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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)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 and in the process the larvae accumulate proteins from the wastes. Hence, the fly finds its place as an ingredient in poultry feed, animal/pet food and fish feed. Since the fly is reared commercially, studies on rearing conditions would help scientists and the industry. The present study was carried out in the Black soldier fly breeding laboratory in Agricultural Research Institute, Rajendgranagar, 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 Gainsville diet at five temperatures *viz.*,15^{0}C, 20^{0}C, 25^{0}C, 35^{0}C and 42^{0}C. 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 Gainsville diet at 20^{0}C,25^{0}C and 35^{0}C recorded least larval duration while sesamum cake at 15^{0}C recorded longest larval and prepupal duration. Shortest pupal period was noticed in groundnut cake at 15^{0}C and longest pupal period in sesamum cake at 25^{0}C.

Keywords: Diet combinations; population; larval rearing temperature; black soldier fly.

1. INTRODUCTION

The world's population has increased sharply in recent decades and could reach 9.7 billion in 2050 [1]. 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 [2]. The gas released from this landfill contributes to the greenhouse effect and global warming [3,4]. 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 [5]. 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 [6]. 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 [7]. 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 [8,9]. Although the larva gut microbiome remains stable during development when larvae are reared on a uniform diet [10], its composition can change significantly when the diet is varied, as shown for Black Soldier Fly Larvae (BSFL) reared on food waste, cooked rice or calf forage [11] and vegetable or fish meal [12].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 [13].

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 my help us understand the rearing conditions and larval diets better. This manuscript is significant for the scientific community as it provides valuable insights into optimizing the rearing conditions of the black soldier fly, an important species for waste management and sustainable protein production. By elucidating the effects of diet and temperature on the developmental stages of *Hermetia illucens*, the study contributes to improving the efficiency of organic waste conversion and insect farming practices.

2. MATERIALS AND METHODS

2.1 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. [14] and Hoc et al. [15] with slight modifications. The eggs obtained from both the adult populations were used for maintaining the subsequent population.

2.2 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. [16]. The flies were collected in a test tube and released into net cages ensuring a ratio of 1:1 (male: female). 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.

2.3 Data Evaluation and Analysis

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 Gainsville diet was provided to larvae at temperatures *viz*., 15^0C, 20^0C, 25^0C, 35^0C, and 42^0C. The diets were prepared with 70 percent moisture and 5-day-old larvae were released into the trays and exposed to different temperatures for whole larval period.

Observations on larval duration, pre-pupal duration were noted. At pre-pupal stage, they were harvested and reared under ambient temperature (25^0C - 28^0C) 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 AnalysisPlatform Empowered by Statistics (GRAPES) software (version 1.0.0) and values presented as its mean± SE(m).

3. RESULTS AND DISCUSSION

3.1 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 35°C temperature, significantly shorter larval duration was observed (18.50±2.04 days) followed by 25°C (23.00±2.41 days) and 20°C (28.00±1.91 days). Longest larval duration was registered at 15°C (34.2±2.68days).

Interaction effects of diet and temperature were also significant (Fig. 1) with the Ganisville diet at 35°C recording significantly least larval duration of (13.5±0.50 days) followed by the Chicken feed diet at 35°C (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 metabolc processes. However, at 42^0C 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 (Days)						
	Temperature					Mean
Diet	**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	

NA-Not Applicable,
Treatments with same letter are not significantly different

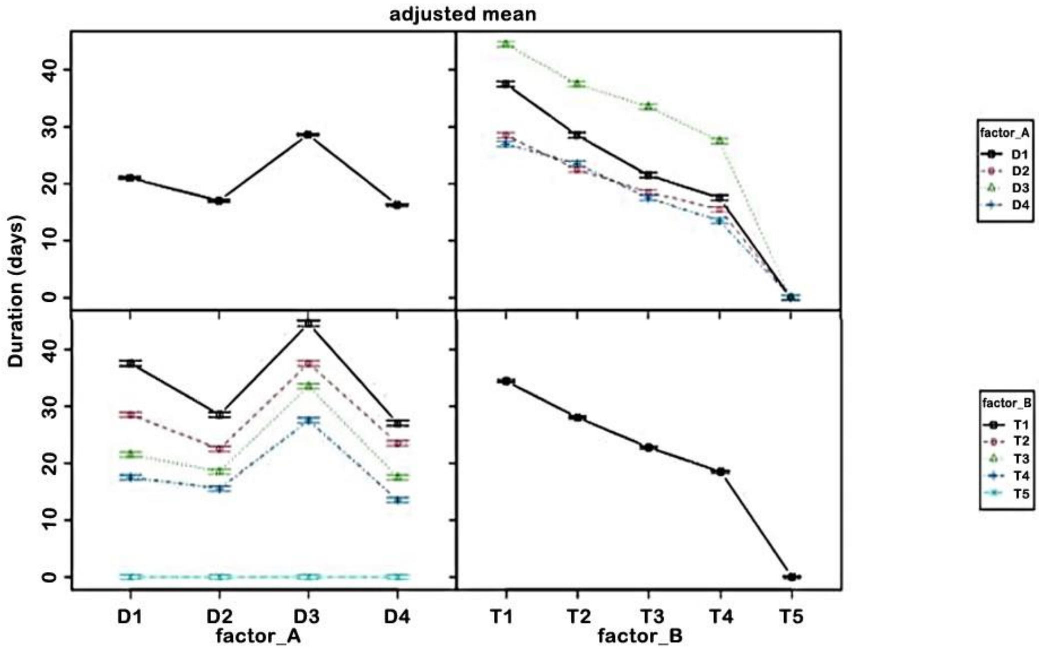

Fig. 1. Graph representing interaction effect of diet and temperature on larval period

Our results are in conformity with those of Shumo et al. [17] who reported that both temperature and substrate type significantly influenced BSF larval development, with the Spent Grain-fed 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. [18] also registered longest (30 days) and the shortest (13 days) larvae growth periods were recorded at 20°C and 30°C respectively.

3.2 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±0.24days. The duration of prepupa was observed to be lesser in Gainsville diet (5.30±0.15days) than any other diets tested. It was also observed that temperatures provided during the larval period had 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).

Interactions between diet and temperature were found to significantly impact 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±0.10days) followed by sesamum cake at 25°C.

Similar results were reported by Fazli et al. [18] 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.

3.3 Pupal Period

The duration of pupae formed from the larvae fed with diets differed significantly (Table 3). Pupae harvested from 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 35°C treatment.

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

Prepupal period (Days)						
	Temperature					Mean
Diet	**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

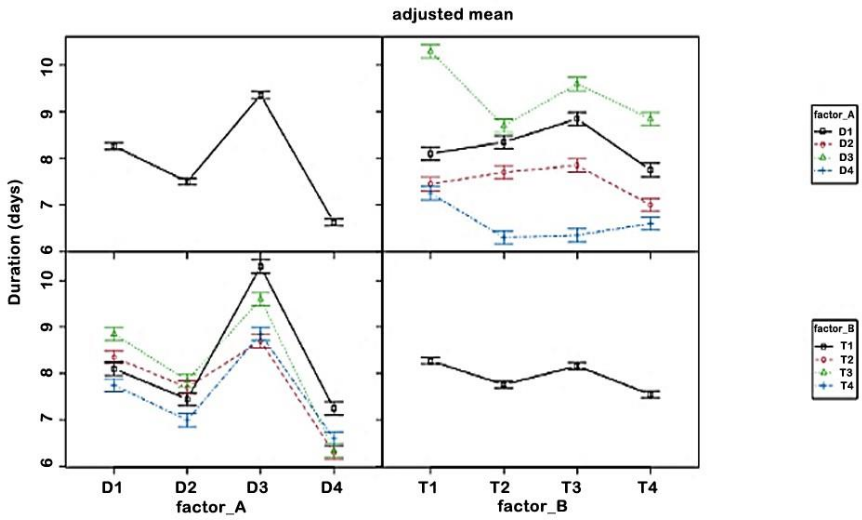

Fig. 2. Graph representing interaction effect of diet and temperature on pre-pupal period

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

Pupal period (Days)						
	Temperature					Mean
Diet	**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±0.15c^{d}	7.85±0.15fg	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±0.15e	8.30±0.1e	9.60±0.15b	7.55±0.15gh	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 $_{(m)}$ (±)		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

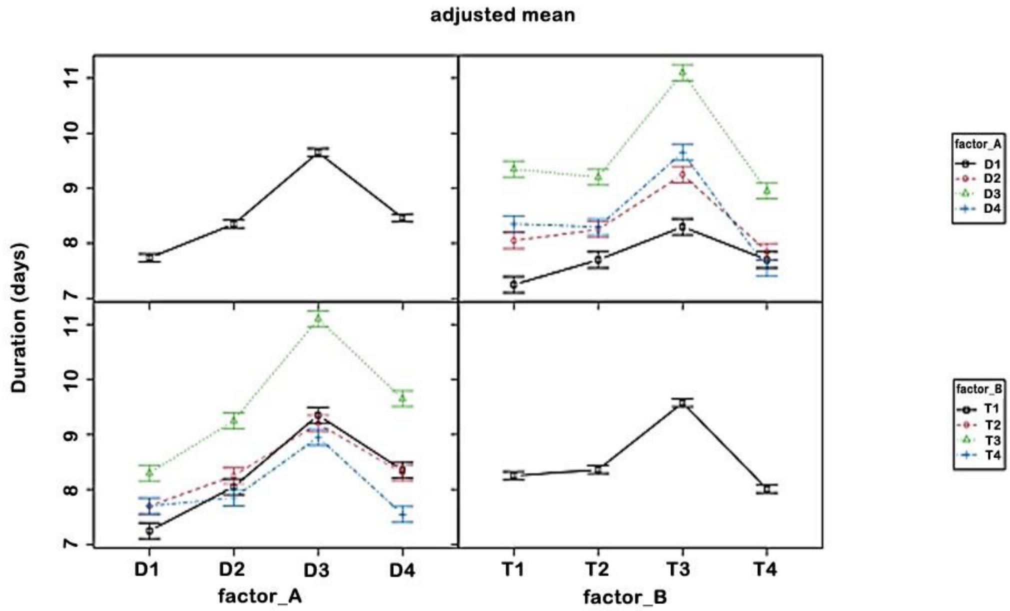

Fig. 3. Graph representing interaction effect of diet and temperature on pupal period

It was observed that interaction effect of temperature and larval diets impacted duration of pupal period significantly (Fig. 3). The shortest pupal duration (7.25±015 days) was observed from the pupae in the groundnut cake at 15°C treatment, 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. [17] 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. [18] 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 [19].

4. 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 is 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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