

**IMPACT OF COMBINATIONS OF DIET AND LARVAL REARING
TEMPERATURE ON DURATION OF LARVAL , PRE PUPAL AND PUPAL
STAGES OF BLACK SOLDIER FLY, *Hermetia illucens* L. (STRATIOMYIDAE:
DIPTERA)**

Abstract

As the world's population increases day by day, the amount of organic waste generated also increases posing huge great threat to mankind and the environment. The black soldier fly is known for its capacity to convert organic wastes to nutrient rich manure. Hence, the fly has been commercially grown world over and is used as an ingredient in poultry feed, animal/pet food and fish feed. The present study was carried out in the Black soldier fly breeding laboratory in Agricultural Research Institute, Rajendranagar, Hyderabad during 2023-24 with an aim to study the impact of diet and temperatures on the developmental periods of larvae, prepupae and pupae in a two factorial completely randomized design with four diets viz., chicken feed, sesamum cake, groundnut cake and Gainesville diet at five temperatures viz., 15⁰C, 20⁰C, 25⁰C , 35⁰C and 42⁰C. Results revealed that diet and temperature had significant impact on durations of larva, prepupa and pupa and the interactions of diet and temperature were also significant. The Gainesville diet at 35⁰C recorded least larval duration, Gainesville diet at 20⁰C, 25⁰C and 35⁰C recorded least larval duration while sesamum cake at 15⁰C recorded longest larval and prepupal duration. Shortest pupal period was noticed in groundnut cake at 15⁰C and longest pupal period in sesamum cake at 25⁰C.

1. Introduction

The world's population has increased sharply in recent decades and could reach 9.7 billion in 2050 (Anonymous, 2019). This has been causing around 931 million tons of food being wasted globally on a daily basis. Such wastage is mainly sent to the landfill (Grossule et al., 2020). The gas released from this landfill contributes to the greenhouse effect and global warming (Cammack and Tomberline, 2017, Scala et al., 2020). The use of Black Soldier Fly (*Hermetia illucens*) in organic waste composting is a novel and an environment friendly approach which holds enormous potential and therefore, is strongly captivating people's attention worldwide (Singh *et al.* 2019). The black soldier fly,

Hermetia illucens (Linnaeus, 1758) belongs to the subfamily Hermetiinae of the family Stratiomyidae. It is a synanthropic, polysaprophagous fly native to the Neotropics, but now found in every zoogeographic region (Marsha *et al.*, 2015). It is renowned as a bio-converter and as an important source of insect protein in aquaculture and livestock farming.

The fly has a dry weight that consists of approximately 50% crude protein (CP), up to 35% lipids and an amino acid profile comparable to fishmeal (Elwert *et al.*, 2010). Hence, large scale farming of the fly is now being done all over the world and the larval meal finds place as an ingredient in poultry feed, animal/pet food, fish/prawn feed. Many workers have reported that the bioconversion efficiency of the larvae is influenced by substrate type, nutritional content, moisture level, toxin presence, temperature and humidity, which all contribute to their growth and conversion capacity. Although the larva gut microbiome remains stable during development when larvae are reared on a uniform diet (Cifuentes *et al.*, 2020), its composition can change significantly when the diet is varied, as shown for BSFL reared on food waste, cooked rice or calf forage (Jeon *et al.*, 2011) and vegetable or fish meal (Bruno *et al.*, 2019). The BSFL microbiome is impacted by diet and other biotic and abiotic factors, explaining substantial differences between the microbiomes of larvae reared in different locations (Wynants *et al.*, 2019).

The present study was carried out to know the impact of a combination of temperatures and diets on the larval, prepupal and pupal characteristics and the outcome may help us understand the rearing conditions and larval diets better.

2. Materials and Methods

Rearing of Black soldier fly

Thrissur and Medipalle populations of black soldier fly were collected from various sources and reared in the laboratory by the rearing methods described by Sheppard *et al.* (2002) and Hoc *et al.* (2019) with slight modifications. The eggs obtained from both the adult populations were used for maintaining the subsequent population.

Crossing of populations

Medipalle male and Thrissur female were crossed. Male and female adults were separated based on the differences described by Julita *et al.*, (2020). The flies were collected in a test tube and released into net cages ensuring a ratio of 1:1 (male: female). The adult feed, attractant and eggies were provided to facilitate oviposition. Populations obtained from the eggs of the crossed population were reared for conducting the experiment further.

Studying the impact of diet and temperature on larval, pre pupaland pupal durations

A total of four larval diets were taken in combination with five different temperatures to study their impact on various life history traits. Each one of the larval diets including groundnut cake, chickenfeed, sesamum cake, and Gainesville diet was provided to larvae at temperatures *viz.*, 15⁰C, 20⁰C, 25⁰C, 35⁰C, and 42⁰C. The diets were prepared with 70 percent moisture and 5-day-old larvae were released into the trays and exposed to different temperatures.

Observations on larval duration, pre-pupal duration were noted. At pupal stage, they were harvested and reared under ambient temperature (25⁰C - 28⁰C) till they emerged as adult stage. The larval, pre-pupal and pupal, duration were measured. The experiment layout in factorial Completely Randomized Design (CRD). The data was analysed by using General R based Analysis Platform Empowered by Statistics (GRAPES) software and values presented as its mean± SE(m)

3. Results and Discussion

Larval period

Results revealed that the type of diet had significant impact on the duration of the larval period (Table 1). Gainesville diet and chicken feed recorded significantly lesser larval duration (16.58±7.00 and 17±7.12days respectively), groundnut cake treatment registered 21.00±9.30 days and sesamum cake showed significantly highest larval duration 28.4±11.43 days. Temperature was also found to have a profound effect on duration of the larval period. At 35⁰C temperature, significantly shorter larval duration was observed (18.50±2.04 days) followed by 25⁰C (23.00±2.41 days) and 20⁰C (28.00±1.91 days). Longest larval duration was registered at 15⁰C (34.2±2.68days).

Interaction effects of diet and temperature were also significant (Fig 1) with the Gainesville diet at 35⁰C recording significantly least larval duration of (13.5±0.50 days) followed by the Chicken feed diet at 35⁰C (15.5±0.50). Significantly longest duration of

43.5±0.50 days was observed in sesamum cake at 15°C, while rest of the treatments registered between 17.50±0.50 and 37.5±0.50days.

Higher temperatures hasten the life cycle of insects by impacting the metabolic processes. However, at 42°C the temperature had a detrimental effect and all the larvae died in less than a day as temperature in the diet tray increased by 8°C to 10°C compared to the air temperature.

Table 1. Interaction effect of diet and temperature on larval period

Larval period						
Diet	Temperature					Mean
	15°C	20°C	25°C	35°C	42°C	
Groundnut cake	37.50±0.50 ^b	28.5±0.50 ^d	21.5±0.50 ^g	17.5±0.50 ^h	NA	21.00±9.30 ^b
Chicken feed	28.5±0.50 ^d	22.5±0.50 ^{fg}	18.5±0.50 ^h	15.5±0.50 ⁱ	NA	17.00±7.12 ^c
Sesamum cake	43.50±0.50 ^a	37.5±0.50 ^b	33.5±0.50 ^c	27.5±0.50 ^{de}	NA	28.40±11.43 ^a
Gainsville diet	27.4±0.50 ^e	23.5±0.50 ^f	18.5±0.50 ^h	13.5±0.50 ^j	NA	16.58±7.00 ^c
Mean	34.20±2.68 ^a	28.00±1.91 ^b	23.00±2.41 ^c	18.50±2.04 ^d	NA	
Factors	SE _(m) (±)		CD (p=0.05)		CV (%)	
A factor	0.22		0.64		3.33	
B Factor	0.24		0.72		3.33	
AXB Interaction	0.49		1.44		3.33	

Our results are in conformity with those of Shumo et al., (2019) who reported that both temperature and substrate type significantly influenced BSF larval development, with the Spent Grain-fed BSF larvae needing significantly less time (to reach prepupal stage in the temperature treatments tested. The time needed for larval development decreased gradually with the increasing temperatures and was the shortest at 30 °C for the SG-fed larvae (24.700 ± 3.590 days) and 35°C for the Cowdung -fed larvae (83.400 ± 4.040 days). Fazli et al (2021) also registered longest (30 days) and the shortest (13 days) larvae growth periods were recorded at 20°C and 30°C respectively.

NA-Not Applicable,

Treatments with same letter are not significantly different

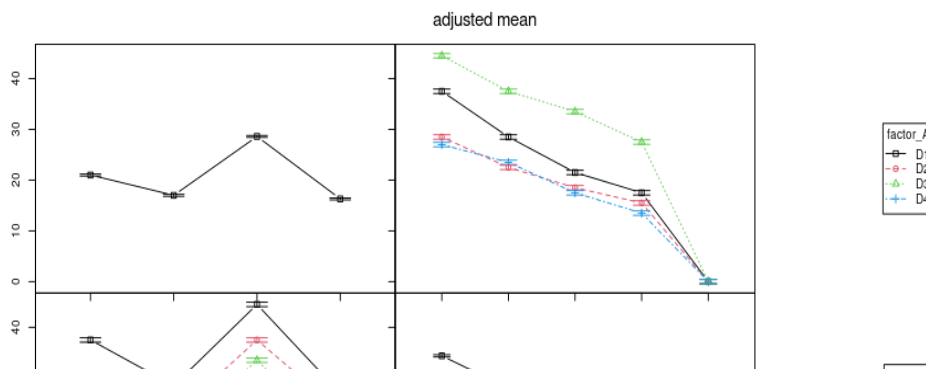


Fig 1. Graph representing interaction effect of diet and temperature on larval period

Prepupal period

It was evident from the results (Table 2) that diet has exerted a significant impact on the duration of pre-pupal period. The highest period was registered in sesamum cake with 7.49 ± 0.24 days. The duration of prepupa was observed to be lesser in Gainsville diet (5.30 ± 0.15 days) than any other diets tested. It was also observed that temperatures provided during the larval period have a significant impact on the pre-pupal duration. It was recorded to be highest and on par for both the pre-pupal stages (8.275 ± 0.45 , 8.162 ± 0.46 days respectively) exposed to 15°C and 25°C . Lowest pre-pupal duration was noticed at temperature 35°C (7.55 ± 0.93 days).

There were also significant interactions between diet and temperature on the duration of pre-pupal period (Fig 2). The pre-pupae of the larvae that were fed with Gainsville diet and exposed to 20°C , 25°C and 35°C temperatures recorded least pre-pupal duration (6.30 ± 0.20 , 6.35 ± 0.25 , 6.6 ± 0.10 days respectively), while longest pre-pupal period was observed in sesamum cake at 15°C (10.3 ± 0.10 days) followed by sesamum cake at 25°C .

Similar results were reported by Fazli *et al* (2021) who noted that pre-pupal growth duration was obtained as 35 days at 20°C , 14 days at 25°C , 10 days at 30°C , and 20 days at 35°C . The fastest and lowest pre-pupal growth paces were recorded at 20°C (35 days) and 30°C (10 days), respectively.

Table 2. Interaction effect of diet and temperature on prepupal period

Prepupal period (Days)						
Diet	Temperature					Mean
	15°C	20°C	25°C	35°C	42°C	
Groundnut cake	8.10±0.10 ^{ef}	8.35±0.15 ^{de}	8.85±0.15 ^c	7.75±0.15 ^{fgh}	NA	6.61±0.16 ^b
Chicken feed	7.45±0.05 ^{hi}	7.70±0.10 ^{gh}	7.85±0.15 ^{fg}	7.00±0.20 ^j	NA	6.00±0.13 ^c
Sesamum cake	10.30±0.10 ^a	8.70±0.10 ^{cd}	9.60±0.15 ^b	8.85±0.15 ^c	NA	7.49±0.24 ^a
Gainsville diet	7.25±0.05 ^{ij}	6.30±0.20 ^k	6.35±0.25 ^k	6.60±0.10 ^k	NA	5.30±0.15 ^d
Mean	8.27±0.45 ^a	7.76±0.35 ^b	8.16±0.46 ^a	7.55±0.00 ^c	NA	
Factors	SE _(m) (±)		CD (p=0.05)		CV(%)	
A factor	0.06		0.17		2.84	
B Factor	0.06		0.19		2.82	
AXB Interaction	0.13		0.38		2.82	

NA-Not Applicable

Treatments with same letter are not significantly different

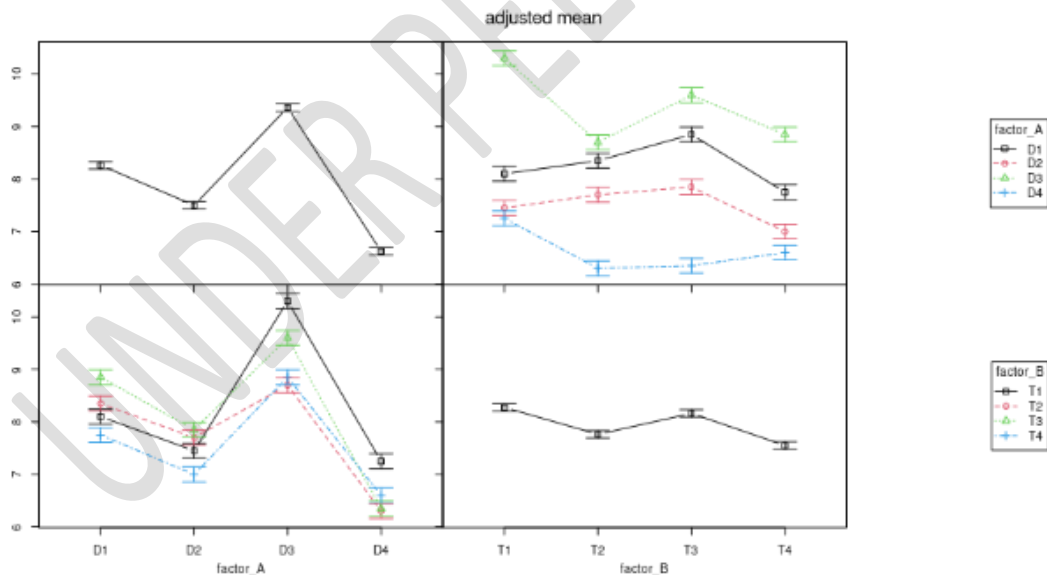


Fig 2. Graph representing interaction effect of diet and temperature on pre-pupal period

Pupal period

The duration of pupae formed from the larvae fed with diets differed significantly (Table 3). Pupae harvested from the sesamum cake exhibited the longest duration 7.72 ± 0.32 days, while shortest pupal duration was seen in the groundnut cake treatment (6.19 ± 0.15). The longest pupal duration was recorded at temperature 25°C (9.57 ± 0.38 days), while shortest duration (8.01 ± 0.21 days) was seen in the pupae from the larvae exposed to 35°C .

It was observed that there was significant difference in the interaction effect of temperature and larval diets on pupal duration (Fig 3). The shortest pupal duration (7.25 ± 0.15 days) was observed from the pupae that were formed from the groundnut cake at 15°C , while longest pupal period was observed in the pupae of larvae obtained from the sesamum cake at 25°C (11.1 ± 0.20 days).

Similar results were reported by Shumo *et al* (2019) who observed that pupal developmental time decreased gradually with increasing temperatures and was shortest at 35°C (48.256 ± 15.490) and 30°C (13.000 ± 2.110) for prepupae reared on Cowdung and Spent Grain substrates, respectively. Fazli *et al* (2021) also found that the pupal growth period was found to be 15 days at 20°C , 9 days at 25°C , 7 days at 30°C , and 6 days at 35°C .

Table 3. Interaction effect of diet and temperature on pupal period

Pupal period (Days)						
Diet	Temperature					Mean
	15°C	20°C	25°C	35°C	42°C	
Groundnut cake	7.25 ± 0.15^h	7.70 ± 0.20^{fg}	8.3 ± 0.20^e	7.70 ± 0.1^{fg}	NA	6.19 ± 0.15^c
Chicken feed	8.05 ± 0.15^{ef}	8.25 ± 0.05^e	9.25 ± 0.15^{cd}	7.85 ± 0.15^{fg}	NA	6.68 ± 0.20^b
Sesamum cake	9.35 ± 0.15^{bc}	9.20 ± 0.10^{cd}	11.10 ± 0.2^a	8.90 ± 0.05^d	NA	7.72 ± 0.32^a
Gainsville diet	8.35 ± 0.15^e	8.30 ± 0.1^e	9.60 ± 0.15^b	7.55 ± 0.15^{gh}	NA	6.77 ± 0.29^b
Mean	8.25 ± 0.28^b	8.36 ± 0.20^b	9.57 ± 0.38^a	8.01 ± 0.21^c	NA	
Factors	SE _(m) (\pm)		CD (p=0.05)		CV (%)	
A factor	0.058		0.17		2.68	
B Factor	0.065		0.19		2.68	
AXB Interaction	0.120		0.38		2.68	

NA-Not Applicable,

Treatments with same letter are not significantly different

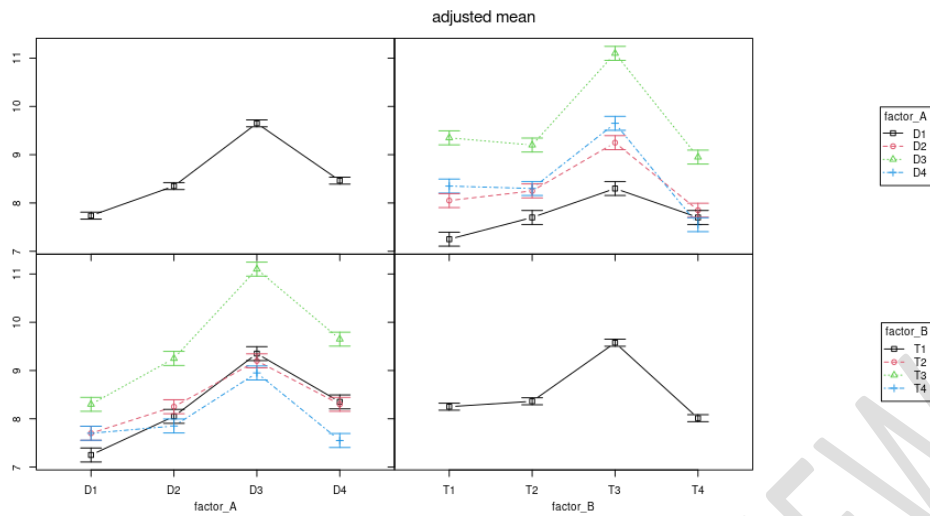


Fig .3. Graph representing interaction effect of diet and temperature on pupal period

Conclusion

Our study helped to understand the durations of larval, prepupal and pupal periods at wide range of temperatures and various locally available diets viz., chicken diet, sesamum and groundnut cake. Oilcakes recorded lesser durations in the insect as they suffice protein requirement, and help the insect to reach the next stage earlier, thus shortening its life cycle. Cammack and Tomberline (2017) reported that protein and carbohydrates in diet fed to the larvae of black soldier fly had a positive impact on its larval development. The findings of the present study would help Black soldier fly researchers and commercial growers to know the blend of rearing temperature and diet for higher larval and pupal yields.

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