

## Impact of daylight duration on pupation and adult emergence of black soldier fly (*Hermetia illucens*)

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### Abstract

The black soldier fly (*Hermetia illucens*) has been adopted as a new resource for the animal feed sources for its high nutritional value and easily cultured on organic streams. However, black soldier fly larvae are very sensitive to abiotic factors such as light. Currently, there are scarcity of information about the impact of light on pupation. This study examined the effect of daylight duration on the pupation time and adult emergence of black soldier fly prepupae. The pupation time and adult emergence were estimated for new prepupae treated at 0 h, 04 h, 8 h, and 12 h of light, under 29°C and 68% relative humidity. Adult successfully emerged in all treatments. Prepupae 0 h treated took shortest time to emerge adult, although 12 h treated prepupae needed longest time. Adult emergence percent was highest in 0 h treatment among other treatments and lowest in 12 h.

**Keywords:** daylight, prepupae, organic waste, black soldier fly, fecundity, adult emergence

### Introduction

Recently, black soldier fly, *Hermetia illucens* (L.), larvae have pulled attention due to their high efficiency for bioconversion of a variety of organic wastes into high-quality nutrient biomass, which can be utilized as livestock feed ingredient [3, 17, 18, 21] and the residues of organic waste can be manipulated as bio-fertilizers [5, 7, 26]. The black soldier fly distributes closely resources includes various organic compositions with an available pupation ambient environment [8, 20]. These dwelling place can simplify immature growth [29-30]. Prepupae of black soldier flies tomb themselves in a comfortable pupation place for protection from enemies while they pass metamorphosis [14]. The movement of prepupae afar from feeding media is stimulated by intrinsic behavioral sequence and the abiotic factors including temperature, light, and soil moisture that they migrate toward cool, dark and dry place [10]. In addition, the pupation time carry out about 50% of the immature developmental stage [32].

Former investigations on black soldier flies has been focused on their utilization in waste management [2, 27-30] for year-round production. There are scarce of information on the effect of light on pupation of black soldier fly development and adult emergence. However, some research carried out the impact of light on mating and egg production, including minimum light intensity [29, 33] and spectral light composition [22] where it is argued that light intensity plays a secondary role to light spectral composition [22]. Diapause related research is very limited with one literature [25]. First, Holmes *et al.* [11] showed the effect of photophase duration on *H. illucens* development in tropical region species that is not enough for another region including warmer world. In this study, we compared the effects of four different daylight duration on wild BSF

prepupae and adult emergence from Bangladesh. We also measured the relation between daylight and pupation time. This knowledge will play important role in establishing mass culture of black soldier fly through optimizing the pupa development environment with high adult emergence rate.

### Materials and methods

#### *H. illucens* prepupae collection

Eggs were collected from wild BSF from the campus area of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. Our study area located in 23°44'30" N and 90°22'49" E. A ten liter container containing small pieces domestic organic waste with lid had been used to attract the adult flies to lay eggs. This container carried four holes (2 inch diameter) on the upper portion to allow adult flies. A cluster of corrugated cardboards of 4 inches long tied together and attached in a bamboo splits establishing in the wastes inside the container for laying eggs of BSF (Booth and Sheppard 1984). Two days later, egg clutches of BSF were collected with corrugated sheets for next studies. The experimental culture of BSF was conducted in the insectary of Biological Research Division, BCSIR in June to August, 2018. The experimental diets were grinding organic waste including spoiled fruits, potato and vegetables which was kept in a plastic container having cemented ladder from the bottom to collection pipe at angle of 45° that facilitates the self-harvesting of mature larvae or prepupae. The corrugated sheets with egg clutches were directly established in the experimental diets. Larvae were maintained at 29.40±1.77° C, RH 68.25±2.32 %, 14:10 (L: D) photoperiod. When larvae reached in the prepupae stage of development that they were collected for next experiment.

### Growth Chamber

The dark treatment was carried out in a wood box with a 1.5 × 1.5 × 2 ft which was positioned in a chamber to prevent light contamination when opening the growth chamber door during experimental observations and data collection. The inner part of ventilation and windows of growth chamber were covered by 1 cm thick paper boards and outer part of windows closed by wood cover which prevented both sides light penetration. The data of the 0 h light treatment were collected by night vision goggles in stealth mode (no visible light emitted). Other treatments were positioned another room in the same height wood boxes. This room had two windows with glass cover which allow light penetration.

### Experimental design

Collected post-feeding larvae were divided into four groups. The groups were treated with daylight durations of T1 = 0 h, T2 = 4 h, T3 = 8 h, and T4 = 12 h. Each group had ten replications and post-feeding larvae placed into a 500 ml clear plastic rectangular container. Each container contained 100 individuals and labeled. Containers monitored daily for pupation. Pupated larvae were transferred into another 500 ml clear plastic rectangular container with a clear and porous plastic lid<sup>[11]</sup> and kept respected growth chambers. Daily observed and record adult eclosion. Upon adult emergence, individual adults were sexed and monitored daily until death. Emerged adults were placed in pairs in ten sets of identical transparent plastic containers (10L) from different groups for determining their fecundity. This setups were positioned in the insectary with artificial lighting (60W) and 29.40±1.77° C, RH 68.25±2.32 % under a 14:10 (L:D) photoperiod where they could mate. The oviposition substrates (poultry diet mixed with water) with cardboard as attractant for females<sup>[2]</sup>. were provided in adult rearing containers. Cardboard strips were checked every day for egg masses. Egg clutches were collected and the eggs counted under a microscope.

### Data analysis

The observed data were analyzed using Statistical software, SPSS with version 22.0. Firstly, we calculated descriptive statistics, mean and standard deviation (SD). Then we compared the data by analysis of variance (ANOVA). Lastly, measuring of significance through Duncan Multiple Rank Test (DMRT) of Post Hoc series of tests were performed.

### Results and Discussion

Temperature and RH were same across T2, T3 and T4, but varied across T1 with other treatments ( $F = 20.512$ ,  $df = 3$ ,  $P < 0.05$  and  $F = 22.507$ ,  $df = 3$ ,  $P < 0.05$ , respectively, see Table 1). The T1 of daylight treatment was 1.44°C lower in temperature and 3.67% higher in RH compared to the other daylight treatments. Lastly, as a result of different day lengths in each treatment, light intensity measured in Lux was also different across treatments ( $F = 546906.785$ ,  $df = 3$ ,  $P < 0.05$ ). Specifically, the T1 treatment had the lowest light intensity compared to the other treatments. In this study, the light intensity increased with the duration of daylight which was similar with previous findings<sup>[11]</sup>. The effect of daylight on the mean time to reach adult stage varied among treatments (Table 2;  $F = 1172.334$ ,  $df = 3$ ,  $P < 0.05$ ) with Prepupae of T1 taking short time to pupate than

all other treatments. There was small difference in the length of the pupation stage prepupae placed between T1 and T2. T4 needed longest time to emerge adult among other treatments. T3 need less time than T4. Our current findings differed with previous investigation founding to pupate shortest time in 12 h daylight duration and longest time in 0 h daylight<sup>[11]</sup>. The obtained divergences may cause for in methodologies, experimental set-ups and temperatures at which the BSF larvae were reared. Holmes *et al.*<sup>[11]</sup> exposed larvae in daylight from egg to until death. Other study suggested the effects of day length on insect development inclined that insects develop faster at decreasing photoperiods resulting in diapausal adults as occurs in the eight-toothed spruce bark beetle, *Ips typographus* (L; Coleoptera: Scolytidae)<sup>[9]</sup>. However, another findings found that insect growth does not change while cultured under decreased day length and diapause is not initiated such as in *Orius niger* (Wolff; Hemiptera: Anthocoridae)<sup>[1]</sup>. However, these results may vary extremely depending on the experimental design. Phenotypic plasticity is another factor that may have stimulated the overall development of BSF. It has the ability of an organism to change its phenotype or to reform developmental events with changing in environmental conditions<sup>[13, 19]</sup>. "Phenotypic plasticity leads organismal diversification within species without having to couple it with speciation through the evolution of environment-specific responses in phenotype expression<sup>[19]</sup>. This may clear that the lower days requirements for black soldier fly larvae treated in 0 h and 4 h (Table 2), should black soldier flies undergo diapause and later development. In this study, we found that the effect of treatments on adult longevity differed between day length and sex ( $F = 50.956$ ,  $df = 3$ ,  $P < 0.05$  for male and  $F = 9.764$ ,  $P < 0.05$  for female) (Table 2) which is supported by previous investigation<sup>[11]</sup>. Here, we observed that there were no difference between adult longevity of T1 and T2 treatments, and between T3 and T4 treatments. Overall the differences of adult longevity among treatments were not prominent. These findings was similar with Holmes *et al.*<sup>[11]</sup>. In this study, adult emergence rate greatly depended on daylight ( $F = 452.634$ ,  $df = 3$ ,  $P < 0.05$ ) (Table 2) that the adult emergence percentage of T4 was lowest and highest in T1 among other treatments. There were no difference of adult emergence percentage between T1 and T2. These findings were not similar with previous findings<sup>[11]</sup>. In the same study<sup>[11]</sup>, the adult emergence rate was lowest in 0 h treatment and highest in 12 h treated prepupae. However, these results may vary due to the experimental design and methodology. The fecundity was lowest in T4 among treatments and highest in T1 (Table 2). The differences of fecundity between T1 and T2 were low but so far with T3 and T4. Some suggested that in stressful condition, female insects can resorb their eggs<sup>[23]</sup>, possibly ingesting nutrients. However, the exact cause of egg resorption is unknown<sup>[24]</sup>. Still there was no data on egg resorption in the black soldier fly, Tomberlin *et al.*<sup>[29]</sup> mentioned females typically develop a single clutch over their lifetime and in the absence of mating will oviposit infertile eggs<sup>[30]</sup>. Additionally, late oocyte development has reduced the realized fecundity<sup>[31]</sup>. When post-diapausing females decreased fecundity, feeding hibernal diapausing larvae can display extended fecundity in post-diapausal females<sup>[4, 6, 12]</sup>. These will help to explain our findings.

**Table 1:** Mean ( $\pm$ SD) for temperature, relative humidity and light intensity from each day length treatments.

Treatments	Temperature ( $^{\circ}$ C)	Relative Humidity (%)	Light intensity (lux)
T1	28.05 $\pm$ 1.19b	72.52 $\pm$ 2.70a	2.88 $\pm$ 0.46d
T2	29.49 $\pm$ 0.56a	68.85 $\pm$ 1.47b	5478.22 $\pm$ 11.94c
T3	29.49 $\pm$ 0.56a	68.85 $\pm$ 1.47b	7254.79 $\pm$ 39.75b
T4	29.49 $\pm$ 0.56a	68.85 $\pm$ 1.47b	8450.44 $\pm$ 24.96a

All statistical comparisons mean difference is significant at the 0.05 level\*.

**Table 2:** Mean ( $\pm$ SD) length of black soldier fly pupation time and percent successful of adult emergence across light treatments.

Treatments	First adult emergence (days)	Male Longevity (days)	Female Longevity (days)	Adult Emergence Percentage	Fecundity
T1	13.37 $\pm$ 1.43d	9.33 $\pm$ 1.52a	8.17 $\pm$ 1.15a	90.70 $\pm$ 3.71a	433.20 $\pm$ 29.65a
T2	15.17 $\pm$ 1.46c	9.17 $\pm$ 1.53a	8.03 $\pm$ .10a	82.70 $\pm$ 3.02b	416.20 $\pm$ 28.15b
T3	22.60 $\pm$ 2.357b	7.23 $\pm$ 1.01b	7.13 $\pm$ 1.04b	35.90 $\pm$ 10.42c	373.80 $\pm$ 53.28c
T4	56.93 $\pm$ 5.71a	6.80 $\pm$ 1.21c	7.10 $\pm$ 1.40b	6.00 $\pm$ 3.16d	239.40 $\pm$ 36.31d

All statistical comparisons mean difference is significant at the 0.05 level\*.

## Conclusions

In summary, the daylight duration has a significant effect on facilitating prepupae to pupate. In the complete darkness, prepupae took shortest time to pupate and high rate of successful adult emergence. For the aim of mass rearing, complete darkness is standard for black soldier fly prepupae to pupate which would be most suitable with significant pupation, high rate of adult emergence, high fecundity and longevity.

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