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Sustainable Waste Management: Green Concept of Black Solider Fly Larvae (*Hermetia illucens*) as Bio-degradable Waste Convertor; Comparison of Life Cycle and Growth Performances in Two Different Substrates.

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Abstract: Viable waste management using insects are in trend, nevertheless no significant practices available locally. The black soldier fly larvae (BSFL; Hermetia illucens) have proven successfully the decomposition of organic matter savagely, enriched with favorable amino acid source for animal feed. The study intended to compare the life cycle modalities of BSFL with two different substrates, swill (T1) and poultry starter feed (T2), by assessing the days taken for completion of life cycle, egg and larval characteristics and crude protein (CP) percentage under IM3 agro climatic region in Belihuloya, Sri Lanka. There was no substrate-dependent effect on the egg characteristics. The total time period taken to complete the life cycle in T2 (37 - 45 days) was quicker than the T1 (46 - 57 days). Substantial length and width variation in different larval instars were observed whereas higher values were recorded in T2 with compared to T1. The CP percentage of pre-pupae stage was 51.99% in T2 while 39.46% in T1. The latter instars of BSF larvae CP percentage were recorded as 48.88% and 33.11% in T2 and T1 respectively. In conclusion, poultry starter feed (T2) that consist of balanced nutrient composition could be used as the most appropriate substrate for early life cycle completion with compared to swill (T1). T2 may effectively utilize as artificial media for initial propagation of BSFL. Larva growth under T2 conditions recorded the highest CP levels but it need to be further studied prior to use as protein substitution in animal feed formulation.

Keywords: Black solider fly larvae, *Hermetia illucens*, Crude protein, Swill, Waste

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Introduction

Solid waste management has become a terrific issue where all the nations are struggling to implement more effective methodologies to make sure cleanliness, healthy and productive nation coupling with globalization. It is having a positive correlation with waste generation and the raise of income levels among the population (Hoonweg *et al*., 2012). It is predicted that average waste generation is 0.74 kg /day person where global waste is expected to be raised to 3.4 billion tons by 2050 along with double population growth, especially in South Asia (Kaza *et al*., 2018). Unplanned urbanization is in trend where more than half of the generated waste is open dumped, creates the worst damage to the environment. According to the literature middle income countries counts 53% of food and green waste from their total waste (Kiran *et al*., 2014; Levis *et al*., 2010).

Emission of volatile odor from decomposing biodegradable waste has many adverse effects, especially animal waste. A higher greenhouse gas emission; ammonia in major and other volatile compounds are generated resulting severe health hazards. The people who live proximity to the waste collected areas are undergoing higher levels of depression, tension and anger (FAO, 2009 and Baskin *et al*., 2017).

Previous studies indicated that small and medium scale solid waste management using aerobic treatments prior to open dumping would be an appropriate method to a country like Sri Lanka, since more than 70% of the waste fraction is comprised with bio-degradable waste (Menikpura *et al*., 2012). Bio degradation using Black Soldier Fly Larvae (BSFL) is a proven success method worldwide (Diener *et al*., 2011). It is a harmless insect and widely spread in warmer areas of the world. Which is having a short complete life cycle with the especial ability of controlling house fly (Newton *et al*., 2005). The odor generated by bio waste inhibits the oviposition of house fly. They composed with natural antibiotics and able to reduce harmful bacteria like Escherichia coli 0157:H7 and Salmonella enterica (Erickson *et al*., 2004; van Huis *et al*., 2013; Sheppard *et al*., 1994). Few literatures presented their environmental impact by insect production (Salmone *et al*., 2016, Hackstein and Stumm, 1994; Oonincx *et al*., 2010).

The developing countries are mainly struggle to achieve the nutrient requirement where dietary preferences are shifting towards animal based products. The livestock production's profit is marginalized due to high expense of Crude Protein in animal feed which is accounting 60% -70 % of the total production cost. Another advancement is, BSFL are ferocious in transforming organic matter into livestock feed crude protein (Lalander *et al*., 2019).

Same as globally, Sri Lanka also standing behind the waste processing requirement. Locally it is predicted that municipal waste generation will be accelerated 1kg/person day by 2025 (Vidanarachchi *et al*., 2006). Most of the local waste management is carried out by local authorities and failed to provide sufficient waste management service due to lack of efficient procedure and skilled workforce. It results that approximately 90% of the collected waste is open dumped in an uncontrolled manner (Menikpura *et al*., 2012). Technology may not be the only solution since other effective alternative methods are in an urgent need (Kuruppuge *et al*., 2013).

Locally the livestock sector is a powerful tool in uplifting rural economy for centuries (Alahakoon *et al*., 2016). In Sri Lanka it is less known on BSFL production and lifecycle analysis. Since fewer literatures existed over insect life cycle assessments on this specific field, this study intends to compare the life cycle of BSF with the standard life cycle and growth performances in two different substrates conditions under Belihuloya IM3 agro ecological zone in Sri Lanka. Research outcomes serve best in sustainable insect production for environmental management and animal feed production.

Materials and Methods

For the BSF larvae breeding purpose, a special container was designed that facilitating aeration, leachate extraction, self-harvesting and corrugated surfaces for laying eggs with a dark atmosphere. 100% Swill (T1) and poultry starter feed (T2) were used using three replicates as experimental substrates. After initiation of brooding, swill was top up once in two days. Initially 700 g of poultry starter feed was mixed with Chlorine free water to de-crumble and made as pellet. Both substrates were stored in the dark and observed daily. Sufficiently moist state was ensured in both substrates.

Favorable breeding climatic requirements were maintained for BSF, ensuring the temperature range of 25 °C to 30 °C and relative humidity 50% to 80%. Since BSFL do not prefer direct sunlight the experiment set up was maintained under shade (Zhang, 2010; Givens *et al*., 2013).

Adult behavior and the larval performances of BSF were recorded daily in two different substrates. Special attention was paid towards the adult's oviposition behavior. Egg characteristics were evaluated by measuring the egg length using electron microscope and volume of egg mass using a ruler. The larval growth performances of both substrates were compared by measuring length and width of larval stages using three replicates. The life cycle completion days considering life stages were recorded respectively. In addition, the percentage of crude Protein percentage (CP%) was calculated performing Proximate analysis and multiplying the resulted Nitrogen percentage by 6.25 in terms of latter stages of larvae and the pre-pupae. All the parameters were statistically analyzed for the standard deviation and Standard Error.

Results and Discussion

It was observed that the large number of egg masses (3 egg masses/day) were placed in poultry starter feed, with compared to small number of egg masses in swill (1-2 egg masses / day). Measurements of the average egg length was recorded around 1mm (Caruso et al., 2014) where 0.996 µm in swill (SD = 0.006) and 0.997 µm in poultry starter feed (SD = 0.007). The average eggs volume in swill was recorded as 0.973 eggs / mm3 (SD = 0.005) and in poultry starter feed with 0.95 eggs / mm^3 (SD = 0.024). The results showed that there was no substrate-dependent influence on egg characteristics, although the oviposition of the eggs showed their attraction to the smell of substrate.

The occurrence of the first instar larvae was registered in poultry starter feed after two days of oviposition, and swill substrate followed the hatching of the eggs after the third day of oviposition. The length of the first instar larvae was recorded as 932.49 µm (SD = 2.174) under swill subtrate where 893.668µm (SD = 0.611) length was recorded in poultry starter feed substrate.

Once the larval growth was initiated, there was a significant variation in larval length and width in terms of substrate specificity (Figures 1 and 2). Larval length measurements revealed that larvae brood in poultry starter feed indicated a higher larval length values than swill substrate. This may be due to high nutrient value of substrate supplement with poultry feed. There was a gradual increase in larval length from L1 to L5 in poultry starter feed. Even though larval length in swill also followed the same, a declining was encountered from L4 to L5.

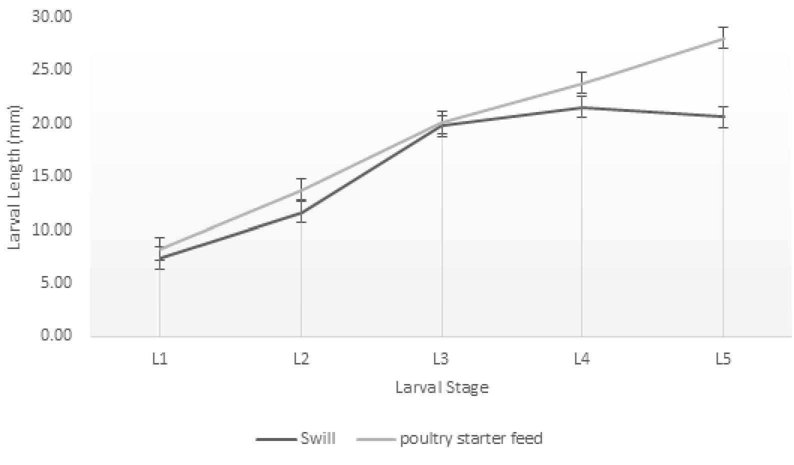

Figure 1: BSFL Larval length of different larval stages

Irrespective of brooding media the larval width was increased up to L3 stage. Nevertheless L1-L3 growth has increased in swill much slower phase than poultry starter feed. Poultry starter feed substrate has shown increased width between L3 and L4 with compare to the larvae brood in swill. The larval width was increased up to L4 and showed a decrease width in L4 to L5 in poultry starter feed. Larvae brood in swill has shown maximum growth up to L3 and started decreasing width from L3 to L5.

Irrespective of brooding media the widths of the various larval stages, increased up to L3, followed by steady growth up to L4. The increase in larval width was leveled in T1 from L3 to L4, followed by a decrease in growth to L5. The larvae brood in the T2 diet was wider than the T1 stage except the L3 stage.

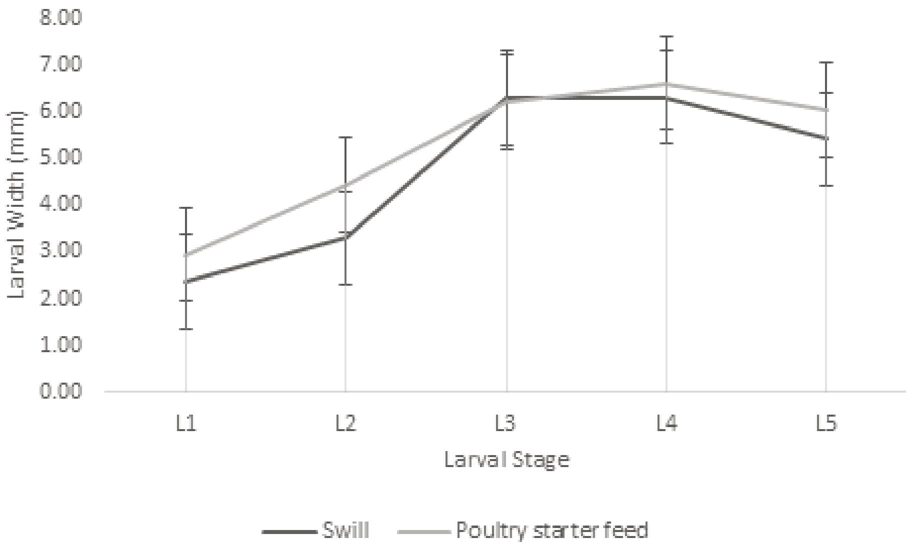

Figure 2: BSFL larval width of different larval stages

BSFL life cycle completion under poultry starter feed indicated early with compared to the swill. Five days were taken to initiate egg laying in poultry starter feed and 2 days to hatch and emergence of first instar. Further another 10-13 days were taken to attain 1st instar to 6th instar stage (Pre-pupae stage) in poultry starter feed. Time taken for emergence of pre-pupae to adult was exhibited 20 - 25 days. Collectively the total BSFL life cycle period brood in poultry starter feed was indicated around 37- 45 days.

Three days were taken to initiate laying eggs in swill. Eggs hatching were extended by another 3 days. After emergence of 1st instar at the 6th day of the experiment the larval stage continued up to 12 -16 days until attain the pre-pupae stage. From the pre-pupae stage to emergence of adult stage the life cycle in swill recorded as 28 - 35 days. Collectively the BSFL life cycle completion in swill was exhibited 46 - 57 days.

The results envisaged that larvae brood in poultry starter feed showed an accelerated life cycle completion than swill. After 3 days of the experimental setup in which swill odor may influence the attraction of BSF to the substrate much earlier than poultry starter feed. Second incidence of oviposition was detected after 5 days of experimental setup where substrate odor emission was delayed. According to Caruso *et al*., 2014, the standard life cycle is around 45 days. The proximate values indicated that pre-pupae stage, under swill substrate: 39.46% (SD= 0.84) crude protein and under poultry starter feed substrate: 51.99% (SD=2.22) crude protein was recorded. The CP levels were recorded in latter level instars 33.11% (SD= 1.68) and 48.88% (SD = 1.27) under swill and poultry starter feed respectively (Figure 3).

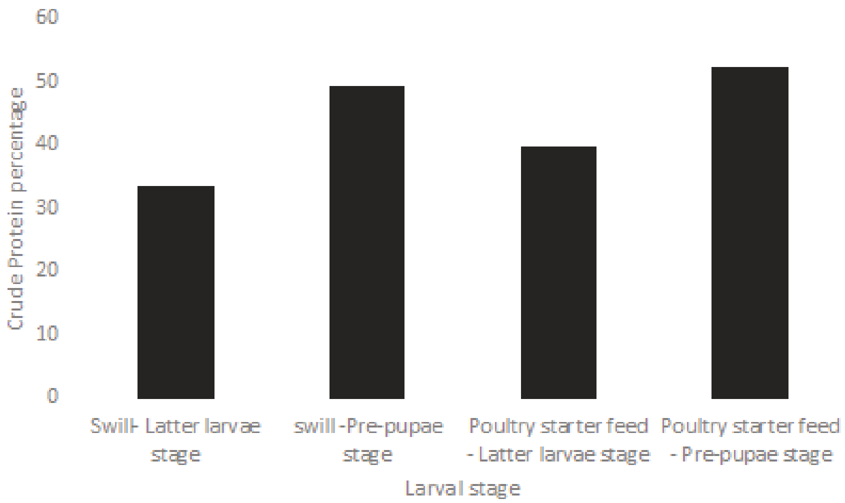

Figure 3: CP % of different life stages in different substrates

Conclusion

Results of the study revealed that, poultry starter feed envisioned the most suitable substrate for an accelerated life cycle compared to 100% swill and the standard BSFL life cycle. The higher nutrient composition of poultry starter feed enhanced the growth performances of larvae with respect to the 100% swill. The larvae brood in poultry starter feed was contained high levels of CP where other respective characteristics of larvae need to be further studied to substitute animal feed protein component.

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