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Role of pupation substrate on post-feeding development of black soldier fly larvae, *Hermetia illucens* (Diptera: Stratiomyidae)

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Abstract
Recycling organic wastes using black soldier fly based technology into valuable products has recently attracted special attention worldwide. However, black soldier flies only convert waste during their feeding stage. Therefore, it is important to optimize the non-feeding stages, especially the pupal stage, in order to improve the entire life cycle of the insect. This study aims to assess the influence of pupation substrates on the post-feeding development of the black soldier fly. Four-days-old larvae were reared from a cohort of eggs laid the same day using commercial chicken feed until they stopped feeding, and 1600 post-feeding larvae were collected and transferred to four different substrates consisting of wood shavings, fine sand, wheat bran and nothing for pupation and emergence. Each treatment consisted of four replicates of 100 post-feeding larvae. They were daily monitored to record the appearance of the pupae and adult emergences. Regardless of the type of treatment, pupation started four days after the introduction of post-feeding larvae in the pupation substrate. The pupation and emergence times of those subjected to wood shavings were relatively short (7.25±0.50 days), compared to others. The pupation/emergence rate was significantly low in the absence of pupation substrate ($p<0.05$) as compared to the pupation/emergence rate recorded with post-feeding larvae subjected to wheat bran (91.50±1.29%) and wood shavings (91.58±6.45%), respectively. These results of this study suggest that the best substrate for pupation and adult emergence is wood shavings and could be considered in good management of a black soldier fly production system.

Keywords: Adult emergence, animal feed, pupation substrate, waste management

1. Introduction

Processing of organic wastes with the fly larvae is an emerging waste treatment technology. Larvae grown on organic wastes can be an alternative source of proteins for animal feed production and can therefore provide revenues for financially viable waste management systems [1-3]. Black soldier fly, *Hermetia illucens* is a holometabolous insect naturally encountered in the tropical and sub-tropical areas between the latitudes of 40°S and 45°N [4]. Females mate once with one oviposition event in their lifetime and selectively oviposit their eggs in dry sheltered cavities near to decomposing organic matter approximately two days after a successful copulation [5]. Shortly after having laid their eggs, the female dies. The closeness of the eggs to the decomposing organic matter ensures that the larvae have their first food source nearby after hatching. The sheltered cavities protect the eggs mass from predators and prevent dehydration by direct sunlight. The eggs hatch four days after oviposition and the emerged larvae will search for food and start feeding on the organic nearby waste [4].

Black soldier fly larvae are voracious and require an average of two weeks to complete their life cycle in suitable conditions [6-9]. The larvae feed on different decaying organic materials, such as rotting fruits and vegetables, animal manure and human excreta [10-12], and can be used commercially to solve a number of environmental problems associated with manure and other organic waste (e.g. reducing manure mass, moisture content and offensive odours) [11, 13]. At maturity, the last instar larvae also called post-feeding larvae or pre-pupae migrate from the feed source in search of a dry and protected pupation site [14]. This migrating behaviour of the post-feeding larvae away from feeding sites is common in most holometabolous insects that search for suitable pupation substrates to bury themselves for protection from predation and desiccation while they undergo metamorphosis [15]. This behaviour is initiated by an innate behavioural sequence and mediated by the abiotic environment factors including temperature, light, and substrate moisture, such that the larvae tend to migrate toward cool, dark, and dry substrates [16]. Pupation occurs within the larval skin also called puparium and the adult emerges after about two weeks [17].

The interest in black soldier fly larvae organic waste processing has been largely attributed to the production of larval biomass for animal feeds with a high market value. Black soldier fly larval biomass contains about 32–58 % proteins and 15–39 % lipids, which are valuable for the production of animal feeds for livestock [18-21]. As previously described, in a black soldier fly facility, the bioconversion of wastes into larval biomass takes place only at the larval stage. Therefore, for a continuous availability of larvae in a facility, it is necessary to optimize the other stages of development that depend more or less on abiotic factors. During the pupa stage for instance, holometabolous insects are completely immobile and do not feed. However, it is a most important stage during which the metamorphosis takes place. Previous studies indicate that the success of this stage may be strongly affected by the substrate in which the post-feeding larvae are found after completing the larval stage [22, 23]. The aim of this study is therefore to determine the role pupation substrates on post-feeding development, pupation and emergence of black soldier fly.

2. Materials and methods

2.1. Study area

The present study was conducted in a greenhouse at the Agri-Business vocational training Center (ABC), Dschang. The Center is situated in the Western Region of Cameroon between 5°25'–5°30' North Latitude and 10°-10°5' East Longitude and at an average Altitude of 1410m, with an equatorial climate. Data of the meteorological station of Dschang from 2001 to 2009 shows that there are two seasons: a long rainy season from March to October and a short dry season from November to February. The rainfall varies between 1500 – 2000mm per year. The average annual temperature is around 21 °C with average annual sunstroke of 1800 hours and a relative humidity varying between 40–97%. The air is perpetually fresh and tends to saturation early in the morning, hence the regular presence of fog or mist in the atmosphere before sunrise.

2.2. Origin of black soldier fly post-feeding larvae

Eggs were collected from a black soldier fly colony maintained in a greenhouse at the Agri-Business vocational training Center (ABC), Dschang. ABC's colony originated from young larvae outcome from a local strain of black soldier fly breed since 2016 at the International Institute of Tropical Agriculture (IITA), Cameroon. For the collection of eggs, "Eggies" were made with five clean wooden sheets (2×4 ×0.3cm) separated with pushpins and held together by two rubber bands on both ends of the bundle (the pushpins created a small gap of about 2 mm between the wooden sheets, allowing space for egg packages), and placed inside a mating cage over an attractant container half filled with fermented chicken feed mixed with water at 70% to encourage females to lay eggs. Eggs harvested the same day were transferred into an incubation unit where temperature and relative humidity were in ranges 25–35 °C and 40–80% RH, respectively. The incubation unit consisted of a hatching crate, a hatching container, a shelf (10×5×5cm) made with wood and sieve, and a hygro-thermograph. The hatching container was filled with 2cm thick chicken feed moistened to 70% with fresh water, above which the shelf with the sieve was put. The harvested eggs were spread on the sieve, and the dry feed was scattered around the container to prevent the hatched larvae from escaping. The whole device was finally put into the hatching crate. The eggs hatched after 3–4 days and the young larvae were fed in the same container for four days before using for further growth.

Two thousand (2000) four-days-old larvae hand-counted were collected and transferred into a plastic container (Ø32x11.25cm), and were fed *ad libitum* using commercial chicken feed until they reach the post-feeding stages, distinguished by the characteristic black cuticle, contrasting with the white larvae. The rearing container was covered with a perforated lid fitted with a mesh to prevent larvae from escaping and for ventilation.

2.3. Experimental design

One thousand six hundreds (1600) black soldier fly post-feeding larvae were removed from the larval food and were divided into four groups of 400 each for pupation and emergence. As pupation substrate, the first group received the wood shavings, the second the fine sand, and the third the wheat bran. The fourth group (control group) received no pupation substrate. The pupation substrates were chosen based on their availability and current uses in various black soldier fly production units. The distribution was random such for each group to have equal opportunity of being selected for any treatment. The experiments were conducted in a dark room using plastic containers (Ø11.30×5.53cm) covered with a perforated lid fitted with a mesh to prevent pupa predators and for ventilation. The containers were filled with 20mm depth of their respective pupation substrate, and were each inoculated with 100 of post-feeding larvae respectively. Four repetitions were established for each treatment and were placed in the dark room where temperature and relative humidity were in ranges 25–35 °C and 40–80% RH, respectively until the emergence of adults.

2.4. Data collection and statistical analyses

For each treatment, the date of inoculation into the pupation containers was recorded and the post-feeding larvae were monitored every day in the afternoon to record the appearance of pupae distinguished when they becomes completely immobile with a puparium rigid without elasticity and the last abdominal segment bent in ventral position. The date when the first pupae appear was noted. From this date, the number of pupae appearing per pupation container and per day was recorded in order to analyse the pupation dynamics. Pupae were collected progressively and placed in a new container with a layer of their respective pupation substrate for adult emergence. Pupae were also monitored every day in the afternoon to record the appearance of adult, and the same procedure was used to determine the emergence dynamics. The pupation/emergence time were defined as the number of days between the start of the experiment and the observation of half of the pupae/adults flies in each container. The pupation/emergence rate were determined by dividing the recorded number of pupae/adult flies in each container at the end of the experiment by the initial number of post-feeding larvae/pupae in the same container at the beginning times hundred.

The statistical analysis was performed by R version 3.5.0 software. The collected data were submitted to Shapiro normality test. Therefore, the results of the pupation and emergence time of black soldier fly post-feeding larvae under different pupation media were analysed by Kruskal-Wallis non-parametric test (Pairwise Wilcoxon test was used to determine the difference in rank means), while the pupation and emergence rate were analysed using one-way analysis of variance (ANOVA), followed by Tukey post-hoc test. All statistical analyses were carried out with 95% confidence interval and $p<0.05$ was considered to indicate a significant difference between the values compared.

3. Results

3.1. Pupation dynamics

The pupation dynamics of black soldier fly post-feeding larvae under different pupation substrates showed significant variations (Figure 1). Regardless of the type of treatment, the first pupae appeared after four days in the pupation substrate. The pupation time of post-feeding larvae subjected to wood shavings was significantly short (7.25±0.50 days) compared to the other substrates (p=0.0155). No significant difference in the pupation time was observed between the post-feeding larvae placed in fine sand, wheat bran and no substrate ($p>0.05$). The pupation rate was also affected by the pupation substrate (p=0.0001), and that of post-feeding larvae in no substrate was significantly low compared to other substrates (76.75±3.86%). The highest pupation rate was recorded with the wheat bran (91.50±1.29%) (Table 1).

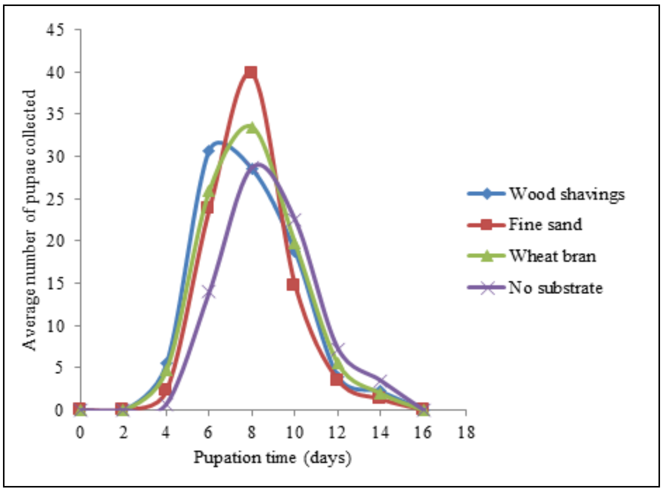

Fig 1: Pupation dynamics of black soldier fly post-feeding larvae under four pupation substrates

Table 1: Effects of pupation substrate on the pupation and emergence success of black soldier fly post-feeding larvae

Pupation substrate	Pupation time (day)	Emergence time (day)	Pupation rate (%)	Emergence rate (%)
Wood shavings	7.25 ± 0.50^{b}	7.25 ± 0.50^{c}	89.75 ± 3.40^{a}	91.58 ± 6.45^{a}
Fine sand	8.00 ± 0.00^{a}	8.25 ± 0.50^{b}	87.50 ± 3.50^{a}	68.66 ± 10.42^{b}
Wheat bran	8.00 ± 0.00^{a}	8.00 ± 0.00^{bc}	91.50 ± 1.29^{a}	78.66 ± 10.99^{ab}
No substrate	8.00 ± 0.00^{a}	9.75 ± 0.50^{a}	76.75 ± 3.86^{b}	63.72 ± 13.40^{b}

Values followed by the same letter are not significantly different ($p<0.05$).

3.2. Emergence dynamics

The emergence dynamics of black soldier fly pupae was also affected by the different pupation substrates (Figure 2). Regardless of the type of pupation substrate, the first flies started emerging after 10 days of incubation and subsequently continued until the 22nd day. The emergence time was significantly affected by the pupation substrate (p = 0.0054), and the pupae subjected to wood shavings still recorded the lowest (7.25±0.50 days). The emergence time of pupae without pupation substrate was longer (9.75±0.50 days), compered with wheat bran and fine sand. The emergence rate ranged from 63.72±13.40% to 91.58±06.45% and also showed significant variation (p=0.0141). The pupae under wood shavings recorded the highest emergence rate while those without pupation substrate recorded the lowest rate (Table 1).

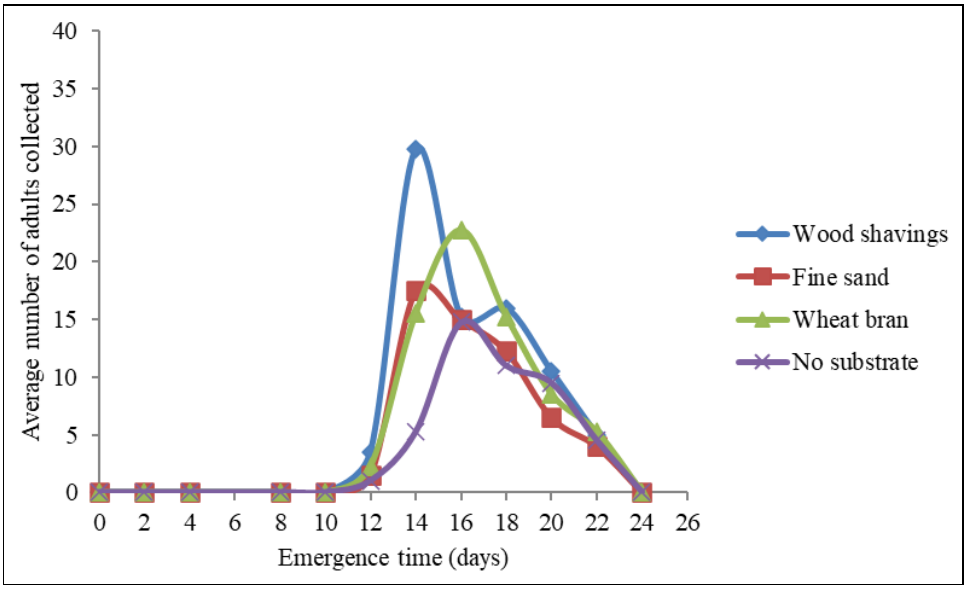

Fig 2: Emergence dynamics of black soldier fly pupae under four pupation substrates

4. Discussion

Regardless of the pupation substrates, the first pupae started appearing after about four days. Then the appearance of the pupae followed a bell-shaped curve and ends after about 15 days later. The pupation time and pupation rate were significantly influenced by pupation substrate. Post-feeding larvae in wood shavings took less time to pupate, while those in wheat bran recorded the highest pupation rate. These results suggest that substrate density and compaction may have impacted post-feeding larvae dispersal and pupation. Dimou *et al*. [24] reports that substrate compaction can have significant effects on a post-feeding larva's ability to pupate. The denser the substrate, the more impenetrable it may be for post-feeding larvae. This impenetrability may prevent the insect's innate behaviour to bury itself during pupal development [15]. The lowest pupation rate was recorded with post-feeding larvae in no pupation substrate, followed by those in fine sand, which was the densest and most compact substrate. In no pupation substrate, post-feeding larvae aggregated together after searching for a moment. Holmes *et al*. [23] report that this behaviour may be an effort to increase the temperature due to the lack of a pupation substrate that can serve as an abiotic cover. It can also be an adaptive behaviour to survive, because the behavioural strategy to bury itself during pupal development is generally known as a protective mechanism to prevent predators and desiccation [15]. The pupation and emergence success could also be affected by the difference in moisture content of the pupation substrates used. Chen and Shelton [22] indicated that soil moisture content can significantly affect swede midge (Diptera: Cecidomyiidae) emergence, regardless of soil type. Similarly, Hulthen and Clarke [25] reported that soil moisture content, especially the extreme of soil moisture, is a major pupal mortality factor of *Bactrocera tryoni* (Diptera: Tephritidae) pupae in soil. However, this parameter has not yet been evaluated in black soldier fly.

The pupation substrates also significantly affected the emergence dynamics of the black soldier fly pupae. The first flies started emerging after 10 days of incubation regardless of the type of substrate. Subsequently, the appearance of the flies followed a bell-shaped curve. This trend was also observed by Caruso *et al*. [26] and Dortmans *et al*. [4]. The average emergence time of pupae without pupation substrate was relatively long, and the treatment recorded the lowest emergence rate. These results confirmed the importance of pupation substrate in the pupation and emergence process of black soldier fly. Tomberlin and Sheppard [27] reported that insects like black soldier fly that do not feed as adults have a limited amount of stored energy that is allocated to adult fitness, including mating, flight and ovarian development. Several important catabolic reactions occur during post-feeding stage and are responsible for energy depletion [28]. Therefore, it is likely post-feeding larvae in the no substrate treatment that did not emerge, expended too much energy in pupation substrate researching and body temperature regulation, and as a result, could not energetically afford metamorphosis and died. The highest emergence rate was recorded with the pupae under wood shavings with a record time. As previously described the less dense and less compact structure of this substrate would have favoured the pupation and emergence process of the black soldier fly post-feeding larvae. Dortmans *et al*. [4] reported that, after two to three weeks, a suitable pupation substrate dries slightly, making it easier for the flies to crawl out of the pupal skin to the top of the substrate and fly out of the pupation medium.

5. Conclusion

This study confirms the importance of the pupation substrate in the black soldier fly metamorphoses. Regardless of the type of substrate, the pupation and emergence process was considerably improved with the pupation substrate, and the best performances were recorded with wood shavings. In the absence of pupation substrate, pupation and emergence success were negatively affected. This information could be useful and offer optional solutions for sustainable wastes management using black soldier fly based technology.

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