data for F_1 generation. In agreement with Sharma and Dwevedi (1997) for *C. chinensis*, Faruki *et al*. (2005) for *A. diaperinus* and Faruki *et al.* (2007) for *T. castaneum* and *T. confusum*, fecundity, iimature developmental

stages and adult emergence in *C. maculatus* were significantly reduced by 254nm UV treatments. In addition, as demonstrated by Begum *et al*. (2007; 2009) for *A. diaperinus*, the present results on lengthened immature duration and reduced number of adult emergence from UV-treated lines are obvious signs of population suppression in *C. maculatus* under study.

The most likely explanation of the UV irradiation effects on the adult experimental insects is that UV-C at 254nm causes adjacent thymine (T) molecules of the DNA strands to dimerize, and further accumulation of these defects inhibits DNA replication, thereby rendering its harmful impacts on the exposed organism (Allen 2001). Adult mortality and reduced longevity in the UV irradiated insects might result from structural changes in the haemolymph as well as reduction in the total haemolymph count as demonstrated in the flesh fly *Parasarcophaga ruficornis* by Krishna and Srivastava (1991). It may be concluded that the present results are very much encouraging in view of designing an effective phytosanitary treatment protocol against *C. maculatus* that inflicts a considerable damage to a number of stored pulses in many tropical and sub-tropical countries including Bangladesh.

Conclusion

This study clearly demonstrated UV irradiation induced adult mortality and alterations in various reproductive parameters in parental through F_1 generation in *C. maculatus*. Irradiation significantly reduced egg-laying, lengthened immature durations, decreased adult emergences as well as longevity in both sexes. In relation to prospective phytosanitary treatments of the stored pulses with UV-rays, the present findings are promising.

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