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# EFFECT OF ULTRAVIOLET RADIATION AND SOME BOTANICALS ON DEVELOPMENT OF *CALLOSOBRUCHUS*

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**KEY WORDS**
*Callosobruchus chinensis*
*Callosobruchus maculatus*
Mung bean
Plant extracts
Pulses
Ultraviolet irradiation

**ABSTRACT:** Damage of stored pulses by the bruchid beetles, *Callosobruchus* species are of great concern in India. The objective of this study, therefore, was to determine the insecticidal potential of plant extracts and ultraviolet (UV) radiation on or inside the pulses without affecting the quality. Three days old eggs on pulses and newly emerged adults of *Callosobruchus maculatus* were exposed to UV radiation (253.7 nm wavelength) for different durations at room temperature, without humidity control. Increases in time of exposure of UV-rays gradually decrease the rate of hatching and development of *C. maculatus* at different UV exposure time. The percentage of hatched eggs and lengthened the duration of immature stages were determined to be 6 and 12 days, 12 and 20 days (3 days old and newly emerged adults) in control and UV-treated, respectively. The longest mean generation time was recorded in 3-day-old eggs and newly emerged adults at 60 min exposure time. Here, we also evaluated the potential of plant extracts of *Syzygium aromaticum* (Clove floral buds), *Allium sativum* (Garlic bulbs), *Piper nigrum* (Black pepper seeds), and *Azadirachta indica* (Neem leaves) to control *Callosobruchus chinensis*. Furthermore, plant extracts increments proportionately decreased the growth rate, and offspring emergence was 50% abolished when parents were treated with 3.5 ml of each plant extracts. Thus, by examining similar insecticidal activities as synthetic insecticides on the mortality of *C. chinensis* by *A. sativum* extract represents a better alternative for the management of *Callosobruchus* infestations.

## INTRODUCTION

*Callosobruchus* species is considered as the most serious stored product pest in India, Asia, and Africa (Park *et al.*, 2003). Maximum damage occurs from February to August when all the developmental stages of insect exist simultaneously (Devi and Devi, 2014). Several factors affect the extent of damage such as storage conditions, containers, and grain legume varieties (Nchimbi-Msolla

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and Misangu, 2002; Mebeasilassie, 2004). The primary infestation occurs in the field, where bruchid adults lay eggs on pods after which larvae hatch, penetrate the seed and feed on cotyledonary and/or embryonic tissues (Mohan and Subbarao, 2000). The damaged seeds are not fit for human consumption (Deshpande *et al.*, 2011; Bae *et al.*, 2014) or planting and also reduce their aesthetic and market value (Singh, 2011; Sarwar, 2015). The use of chemicals to control the insects is very common, but the chemical insecticides are expensive and have adverse effects on human health and the environment (Bindhu *et al.*, 2015). Moreover, the insects are developing resistance to insecticides and are not effective in controlling them. Temperature management is also an important aspect for control of stored grains in storage godowns and processing industries (Fields, 1992; Dosland *et al.*, 2006; Phillips and Throne, 2010; Verma *et al.*, 2018). Ultraviolet (UV) radiation is germicidal as used for insect management (Hori *et al.*, 2014; Sheeja and Jones, 2015). It is used as a surface disinfectant of insect eggs and as an attractant for beetles in physiological and embryological studies (Sedaghat *et al.*, 2014). Radiations can produce a variety of damage in terms of mutations, chromosomal aberrations, etc (Tandon *et al*., 2009). Penetrating effect is less in UV treatment than ionizing radiations, so for large scale use of UV radiation is less (Hasan and Khan, 1998; Hallman, 2013). Irradiation is the great phytosanitary treatment used to manage stored-product insects (IAEA, 2002). Bakri *et al.*, 2005, used another method, i.e., sterile insect technique to control bruchid beetles. *Callosobruchus maculatus* newly emerged adults show inherited deleterious effects on developmental biology, sterility and various other histopathological effects in sperm cells such as cell membrane rupture and abnormal mitochondria when irradiated with low doses of gamma radiations (Ibrahim *et al.*, 2017). Irradiation technology provides new information on possible methods for controlling *C. maculatus*.

Plants and their products are also better option as they show insecticidal potential and toxicity against many stored product insects. (Regnault-Roger *et al.*, 2012; Teodoro *et al.*, 2017; de Araújo *et al.*, 2017; Jumbo *et al.*, 2017). These oils have neurotoxic effects on these insect-like inhibitions of acetylcholinesterase or disturbing the functions of GABAergic and aminergic systems (Jankowska *et al.*, 2017). The aim of the present study is to assess the effect of UV treatment on adult and newly laid eggs at different time intervals on the life cycle and developmental biology of the *C. maculatus* and aqueous extracts of four medicinal plants on mortality of *Callosobruchus chinensis* for the purpose of pest management or pest control strategies.

## MATERIALS AND METHODS

All experiments were conducted in a laboratory at the Department of Zoology, M. D. University, Rohtak. The cultures of *C. maculate* F. and *Callosobruchus chinensis* L. used in the present study were already present in the laboratory.

### Laboratory Maintenance of the Experimental Insect

Bruchid beetles were reared on mung bean seeds inside a BOD at 27 ± 2°C, 12:12 L:D, and with 70 ± 5% RH. Newly emerged adults from culture were allowed for mating and oviposition on mung bean seed under laboratory condition. Then, parental insects were separate and eggs containing mung bean seeds were transferred to fresh seeds in the glass bottles covered with a muslin cloth to avoid the escape of bruchids beetles and contamination.

### Preparation of Seeds

Packed mung bean seeds were purchased from the local market. The seeds were cleaned and dried in an oven at 27°C. Now, seeds were disinfected by keeping them at 0°C for 15-20 days before use.

### Treatment of Insects with UV

Newly emerged insects were released on new seeds for egg laying after 24 h insects were removed and seeds with one or two eggs were taken and kept separately in a beaker to obtain 3 days old eggs. All experimentation procedures were completed under the same environmental conditions, as mentioned above. Effects of various irradiation times (0, 10, 20, 30, 40, 50, and 60 min) of UV beams on the most important biological traits of this pest were evaluated. The radiation source was a Philips TUV 30 watt T8 UV-C germicidal light bulb (G30T8) emitting radiations of 253.7 nm wavelength. Bioassays were conducted at room temperature. Toward the finish of the exposure time, the UV-lamp was turned off and the Petri dishes were removed immediately. To observe the effects of UV radiation on the development of *C. maculatus*, the seeds were placed at 15 cm distance from the TUV bulb. Eggs on seeds and adults insects (newly emerged male and female), both were irradiated for 10, 20, 30, 40, 50, and 60 min by a TUV bulb.

*UV treatment for eggs*

Eighty mung bean seeds were placed in three Petri plates, and eight pairs of newly emerged insects were introduced into each Petri plates. Now, these three replicas were kept in BOD for 24 h at favorable conditions for mating and egg laying. After 24 h seeds having one or two eggs were selected and collected in a beaker, then these seeds were kept again in BOD for 2 days. The 3 days old seeds having 1 or 2 eggs were utilized for UV exposure at a different time interval. There are total seven sets (10-60 min with control) each containing ten seeds which were placed at a distance of 15 cm from the UV source. At that point, UV exposure was given to all seven sets (10 seeds containing at least two eggs) as indicated by their time interval. After exposure, the irradiated and control eggs of various age groups were kept separately at 27 ± 2°C until bring forth.

Later to check the larval and pupal stages of *C. maculatus,* a couple of seeds were taken from each set and dipped in water for overnight separately. After 24 h, all seeds soaked in water were dissected with the help of sharp needle and forceps to observe the larval and pupal stages whether these were 1$^{st}$, 2$^{nd}$, 3$^{rd}$, and 4$^{th}$ instar larva or pupa and also measured the length and breadth of larva and pupa with the help measuring scale and were protected in the 70% ethanol.

*UV treatment for adults*

Newly emerged pairs of adult *C. maculatus* were irradiated with UV radiations for 10, 20, 30, 40, 50, and 60 min. After exposure, the irradiated and control adults were kept separately in BOD at optimal conditions on the fresh mung bean seeds for egg laying followed by the observation of the larval and pupal stages of these insects.

## Mortality Effects of Some Medicinal Plants

The present experiment was conducted for controlling the pulse beetle, *C. chinensis* by different plants extracts on mung bean seeds. The insecticidal activity of four endemic species and day by day mortality effects of these plants against *C. chinensis* were recorded for 1 week.

*Preparation of aqueous extract of plants*

For the extraction, fresh leaves, seeds, and bulbs of selected plants were collected and dried properly under shade to prepare fine powder by the help of mortar pestle/electric grinder. The powder was sieved through the muslin cloth; 10 g of powdered was dissolved in 33 ml of distilled water. The resulting mixture/solution was centrifuged at 3000 rpm for 15 min and allowed to settle for 24 h. After settling down, the solution again filtered through Whatman filter paper no. 1 in a conical flask. The concentration of the extract was taken in 50 ml measuring cylinder and made the volume of each plant extract up to 33 ml with distilled water. The extracts were covered by aluminum foil properly and stored separately at 4°C until use (Chudasama *et al.*, 2015).

*Treatments of extracts on C. chinensis*

The concentration of extracts was applied at various dosages on Whatman No. 1 filter paper and air-dried for 5–10 min. The controls were treated with distilled water. The treated filter paper was placed in Petri plates and 12 g of mung bean seeds were placed on the paper. The five pairs of insects were released in each Petri plates. The observations were recorded daily after 24 h for 1 week.

## RESULTS

### Effect of UV Irradiation on Development of Bruchid

*On eggs*

UV radiations delayed the hatching and increase the duration of developmental stages (larval and pupal periods) in *C. maculatus* (Table 1). UV radiations increased the time of development, i.e., the life cycle of insect from 23-28 days (in control) to 35-40 days in UV treated eggs.

UV treated eggs (60 min) were dissected and studied at different days up to 40 days. On the 12$^{th}$ day, there was no appearance of larvae in the seeds as compared to control eggs. The morphological measurements, i.e., the length and breadth of 3$^{rd}$ instar of control on the 12$^{th}$ day were found to be 2.43 mm and 1.22 mm, respectively, and of 2$^{nd}$ instar larvae of UV treatment were found to be 1.5 mm and 1.0 mm, respectively, on the 20$^{th}$ day (Table 1). The various developmental stages of UV irradiated eggs of *C. maculatus* were found to be of almost equal in size compared to the control (Table 1). These results showed a delay in hatching and development of UV treated eggs. After 28 days, the adults emerged out from the control seeds, but in case of UV irradiated eggs, there were existed different stages of insect (larvae and pupae), indicating the delayed development (Table 2 and Figure 1).

Further, there was no noticeable increase or decrease found in the size of the larva with increasing exposure time of UV up to the all (10-60 min) doses, and the size was stabilized at 2$^{nd}$ instar stage (Table 2). Morphological measurements showed slight variations according to developmental stage existed. Results indicated the delayed development and prolonged duration of immature stages (larval and pupal stage) with an increase in the duration of the exposure period.

*On newly emerged adults*

Similar results were obtained from the eggs laid on the seeds by the UV treated adults. No stages were found in the seeds dissected on the 12$^{th}$ day of egg laying. After 20 days from egg laying, only

**Table 1: Duration of life cycle and morphometric measurement of different stages of *Callosobruchus* in control and UV treated for 1 h.**

| Life stage | Duration (days) | | Length+breadth (mm) | |
|---|---|---|---|---|
| | Control | UV treated | Control | UV treated |
| Egg | 6-7 | 8-12 | 0.47±0.08; 0.12±0.21 | No emergence |
| 1$^{st}$ instar larva | 8-9 | 13-17 | 0.60±0.03; 0.22±0.03 | 0.3±0.06; 0.15±0.34 |
| 2$^{nd}$ instar larva | 10-11 | 18-20 | 1.19±0.05; 0.77±0.02 | 1.5±0.5; 1.0±0.5 |
| 3$^{rd}$ instar larva | 12-13 | 21-25 | 2.43±0.15; 1.22±0.08 | 1.8±0.05; 0.85±0.04 |
| 4$^{th}$ instar larva | 13-16 | 26-28 | 3.64±0.18; 2.00±0.11 | 2.25±0.45; 1.5±0.65 |
| Pupa | 17-22 | 29-33 | 4.57±0.07; 2.60±0.06 | 4.0±1.0; 2.0±1.0 |
| Adult | 23-28 | 33-40 | 4.25±0.12; 3.34±0.05 | 3.85±0.09; 2.86±0.62 |

UV: Ultraviolet.

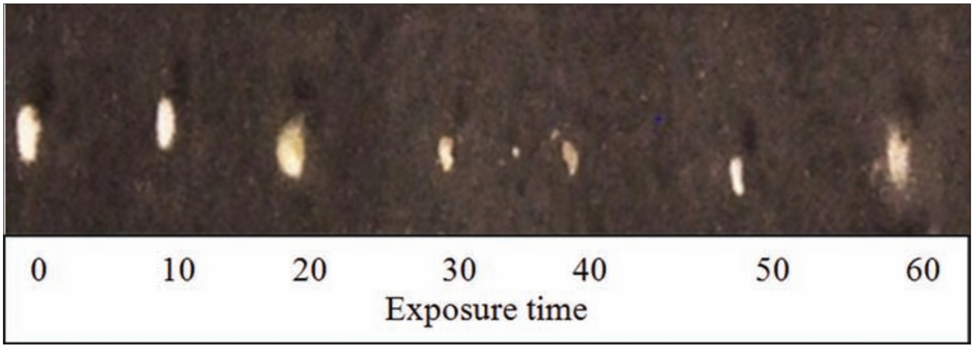


**Figure 1: Effect of ultraviolet irradiation on eggs of *Callosobruchus maculatus* after 20 days from egg laying.**

**Table 2: The effect of different doses of UV irradiation on 3 days old eggs of *C. maculatus* at different exposure time. The seeds were dissected on 12$^{th}$, 20$^{th}$, and 33$^{th}$ days to observe developmental stages of the insect.**

| **Exposure time** | **Developmental stage** | | |
|---|---|---|---|
| | **14$^{th}$ day** | **20$^{th}$ day** | **33$^{th}$ day** |
| Control | 2$^{nd}$ instar (L=1.19; B=0.77 mm) | Adult | Adult |
| 10 min | No emergence | 2$^{nd}$ instar (L=2 mm; B=1.8 mm) | Pupa male (L=4 mm; B=2 mm) |
| 20 min | No emergence | 2$^{nd}$ instar (L=1.5 mm; B=1 mm) | Pupa female (L=5 mm; B=3 mm) |
| 30 min | No emergence | 2$^{nd}$ instar (L=2 mm; B=1 mm) | Pupa male (L=4 mm; B=2 mm) |
| 40 min | No emergence | 2$^{nd}$ instar (L=1 mm; B=1.5 mm) | Pupa male (L=4 mm; B=2 mm) |
| 50 min | No emergence | 3$^{rd}$ instar (L=2 mm; B=0.5 mm) | Pupa male (L=4 mm; B=1.5 mm) |
| 60 min | No emergence | 2$^{nd}$ instar (L=2 mm; B=1 mm) | Pupa male (L=4 mm; B=2 mm) |

*C. maculatus*: *Callosobruchus maculatus*, UV: Ultraviolet.

2$^{nd}$ instar stages were observed in all the UV treated groups, in comparison with control, where adults have been emerged out (Table 3). Results indicate highly delayed hatching, delayed, and expanded the duration of immature stages with an increase in duration of exposure time to adults.

After 33 days from egg laying, pupal stages were present with an increase in exposure time (Figure 2; Tables 2 and 3). Exposure periods of 30, 40, 50, and 60 min resulted in the appearance of 2$^{nd}$ instar larvae (Figure 2 and Table 3). The life cycle of bruchid was prolonged by 10-12 days, with a high degree of suppression of development, and extended duration of immature stages due to the increased UV exposure time.

### Effect of Medicinal Plant Extracts on Mortality of Bruchid

The total number of insects which survived after the treatment with natural plants products was recorded for 7 days. The final mortality at 7$^{th}$ day of treatment is shown in Table 4. Fifty percent mortality was recorded for the *Piper nigrum* (pepper), *Syzygium aromaticum* (clove), and *Azadirachta indica* (neem) at 3.5 ml dose

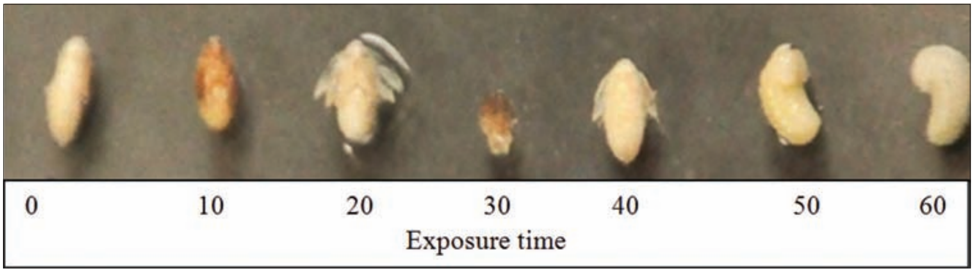


**Figure 2: Effect of ultraviolet irradiation on the adults of *Callosobruchus maculatus* after 20 days from egg laying.**

**Table 3: The effect of UV irradiation on newly emerged adults of *C. maculatus* at different exposure time. The seeds were dissected on 14th, 35th, and 44th days to observe developmental stages of the insect.**

| Exposure time | Developmental stage | | |
|---|---|---|---|
| | 12th day | 20th day | 33th day |
| Control | 2nd instar (L=1.2 mm; B=0.8 mm) | Adult | Adult |
| 10 min | No emergence | 2nd instar (L=1 mm; B=0.5 mm) | Pupa female (L=5 mm; B=3 mm) |
| 20 min | No emergence | 2nd instar (L=2 mm; B=1 mm) | Pupa male (L=3 mm; B=2 mm) |
| 30 min | No emergence | 2nd instar (L=1 mm; B=1 mm) | Pupa female (L=5 mm; B=3 mm) |
| 40 min | No emergence | 2nd instar (L=1 mm; B=0.5 mm) | Pupa female (L=5 mm; B=2 mm) |
| 50 min | No emergence | 2nd instar (L=1 mm; B=0.5 mm) | Pupa female (L=5 mm; B=3 mm) |
| 60 min | No emergence | 2nd instar (L=1 mm; B=0.5 mm) | Pupa male (L=4 mm; B=2 mm) |

UV: Ultraviolet, *C. maculatus*: *Callosobruchus maculatus*.

but for *Allium sativum* L (garlic) it was at 2.5 ml dose. Results showed that with increased concentration of plant extracts, percent mortality increased.

## DISCUSSION

UV radiation is widely used as germicidal, surface disinfection of insect eggs and as an attractant for insects in physiological and embryological studies. Many researchers have considered that UV rays were useful for controlling the development of many stored products insects and also cause mortality or sterility in bruchid beetles depends on the dose and exposure time. Increase in an exposure time of UVC radiation gradually decreases the percentage of eggs hatching. UV radiation increased the time of development from 25-30 days (control) to 44-52 days of the life cycle in case of UV treated eggs. These results are in agreement with the findings of Islam and Mustari (2010) who suggested that the duration of the immature stages in *C. maculatus* was significantly increase both in parental and F1 generation, showing that UV radiations extended the developmental period.

Highly delayed hatching was found in eggs oviposited by UV irradiated adults, and even after a month there was no hatching in eggs with 30 and 60 min exposure of UVC. In case of UV treated adults, the life cycle was highly delayed with a high degree of

**Table 4: The effects of medicinal plants extracts on mortality of *Callosobruchus chinensis.***

| Plants | Dose (ml) | Mortality (%) at 7th day |
|---|---|---|
| *Piper nigrum* | 0.5 | 16.6 |
| | 1.5 | 19.8 |
| | 2.5 | 33.3 |
| | 3.5 | 50 |
| *Allium sativum* | 0.5 | 33.3 |
| | 1.5 | 40.4 |
| | 2.5 | 50 |
| | 3.5 | 66.6 |
| *Syzygium aromaticum* | 0.5 | 23.5 |
| | 1.5 | 35.3 |
| | 2.5 | 39.3 |
| | 3.5 | 50 |
| *Azadirachta indica* | 0.5 | 16.6 |
| | 1.5 | 33.3 |
| | 2.5 | 37.6 |
| | 3.5 | 50 |

suppression of development and prolonged durations of immature stages with increasing duration of UV exposure as was found in studies of Islam and Mustari (2010). Results also indicated that newer eggs were more resistant than the older eggs, which is similar to the result of Faruki (2007). Effects of UV irradiation on adult insects have been explained by Allen, 2001. He suggested that UVC at 254 nm dimerize the adjacent thymine molecules of the DNA strands which inhibited DNA replication, thereby show the effect on exposed insects. The UV radiation is a clean form of pest control (Shimoda and Honda, 2013).

The plant materials have many properties such as insecticidal, repellent, and insect growth regulator (Tabu *et al*., 2012). The result presented in this study showed that extracts from *A. indica, S. aromaticum, P. nigrum*, and *A. stadium* negatively affected the longevity of *Callosobruchus*. Extract from *A. sativum* shows highest, i.e., 66.6% mortality at 3.5 ml dose after 6 day treatment. Jung and Yong, 2014, showed the insecticidal effect of clove derivatives against aphid. The powder of black pepper, garlic, and clove considerably lower the life cycle of *Callosobruchus* species (Singh, 2011; Devi and Devi, 2015). Similar effects were reported in the present study on *Callosobruchus* due to the presence of bioactive compounds in these extracts. Neem derivatives also show high anti-ovipositional activity and also control the infestation caused by bruchid beetles (Chandrakala *et al.*, 2013). To reduce the damage caused by beetles botanicals are a better option compared to other pest control methods, as they are eco-friendly and effective.

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