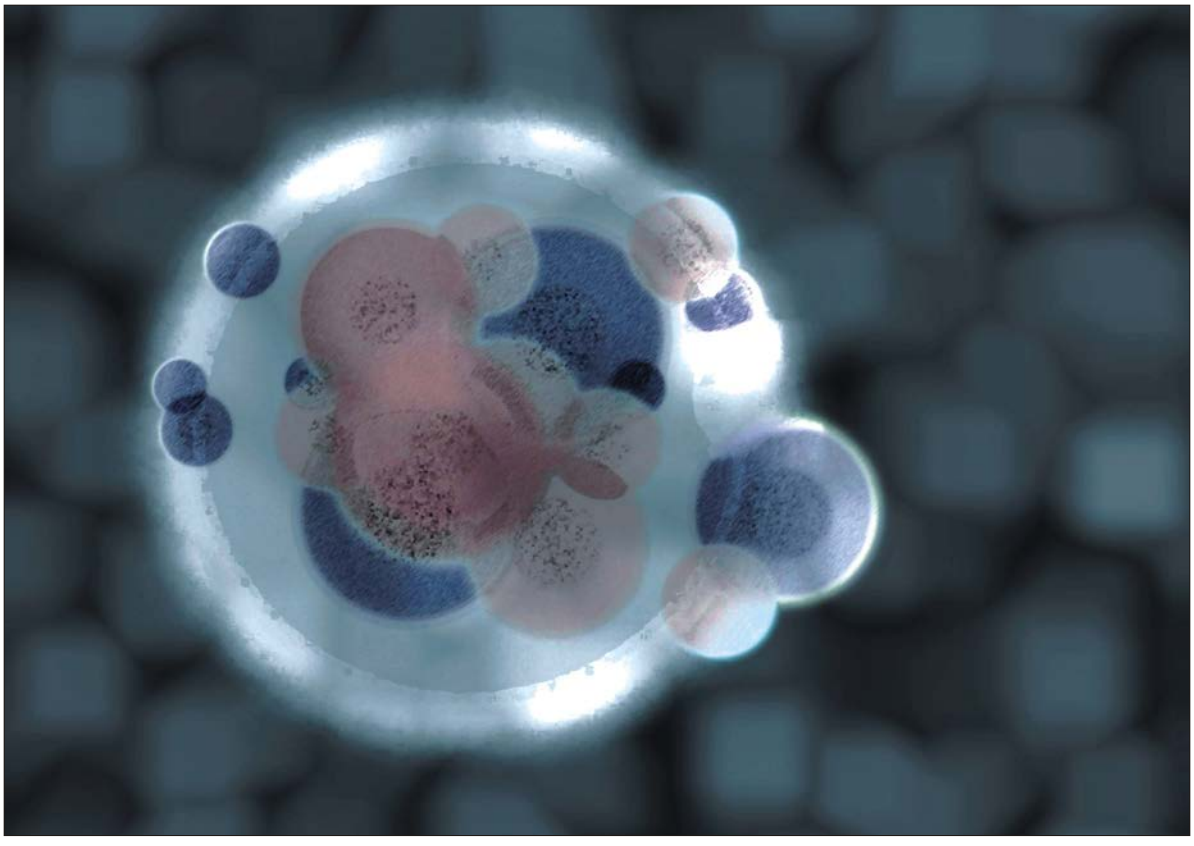

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Stress Response of Different Exposure Time by UV 254 on the Biology and Body Total Protein and Genomic DNA Content of Red Cotton Bug

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Abstract: UV 254 nm radiation generally considered as a germicide and its impact has not been studied against highly mobile agricultural pest, *Dysdercus cingulatus* Fab. Newly hatched egg of *D. cingulatus* Fab. was exposed with UV 254 nm radiation and the consequence such as nymphal development and survival, morphogenetic effect and whole body protein and genomic DNA content were evaluated under laboratory conditions. Exposure of UV 254 nm radiation prolonged the nymphal developmental period from 1-7 days, reduced the nymphal survival rate and caused both in nymphs and adults morphological deformities. However, the total body protein contents increased up to third instar of *D. cingulatus* and then it decreased. Whereas, the whole body genomic content increased from 5-20 min UV-C exposure. Hence, UV 254 nm radiation has been considered for its impact on terrestrial animals including insect pests.

Key words: UV-C radiation, exposure time, red cotton bug, biology, protein, total genomic DNA

INTRODUCTION

In the visible spectra (VIBGYOR) of the light, the region of radiation from 280-400 nm is called Ultraviolet Radiation (UV). Within the UV portion is possible to distinguish three types of wavelengths: UV-C (100-280 nm), UV-B (290-320 nm) and UV-A (320-400 nm) (Diaz and Fereres, 2007). Unlikely the radiation present in the terrestrial sunlight, the division between the UV-B and UV-C of 290 nm has shorter wavelength (Hollosy, 2002). It is a general observation recorded by many scientist that shorter wavelength light has more energetic and cause maximum damaging to microbes (Ozcelik, 2007; Sini *et al*., 2007; Rachh *et al*., 2010); plants (Yuan *et al*., 2006; Farokh *et al*., 2010) and animals. However, it has been used for germicidal use (Wells *et al*., 2011).

UV-A, UV-B and UV-C radiations causes damage to all the living organisms. UV-C radiation is the most potent radiation among these three types of UV radiation and acts at short range. Though, UV-C caused maximum damage, however, it does not pose any risk to earth life creatures (Smith *et al*., 2009). Orientation, navigation, host finding and feeding behaviors have been altered by various wavelength of UV regions (Antignus and Ben-Yakir, 2004). It is essential to evaluate the impact of various UV regions exposure on insects. Because they are always crawling and moving over the plants both in day and night time. Impact of UV radiation affects both freshwater (Hader and Sinha, 2005) and marine water dwelled organisms (Hernandez *et al*., 2007) have been documented elsewhere. Impact of UV-C on stored products pests (Faruki, 2005; Faruki *et al*., 2005, 2007) and on other insect pests (Zhou and Steller, 2003; Needham *et al*., 2006; Weintraub *et al*., 2008) including *Dysdercus koenigii* heteroptera were available in the literature.

Dysdercus cingulatus Fab. (Pyrrhocoridae) is the most serious and predominant insect pest of cotton in Southeast Asian countries and having many alternative hosts belongs to Malvaceae and Bombacaceae family (Kohno and Thi, 2004; Pandey *et al*., 2010). Both nymphs and adults are moving from one part of the plant to another for feeding and hence, there are a lot of possibilities of phasing sunlight directly. Usually neem has been used for the management of this pest (Pandey and Tiwari, 2011). Moreover, the possibility of direct impact of UV-C to insect biology, morphology and whole body total protein content has received little attention. This study was undertaken under the laboratory conditions, to study the impact of UV-C radiation on the biology, morphology and total protein content of a common cotton pest, red cotton bug *D. cingulatus*.

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MATERIALS AND METHODS

Insects: Life stages of *D. cingulatus* were collected from cotton and bhendi fields of Tirunelveli District, Tamil Nadu, India and were maintained under laboratory condition 27±2°C temperature, 70-75% RH, 11 L and 13 D h Photoperiod in plastic containers 20×10×15 cm. on water soaked cotton seeds. The laboratory emerged first to fifth instar nymphs were used for the experiments.

UV-C light exposure: Twenty newly emerged first, second, third and fourth instar *D. cingulatus* >6 h. nymph was randomly selected from the stock culture and expose them directly to UV-C lamp of 254 nm (G30T8 TUV 30W, Philips) were 895 mm length, applied at a distance from the lamb of approximately one foot for different duration of 05, 10 and 20 min. Previously insects were exposed to 1, 2 and 4 min, since no impact was recorded, we selected 05, 10 and 20 min. For exposure six replications n = 120 were maintained for each nymphal instar. After the UV-C treatment, insects were transferred into a plastic container (20×10×15 cm) and maintained till their death using water soaked cotton seeds. Molting times, abnormalities if any, mortality of the insect was recorded from all the exposure categories.

Total body protein and genomic DNA analysis: Moribund/dead insects were collected, maintained at -4°C in a freezer and used for the total protein estimations. Randomly select 6 dead and alive insects each and homogenised with 1.0 mL of phosphate buffer, in a chilled pestle and mortar and centrifuged at 12,000 rpm for 6 min at 4°C. The supernatant was used for the estimation of total body protein (Bradford, 1976). Whole body total genomic DNA was extracted from 4-6 insects from each treatment using a modification of a general procedure for extraction with phenol (Sambrook *et al*., 1989). The insects were crushed and incubated at 40°C in 0.6 mg mL^{-1} Proteinase K and 300 µL TNES buffer 50 mM tris-HCl, pH 7.5, 0.4 M NaCl, 20 mM EDTA, 0.5% SDS for 4-8 h. DNA was then purified by washing with organic solvents: once with a chloroform: isoamyl mix 24:1 v/v.; once with a chloroform: phenol mix 1:1 v/v. and once with chloroform only. DNA was then precipitated with absolute ethanol and OD was taken in UV-Visible spectrophotometer at 580 nm.

Data analysis: Statistical comparison was made between control category to 0.5, 10.0 and 20.0 min UV-C radiation exposed individually by Tukey Multiple Range Test (TMRT). and the significance were expressed at 5% level using SPSS ver 11.5.

RESULTS AND DISCUSSION

Egg hatching: Firstly, analyze the hatchability of the irradiated *D. cingulatus* eggs. The egg hatchability of the irradiated egg was drastically reduced 45.0, 5.0 and 0% for 5, 10 and 20 min exposure, respectively when compared to non-irradiated eggs (91.66%). Similarly, an increase in UV-C exposure time gradual decreased the percentage of *Tribolium castaneum* and *T. confusum* egg hatching (Faruki *et al*., 2007). These results suggest that the irradiation to the eggs may induce some stresses in hatchability of the eggs.

Nymphal development and survival: Nymphal developmental period of *D. cingulatus* was gradually increased from first instar to fifth nymphal instars (Table 1). Irrespective of the UV-C exposure time, *D. cingulatus* total nymphal developmental period was significantly increased (3, 4 and 6 days for 5, 10 and 20 min exposure, respectively; $p<0.05$) than the control category. However, no difference was observed in the total nymphal developmental time of *Myzus persicae* Sulzer. (Homoptera: Aphididae), when reared under various UV-absorbing films methods (Chyzik *et al*., 2003). They further reported that the elimination of UV from the light spectrum has no significant effect on the generation development time of *Myzus persicae*. However, it is interesting that the death rates of the aphids were 1.5-2 times higher under the regular films than under the UV-absorbing ones, both in cages and in walk-in tunnels. To the best of our knowledge this is the first report available about the inhibitory effect of UV-C radiation on *D. cingulatus*.

Nymphal survival following irradiation was 78.3, 72.6 and 70.6% for 5, 10 and 20 min UV-C exposure, respectively and non-irradiated nymphal survival was 94.9%. Overall manner, there was does and time of exposure dependent radiation effect on hatchability and nymphal survival (Fig. 1). It was previously reported that adult emergence was significantly decreased when larvae of *T. castaneum* (Faruki, 2005) and *Alphitobius*

Table 1: Impact of UV-C radiation exposure time (min) on the nymphal developmental period (days) of red cotton bug

Nymphal developmental stage	Exposure time (min) 0	5	10	20
I	2.5±0.02	5.8±1.6*	5.8±1.5*	6.0±0.1*
II	3.6±0.1	3.5±0.8^{NS}	3.8±0.9^{NS}	3.9±0.8^{NS}
III	3.2±0.1	4.5±1.60*	5.0±1.2*	5.4±1.5*
IV	4.5±0.7	3.9±1.7^{NS}	4.0±2.0^{NS}	4.3±1.5^{NS}
V	6.5±0.1	5.6±1.2*	5.6±0.9*	6.2±1.4*
Total	20.2±0.1	23.34±0.7*	24.5±0.5*	26.1±0.9*

Different exposure period data of individual nymphal instar compared with the control data, Significance at 5% level by TMRT, NS: Significant

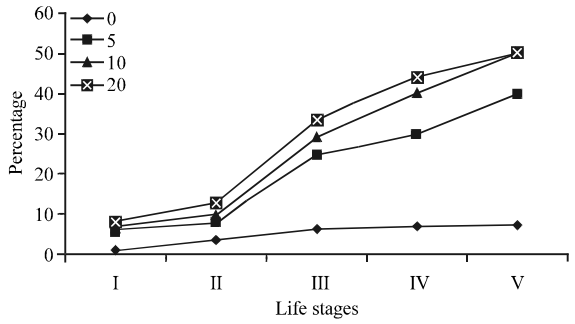

Fig. 1: Impact of UV-C radiation exposure periods (min) on the nymphal mortality percent of red cotton bug

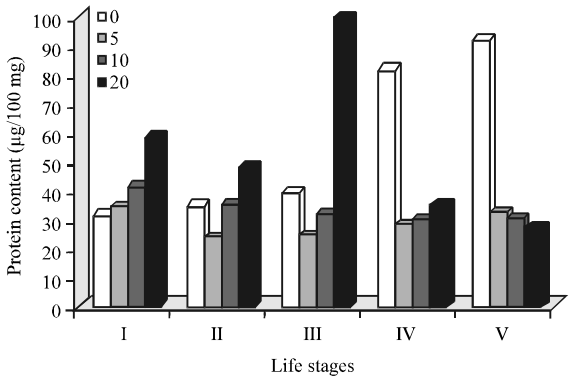

Fig. 2: UV-C radiation exposure time (min) on the total body protein content (µg/100 mg) of red cotton bug nymphal status

diaperinus were exposed to UV radiation as observed in the present study 94.8, 78.0, 72.6 and 70.2% for control, 5, 10 and 20 min UV exposed insects, respectively. A study by Needham *et al.* (2006) using dust-mite *Dermatophagoides farinae*, has shown that an exposure of 5 sec is sufficient to kill 100% of newly laid eggs. This is likely to be due to the induction of self-destruction as demonstrated using UV-C and eggs of the fly *Drosophila* (Zhou and Steller, 2003).

Several studies have shown that whiteflies (Matteson *et al.*, 1992), thrips (Ben-Yakir *et al.*, 2008), *Myzus persicae* and its hymenopteran parasite (Chyzik *et al.*, 2003) and leafhoppers (Weintraub *et al.*, 2008) either chooses environments where UV radiation is present or disperse less where there is no UV radiation. It has been shown that thrips have maximum visual acuity in the UV range and move toward UV-A radiation source (Sinha and Hader, 2002). Thus, these physiological data and recent reports (Ben-Yakir *et al.*, 2008), strengthen our results, where thrips invasion is limited in UV-absorbing covers. The general effect of UV-absorbing covers that we observed, the reduction in the immigration rate of insects, is in agreement with other studies (Chyzik *et al.*, 2003).

Morphogenesis: When UV-C radiation exposure time was increased, 18.02% adults were emerged with abnormalities in wings, legs and antennae. 7.66% of nymphs are unable to molt their exoskeleton and it leads to the death. In addition to the wing abnormalities, the wing color was also changed from red to pale red and this might be due to the interruption of UV-radiation in pigment production. Further studies are needed to confirm this hypothesis. Moreover, Raviv and Antignus (2004) and Krizek *et al.* (2005) have also reported that UV radiation interrupts the life cycle of several pathogenic fungi and alters the visual behavior of many insects.

Whole body protein and genomic DNA content: Impact of UV-C radiation on the whole body protein content of *D. cingulatus* life stages is presented in Fig. 2. It was very clear from the results that, protein content was gradually increased from the first instar to fifth instar. Though maximum DNA content was observed in the fifth nymphal instar of *D. cingulatus,* it was highly reduced (70%) in 5 min UV-C radiation treatment. It was further reduced 71.4 and 75.53 times when *D. cingulatus* fifth instar exposed to UV-C for 10 and 20 min, respectively. Similar trend was also observed for fourth instar too. Both in first and second instars also the total DNA content was gradually increased from control to 20 minutes UV radiation exposed insect. UV-damaged DNA and induce the alternation of binding proteins (Sugasawa *et al.*, 2005).

UV is able to create two of the most mutagenic and cytotoxic DNA lesions: cyclobutane-pyrimidine dimers and 6-4 photoproducts (Sinha and Hader, 2002). Organisms have therefore developed a number of repair mechanisms to counteract the DNA damage caused by UV. These repair mechanisms will however be unable to cope if the UV dose applied is higher than the repair capacity. As the result total genomic content has been gradually decreased from 5 min UV-C exposure times to 20 min in all the nymphal instars of the pest except the first instars. Furthermore, the whole body DNA content was gradually increased from the first instar to the fifth instar in the non-irradiated *D. cingulatus*. However, the DNA content was gradually increased from 5-20 min irradiated first to fourth instar nymphs. However, DNA content in 5 minutes treatment was lesser ($p<0.05$) than control categories (Table 2). Though, maximum DNA content was observed in the fifth nymphal instar of *D. cingulatus,* it was highly reduced (70%) in 5 min UV-C radiation treatment. It was further reduced 71.4 and 75.53 times when *D. cingulatus* fifth instar exposed to

Table 2: Influence of UV-C exposure time (min) on the whole body genomic DNA content (μg mL^{-1}) of *D. cingulatus* nymphal instars

	UV-C exposure time (min)			
Nymphal stage	0	5	10	20
I	31.50	33.45*	37.80*	61.50**
II	32.85	23.25*	36.90*	42.15**
III	39.00	23.23*	31.80*	39.95^{NS}
IV	91.05	28.80**	30.60**	34.65**
V	106.05	31.20**	30.30**	25.95**

Different exposure period data of individual nymphal instar compared with the unexposed insect data, Vlalues are significant at *5 and **10% level by TMRT, NS: Not significant

UV-C for 10 and 20 min, respectively. The DNA is one of the key targets for UV-induced damage as reported by Sinha and Hader (2002).

CONCLUSION

In conclusion, *Dysdercus cingulatus* is a serious pest of cotton and distributed all the cotton growing region of many Asian countries. It is highly mobile pest, moving from place to place and exposed to sun light frequently where UV-C is reported. Results revealed that UV 254 irradiated eggs altered the developmental parameters, drastically reduce the total genomic DNA content except in the first instar and total protein content which leads to morphological changes wing deformity which lead to the death of the organisms. This study highlighting that germicidal radiation UV 254, not only having surface sterilization capacity, it would have penetration capacity to affects higher organisms which would provide a technical or researchable advance in a irradiation impact on higher organisms study.

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