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Effect of ultra-violet rays on growth and development of pulse beetle, *Callosobruchus chinensis* (L.) Bruchidae, Coleoptera

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Abstract
The Pulse beetle, *Callosobruchus chinensis* L. is considered as economically important pest of storage grain legumes because of its polyphagous feeding habit and quantitative loss caused by it. The pesticidal impact on environment necessitates the development of irradiation technique for its management. The current study aimed to study the effect of ultra violet rays on growth and development of pulse beetle. The adults of pulse beetle were exposed to ultraviolet (UV) light (254 nanometres) for different time periods (2, 4, 6, 8, 10, 12, 14, and 16 minutes) and each replicated thrice. These were transferred into the Petri dishes having black paper in the bottom and 3 gram sterilized and conditioned chickpea grains on it. The culture of unexposed beetles was maintained for comparison. The different time period levels were maintained in the laminar flow cabinet using ultra-violet radiations (254 nanometres). The fecundity (180.00/ 2 females), hatchability (87.41%), pupation (81.77%) and adult emergence (F_1) (124.66) were maximum in the unexposed beetles, whereas, minimum in beetles exposed for 16 min (42.00 eggs/ 2 female, 34.13%, 25.46% and 3.00 respectively) under UV radiation.

Keywords: *Callosobruchus chinensis,* ultraviolet (UV) radiation, laminar flow cabinet, fecundity and adult emergence

Introduction
India has made remarkable progress in enhancing production of pulses during the past 15 years. During 2005-06, the total production of pulses in India was 13.38 million MT, which increased to 25.58 million MT during 2020-21. This shows an impressive growth of 91% or a compound annual growth rate (CAGR) of 4.42%. During 2020-21, chickpea had a lion's share of 49.3% in the total pulses production [1]. India contributes 70 per cent of total world Bengal gram production of 116.2 lakh tonnes cultivated under 112 lakh hectares with productivity of 1036 kg/hectare in 2020- 21. In India, Bengal gram takes first position in total pulse production followed by Black gram [2]. Chickpea is an important source of protein, enriched human food and animal feed particularly for the low income population in India. It contains 38-59% carbohydrate and 25.30-28.90% protein which is the maximum nutrients provided by any pulse having no any specific major anti-nutritional factor. *Callosobruchus chinensis* L. is an important major insect pest for grain legumes in many tropical and subtropical countries. Even upto 100% loss of pulse seeds was found due to infestation of pulse beetle in several experiments. It not only causes qualitative and quantitative losses but also reduce germination ability of seeds [3]. The larvae of this species feed and develop exclusively on the seed of chickpeas, while the adults do not require food or water and spend their limited lifespan (1-2 weeks) in mating and laying eggs on seeds. The first instar larvae burrow and feed on the endosperm and embryo, undergo a series of moults and burrow to a position just underneath the seed coat prior to pupation [4].

The use of insecticides and its concomitant impact on environment has necessitated exploration of alternative non-toxic pest control methods. Irradiation becomes an established technique for controlling stored product insects because of residue free advantages over chemical fumigation [5]. Ultraviolet (UV) radiation is germicidal as used for insect management. It is used as a surface disinfectant of insect eggs and as an attractant for beetles in physiological and embryological studies [6]. Among various ionizing and non- ionizing radiations, UV-C is less harmful to living organisms and therefore, could be considered as eco friendly methods in combating stored product pests [7]. UV radiation causing impact on biological parameters of pulse beetle was reported by Heidari *et al*. [8] this irradiation Along with fumigation, technique can be considered as integral component of IPM. The development of C. *maculatus* is slower following UVC treatment compared to untreated control. Irradiation is developing into an established technique for controlling stored grain insects because of residue free advantages over chemical fumigation [9]. There was a negative correlation between *Callosobruchus maculatus* eggs and UV rays irradiation. An increase in time of exposure to irradiation caused a gradual decrease in percentage of hatching of pulse beetle eggs [10]. Significant reduction in egg hatching and adult emergence caused by UV irradiation in *E. cautella* (Walker) are promising from pest management point of view. There was also increase in adult mortality of the UV irradiated adults [11]. UV radiation treatment as effective management tool was exploited in pests other than pulse beetle, such as *Tribolium castaneum, T. confusum* and *Cadra cautella.* The percentage adult mortality for the exposure periods significantly increased as the exposure period increased [12]. UV rays and triflumuron treatments deleteriously reduced the populations of all the developmental stages of *Alphitobius diaperinus* [13]. The present study aimed to find out the effect of UV-rays with the search of a non-chemical method against fecundity, hatchability, pupation percentage and adult emergence of *Callosobruchus chinensis* L. which is a primary storage pest and causes more qualitative and quantitative damage.

Materials and Methods

To maintain the stock culture of *Callosobruchus chinensis* L, it was procured from the pure culture already maintained in Department of Entomology, S. K. N. College of Agriculture, Jobner**.** The chickpea grains were cleaned, rinsed in water, sun dried and were subjected to sterilisation at 60 °C temperature for five hours to eliminate any insect infestation, hidden or otherwise. These grains were conditioned for 48 hours at 29 ± 1.5 °C temperature and 70 ± 5.0% relative humidity. For maintaining subsequent insect culture, 20 pairs of newly emerged adults were released for oviposition in the glass jar (size 18 × 10 cm) containing 200 g chickpea grains. For handling the infested grains and insects, a forcep and a camel hair brush was used. Subsequent experiment was conducted at 29 ± 1.5 °C temperature and 70 ± 5.0% relative humidity. The newly emerged adults of *Callosobruchus chinensis* (immediately after hatching out from the eggs, unmated) were exposed to UV light for different time periods (2, 4, 6, 8, 10, 12, 14, and 16 min) and each replicated thrice. The culture of unexposed beetles was maintained for comparison. The different time period levels were maintained in the laminar flow cabinet using UV radiations (254 nm) (Figure 1).

The UV light tube was of Philips make (30 Watt) and the equipment to hold the same was manufactured by Kirloskar Electrodyne. The distance of beetles and the UV tube was 50cm emitting energy at the 254 nm at an intensity of 600 uw/cm^2. Two different pairs of adults immediately after emergence (one pair male and one pair female) were exposed to the ultra-violet radiations (for each time periods mentioned above) and were transferred into the Petri dishes having black paper in the bottom and 3 g sterilized and conditioned chickpea grains on it (Figure 2). The beetles were allowed to mate and oviposit. Three replications of the culture were maintained. The eggs laid by the beetles were separated every 24 h by sieving (50 mesh sieve) and were counted under the microscope. The survival of the eggs was recorded after examining the egg chorion and by recording the number of larvae that hatched out. The pupation percentage was worked out on the basis of larvae pupated out of the total larvae. The number of adults emerged were counted every 24 h. After counting, the newly emerged adults were discarded from the samples so as to avoid further counting.

The percentage data on hatching and pupation were transformed into angular values (arc sine √percentage) to convert the percentage into degrees. The data on fecundity were converted into log X values and that of adult emergence into √X for ease in analysis of variance.

Fig 1: UV radiation treatment using laminar flow cabinet

Fig 2: UV radiation treated adults were transferred to petridish with chickpea seeds in it.

Table 1: Fecundity, hatchability, pupation percentage and adult emergence (F_1) of *C. chinensis* as influenced by different exposure periods of UV rays

S. No	Exposure time to UV rays	Fecundity/ 2 female*	S.D.	Hatchability (%) **	S.D.	Pupation (%) **	S.D.	Adult emergence (F1)***	S.D.
1.	2 min	104.00(2.02)	1.00	51.91(46.09)	0.47	49.98(44.99)	0.93	23.66(4.91)	0.58
2.	4 min	95.00(1.98)	1.00	48.06(43.89)	0.31	45.24(42.27)	0.70	19.66(4.49)	0.58
3.	6 min	85.00(1.93)	1.73	45.48(42.41)	0.25	43.08(41.02)	0.85	16.00(4.06)	1.00
4.	8 min	75.00(1.88)	2.65	44.00(41.55)	0.53	41.40(40.04)	0.92	13.00(3.67)	1.00
5.	10 min	66.00(1.82)	3.00	40.95(39.78)	2.59	34.50(35.97)	1.19	9.00(3.07)	1.00
6.	12 min	54.00(1.73)	4.00	38.42(38.30)	1.51	33.22(35.19)	1.61	6.66(2.67)	0.58
7.	14 min	48.00(1.69)	1.00	36.80(37.34)	0.67	26.13(30.72)	2.28	4.66(2.27)	0.58
8.	16 min	42.00(1.63)	3.46	34.13(35.74)	0.07	25.46(30.29)	2.07	3.00(1.87)	0.00
9.	Unexposed	180.00(2.25)	5.00	87.41(69.22)	0.82	81.77(64.72)	0.12	124.66(11.18)	1.53
	S.Em±	0.0109		0.383		0.494		0.061	
	CD at 5%	0.0325*		1.138*		1.468*		0.1818*	

Data based on two pairs of adults (three replications). *Figures in the parenthesis are log X values. **Figures in the parenthesis are angular transformed values. *** Figures in the parenthesis are √X values. *Significant at 5% level

Results and Discussion

The results on fecundity (Table 1, Figure 3) indicated that the treatments on which *Callosobruchus chinensis* was allowed to feed and reproduce differed significantly with each other. The maximum number of fecundity was observed in control (180 eggs/ 2 females) which differed significantly (p 0.05= 0.0325) with other treatments. The minimum number of eggs was laid on treatments exposed to 16 min (42 eggs/ 2 females) followed by 14 min (48 eggs/ 2 females), 12 min (54 eggs/ 2 females), 10 min (66 eggs/ 2 females) and 8 min (75 eggs/ 2 females). The descending order of egg laying potential recorded in different time periods exposure was control, 2 min, 4 min, 6 min, 8 min, 10 min, 12 min, 14 min and 16 min.

The hatchability percentage on different treatments varied between 34.13% and 87.41% and differed significantly (p 0.05= 1.138) with each other. The maximum hatchability percentage was observed on unexposed treatment (87.41%) followed by 2 min (51.91%). The most effective treatment in which minimum hatchability observed was 16 min (34.13%) followed by 14 min (36.80%), 12 min (38.42%), 10 min (40.95%) and 8 min (44.00%). The least effective to most effective treatments can be ranked as 2, 4, 6, 8, 10, 12, 14 and 16 min. (Table 1, Figure 4)

The maximum pupation percentage was observed in control (81.77%) and minimum in treatment exposed to 16 min (25.46%) that differed significantly (p 0.05= 1.468) with other treatments. The pupation percentage in ascending order can be ordered as 16 min (25.46%), 14 min (26.13%), 12 min (33.22%), 10 min (34.50%), 8 min (41.40%), 6 min (43.08%), 4 min (45.24%) and 2 min (49.98%) (Table 1, Figure 5)

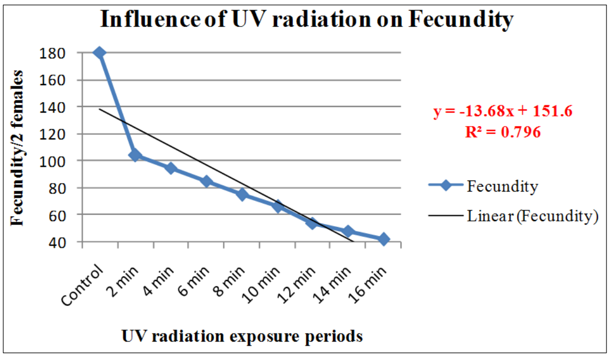

Fig 3: Influence of UV radiation on fecundity of *Callosobruchus chinensis* (L.)

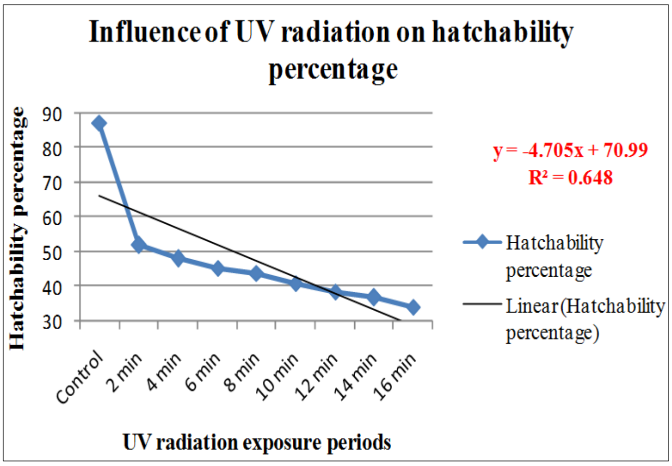

Fig 4: Influence of UV radiation on hatchability percentage of *Callosobruchus chinensis* (L.)

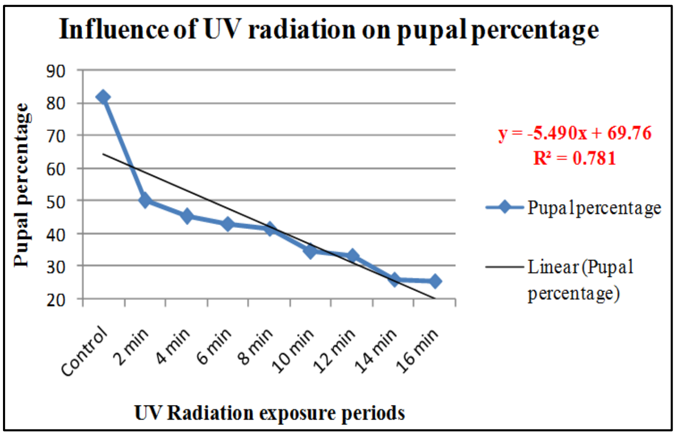

Fig 5: Influence of UV radiation on pupal percentage of *Callosobruchus chinensis* (L.)

The adult emergence (F_1) varied between 3.00 and 124.66 among the treatments used. The maximum adult emergence was observed in control (124.66) and differed significantly (p 0.05= 0.1818) with other treatments. The minimum adult emergence was observed in treatments exposed to 16 min (3.00). Rest of the treatments were ranked in the middle order. The adult emergence in ascending order can be ordered as 14 min (4.66), 12 min (6.66), 10 min (9.00), 8 min (13.00), 6 min (16.00), 4 min (19.66), and 2 min (23.66) (Table 1, Figure 6).

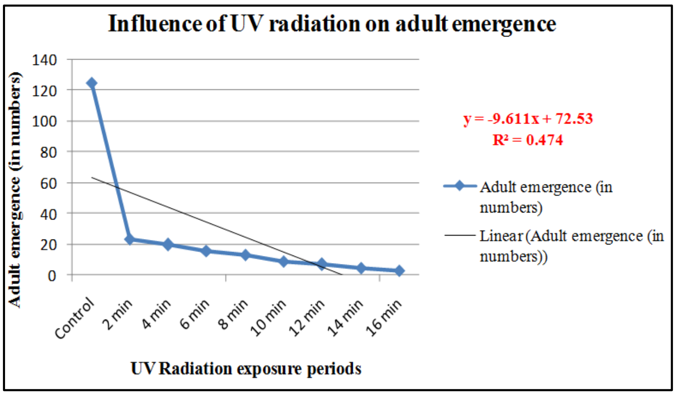

Fig 6: Influence of UV radiation on adult emergence of *Callosobruchus chinensis* (L.)

These findings suggested that increase in exposure time decreased fecundity, hatchability, pupation and adult emergence which was in accordance with the findings of Singh and Das [9] which may be due to decrease in the hatching rate of the irradiated eggs of pulse beetle with increasing period of exposure. The findings of the study agreed with the results of Sedaghat *et al*., [14] who reported the percentage of *Callosobruchus maculatus* hatched eggs were found to be higher (95%) in control while it significantly reduced in different UV radiation exposure time periods. Faruki *et al*. [12] also reported the reduction in the rate of hatching of eggs of the flour beetles (*Tribolium castaneum* and *T. confusum*) and the almond moth (*Cadra cautella*). They also observed gradual decrease in adult eclosion from the UV irradiated eggs of *T. castaneum, T. confusum* and *C. cautella* as the the duration of exposure to irradiation increased, which is in agreement with the result of the present findings. Sorungbe *et al*. [11] found that adult eclosion from UV irradiated larvae of *E. cautella* gradually decreased as the duration of exposure to irradiation increased and it also had significant effect on adult mortality if adults were irradiated with UV radiation which was in accordance with our results. Sedaghat *et al*. [10] reported that UV-irradiation is a safe and clean method for stored product preservation and pest control since all exposure periods of UV-irradiation reduced the hatching of eggs of cowpea weevil in comparison to control. An increase in time of exposure to irradiation caused a gradual decrease in percentage of hatching of eggs in all age groups of eggs. Similar effects were reported in the present study. Increase in time of exposure of UV-rays gradually decrease the rate of hatching and development of *C. maculatus* at different UV exposure time as reported by Bhardwaj *et al*. [6] and Sedehi *et al*. [5] which was in close agreement with present study. The results of the study revealed the complex interactions between growth parameters and UV radiation which was similar to the report of Heidari *et al*., [8] whom found that net reproductive rate (R_0) and intrinsic rate of increase (r) of *C. maculatus* were significantly decreased with increase in exposure period of UV-C radiation.

The correlation analysis indicated that there was a significant negative correlation between UV radiation exposure time period and fecundity (-0.8921), hatchability (-0.8049), pupation (-0.8837) and adult emergence (-0.6884). The regression equation obtained for fecundity, hatchability, pupation, and adult emergence include Y= 151.6- 13.68X, Y= 70.99- 4.705X, Y= 69.76-5.490X and Y= 72.53-9.611X respectively (Table 2). The equations indicated that 151.6, 70.99, 69.76 and 72.53 are intercepts and exposure to UV rays for one minute was responsible to cause reduction in fecundity, hatchability, pupation and adult emergence by 13.68%, 4.705%, 5.490% and 9.611% respectively. A decreasing trend in these parameters was evident when period of exposure was increased.

Table 2: Correlation and regression coefficient between exposure time of UV rays and life processes of *C.chinensis*.

S. No.	Aspects	Correlation coefficient (r value)	Regression equation (y = α + βx)	Coefficient of determination (R^2)
1.	Exposure to UV rays vs. fecundity	-0.8921	Y= 151.6- 13.68X	0.796
2.	Exposure to UV rays vs. hatchability	-0.8049	Y= 70.99- 4.705X	0.648
3.	Exposure to UV rays vs. pupation	-0.8837	Y= 69.76-5.490X	0.781
4.	Exposure to UV rays vs. Adult emergence	-0.6884	Y= 72.53-9.611X	0.474

*Significant at 5% level.

Conclusion

Although various methods such as insecticide treatment, diatomaceous earth, fumigation, heating and grading systems are available irradiation treatment with UV radiation gains importance because of its potential to reduce the biological potential of pulse beetle and its non-residual impact on environment. The results showed that fecundity, hatchability, pupation and adult emergence were significantly reduced when adults of pulse beetle were exposed to different periods of UV radiation (254nm). The results in compilation demonstrated that UV radiation treatment for 16 minutes is a promising one from pulse beetle management point of view. Thus UV irradiation can be used with other control methods such as biological control in integrated pest management (IPM) of stored products pests and it may also provide a sustainable alternative for the control of other storage insect pests in near future.

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