BLACK SOLDIER FLY LARVAE FRASS PRODUCTION FOR MANAGING WATER HYACINTH ENCROACHMENT

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ABSTRACT

Water hyacinth (WH) is one of the most invasive and toxic aquatic weeds which detrimentally affects freshwater quality and ecosystem via hindering sunlight from reaching aphotic zone and depleting oxygen level in water bodies. Black soldier fly larvae (BSFL) as degradation agent that feed on WH is an approach to manage the invasive plant. The objectives of this study were to: (i) produce frass from BSFL which is mature and suitable for improving soil and seed germination by using WH as feeding substrates and (ii) characterize the agronomic properties of the frass produced from BSFL after being fed on WH. The frass production was carried out by rearing BSFL using WH at a feeding rate of 200 mg larvae-1 day1 for 48 days. The rearing of BSFL using WH was carried out and monitored for the changes of BSFL to pupae. Water hyacinth was fully digested by BSFL at 48 days. The BSFL frass were sieved to pass a 2 mm sieve and characterized for the physical, biological, and chemical properties. The BSFL colour was dark, and the odour became earthy, suggesting that the frass had reached maturity. The BSFL frass resulted in 100% germination and showed 86.46% germination index of maize seeds in frass produced from WH revealing that the frass had no toxic effect, but rather promoted seedling growth. The BSFL frass is a slightly alkaline material (pH =7.64), and its electrical conductivity (6 µS/cm) was in the range of permissible level as an organic fertilizer. The BSFL frass could be considered as an organic fertilizer with an appropriate range of organic matter (50.31%), organic carbon (29.18%), 2.18% total N, 1270 mg kg-1 ammonium, 1370 mg kg-1 and nitrate. The C/N ratio of BSFL frass was 13 suggesting that the frass can decompose rapidly to release essential nutrients for crop uptake. The BSFL frass produced from WH has potential to be used as organic fertilizer to promote crop growth and soil productivity. A pot experiment study is in progress to determine the effects of BSFL frass on soil productivity and crop growth.

Keywords: *digestion, environmental conservation, organic fertilizer; plant growth enhancer, waste management*

INTRODUCTION

Water hyacinth (WH) is one of the invasive floating and blooming plants found in water bodies all over the world. It originated in the Amazon Basin of South America and its invasiveness has expanded throughout the tropical and subtropical regions (Dersseh et al., 2019). In the areas whereby the community depends on the rivers for food sources and transportation, the invasion of WH had affected the community wellbeing and aquatic ecosystem. This is because the plant has an aggressive growth rate that can double its population as early as two weeks by producing short runner stems that can generate new plants and it can also proliferate through seeds. A single WH may generate up to 140 million new plants every year; these aquatic plants span 1.40 km2 of water area and produce 28,000 tonnes of biomass (Tao et al., 2016). To end this, it is crucial to manage the invasion plant such as WH and turn them into valuable products, for example organic fertilizer to reduce over reliance on chemical fertilizers in line with the increasing prices and demands of chemical fertilizers.

The use of black soldier fly larvae (BSFL) as degrader agent to digest on WH may help to reduce invasion of WH and produce the insect biomass (protein, chitin, and fat), which is known as frass (Bortolini et al., 2020). Black soldier fly larvae (Hermetia illucens L.) are considered as a bio-waste recycler within the insect category which has been used in waste management technology known as bioconversion. The bioconversion of organic wastes using BSFL is one of the most efficient insect-based bio converters because the larvae are voracious digesters on organic waste such as crop, animal, and human wastes and turn the wastes into frass (Isibika et al., 2019). Frass is the excreta of BSFL, indigestible material, metabolic products, and residues from different BSFL development stages, which can be used as a plant growth enhancer. Schmitt and Vries (2020) reported that frass production is a profitable method to manage degradable waste because frass can be sold at high prices. According to Caruso et al. (2020), Beesigamukama et al. (2020), and Bortolini et al. (2020) the BSFL frass has high degradation efficiency, rich in macronutrients, micronutrients, and organic matter (OM) that increased soil fertility and more environmentally friendly compared with chemical fertilizers. However, the quality of the frass excreted by BSFL during the degradation of organic wastes depends on the nature of the organic wastes consumed by the BSFL and the stabilization during the process of degradation which involved biological, physical, and chemical factors. The research questions that need to be addressed in this study were: (i) does frass from BSFL can be produced using WH as feeding substrates? and (ii) are the agronomic properties of BSFL frass produced from WH has potential to be used as an organic fertilizer? Thus, the objectives of this study were to: (i) produce frass from BSFL which is mature and suitable for improving soil and seed germination by using WH as feeding substrates (ii) characterize the agronomic properties of the frass produced from BSFL after being fed on WH.

Water hyacinth was used as the main substrate for the frass production because the plant became a nuisance for the community and aquatic ecosystem and easily found in most parts of Sarawak especially in Mukah. The BSFL serves as bio converter agent and assist in overcoming the invasion of the WH. It is hypothesized that the frass produced from BSFL after being fed on WH has good agronomic properties and is free of toxicity and could be used as an organic fertilizer to improve soil properties and enhance seed germination rate. The impacts of this study to the science community are to provide the information on managing WH by using them as rearing substrates for BSFL to produce frass and revealed the characteristics of BSFL frass produced after being fed on WH that could be considered as one of the organic fertilizers and eventually initiate farmers in managing their crops through utilizing the macrophyte into organic fertilizer.

MATERIALS AND METHODS

REARING BLACK SOLDIER FLY LARVAE FOR FRASS PRODUCTION

The eggs of black soldier fly were supplied by BSF Farming and Trading, Bintulu, Sarawak, Malaysia and incubated for four days in the tray filled with rejected sago starch flour as feeding starter for BSFL. The rejected sago starch flour that did not meet the premium quality for domestic market and export was collected from Sago Link Sdn. Bhd. Mukah, Sarawak, Malaysia. The WH as main feeding substrate was collected from Rejang River along Dalat and Mukah and canals in Mukah coordinated (2°44'52.7"N 111°56'19.3"E). Dalat situated on peat land that is classified as deep peat (greater than 150 cm), similar to other regions in the Rajang delta with annual temperature of 25.3 °C to 32 °C and annual rainfall of 3000 to 4000 mm (Isia et al., 2020). High nutrient content waterbodies prevalent due to agricultural runoff and inadequate wastewater treatment in Dalat river favour condition for WH to thrive and grow vigorously (Figure 1).

The black soldier fly eggs hatched into larvae after four days and then the larval stage begins and consumed on starter diet because at the first instar the BSFL have small projecting head which containing chewing mouthparts which help in consuming their feeds. Larvae need to go through six instars and complete their development in approximately 14 days (Hall and Gerhardt, 2002). As reported by Hall and Gerhardt (2002), when the larvae development is progressing, they are insatiable feeders which are able to consume feeds that are double or even triple their weight each day in this stage. The BSFL fed on starter diets such as rejected starch and left over kitchen wastes (bread, fruits peel, and grinded coffee wastes) for 10 days after which at 10 days to 48 days the BSFL were fed with 5 kg of WH. The WH (feeding substrates) were sprinkled with 20 mL of water to maintain moisture content, humidity, and temperature. The temperatures of the feeding substrates were taken daily to avoid an increase of the temperature that restricted the growth of BSFL. The rearing of BSFL fed on WH was carried out for 48 days whereby the larvae turn into pupae. The separation of the pupa from the frass was carried out using the hand sieve after which the frass was further analyzed for selected physico-chemical properties using standard procedures (Tan, 2005) and phytotoxicity test (Sullivan and Miller, 2001).

Figure 1. Water hyacinth invasion in Rejang River (left) and water hyacinth sampling for rearing black soldier fly larvae (right)

PHYSICAL, CHEMICAL, AND PHYTOTOXICITY ANALYSES OF FRASS

The temperature of the WH as feeding substrates and the ambient temperature were recorded from day one to day 48 whereby the BSFL completed larvae stage and reached into pupae stage. The temperature of substrate was determined using Reotemp compost thermometer (Reotemp Instrument Co, Van Nuys, Calif). The electrical conductivity (EC) and pH of the frass were determined in distilled water using a ratio of 1:10 (w/v) (Ramli et al., 2012). The colours of frass were determined based on colours categorized in Munshell Colour Chart. Total organic matter (OM) and carbon (C) of the BSFL frass were determined using loss-on-ignition method (Tan, 2005). Total N was determined using Kjedhal method (Bremner, 1965), whereas the method described by Keeney and Nelson (1982) was used to extract exchangeable ammonium and available nitrate in the frass after which the ions were determined using steam distillation (Tan, 2005). Cation exchange capacity (CEC) was determined using leaching method (Tan, 2005), followed by steam distillation and colourimetric titration (Bremner, 1965). Available P and exchangeable K were extracted using the double acid method described by Tan (2005) and their extractants were determined using Atomic Absorption Spectrometry (AAnalyst 800, PerkinElmer, Norwalk, CT) and UV-VIS Spectrophotometry, respectively. There were three replications for each analysis.

The phytotoxicity test was carried out using the germination bioassay method described by Zucconi et al. (1985). The frass extractant was prepared by mixing 5 g of frass with 50 mL of distilled water (1:10 w/v). The suspensions were shaken using an orbital shaker at 180 rpm for an hour. The suspensions were filtered using Whatman filter paper no. 2 and the extractants were further diluted to a dilution series of 10, 100, and 1000, respectively. Each extractant of 5 mL (1×, 10×, 100×, 1000×) was placed in a petri dish which contained filter paper to retain the moisture for seed germination. Thereafter, each petri dish was placed with 10 maize seeds as test crop due to its fast germination rate and sensitivity towards toxicity level and left for five days. Each treatment had four dilution factors and each dilution factor had three replications. The maize seeds germination was prepared in distilled water served as the positive control for comparison against the BSFL frass extractants. After five days of germination, the seed germination rate, radicle length, and shoot length were determined using a measuring tape.

The percentage of germination index (GI), germination (G), relative shoot growth (RSG), relative root growth (RRG) was calculated using given formula:

Germination Index (%) = (G × RRG) × 100
where, G (%) = (Number of seeds germinated in the extract / Number of seeds germinated in the control) × 100%
RRG (%) = (Mean root length in extract/Mean root length in control) × 100%
RSG (%) = (Mean shoot length in extract/Mean shoot length in control) × 100%

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

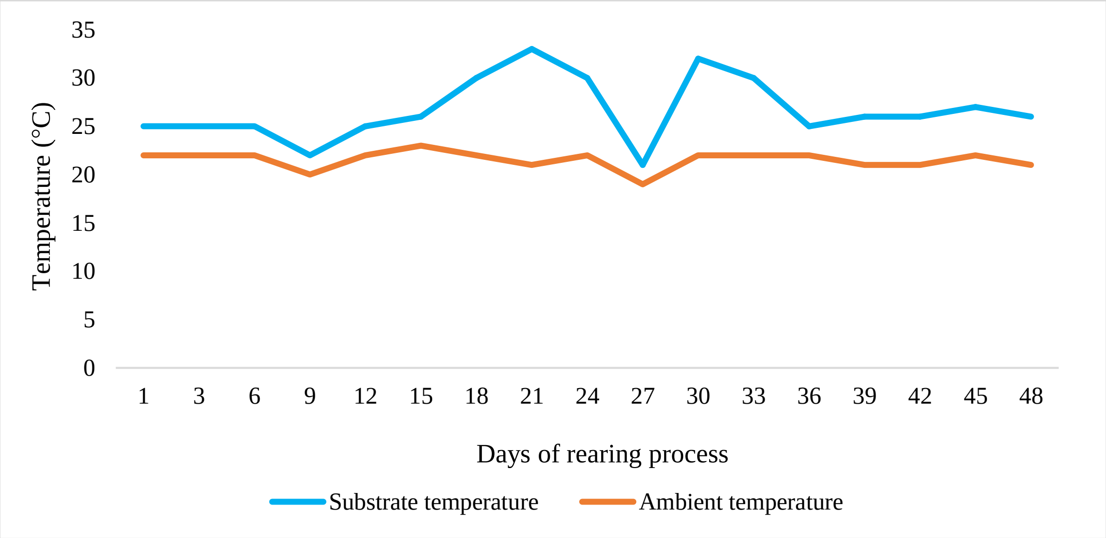
The BSFL rearing trays were arranged in a completely randomized design (CRD) with three replications for each treatment. One-way analysis of variance (ANOVA) was used to determine the extractants effects consisted of BSFL frass extractants and distilled water on maize germination, whereas Tukey's test was used to compare extractant means at $P \leq 0.05$ using the Statistical Analysis System software version 9.4.

RESULTS AND DISCUSSION

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FRASS

The temperature of WH substrate showed slight changes throughout the degradation process of WH by BSFL (Figure 2). The slight fluctuate of the WH substrate temperature from 30 °C to 33 °C at day 15 to day 24 was because the BSFL were actively degraded the substrate within two weeks as agreed by Bullock et al. (2013). The authors stated that 35 °C is the optimum temperature when the BSFL consume their food. Whereas, the decreased of the substrate temperature at day 27 was because the substrate were sprinkled with water to moistened the substrate. Humidity influences the growth of the BSFL, for instances, 70% humidity improved the growth and development of BSFL (Bullock et al., 2013). In addition, the feeding substrate temperature need to be monitored during the degradation process as according to Lalander et al. (2013) the BSFL prefers a temperature range of 25 °C to 35 °C for rapid degradation within two weeks to digest the feeding substrate. The survival rate of BSFL larvae will drastically decrease when the temperature exceeds 35 C, therefore unlike the composting process, the rearing trays did not reach thermophilic phases that exceed 40° C (Shaphan et al., 2018). The temperature of feeding substrate decreased and almost similar to ambient temperature towards the last stage of larvae phase, whereby the BSFL turn into pupae on day 48 (Figure 2).

Figure 2. Temperature profile of rearing black soldier fly larvae fed on water hyacinth for forty-eight days



The colour of the WH was green at first day of BSFL rearing, after digested and excreted by the BSFL, the substrate turns into dark colour as compared against Munsell colour chart (Figure 3, Table 1). The dark colour of the BSFL frass after 48 days of rearing was related to the appropriate amount of aeration and humidity throughout the degradation and digestion of WH by the BSFL. The BSFL frass produced after the degradation of WH on days 48 does not emits unpleasant odours suggested that the frass was fully digested and stabilized. Odourless mixture of degradation products is one of the maturity indicators (Sullivan and Miller, 2001).

Figure 3. The changes of water hyacinth colour during the progress of rearing the black soldier fly larvae

In addition, Lalander et al. (2013) stated that the BSFL is able to process organic wastes rapidly, aerate, dry the substrate and reduce the foul odour. The electrical conductivity (EC) of the BSFL frass was in the range of permissible level as an organic fertilizer (Table 1). Whereas, pH of the BSFL frass is a slightly basic (Table 1). The BSFL frass could be considered as an organic fertilizer with an appropriate range of organic matter (50.31%), organic carbon (29.18%), 2.18% total N, 1270 mg kg-1 ammonium, and 1370 mg kg-1 nitrate. The C/N ratio of BSFL frass produced from WH was 13 suggesting that the frass can decompose rapidly to release essential nutrients for crop uptake. The CEC of BSFL frass (35.06 cmol(+) kg¬¹) indicate that the BSFL frass can be used to retain base exchangeable cations such as K+, Ca2+, Mg2+, and Na+ in soils. High content of available P (0.10 mg kg-1) and exchangeable K (895.9 mg kg-1) suggest that the BSFL frass could be used as soil organic amendment to improve P and K availability soil. One of the advantages using BSFL as decomposing agent to digest WH is the larvae able to digest the plant thoroughly despite of low nutritional content in WH as according to Bonelli et al. (2020), BSFL depicts greater adaptation to low or unbalanced nutritional substrates. In previous studies found that the quality of the substrates significantly affects the nutritional and chemical properties of the frass produced by the BSFL (Ewald et al., 2020; Galassi et al., 2021; Meneguz et al., 2018). However, in this study, the BSFL frass produced from WH as main feeding substrate show good agronomic properties that could be used as organic fertilizer as depicted in Table 1.

Table 1: Selected physico-chemical properties of black soldier fly larvae frass after forty-eight days of rearing

Variable	Black soldier fly larvae frass
Colour	7.5YR very dark brown
Electrical conductivity (µS cm^{-1})	6 ± 0.35
pH	7.64 ± 0.026
Total organic carbon (%)	29.18 ± 1.54
Total organic matter (%)	50.31 ± 2.66
Total nitrogen (%)	2.18 ± 0.58
C/N ratio	13 ± 4.24
Exchangeable ammonium (mg kg^{-1})	1270 ± 0.017
Available nitrate (mg kg^{-1})	1370 ± 0.016

PHYTOTOXICITY TEST OF BLACK SOLDIER FLY LARVAE FRASS

Seed germination of maize in distilled water (control) and extractants of BSFL regardless of the dilution, indicated no significant difference in term of root length, shoot length, seed germination percentage, and relative seed germination (Table 2). The germination index values were above 80% in all BSFL extractants suggests that the BSFL frass is matured, stabilized, safe, and suitable to be used as growing media. The BSFL frass could be considered as soil amendment for crop germination and growth as evidenced by the value of germination index which is in accordance to Teresa et al. (2011). The value of the germination index above 80% is categorized as free from plant toxicity (Teresa et al., 2011). Furthermore, a facile and accepted definition refers to the compost's compatibility for plant growth, which includes the combination of stability and humification of organic matter followed by the absence of toxic elements that could harm or prevent plants from germinating or developing (Smith, 2009).

Table 2. Phytotoxicity test of black soldier fly larvae frass at different extractants compared against distilled water (control)

Media	Root Length (cm)	Shoot Length (cm)	Seed germination (%)	Relative Seed Germination (%)	Germination index (%)
Distilled water	1.26	2.01	100	100	100
E1	3.57	2.42	100	100	84
E2	2.88	2.83	100	100	83
E3	3.67	2.88	100	100	84
E4	1.99	2.66	100	100	91

CONCLUSIONS

The BSFL frass fed on WH were produced for 48 days, whereby the colour of WH as feeding substrate was turn into dark and the odour of the BSFL frass became earthy, suggesting that the frass had reached maturity. The BSFL frass is alkaline with pH 7.64, and its electrical conductivity (6 µS/cm) was in the range of permissible level as an organic fertilizer. The BSFL frass could be considered as an organic fertilizer with an appropriate range of organic matter (50.31%), organic carbon (29.18%), 2.18% total N, 1270 mg kg-1 ammonium, 1370 mg kg-1 nitrate, and 1400 mg kg-1 available phosphorus. The C/N ratio of BSFL frass produced from WH was 13 suggesting that the frass can decompose rapidly to release essential nutrients for crop uptake. The BSFL frass resulted in 100% germination and showed 86.46% germination index of maize seeds in frass produced from WH revealing that the frass had no toxic effect to the maize seeds, but rather promoted seedling growth. The BSFL frass produced from WH has potential to be used as organic fertilizer to promote crop growth and soil productivity. A pot experiment study is in progress to determine the effect of BSFL frass from WH on soil productivity and crop growth.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Ministry of Higher Education Malaysia and Universiti Putra Malaysia Bintulu Sarawak Campus for the collaborative research.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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