

Augmenting black soldier fly (BSF), *Hermetia illucens*, production on various agricultural waste streams, and the beneficiation of BSF frass

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Augmenting black soldier fly (BSF), *Hermetia illucens*, production on various agricultural waste streams, and the beneficiation of BSF frass

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***"Obstacles are those frightful things you see when you take your eyes off the goal."- Dr.
Irvin Khoza***

This thesis is solely dedicated to the late *Professor Stefan Hendrick Foord*

Declaration

I, **Kubayi Consol** (Student number: **15010691**), declare that this thesis is submitted to the University of Venda for the Doctor of Philosophy degree. This work has not been submitted previously for any other degree at this or any other institution, and it is my own work in design and execution. All materials contained therein have been duly acknowledged.



27/11/2024

Signature

Date

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Summary

A sustainable and innovative approach to managing organic waste is presented by Black Soldier Fly Larvae (BSFL), *Hermetia illucens*, flowing from previously underutilised biological waste streams in agricultural systems. These waste streams are regularly disposed of irresponsibly or recycled inappropriately that lead to environmental problems characterised by emission of greenhouse gases, nutrient leaching, and inefficient waste reduction methods. BSFL's capacity to convert organic material into valuable protein-rich biomass and nutrient-rich frass have great potential in sustainable waste management systems, especially in handling high moisture organic waste streams and contributing to a circular-loop economy.

Thus, the objective of this study was to evaluate the BSFL performance in converting three economically abundant organic waste streams in Limpopo, Vhembe district, South Africa (avocado waste, orange waste, and potato peels) and to evaluate the effect of frass from those substrates on agricultural productivity. This was achieved through three main specific objectives: 1) to determine the growth and survival of BSFL in response to sawdust as a bulking agent for managing high-moisture potato peels; 2) to investigate the effect of nutrient composition of the three organic wastes on BSFL survival, growth rate, larvae yield, waste reduction and the efficiency to convert waste into useful biomass ; and 3) to evaluate the agronomic potential of BSFL frass as an organic fertiliser for improving the growth and yield of beetroot (*Beta vulgaris*).

This study also contributed to a better understanding of how BSFL performance can be optimised in various organic waste streams, illustrating the potential for sawdust to provide effective bulking more quickly, thus improving larval growth and subsequent management of the high moisture substrates during the bioconversion process. Results showed that sawdust effectively enhanced larval yield and growth rates by maintaining the aeration and moisture balance of the substrates. Moreover, the results of bioconversion in the three waste streams indicated that larval biomass and frass production were highest when potato peels were used, and some wastes were more efficiently reduced than others, which is mainly related to their composition. In agronomic scales, application of frass has improved beetroot growth and yield, thus showing that the frass also serves as a biopesticide that can protect crops.

This will advance the knowledge base of BSFL bioconversion systems and allow practical guidance identifying conditions to optimise substrates and possibly render frass to be integrated with agriculture. It highlighted that BSFL not only provides a means for organic waste reduction, but when used as a component of organic fertilisers, increases crop yields and improves soil health, thus presenting an eco-friendly agricultural opportunity. This research contributes to offsetting both ecological and economic problems and creates a step towards broader and more effective application of the insect industry on BSFL for this niche in the waste valorisation system.

Keywords: Black soldier fly larvae, organic waste management, bioconversion, sawdust, bulking agent, frass, circular economy, sustainable agriculture, waste valorisation, high-moisture substrates.

Preface

Dissertation format

This dissertation is presented in a paper-based format, with references included at the end of each chapter. Consequently, some repetition of information occurs, as each chapter is structured to stand alone as an independent piece.

The term “we” represents a collaborative reflection of the research team, even though the work was conducted individually by me, Consol Kubayi.

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Ethical clearance certificate

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Chapter 1

Literature Review and Introduction

1.1 Introduction

1.1.1 Biological waste management

The exponential growth of organic waste has emerged as a global problem that can intensify environmental degradation and has severe economic implications and public health challenges (Giusti, 2009). The irresponsible disposal of organic waste, especially using the traditional methods involving landfilling and incineration, has contributed to greenhouse gas emissions (methane and carbon dioxide) (Rehman et al., 2023) and water contamination through leaching (Schmitt & de Vries, 2020). Innovative and sustainable waste management practices are needed since global waste generation is proportional to population growth (Gold et al., 2020). One such practice is the application of biological waste management methods such as the black soldier fly larvae (He et al., 2021; Salam et al., 2023).

1.1.2 Role of insects in waste management

Insects are essential in breaking down organic waste, contributing to nutrient cycling and reducing environmental pollutants (Liu et al., 2022). Amongst many insects, such as mealworm (*Tenebrio molitor*) and domestic fly (*Musca domestica*), the black soldier fly larvae (*Hermetia illucens*, BSFL) have attracted scientific attention due to their ability to decompose high volumes of organic waste in a short period (Čičková et al., 2015). BSFL is a voracious insect that can consume large quantities of organic waste ranging from poultry manure (Parry & Weldon, 2023), fruits and vegetables (Scala et al., 2020; Scieuzo et al., 2023), and human faeces (Banks, 2014), producing valuable agricultural products such as frass and protein-rich larvae. Using BSFL in waste management systems offers an innovative approach that aligns with sustainable development goals (Sable et al., 2024). The ability to reduce volumes of organic waste while producing protein-rich larvae and frass that can be utilised as animal feed and organic fertilisers makes the insect a versatile tool in a circular loop economy (Liu et al., 2022).

1.2 Research Problem Statement

Sustainable organic waste management remains a key global challenge when accounting for the growing volumes of agricultural waste generated by population growth and urbanisation (Giusti, 2009). In South Africa, the production of organic waste is estimated to be 9 million tons, representing 31 % of average annual agricultural production waste (Oelofse & Nahman,

2013). Particularly, in Limpopo Province, Vhembe District, South Africa, this region encompasses extensive agricultural zones with farms that predominantly cultivate these crops, generating substantial agricultural waste streams. These waste streams offer a consistent and abundant organic waste source, making them suitable for BSFL bioconversion. While the BSFL shows the ability to manage organic waste streams through bioconversion, this strength is also hindered by the physical properties of substrates with high moisture content in the waste streams. Excessive moisture content results in challenges involving larval survival, growth, yield and bioconversion efficiency by limiting aeration and hindering larval respiration (Diener et al., 2011).

Furthermore, other studies explored the application of non-nutritive bulking agents, for instance, sawdust, to manage substrate moisture and enhance larval development (Laksanawimol et al., 2024; Nyakeri et al., 2017). However, their direct impact on BSFL performance remains underexplored. The application of these bulking agents at different doses on an industrial scale is still understudied. Moreover, frass produced from different waste streams can affect crop yields and soil health differently (Abiya et al., 2022). Thus, evaluating frass from various substrates and its effects on plant growth is essential for optimising BSFL rearing and enhancing the quality of both the larvae and the organic fertilisers produced.

1.3 Rationale for the Study

South Africa faces mounting challenges in waste management, with significant amounts of organic waste generated from agricultural activities (Oelofse & Nahman, 2013). Utilising BSFL for organic waste bioconversion offers a sustainable approach to reducing waste while producing valuable byproducts such as protein-rich larvae and nutrient-rich frass. However, high-moisture substrates such as potato peels present barriers to efficient BSFL performance due to moisture-induced challenges such as poor aeration, substrate compaction, and leachate production.

Sawdust was chosen as a bulking agent due to its moisture-absorbing properties, affordability, and local availability. Its ability to improve substrate structure and aeration makes it a practical solution for enhancing BSFL bioconversion performance (Diener et al., 2011). Additionally, focusing on potato peels was necessary due to their significantly higher moisture

content and abundance, which presents a unique challenge in waste management. Therefore, this study also explores the application of BSFL waste management strategies at an industrial scale, aiming to test the effect of sawdust at different application rates to manage moisture content challenges. Consequently, assessing the performance of the larvae in different waste streams on larval performance and bioconversion efficiency since they are direct biological responses than the chemical nutrient profiling. The use of different frasses as potential organic fertilisers is essential since it could contribute to sustainable agricultural practices and support South Africa's circular economy goals.

1.4 Research Objectives

The primary objective is to assess the ability and performance of BSFL in converting substrates with high moisture organic waste streams that differ in nutrient composition and the assess the effectiveness of these waste streams as frass on leafy vegetables. Therefore, to achieve this, the following specific objectives were assessed:

1. To determine the growth and survival of black soldier fly larvae in response to varying sawdust proportions as a bulking agent in potato peels.
2. To evaluate the impact nutritional composition of avocado, orange waste, and potato peels on larval growth, survival, bioconversion efficiency, and waste reduction.
3. To assess the effectiveness of frass derived from locally common organic waste streams (avocado, orange waste, and potato peels) in Thohoyandou, South Africa, as an organic fertiliser.

1.5 Research Hypotheses

This study is guided by the following hypotheses:

1. Adding sawdust as a bulking agent will improve BSFL growth performance by enhancing substrate aeration and moisture management (Nyakeri et al., 2017).
2. BSFL will exhibit different growth rates and biomass yields depending on the nutrient composition of the substrates used (Sripontan et al., 2020).
3. The application of BSFL frass will positively affect the growth and yield of beetroot plants, demonstrating its potential as an organic fertiliser (Abiya et al., 2022).

1.6 Significance of the Study

This research contributes to the growing body of knowledge on sustainable waste management through biological waste conversion. By optimising the use of BSFL in converting high-moisture organic waste streams, the study offers solutions for addressing environmental and economic challenges associated with agricultural waste. It highlights the practical benefits of incorporating sawdust as a bulking agent to enhance BSFL performance, thereby improving waste reduction efficiency.

Furthermore, the study demonstrates the agronomic potential of BSFL frass as an organic fertiliser, promoting soil health, increasing crop yields, and offering a sustainable alternative to inorganic fertilisers. The findings have implications for circular economy initiatives, providing valuable insights for policymakers, researchers, and agricultural practitioners seeking to develop eco-friendly waste management and agricultural productivity strategies in South Africa and beyond.

1.7 Literature Review

1.7.1 Recent advances in organic waste valorisation

Recent studies have shown interest in using biological waste management methods in valorising large volumes of organic waste. Furthermore, scientific research has shown that organic waste streams have untapped potential to be used as resources that yield high-value products such as bioenergy (Li et al., 2011) and organic fertilisers (Chavez et al., 2024; Jasso et al., 2024). Technologies such as waste composting and anaerobic digestion have been used for the longest time, but they remain costly, have limited scalability, and cause negative environmental impacts (Tiwary et al., 2015; Wei et al., 2017). Therefore, insect-based waste management has emerged as a promising alternative, offering an environmentally friendly and economically viable method for transforming organic waste into useful outputs (Salam et al., 2023).

The utilisation of biological agents, specifically insects, in waste degradation does not only align with global sustainability goals but also plays a significant role in contributing towards nutrient cycling and greenhouse gas reductions (Beesigamukama et al., 2021; Qazi et al.,

2016). Compared to traditional waste management methods such as incineration, insect-based systems offer higher conversion rates (Gold et al., 2020) and lower operational costs (Beesigamukama et al., 2021), positioning them as suitable solutions for low-resource settings and developing economies.

1.7.2 The growing significance of insect-based bioconversion

These insects have shown the important potential for waste reduction, nutrient recycling, and the generation of valuable resources such as protein-rich biomass and organic fertilisers (Liu et al., 2022). These insects are specified as suitable bio-converters of low-value organic matter by looking at the abiotic attributes (moisture content and nutrient load) and functional characteristics such as feeding behaviour and morphology (i.e mouthpart) (Fowles & Nansen, 2020). The combination of functional characteristics and abiotic attributes is the main determinant of the insect suitable for organic waste conversion. Among the most studied insect species for bioconversion are the black soldier fly larvae (*Hermetia illucens*), Mealworms (*Tenebrio molitor*), Housefly larvae (*Musca domestica*), Superworms (*Zophobas morio*), and House crickets (*Acheta domesticus*) (Siddiqui et al., 2024). Moreover, different waste streams differ in nutritional composition, significantly contributing to the challenge of waste management when considering the type of species to apply in the bioconversion process.

The nutritional composition of organic waste streams is critical in determining bioconversion efficiency using BSFL (Siddiqui et al., 2022). Diverse waste types, including livestock manure (Čičková et al., 2015), food waste (Nguyen et al., 2015), fruit and vegetable residues (Scala et al., 2020), and mixed industrial organic waste (Lee et al., 2024), vary significantly regarding nutrient content, moisture levels, and degradability. Such varying nutritional composition impacts BSF growth and development. For example, optimised BSFL conversions occur when wastes are palatable with less cellulose and lignocellulose (Rehman et al., 2019; Xiao et al., 2018). Furthermore, the substrate with fewer nutrients improves bioconversion efficiency when topped with nutritional substrates such as wheat bran due to its high protein nutritional content (Parry & Weldon, 2021).

Some organic wastes have compounds that are not digestible by insects (Manurung et al., 2016). For instance, due to high lignocellulose content, chicken and cow manure has very low

bioconversion rates (Rehman et al., 2019). By using *Bacillus subtilis* bacteria to inoculate chicken manure to digest lignin, BSFL bioconversion can improve (Xiao et al., 2018) in a short period of time (14 days). Similarly, the fermentation of maize straw (lignin-rich substrate) with *Aspergillus oryzae* and *Trichoderma reesei* fungi is capable of allowing for successful larval development with reduced mortality recorded (Gao et al., 2019). Furthermore, inoculating different lignocellulolytic exogenous bacteria strains in a mixture of dairy and chicken manure has seen the bacteria break down at least 46.85 % to 72.90 % of cellulose, resulting in palatability, leading to the highest waste reduction of 48.77 % and bioconversion of 10.84 % in 19 days (Rehman et al., 2019)

1.7.3 Optimising bsfl for waste management-related biological and ecological considerations

The bioconversion process in waste management is influenced by environmental factors, including temperature, humidity, and substrate composition (Barragán-Fonseca et al., 2018; Yakti et al., 2023). The optimal condition for larval development includes a temperature range between 29 and 31 °C, with a relative humidity range of 60-70 % (Holmes, 2010). The larvae exhibit rapid growth and high consumption rates during their feeding stage, which is crucial for effective organic waste reduction (Arabzadeh et al., 2022).

Enzymatic activity within BSFL plays a critical role in decomposing complex organic materials. The BSFL gut produces enzymes such as amylases, proteases, and lipases, which facilitate the breakdown of carbohydrates, proteins, and fats (Barragan-Fonseca et al., 2017). Additionally, BSFL larvae have shown the ability to neutralise harmful pathogens present in waste, further enhancing their potential for safe and efficient waste processing (Lalander et al., 2013).

1.8 Challenges of high-moisture waste

Moisture balance is essential for optimising the performance of BSFL in waste conversion, as it affects the aeration, structural integrity, and biological activity within the waste substrate (Yakti et al., 2023). High moisture content in organic waste results in several challenges, including increased risks of spoilage, odour production, leachate formation, and slow decomposition (Lalander et al., 2020; Popa & Green, 2012). Also, decomposition can become anaerobic without proper moisture control, producing foul-smelling gases and other negative environmental impacts (Klüber et al., 2022; Siddiqui et al., 2022). High moisture content can

also impact the BSF lifecycle and performance. In BSFL rearing, excessive moisture can lead to poor larval development, survival, and decreased waste conversion efficiency (Chen et al., 2019; Cheng et al., 2017). Dzepe et al. (2020) reported that BSFL showed optimal growth on substrates with 60 % moisture content, with growth decreasing at 80 %. While moisture levels below 80 % did not affect larval survival, survival rates declined when moisture exceeded 90 % (Dzepe et al., 2020; Lalander et al., 2020). However, Bekker et al., (2021) reported that while moisture content below 80 % effectively improves larval weights, substrates with only 40 % moisture content result in high mortality and reduced growth. High moisture content also impacts the mechanical management of larvae, and sieving of the substrates during harvest becomes difficult due to the compacted wet substrate combined with dead larvae, which produce unhealthy smells (Chen et al., 2019). Therefore, introducing bulking agents to simplify the compacted wet substrate structure can potentially solve this challenge.

1.9 Use of bulking agents

Bulking agents are materials added to substrates to improve their physical properties, particularly aeration and moisture content balancing (Peguero et al., 2022; Rincón-Betancurt et al., 2021). The primary application of these agents is to assist with creating void spaces in the substrate that improve the larval respiration process and faster decomposition (Lee et al., 2024). Bulking agents such as sawdust (Lee et al., 2024), rice (Laksanawimol et al., 2024), straw (Grossule et al., 2020), and other organic materials are commonly used in waste management. For example, Lee et al. (2024) showed that applying sawdust can effectively promote larval growth but has no significant differences in survival. Similarly, Laksanawimol et al. (2024) demonstrated that bulking agents such as coconut coir dust, spent coffee grounds, rice husk ash and rubberwood sawdust were effective in smaller quantities. Still, their use required pretreatment to eliminate toxic substances that could hinder larval growth, while rice husk was the most effective at improving BSFL survival and growth (Laksanawimol et al., 2024). Different bulking agents have different properties towards larval development and survival since some agents should be pretreated for their usefulness in BSFL rearing to avoid toxic compounds that can hinder growth performance (Laksanawimol et al., 2024; Peguero et al., 2022). Other than the moisture-absorbing capacity benefit of bulking agents, most of these bulking agents are utilised due to low cost and widespread availability (Grossule

et al., 2020). As low cost and widely available materials, these agents offer a practical solution to improve the efficiency of BSFL-based waste management systems.

1.10 The production and use of frass as an organic fertiliser

Organic fertilisers are eco-friendly and supply nutrients into the soil at a gradual rate, promoting long-term soil fertility (Houben et al., 2020; Kagata & Ohgushi, 2012). Frass, a byproduct of insect farming, has gained attention as an organic fertiliser, particularly among farmers concerned with the environmental impacts of inorganic (Chavez et al., 2024; Schmitt & de Vries, 2020). During production, the amount of frass produced can exceed about 30 % of the total waste used, depending on the substrate type, and the frass weight relies on the substrate type (Basri et al., 2022). For instance, Salomone et al. (2017) fed the larvae with ten tonnes of food waste and produced 3,346 kg of frass (33.64 %) and 300 kg (3 %) of dried larvae. Quilliam et al. (2020) fed the larvae with 2,624 kilograms of watermelon, avocado, mango, or papaya, which yielded 1.506 kg (57.39 %) of usable frass as an organic fertiliser. Meanwhile, Ravi et al. (2020) used 1,000 kg of mixed vegetable waste, yielding approximately 250 kg (25 %) of frass. Moreover, Cai et al. (2019) used 1,000 kg of 60 % mushroom waste topped with 40 % bran or kitchen waste to catalyse the bioconversion process, resulting in maximum production of 72 kg (7 %) and 129 kg (12 %) of frass, respectively. These variable findings underscore the importance of choosing the appropriate waste substrate maximised for the larvae to maximise the efficiency and sustainability of the bioconversion process.

While organic fertilisers are used in crop production, studies have shown differences in plant nutrient utilisation between organic fertilisers. For example, lettuce (*Lactuca sativa* var. *Crispa*) grown in cow manure yielded higher biomass, plant height, more leaves, a larger leaf area, and increased chlorophyll content compared to plants treated with insect frass (Putra et al., 2017). Nitrogen uptake was more efficient in plants grown with cow manure, while phosphorus and potassium were better utilised in frass-treated plants (Putra et al., 2017). Several other studies have highlighted the ability of insect frass to improve the growth performance of French beans (*Phaseolus vulgaris* L.), tomatoes (*Solanum lycopersicum* L.) (Anyega et al., 2021), and kales (*Brassica oleracea* L. var *acephala*) (Anyega et al., 2021), maize (*Zea mays* L.) (Tanga et al., 2022), and Swiss Chard (*Beta vulgaris*) (Abiya et al., 2022). Frass is crucial for plant growth, yet it contains biopesticide properties (Kawasaki et al., 2020; Quilliam et al., 2020). Several studies have further shown that frass can suppress photogens and fungi

on lettuce (*Lactuca sativa*) (Dzepe et al., 2022), sugar beet (*Beta vulgaris*), cress (*Lepidium sativum*) (Elissen et al., 2019), and Pakchoi (*Brassica rapa* L.) (Agustiyani et al., 2021). Frass has an impact on different crops, yielding positive results (Abd Manan et al., 2024). This suggests that frass can suppress harmful organisms while improving crop yield.

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Chapter 2

Evaluating sawdust as a bulking agent to enhance Black Soldier Fly Larvae (*Hermetia illucens*) performance and survival in potato peels

Evaluating sawdust as a bulking agent to enhance Black Soldier Fly Larvae (*Hermetia illucens*) performance and survival in potato peels

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Abstract

The rearing of black soldier fly larvae (BSFL) offers a sustainable solution for producing high-protein larvae suitable for animal feed, which contributes to waste reduction, nutrient recovery, and improved livelihoods. While substantial research has been conducted on the basic requirements for rearing BSFL at both large and small scales, there is limited knowledge on using non-nutritive bulking agents such as sawdust to enhance larval performance and survival. This study explored the growth and survival of BSFL in response to varying proportions of sawdust as a bulking agent applied at 5, 10, and 15 % of the total substrate. The results demonstrated that the application survival percentage was not significantly affected by sawdust application, with all the treatments yielding survival above 65 %. Sawdust significantly improved larval harvest yields, with the 15 % sawdust application (Sawdust15) yielding 682.97 g and the 10 % application (Sawdust10) yielding 648.91 g on a dry biomass basis, with Sawdust5 resulting in little or no effect as compared to the control population. Larval yield was directly proportional to growth rates, where Sawdust15 showed the highest growth rate (0.1233 g/day) compared to Sawdust10 (0.1189 g/ day). In contrast, larvae in the control treatment exhibited the lowest growth rate (0.1005 g/ day) and quickly lost weight after reaching their growth asymptote two days earlier than the sawdust-induced treatments. These findings indicate that sawdust application at 10–15 % significantly enhances BSFL growth and biomass yield, with the 15 % inclusion level yielding the highest larval weight and biomass conversion efficiency. However, the 5 % sawdust application did not improve performance, suggesting that lower inclusion rates may not be optimal for BSFL rearing on high-moisture substrates. Therefore, sawdust likely enhances movement flexibility for the larvae, allowing them to conserve energy for later utilisation during the metamorphosis process between the 4th and 5th instars.

Keywords: black soldier fly, bioconversion, Bulking agents, value addition, organic waste

2.1 Introduction

The global population is rapidly increasing and could double by 2050 (Shumo et al., 2019), driving a rising demand for food. Additionally, urban populations generate large volumes of organic waste, with projections suggesting this output will double by 2025 compared to 2016 levels reported by the Food and Agriculture Organisation (Sarpong et al., 2019). Managing organic waste, therefore, remains a key challenge for societies.

In the last decade, the BSFL has gained attention due to its ability to manage and recycle waste in a short period (Barragán-Fonseca et al., 2018; Lalander et al., 2019). The voracious appetite of the larvae allows them to rapidly consume organic waste and reduce tonnes by converting the waste into valuable agricultural products used as animal feed (Fu et al., 2022) and as a biofertiliser (Boudabbous et al., 2023). However, waste reduction and bioconversion processes depend on the substrate type (Gold et al., 2020; Nyakeri et al., 2017a). For example, Gold et al. (2020) demonstrated that BSFL managed to reduce only 12 % of cow manure and bioconverted only 3.8 % into valuable biomass, while the larvae managed to reduce at least 58.40 % of vegetable canteen waste, with only 22.70 % that was turned into valuable biomass. Moreover, Nyakeri et al. (2017) have shown that restaurant waste was easily reduced up to 81.80 % with a bioconversion rate of 20.80 % compared to brewers' waste with a substrate reduction of 44.70 % and a bioconversion rate of 16.60 %. This process is essential in reducing waste and recovering nutrients from the waste streams by preventing them from leaching into water sources (Popa & Green, 2012). Despite the ability to convert a wide variety of waste streams into valuable agricultural byproducts that promote circular loop economy benefits (Sable et al., 2024), the larval performance of BSFL depends on the substrates' physical properties.

During rearing or mass production, the physical properties are key to BSFL development and productivity (Bekker et al., 2021; Chen et al., 2019; Lalander et al., 2020). Physical properties include larval and feed density (Barragán-Fonseca et al., 2018; Lopes et al., 2023), temperature (Yakti et al., 2023), and moisture content (Cheng et al., 2017; Chen et al., 2019). For example, the substrate moisture content is a key factor influencing larval performance (Lalander et al., 2020). Studies have reported that optimal larval growth and high survival percentage occur on substrates with a moisture content ranging from 70 to 80 % (Bekker et al., 2021; Lalander et al., 2020). However, more than 80 % moisture content can increase

mortality but potentially enhance larval weight (Bekker et al., 2021; Lalander et al., 2020). In contrast, Nguyen et al. (2013) recorded lower survival and longer development time in thick substrates with lower moisture content. Excessive moisture in substrates affects larval mobility, feeding behaviour, and survival, as larvae cannot feed from the surface down to the substrate depths. (Lopes et al., 2023). Studies by Amrul et al. (2022) and Nayak et al. (2023) highlighted that the substrate's viscosity impacts BSFL's performance. When the substrate is too thick and sticky, the larvae use more energy in mobility than in feeding, leading to high mortality rates, thus allowing other Diptera species to invade the substrate (Lopes et al., 2023). Stabilising the high moisture content in the substrate environment remains a key goal in BSFL-rearing programs.

Non-nutritive substrates such as powdered cellulose, mixed straws, and concentrated lignocellulose are emerging as important components in BSFL-rearing programs (Yakti et al., 2023). Collectively, these materials are known as bulking agents for creating void spaces in the substrate, facilitating aeration, improving moisture management, and assisting in heat dissipation (Rincón-Betancurt et al., 2021; Yakti et al., 2023). Substrate moisture critically influences BSFL performance, with optimal moisture levels facilitating enhanced larval growth and increased yields (Chen et al., 2019; Cheng et al., 2017). Materials such as rice husk enhance larval performance by preserving structural integrity, facilitating larval development through decreased substrate compaction and establishing a more aerated environment (Laksanawimol et al., 2024). Furthermore, bulking agents such as sawdust stabilise moisture levels and enhance substrate bioconversion efficiency, as BSFL demonstrates increased biomass yields and accelerated development in optimised substrate mixtures (Lee et al., 2024). Augmenting substrate porosity with bulking agents, especially under-regulated moisture conditions, can substantially elevate BSFL productivity (Nyakeri et al., 2017b).

Sawdust as a bulking agent highlights its ability to create an ideal substrate texture that supports larval feeding behaviour, allowing larvae to access nutrients more readily while reducing compaction issues Nyakeri et al. (2017). For instance, substrates supplemented with sawdust enhanced larval biomass yield and survival rates by stabilising the larval environment (Laksanawimol et al., 2024; Lee et al., 2024). The wood-derived particles in sawdust effectively manage moisture and improve the properties of the substrate, thus allowing for better water retention (Nyakeri et al., 2017). This study investigated the performance of BSFL under

varying ratios of a non-nutritional bulking agent, sawdust. We hypothesised that increasing sawdust in the standardised diet would benefit BSFL performance, since several studies have highlighted the benefit of adding bulking agents (Nyakeri et al., 2017; Laksanawimol et al., 2024; Lee et al., 2024).

This study contributes to using sawdust as a non-nutritive bulking agent to determine its effectiveness in mass rearing of BSFL, particularly in high moisture substrates. Unlike previous studies (Nyakeri et al., 2017; Lee et al., 2024) that used sawdust as a bulking agent without any dose, this study quantifies explicitly the effectiveness of sawdust inclusion levels on BSFL survival and growth. The results provide a framework for optimising BSFL-rearing conditions, particularly for substrates prone to excess moisture, offering practical applications for large-scale BSFL bioconversion systems and contributing to sustainable organic waste management.

2.2 Methods and materials

2.2.1 Starter mix preparation

Newly hatched neonates were collected from a stock colony at the University of Venda, South Africa. These larvae were placed on a nutrient-rich starter mix for five days and incubated in the dark at 28 °C to ensure their survival and proper development before beginning further experiments (Nyakeri et al., 2017). The starter mix comprised equal parts by weight (1:1:1:1) of pulverised potato peels, orange waste, avocado waste, pullet starter for broilers, and water. Three hundred grams of this mixture were placed in a 2,000 ml container with a ventilated lid, and a 10 mm layer of sawdust was added on top to reduce rapid moisture loss.

2.2.2 Experimental design and substrate preparation

Three treatments were developed with sawdust additions of 5, 10, and 15 %, in contrast to a control with no sawdust [(0 %; Table 2.1); (Table S2.1)]. Due to the tray size limitation, the study could not add sawdust application rates more than 15 %. A standard diet with 82 % moisture content consisting of 40 kilograms of the substrate was enriched with nutrient-rich 10 kilograms of spent grain collected from local households producing small-scale African beer (Table 2.1). The sawdust, used as a bulking agent, was obtained from a local sawmill processing raw gum tree (*Eucalyptus mannifera*). Potato peels (*Solanum tuberosum*) were collected from the Fish and Chips shops in Thohoyandou and the cafeteria at the University

of Venda. The potato peel waste was crushed into fine particles using a hammermill with a 25 mm sieve. Each experimental tray held 50 kg of substrate, comprising a pulverised potato peel-to-spent grain ratio of 40:10 (w/w). The spent grain provided a protein source. The standardised moisture content for each treatment was determined by utilising the control diet moisture value (potato peels) while considering the influence of the bulking agent (Table 2.1).

Table 2.1: Diet composition and water additions of control and three bulking agent treatments.

Treatment (Sawdust application %)	Potato peels (kg)	Spent grain (kg)	Sawdust amount (kg)	Water amount (L)	Total moisture contributions (%)
Control (0.00)	40	10	0	0	82
5	40	10	2.5	0.45	82
10	40	10	5	1.15	82
15	40	10	7.5	1.82	82

2.2.3 Moisture balance and calculations

Ten grams of each substrate were weighed using a four-decimal scientific scale (ADAM scale PW214) in an empty foil container to ensure consistent moisture levels. The samples were then dried in an oven at 100°C for six hours, and equation [1] was applied to standardise the moisture content. Equation [2] calculated the water inclusion needed to standardise the moisture content across the treatments. Moisture balancing was conducted to eliminate variations between treatments, ensuring that the results reflect the bulking agent's impact.

$$\text{Standard moisture content} = \frac{(\gamma_i - F) - (\gamma_f - F)}{\gamma_i - F} \times 100 \quad [1]$$

Where:

γ_i = Initial wet substrate in the foil container

γ_f = Final dry weight in the foil container

F = Empty foil container

$$TM \% = \sum (MC_{\text{substrate}} \times M_{\text{substrate}} \text{Total tray weight}) + (MC_{\text{sawdust}} \times MI_{\text{sawdust}} \text{Total tray weight}) \\ + (MC_{\text{spent grain}} \times MI_{\text{spent grain}} \text{Total tray weight}) + (100\mu \text{Total tray weight})$$

[2]

Where:

TM = Total Moisture content

MC = Moisture content of the substrate.

MI = Mass inclusion of the substrate component.

100μ = 100 % moisture for water, and μ , the amount of water, must be included to balance the standardised moisture.

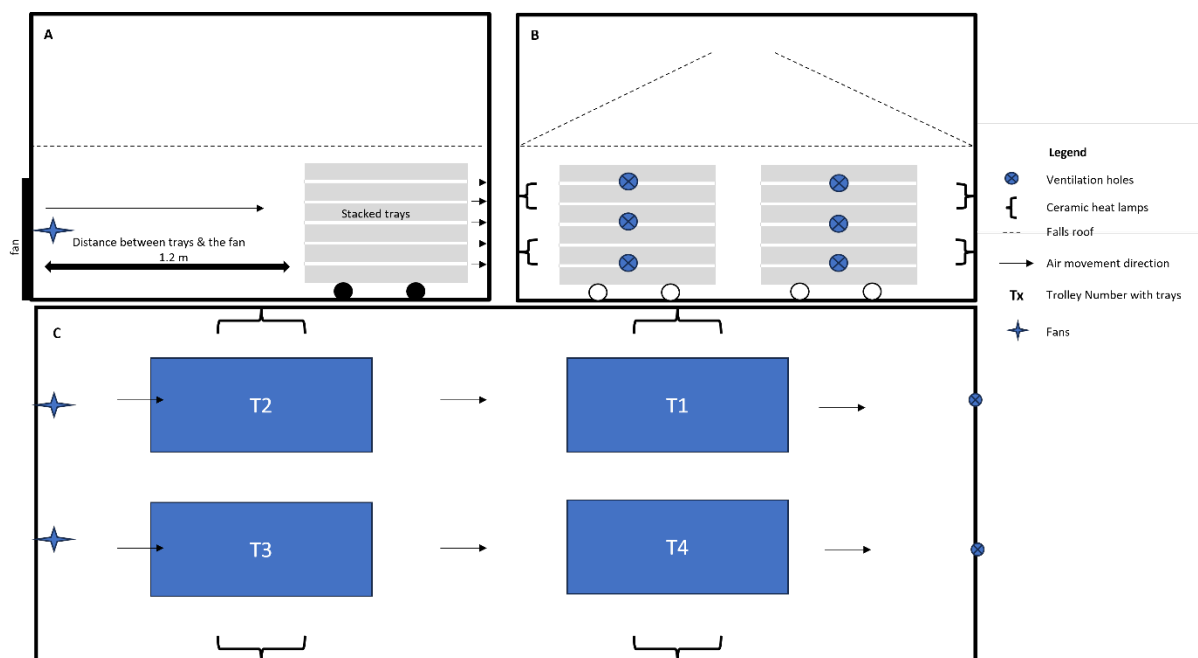


Figure 2.1: Schematic diagram of the rearing chamber illustrating the side view (A), front view (B), and top view, rotating continuously in a circular loop until harvest.

The prepared substrates were used to fill each experimental tray with 50 kg of material (see Table 1 for complete composition). Each treatment was replicated eight times, with one tray representing each replicate. The experiment took place in a rearing chamber measuring 4,000 x 3,500 x 1,000 mm (Figure 1), equipped with a false roof to ensure even airflow and heat distribution through fans and ceramic infrared heaters (FTE-250W-230V). The ambient

temperature in the chamber was monitored using SONOFF (SNZB-02P) sensors (Figure 2.1). Four stackable aluminium trays (with a 33.5 mm gap between them) were arranged randomly on a trolley, with one replicate per treatment. The experiment spanned four weeks, employing a staggered entry system. Every second day, a new set of trays (four trays in total; one replicate per treatment) was placed on a trolley, reaching eight replicates per treatment by the end of the 15-day period. To manage the high moisture content of the feeding substrate as the larvae neared the prepupal stage, the trays were initially positioned about one meter away from the fans. They were progressively moved closer to create a moisture gradient in the rearing chamber.

2.2.4 Larvae sampling

We selected a random sample of twenty BSFL individuals from each tray every second day, weighed them using a four-decimal precision scientific balance (ADAM scale PW214), and returned them to their respective trays.

2.2.5 Larval yield

The total mass of harvested prepupae was weighed with an industrial-grade platform scale. The final individual prepupae weight per tray was determined by sampling the weights of twenty individuals on the harvest day, reporting the larvae yield as dry biomass. Moisture content of the larvae was measured by drying a ten-gram sample in an oven at 100 °C for six hours. Dry biomass was calculated using equation [3]:

$$\text{Dry biomass} = [(\text{Larvae wet biomass}) \times (1 - \text{Moisture Content})]$$

[3]

2.2.6 Survival percentage

We collected all live prepupae on day 14 to estimate the total prepupae in each tray. The initial number of neonates was estimated based on the assumption that a single neonate weighs approximately 0.000015 g and consumes around 2 g of substrate throughout its lifetime (Parry and Weldon, 2021). Consequently, a starter mix of 0.375 g of newly hatched neonates was estimated to contain approximately 25,000 individuals. To calculate the total number of prepupae harvested per treatment, the total prepupae mass was divided by the mean weight of the prepupae, following equation [4] as done by Diener et al. (2011). The survival rate was estimated using equation [5], as described by Deng et al. (2022).

$$\text{Number of prepupae during harvest} = \frac{\text{Total prepupae mass during harvest}}{\text{Prepupae mean weight}} \quad [4]$$

$$\text{Survival (\%)} = \frac{\text{Number of prepupae during harvest}}{\text{Number of neonates at start}} \times 100 \quad [5]$$

2.2.7 Statistical analysis

2.2.7.1 Larval survival

Outliers characterised larval survival data. We first fitted two models to deal with normality violations caused by outliers (Figure S2.2). Model 1 was constructed using a generalised linear mixed-effects regression that included treatment as a fixed effect and Tray ID as a random effect to deal with non-random survival data. Model 2, a generalised linear model, included only the fixed effect (Harrison et al., 2018; Silk et al., 2020). These models were compared using a likelihood ratio test (LRT) to assess whether including the random effect significantly improved the model (Anisimova & Gascuel, 2006). We found that including the random effect significantly improved model fit ($\chi^2 = 23635$, Df = 1, $p < 0.001$). We then evaluated the significance of the treatment effect and did a Tukey pairwise comparison between treatment levels (Lenth & Lenth, 2018).

2.2.7.2 Larval weights

We investigated the effects of treatment (sawdust), time (growth days), main effects (Day and treatment) and their interaction on larval weight. We applied a mixed-effect generalised linear model (Harrison et al., 2018; Silk et al., 2020) to account for the sequential sampling of the same tray across multiple days. We included replicates nested within days as random effects to capture variation from tray placement in the rearing room and across sampling days (Harrison et al., 2018; Silk et al., 2020).

The fitted models include: (1) treatment only, (2) day only, (3) treatment + day, (4) treatment $\times$ day interaction, (5) a quadratic response of larval weight to increasing treatment levels, and (6) a null model (intercept + random effect only), hypothesising that treatment, sampling day, their interaction, or the day and treatment affect larval weight. The quadratic model tested for a non-linear larval response across the treatment gradient by assigning numeric scores to treatment levels and including linear and squared terms as fixed effects.

Model selection was based on the Akaike Information Criterion (AIC) to identify the most parsimonious model and assess covariate effects (Symonds & Moussalli, 2011). Models with $\Delta AICc < 2$ were considered equally supported (Mazerolle & Mazerolle, 2017). We calculated pseudo- R^2 values for the selected model, including conditional (R^2c) and marginal (R^2m) values (Nakagawa & Schielzeth, 2013). Subsequently, we used a likelihood ratio test to evaluate variable importance if model uncertainty was present (Anisimova & Gascuel, 2006).

We conducted post hoc comparisons of marginal means across treatments using a Tukey test (Graves et al., 2015). To assess growth trends in larval weight changes over time, we used the “emtrends” function from the “emmeans” package to contrast estimated slopes (Lenth & Lenth, 2018). Model fit was verified through Pearson residuals, confirming that all assumptions were met (Figure S2.3). The models were fitted using the ‘glmer’ function from the ‘lme4’ package (Bates et al., 2015) with a log link function and a Gamma distribution (Lo & Andrews, 2015).

2.2.7.3 Larval yield

We investigated the effects of treatments on total larvae yield by fitting a generalised linear mixed-effects regression that included a fixed effect (treatment) and a random effect (Tray ID). (Harrison et al., 2018; Silk et al., 2020). A likelihood ratio test was applied to evaluate the importance of variables in cases where model uncertainty was present (Anisimova & Gascuel, 2006). When the treatment effect was significant, we conducted post hoc comparisons using the Tukey test to compare treatment means (Graves et al., 2015). Robust regression was carried out with MM-estimation using the "robustbase" package in R (Maechler et al., 2021). All analyses executed were in R Software 4.3.1 (R Core Team., 2023).

2.4 Results

2.4.1 Larvae survival

Sawdust treatments did not significantly influence BSFL survival ($\chi^2 = 1.53$, $df = 3$, $p = 0.67$). Despite the lack of statistical significance, Sawdust5 exhibited the highest mean survival ($72.00 \pm 1.87\%$), followed by Control ($68.30 \pm 2.01\%$), Sawdust15 ($67.00 \pm 2.80\%$), and Sawdust10 ($66.50 \pm 2.80\%$) (Table 2). Table 2.2: Larvae harvest yield, growth rate and survival percentage. The values are expressed in mean and standard error. Different letters show significant differences (Tukey post hoc test, $p < 0.05$).

Treatment	Survival percentage (%)	Larvae harvest yield	Growth rate (g/2days)
Control	68.30 ± 2.01 ^a	418.10 g ± 33.10 ^a	0.1005 ± 0.02 ^a
Sawdust5	72.00 ± 1.87 ^a	499.46 g ± 47.26 ^a	0.1007 ± 0.02 ^a
Sawdust10	66.50 ± 2.80 ^a	648.91 g ± 63.37 ^b	0.1189 ± 0.02 ^{ab}
Sawdust15	67.00 ± 2.80 ^a	682.97 g ± 63.15 ^b	0.1233 ± 0.02 ^b

2.4.2 Larvae growth rates over time

There is overwhelming support that the main effects (day and treatment) impacted larval growth (AIC = -20501.11; AICc Weight = 0.65; Table S2.2). A subsequent LRT indicated that adding the interaction between days and treatment did not significantly improve model fit ($\chi^2 = 1.91$, df = 3, p = 0.59), but adding a day to the treatment significantly improved model fit ($\chi^2 = 6.02$, df = 1, p = 0.01).

We found day and treatment to significantly impact larval growth ($\chi^2 = 11.51$, df = 3, p = 0.009), with fixed effects explaining 78 % of the variation in larval weight. Adding random effects increased the explained variation to 88 %. Multiple comparisons indicated no significant difference in the growth trend slopes between the Sawdust10 (0.1189, 95 % CI = [0.0974–0.1450]) and Sawdust15 treatments (0.1233, 95 % CI = [0.1010–0.1504]) (Table 2.2). Sawdust15 growth trend was significantly different compared to Sawdust5 (0.1007, 95 % CI = [0.0826–0.1230]) and the Control (0.1005, 95 % CI = [0.0824–0.1227]) (Figure 2.2; Table 2.2).

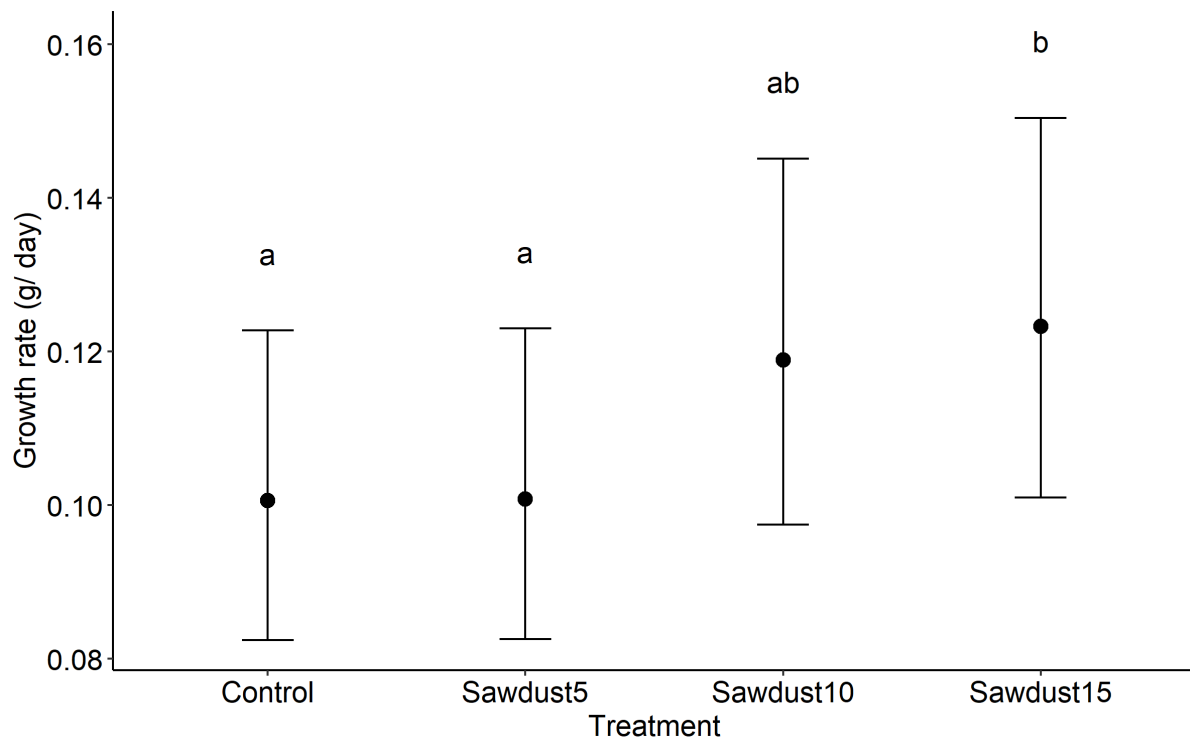


Figure 2.2: Larval growth rate between treatments. The results are presented as the mean $\pm$ confidence intervals (CI). The growth rates are measured in grams per day. Significant differences are shown through distinct letters, as determined by the Tukey post hoc test ($p < 0.05$). The error bars indicate the 95 % confidence intervals.

2.4.3 Larvae harvest yield

There was strong support that the tray ID impacted larval yield (AIC = -141.10; Table S2.3). Sawdust treatments significantly affected larval yields ($\chi^2 = 24.67$, $df = 3$, $p < 0.001$). A Tukey post-hoc multiple comparison showed that sawdust treatments exceeding 10 % had significantly higher larval numbers than the Control (Figure 2.3; Table 2.2). Sawdust15 produced the highest yield ($682.97 \text{ g} \pm 63.15$), followed by Sawdust10 ($648.91 \text{ g} \pm 63.37$), Sawdust5 ($499.46 \text{ g} \pm 47.26$), and the Control ($418.10 \text{ g} \pm 33.10$).

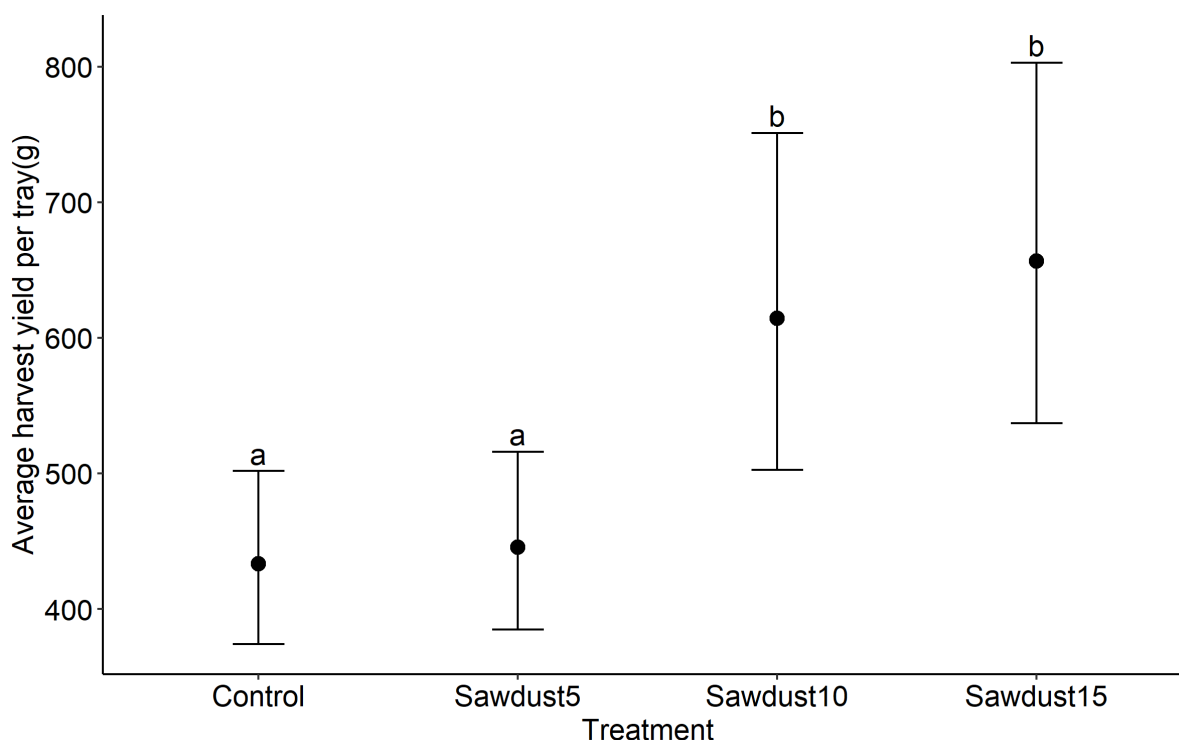


Figure 2.3: Larvae yield during harvest on day 14. The results are presented as the mean $\pm$ standard error (SE). The weight of the larvae was quantified in grams. Significant differences are shown through distinct letters, as determined by the Tukey post hoc test ($p < 0.05$). The error bars indicate the 95 % confidence intervals.

2.5 Discussion

This study investigated the role of sawdust as a bulking agent in enhancing the growth and survival of black soldier fly larvae (BSFL), focusing on its impact on larval yield. The findings demonstrated that a 15 % sawdust inclusion significantly improved BSFL growth and harvest yield, aligning with previous studies on the benefits of bulking agents in optimising BSFL rearing conditions (Lee et al., 2024; Nyakeri et al., 2017b).

Despite improvements in growth and yield, sawdust application did not significantly affect BSFL survival, with survival rates consistently above 65% across all treatments. This result suggests that the inclusion of eucalyptus sawdust, even though it is known to contain secondary metabolites such as tannins, terpenoids, and alkaloids (Thomas Alamu et al., 2025), did not negatively impact larval survival in this study. These compounds are often associated with reduced insect performance in other substrates, such as coconut coir dust, which releases tannins that may lower survival (Laksanawimol et al., 2024). However, in this case,

the concentration or bioavailability of such compounds may have been insufficient to elicit a toxic effect, or the larvae may have demonstrated resistance (Thomas Alamu et al., 2025). Moreover, this further aligns with earlier findings that moderate bulking agent inclusion improves substrate structure and aeration without compromising larval viability (Lee et al., 2024; Laksanawimol et al., 2024), highlighting the potential for eucalyptus sawdust use without detrimental effects on survival (Thomas Alamu et al., 2025).

In contrast, several studies have highlighted that bulking agents effectively control moisture, gas exchange, thermal control and C:N ratios (Čičková et al., 2013; Laksanawimol et al., 2024; Rincón-Betancurt et al., 2021). For instance, Čičková et al. (2013) found that adding sawdust to pig manure improved the survival of the Domestic house fly (*Musca domestica*), attributing this to the substrate's C:N ratio, nutrient availability, and physical structure. While bulking agents can affect survival, their impact also transfers to growth rates and larval weights.

Regarding growth performance, sawdust inclusion significantly enhanced BSFL growth rates and yield. Sawdust10 and Sawdust15 treatments yielded significantly higher biomass compared to the control, suggesting that improved substrate porosity facilitated larval feeding and movement (Rincón-Betancurt et al., 2021). Growth over time indicated that larvae grew faster and attained higher weights in treatments with higher sawdust applications. This supports previous findings that bulking agents enhance substrate aeration, reducing compaction and creating an optimal environment for larval feeding and development (Tschirner & Simon, 2015). Furthermore, bulking agents seem effective in moisture control and moisture balance. The effectiveness of sawdust as a bulking agent is due to its ability to manage moisture content rather than its nutritional value (Grossule et al., 2020; Lee et al., 2024). This is attributed to cellulose's role as a moisture trap, where water is initially captured on cellulose surfaces, creating a stable environment for BSFL (Belbekhouche et al., 2011).

The larvae reaching their peak at 10 days across all treatments and then dropping in weight suggests that 10 days was the point at which feeding stopped and transition to the prepupal stage started. Similar tests from other studies show that feed composition is key in growth speed (Broeckx et al., 2021; Meneguz et al., 2018). The results further indicate that sawdust-enhanced substrates supported larval development to the prepupae stage within 15 days, consistent with Yakti et al. (2023). This suggests that substrate modifications via bulking

agents can influence larval development timelines. In contrast, some studies found that excessively high bulking agent inclusion hindered larval progression to the prepupae stage due to increased substrate dryness (Laksanawimol et al., 2024). This study only investigated saw-dust inclusion up to 15 % due to space limitation in the rearing trays; however, the trend suggests that higher sawdust inclusion could potentially further improve growth and yield. This would, however, come at a trade-off on the available dietary substrate or even spreading the substrate too far apart for adequate growth performance. This could be circumvented by using consumable bulking agents such as spent grain. Thus, balancing bulking agent application is crucial to optimising growth conditions.

2.6 Conclusion

Sawdust effectively improved BSFL growth and yield, primarily by enhancing substrate structure and moisture regulation. While survival percentages were not significantly affected, growth performance significantly improved with sawdust inclusion above 10 %, demonstrating its potential as a bulking agent in high-moisture organic waste substrates. These findings suggest that sawdust is a viable additive for optimising BSFL rearing conditions, particularly for substrates with excess moisture. Further research should explore the long-term effects of sawdust on nutrient composition and bioconversion efficiency across diverse organic waste streams since the study did not primarily focus on the nutritional composition of the substrate, but rather the physical structure of the substrate. This study did not explore the effect of phytochemicals involved in sawdust on the substrate; therefore, more research should explore detoxification strategies and microbial conditioning to enhance the suitability of sawdust as a bulking agent in BSFL rearing.

2.7 References

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2.8 Supplementary data

Tables

Table S2.1: Moisture contribution of all the used components to formulate the experimental diet for uniformity during the start.

Diet component	Control		Sawdust5		Sawdust10		Sawdust15	
	Mass (kg)	Moisture contribution (%)	Mass (kg)	Moisture contribution (%)	Mass (kg)	Moisture contribution (%)	Mass (kg)	Moisture contribution (%)
Potato peels	40.00	68.00	40.00	65.00	40.00	62.00	40.00	59.20
Spent grain	10.00	14.00	10.00	14.00	10.00	13.00	10.00	12.50
Sawdust	0.00	0.00	2.50	3.00	5.00	5.00	7.50	7.10
Water	0.00	0.00	0.45	1.00	1.15	2.00	1.80	3.20

Table S2.2: Akaike Information Criterion summary table on modelling larvae weight between treatments.

Model names	K	AICc	Delta_AICc	AICcWt	LL	Cum.Wt
Treatment+Day	8	-20501.11	0.00	0.65	10258.57	0.65
Quadratic	6	-20497.67	3.44	0.12	10254.84	0.77
Treatment	7	-20497.10	4.02	0.09	10255.56	0.86
Treatment*Day	11	-20497.00	4.12	0.08	10259.53	0.94
Day	5	-20496.08	5.04	0.05	10253.04	0.99
Null model	4	-20492.06	9.05	0.01	10250.04	1.00

Table S2.3: Likelihood Ratio Test summary for larvae harvest yield

Model names	K	AICc	BIC	LogLik	Deviance
Larvae harvest yield	6	-141.1	-132.3	76.55	-153.1
Null model	5	419.48	426.81	-20474	409.48

Figures

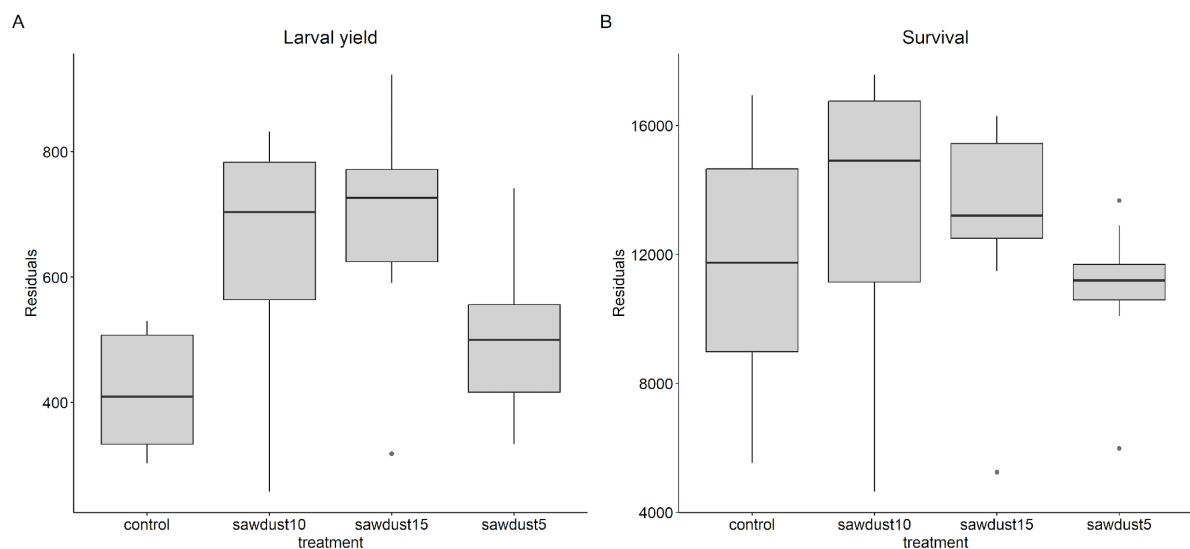


Figure S2.1: Outliers affecting linear model assumption for (A) larvae yield and (B) survival

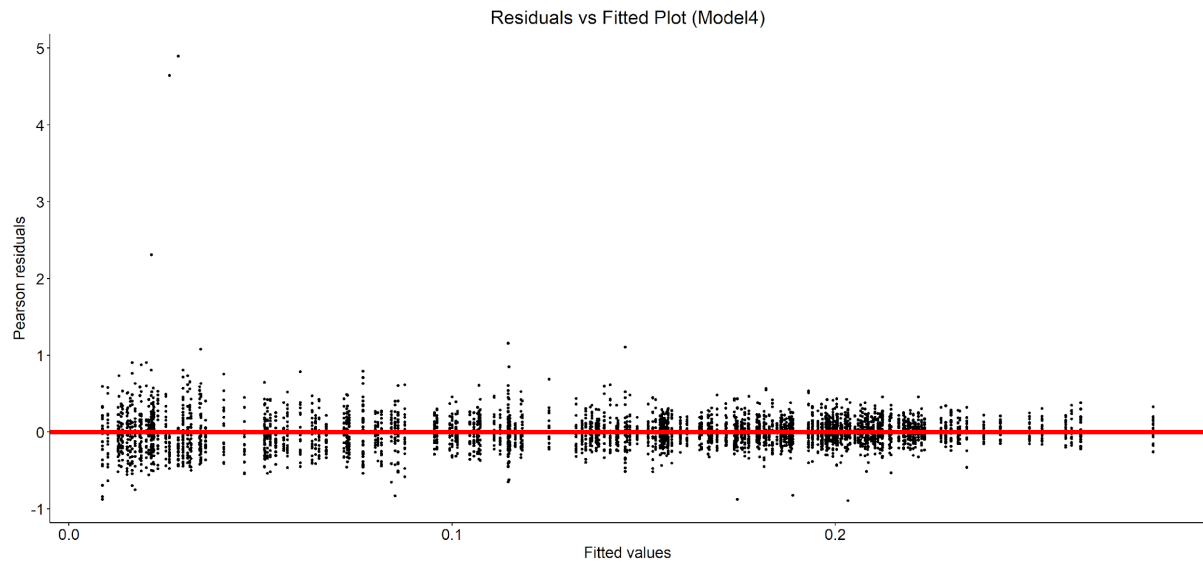


Figure S2.2: Residuals vs. Fitted values for the most parsimonious model showing Pearson residuals against fitted values, with a red horizontal line at $y = 0$ indicating the baseline.

Chapter 3

Augmenting the bioconversion efficiency of black soldier fly larvae using avocado, orange and potato peels as organic waste streams

Augmenting the bioconversion efficiency of black soldier fly larvae using avocado, orange waste and potato peels as organic waste streams

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Abstract

The generation of excess organic waste poses significant environmental challenges globally. As population growth and urbanisation increase, food waste, including fruits and vegetables, rises substantially. Approximately one-third (1.3 billion tons) of all food produced globally is wasted annually. Improving waste management and reduction strategies is crucial to address these environmental impacts, particularly in developing regions with inefficient supply chains. Black soldier fly larvae (BSFL) are a promising, eco-friendly solution for converting large volumes of organic waste into valuable resources, including high-protein biomass for animal feed and nutrient-rich organic fertiliser. Therefore, this study evaluated how different types of fruit and vegetable waste affect black soldier fly larval performance and the efficiency of converting organic waste into valuable biomass, using 1,000 × 1,000 × 100 mm aluminium trays housed in a controlled growing chamber with a false roof. This study evaluated the potential of BSFL to valorise waste through: (i) determining the effect of three different waste streams (potato peels, orange waste, and avocado waste) on larval growth and survival, and (ii) assessing the efficiency of the larvae in reducing waste and converting it into valuable biomass. Results showed that potato peels produced the highest dry larval biomass (919.45 g

per tray) and growth rate (0.2051 g/day), with significant waste reduction (57.63 %) and bioconversion efficiency (5.61 %). Orange waste yielded moderate results, while avocado waste lagged in larval development and waste reduction but excelled in the efficiency of feed digestion (14.20 %). These findings support BSFL as an effective bioconversion agent for organic waste streams, particularly potato peels. The study emphasises the importance of substrate selection and highlights BSFL's potential in sustainable waste management systems, contributing to a closed-loop circular economy.

Keywords: Circular economy, Waste valorisation, Insect biomass, Larval growth optimisation, Sustainable agriculture, Bulking agent, Sawdust.

3.1 Introduction

The emergence of organic waste generated globally is promising to be a serious threat not only to humans but also to the environment (Siddiqui et al., 2024). The amount of waste generated causes negative environmental effects such as air contamination from organic waste and bad odour from leachates that directly run into riverine streams (Schmitt & de Vries, 2020). This is a pressing issue, especially from developing countries that rely on landfilling and incineration as traditional organic waste management methods (FAO, 2014). These methods have negative consequences, such as using more land as part of the decomposition process that takes longer, thus contributing to greenhouse gases such as methane generated from the waste due to the anaerobic process taking place (FAO, 2014; Kim et al., 2021). Therefore, there is a need for alternative waste management methods, such as the use of biological species to mitigate these challenges.

The use of insects to manage organic waste has gained scientific interest and the black soldier fly larvae stand out in managing organic waste due to its ability to thrive on diverse organic wastes such as plant-based wastes (corn stover, fruit and vegetable waste, wheat-mill by-products, mushroom root waste, and soybean curd residue) and animal-based wastes (kitchen waste, municipal organic waste, and abattoir waste), highlighting its potential to address global food waste challenges in an environmentally sustainable way (Surendra et al., 2020; Xiang et al., 2024). The quality and nutritional composition of waste significantly affect larval development, growth rate, and bioconversion efficiency, ultimately influencing the conversion of waste into useful biomass (Fuso et al., 2021; Nyakeri et al., 2017b; Nicholas Romano et al., 2023). Beyond addressing organic waste management, BSFL biomass has numerous applications. Larvae can be used as high-protein feed for aquaculture, reducing reliance on traditional feed sources (Mikołajczak et al., 2022; Tippayadara et al., 2021). Additionally, frass, a byproduct of BSFL waste conversion, serves as an organic fertiliser, enhancing soil health and supporting sustainable agricultural practices (Basri et al., 2022).

Although South Africa is not a leading global producer, it produces significant quantities of waste from processing citrus and avocados, attributed to its strong agricultural and hospitality sectors (Chisoro-Dube & Roberts, 2021; Sibulali, 2020). High volumes of organic waste result from processing avocado oil (Permal et al., 2020; Rashama et al., 2021) and orange juice (Castro et al., 2020). For example, South Africa produced an average of 131,049 tons of

avocados annually from 2013 to 2023, yielding approximately 11,000 tons of waste by-products yearly (SAAGA, 2024). In the 2022-23 season, orange production was estimated at 1.62 million metric tons, with 40-60 % of the fruit considered waste after processing (Citrus Industry, 2023). Similarly, the restaurant and hospitality sectors, both formal and informal, contribute substantial amounts of organic waste, primarily from potatoes (Hadidi et al., 2023).

Scientific research studies have implemented waste management using BSFL to convert a mixture of fruits and vegetables, including oranges, peppers, pears, and tomatoes (Meneguz et al., 2018), avocado or guava with bananas (Mentari et al., 2020). This necessitates the need to assess larval performance on individual substrates, as illustrated by Scieuzo et al. (2023). These organic waste fed to the larvae do not have a balanced nutrient composition and this challenge impacts larval performance and nutrient assimilation (Barragan-Fonseca et al., 2018; Nguyen et al., 2013). High-protein and carbohydrate-rich substrates is said to promote larval growth and improve waste conversion efficiency, whereas nutrient-deficient wastes hinder development unless supplemented with additional nutrients (N Romano et al., 2023; Scieuzo et al., 2023; Siddiqui et al., 2022). For instance, Oonincx et al. (2015) observed that BSFL fed on high-protein vegetable substrates completed their development in three weeks, compared to five weeks on low-protein substrates. Therefore, this necessitates the importance of assessing the performance of BSFL in individual waste streams such as potato peels, avocado and orange waste.

Due to the large agriculture and hospitality industries, potato peels, avocado, and orange waste streams are prevalent in the Vhembe district, Limpopo, South Africa. This study aims to assess BSFL performance on these individual waste streams by enhancing high-grade agricultural products and supporting a closed-loop circular economy by minimising and reusing organic waste. It was hypothesised that BSFL would exhibit differing growth rates and biomass yields depending on the nutrient composition of each substrate (Sripontan et al., 2020). This hypothesis was tested by (i) determining the effect of the nutritional composition of three different waste streams on larval growth and survival and (ii) evaluating the efficiency of the larvae in reducing waste and converting it into valuable biomass.

3.2 Methods and materials

3.2.1 Neonates' preparation

Newly hatched neonates were harvested from a stock colony maintained at the University of Venda, South Africa. These neonates were placed on a nutrient-rich starter mix diet for five days (5-DOL) and stored in a dark incubator at 28 °C and 65 % relative humidity to ensure survival and development before further experimentation (Nyakeri et al., 2017a). The starter mix was prepared using pulverised potato peels, orange, avocado waste, pullet starter for broilers, and water in equal parts by weight (1:1:1:1:1). Three hundred grams of the starter mix were placed in a 2,000 ml container with a ventilated lid, covered with a 10 mm layer of sawdust to minimise rapid evaporation.

3.2.2 Experimental design and diet preparation

Three substrates (treatments) were prepared: avocado waste, orange waste, and potato peels. The pulverised avocado waste (*Persea americana*) was sourced from a local farm specialising in oil extraction. Potato peels (*Solanum tuberosum*) were collected from a Fish and Chips supermarket in Thohoyandou and the University of Venda cafeteria. The citrus waste was obtained from Limpopo Dairy outside Louis Trichardt town. The avocado waste was already pulverised during the collection, while orange waste and potato peel waste were pulverised into small particles using a hammermill with a 25 mm sieve. Each substrate was supplemented with 20 % nutrient-rich spent grain (included as a protein source for larvae). The spent grain was collected from households in local rural areas where African beer is brewed on a small scale. A 15 % proportion of the total substrate was supplemented with a non-nutrient bulking agent, sawdust. A bulking agent was added to improve larval movement and heat dissipation in the substrate (Rincón-Betancurt et al., 2021). The sawdust was sourced from a local sawmill that processes untreated gum trees (*Eucalyptus mannifera*).

The moisture content of treatments was standardised to the substrate with the highest moisture level (75% [potato peels]; Table 3.1; Table S3.1). While the moisture content of the waste stream can vary, the optimal range of 70-80 % enhances larval performance (Cheng et al., 2017; Lalander et al., 2020). Ten grams of each substrate were weighed using a four-decimal scientific scale (ADAM scale PW214) in an empty foil container to calculate the

moisture content. The samples were then dried in an oven for six hours at 100 °C, and equation [1] was used to calculate the moisture content.

$$\text{moisture content} = \frac{(D_i - F) - (D_f - F)}{D_i - F} \times 100 \quad [1]$$

Where:

D_i = Initial wet substrate in the foil container

D_f = Final dry weight in the foil container

F = Empty foil container

Equation [2] was used to calculate the water volume (in kilograms) required to balance the moisture across the treatments

$$\text{TM \%} = \sum \left(\frac{\text{SMC}_{\text{feed}} \times \text{MI}_{\text{feed}}}{\text{Total tray weight}} \right) + \left(\frac{\text{SMC}_{\text{sawdust}} \times \text{MI}_{\text{sawdust}}}{\text{Total tray weight}} \right) + \left(\frac{\text{SMC}_{\text{spent grain}} \times \text{MI}_{\text{spent grain}}}{\text{Total tray weight}} \right) + \left(\frac{100\beta}{\text{Total tray weight}} \right) \quad [2]$$

Where:

TM = Total Moisture content

SMC = Feeding substrate moisture content.

MI = Mass inclusion of the component of the substrate.

100β = 100 % moisture for water, and β , the amount of water must be included to balance the standardised moisture.

Table 3.1: A complete diet composition for all treatments, including the water volume, was adjusted to achieve a standardised moisture content.

Substrate	Substrate inclusion (kg)	Spent grain inclusion (kg)	Sawdust inclusion (kg)	Water inclusion (kg)	Substrate Moisture content (%)	Balanced Moisture content (%)
Avocado waste	40.00	10.00	7.50	3.50	69.00	75.00
Orange waste	40.00	10.00	7.50	1.50	73.00	75.00
Potato peels	40.00	10.00	7.50	0.00	75.00	75.00

Each experimental tray was then filled with 50 kg of substrates prepared according to the complete composition as shown in Table 1. There were eight replicates (trays) per treatment. The experiment was conducted in a rearing chamber (4,000 x 3,500 x 1,000 mm) with a false roof (Figure 3.1) to level airflow and temperature from fans and ceramic infrared heaters (FTE-250W-230V). Ambient temperature in the rearing chamber was monitored by SONOFF (SNZB-02P) sensors. On a trolley (Figure 3.1), the trays were placed randomly as six stackable aluminium trays (one substrate per tray with a 33.50 mm gap between trays. Three treatments with a minimum of two replicates per treatment (2 trays per treatment) per trolley. The trial was executed over a weeklong period, with trays (treatments) entering the system in a staggered manner. A new set of staggered trays (six trays in total; two replicates per treatment) was added to a trolley every second day until there was a total of eight replicates for each treatment by the end of the 7-day period. In the beginning, the trays were placed more than a meter from the fans to avoid excessive moisture loss from the feeding substrate. Once the larvae neared the prepupal stage, the trays were slowly moved up to the fans to prevent moisture buildup on the back trays.

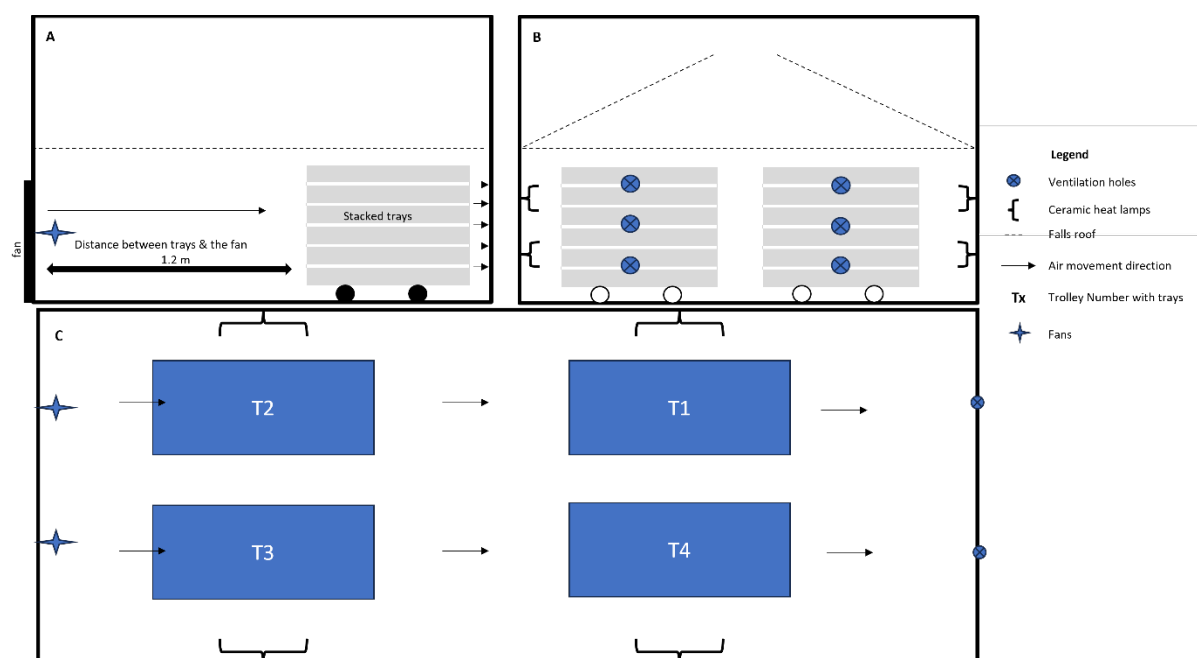


Figure 3.1: Schematic diagram of the rearing chamber showing the side view (A), front view (B) and top view rotating in a circular loop until harvest.

3.2.3 Larvae sampling

Every second day, 20 BSFL individuals were randomly selected from each tray, weighed (using a four-decimal precision scientific balance; ADAM scale PW214), and returned to their respective trays.

3.2.4 Larval yield and total weights

On day 14, we separated prepupae from the frass using a 5 mm hand sift. The total mass of harvested prepupae was weighed using an industrial-grade platform scale. The final individual prepupae weight per tray was estimated by averaging the weights of the twenty sampled individuals on harvest day. The larval yield mass was reported on dry biomass. To obtain dry biomass, the amount of water in the larvae was determined by drying a ten-gram sample ($n = 3$) in an oven at 100 °C for six hours using equation [1]. The dry biomass was then calculated using the equation [3]:

$$\text{Larval dry biomass} = [(\text{Larvae wet biomass}) \times (1 - \text{Moisture Content})] \quad [3]$$

3.2.5 Survival percentage

All live prepupae were collected on the day of harvest (14 days) to estimate the number of prepupae in each sample tray. A single neonate weighs approximately 0.00015 g (Parry and Weldon, 2021). With an estimated consumption rate of 2 g per larva over its lifetime, the initial number of neonates added to each tray was calculated as 25,000 to achieve the desired feed consumption per tray. Therefore, we introduced a starter mix of 0.375 g of newly hatched neonates per treatment. The total number of prepupae during harvest per treatment was estimated by dividing the total prepupae mass by the mean weight of the prepupae using Equation [4], and survival was estimated using Equation [4], and survival percentage was calculated using Equation [5].

$$\text{Number of prepupae during harvest} = \frac{\text{Total prepupae mass during harvest}}{\text{Prepupae mean weight}} \quad [4]$$

$$\text{Survival (\%)} = \frac{\text{Number of prepupae during harvest}}{\text{Number of neonates at start}} \times 100 \quad [5]$$

3.2.6 Waste reduction, efficiency of conversion of digested, and bioconversion

The waste reduction, efficiency of conversion of digested (ECD), and bioconversion were calculated on a dry matter basis. To obtain these parameters, we estimated the moisture

content at 5-DOL, the initial and final dry matter of the prepupae, the initial and final dry matter of the substrate and the frass, using Equation [1]. Triplicate samples for moisture content were determined by drying a 10 g sample from each tray within each treatment to constant weight in an oven at 100 °C for six hours. We subtracted the amount of bulking agent (sawdust) from the substrate before performing calculations on a dry matter basis, as it does not contribute to the nutrient composition of the larvae. waste reduction, ECD and bioconversion parameters were then estimated using equations outlined in Diener et al. (2009), specifically Equations [6], [7], and [8]. Initial substrate dry biomass values were obtained using Equation [3]:

$$\text{Waste reduction} = \frac{\text{Total substrate added} - \text{Total residue harvested}}{\text{Total substrate added}} \times 100 \quad [6]$$

$$\text{Efficiency of conversion of digested feed (ECD)} = \frac{\text{Prepupae biomass (f)} - \text{Prepupae biomass(i)}}{\text{Total substrate added} - \text{Total residue harvested}} \times 100 \quad [7]$$

$$\text{Bioconversion} = \frac{\text{Prepupae biomass (f)} - \text{Prepupae biomass(i)}}{\text{Substrate mass (i)}} \times 100 \quad [8]$$

Where:

Prepupae biomass (i) = initial prepupae biomass

Prepupae biomass (f) = final prepupae biomass

Substrate biomass (i) = initial substrate biomass

Nutritional composition data for avocado, orange, and potato peels were obtained from previously published literature (Supplementary Table S3.1). We selected studies that reported full proximate analyses and, where possible, prioritised those conducted in similar agro-ecological contexts. This approach was adopted due to practical constraints that precluded laboratory analysis. Reported values were compiled and presented as means $\pm$ SE (Table 3.2), and individual values were visualised using grouped bar graphs (Supplementary Figure S3.1) to evaluate consistency and variability across sources.

Table 3.2: Nutritional composition (mean $\pm$ SE) of avocado, potato peels, and orange based on data compiled from different literature sources. Values are reported on a wet biomass basis. This summary reflects the average composition and associated variability across sources used in this study.

	%			mg/100g		
Substrate	Carbohydrates	Protein	P	K	Ca	Mg
Avocado waste	7.83 $\pm$ 0.76	1.95 $\pm$ 0.03	52.80 $\pm$ 0.50	464.60 $\pm$ 31.80	12.40 $\pm$ 0.25	29.00 $\pm$ 0.00
Orange waste	11.87 $\pm$ 0.90	0.90 $\pm$ 0.06	17.20 $\pm$ 0.80	206.40 $\pm$ 12.80	41.80 $\pm$ 2.62	12.00 $\pm$ 0.84
Potato peels	15.74 $\pm$ 1.35	3.05 $\pm$ 0.03	102.00 $\pm$ 15.0	497.36 $\pm$ 121.00	19.16 $\pm$ 3.17	32.44 $\pm$ 2.37

3.3.3 Statistical analysis

3.3.3.1 Larval survival

To analyse the effect of treatment on larval survival, we fitted a generalised linear mixed-effects model (GLMM) using the “glmer” function and compared it to a generalised linear model (GLM) using the “glm” function from the “lme4” package using a binomial distribution and a log as a link function (Harrison et al., 2018; Silk et al., 2020). The mixed model included tray ID as a random effect to account for variation arising from differences between trays. In cases of model uncertainty, the likelihood ratio test (LRT) was used to evaluate the significance of the random effect (Anisimova & Gascuel, 2006). Model assumptions were further assessed using Pearson residuals, and the presence of influential outliers prompted the application of a robust regression model (Figure S3.2A). Robust regression was performed using the MM-estimation method in the “robustbase” R package (Maechler et al., 2021). When the treatment effect was significant, a post hoc Tukey test was applied to compare treatment means (Graves et al., 2015).

3.3.3.2 Larval weights

We examined the effects of treatment (waste stream), time (growth days), and their interaction on larval weight, while also considering the main effects of treatment and time. To account for sequential sampling from the same tray across multiple days (repeated measures), we employed a generalised linear mixed-effects model (Harrison et al., 2018; Silk et al., 2020). Replicates nested within days were included as random effects to capture variation arising from tray placement in the rearing room and differences across sampling days (Harrison et al., 2018; Silk et al., 2020).

Six candidate models were fitted to evaluate the effects of treatment, sampling day, their interaction, or both on larval weight: (1) treatment only, (2) day only, (3) additive effects of treatment and day, (4) treatment $\times$ day interaction, (5) a quadratic treatment response, and (6) a null model with random effects only. The quadratic model assessed potential non-linearity by numerically coding treatment levels and incorporating both linear and squared terms as fixed effects.

Model selection was guided by the Akaike Information Criterion (AIC), focusing on identifying the most parsimonious model and evaluating covariate effects [(Table S3.3); (Symonds &

Moussalli, 2011)]. Models with $\Delta AICc < 2$ were considered to have equal support (Mazerolle & Mazerolle, 2017). In cases of model uncertainty, likelihood ratio tests (LRT) were used to assess variable importance (Anisimova & Gascuel, 2006). Post hoc comparisons of marginal means across treatments were performed using the Tukey test (Graves et al., 2015). To analyse growth trends in larval weight over time, we applied the “emtrends” function from the “emmeans” package to compare estimated slopes (Lenth & Lenth, 2018). The models were fitted with the “glmer” function from the “lme4” package using a log link function and a Gamma distribution (Lo & Andrews, 2015).

3.3.3.3 Total harvest yield

To analyse the effect of treatment on larval survival, we utilised a generalised linear mixed-effects model (GLMM) fitted with the “glmer” function and compared it to a generalised linear model (GLM) fitted with the “glm” function, both from the “lme4” package. The models were built using a binomial distribution and log as a link function (Harrison et al., 2018; Silk et al., 2020). The mixed model incorporated tray ID as a random effect to account for variation between trays. To assess model uncertainty, an LRT was conducted to evaluate the significance of the random effect (Anisimova & Gascuel, 2006). The mixed model was then analysed using ANOVA adjusted to a 95 % confidence interval. When the treatment effect was found to be significant, a post hoc Tukey test was applied to compare treatment means (Graves et al., 2015).

3.3.3.4 Waste reduction, efficiency of conversion of digested, and bioconversion

We fitted linear regression mixed models to assess the impact of the random effect (Tray ID) against the generalised regression models for bioconversion, efficiency of conversion of digested material, and waste reduction. We further used the likelihood ratio test (LRT) to compare the models. Subsequently, the mixed models explained a lot of variation by adding tray ID. However, due to outliers [Figure S3.2(B-D)], a robust regression model was fitted to account for the effect of outliers (Maechler et al., 2021). Robust regression was performed using the MM-estimation method in the “robustbase” R package (Maechler et al., 2021). If the treatment effect was significant, a post hoc Tukey test was applied to compare treatment means (Graves et al., 2015). All analyses were performed using R software, version 4.3.1 (R Core Team, 2023).

3.4 Results

3.4.1 Larval survival

The waste stream significantly affected larvae survival ($\chi^2 = 18.50$, $df = 2$, $p < 0.001$). Larvae fed on potato peels had a significantly higher survival compared to avocado waste ($\beta = -0.15593$, $SE = 0.06552$, $p = 0.0268$) and orange waste ($\beta = -0.1749$, $SE = 0.0416$, $p < 0.001$). There was no significant difference between avocado and orange waste ($\beta = -0.0189$, $SE = 0.0636$, $p = 0.9511$). Tukey's multiple comparison test showed that BSFL survival on potato peels significantly differed ($66.63 \pm 4.20\%$) compared to avocado ($49.20 \pm 4.2\%$) and orange waste ($46.00 \pm 4.20\%$), but there were no significant differences between avocado and orange waste.

3.4.2 Rate of change in weight over time

The most parsimonious model included the interaction between waste streams and days ($AIC = -13566.67$; $\chi^2 = 1086.68$, $df = 2$, $p < 0.001$; Table S3.3). Growth trend revealed that potato peels (0.2051 g/day, 95% CI = $[0.1817, 0.2317]$, $p < 0.001$) were the most significant substrate for promoting BSFL growth, followed by orange waste, with avocado waste being the least influential (Figure 2; Table 3). Although larvae weight increased across all treatments, potato peels consistently outperformed both orange (0.1259 g/day, 95% CI = $[0.1115, 0.1421]$, $p < 0.001$) and avocado waste (0.1231 g/day, 95% CI = $[0.1091, 0.1391]$; (Figure 2; Table 3)).

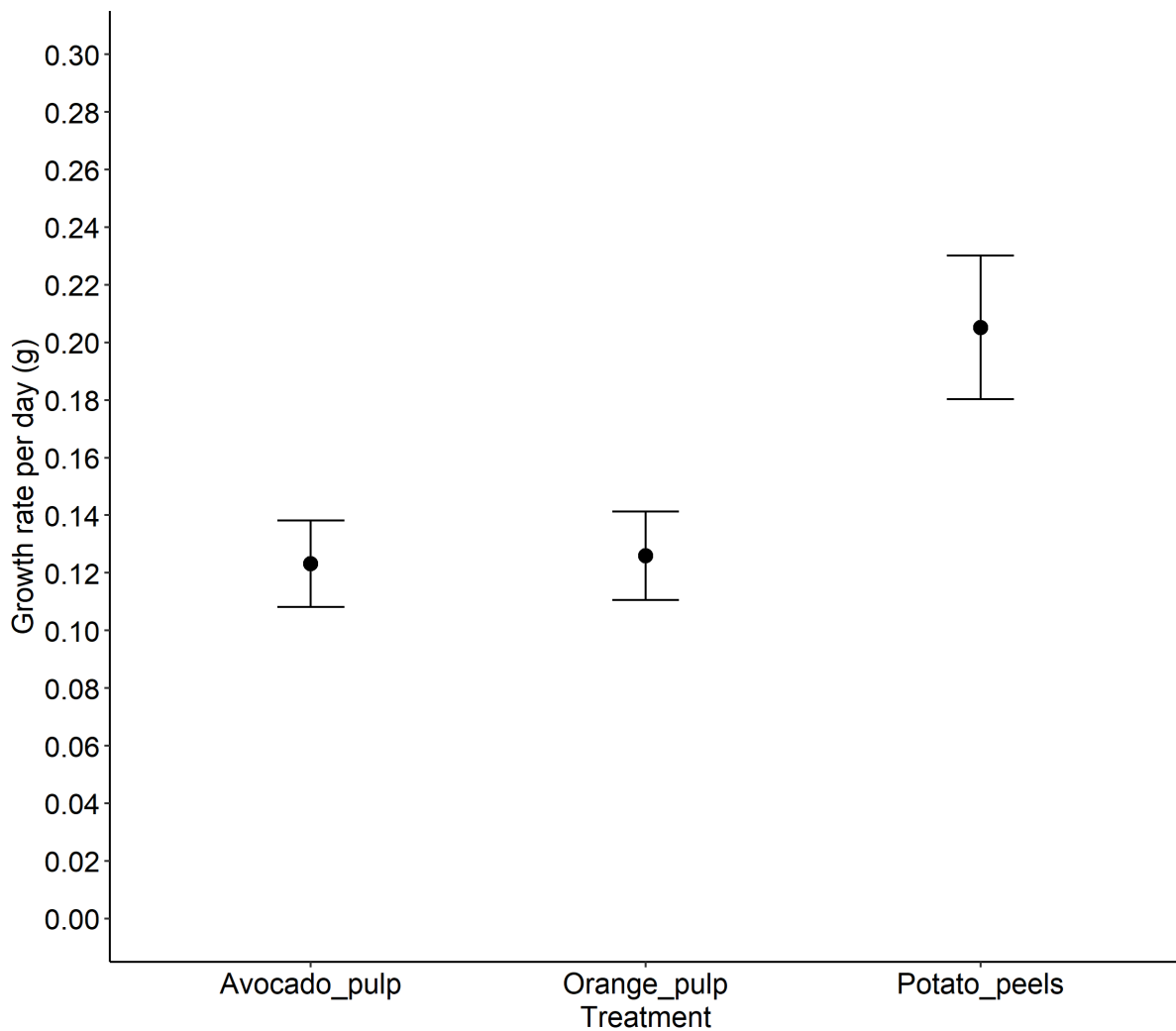


Figure 3.2: Larval growth rates (g/ day) feeding on avocado waste, orange waste, and potato peels after inoculation into the substrates over 14 days period. Error bars represent confidence intervals (CI).

4.4.3 Total larval harvest yield

Overwhelming support was found (AIC = -102.03; Table S3.2), indicating that treatment affected larval harvest yield ($\chi^2 = 57.59$, $df = 2$, $p < 0.001$), with potato peels achieving higher yield per tray harvest compared to avocado and orange waste (Table 3.3; Figure 3.3). A Tukey post-hoc multiple comparison test has shown that larval harvest yield significantly differed from avocado and orange waste (Table 3.3). However, there was no significant difference between avocado and orange waste harvest yield (Table 3.3).

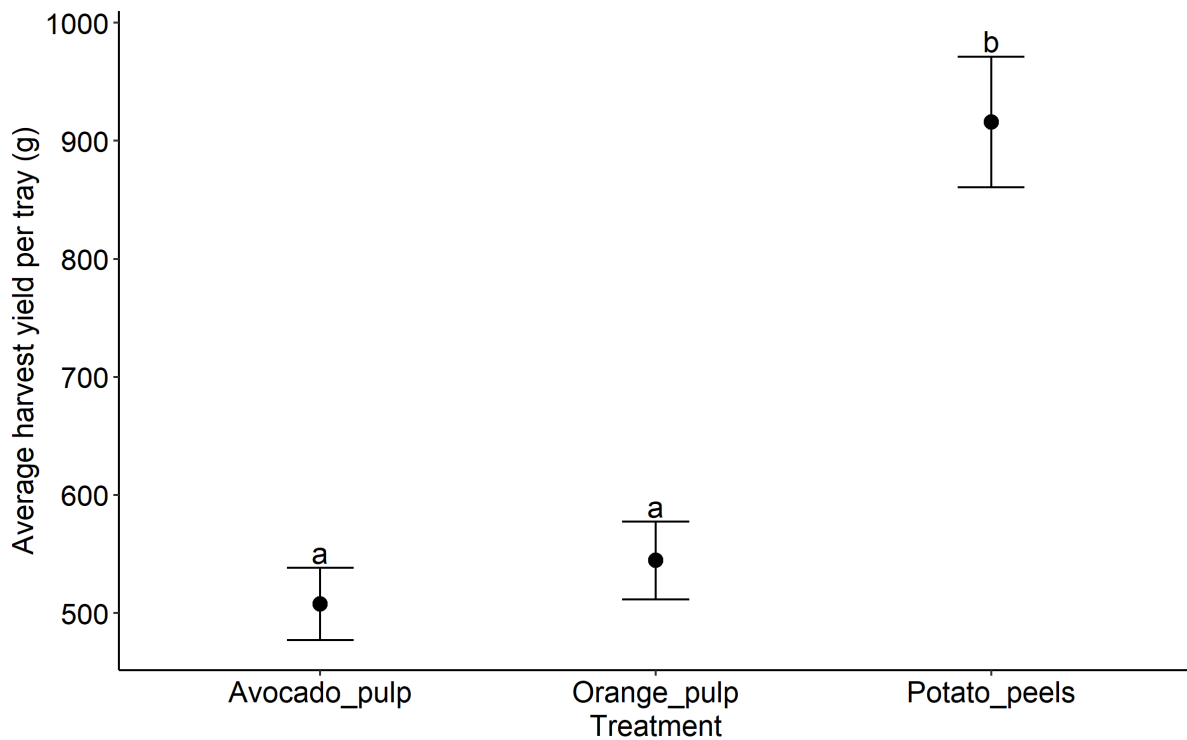


Figure 3.3: Average larval harvest yield per tray from eight experimental trays in avocado, orange, and potato peels. Bars with different letter codes indicate significant differences (Tukey's multiple comparison test, $P < 0.05$).

3.4.4 Bioconversion, efficiency of conversion of the digested feed and waste reduction

The impact of substrate significantly influenced waste reduction ($\chi^2 = 550.53$, $df = 2$, $p < 0.001$). Significant waste reduction was observed in potato peels than those fed on avocado waste ($\beta = -46.7387$, $SE = 2.0261$, $p < 0.001$). In contrast, there was no significant difference observed between potato peels and orange waste treatment ($\beta = -0.4645$, $SE = 8.2131$, $p = 0.998$). The highest waste reduction was observed in potato peels treatment at 57.63 % (± 3.44), followed by orange waste at 50.61 % (± 5.53) and avocado waste at 8.33 % (± 1.02 ; Table 3.3).

Table 3.3: Mean larval weight, substrate harvested, Waste reduction, larval harvest yield, bioconversion, and efficiency of conversion of feed digested (ECD) of BSFL fed on avocado, orange waste, and potato peels. The data was all recorded during harvest.

Treatment	larval mean weight(g)	Substrate harvested (g)	Substrate reduction (%)	Larval harvest yield (g)	Bioconversion (%)	Efficiency of conversion digested
Avocado waste	0.1549 ± 0.0032	11 642	8.33 ± 1.02^a	507.76 ± 30.72^a	1.00 ± 0.23^a	14.2 ± 3.87^a
Orange waste	0.1795 ± 0.0037	5 285	50.61 ± 5.53^b	544.57 ± 32.95^a	1.62 ± 0.40^a	4.18 ± 1.34^b
Potato peels	0.1970 ± 0.0024	4 025	57.63 ± 3.44^b	915.99 ± 55.42^b	5.61 ± 0.12^b	9.93 ± 0.50^c

Similarly, waste stream type significantly impacted ECD ($\chi^2 = 83.34$, $df = 2$, $p < 0.001$). Larvae fed on avocado showed the highest ECD compared to orange waste ($\beta = -10.3663$, $SE = 1.3003$, $p < 0.001$) and potato peels ($\beta = 4.3082$, $SE = 0.7052$, $p < 0.001$), respectively. There was a significantly higher ECD in potato peels-fed BSFL than orange waste ($\beta = -6.0580$, $SE = 1.3344$, $p < 0.001$). The avocado waste recorded the highest ECD of ($14.2 \pm 3.87\%$), followed by potato peels at 9.93 % and orange waste at 4.18 % respectively (Table 3.2).

Bioconversion was also significantly influenced by the type of waste stream ($\chi^2 = 662.25$, $df = 2$, $p < 0.001$). Larvae fed on potato peels exhibited a higher bioconversion compared to those fed on avocado waste ($\beta = -4.4367$, $SE = 0.1911$, $p < 0.001$) and orange waste ($\beta = -3.7205$, $SE = 0.3338$, $p < 0.001$). The bioconversion difference between orange and avocado waste was insignificant ($\beta = 0.6547$, $SE = 0.3889$, $p = 0.1970$). The potato peel treatment group observed the highest bioconversion at 5.61 %, followed by orange waste at 1.62 %, and avocado waste at a much lower percentage (Table 3.3).

3.5 Discussion

The BSFL's voracious appetite makes the larvae a potent organic waste management tool since it can valorise different organic waste, ranging from animal-based, such as chicken manure (Parry & Weldon, 2023) and pig manure (Čičková et al., 2013), to plant-based, such as fruits (Scala et al., 2020; Scieuzo et al., 2023) and vegetables (Lindberg et al., 2022; Magee et al., 2021). This literature concurs with our findings in this study, with the larvae showing significant differences in survival, larval yield, and conversion efficiency of potato peels, orange and avocado waste.

In this study, we observed that the substrate type influenced larval survival, with potato peels outperforming avocado and orange waste, respectively. The nutrient composition of the waste could explain the differences in larval survival across the different wastes. Based on the literature, potato peels have a better protein value than avocado and orange waste, which explains the better survival percentages observed (Barragán-Fonseca et al., 2018). The substrate texture of the potato peels may have improved aeration, which could also influence larval survival, as Tschirner and Simon (2015) reported. In contrast, literature shows that orange has low protein content and a carbohydrates-to-protein ratio, which may have been

a limiting factor to larval survival of the BSFL (Oonincx et al., 2015). Similarly, avocado waste contains more protein than an orange. However, the oily texture of avocado may have caused substrate compaction, potentially reducing larval movement to acquire nutrients despite adding bulking agents across all treatments to improve substrate structure (Isibika et al., 2021). Since the nutritional composition of the waste streams was not directly analysed in the present study but was instead inferred from existing literature, these inferences should be interpreted with appropriate caution and regarded as preliminary hypotheses requiring further experimental validation.

Regarding growth rates, potato peels grown larvae continued to outperform larvae grown in avocado and orange substrates by showing a significant growth rate and larval yield during harvest. Other studies have shown that substrates with a balanced carbohydrate-to-protein content significantly improve larval development and metabolism (Cammack & Tomberlin, 2017; Oonincx et al., 2015). The synergistic relationship of these macronutrients has been shown to be associated with rapid improvement in larval development and waste degradation in the literature (Diener et al., 2009).

In our study, the larvae reared in the potato peels substrate reached growth peak earlier (day six) than those reared in avocado and orange waste, which required six more days (day twelve) to reach the peak. Although we did not measure nutrient content directly, these differences may be linked to the improved nutrient balance suggested in the literature for potato peels.

Furthermore, micronutrients may also influence BSFL performance. According to the literature, the potato peels substrates are rich in phosphorus (P), potassium (K), and magnesium (Mg), which are important for physiological functions in insects (Nocelli et al., 2016; Tavabe et al., 2013). In this study, we observed that the BSFL partially utilised potassium, indicating a nutrient retention pattern. At the same time, there was active uptake of phosphorus and magnesium, particularly in the potato and avocado waste, compared to the orange waste. These interpretations, while plausible, remain speculative in the absence of direct mineral analyses from our study (Nocelli et al., 2016; Tavabe et al., 2013).

The average larval harvest yield reinforces the importance of substrate quality. Potato peels yielded the highest biomass among the tested substrates on a dry biomass basis, suggesting

that substrates with a balanced nutrient profile and favourable physical properties enhance BSFL performance (Nyakeri et al., 2017). Our results in this study concur with previous studies showing that moderate nutrient substrates, including strawberries and bananas, have improved bioconversion rates (Scieuzo et al., 2023; Scala et al., 2020). In contrast, the physical properties of orange and avocado waste could have impacted larval yield as observed with growth rates rather than the nutrient profile.

A higher bioconversion percentage indicates the ability of the larvae to assimilate nutrients from the waste, while a higher ECD shows the efficiency of converting waste into valuable biomass. This study observed the inverse relationship between bioconversion and ECD in avocado waste, whereas potato peels and orange waste had higher bioconversion and ECD. The literature has shown that nutrient-poor or less digestible substrates, such as avocado, can lead to higher ECD values due to larval selection in assimilating the limited available nutrients (Parry and Weldon, 2021). Furthermore, a plausible explanation for the inverse pattern observed is that the avocado waste contained the skins as part of the waste, which could have affected the larvae in nutrient assimilation due to the hardness of the skin. In contrast, potato peels waste had a better nutritional balance, which could be the plausible explanation for an improved bioconversion process compared to the other two waste streams (Isibika et al., 2023). These findings, however, remain interpretative due to the lack of direct compositional analysis.

Regarding waste reduction, the substrate type further demonstrated that it could influence BSFL performance. This study observed the highest waste reduction in potato peels, followed by orange and avocado waste. The waste reduction trends in this study align with other studies in the literature that elaborated on the poor performance of the larvae when fed with a single type of waste, which affects the larvae due to poor nutritional content (Scala et al., 2020; Scieuzo et al., 2023). Moreover, Parry and Weldon (2021) have shown that a mixture of waste streams can improve waste reduction due to improved nutritional content of the substrate. The low reduction in avocado waste may stem from the substrate's physical condition due to a mixture of the seeds, waste, and skins, which may be challenging to break down due to the high fibre content (Scieuzo et al., 2023). Literature also suggests that substrates with high phenolic compounds, such as orange peels, reduce overall digestibility and mineral uptake (Isibika et al., 2023; Lattanzio et al., 2006).

In contrast, potato peels substrate likely offered a more accessible nutrient profile, enhancing larval waste consumption and degradation rates (Tschirner and Simon., 2015; Barragán-Fonseca et al., 2018). Nonetheless, these explanations remain hypothetical without chemical profiling. Future studies should explore the role of substrate processing, such as drying or fermenting, which could improve waste reduction outcomes in fibre- and oil-rich wastes such as avocado (Parry and Weldon., 2021).

3.6 Conclusion

This study has shown that both macro- and micro-nutrient compositions of the substrates play an important role in influencing the larval performance of BSFL in waste valorisation systems. The potato peels outperformed orange and avocado waste streams, supporting the highest survival, growth rate, and overall bioconversion efficiency. The results are focused on protein-carbohydrate balance and the impact of micronutrient profile, since they have shown their importance in supporting larval metabolism and development.

Orange and avocado waste showed moderate larval performance, likely due to substrate compaction and phenolic compounds that limited nutrient assimilation. Lower potassium retention in orange waste and mineral depletion in avocados suggest nutrient-binding or restricted bioavailability. Despite these limitations, each waste stream offers unique valorisation potential: potato peels for high-yield feed production, orange waste for efficient waste volume reduction, and avocado waste for gradual, eco-friendly decomposition. Future work should prioritise in-depth chemical characterisation of waste substrates and frass to elucidate better the relationships between substrate composition and larval physiological responses. Moreover, pre-treatment methods should be explored to improve digestibility and enhance nutrient bioavailability for more sustainable BSFL-based waste management systems.

3.7 References

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3.8 Supplementary data

Tables

Table S3.1: Nutritional composition of avocado, potato peels, and orange from different literature sources.

Substrate	Author	%			mg/100g		
		Carbohydrates	Protein	P	K	Ca	Mg
Avocado	Marcel et al., 2022	4.80	1.82	52.00	339.00	12.00	29.00
Avocado	Stephen & Radhakrishnan., 2022	8.64	1.96	54.00	507.00	13.00	29.00
Avocado	Zafar & Sidhu., 2018	8.64	1.96	54.00	507.00	13.00	29.00
Avocado	Lye et al., 2022	8.53	2.00	52.00	485.00	12.00	29.00
Avocado	Hurtado-Fernandez et al., 2018	8.53	2.00	52.00	485.00	12.00	29.00
Orange	Marcel et al., 2022	15.00	1.00	18.00	237.00	52.00	13.00
Orange	Liu et al., 2012; Lu et al., 2023	11.75	0.94	18.00	181.00	40.00	10.00
Orange	Etebu & Nwauzoma., 2014	9.35	0.94	14.00	181.00	40.00	10.00
Orange	Lu et al., 2023	11.50	0.67	18.00	196.00	37.00	14.00
Orange	Guo et al., 2023; Marcel et al., 2022	11.75	0.94	18.00	237.00	40.00	13.00
Potato	Schieber & Saldaña, 2009; Mouillé et al., 2008	12.44	2.57	126.00	694.00	24.00	37.60
Potato	Beals., 2019	12.44	4.00	126.00	620.00	20.00	33.00
Potato	Burlingame et al., 2009; Bellumori et al., 2020	18.30	3.20	126.00	693.80	27.80	37.60
Potato	Robertson et al., 2018	17.50	2.10	57.00	425.00	12.00	27.00
Potato	Bellumori et al., 2020; King & Slavin., 2013	18.00	3.40	75.00	54.00	12.00	27.00

Table S3.2: Moisture contribution of all the used components to formulate the experimental diet for uniformity during the start.

Diet component	Avocado waste		Orange waste		Potato peels	
	Mass (kg)	Moisture contribution	Mass (kg)	Moisture contribution	Mass (kg)	Moisture contribution
Potato peels	40.00	53.00	40.00	57.00	40.00	59.00
Spent grain	10.00	11.00	10.00	11.00	10.00	11.00
Sawdust	7.50	4.00	7.50	4.00	7.50	4.00
Water	3.50	6.00	1.50	3.45	0.00	0.00

Table S3.3: Model comparison for larval growth over time.

Model names	K	AICc	Delta_AICc	AICcWt	LL	Cum.Wt
Treatment*Day	9	-13566.67	0.00	1.00	6792.36	1.00
Treatment + Day	7	-12631.41	935.27	0.00	6322.72	1.00
Treatment	6	-12628.52	938.15	0.00	6320.27	1.00
Quadratic	6	-12628.52	938.15	0.00	6320.27	1.00
Day	5	-10407.77	3158.91	0.00	5208.89	1.00
Null model	4	-10405.37	3161.30	0.00	5206.69	1.00

Table S3.4: Model comparison for larval harvest yield of black soldier fly larvae (BSFL).

Model names	K	AICc	BIC	LL
Mixed model	4	-102.03	303.04	-145.17
Glm model	2	298.34	-9614	56.02

Figures

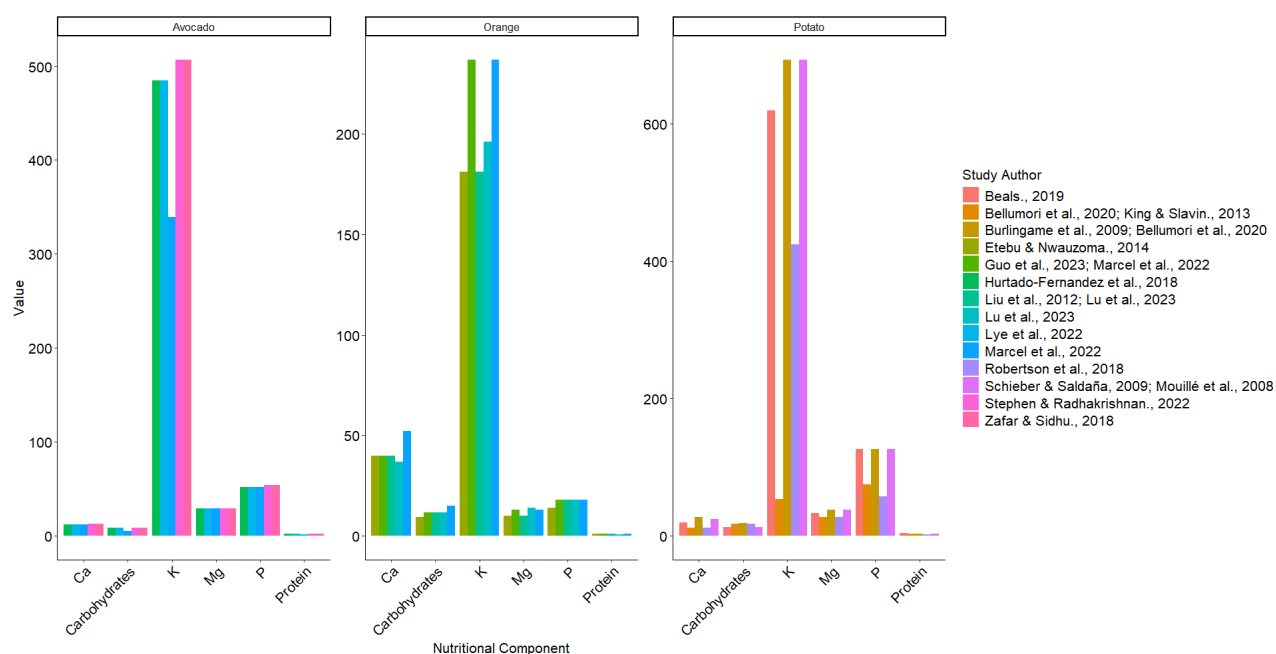


Figure S3.1: Comparative nutritional composition of avocado, orange, and potato waste streams across published literature sources.

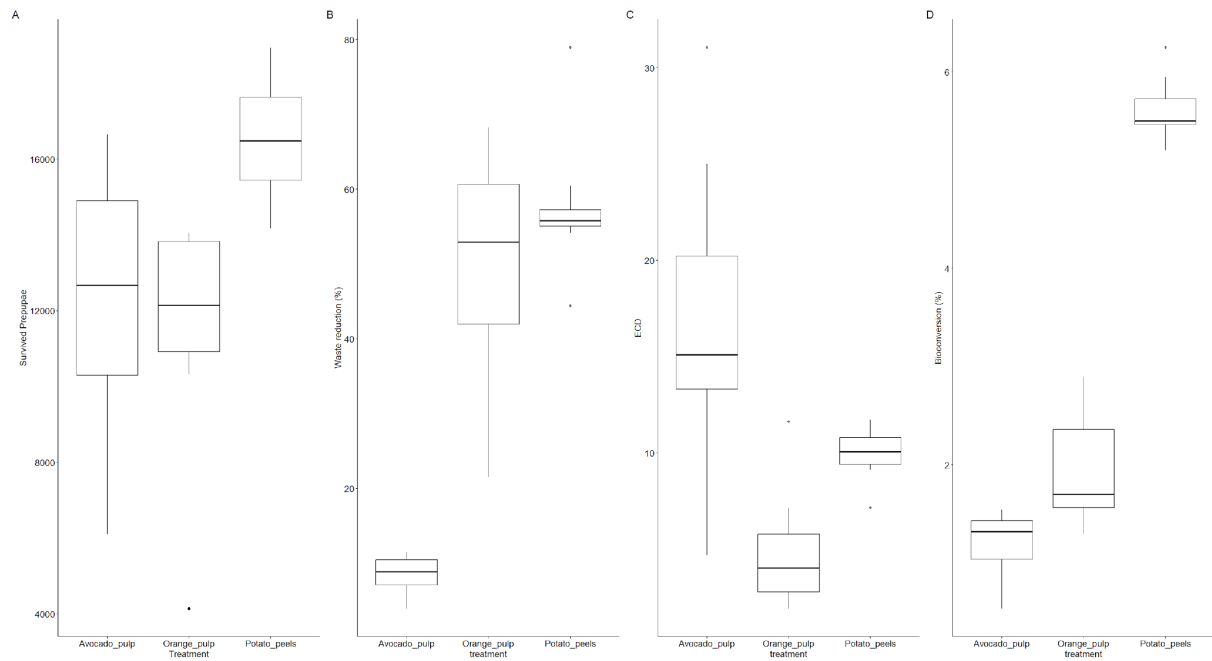


Figure S3.2: Residual outliers of the (A) larval survival, (B) waste reduction, (C) efficiency of conversion of the digested, and (D) bioconversion by the black soldier fly larvae.

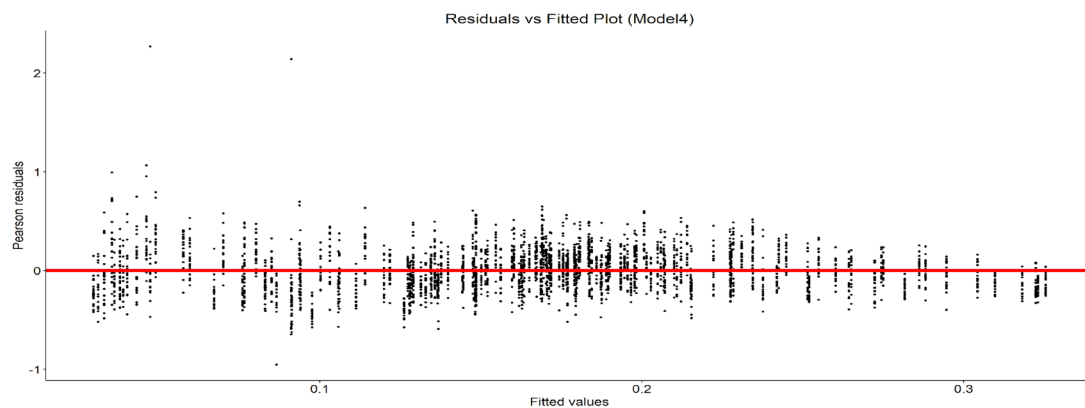


Figure S3.3: Pearson residual of the most parsimonious model (model 4)

Chapter 4

The impact of black soldier fly (*Hermetia illucens*) composted frass on Crimson Globe beetroot (*Beta vulgaris*) growth performance

The impact of black soldier fly (*Hermetia illucens*) frass on Crimson Globe beetroot (*Beta vulgaris*) growth performance

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Abstract

Black soldier flies (*Hermetia illucens*, BSF) are gaining popularity due to the larvae's ability to process or consume organic waste efficiently. These insects consume a wide variety of waste materials, transforming them into protein-rich larvae and nutrient-dense by-products known as frass. While BSF production is mainly popular due to low-cost and effective organic waste management and production of protein, frass, which is a by-product, has the potential to create an additional income stream and provide a high-quality organic fertiliser. However, the quality of the frass can vary due to the quality of the organic waste, storage and post-production processes. For this reason, the study evaluated the effectiveness of frass from three common organic waste streams local to Thohoyandou, South Africa. A composted frass was used from larvae fed on three waste streams: potato peels, avocado, and orange waste. Three frass application levels were used: i) optimal recommended nutrient level, ii) half of optimal and iii) one and a half of optimal. Additionally, two control treatments were included, a zero application and optimal inorganic NPK fertiliser. Beetroot (*Beta vulgaris*) was used as an indicator crop and was harvested after a 10-week growth period. Results showed that increasing frass application significantly improved leaf weight, bulb weight, and bulb diameter and reduced leaf damage after ten weeks. However, no significant differences were observed in leaf number and height between frass and inorganic fertiliser treatments, suggesting frass could serve as a partial or full substitute for inorganic fertilisers. Plant damage decreased as frass application increased, while the control and inorganic treatments exhibited higher damage. This study has shown that frass as an inorganic fertiliser is viable for agricultural purposes in terms of crop growth parameters and could even improve the quality of crops by decreasing potential insect or pest damage.

Keywords: BSFL, compost maturity, sustainable fertiliser, soil amendment, soil health, food security

4.1 Introduction

The increasing demand for sustainable agricultural practices is driving initiatives to reduce reliance on chemical fertilisers, which are associated with various environmental problems, such as soil degradation, eutrophication, and greenhouse gas emissions (Kihara et al., 2020; Schmitt & de Vries, 2020; Swanepoel et al., 2016). The overuse of inorganic fertilisers contributes to nutrient leaching, soil acidification, and water pollution, causing environmental degradation and a decline in biodiversity (Poveda, 2021). In contrast, organic fertilisers release nutrients gradually and enhance soil ecosystems by promoting microbial diversity and activity (Houben et al., 2020; Kawasaki et al., 2020). One emerging source of organic fertilizer is frass, a by-product generated by black soldier fly (BSF; *Hermetia illucens*) larvae during the breakdown of organic waste.

Black soldier fly larvae (BSFL) are amongst the biological favourites for waste management, while the agricultural by-products, larvae and frass, can be used as animal protein supplement and fertiliser (Barragan-Fonseca et al., 2017). The BSFL can volarise wastes such as food scraps, manure, and agricultural by-products, transforming the larvae into protein-rich biomass, which can serve as a sustainable animal feed alternative, especially for poultry and fish (Čičková et al., 2015; Fowles & Nansen, 2019). Frass is a combination of insect droppings, exoskeleton, and undigested substrate that is rich in nutrients (Song et al., 2021b). It serves as a biofertiliser, providing crucial macro-nutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as increasing beneficial microbial activity (Houben et al. 2020; Terfa 2021, Dzepe et al., 2022).

More from being an eco-friendly biofertiliser, frass contains important biopesticide properties, such as the chitin which promotes plant growth and suppresses pests and plant diseases, thereby serving as both a fertiliser and a biopesticide (Quilliam et al., 2020). BSFL frass has been observed to inhibit pathogens such as *Fusarium oxysporum*, responsible for fusarium wilt in cow peas (Quilliam et al., 2020). The presence of chitin and other microbe-stimulating compounds in frass likely triggers plant immune responses, thereby decreasing disease incidence (Dzepe et al., 2022). This distinctive property makes frass a biological amendment that can facilitate pest and disease management in sustainable agricultural systems (Chavez et al., 2023; Gurung et al., 2024).

However, fresh frass contains nutrients that are not readily plant available (Houben et al., 2020) and might need to be composted to increase its stability, reduce pathogen content (Lomonaco et al., 2024), decrease phytotoxicity levels and decrease N immobilisation (Gebremikael et al., 2022; Song et al., 2021). Therefore, this study aims to assess the effectiveness of three different organic frass fertilisers compared to inorganic fertilisers. This will be achieved by (i) comparing the growth indicators in inorganic vs frass fertiliser and (ii) assessing plant damage under frass fertiliser vs inorganic fertiliser.

4.2 Methods and materials

4.2.1 Frass production

Frass from three different organic waste streams consumed by BSF were evaluated. These include avocado, orange waste, and potato peels independently supplemented with a protein source (spent grain) at a ratio of 80:20 % in a 50 kg total diet topped with 7.5 kg sawdust as a bulking agent. The byproduct, frass, was sieved through a 5 mm sieve, with a 35 % moisture for all treatments. The chemical analysis of raw frass is given in Table 1.

Table 4.1: Analysis of the frass macro-and-micro nutrients before and after composting.

Nutrients	unit	Pre-composting			Post-composting		
		Avocado Waste	Potato Waste	Orange Waste	Avocado Waste	Potato Waste	Orange Waste
		Frass	Frass	Frass	Frass	Frass	Frass
N	%	1.6	2.94	2.96	1.7	0.95	1.06
Ca	%	0.05	0.1	0.45	0.12	0.1	0.75
Mg	%	0.07	0.16	0.1	0.13	0.17	0.19
K	%	0.88	2.27	0.89	1.22	2.52	1.31
Na	%	119	702	160	306.5	577.5	429.5
S	%	0.1	0.17	0.14	0.14	0.2	0.18
P	%	0.12	0.29	0.15	0.16	0.34	0.22
Fe	mg/kg	547	509	773	7556	1073	12911
Mn	mg/kg	28	37	38	99	40.5	190
Cu	mg/kg	10	7	6	28	15	36
Zn	mg/kg	16	21	13	26	24.5	29.5
Mo	mg/kg	0.24	0.44	0.43	0.2	0.49	0.25
B	mg/kg	32	14	30	37	12	32
Al	mg/kg	148	465	668	3178	795	5633
Cl	%	0.03	0.85	0.05	0.03	0.62	0.09

pH	pH units	6.7	7.9	5.4	7.25	8.5	8.05
Calc. C	%	67.21	62.91	60.76	51.54	54.07	50.6
C (Total)	%	56.58	54.78	55.62	49.3	48.6	44.83
C:N	-	35.36:1	18.63:1	18.79:1	30.315:1	57.69:1	48.44:1
Moisture	%	33.71	36.68	34.47	27.14	32.42	33.65
Ash	%	2.69	5.77	4.34	11.36	7.01	12.97

4.2.2 Frass composting

A volume of 250 L of frass from each waste stream was composted. First, the moisture content of the frass was adjusted to 60 % and maintained by adding water during the composting process (Misra et al., 2003). The frass was placed on a heap and was turned every four days until it reached a consistent temperature below 25 °C. The compost was then maintained for ten weeks (Figure S4.1). Chemical analysis was done on non-composted and composted frass (Table 4.1) to determine nutrient content.

4.2.3 Treatment application rate

To calculate the frass and fertiliser application rate, optimal fertiliser recommendations (NPK ratio of 4:3:4, ARC, 2013) for beetroot were used to calculate the relative applications per pot. Frass analysis indicated that frass is mostly deficient in P (Tables 4.1 and 4.2), with nutrients from the different waste streams showing slight variation (Tables 4.1 and 4.2). All the treatments were aligned to have similar N levels since crops usually respond well to N fertiliser, but due to the very low P levels, it was supplemented in the experiment based on nutrient analysis to improve nutrient availability (Kubayi, 2022).

First, the conversion of field application to pot application by estimating a mass ratio of soil was calculated:

The soil volume per single (1 *ha*) with a field depth of 0.15 m:

$$\begin{aligned} \text{Length} \times \text{Width} \times \text{Depth} & \quad [1] \\ &= 100 \text{ m} \times 100 \text{ m} \times 0.15 \text{ m} \\ &= 1\,500 \text{ m}^3 \end{aligned}$$

Obtained the mass of the soil in 1 ha, given the soil volume and bulk density of the soil used.

$$\begin{aligned} \text{BD} \times \text{Vol} & \quad [2] \\ &= 1.5 \text{ Mg m}^{-3} \times 1500 \text{ m}^3 \\ &= 2\,250 \text{ Mg} \\ &= 2\,250\,000\,000 \text{ g of soil/ 1 hectare and 0.15 m soil depth} \end{aligned}$$

Now calculate the N requirement for treatments:

$$\frac{\text{Pot soil (g)}}{\text{Field soil (g)}} \times N \text{ application per hectare (g)} = N \text{ application per pot (g)} \quad [3]$$

$$\frac{5\,000\text{ g}}{2\,250\,000\,000\text{ g}} \times 140\,000\text{ g} = 0.31\text{ g of N fertilizer per pot}$$

The nitrogen fertiliser application in all the waste streams was calculated individually. For example, N application for avocado waste should be 0.31 g per treatment, and the average N content in the avocado waste frass is 1.7 g (Table 2), dry mass.

$$\frac{0.31\text{ g N per pot}}{1.7\text{ g N in }100\text{ g frass}} \times 100\text{ g frass} = 18\text{ g of dry frass per pot}$$

However, the frass applied in avocado waste has nitrogen moisture content of 27%. Therefore, to correct for the moisture content, it was adjusted as follows:

$$x - x(0.27) = 18$$

$$0.73x = 18$$

$$x = 25\text{ g frass to apply per pot}$$

Thus, avocado waste frass at half rate will be 12.5 g, and frass at optimal x 1.5 will be 37.5 g. Table one summarises the ratios and application of the different waste streams.

Table 4.2: Frass and inorganic fertiliser application rates

	N	P	K	Dry mass (g)	Optimal application rate (g)
Ideal ratio (Fertilizer guidelines)	4	3	4		
Ratio of avocado frass	11	1	8		
Ratio of potato frass	5	2	13		
Ratio of orange frass	8	2	10		
Nutrients required kg/ha	140	105	140		
Nutrients required per pot (g)	0.3	0.2	0.3		
Mass of frass according to optimal N requirements					
Avocado frass (g/pot)				18.2	25
Potato frass (g/pot)				32.6	48
Orange frass (g/pot)				29.2	44
Nutrients provided per pot from above applied frass					
Avocado frass (g/pot)	0.3	0	0.2		
Potato frass (g/pot)	0.3	0.1	0.8		
Orange frass (g/pot)	0.3	0.1	0.4		
Inorganic fertiliser rate					
NPK 2:3:4 (required at planting)	70	105	140		
KAN (required after 2 weeks)	70				
Total inorganic fertiliser nutrients (kg/ha)	140	105	140		
Mass of fertiliser per pot					
NPK 2:3:4 (gram at planting)					2.3
KAN (gram after 2 weeks)					0.3

Frass nutrient composition is 1.23 % N, 0.24 % P, and 1.68 % K, resulting in a nutrient ratio of approximately 4:1:5. This might result in lower P levels; however, most guidelines suggest higher N and K application.

4.2.4 Fertiliser application

Inorganic fertiliser application followed optimal recommendations, which vary based on soil analysis. For the NPK treatment, the ARC Production Guidelines were followed (ARC, 2013), applying NPK in a 2:3:4 ratio with an additional N top-up after three weeks, resulting in a total NPK ratio of 4:3:4. fertiliser quantities were calculated using these ratios and formulas. Each pot received 2.3 g of 2:3:4 fertiliser during transplant and 0.3 g of KAN after two weeks (Table 4.2).

4.2.5 Experimental layout

The experiment was done in a growing tunnel and followed a completely randomised block design lasting ten weeks. There were five treatments: (i) control (no application), (ii) inorganic NPK fertiliser treatment, (iii) composted avocado waste frass, (iv) composted orange waste frass, and (v) composted potato peels frass. Three application levels were applied from the three waste streams: half-optimal, optimal, and one-and-half-optimal. Each treatment was replicated twenty-five times, resulting in a total of 275 pots in the experiment. Pots were prepared by adding 5 kg of course sandy soil sieved through a 5 mm sieve (Tanga *et al.*, 2022). One week before introducing the seedlings, the frass treatments were applied to ensure that the nutrients from the treatments were blended with the soil (Anyega *et al.*, 2021; Tanga *et al.*, 2022).

Beetroot seedlings were cultivated in controlled greenhouse conditions in seedling trays for six weeks, and seedlings with a minimum of two mature leaves were then transplanted into the prepared pots. Two plants were planted per pot. After one week, the weakest plant was removed to ensure successful crops. The pots were placed in a growing tunnel, 600 x 600 mm apart. Regular weeding was carried out throughout the trial. The beetroots were irrigated once daily with at least 300 mm of water.

4.2.6 Sampling parameters

Measurements were taken to assess the growth performance of beetroot. The above-ground and below-ground biomass was determined during the growth period and after the plants were harvested on week 10. The number of leaves developed per bulb and the longest leaf length were recorded as indicators of plant height. Plant height was recorded on the tallest leaf of each plant. The fresh weight and diameter of the bulbs were measured for yield parameters upon harvesting after 10 weeks. The beetroot was uprooted, grouped by treatments, cleaned and dried before measurements were taken. The leaves were cut and dried at 60 °C for five days to obtain the dry biomass. The bulb diameter was measured using a vernier calliper held horizontally around the circumference of the beetroot bulb.

4.2.7 Statistical analysis

The effect of fertilizer treatments on plant growth and yield parameters was assessed by fitting generalised linear models (GLMs) using the ‘lme4’ package (Bates et al., 2015). Before analysis, data were checked for homogeneity of variance using Pearson residual plots. GLMs were fitted to the measured crop parameters, including plant height, leaf number, leaf weight, bulb weight, bulb diameter, and leaf damage. Gamma distribution and log as a link function were used for plant height, leaf weight, bulb weight, and bulb diameter. In contrast, leaf number (a count variable) was modelled with a Poisson distribution, and leaf damage was analysed using a binomial distribution with log as a link function. Each model was compared to a null model (parameter ~ 1) using the corrected Akaike Information Criterion (AICc), with less than two AICc units of the top model ($\Delta\text{AICc} < 2$) considered to have equal support (Table S4.1) (Mazerolle, 2017). Analysis of Variance (ANOVA) was conducted on the fitted models to evaluate the significance of the fertiliser treatments. Following Anova, least square means were computed using the ‘emmeans’ package, with mean separation carried out using Tukey’s Honest Significant Difference (HSD) test, adjusted for multiple comparisons. The ‘cld’ function from the ‘multcompView’ package was used to visualise significant differences between treatment groups (Graves et al., 2015). All analyses were performed R software, version 4.3.1 (R Core Team, 2023).

4.3 Results

Treatment application resulted in highly significant results for leaf weight, bulb weight, bulb diameter, and leaf damage after a ten-week sampling period (Tables 4.3 and 4.4).

Table 4.3: Summary results from Anova using plant parameters as response variables

Response	Chi-square	Degrees of freedom	Pr(>Chisq)
Leaf height	2.13	10	0.99
Leaf number	12.13	10	0.28
Leaf weight	33.17	10	<0.0001***
Bulb weight	44.74	10	<0.0001***
Bulb diameter	69.26	10	<0.0001***
Leaf damage	138.9	10	<0.0001***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

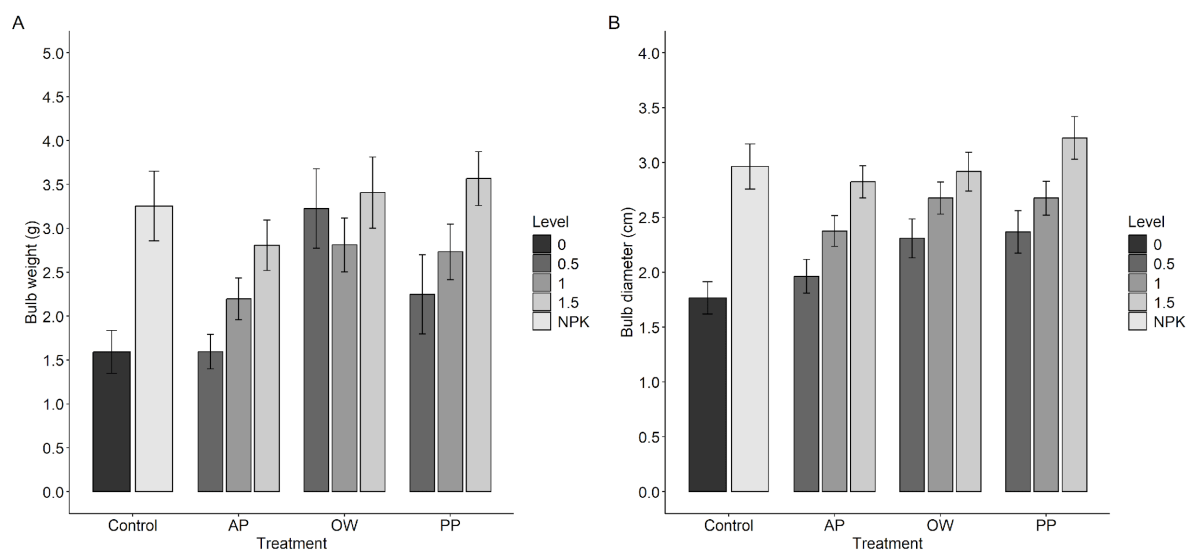


Figure 4.1: Bulb weight (A) and diameter (B) across different treatments (control, avocado waste, orange waste, and potato peel) applied at half-optimal, optimal, and one-and-half-optimal levels. Values are expressed in mean and standard error bars.

Measuring bulb weight ($\chi^2 = 44.74$, $df = 10$, $p < 0.001$) indicated that the control NPK, OW High, and PP High treatments resulted in significantly higher bulb weights (Figure 4.1A, Tables 4.3 and 4.4). The control zero treatment produced the lowest bulb weight, while a positive

trend can be observed within waste streams, where increased application level resulted in increased bulb weight (Figure 4.1B).

Bulb diameter is linked to bulb weight, and similar results have been observed. The treatments had a highly statistically significant effect on bulb diameter ($\chi^2 = 69.26$, $df = 10$, $p < 0.001$) (Figure 4.1B, Tables 4.3 and 4.4). A Tukey's HSD post hoc test indicated that PP High resulted in the largest diameters (Figure 4.1B, Table 4.4). Significant differences were only measured between control NPK and control zero and AP low, Table 4). The increase in frass application level corresponded to an increase in bulb diameter within each waste stream, although not significantly (Table 4.4).

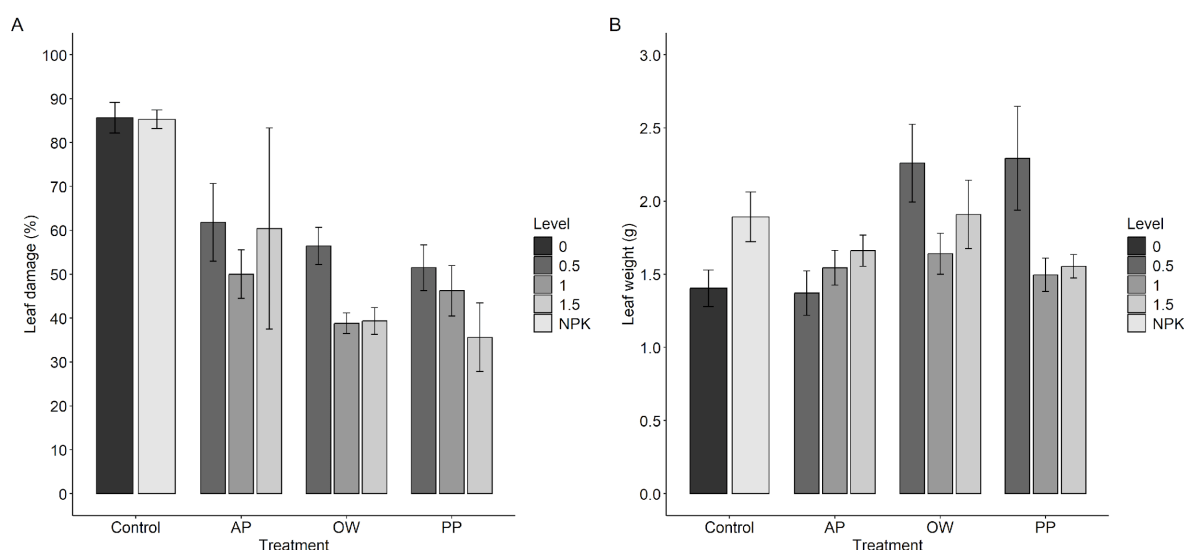


Figure 4.2: Leaf damage (A) and leaf weight(B) across different treatments (control, avocado waste, orange waste, and potato peel) applied at half-optimal, optimal, and one-and-half-optimal levels. Values are expressed in mean and standard error bars.

Leaf damage showed significant results (Figure 4.2A; Table 4.4). Treatment level had a highly statistically significant effect on leaf damage ($\chi^2 = 138.9$, $df = 10$, $p < 0.001$), indicating substantial differences in leaf damage across the different treatments (Figure 4.2A, Tables 4.3 and 4.4). Tukey's HSD post hoc test comparisons showed that the Control and Control NPK treatments resulted in significantly higher levels of leaf damage than all other treatments. In particular, the treatments with higher frass application also resulted in the lowest leaf damage (such as OW high and (Figure 4.2A, Table 4). While Control and Control NPK exhibited

the highest levels of damage, treatments such as PP High, OW Optimal, and OW High significantly reduced leaf damage (Figure 4.2A, Table 4.4). Moreover, frass fertiliser treatments significantly impacted leaf weight ($\chi^2 = 33.17$, $df = 10$, $p < 0.0001$), and a Tukey HSD post hoc test showed that the OW Low and PP Low treatments produced the highest leaf weights (Figure 4.2B, Table 4.4).

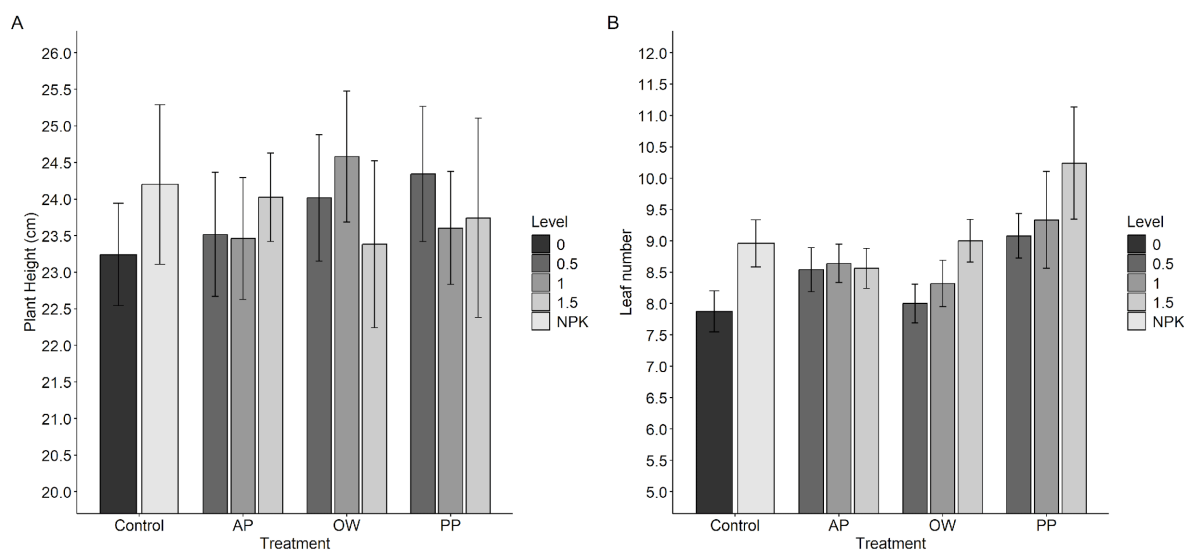


Figure 4.3: Plant height (A) and Leaf number (B) across different treatments (control, avocado waste, orange waste, potato peel) applied at half-optimal, optimal, and one-and-a-half-optimal levels. Values are expressed in mean and standard error bars.

Neither plant height ($\chi^2 = 21.32$, $df = 10$, $p = 0.99$; Figure 4.3A, Tables 4.3 and 4.4) nor leaf number ($\chi^2 = 21.12.13$, $df = 10$, $p = 0.28$; Figure 4.3B, Tables 4.3 and 4.4) were significantly affected by the treatments after ten weeks. The Control zero treatment consistently performs lower than other treatments, although not significantly in the case of plant height and leaf numbers (Tables 4.3 and 4.4). Another general trend that is observed is an increase in crop parameters with an increase in application levels within waste streams. Higher application levels usually performed better than lower levels. A low increase was observed in leaf numbers corresponding with an increase in frass application (Figure 4.3B).

Table 4: The impact of different types of fertilizers and varying applications on yield and growth parameters of beetroot bulbs and their leaves.

Treatment		Plant height (cm)	Leaf number	Leaf weight (g)	Bulb weight (g)	Bulb diameter (cm)	Leaf damage (%)
Waste stream	Application level						
Control zero	Control	23.24 ± 0.70	8 ± 0.32	1.40 ± 0.19 ^a	1.59 ± 0.24 ^a	1.77 ± 0.14 ^d	0.86 ± 3.00 ^{ab}
Control NPK	Control_NPK	24.20 ± 1.09	9 ± 0.37	1.89 ± 0.19 ^{ab}	3.25 ± 0.40 ^b	2.96 ± 0.21 ^{ab}	0.85 ± 0.02 ^a
Avocado waste	Low	23.52 ± 0.85	9 ± 0.35	1.37 ± 0.15 ^a	1.60 ± 0.97 ^a	1.96 ± 0.15 ^{cd}	0.62 ± 0.22 ^b
Avocado waste	Optimal	23.46 ± 0.83	9 ± 0.30	1.54 ± 0.11 ^{ab}	2.20 ± 0.34 ^{ab}	2.38 ± 0.14 ^{ad}	0.50 ± 0.05 ^{bc}
Avocado waste	High	24.2 ± 0.60	9 ± 0.32	1.66 ± 0.11 ^{ab}	2.80 ± 0.28 ^{ab}	2.82 ± 0.15 ^{ab}	0.60 ± 0.23 ^{ab}
Orange waste	Low	24.01 ± 0.86	8 ± 0.31	2.26 ± 0.27 ^b	3.22 ± 0.45 ^b	2.31 ± 0.18 ^{bd}	0.56 ± 0.04 ^{bc}
Orange waste	Optimal	24.58 ± 0.90	8 ± 0.37	1.64 ± 0.14 ^{ab}	2.81 ± 0.31 ^{ab}	2.68 ± 0.15 ^{ab}	0.38 ± 0.02 ^b
Orange waste	High	23.38 ± 1.14	9 ± 0.34	1.91 ± 0.23 ^{ab}	3.41 ± 0.40 ^b	2.92 ± 0.18 ^{ab}	0.39 ± 0.03 ^b

Potato peels	Low	23.34 ± 0.92	9 ± 0.36	2.29 ± 0.36 ^b	2.25 ± 0.45 ^{ab}	2.37 ± 0.19 ^{bd}	0.51 ± 0.05 ^b
Potato peels	Optimal	23.60 ± 0.77	9 ± 0.77	1.50 ± 0.11 ^{ab}	2.73 ± 0.34 ^{ab}	2.68 ± 0.19 ^{abc}	0.46 ± 0.06 ^b
Potato peels	High	23.74 ± 1.36	10 ± 0.90	1.55 ± 0.08 ^{ab}	3.57 ± 0.31 ^b	3.22 ± 0.19 ^a	0.35 ± 0.08 ^b
		ns	ns	***	***	***	

Control= No fertiliser application, Control NPK= Inorganic NPK fertiliser, Low= Half optimal frass application, Optimal= optimal frass application, High= 1.5 x optimal frass application within the same column, values denoted by the same letter, along with their respective means and standard errors, are not statistically different at a significance level of $P \leq 0.05$.

5.4 Discussion

Organic fertilisers contribute to soil fertility and health with less negative environmental impacts (Choi et al. 2009). However, using frass to enhance plant growth has encountered challenges, including the slow release of nutrients due to mineralisation and potential chemical components, such as salts and phenolic compounds, that might negatively affect plant growth. This study assessed the effectiveness of frass as an organic fertiliser, as compared to commercial NPK and a control with no added fertiliser. The frass was derived from common organic waste products in the local area of Thohoyandou, South Africa including avocado, orange waste, and potato peels. Growth parameters and leaf damage were measured using a beetroot crop. To allow for variable availability of nutrients from organic fertiliser, the frass treatments were applied at three levels. The optimal frass level had a nutrient content similar to the NPK treatments. Although the nutrients were not all immediately available.

This study demonstrated that composted frass as an organic fertiliser can perform equally well, or even better than inorganic fertilisers, in terms of beetroot growth and yield. This was evident in the positive impact of frass on growth performance. These findings align with other studies (Anyega et al., 2021; Beesigamukama et al., 2021; Tanga et al., 2022) that have reported enhanced vegetable growth, yield, and nutritional quality following frass amendments.

For improved performance, Tanga et al., (2022) reportedly combined frass with NPK fertilisers to synergistically enhance plant growth, with inorganic fertilisers providing readily available nutrients and frass releasing nutrients gradually after mineralisation (Abiya et al., 2022). In this study, increasing the application levels of composted frass resulted in higher beetroot yield and bulb diameter, surpassing those grown with inorganic fertilisers. The lack of significant differences in leaf number and height between plants treated with frass and those treated with inorganic fertilisers further supports the potential of frass as an organic fertiliser for promoting plant growth (Beesigamukama et al., 2020). This observation is consistent with Abiya et al., (2022), and Anyega et al., (2021), who also reported better growth and yield performance in various vegetables.

Nonetheless, the substrate type fed to the BSFL resulted in differences in crops performance, as substance nutrient composition varies in relation to substrate fed to the larvae, that directly impacts on plant growth and nutrient availability (Gärttling et al., 2020; Gärttling & Schulz, 2022). In our study, we harvested heavy bulbs from potato peels in all application levels and highest yield was observed in the treatment at optimal application levels. Orange waste exhibited a similar trend but produced moderate bulbs with lower weights than potato peels, while there was a lowest bulb weight found in the avocado waste in all organic treatment application levels. The higher growth and yield supported by frass from high-nutrient substrates is consistent with previous findings of other studies conducted on maize and coleus (Beasly et al., (2023; Beesigamukama et al., 2020).

Increasing the application of frass resulted in reduced plant damage across all treatments compared to the control (no fertiliser) and NPK fertiliser application, since frass, contains anti-pest and pathogen properties (Dzepe et al., 2022; Kawasaki et al., 2020). Insect chitin, which is a component of frass, is known for activating plant defence mechanisms that enhance resistance to pests and other plant-related infections (Quilliam et al., 2020). In this study, *Cercospora* leaf spot caused significant leaf damage in beetroot compared to inorganic fertiliser and the control treatment. Dzepe et al. (2022) identified two fungal genera, *Cercospora* and *Aspergillus*, which affected lettuce (*Lactuca sativa*), and their findings align with this study, where leaf damage decreased as frass application levels increased. Dzepe et al. (2022) hypothesised that *Cercospora* sp have been transmitted from the frass to plants, while the control treatments were likely affected by wind circulation. This may explain the beetroot damage in the growing tunnel where extraction fans were operating. Elissen *et al.*, (2019) reported that an increase in frass application suppressed a pathogenic fungus *R. solani*, which is in line with the findings in this study. These findings suggest that frass can be a natural anti-pest or pathogen agent for plants.

4.5 Conclusion

This study highlights that frass derived from avocado, orange waste, and potato peels is effective organic fertiliser for beetroot cultivation. The results showed that frass matches and can surpass the performance of inorganic fertilisers in enhancing growth and yield, particularly in leaf weight, bulb weight, and bulb diameter. Composting improved frass maturity and nutrient availability, contributing to the observed plant performance.

Additionally, using frass helped reduce plant damage, likely due to the anti-pest and pathogen properties. It further supports its potential as a sustainable alternative to inorganic fertilisers. The substrate used for feeding BSFL influence the quality of the frass and nutrient composition, resulting in variation of performance of as organic fertilisers. Overall, the study proves that when frass is applied at optimal levels, it can be used as an efficient, environmentally friendly organic fertiliser. These results further contribute to exploring frass in sustainable agriculture, particularly in integrated nutrient and pest management strategies.

4.6 References

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4.7 Supplementary data

Tables

Table S4.1: AIC Tables ranking models within each parameter.

Model names	Parameter	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
Null Model	Leaf height	2	1611.9	0	1	1	-803.93
Model 1		12	1631	19.13	0	1	-802.91
Null Model	Leaf Number	1	1237.6	0	0.99	0.99	-617.81
Model 2		11	1246.5	8.88	0.01	1	-611.74
Model3	Leaf weight	12	640.08	0	1	1	-307.43
Null Model		2	651.52	11.44	0	1	323.74
Model4	Bulb weight	12	984.73	0	1	1	-479.76
Null Model		2	1005.2	20.47	0	1	-500.58
Model5	Bulb diameter	12	722.31	0	1		-348.55
Null Model		2	754.67	32.36	0	1	-375.31
Model6	Leaf damage	11	342.13	0	1	1	-158.69
Null Model		1	458.32	116.18	0	1	-228.14

Synthesis and Conclusion

5.1 Introduction

This chapter summarises the key research findings and evaluates their alignment with the study's objectives. It also highlights the challenges encountered during the research and offers recommendations for future studies on optimising black soldier fly larvae (BSFL) use in waste management. With organic waste management a pressing global issue, BSFL presents a sustainable solution by transforming organic waste into valuable biomass and frass fertiliser.

Waste generation is an inevitable result of human activity, posing challenges to both human health and the environment (Paramashivam et al., 2017). Managing organic waste is particularly critical in both developed and developing countries due to its impact on environmental sustainability and agricultural productivity (Despoudi et al., 2021). The reliance on traditional waste management practices, such as landfilling and incineration, has contributed to greenhouse gas emissions and environmental degradation (Qazi et al., 2016). These issues underscore the urgent need for innovative and sustainable waste conversion strategies that align with global sustainability goals.

The BSFL are widely recognised for converting organic waste into valuable biological resources. They efficiently process a variety of waste streams, including kitchen waste, brewers' spent grain, and chicken manure (Shumo et al., 2019). Additionally, BSFL systems have transformed vegetable waste and poultry manure mixtures into protein-rich larvae and biofertilisers (Parry & Weldon, 2023). Domestic biodegradable waste, such as food, fruit and vegetable residues, has also been processed into larval biomass and biofertiliser, showcasing BSFL as a promising solution for waste valorisation (Yang-Jie et al., 2022). Moreover, organic municipal solid waste, animal manure, and agricultural residues have been converted into nutrient-rich larvae and organic fertilisers, advancing sustainable waste management practices (Surendra et al., 2020).

The by-products of these processes have diverse applications: frass is widely used as an organic fertiliser, while BSFL biomass, rich in protein and fat, is incorporated into animal feed production (Shumo et al., 2019; Surendra et al., 2020). However, managing high moisture

content in some waste streams remains challenging, requiring bulking agents to enhance aeration and optimise larval feeding conditions (Parry & Weldon, 2023; Rehman et al., 2019).

Chapter 2 evaluated sawdust as a non-nutritive bulking agent to optimise BSFL performance in high-moisture substrate, potato peels, addressing compaction and nutrient imbalance. The research aimed to overcome challenges posed by high-moisture organic waste streams, which hinder larval growth and reduce waste decomposition efficiency.

The results demonstrated that incorporating sawdust significantly improved larval growth and biomass yield, with the 15 % sawdust treatment achieving the highest growth rates and biomass production compared to the control. While BSFL survival remained consistent across treatments, sawdust-enhanced substrate aeration and moisture balance provided optimal larval movement and feeding conditions. These findings highlight the role of bulking agents in improving substrate physical properties without affecting larval survival, as confirmed by the literature (Lee et al., 2024). Additionally, sawdust incorporation facilitated nutrient assimilation, particularly carbohydrates and proteins from potato peels, leading to feasible feeding and increased larval biomass as sawdust application increased.

We further emphasised the importance of substrate composition and pretreatment, such as milling and adding bulking agents, to enhance larval performance. Compared to a study by Laksanawimol et al. (2024), our research confirmed that sawdust effectively stabilises high-moisture substrates that allow the larvae to reach the pupal stage, aligning with prior findings on improved BSFL performance under optimal physical conditions (Nayak et al., 2023; Nyakeri et al., 2017; Yakti et al., 2023). Overall, the study highlights the potential of bulking agents such as sawdust in scaling up BSFL-based bioconversion processes for sustainable organic waste management.

In Chapter 3, we were interested in assessing BSFL performance on growth rates, biomass yields, waste reduction efficiency, and bioconversion process on whether it varies depending on the nutrient composition properties of three locally abundant organic waste streams (avocado waste, orange waste, and potato peels) in Limpopo, Vhembe district, South Africa. The assessment contributed to efficient waste valorisation and addressing critical environmental and agricultural challenges.

The results revealed significant differences in BSFL performance across the substrates. Potato peels supported the highest larval growth rate, biomass yield, and waste reduction, attributed to their balanced macronutrient composition and favourable physical properties. Orange waste showed less performance than potato peels, yet it was better than avocado waste, which lagged due to structural and compositional challenges, including residual oil and high fibre content. In this study, we observed that the inclusion of the complete avocado as BSFL substrate affected the valorisation process of the waste since the avocado skins were challenging to be ingested by the larvae. The larvae might have utilised more of the nutrients from the waste than the seeds and skins, since our results have shown higher ECD from the avocado substrate. An inverse relationship was observed between bioconversion efficiency and feed conversion efficiency (ECD), with avocado waste achieving the highest feed conversion efficiency but the lowest bioconversion. The results further demonstrated that the fruits (avocado and orange waste) yield poor performance compared to the potato, a vegetable. This could be better explained by the availability of excessive phenols and polyphenols in their leaves that plants use as a defence mechanism against pests (Isibika et al., 2021; Lattanzio et al., 2006). Therefore, we suspected this mechanism could have caused the critical driver to lower the performance of BSFL.

These findings align with and expand on existing literature, highlighting the critical role of substrate composition in BSFL performance (Lalander et al., 2019; Nyakeri et al., 2017; Scieuzo et al., 2023). Nutrient-rich and physically optimal substrates, such as potato peels, enhanced larval metabolism, nutrient assimilation, and biomass production (Barragán-Fonseca et al., 2018). Conversely, substrates with lower protein content or unfavourable structural properties, such as avocado and orange waste, have limited larval efficiency (Oonincx et al., 2015). This chapter highlighted the importance of targeted substrate selection and optimisation, providing a framework for improving BSFL-driven waste management systems and advancing circular economy objectives.

In Chapter 4, we evaluated the effectiveness of BSFL-derived frass as an organic fertiliser for beetroot cultivation, hypothesising that frass could enhance crop growth and reduce plant damage compared to inorganic fertilisers. Frass from the three waste streams was applied at varying levels to assess its impact on plant growth parameters, including bulb weight, bulb

diameter, and leaf damage. This chapter contributes to sustainable agriculture by exploring frass as a viable alternative to inorganic fertilisers.

The findings revealed that frass significantly improved vital growth parameters. High application levels of potato peel frass produced the largest bulbs and diameters, outperforming orange and avocado waste frass. Increasing frass application levels consistently enhanced crop performance, with optimal and high levels of potato peel frass matching or exceeding the effects of inorganic fertilisers. While no significant differences were observed in leaf number and height between frass and inorganic fertiliser treatments, frass consistently improved overall crop growth and yield.

Frass also reduced plant damage, likely due to its anti-pest and pathogen properties (Quilliam et al., 2020), which were more effective than those of inorganic fertilisers. The chitin content in frass may have stimulated plant defences, reducing damage from *Cercospora* leaf spot. These findings align with previous research demonstrating the dual role of frass as both a fertiliser and a biopesticide (Dzepe et al., 2022; Elissen et al., 2019). This study highlights the potential of BSFL frass to replace inorganic fertilisers, offering an environmentally friendly solution for improving crop yield and quality. The results also emphasise the importance of substrate selection in determining frass nutrient quality, reinforcing its role as a sustainable option for integrated nutrient and pest management.

5.2 Recommendations for future studies on mass rearing of BSFL

This study is one of the few studies (Parry & Weldon, 2023; Scala et al., 2020; Scieuzo et al., 2023). To evaluate BSFL-driven waste management at a production scale. This approach provides valuable insights into practical challenges and opportunities not evident in smaller-scale experiments. Future studies should build on these findings by addressing limitations encountered in production-scale systems to optimise BSFL performance and scalability. For example, environmental monitoring in rearing chambers could be improved by using precise tools, such as iButtons, to capture microclimatic variations within rearing trays that affect larval growth and waste bioconversion. Additionally, tray design improvements are crucial for maintaining optimal conditions for larval performance. Although shallow trays are beneficial in reducing compaction, improving aeration, and aiding drainage, limited experimentation with higher bulking agent application rates beyond 15 %. Future designs should balance these

advantages with greater flexibility by incorporating better tray designs or adjusting feed-to-bulking ratios to accommodate higher application levels.

Further research in the case of frass-based fertilisers should compare composted and uncomposted frass in order to provide better insight into impacts on soil health and crop performance. Although this study showed an increase in crop yield and a decrease in plant damage with a composted frass material, direct comparisons of frass amendment types would better inform its use. In addition, the hand sifting of frass created difficulty in high-moisture substrates, resulting in rough granules, especially for the treatments with orange waste. In comparison, potato peel and avocado waste frass had finer particle sizes that were easier to sieve, illustrating the major impact that substrate type has on frass handling. Better moisture content and sift quality management are explained as a way to alleviate these issues, greatly increasing the practical usability of frass as a fertiliser. Therefore, the amount of heat extraction and aeration systems must be improved in the growing chamber as this ensures optimal composting and homogeneous quality of the frass. Thus, it becomes a potential, sustainable alternative to inorganic fertilisers in agriculture.

5.3 Conclusion

This study evaluated the optimisation of BSFL systems for sustainable organic waste management through three interlinked experimental components. The first experiment demonstrated that the incorporation of sawdust as a non-nutritive bulking agent into high-moisture substrates improved substrate aeration and moisture balance, leading to enhanced larval growth and biomass yield. The second experiment assessed BSFL performance on three locally available organic waste streams, revealing that substrate composition plays a critical role in larval development, waste reduction, and bioconversion efficiency. Among the tested substrates, potato peels exhibited the highest performance, likely due to their balanced nutrient content and suitable physical properties, while avocado and orange wastes presented challenges related to compaction, residual lipids, and phenolic compounds that may have interfered with nutrient assimilation. The third experiment investigated the fertiliser potential of BSFL-derived frass in beetroot production. Composting improved frass maturity, resulting in higher crop yield and reduced foliar damage. The study confirmed that properly processed frass can be a valuable organic fertiliser with additional plant-protective

properties. Overall, the findings provide new insights into how bulking strategies, substrate selection, and frass processing influence BSFL performance and downstream applications. This integrated approach contributes to the development of efficient and scalable BSFL-based waste management systems aligned with the goals of sustainable agriculture and the circular-loop economy.

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