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Research Article

Influence of agricultural wastes on larval growth phases of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): An integrated approach

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Abstract

Insects are an effective tool for converting nutrients in agricultural by-products into protein-rich biomass and compost. Black soldier fly (BSF) (*Hermetia illucens*) larvae are currently one of the insect species widely used as a protein source in aquafeed globally. Although much effort has been spent on the use of BSF in aquafeed, there is not much information on the biology of the insect, especially with the morphology of the BSF. This study aimed to evaluate the influence of various organic wastes, such as fruit wastes (FW) and vegetable wastes (VW), on different growth phases of BSF larva (BSFL), using morphometric and scanning electron microscopic examinations, and the composition of the compost produced, as well as a method for up-scaling of larval production of BSFL. Faster growth was observed in BSFL fed with VW substrate (40 days) compared to the FW (46 days). Based on the morphometric measurements such as length, larval head length, total length etc., five larval stages, prepupal and pupal stage of BSFL were differentiated and described. In addition, SEM imaging of BSF mouth parts found that the mouth morphology of the BSF larvae and prepupal stage differed, and the BSF prepupa had reduced mouthparts. Also, the mandibular-maxillary complex was well developed than the BSF prepupa. BSFL larvae have proven to convert fruit and vegetable waste into high-quality residue fertilizer for the soil. The BSF compost showed optimum nitrogen, phosphorous, potassium, calcium and sulphur content. This research establishes a baseline knowledge and guidance on the BSF-rearing facilities.

Keywords: Growth phases, Insects, Morphology, Sustainability, Waste management

INTRODUCTION

Agricultural waste can be bio-converted into valuable materials such as animal feed, human food, secondary industrial compounds, compost, and so on, and leftover food waste can be used as organic matter and nutrient-rich soil amendments (Rehman *et al.,* 2023). Insect-based bioconversion offers marketable solutions for reducing agricultural waste that is fiscally manageable, have low space and energy requirements, are environmentally friendly, have real market/commercial opportunities, and yield higher feed conversion ratios than conventional livestock (Babarinde *et al.,* 2020). Aquaculture is one of the main sectors engaged in producing high-quality protein. Aquaculture's success depends entirely on a reliable supply of quality feed. Due to the limited availability of soybean meal and fishmeal as the main protein sources, the demand for alternative sustainable feed ingredients has grown over time, and this demand is being seen globally (Sogari *et al*., 2019; Law and Wein, 2018; Van Huis, 2013; Tacon *et al*., 2011). In the quest for substitute feed, insects have been recognised and identified as a high protein source, so they can be considered to meet the demands and address the issues with the feed market (van Huis and Oonincx, 2017). Among the many insect species that are mass-produced, the black soldier fly (*Hermetia illucens*), house fly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*) are capable of converting organic wastes into high-quality protein for pig, chicken, and fish feed (Onsongo *et al*., 2018).

Due to its voracious appetite for organic waste, the black soldier fly (BSF), *H. illucens* (Linnaeus, 1758: Diptera: Stratiomiydae), is used as a new green trend technique to treat organic wastes (Lalander *et al*., 2013; Nguyen *et al*., 2013; Diener *et al*., 2009). The creation of commercially valuable products like compost, animal feed and biodiesel is stimulated by the processing of biowaste by this insect, increasing the bar for a circular economic strategy (Mertenat *et al*., 2019). In addition, the BSF larvae (BSFL) are regarded as a suitable substitute for fishmeal or soybean meal in life stock feeds (Barragan-Fonseca *et al*., 2017; Tran *et al*., 2015; Van Huis, 2013) because of their nutritional makeup (an average of 43% crude protein, 33% fat and other micronutrients) (St-Hilaire *et al*., 2007). There has been an increasing interest in creating novel techniques for the mass production of BSFL to replace organic waste management techniques and composting with a protein-rich animal diet (Beyers *et al.,* 2023). However, a viable innovative method for BSFL upscaling has not yet been developed. In order to develop a mass production method, the biology of BSF needs to be explored. The goal of the current study was to characterize the different larval stages (including morphometric measurements, developmental duration, pre-oviposition time, fecundity and adult longevity) as well as the viability of upscale production of BSFL on two different organic wastes, including vegetable and fruit wastes that are locally accessible.

MATERIALS AND METHODS

Collection and rearing of *H. illucens*

The study was conducted under laboratory and outdoor conditions at the Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru, Karnataka, India. The BSFL used in this study were collected from the field using the compost container kept in an open source. The COI gene's barcode fragment was amplified by LCO and HCO primers to validate the species identification (Marien *et al*., 2018). DNA sequencing reaction of PCR amplicon was conducted using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer (Daniso *et al*., 2020). The results were compared with the data stored in the online repositories. The barcoding was conducted at Barcode Biosciences, Bengaluru, India.

Substrate for rearing the BSFL

Two different organic waste diets, such as fresh fruit waste (FW) (mix of banana peel, pineapple peel, watermelon peel and papaya peel in the ratio of 1:1:1:1) and fresh vegetable waste (VW) (includes a mix of brinjal, cucumber peel, radish peel, carrot peel and cabbage in the ratio of 1:1:1:1:1) were collected from the juice shops and hotels respectively, in two different plastic containers and brought to the BSFL rearing facility at the Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru.

Small scale culture unit

The above-collected wastes were immediately transferred to two different plastic containers (35x51 cm) in an aerobic condition, separately, for rearing the BSFL. The plastic containers were covered with muslin cloth to avoid any source of contamination. For rearing the adult male and female BSF, a nylon mesh cage called ‘love cage’ was made with a mosquito net (70x140 cm) and was hung (Fig. 1). Adult male and female BSF were introduced into the love-cage and allowed to mate in daylight. A mixture of regular tap water and honey water was sprayed twice daily. The love-cage also received a supply of attractant that mimicked the decomposing organic matter for egg deposition. The attractant was covered with a folded piece of cardboard to make it easier for the adult females to lay eggs (Fig. 2). The harvested eggs (1g on each container, containing approximately 2000 BSFL) were spread out on a stainless-steel mesh to hatch in a hatchling container (Dortmans *et al*., 2017). In the plastic containers, newly hatched larvae were raised on distinct diets of mixed fruit and vegetable waste (performed in 3 replicates).

Large scale culture unit

The BSF outdoor cultural unit is shown in Fig. 3 and the size is 2.5 m by 1.8 m by 1.2 m. There are two tanks to keep the VW and FW apart. Dried leaves and wastes (VW and FW) were alternately layered in the tanks to keep the moisture level constant. The system's top was fitted with an iron cover evenly sprinkled with charcoal to reduce unpleasant odours from the unit. Blowers (2HP) were installed at the bottom of the tanks to provide optimal aeration. At first, the FW and VW were independently introduced in two different tanks.

The larval growth rate depends on several factors; the temperature and humidity in the study were recorded in the range of 22°C to 30°C and 39 to 48%, respectively (Lohri *et al*., 2017). The substrate's moisture content and pH calculated are represented in Table 1. The stable environmental conditions assist in the metamorphosis of the BSFL. Fluctuations in these conditions can delay their development. Unsuitable conditions of the rearing can affect the behaviour of the BSFL (Meneguz *et al*., 2018).

Morphometric measurements

After hatching, the BSFL were randomly collected instar-wise (10 numbers from each instar in 3 replicates) and stored in 70% ethanol for 24 hours for morphometric assessment. Using the MIA-IMAGE IR software connected to the optical microscope, morphometric features, including the total length (TL), head capsule measurement, head length (HL) and head breadth (HB), antennae length (AL), antenna breadth (AB), eye length (EL), eye breadth (EB), and individual segments length (SL) were measured (Gligorescu *et al*., 2019) (Fig. 4 and Fig. 5). Thus, based on the presence of setae and the length of the antennae, Dyar's rule of geometric growth in head measurement was used to distinguish between five larval phases.

Determination of growth performance

The BSFL developmental characteristics on the FW and VW substrates were observed, and the BSF larval counts, weights, residue masses, and feed masses were computed to assess the BSF's ability to grow from the third larval instar onward in accordance with (Gold *et al*., 2020). Waste reduction efficiency is determined as the ratio of the dry mass of the given feed to the dry mass of the dry residue (residue mass), along with the survival rate, waste reduction, bioconversion rate and waste conversion efficiency as per the following formula,

$$\text{Survival rate (\%)} = \frac{\text{larvae}_{\text{end}}}{\text{larvae}_{\text{beg}}} \times 100 \quad (1)$$

$$\text{Waste reduction (\%)} = \left(1 - \frac{\text{residue mass (g)}}{\text{feed mass (g)}}\right) \times 100 \quad (2)$$

$$\text{Bioconversion rate (\%)} = \frac{\text{larvae gain(g)}}{\text{feed mass(g)}} \times 100 \quad (3)$$

Waste conversion efficiency (%) = larvae weight gain (g) / {feed mass (g) - residue mass (g)} x 100 (4)

Hatching rate (%) = (Number of BSF eggs hatched / Total number of BSF eggs) x 100 (5)

Scanning electron microscopic analysis

The BSFL samples (fifth instar and prepupae) were preserved in 70% ethanol for the Scanning electron microscopic (SEM) examination. By dehydrating the insect tissue in ethanol at varying concentrations (30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%), followed by 48 hours of shade drying, the samples were rendered moisture-free (Kim *et al*., 2015). The sample was studied using a German-made ZEISS EVO 18 scanning electron microscope with 5 kV voltage.

Composting parameters

Various physicochemical characteristics of the compost made from the BSFL, cultivated on various substrates, including moisture content, water holding capacity (WHC), pH, total nitrogen, total phosphorus, potassium, calcium, total magnesium, and total sulphur, were examined (Ahn *et al*., 2008). The NPK and mineral analysis of the compost was conducted by ICAR -Indian Institute of Horticultural Research, Karnataka, India. The moisture content was assessed using the moisture analyser (Uni Bloc MOC63u model). For the WHC, a sample amount of known moisture content waste weighed (w_i) and placed in a beaker, soaked for two days, and the excess water was later drained through Whatman filter paper 1. The dried sample was weighed again (w_s). The amount of water retained in the dry sample was calculated as the WHC of the compost and was calculated according to the formula

$$\text{WHC} = \frac{[(\text{Ws}-\text{Wi})+\text{MC}\times\text{Wi})]}{[(1-\text{MC})\times\text{Wi}]} \quad (5)$$

Where MC – Moisture content; Wi – initial weight; Ws – final weight

Statistical analysis

The statistical analysis was performed by IBM SPSS software ver. 25 (IBM Corp), the results of the morphometric analysis were statistically analysed by one–way analysis of variance (ANOVA), followed by Duncan homogenous subsets and Tukey's HSD (honestly significant differences) post Hoc test for multiple comparisons to assess and compare the significant difference between the samples. The $P < 0.05$ was chosen to indicate the significant difference.

RESULTS

Biometrics of *H. illucens*

The life cycle of *H. illucens* begins with an egg (Fig. 6). The adult female laid 542.9±11.62 eggs in a single clutch, irrespective of the substrate, on the corrugated cardboard eggies placed in the love-cage (Fig. 1). The fresh eggs appeared pale yellow (Fig. 7). As they age, the colour changes to a darker beige analogous to the rice grain. A black colour spot was observed at the tip of the egg. The eggs were measured to be 1.21±0.012mm long. The BSF eggs' hatching rate on the FW and VW substrates was 95%. The eggs were hatched into the first instar after four days. BSFL showed colour differentiation in the successive larval stages; the first instar larvae were pale beige in appearance; however, the larvae colour intensified as the larvae aged; the fifth instar turned slightly brownish, and the prepupal and the pupal stage, charcoal black. The larvae passed through five instar successive stages, namely the first, second, third, fourth, fifth instar stages, prepupae and pupae stages, to reach adulthood.

Morphometric measurements

The BSF larvae were observed to feed until they reached the fifth instar actively. The development of the BSFL from the first instar to the prepupal stage was fast in the VW substrate based on the number of days the BSFL took to reach the prepupal stage (40 days to reach the prepupal stage) when compared to the BSFL reared on the FW substrate (46 days to reach the

Table 1. Moisture content and pH of the fruit substrate

Agri-food by-products		Moisture (%)	pH
Fruit wastes	Papaya peel	88.50±0.35	5.98±0.04
	Banana peel	89.80±0.60	9.91±0.01
	Pineapple peel	81.74±1.96	3.92±0.16
	Watermelon peel	90.03±0.46	5.37±0.11
Vegetable wastes	Carrot peel	86.24±0.17	5.96±0.11
	Brinjal peel	89.17±0.49	5.75±0.04
	Cabbage	80.78±0.21	6.56±0.02
	Cucumber peel	93.15±0.94	6.52±0.03
	Radish peel	92.35±0.19	5.87±0.03

Results are expressed as mean ± SD (3 replicates)

prepupal stage). The morphometric measurements of each stage of the BSFL are presented in Table 2 and Table 3, suggesting that the BSFL passed through five instars. It is difficult to distinguish between the first and the second instar, based on the larvae's size and weight. The results of the larval growth performance were statistically analysed for Tukey HSD multiple comparisons between the samples for various parameters. The morphometric variables, such as TL and EL, showed significant variations among the larval stages, whereas other stages did not.

Determination of growth performance

The growth of BSF larvae was found to be different on different substrates. The larvae reared on the fruit substrate showed a better growth response than those reared on the vegetable substrate, which could be due to the impact of diet qualities on BSFL. The antennae length (AL) and antenna breadth (AB) of the BSFL are short in the younger instar and become proportionally longer as the larvae grow old. The AL of the BSFL reared on FW ranged from (0.07±0.002 mm to 0.08±0.001 mm at $P<0.05$) and the AL of the BSFL reared on VW ranged from (0.04±0.002 mm to 0.09±0.003 mm at $P<0.05$). The total number of BSFL at the end of the rearing in the FW substrate container was ~1630 and in the VW substrate was 1580.

The survival rate, waste reduction, bioconversion rate, waste conversion efficiency and protein conversion efficiency are tabulated in Table 4 for fruit and vegetable substrate. The mean results of digestibility rates were not significantly different between the fruit and vegetable substrates. The comparisons of results for survival rate, waste reduction, bioconversion rate, waste conversion efficiency and larval weight were higher for the larvae reared on fruit substrate. These results suggest that the present experimental conditions are suitable for culturing BSF in a small rearing facility and that these larvae can be developed on different fruit and vegetable substrates.

Scanning electron microscopy (SEM) analysis

To gain further knowledge about the mouth morphology of the polyphagous and saprophagous BSFL, the mouth parts of the fifth instar and the prepupa were examined using a Scanning electron microscope (SEM). The head of the BSF was conical in shape, less rounded, and mostly a hemicephalic head. The mouth part of the larvae had a mandibular-maxillary apparatus protected by the labrum. The mandibular-maxillary apparatus is the fusion of the mandible and maxilla. The mouthparts of the prepupa had reduced mouthparts, and the head and eye prominently looked different from the BSF larval instars. However, the morphological characteristics of the prepupa head looked different from the other stages of the larvae. This helped us understand that the BSFL from instar one to five were voracious feeders, scavengers that fed on the organic

Fig. 1. *Hanging 'Love cage' for the indoor culturing of adult BSF*

Table 2. Morphometric measurements of BSFL grown on different subtracts like fruit waste (FW) and vegetable waste (VW) with relation to head capsule region

Growth Parame-	BSFL1		BSFL2		BSFL3		BSFL4		BSFL5		BSF Prepupa	
	FW	VW	FW	VW	FW	VW	FW	VW	FW	VW	FW	VW
TL	7.17±0.83^{e}	8.43±0.10^{f}	12.83±0.05^{d}	9.62±0.05^{e}	17.38±0.83^{c}	11.02±0.42^{d}	18.92±0.60^{b}	14.47±0.36^{c}	22.43±1.35^{a}	17.47±0.32^{b}	21.66±0.15^{a}	21.55±0.04^{a}
HL	0.82±0.01^{d}	0.82±0.01^{e}	0.97±0.01^{c}	0.90±0.002^{d}	1.29±0.06^{a}	0.97±.05^{d}	1.44±0.08^{b}	1.06±.03^{c}	1.51±0.08^{a}	1.22±0.13^{b}	1.45±0.04^{a}	1.46±0.05^{a}
HB	0.71±0.01^{c}	0.73±0.02^{e}	0.97±0.004^{b}	0.84±0.02^{d}	1.01±0.03^{b}	0.91±0.05^{c}	1.02±0.08^{b}	0.94±0.01^{c}	1.22±0.05^{a}	1.06±0.02^{b}	1.25±0.01^{a}	1.26±0.03^{a}
AL	0.07±0.002^{d}	0.04±0.002^{e}	0.08±0.001^{c}	0.05±0.001^{d}	0.10±0.002^{b}	0.05±0.003^{c}	0.1±0.01^{b}	0.06±0.003^{b}	0.11±0.01^{a}	0.09±0.01^{a}	0.08±0.001^{c}	0.09±0.003^{a}
AB	0.04±0.01^{c}	0.02±0.001^{f}	0.04±0.003^{c}	0.02±0.001^{e}	0.05±0.004^{ab}	0.02±0.002^{d}	0.05±0.003^{c}	0.03±0.002^{c}	0.05±0.01^{bc}	0.05±0.002^{b}	0.06±0.004^{a}	0.05±0.003^{a}
EL	0.10±0.001^{f}	0.09±0.003^{e}	0.11±0.003^{e}	0.10±0.002^{e}	0.18±0.02^{d}	0.14±0.01^{d}	0.21±0.01^{c}	0.17±0.01^{c}	0.23±0.01^{b}	0.21±0.01^{b}	0.41±0.005^{a}	0.36±0.02^{a}
EB	0.05±0.001^{d}	0.04±0.001^{e}	0.06±0.003^{d}	0.05±0.0002^{d}	0.09±0.004^{c}	0.05±0.003^{d}	0.10±0.01^{bc}	0.07±0.003^{c}	0.11±0.01^{b}	0.08±0.004^{b}	0.15±0.02^{a}	0.13±0.003^{a}
ML	0.55±0.02	0.41±0.01	0.71±0.01	0.46±0.005	0.76±0.03	0.54±0.02	0.92±0.04	0.63±0.02	0.99±0.03	0.73±0.02	-	-

Results are expressed as mean ± SD of five replicates followed by Duncan value in the same criterion that are significantly different from each other by Duncan test at probability 0.05TL: Total length, HL: Head length, HB: Head breadth, AL: Antenna length, AB: Antenna breadth, EL: Eye length, EB: Eye breadth, ML: Mouth length

Table 3. Larval growth of *Hermetia illucens* over time on fruit substrate with relation to segments

Substrate	FW	VW	FW	VW	FW	VW	FW	VW	FW	VW	FW	VW
Instar	BSFL1		BSF L2		BSFL3		BSFL4		BSF L5		BSF Prepupa	
S1L (mm)	0.69±0.003^{d}	0.71±0.01^{d}	1.26±0.02^{c}	0.80±0.005^{c}	1.36±0.03^{b}	0.84±0.04^{c}	1.40±0.11^{b}	1.01±0.01^{b}	1.62±0.04^{a}	1.26±0.05^{a}	0.56±0.02^{e}	0.56±0.03^{e}
S2L (mm)	0.51±0.003^{e}	0.51±0.01^{f}	0.81±0.004^{d}	0.59±0.01^{e}	1.01±0.05^{c}	0.65±0.04^{d}	1.04±0.07^{c}	0.83±0.02^{c}	1.24±0.06^{b}	1.03±0.04^{b}	1.44±0.03^{a}	1.36±0.04^{a}
S3L (mm)	0.56±0.004^{f}	0.66±0.03^{f}	0.96±0.01^{e}	0.75±0.03^{e}	1.24±0.04^{d}	0.81±0.01^{d}	1.33±0.01^{c}	1.08±0.01^{c}	1.57±0.02^{b}	1.33±0.02^{b}	1.64±0.03^{a}	1.63±0.05^{a}
S4L (mm)	0.48±0.003^{f}	0.58±0.01^{f}	0.98±0.01^{e}	0.67±0.03^{e}	1.11±0.02^{d}	0.76±0.04^{d}	1.22±0.05^{c}	1.01±0.07^{c}	1.57±0.05^{b}	1.26±0.02^{b}	1.66±0.03^{a}	1.67±0.04^{a}
S5L (mm)	0.49±0.003^{e}	0.67±0.02^{f}	0.96±0.01^{d}	0.76±0.02^{e}	1.24±0.05^{c}	0.85±0.03^{d}	1.48±0.08^{b}	1.19±0.07^{c}	1.92±0.09^{a}	1.53±0.04^{b}	1.90±0.01^{a}	1.86±0.02^{a}
S6L (mm)	0.49±0.003^{f}	0.65±0.03^{f}	0.96±0.02^{e}	0.76±0.02^{e}	1.32±0.05^{d}	0.86±0.05^{d}	1.57±0.06^{c}	1.17±0.04^{c}	1.95±0.02^{b}	1.49±0.07^{b}	2.07±0.04^{a}	2.03±0.02^{a}
S7L (mm)	0.54±0.003^{f}	0.67±0.03^{f}	0.92±0.01^{e}	0.76±0.03^{e}	1.40±0.05^{d}	0.89±0.07^{d}	1.71±0.12^{c}	1.30±0.02^{c}	2.11±0.10^{b}	1.51±0.07^{b}	2.24±0.03^{a}	2.28±0.01^{a}
S8L (mm)	0.53±0.002^{e}	0.74±0.02^{f}	1.02±0.01^{d}	0.83±0.01^{e}	1.55±0.01^{c}	0.93±0.08^{d}	1.84±0.04^{b}	1.33±0.07^{c}	2.16±0.16^{a}	1.60±0.07^{b}	2.25±0.03^{a}	2.26±0.04^{a}
S9L (mm)	0.58±0.01^{e}	0.74±0.03^{f}	0.92±0.004^{d}	0.84±0.02^{e}	1.43±0.06^{c}	1.05±1.14^{d}	1.85±0.02^{b}	1.28±0.05^{c}	2.24±0.14^{a}	1.51±0.07^{b}	2.27±0.02^{a}	2.27±0.02^{a}
S10 (mm)	0.63±0.01^{e}	0.75±0.03^{e}	1.02±0.005^{d}	0.87±0.02^{d}	1.33±0.06^{c}	0.85±0.11^{d}	1.57±0.06^{b}	1.13±0.05^{c}	2.17±0.17^{a}	1.51±0.06^{b}	2.25±0.04^{a}	2.26±0.03^{a}
S11L (mm)	0.78±0.01^{d}	0.89±0.01^{e}	1.97±0.01^{c}	1.04±0.02^{e}	2.57±0.19^{b}	1.63±0.13^{d}	2.90±0.06^{a}	2.03±0.07^{b}	3.07±0.47^{a}	2.51±0.27^{a}	1.85±0.03^{c}	1.83±0.04^{c}

Results are expressed as mean ± SD of five replicates followed by Duncan value in the same criterion that are significantly different from each other by Duncan test at probability 0.05; S1L – Segment 1 length, S2L – Segment 2 length, S3L – Segment 3 length, S4L – Segment 4 length, S5L – Segment 5 length, S6L – Segment 6 length, S7L – Segment 7 length, S8L – Segment 8 length, S9L – Segment 9 length, S10L – Segment 10 length, S11L – Segment 11 length

Fig. 2 *Rearing facility for BSFL (Photomicrograph) attractant was covered with a folded piece of cardboard to make it easier for the adult females to lay eggs.A: Small scale rearing setup (at laboratory level); B: Mass production unit; C: Eggies; D: BSF eggs laid on the attractant by the female adult*

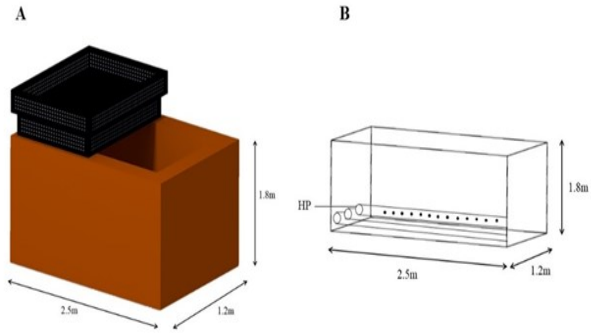

Fig. 3 A, B. *Schematic diagram of BSFL outdoor culturing unit. The tank is closed with an iron rod (black coloured), for charcoal plying. The blowers (HP) installed in the bottom of the tank for hot aeration*

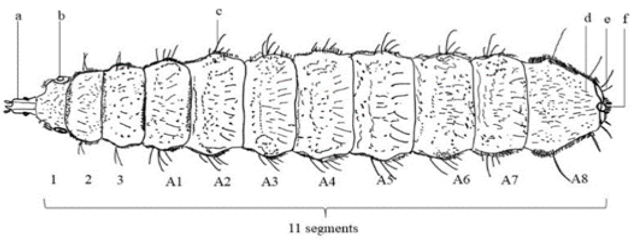

Fig. 4. *Schematic diagram of BSFL (with labelling). a: head; b: prothoracic segment; c: dorsolateral bristle; d: dorsal bristle; e: apical bristle; f: back stigma; 1, 2, 3: 1st, 2nd, 3rd thoracic segment; A1- A8: abdominal segments*

waste and the prepupal stage did not feed and had a hook-like structure on the head region. This differentiation could be seen through the SEM images (Fig. 8).

Composting parameters

A better understanding of the compost enabled us to understand the compost system's characteristics and its structural stability. The compost quality data from BSFL were compared with the organic manure specifications as per The Fertilizer Control Order 1985, FAI, New Delhi. The final compost from FW and VW obtained from the rearing of BSF was blackish brown. Table 5 shows the physicochemical properties of the compost obtained from FW and VW. The compost's texture was fine, crumbled and had a pleasant smell. The NPK value, total nitrogen (2.24%), total potassium (5.20%), and total phosphorous (1.02%) were higher in the compost from the vegetable substrate. The microbes need the nitrogen content in the compost to fulfil

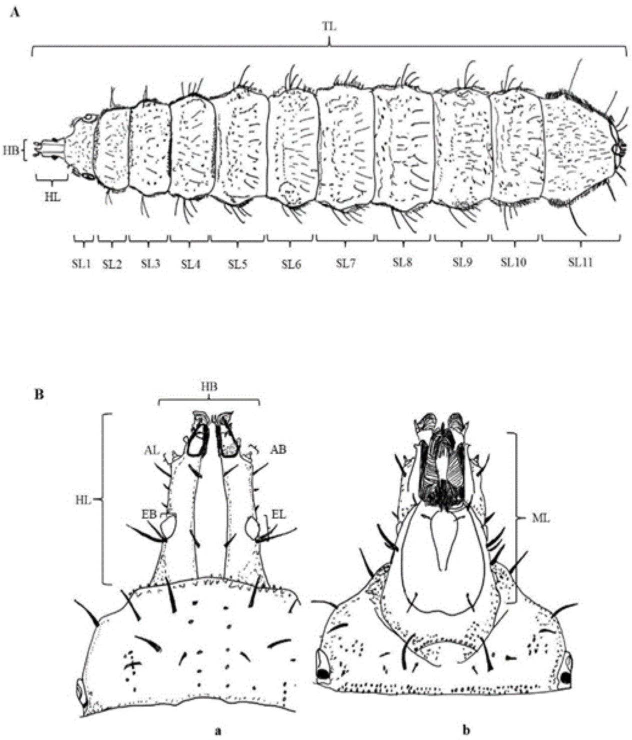

Fig. 5. *Morphometric measurements of BSFL;A: Entire larvae; TL: Total length, HL: Head length, HB: Head breadth, SL1 - SL11: Body segment length from 1 to 11; B: Head region magnified; a: dorsal view, b: ventral view; HL: Head length, HB: Head breadth, AL: Antenna length, AB: Antenna breadth, EL: Eye length, EB: Eye breadth, ML: Mouth length*

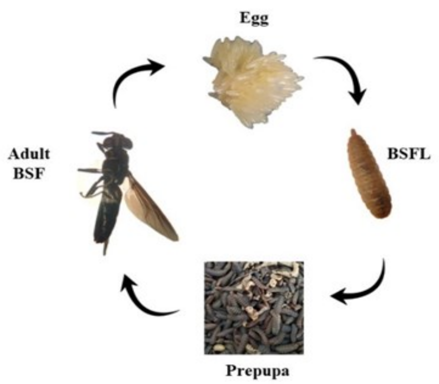

Fig. 6. *Lifecycle of Hermetia illucens*

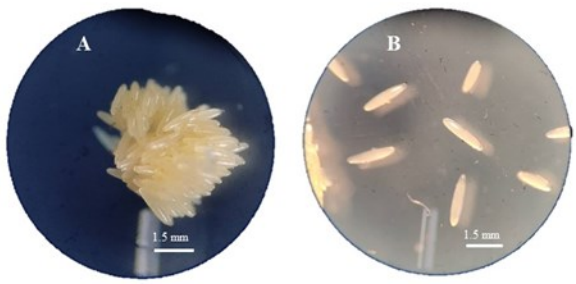

Fig. 7. *Microscopic images of BSF eggs A: egg cluster; B: single egg*

the need for a food source for the body's cell formation. The moisture content of the VW compost was higher than the FW compost.

DISCUSSION

Even though many studies regarding the lifecycle of BSFL have been made, there is confusion regarding the number of instars; the BSFL passes through, as the number of BSFL stages have been reported differently in the studies. Studies by Gligorescu *et al*. (2019) reported that the BSFL passes through seven instars; however, work by (Barros-Cordeiro *et al*., 2014) suggested that the BSFL passes through six instars. Hence, an effort was made to confirm and distinguish the BSFL instars based on the various morphometric measurements. The morphological details of the BSFL are illustrated in Fig. 4. In the present study. The BSFL were sampled during the moulting stages. The consistency of larval stages was determined by observing the head capsule width. The head of the BSF larvae

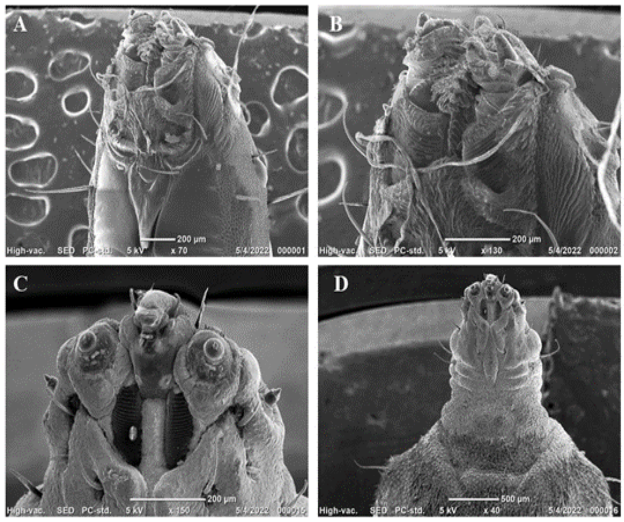

Fig. 8. *Scanning Electron micrographs of anterior ventral side of head of BSFL. Mouth parts of the fifth instar (A: x70; B: x130); Prepupal mouth part (C: x150; D: x40)*

was dark reddish brown. The head capsule of *H. illucens* has a proportional increase in size from the earlier larval stages to the prepupal stage, showing that the larvae pass through five instars, a prepupal and pupal stage, indicating that each larva moults five times before reaching the prepupal stage, these results were in comparison with the studies conducted by May (1961) who had claimed that the BSFL passes through five instars before entering the prepupal stage. The findings provided here support prior findings (Singh *et al*. 2019). The head capsule length of the fifth instar (1.51±0.08 mm) (table 2) and the prepupa (1.45±0.04 mm) (table 2) of the larvae reared on FW did not show much significant difference as compared with the fifth larvae (1.22±0.13 mm) (table 2) and prepupa (1.46±0.05 mm) (table 2) raised on the VW. The transition from one instar to the other, i.e., the moulting, was distinctly observed on the fifth day in all the instars of the BSFL.

The growth of BSFL took 40 days in FW and 46 days in VW which considerably was a slower development contrary to the studies by (Jucker *et al*., 2017) that showed development in 24 days, which could be explained due to the different environmental conditions, dietary plan and the method adopted in the study. The BSFL, after a few days, turned into prepupa, charcoal black colour in both the substrate crate and stopped feeding and started to migrate away from the substrate, leaving

Table 4. Feeding rate and digestibility of BSF reared on fruit and vegetable substrate

Substrate	Survival rate (%)	Waste reduction (%)	Bioconversion rate (%)	Waste conversion efficiency (%)	Larval weight (mg)
FW	91.43±2.64	91.91±4.34	3.28±0.54	3.57±0.73	54.76±1.02
VW	88.39±3.84	85.93±2.74	3.11±0.96	3.35±0.54	50.59±1.28

Results are expressed as mean ± SD of triplicates

Table 5: Physico-chemical properties of the different compost of BSFL

Physicochemical Properties	Fruit substrate compost (%)	Vegetable substrate compost (%)	FAI standard (1985)
Moisture	46.94±1.78 ^{b}	49.37±1.92 ^{a}	25.0 -
Water holding capacity (WHC)	97.17±0.31 ^{a}	92.48±0.93 ^{b}	-
pH	8.94 ± 0.02 ^{a}	7.56±0.45 ^{b}	6.5 – 7.5
Total Nitrogen	2.07^{b}	2.24^{a}	0.5 -
Total Phosphorus	0.67^{b}	1.02^{a}	0.5 -
Total Potassium	2.50 ^{b}	5.20 ^{a}	0.5 -
Total Calcium	0.28 ^{b}	0.46 ^{a}	-
Total Magnesium	0.20 ^{b}	0.35 ^{a}	-
Total Sulphur	0.27 ^{b}	0.35 ^{a}	-

Results are expressed as mean ± SD of triplicates followed by Duncan value in the same criterion that are significantly different from each other by Duncan test at probability 0.05

their feeding environment, searching for a dry, shady area to metamorphose into a pupa. The propulsion of the bodies of these voracious scavengers through the substrate occurs through body contractions (Nakamura *et al*., 2016). The pupae were observed to be photophobic and moved away from the feed source to metamorphose into adults (Yang, 2022). The pupae emerge into an adult after ten days of completing their life cycle. The different waste supported the growth of the individual black soldier fly larvae, and the results of the digestibility rates were similar to other studies (Lalander *et al*., 2019; Jucker *et al*., 2020; Diener *et al*., 2011; Yang and Tomberlin, 2020). The survival rates were comparable with that of the previous studies by Gold *et al*. (2020), which showed a survival rate above 80%, ranging from 85-92% (table 4) in the present study.
The ability of the BSFL to grow and develop on any organic waste is highly supported by its mouthparts and the alimentary canal. To gain an insight on the functional parts of the BSFL mouthparts, a SEM analysis was performed. The BSFL mouth morphology is similar, as documented by (Bruno *et al*., 2020; Schremmer, 1986). The *H. illucens* larvae conserve several primitive elements, particularly like the prostigma, i.e., the spiracle on the BSFL, present on the first segment of the body. The function of the spiracle is to provide an airway into the insect's muscular thorax (Oliveira *et al*., 2016). The body of the BSFL has 11 segments covered with setae all over the larval body, which can be observed through the microscope; the 11th segment is the longest, whose posterior end shows the presence of two long bristles curved in the opposite direction to that of the body setae; the lateral side of the larval body have minute hair-like structures. The first instar larvae showed the presence of three setae which later on, with the increasing instar, reduced to two on the lateral setae. The adult body can be distinguished into a head, thorax and an abdomen region, the abdomen has five segments, and the second segment is translucent. The morphology of *H. illucens* larvae is particularly similar to the other Dipterans. In addition, a detailed study on the morphology of the BSFL and understanding how different diet regimes and environmental conditions can affect the body of the BSF can represent an important topic of further research.
In aerobic circumstances, organic matter is biologically degraded to produce compost which is commonly used in agriculture as a soil fertilizer. The compost has several benefits, like nutrient availability to plants, soil-borne pathogen suppression, etc. The BSFL is an excellent candidate for the biodegradation of organic matter as it takes less time than conventional composting for decomposition (Widyastuti *et al*., 2021).
A step towards implementing the bioconversion system, the *H. illucens* green trend technology can be considered a promising tool as this approach is affordable and efficient, and treatment facilities can be installed on a small scale. A complete knowledge of understanding the process of the *H. illucens* population and its development creates an ideal model for waste conversion efficiency.

Conclusion

The results presented in this study confirmed that the BSFL had five actively feeding instars based on the growth parameter, i.e., using the head capsule measurement, all the instars can be easily identified. The BSFL reared on the FW showed better growth than the BSFL reared on the VW. The prepupa of the BSF is a non-feeding stage that can be distinguished from the other instars based on morphological characteristics. There are very few studies relating to the biology of the BSFL; this study on the life cycle and larval growth is a great model for understanding the dynamics of the BSFL. Apart from the growth of the larvae, this research also highlighted the mouth morphological difference between the larvae and the prepupa. Furthermore, the results provided a suitable method for mass production of BSFL and using these as sustainable protein-rich aqua feed, successfully replacing conventional feeds like fishmeal and soymeal in the aquaculture industry. The outcome of this study also offered a practical way to produce BSFL in large quantities for environmentally friendly waste management along with sustainable aquaculture and agricultural practices.

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Conflict of interest

The authors declare that they have no conflict of interest.

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