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Comparison of the Life Cycle and Bioconversion Efficiency of Medipalle (Telangana) and Thrissur (Kerala) Populations of Black Soldier Fly (*Hermetia illucens*) (Stratiomyidae: Diptera)

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ABSTRACT: The black soldier fly is an ideal insect for waste treatment as it offers an ecofriendly solution for converting waste to nutrient-rich manure while also providing a reliable source of protein, fat, fibre, lauric acid, calcium and other minerals, helping alleviate rising global demand for poultry and animal feed. The soldier flies are found occurring naturally throughout the country. The present study was carried out in the laboratory between November 2022 to February 2023 to study and compare the life cycle, morphometrics and bioconversion efficiency of two geographically different populations *viz*. Medipalle (Telangana) and Thrissur (Kerala) populations of black soldier fly. Results revealed that duration of larval period (21.57 ± 0.79 days), prepupal period (8.60 ± 0.79 days), pupal period (7.30 ± 0.49 days) and total life period (53.86 ± 1.07 days) was significantly lesser in the Thrissur population (19.57 ± 0.79 days, 7.00 ± 1.29 days, 6.40 ± 0.53,48.57 ± 2.51 days respectively) compared to the Medipalle population. Length of 10, 14 and 18day old larvae was significantly higher in Thrissur population (1.82 ± 0.08 cm, 1.89 ± 0.06 cm, 2.01 ± 0.05 cm), whereas in Medipalle population lengths were 1.31 ± 0.08 cm, 1.62 ± 0.06 cm, 1.70 ± 0.04 cm respectively. Thrissur population was found to be more robust than Medipalle population with length of prepupae and weight of pupae significantly higher (1.90 ± 0.09 cm and 0.12 ± 0.013g/pupa respectively) whereas in Medipalle population it was 1.69 ± 0.03 cm and 0.04 ± 0.006 g/pupae respectively. Crude fat content (%) and ash content (%) were significantly higher in Thrissur population (28.29 ± 0.05%,7.43 ± 0.01% respectively) compared to Medipalle population (20.23 ± 0.07%, 5.97 ± 0.005 % respectively). Thus, it could be concluded that Thrissur population of Black soldier fly held more promise for commercial farming systems owing to its shorter life cycle, heavier larvae, prepupae and pupae and enhanced crude fat and ash content.

Keywords: Black soldier fly, bioconversion, waste management, lifecycle, proximate.

INTRODUCTION

Annually, a third of all food produced (1.7 billion metric tons) is lost or wasted along the food chain, which has a significant environmental (3.3 billion metric tons of CO2-equivalent greenhouse gas (GHG) emissions per year) and economic (total social costs of $ 1.2 trillion per year) footprints (Scialabba *et al*., 2014). Moreover, high moisture and nutrient contents, biological instability due to biodegradation and enzymatic activity, and the potential presence of pathogens make the organic waste management more challenging in comparison to other waste streams (Russ and Meyer-Pittroff 2004; Varelas, 2019; Yin *et al*., 2014). Cities of low and middle-income countries face severe challenges in managing the increasing amount of waste produced, especially the organic fraction. Black Soldier Fly (BSF) biowaste treatment is an attractive treatment option as it offers a solution for waste management while also providing a protein source to help alleviate the rising global demand for animal feed (Mertenat *et al*., 2019). The black soldier fly, *Hermetia illucens* (Linnaeus, 1758) belongs to family Stratiomyidae under Order Diptera and is a significant organic waste management agent as well as a domestic animal feed supplement.

Treatment of biodegradable waste using the black soldier fly larvae (BSFL) is an emerging waste treatment technology attracting entrepreneurial activities around the globe, mainly because the larvae grown on the waste have the potential to become starting material for several commercial products such

as animal feed, biodiesel, chitin as a biopolymer, and soil fertilizer (Purkayastha and Sarkar 2022). The larvae are also rich source of proteins and lipids and can be used as diets or diet components for animals such as pigs, fish, and poultry. Ebeneezar *et al*. (2021) reported that crude protein content was 41.44±0.17 g per100g, and the crude lipid content was 35.69±0.24 g per 100 g. Calcium (22.95 ± 0.91g per kg) was the most abundant mineral content in Black soldier fly larva, followed by phosphorus (5.47 ± 0.06g per kg) and potassium (4.78 ±0.081g per kg). Fatty acid profile from the oil revealed a larger concentration of saturated fatty acids than unsaturated acids with the highest concentration of Lauric acid (C12:0).

The black soldier fly offers a cheaper alternative to the protein sources used for animal and poultry diets around the world and hence has gained popularity among entrepreneurs. Commercially, the insect is reared throughout the country as a component of animal feed or directly as animal feed, as a source of lipids, carbohydrates, chitinase and the nutrient-rich frass it produces. The fly is amenable to rearing in the laboratory. Sharanabasappa *et al.* (2019) evaluated the biology of the black soldier fly on muskmelon fruit and found that the incubation, total larval, and pupal periods were 5-7, 25-30, and 10-60 days, respectively. In a single clutch, 555-650 eggs were deposited, with a hatchability of 40-70%. Male and female life cycles lasted 47-107 and 57-120 days, respectively.

Populations that occur close to each other are more alike, linked by greater amounts of gene exchange, than populations that occur further apart; that is the probability of causing gene flow by dispersion decreases with distance between the source and recipient populations(sink) (Bayne,2017). Hence, the present study was carried out to understand the biology and bioconversion efficiency of two geographically isolated populations (Medipalle, Telangana and Thrissur, Kerala) of black soldier fly.

MATERIALS AND METHODS

The studywas carried out in the laboratory of AICRP on Biological Control for Crop Pests and Diseases, ARI, Rajendranagar, PJTSAU, Hyderabad, Telangana during 2022-23. Fly population was collected from a black soldier fly farmer from Medipalle village in Rangareddy district of Telangana state and this was designated as "Medipalle population". Rearing methods of black soldier fly were adopted mainly from Sheppard *et al*. (2002) and Hoc *et al.* (2019) with some modifications.

Maintenance of BSF population. The insects were maintained at 26-28 ^{0}C temperature and 70 % RH in a glass house attached to the laboratory. Eggs collected were incubated in open petri dish placed above nursery feed (Gainesville diet 70 % moisture). Standard diet used Gainesville diet for rearing of Black soldier fly larvae for scientific experiments in laboratory condition. Constituents of Gainesville diet are Alfalfa meal (30 %): wheat bran (50 %) and Corn meal (20 %). To incubate 1 g of egg, 200 g GV diet (70 % moisture) was prepared by mixing 30g of wheat bran, 18g of alfalfa meal and 12g of cornmeal in 140 ml of water (Hogsette, 1992). After 5 days of hatching, 6 day old larvae were separated and the average weight of 6 day old larvae were recorded. For maintaining larval stock, Gainesville diet was prepared and placed in plastic trays (50cm ×30cm×15cm). In each tray 3 kg of feed were placed and 3000 6 day old larvae were released for feeding. After 5 days again 3 kg of feed was added. When 90 % population transformed into pre-pupae, the frass and undigested material were separated (Diener *et al*., 2009; Yang and Tomberlin 2020).

Separated pre-pupae were pupated in moist cocopeat placed in plastic tray and kept inside the cage made of mosquito nets. After adults emerged, water trays (for moisture and humidity), attractant (mixture of 100g dead flies, 200g nursery residue, 200g of old attractant residue in 1 L of water) and egg collecting devices (Eggies) were kept for oviposition (Dortmans *et al.,* 2021). After egg laying, eggies were removed from the love cage (net cages), eggs were scrapped and incubated for next generation.

One gram eggs of Thrissur Black soldier fly population were collected from Black Soldier Fly farmer in Palakkad district of Thrissur and the population was maintained in the same way as the Medipalle population.

Experimental set up. Seven rearing trays per treatment were taken and 3 kg of diet was added to the trays in which 3000 larvae (6 DOL) were released. After 6 days 3 kg diet/tray was added and reared until it reached pre-pupal stage. After pre-pupation, 100g pre-pupae from each replication were separated, washed with tap water and oven dried (84°C for 4 hours) and preserved at -20°C for proximate analysis. Remaining pre-pupae were reared individually in test tubes provided with cocopeat to study the pre-pupal and pupal period. After emergence, 10 pair of adults were released in rearing cage, provided with oviposition attractant and egg collection device to study adult life characteristics. Eggs were collected from rearing cage within 24 hours of egg laying and incubated for hatching.

Observations on the following parameters were taken:

1. Incubation period (days): number of days needed for hatching of eggs from the date of laying.
2. Larval period (days): number of days needed for transformation of 50 % larvae to prepupa from hatching.
3. Pre-Pupal period (days): pre-pupa changed to pupae with curved abdomen.
4. Pupal period (days): no of days required for the adult emergence from pupae.
5. Adult longevity (days): total number of days the adult flies were alive.
6. Fecundity: Total number of eggs laid by a female during its life span.
7. Adult emergence (%): (Number of adults emerged /Total number of pupae×100)
8. Hatching percentage (%): (Number of hatched eggs/total number of eggs).
9. No. of egg clutches laid by the female in each net cage were recorded for mating experiment.

10. Morphometric observations on the following parameters were taken for Medipalle and Thrissur population:
11. Length of the larvae (6 DOL,10 DOL,14 DOL,18 DOL), length of prepupae and pupae, Weights of the larvae (6 DOL,10 DOL,14 DOL, 18 DOL), prepupae and pupae were taken.
12. Total life period of Medipalle and Thrissur populations.
13. Proximate analysis of pre-pupal samples of Thrissur and Medipalle was done using standard protocol for bioconversion efficiency for the following parameters in Central Instrumentation Cell, College of Agriculture, Rajendranagar.
i. Moisture (Horwitz and Latimer, 2005)
ii. Ash (Horwitz and Latimer, 2005)
iii. Crude protein (Horwitz and Latimer, 2005)
iv. Crude fat (Horwitz and Latimer, 2005)
v. Crude fibre (AOAC, 1995)

Preparation of material for proximate analysis. 100 grams of prepupae were collected from each 7 replications per treatment and were placed in glass beakers with hot water for 30 min to kill them. After sometime the killed prepupae were shade dried. Next day the prepupae were kept in hot air oven at 55 °C for 6 hours. Then they were removed and ground to fine powder to carryout proximate analysis.

RESULTS AND DISCUSSION

Results revealed that incubation period of eggs in both the populations was statistically similar (5.60 ± 0.79 days). Duration of larval period (21.57 ± 0.79 days), prepupal period (8.60 ± 0.79 days), pupal period (7.30 ± 0.49 days), total life period (53.86 ± 1.07 days) was significantly higher in Medipalle population than in the Thrissur population (19.57 ± 0.79 days, 7.00 ± 1.29 days, 6.40 ± 0.53,48.57 ± 2.51 days respectively).This indicated that Thrissur population was more amenable for the commercial production units than the Medipalle population though duration of adult stages did not differ significantly between Medipalle (11.20 ± 1.64 days) and Thrissur populations (10.00 ± 0.71 %).Adult emergence and fecundity were on par in Medipalle (72.00 ± 17.89 % and 582 ± 166.66 respectively) and Thrissur population (68.00 ± 22.80 % and 570 ± 79.67 respectively). Egg hatching (%) was significantly higher in the Medipalle population (42.00 ± 8.00 %) than in the Thrissur population (30.00 ± 2.00 %) (Table 1). Samayoa *et al*. (2016) noticed that incubation period, larval duration, prepupal duration, pupal duration and fecundity of Black soldier fly was 3.00 ± 0.00 days, 23.03 ± 0.46 days, 10.94 ± 0.46 days,10.69 ± 0.29 days, 259.90 ± 98.85 when fed on artificial diet. Harnden and Tomberlin (2016) observed that black soldier fly hatching to prepupal duration on Gainesville diet was 4969.2 ± 228.5 Accumulated degree hours (ADH) at 32.2 ºC, 5928.0 ± 0.0 ADH at 27.6 ºC, 5237.4 ± 0.0 ADH at 24.9 ºC and pupa to adult duration was 2747.2 ± 228.5 ADH at 32.2 ºC, 2870.4 ± 176.5 ADH at 27.6ºC, 3715.2 ± 0.0 ADH at 24.9ºC. Franco *et al*. (2022) observed that black soldier fly larvae fed on Gainesville diet had lowest larval duration of 13-14 days compared with mature dairy manure (25 days) and fresh dairy manure (20 days). Miranda *et al*. (2020) observed that time required for prepupation of Black soldier fly when fed on Gainesville diet was 13 days. Tomberlin *et al*. (2002) reported that black soldier fly adult longevity when fed on Gainesville diet was 9.3 ± 0.4 days for male while for female it was 7.9 ± 0.2 days while the adults provided with water.

Length and weight of larva. It was observed that length of 6 day old larvae (DOL) was significantly higher in Medipalle population (0.51 ± 0.04 cm) compared to Thrissur population (0.39 ± 0.04 cm) (Table 2). But length at 10, 14 and 18day old larvae was significantly higher in Thrissur population (1.82 ± 0.08 cm, 1.89 ± 0.06 cm, 2.01 ± 0.05 cm), whereas in Medipalle population lengths were 1.31 ± 0.08 cm, 1.62 ± 0.06 cm, 1.70 ± 0.04 cm respectively. Moreover, though weight of the 6 day old larvae did not differ significantly between Medipalle (0.005 ± 0.001g/larvae) and Thrissur population (0.004 ± 0.00g/larvae), weight of the 10, 14 and 18 DOL was significantly higher in Thrissur population (0.14 ± 0.010 g per larvae, 0.23 ± 0.023 g/larvae ,0.20 ± 0.022 g/larvae) than in Medipalle population (0.062 ± 0.012g/larvae, 0.121 ± 0.010g/larvae, 0.120 ± 0.007g/larvae respectively) indicating better yield of larvae in the Thrissur culture. (Table 2 and 3). Harnden and Tomberlin (2016) observed that length of black soldier fly larvae fed on grain diet (Gainesville diet) was 16.53 ± 0.49 mm at 32.2°C, 18.78 ± 1.24 mm at 27.6°C, 16.79± 0.60 mm at 24.9°C. Tomberlin *et al*. (2002) observed that final larval weight of Black soldier fly fed on Gainesville diet was 0.157 ±0.077 grams. Miranda *et al*. (2020) observed that larvae reared on Gainesville diet reached the maximum weight (180 mg) on 7th day.

Length and weight of prepupae and pupae. Results revealed that weights of prepupae and pupae was significantly higher in Thrissur population (0.16 ± 0.017 g/prepupa and 0.12 ± 0.013g/pupa respectively) while in Medipalle population they were 0.10 ± 0.006g/prepupa and 0.04 ± 0.006 g/pupae respectively. Length of pupae was significantly higher in Thrissur population (1.87 ± 0.06 cm) whereas in Medipalle population it was 1.61 ± 0.04 cm respectively. The length of prepupae was significantly higher in Thrissur population (1.90 ± 0.09 cm) whereas in Medipalle population it was 1.69 ± 0.03 cm respectively suggesting that the Thrissur population was more robust than the Medipalle population highlighting its suitability for commercial farming units (Table 2 and 3).

Tomberlin *et al.* (2002) observed that weight of black soldier fly prepupa fed on Gainesville diet was 0.104 ± 0.027 g. Saadoun *et al.* (2020) observed that mean weight of prepupae when fed with Gainesville diet was 0.22 ± 0.01 g. Meneguz *et al.* (2018) observed that pupal weight of Black soldier fly which fed on Gainesville diet at pH 4.0 was highest 0.100 ± 0.0096 g, compared with pH 6.1 (0.094 ± 0.0120 g), pH 7.5 (0.094 ± 0.0160 g), pH 9.5 (0.095 ± 0.0012 g).

Proximate. Data obtained revealed that crude protein content (%) and crude fibre content (%) did not significantly differ between Medipalle population (40.33 ± 0.08%,26.57 ± 0.05% respectively) and Thrissur population (41.16 ± 0.06 %,29.71 ± 0.02 % respectively.). Crude fat content (%) and ash content (%) were significantly higher in Thrissur population (28.29 ± 0.05%,7.43 ± 0.01% respectively) compared to Medipalle population (20.23 ± 0.07%,5.97 ± 0.005 % respectively). However, moisture content (%) was significantly higher in Medipalle population (51.14 ± 0.03 %) while in Thrissur population was 42.29 ± 0.02 % respectively. Thus, it could be concluded that Thrissur population of black soldier fly held more promise for commercial farming owing to its shorter life cycle, better weight of larvae, prepupae and pupae and enhanced crude fat and ash content (Table 4). Arabzadeh *et al*. (2022) reported that Black soldier fly larvae reared on the Gainesville diet had a crude protein of 51.66 ± 0.24 per cent, crude ash content of 9.60 ± 0.09 %, crude lipid up to 17.26 ± 1.81 per cent. Pliantiangtam *et al.* (2021) showed that Black soldier fly crude protein content when reared on the Gainesville diet was 48 per cent for both larvae and prepupae, crude fat was 20 % for larvae,24 % for prepupae and ash content was 15 % for larvae, 16 % for prepupae. Zulkifli *et al.* (2022) observed that oven dried Black soldier fly larvae had a crude protein content of 47.46 ± 0.14 % and crude fibre of 9.48 ± 0.04 % and moisture content of 3.21 ± 0.03 per cent.

Table 1: Durations of life stages of Medipalle and Thrissur populations of Black soldier fly.

Population	Incubation period (days)	Larval duration (days)	Prepupal duration (days)	Pupal duration (days)	Adult duration (days)	Total life period (days)	Fecundity (no of eggs/ female)	Adult emergence (%)	Hatching percentage (%)
Medipalle	5.60±0.79	21.57±0.79	8.60±0.79	7.30±0.49	11.20±1.64	53.86± 1.07	582±166.66	72.00±17.89	42.00 ±8.00
Thrissur	5.60±0.79	19.57±0.79	7.00±1.29	6.40±0.53	10.00±0.71	48.57±2.51	570±79.67	68.00±22.80	30.00±2.00
t_{obs} value	0	4.76*	2.75*	3.13*	1.5	5.13*	0.17	0.31	3.68*

* Significant at 5% level ($p < 0.05$)

Table 2: Length of the larva, prepupae and pupae of Medipalle and Thrissur populations of Black soldier fly.

Population	Length of Black soldier fly					
	6 DOL	10 DOL	14 DOL	18 DOL	Prepupa	Pupa
Medipalle	0.51±0.04	1.31±0.08	1.62±0.06	1.70±0.04	1.69±0.03	1.61±0.04
Thrissur	0.39±0.04	1.82±0.08	1.89±0.06	2.01±0.05	1.90±0.09	1.87±0.06
t_{obs}value	5.87*	-11.91*	-7.94*	-12.26*	-5.73*	-9.23*

* Significant at 5% level ($p < 0.05$)

Table 3: Weight of the larva, prepupae and pupae of Medipalle and Thrissur populations of Black soldier fly.

Population	Weight of Black soldier fly					
	6 DOL	10 DOL	14 DOL	18 DOL	Prepupa	Pupa
Medipalle	0.005±0.001	0.062±0.012	0.121±0.010	0.120±0.007	0.10± 0.006	0.04±0.006
Thrissur	0.004±0.001	0.14±0.010	0.23±0.023	0.20±0.022	0.16±0.017	0.12±0.013
t_{obs} value	1.20	-13.43*	-8.91*	-9.44*	-8.49*	-14.36*

* Significant at 5% level ($p < 0.05$)

Table 4: Proximate analysis of prepupa of Medipalle and Thrissur population of Black soldier fly.

Population	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Ash (%)	Moisture (%)
Medipalle	40.33±0.08%	26.57%±0.05%	20.23%±0.07%	5.97%±0.005%	51.14%±0.03%
Thrissur	41.16±0.06%	29.71%±0.02%	28.29%±0.05%	7.43%±0.01%	42.29%±0.02%
t_{obs} value	-0.23	-1.49	-2.53*	3.55*	6.57*

* Significant at 5% level ($p < 0.05$)

CONCLUSIONS

The black soldier fly holds a lot of potential for use in aquafeed and aquaculture, pig and poultry feed, livestock food industry and also for the waste management and recycling units. The Thrissur population, which recorded significantly lesser time to complete the life cycle and larvae, prepupae and pupae were found to be more robust could be certainly recommended for the commercial farms. It also recorded significantly more crude fat, ash and lesser moisture making them more preferred for extraction of fat and insect meal preparation processes.

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Conflict of Interest. None.

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