POST-PROCESSING OF BLACK SOLDIER FLY LARVAE FRASS AND PALM OIL FUEL ASH FOR ORGANIC FERTILIZER PRODUCTION

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**A project report submitted in partial fulfilment of the
requirements for the award of the degree of
Bachelor of Engineering (Hons) Civil Engineering (Environmental)**

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled “POST-PROCESSING OF BLACK SOLDIER FLY LARVAE FRASS AND PALM OIL FUEL ASH AS AN ORGANIC FERTILIZER” was prepared by BRANDON LEONG XEYN HIN has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons) Civil Engineering (Environmental) at Universiti Tunku Abdul Rahman.

Approved by,

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Supervisor : Prof. Dr. Mohammed J.K. Bashir

Date : 23-4-2024

Specially dedicated to
my beloved family, supervisor, co-supervisor, lectures, and friends

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ABSTRACT

Inadequately managed biodegradable waste poses a significant environmental, social, and economic hazard, prompting the need for comprehensive waste management strategies. In recent decades, significant attention has been directed towards waste management and valorization efforts, aiming to develop integrated and sustainable waste management approaches. The utilization of black soldier fly larvae (BSFL) for waste treatment has gained traction, offering a pathway to convert waste into marketable goods and foster circularity within the ecosystem. BSF larvae demonstrate voracious feeding habits on a variety of organic waste streams, including food scraps and agro-industrial residues, significantly reducing waste volume in a shorter timeframe compared to traditional composting methods. Among the byproducts generated, frass, the residue of BSF larvae rearing, has garnered interest due to its nutrient-rich composition, positioning it as a potential organic fertilizer. However, the rapid composting process render it biologically unstable, necessitating post-treatment measures for stabilization to enhance its suitability as a fertilizing amendment. Alternatively, blending frass with other fertilizer products could enhance matrix stabilization and augment the effectiveness of the combined fertilizer. The initial analysis of raw frass indicates a high value in nutrient composition of approximately 3.5% nitrogen, 1.1% phosphorus, 3% potassium, but with a low carbon-to-nitrogen ratio of 12 to 1. To address the low C/N ratio of fresh frass, it is blended with palm oil fuel ash, a biowaste rich in carbon, and composted to achieve an optimal C/N ratio and nutrient enhancement. Subsequent analysis of the compost reveals an increased C/N ratio (22:1) and phosphorus content (2.3%). However, there is a notable decrease in nitrogen content to 1%, suggesting nitrogen loss during the composting process. This study evaluates BSF larvae frass's potential as an organic fertilizer for sustainable cultivation, aiming to create a well-balanced fertilizer by incorporating palm oil fuel ash into the frass-based fertilizer to meet specific crop nutrient requirements.

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LIST OF SYMBOLS / ABBREVIATIONS

L	liter
mL	milliliter
BSF	Black soldier fly
BSFL	Black soldier fly larva
BSFLF	Black soldier fly larva frass
POFA	Palm oil fuel ash
EFB	Empty fruit bunches
CFU	Colony forming units
EU	European union
N	Nitrogen
P	Phosphorus
K	Potassium
C/N	Carbon to nitrogen
ppm	Parts per millions, mg/L

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CHAPTER 1

INTRODUCTION

1.1 Background

The exponential rise in food consumption is a result of rising living standards and an expanding global population. As a result, food waste is growing gradually and contributing to a host of social issues. Food waste is described by the Food and Agriculture Organisation (FAO) as food that is discarded, typically in the retail and consumption stages (Liu et al., 2016). The sustainability of our food systems is seriously threatened by food loss and waste. The Food and Agriculture Organisation of the United Nations (FAO) estimates that around one-third of the world's annual 1.3 billion tonnes of food production for human consumption are lost or wasted (FAO, 2011). Each year, more than 2.1 billion metric tons of municipal solid trash are produced worldwide, yet only around 16% of that waste is recycled (Kim et al., 2021). Between 25 and 45 percent of municipal solid trash is made up of food waste in addition to plastic, metal, glass, textile, wood, rubber, leather, and paper waste (**Figure 1.1**) (Brás et al., 2020).

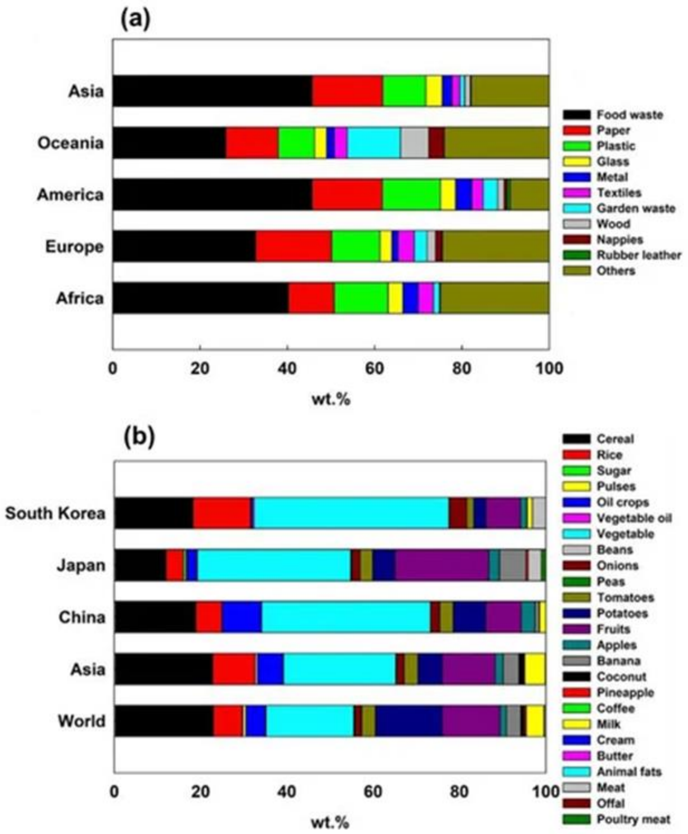


Figure 1.1: a) Municipal solid waste composition in weight percentage (S et al., 2006). b) Average global and Asia-Pacific food waste in weight percentage (Esra Uçkun Kiran et al., 2014).

The continued rise in organic waste production around the world is seen as a growing hazard to ecosystem health as well as to biodiversity and human health. Environmental issues related to this massive amount of garbage include soil, water, and air contamination (Journal of Insects as Food and Feed, 2014). Additionally, it was claimed that food waste had a negative influence on the ecosystem due to the methane gas released from landfills (FAO, 2014). **Figure 1.1** provides a summary of the usual food waste that is disposed of globally and in the Asia-Pacific area. There was a proof that the amount of organic waste in solid garbage declines as a country's income level rises; whereas it is 64% in low-income countries, it is only 28% in high-income nations (Lim et al., 2016). Therefore, efficient treatment solutions are necessary to lessen the financial and environmental costs associated with organic waste.

Due to the overwhelming of waste production, organic waste reduction has gained more attention globally in recent years, and several academics have suggested employing insect-based bioconversion as a commercially viable alternative (Cheng et al., 2017). Common methods for treating organic wastes include landfilling, composting, and incineration (Kim et al., 2021). However, there are several disadvantages associated with the disposal of trash in landfills, including the taking up of valuable space by wastes, the growth of pathogenic organisms, the formation of unfavorable odors, as well as a contribution to greenhouse gas emissions (FAO, 2013). It takes a long time for the wastes to totally breakdown using these standard waste management techniques (Chin et al., 2019). Although the techniques of waste treatment are frequently employed in many cities in developing nations, a solution to the issue of the continually rising amounts of organic wastes is delayed by the lack of interest in investing in strategies to collect, separate, and process the organic wastes (Kinasih et al., 2020). Using insects to treat food waste is becoming more and more popular as an environmentally beneficial way to recycle resources. It also has the benefit of having inexpensive installation costs. Additionally, using a certain extraction method, such insects can be a great source of protein (Lee et al., 2019). However, for the insects to survive and thrive, it is crucial to maintain the right conditions, including feed components, an appropriate temperature, humidity level, and acidity (Yoon et al., 2020).

Several studies have shown the best choice for digesting organic wastes belongs to black soldier fly (Hermetia illucens) larva (Singh et al., 2021). The species' feeding activity has the power to significantly reduce a large amount of organic material while simultaneously producing valuable animal or human feed with a high nutrient composition. (Diener, 2010). One of the five genera in the subfamily Hermetiinae of the order Diptera is the black soldier fly (Hermetia illucens) (Usda.gov, 2023). There are five stages in the life cycle of BSF include eggs, larvae, prepupae, pupae, and adults. The adult and egg-hatching stages are very brief, but the larval and pupal stages contribute the most to the total life cycle (**Figure 1.2**) (Singh and Kumari, 2019). In two weeks, BSFL can pupate under the perfect conditions of food availability, temperature, and humidity.

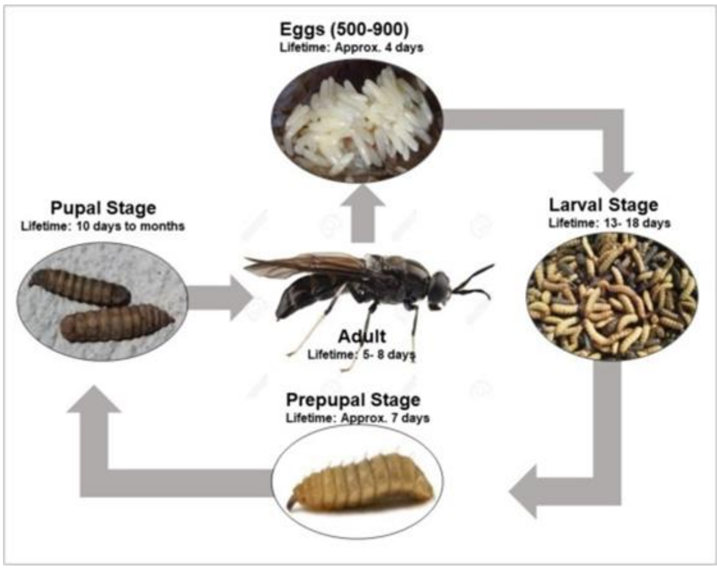


Figure 1.2: Typical lifecycle stages of Black Soldier Fly (Singh and Kumari, 2019).

The larvae efficiently decompose organic wastes like the remains of decaying animals and plants by breaking down a variety of organic wastes utilizing their robust mouth parts and digestive enzymes (Cho et al., 2020). Composting with black soldier fly larvae (BSFL) offers undeniable benefits such as accelerated processing of organic waste and reduced bacterial growth and odor (Journal of Insects as Food and Feed, 2014). According to a study by Ibadurrohman et al. (2020), BSFL has an amazing capacity (75%) to recycle biological wastes, producing 800 g of larval biomass from 4 kg of garbage. Like all other living things, BSFL require nutrients in order to grow. As a result, in order to increase bioconversion performance, BSFL must eat organic wastes that are abundant in nutrients that can be digested (Kinasih et al., 2020). Besides, BSFL is now widely used in a variety of industries for animal feeding, the production of biodiesel, biopolymers (chitin), and soil composting since it has been established that the larval biomass produced through the bioconversion of organic wastes is a high-quality source of fat or oil (Kim et al., 2021). Notably, whole, or processed BSF larvae or pupae can be added to the diets of fish, poultry, pets, and pigs, serving as potential substitutes for typical feed ingredients like soybean- and fish-based meal. Thus, the traditional feed components that insect products could take the place of can be saved for other applications, such as human consumption, thereby promoting food security. Additionally, the bioactive compounds found in BSFL, like antimicrobial peptides, may also provide significant advantages to animal diets (Surendra et al., 2020).

Other than the BSF larvae, its by-products may offer low-income nations' businesses and smallholder farmers a feasible way to generate cash. The highly adaptable and multi-functional byproduct is known as frass, a type of fertilizer that is derived from the waste conversion process by Black Soldier Fly Larvae (BSFL). 'Frass' is a shorthand for the primary by-product in the bioconversion of wastes into high quality protein for animal feedstuff. Insect frass production and sale in the EU are governed by Regulation (EU) 2021/1925, which the European Commission adopted on November 29, 2021 (International Platform of Insects for Food and Feed, Brussels, 2023). In a business setting, frass frequently refers to a mixture of primarily insect feces, substrate residues, and lost exoskeletons. Frass generally refers to insect excretions. It is a natural by-product of mass-raising insects that can make up to 75% of the feeding substrate and is frequently marketed as a fertilizer (Diener, 2016).

The first large-scale field studies offered encouraging perspectives for insect frass' potential use in agriculture, particularly in terms of plant nutrient availability, as more studies began to concentrate on useful applications of insect frass in recent years (Beesigamukama et al., 2020). BSF frass is thought to be a significant output from these production systems, and as BSF production is scaled up, frass valuation is being given more economic and ecological weight. It is anticipated that using frass as fertilizer would be successful and will make the production system more circular (Schmitt and de Vries, 2020). Since this was seen for other manures in livestock farming, marketable frass fertilizers with known application potentials would also prevent the change from a valuable by-product to a waste deposition challenge in upscaling processes of BSF production that may potentially having negative environmental effects (Gärttling and Schulz, 2021).

1.2 Problem statement

Globally, there has been an increase in the efforts to recycle organic wastes utilizing black soldier fly larvae to provide high-quality sustainable protein to replace fishmeal in animal feeds and frass fertilizer for organic farming. However, the quality of the substrates used for larval rearing has a significant impact on the larvae's performance and nutritional quality. Organic wastes with low nutritional quality may contain excessive nitrogen lost through ammonia volatilization. It is crucial to remember that ammonia is a greenhouse gas and that its atmospheric emissions are bad for the environment, human health, and ecosystems of plants, animals, and soil. As more nitrogen is lost throughout the composting process, the frass produced is of a much lower quality and is therefore unsuitable for use as organic fertilizer. Thus, it is necessary to provide the larvae with a feedstock that is balanced in nutrients.

The chemical composition of frass is diverse, and its nutrient levels depend on the feed substrate. There were studies showing that P-dominated frass fertilizers may not provide crops with optimal nutrition. It may be conceivable to add another input which is N-dominated to the frass to produce a well-balanced fertilizer. Future studies will look at whether nutrient addition to frass-based fertilizer is required to meet the specific nutrient needs of the crops. Many aspects of frass are still unknown, such as how it affects plant metabolism, how it behaves in the soil, and what post-processing steps are necessary for better biological stabilization. Additionally, plants' growth will be hindered when BSFL frass is treated in excessive concentrations. Uncertainty existed around whether it was caused by fertilizer burn or phytotoxicity of the quick compost. Heavy metals, phenolic compounds, organic acids, and an excessive build-up of salts are among the phytotoxins that immature compost frequently includes (Luo et al., 2018). These phytotoxins may be harmful to plant growth. To tackle this problem, additional composting of the BSFL frass could lessen its phytotoxicity for plants as well as its net nitrogen immobilization. Besides, to enhance matrix stabilization and boost the combined fertilizer's efficiency, combining the frass with another fertilizer product can be an alternative.

In compared to the larval biomass obtained through the same method, frass has not received nearly as much research. Particularly, there are several information gaps about the application of frass and its advantages in farming and other cultivation-related activities. Furthermore, the lack of characterization of the fresh frass product and standard composting procedures lead to inconsistency in their nutrient value. The full potential of frass should be thoroughly investigated to close knowledge gaps and encourage the uptake of this technology, given the compelling indicators that this biodegradable waste treatment method can be both economically and environmentally viable if carried out properly.

1.3 Aims and Objectives

The objectives of the thesis are shown as following:

i) To characterize both the raw and final product of Black Soldier Fly larvae (BSFL) frass for organic fertilizer production, ensuring suitability for sustainable agricultural practices
ii) To create a well-balanced fertilizer by adding palm oil fuel ash to frass-based fertilizer to meet the specific nutrient requirements of the crops.

1.4 Novelty

The novelty statements of this study are as following:

i) This study focuses on creating a well-balanced frass-based fertilizer to meet the precise nutrients require for crops by adding palm oil fuel ash as a supplementary nutrient into the frass and undergo further composting.
ii) This study concentrates on standardizing the composting process and characterizing the final version of frass fertilizer product.

1.5 Scope of study

This study focuses on evaluating the potential of Black Soldier Fly (BSF) larvae frass as an organic fertilizer for sustainable cultivation practices. Additionally, it aims to enhance the nutrient balance of the fertilizer by incorporating palm oil fuel ash. The characterization of raw frass, microwave-treated frass, and palm oil fuel ash was conducted to assess key parameters such as carbon, nitrogen, phosphorus, potassium content, and the C/N ratio. Based on the nutrient profiles of frass and ash, a suitable mixing ratio was calculated using a blending C/N ratio formula. Subsequently, the compost was prepared according to the determined proportions and subjected to a composting process lasting two weeks. Following composting, the final product underwent characterization to analyze changes in its nutrient profile.

1.6 Thesis Outlines

The thesis outline begins with Chapter 1: Introduction of Research, which delves into the background and issues of the study, as well as the novelty, objectives, and scope of the research. This is followed by Chapter 2: Literature Review, which provides a comprehensive understanding and anticipates the outcomes of the experiment based on past research studies. Chapter 3: Methodology presents the flow of the experiment along with standard procedures. Chapter 4: Results and Discussion presents the experimental outcomes with analysis and discussion. Finally, Chapter 5: Conclusion and Recommendations outlines the final conclusive writing of the study along with recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Waste Management Practices in Malaysia

In Malaysia, waste generation outpaces recycling rates, creating a serious problem with waste management. The amount of municipal solid waste (MSW) disposed of everyday in Malaysia is about 30,000 tons, or 1.17 kg per person (ITA, 2022). MSW was primarily composed of organic waste, with smaller amounts of plastic, paper, mixed organic materials, wood, and other materials also contributing (**Figure 2.1**) (Khamis et al., 2019). Along with the country's population boom, Malaysia has seen a steady increase in rubbish production, with landfills receiving the majority of the waste. 165 landfills, eight sanitary landfills, and three inert landfills for materials like sand and concrete are currently present in Malaysia, according to the National Solid Waste Department. If no action is taken to minimize waste, according to local environmental experts, there won't be any room left by 2050 (ITA, 2022).

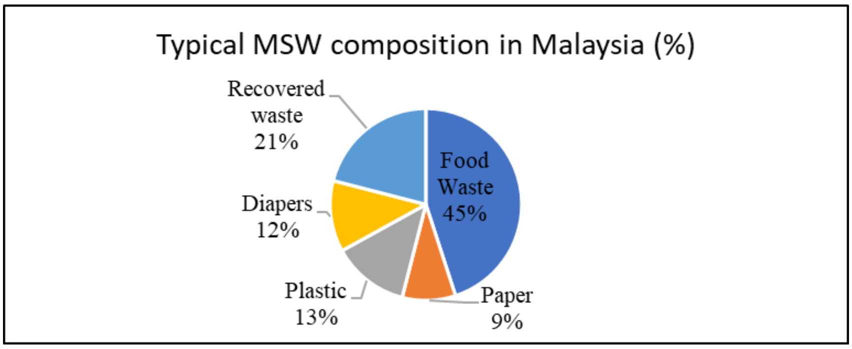


Figure 2.1: Typical MSW Composition in Malaysia (Khamis et al., 2019).

2.2 Conventional Organic Waste Treatment Method

The common methods for treating organic wastes include landfilling, composting, and incineration. Both landfilling and incineration are common methods for disposing of MSW, but landfilling has gained popularity due to the fact that incineration is more expensive, emits significant quantities of harmful gases, and leaves toxic residues that must also be handled (Yu et al., 2022). Furthermore, there were also methods called anaerobic digestion (AD) and mulching for managing and valorizing organic waste that can be used to create useful forms of renewable energy and soil amendments while minimizing trash disposal. Although AD and composting have been suggested as suitable alternatives for handling organic waste, both procedures necessitate strict process control to prevent them from having the same negative environmental effects as landfills, including GHG emissions linked to insufficient process management. To effectively address the issue of waste accumulation, more environmentally friendly techniques are needed. Numerous studies have shown the best choice for digesting organic wastes as black soldier fly (Hermetia illucens) larva (Kinasih et al., 2018).

2.3 Hermetia Illucens or Black Soldier Fly Larvae Treatment

The over-reliance of cities on food imports and excessive trash generation could be reduced by using black soldier fly (Hermetia illucens [L.], Diptera: Stratiomyidae) larvae (BSFL) to upcycle urban solid waste into a growth medium for vegetable cultivation. Despite the fact that there are a variety of insect species that can be utilized in insect farming, the species may eventually affect the materials that can be used as insect substrates as well as the effectiveness of biomass conversion rates from this insect substrate into feed. Black soldier fly (Hermetia illucens L.) production, in particular, is anticipated to be crucial in the development of a circular economy: BSF can be raised on a variety of organic waste substrates, including manures, and the larvae can be processed to be utilized as a protein source in animal diets for pets, poultry, pigs, and other animals as well as in aquaculture (Belghit et al. 2019). Additionally, it is feasible to employ BSF by-products to extract chitinous materials for the economy or to use the oil obtained from the larvae extraction for biodiesel or cosmetics (Rabani et al. 2019).

Black soldier fly- larvae treatment is a promising technique for dealing with biodegradable garbage that might be a factor in the difficulties that have emerged in the last ten years. BSFL are incredibly effective at breaking down organic waste. From 10 tonnes of food waste, they can produce 3346 kg of frass in just 12 days (Salomone et al., 2017). As previously indicated, biodegradable waste is converted into two products in this insect-based treatment: a processing residue known as frass, which can be utilized as fertilizer for a variety of uses, and a larval biomass rich in proteins and lipids that can be used in animal feed (Beesigamukama et al., 2020a). This method adheres to the concepts of a circular bioeconomy, in which the waste from one process becomes the resource in another, because two valuable goods are produced (Slorach et al., 2019). One of the main outcomes from larvae treatment method was frass, which might take the place of conventional N fertilizers and reduce the potential for global warming associated with the usage of any conventional N fertilizers (Salomone et al., 2017). Recently, the valuation of frass is gaining more economic and ecological weight as BSF production scales up. It is anticipated that using frass as fertilizer would be successful. (Kinasih et al., 2018).

2.4 The Byproduct of BSFL Insect – Frass

2.4.1 Frass Production Quality Standard

A paper was created by the International Platform of Insects for Food and Feed (IPIFF) in 2021 to establish the foundation for standard of insect frass throughout the European Union. The EU Regulation 142/2011 was changed as a result of this action, adding insect frass to a new category called "insect excrements" and requiring that frass intended for use as fertilizer undergo a heat treatment at 70 °C for 60 minutes. The quantities of Enterobacteriaceae can be lowered below the detection threshold of 10 (cfu) per gram of frass, eliminate Salmonella spp. from 25 g of product, and lower vegetative forms of Clostridium perfringens (Looveren et al., 2022).

2.4.2 Frass Biofertilizer Research

It has been demonstrated that the frass, or the excrement of the larvae, raises the water-extractable nitrogen concentration in the organic components, which vegetables can use for growth (Green and Popa, 2012). According to reports, BSFL's bioconversion of maize straw resulted in residual frass that met Chinese organic fertilizer standard NY525-2012 (Gao et al., 2019). In field-scale trials, BSFL frass was found to increase the output of shallots and chilies, and the presence of chitin in BSFL frass was suggested to promote plant health (Quilliam et al., 2020).

One area that requires further study is how frass impacts plant growth, its nutrient composition, and variables that determine variation, as well as how it impacts the environment (Berggren et al, 2019). Some helpful bacteria have found in insect frass serve as microorganisms for plant growth, enhancing the health of plants and assisting in their uptake of nutrients. It has a tremendous potential to be upcycled as a fertilizing product such as organic fertilizer, compost material, or soil improver because it has an unusual NPK -nitrogen, phosphorus, and potassium profile (International Platform of Insects for Food and Feed, Brussels, 2023). So, it is crucial to ascertain the precise fertilizing effect of frass when exploring agricultural sustainability and value-added environmentally friendly applications in agriculture or horticulture. As the sector expands quickly, frass will become a significant byproduct; it accounts for between 80 and 95 percent of a BSF manufacturing system's overall output (Devic, 2016).

2.5 Frass Characteristics

2.5.1 Microbial Composition in Frass

Frass's nutritional content and microbial composition are influenced by the feeding substrate (Poveda et al., 2019). Although most insects mostly excrete uric acid, the nitrogen fraction in the frass of terrestrial insects contains only 9–27% ammonium. Frass has a high ammonium content because allantoin decomposes into urea, which urease then converts into NH_4^+ (Green and Popa, 2012). Due to its low moisture content, the conversion of the nitrogen compounds in frass to ammonia should be quite modest. On the positive side, Chitin, another component of insect frass, is usually present, has several beneficial effects on plant growth and health, functions as a nematicide and fungicide, and promotes mycorrhization. BSF frass is also claimed to have insecticidal and insect-repellent properties (Bradley and Sheppard, 1984). However, in some circumstances, it may also appear to be phytotoxic (Sharp, 2013).

In comparison to other agricultural manures and composts, BSF frass had higher nitrogen concentrations due to its high dry matter content, which also increased its conservation qualities. As a result, reduced application rates for the nutrient application are required. Despite being highly high for frass from terrestrial insects, the ammonium nitrogen percentage of total nitrogen was low compared to other agricultural manures, and ammonia volatilization with rising humidity after application was achievable due to the high pH found (Kagata and Ohgushi, 2011).

2.5.2 Frass Nutrient Analyses

In nutrient analyses of BSF frass, it was classified as compound NPK fertilizer with 3.4% N, 2.9% P_2O_5, and 3.5% K_2O on average and a neutral to alkaline pH (Gärttling and Schulz, 2019). The pH level of BSF frass is higher than that of most comparable fertilizers. The maximum growth performance of BSF larvae is only possible on substrates with a pH of 6.0 or higher, and in these treatments, the larvae changed the pH of the substrate to values between 8.0 and 8.5 (Ma et al., 2018). When averaging

14 frass analyses from various sources, Gärttling and Schulz (2019) obtained a mean pH of 7.75.

Comparison of BSF larvae frass with their feed substrate reveals variations in nutrient content, as outlined in **Table 2.1** by Iva Guidini Lopes, Wan, and Lalander (2022). While total carbon and nitrogen levels remain relatively stable at around 37% and 3%, respectively, total phosphorus (1-5%) and potassium (0.5-4.1%) exhibit significant variability, likely influenced by the larvae's diet. Additionally, micronutrient levels differ based on the diet, although pH value (approximately 7.5) and the C/N ratio (around 15) show not much variation, authors observed varying nitrogen concentrations in BSF larvae frass, ranging from 18.3 g kg^-1 (vegetables and fruits) to 25.90 g kg^-1 (chicken feed) when different feedings such as chicken feed, grass clippings, and vegetable and fruit waste were provided. Similarly, when bread waste served as the sole nutrition source for BSF larvae, the resulting frass exhibited a nitrogen content of 15.2 g per kg (Klammsteiner et al., 2020). However, supplementation with protein-rich waste streams, such as fish waste (5-15% addition), increased nitrogen content to a range of 18.4 to 23.8 g kg^{-1} (Lopes et al., 2020). These findings underscore the ability to modify the BSF larvae’s diet to decide the composition of the final product.

Table 2.1: Chemical Characteristics of Various BSF Larvae Frass Derived from Composting Waste Streams.

	Chemical Characteristic											
	C	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Zn**	**pH**	**C:N ratio**	
		(%)				(g kg^-1)		(mg kg^-1)				
Wheat bran and Okara	37.1	4.8	1.0	0.9	1.3	0.1	–	4.2	0.1	7.5	7.7	(Song et al., 2021)
Grain from Distiller	–	3.4	0.8	1.1	13	3.0	5.0	45	90	–	–	(Yildirim-Aksoy et al., 2019)
Brewery spent grain	38.6	3.6	0.5	0.3	9.7	1.0	–	109	182	7.3	10.7	(Anyega et al., 2021)
Gainesville diet	35.2	3.8	5.2	4.1	45	8.0	3.0	−	140	8.8	8.0	(Setti et al., 2019)
Household waste	35.8	2.2	0.5	0.7	10	0.9	0.8	10	10	7.4	16.6	(Kawasaki et al., 2020)
Wheat bran	35.7	2.8	1.4	2.3	–	0.3	–	19.4	15	6.8	16	(Watson et al., 2021)

	Chemical Characteristic											
	C	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Zn**	**pH**	**C:N ratio**	
		(%)			(g kg^-1)			(mg kg^-1)				
Brewery spent grain	35.2	2.1	1.2	0.2	0.2	0.2	–	–	–	7.7	16.8	(Beesigamukama et al., 2020b)
Fresh okara	37.1	5.1	0.3	1.9	16.8	10.5	–	0.2	1.7	7.3	7.3	(Chiam et al., 2021)
Chicken manure	23.6	2.3	1.1	1.8	–	–	–	–	–	8.0	16.4	(Liu et al., 2019)
Pig manure	26.8	2.4	2.1	1.0	–	–	–	–	–	8.7	17.6	(Liu et al., 2019)
Chicken feed	47.9	2.6	–	–	–	–	–	–	–	6.2	18.5	(Klammsteiner et al., 2020)
Grass cuttings	44.3	2.4	–	–	–	–	–	–	–	5.4	18.2	(Klammsteiner et al., 2020)
Vegetables and fruits	48.8	1.8	–	–	–	–	–	–	–	5.6	26.6	(Klammsteiner et al., 2020)

	Chemical Characteristic											
	C	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Zn**	**pH**	**C:N ratio**	
	(%)				(g kg^-1)			(mg kg^-1)				
Cow manure	27.7	1.9	1.0	0.2	–	–	–	–	–	8.4	15.1	(Liu et al., 2019)
Vegetables	38.7	2.8	1.5	3.3	15	7.0	0.3	149	137	8.6	13.8	(Menino et al., 2021)
Average	36.6	2.9	1.6	2.4	11.6	3.7	2.3	42.4	64.0	7.5	14.5	
Median	36.5	2.8	1.1	1.5	9.9	1.0	2.6	14.7	15.0	7.6	15.6	

Frass produced from Black Soldier Fly (BSF) larvae fed on almond byproducts exhibited varying nutrient concentrations, ranging from 12.3 to 22.3 g kg^-1 for nitrogen (N), 17.9 to 44.6 g kg^-1 for potassium (K), and 0.22 to 0.82 g kg^-1 for phosphorus (P), as indicated by research conducted by Palma et al. (2020). These variations were attributed to differences in the compositions of the feed substrates, including glucose, protein, and fiber concentrations. Reduced fiber content and higher amounts of proteins and sugars or starches allowed nutrients to build up in the frass. Moreover, in 2019, Sarpong et al. investigated the feeding of BSF larvae with municipal solid waste of various compositions. Once larvae finished consumption of the waste, they noticed a notable increase in the concentrations of NPK content in the frass, reaching values of 4.8 g kg^-1 for N, 0.9 g kg^-1 for P, and 0.6 g kg^-1 for K. This increase was attributed to the loss of dry matter in the substrate over the course of time and the microorganisms’ activities secreted by the larvae in the growth medium.

The concentrations of potassium in the frass generated by BSFL are generally lower than those of the other essential nutrients. This is because it is difficult to recover the macronutrient K from biological materials, hence it is primarily obtained by mining of geological minerals (Manning, 2010). Regardless of the dietary substrate given to the larvae, this and other elements, such as Mg, Mn, and Cu, are typically present in low amounts in BSF larvae frass.

2.6 Plant Growth Response to Frass Derived Nutrients

Numerous further studies on plant growth were duly carried out to determine whether this product could be utilized as fertilizer once it was discovered that BSF larval frass contained appropriate plant nutrients. In their a study, food waste derived BSFL frass was compared to a commercial fertilizer (of unknown origin) with a comparable nutrient composition in terms of N, P, K, and organic matter (Choi et al., 2009). In addition to plants' reduced absorption of P when fertilized with frass, the authors compared the number of leaves, leaf length and width, and nutrient accumulation in Chinese cabbage plants and discovered that they were all of identical values. In one study, dead adult flies, frass, and larval skins were examined as soil additions in a pot trial with maize plants (Gärttling et al., 2020). Three amounts of fertilizer addition were applied to each byproduct: 180, 215, and 215 kg nitrogen content ha^-1, as well as 75 kg P_2O_5 ha^-1. Comparing the growth outcomes of the frass to the other by-products and the controls—commercial fertilizers composed of organic and chemical substances—suggested that the yield, dry matter generation, leaf surface area, and macronutrient utilization effectiveness from the frass were inadequate. The main reason is because frass is a P-dominated fertilizer rather than an N-dominated fertilizer, the author explained why it has weak fertilization properties. Furthermore, the test crop's low development suggested that the frass could not have an ideal nutritional content for some crops.

After evaluating the fertilizing ability of BSF larvae frass in Brassica rapa, growth-promoting application rates of 1 over 20 or 1 over 30 of frass in relation to soil volume were recommended. Yellow leaves indicated that plant development was impeded when treated at a higher amount (1 over 10) (Kawasaki et al., 2020). BSF larvae frass made from brewery waste, green market trash, and chicken manure was evaluated in Ghana as a fertilizer for producing shallots, pepper, and maize in a field study (Quilliam et al., 2020). The three types of frasses were examined both on their own and in conjunction with NPK chemical fertilizers in various field studies employing 2.5 to 10 t ha each. In general, the authors discovered that fertilization with chicken manure which is the popular local fertilizer produced similar growth responses in plants. Additionally, it was discovered that using frass and chemical NPK fertilizer together improved plant performance.

Frass from Okara-derived BSF larvae was tested as a fertilizer for lettuce plants by being mixed with soil at concentrations of 10, 20, and 30% (v/v) (Chiam et al., 2021). High quantities of NPK values were found in the frass. Surprisingly, lettuce grew poorly when frass was applied widely (20% to 30%), except for 10% of the plants. The low C/N ratio of the fertilizer (7.2), which induced rapid nutrient mineralization in the soil, could be the cause of this undesired growth response at elevated frass proportion. On the other hand, ryegrass responded well and expanded consistently when six dosages of frass were treated as fertilizer, which corresponds to 25–150% of this species' total nitrogen need. Furthermore, soils treated with frass showed higher concentrations of soil organic matter, P_2O_5, and K_2O, indicating increased fertility—a benefit that is commonly recognized when using organic fertilizers instead of chemical inputs.

The rates of nutrients that are available to plants are another advantage of employing frass as a biofertilizer, in addition to the soils' greater organic matter concentrations. In a recent study, after applying composted straw for five weeks in an acric ferralsol, the nutrient decomposition process over 125 days was evaluated. Over time, the soil's release of nitrogen (mostly ammonium), phosphorus, and magnesium was significantly more than that of the control treatment, which did not receive any fertilizer. They detected net immobilization throughout 30 to 60 days in the modified soil (Beesigamukama et al., 2021). These findings suggested that the frass's nutrients had been mineralized. The features of organic fertilizers, like the C to N ratio and biological steadiness, among other aspects, have a significant impact on the mineralization of nutrients from them (Chen et al., 2014).

In a study, different BSF larval frass were sprayed at quantities of 170 and 510 kg nitrogen ha^-1 in a silty loam-textured Haplic Luvisol (Rummel et al., 2021). The frass with the lowest C to N ratio was where nitrogen was mined and accumulated, according to the authors. They also observed that the kinetics of mineralization over time, for both carbon and nitrogen, were influenced by the quality of the diet fed to the larvae. The levels of ammonium nitrogen in the frass are directly correlated with the rates of nitrogen mineralization/immobilization from the soil, possibly because this form of nitrogen is preferred by microorganisms (Beesigamukama et al., 2021). After applying frass, the soil had a high concentration of carbon and nitrogen, which increased N_2O emissions (Rummel et al., 2021).

Another example was, frass from BSF reared on pig slurry increased plant growth for basil and Sudan grass in a pot trial. Using four experimental crops (potato, lettuce, Chinese cabbage and green bean), zahn et al. (2013) evaluated a merchantable BSF frass fertilizer with worm compost and chicken manure in incubation, starter and field trials. The substantially strongest positive yield response showed that frass was preferable than compost and poultry manure because it had high N, P, and K contents and was readily available. Different frass characteristics significantly affected how plant growth. The composted frass displayed greater NH4+ concentrations and a threefold increase in N content when compared to conventional biowaste composts. An ideal effect on plant development was seen when lower rates, e.g., comparable to manure application, were applied (Zahn, 2017). In another trial experiment that was conducted to analyse the effect of frass on pak choi growth. It had proven that the frass type and application rates had an impact on how the BSFL frass affected pak choi growth (Song et al., 2021).

2.7 The Adoption of Post Treatment

Studies on growth using frass have produced both favorable and unfavorable findings. It is conceivable that the weak development in some of the trials could be attributable to the unstable frass-compost. The frass was employed as a freshly created frass in some experiments, while post-composed frass was used in others. When using organic fertilizers during cultivation, the stability and maturity of the compost are key factors to consider. Even while a compost, like frass, provides sufficient nutrients for crop production, if the compost is not stabilized, poor growth can result from a general lack of consistency and phytotoxicity. In general, it is advisable to stabilize compost before adding it to the soil to promote organic matter breakdown and nutrient mineralization. If not, the soil may not have essential minerals for root assimilation, which would leave a plant with abnormally low nutrition (Bernal et al., 2009). The process of organic matter breakdown and transformation, leading to a decrease in the C/N ratio and diminished phytotoxicity, is associated with the stabilization of organic fertilizers. Since BSF larvae compost quickly (12–15 days), the resulting compost is frequently immature and lacks physiological stability (Setti et al., 2019). It is better that some kind of post-treatment be used to stabilize this product and make it usable as a natural fertilizer for agricultural purposes.

One initial study examining the utilization and stability of Black Soldier Fly Larvae frass as a soil amendment. They measured the development of maize (Zea mays) in soil that had been modified with BSF larvae that had been processing kitchen waste (Alattar et al., 2016). Without disclosing the chemical make-up of the frass, the authors mixed the soil and frass in a weight-for-weight ratio of 1:2 and grew the plants for ten weeks. Although the authors did not test these substances, they claimed that the existence of phytotoxic compounds within the frass caused the observed stunted development of plants. Following studies, the phytotoxicity of BSF larvae frass has been investigated (Xiao et al., 2018). It was determined how much frass the Bacillus subtilis-inoculated chicken manure produced after being treated by BSF larvae. After 13 days of BSF larvae treatment, the fertilizer's phytotoxicity levels gradually dropped when tested on Chinese cabbage and rape seeds, yielding a germination index of more than 66%. By using a seed germination test to compare the effects of decomposing chicken, pig, and cow manure using Black Soldier Fly larvae, authors found that the treatment with chicken manure produced a stabilized frass with lower electrical conductivity and a higher germination index (Liu et al., 2019). The lack of maturity in the frass created from chicken excrement, according to the authors, was caused by the feed substrate's high content of N-NH4+ and strong electrical conductivity. By further composting the waste, the initial high content of N-NH4+ might be lowered (Lopes et al., 2019).

In addition to doing bioassays (such as germination tests), there are other methods for evaluating the stability and behavior of organic fertilizers in soil. The C/N ratio can also provide some insightful information. Fertilizers with a C to N ratio of 20 to 40 are recommended to avoid immobilizing certain mineral nutrients, which could result in less-than-ideal development (Chen et al., 2014). The carbon-to-nitrogen ratio of Black Soldier Fly larvae frass varies notably depending on the diet fed to the larvae, consistently staying below that of the specified feed substrate (Sarpong et al., 2019). For instance, the C to N ratio of domestic biodegradable waste decreased from 48 to 17 during the composting process employing BSFL, while the C to N ratio of the byproducts of almond reduced to 20 from 70 (Palma et al., 2020). To produce a stable, non-phytotoxic frass, the diets provided for Black Soldier Fly larvae growth should maintain a C to N ratio ranging between 15 and 30 (Beesigamukama et al., 2021).

According to Chirere et al. (2021), BSF larval frass compost was post-composted for only four days, which led to Swiss chard growing more quickly than in unfertilized soil and similarly to inorganic NPK fertilizer. Although the C/N ratio of the frass was mentioned by the authors (range from 8.2 to 9.0), neither the fresh frass nor any stability-related properties were measured by the authors, nor was any potential phytotoxicity examined. It was demonstrated that exceeding 10% application rates of fresh frass resulted in inhibited growth and reduced biomass production in B. rapa plants compared to those treated with raw BSFL frass (3 weeks old), naturally composted, and aerated composted frass (both aged 8 weeks). Conversely, applications of up to 40% led to enhanced growth following five weeks of frass composting, underscoring the importance of stabilizing the product prior to soil application. Similarly, frass composted for 112 days underwent evaluation for its efficacy in fertilizing crops such as tomatoes, kale, and French beans in both greenhouse and field settings, alongside other soil amendments including commercial organic and chemical fertilizers. Results indicated that a blend of composted frass and NPK fertilizers demonstrated superior performance compared to untreated soil and soil treated exclusively with frass in terms of plant growth, crop yield, nitrogen absorption, and efficiency in nutrient utilization. Nevertheless, the three tested plant species also exhibited positive responses to the application of composted BSF larvae frass alone. The same combinatorial strategy of blending different composts and NPK fertilizers typically resulted in favorable growth outcomes (Anyega et al., 2021) how the BSFL frass affected pak choi growth (Song et al., 2021).

2.8 Key Parameters for Plant Growth

2.8.1 Acidity and Alkalinity (pH value)

Maintaining ideal pH levels is one of the simplest and most crucial elements in all plant-growing systems. Most vegetable crops thrive best in soil with a pH between 6 and 7. The pH of the soil affects the microbial activity, root growth, and solubility of nutrients. High pH (>7.5) encourages mineral weathering, increased bacterial populations, and an increase in the release of cations, but it also reduces the solubility of salts like carbonates and phosphates (Ncsu.edu, 2023). The availability of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and molybdenum is often decreased at low pH levels (5.5). However, with pH values below 5, the availability of aluminum and manganese can rise to dangerous levels. Low pH levels inhibit essential microbial decomposers' activity, which can significantly slow down the biological conversion of organic matter to nutrients useful for plant growth. Low pH reduces legumes' ability to fix nitrogen. Due to the application of manure over time, many soils become acidic; thus, it is crucial to monitor pH levels and, if necessary, add agricultural limestone in accordance with the recommendations of soil tests. pH ranges between 7 and 8.3 in soils encourage microbial activity but may limit the availability of phosphate, iron, manganese, copper, and zinc (Hartz, 2002).

As shown in **Table 2.2**, the pH value of BSFL frass from food waste ranges from 5.6 in fruit and vegetables to 8.0 in a mixture of food waste, chicken faces, and sawdust (3:2:1); and maize straw substrates. The ideal pH range for encouraging plant growth and providing a favorable habitat for the beneficial bacterial populations in BSFL frass is between 7.0 and 8.0, which is normally the range for BSFL frass (Basri et al., 2022).

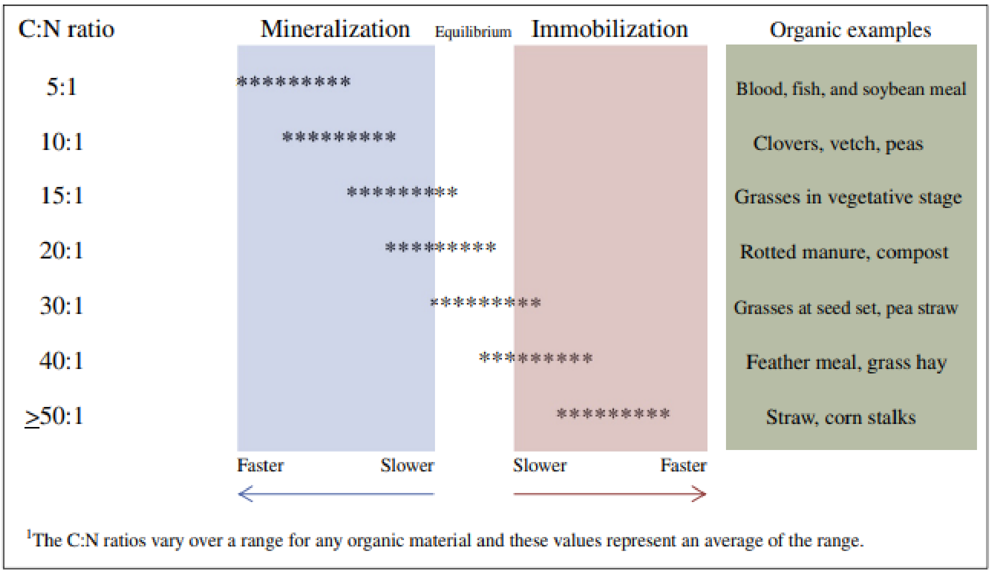
Table 2.2: The physiochemical characteristics of BSFL frass from different food waste types (Basri et al., 2022).

Type of Food Waste	pH	C/N Ratio	Moisture (%)	Temperature (°C)	Total Nitrogen as N (%)	Total Phosphorus as P_2O_5 (%)	Total Potassium as K_2O (%)	References
Kitchen waste	7.0	8:1	50	-	-	-	-	(Ritika, Satyawatiand and Rajendra, 2015)
Kitchen waste	7.4	17:1	63	-	-	-	-	(Liu et al., 2020)
Municipal food waste	7.3–7.4	8:1–9:1	63–65	26.3–26.5	-	-	-	(Sarpong et al., 2019)
Household food waste	7.4	17:1	56	-	2.2	0.1	0.1	(Kawasaki et al., 2020)
Food waste, chicken faeces, and sawdust (3:2:1 ratio)	6.1–8.0	-	72	27.0	1.7	1.1	2.1	(Attiogbe, Yaa and Martey, 2019)
Fruits and vegetables	5.6	27:1	10	-	1.8	-	-	(Klammsteiner et al., 2020)
Maize straw	8.0	-	38	-	0.6	2.5	2.1	(Zeng Liang Gao et al., 2019)

Type of Food Waste	pH	C/N Ratio	Moisture (%)	Temperature (°C)	Total Nitrogen as N (%)	Total Phosphorus as P_2O_5 (%)	Total Potassium as K_2O (%)	References
Okara and wheat bran	7.5	8:1	-	-	4.8	1.0	0.9	(Song et al., 2021)
Okara and wheat bran	7.7	10:1	-	-	3.2	0.8	0.5	(Song et al., 2021)
Brewery spent grain	7.7	17:1	30	-	2.1	1.2	0.2	(Beesigamukama et al., 2020)

2.8.2 Carbon to Nitogen (C/N ratio)

The carbon-to-nitrogen ratio of organic matter refers to the proportion of carbon to nitrogen. In organic matter, carbon constantly predominates over nitrogen. The carbon-to-nitrogen ratio, abbreviated C: N, is typically expressed as a single value (T.C. Flavel and Murphy, 2006). Therefore, a ratio of 20 indicates that the organic matter contains 20 g of carbon and 1 g of nitrogen. When the C:N ratio of an organic substrate is between 1 and 15, N quickly minerals out and is released, making it available for plant uptake. The faster nitrogen is released into the soil for usage by crops, the lower the C:N ratio. Microbial immobilization happens when the C:N ratio exceeds 35. An equilibrium between mineralization and immobilization is reached at a ratio of 20 to 30 (**Figure 2.2**). The C:N ratio of soil microorganisms is around 8. It has been shown that they perform best on a "diet" with a C:N ratio of 24. They must obtain enough carbon and some nitrogen from the soil to sustain that ratio in their cells (Brust, 2019).



[1]The C:N ratios vary over a range for any organic material and these values represent an average of the range.

Figure 2.2: The C : N ratio of some organic material and their mineralization and immobilization rates (Brust, 2019).

2.8.3 Nitrogen, Phosphorus, and Potassium (NPK value)

NPK stands for nitrogen, phosphorus, and potassium, the three essential macronutrients for plant growth. Most fertilizers contain these three nutrients, which are crucial for various phases of plant growth. A fertilizer with an NPK value of 10-5-5 comprises 10% nitrogen, 5% phosphorus, and 5% potassium. Nitrogen is an essential element for photosynthesis and aids in the growth of leaves and stems, giving plants their lovely, deep green hue. In addition, nitrogen contributes to the production of protein, chlorophyll, and nucleic acid in plants, all of which are sources of forages. The soil microorganisms that eventually will benefit the soil itself are also supported by it. Phosphorus is essential for the growth of roots as well as the emergence of flowers and fruits. The all-purpose MVP of nutrients, potassium. It improves the quality of fruits and vegetables, develops the plant's immune system, increases resistance to diseases and pests, and helps control water balance. As an enzyme activator, potassium plays a role in plant metabolism. Lack of potassium will cause an energy shortage, which will result in a poor crop and curled leaf tips, fading veins, or dark or purple patches on leaves. Results of plant growth are significantly influenced by the NPK ratio and rate.

Vascular epiphytes are typically thought to be nutrient-deficient in their native habitats; yet three species of epiphyte bromeliads respond favorably to increased supplies of both N and P independently (Zotz and Asshoff, 2010). For the growth of horticultural crops, nitrates are frequently the preferable supply; yet many horticultural crops are typically harmed by high NH4-N levels. However, ornamental plant growth benefits from the right NO3-N/NH4-N ratio (Stegani et al., 2019).

In epiphytic bromeliads, phosphorus is a limiting nutrient under natural conditions; an increased supply of P significantly improved growth and increased tissue P concentration levels (Zotz and Asshoff, 2010). The epiphytic A. longicaulis demonstrated exceptionally effective P uptake in this investigation, and when given 0.225 mg L^-1 P, the shoot P content increased by 8-fold. N was the most limiting nutrient, as evidenced by the fact that another gesneriad, P. tabacum, growing in the karst zone, had a low N/P ratio (7.6) in the above-ground biomass (Liang et al., 2010).

Fertilisation treatments can enhance plant growth and yield. Plants may compete with one another for different nutrients depending on their availability. Consequently, fertilisation can be a way to supply nutrients that come from the outside environment. A study shows that the fresh production, dry production, and organic matter production of Cichorium intybus can all be increased by applying NPK fertilizer at a rate of 60 kg/ha (Nafiatul Umami, A. Abdiyansah and Agus, 2019). The amount of NPK fertilizer used can be increased to boost Cichorium intybus production.

2.9 Nutrient Enrichment- Industrial biowaste

2.9.1 Palm Oil Fuel Ash

In Malaysia, over 300 palm oil mills are powered by self-generated electricity from palm oil biomass (Hanizam Bt. Awang and Mohammed Zuhear Al-Mulali, 2018). This sustainable energy source not only supports the production of crude palm oil but also supplies electricity to nearby isolated areas. Malaysia, with its vast biomass resources, has the potential to significantly reduce CO2 emissions by utilizing alternative fuels, thereby aligning with its goal of achieving developed nation status without environmental harm. While fossil fuels currently dominate energy generation across Malaysian industries, the shift to burning biomass from palm oil offers a promising solution. However, this practice produces a byproduct called palm oil fuel ash (POFA), which poses environmental challenges. POFA production is expected to increase due to rising energy demand and the expansion of the palm oil sector. Annually, Malaysia and Thailand generate over 3 million and 100,000 tons of POFA, respectively (Hossein Noorvand et al., 2013).

In general, POFA's colour can range from a light grey to a darker shade based on how much carbon it contains. The physical characteristics of POFA are significantly impacted by the operations of the palm oil factory. Within the POFA, oil palm empty fruit bunch waste has the highest number proportion with a total of 23% for each fresh fruit bunch among the various solid waste, refer to **Figure 2.3** (Tjokorde Walmiki Samadhi, Winny Wulandari and Kezia Rembula Tirtabudi, 2020).

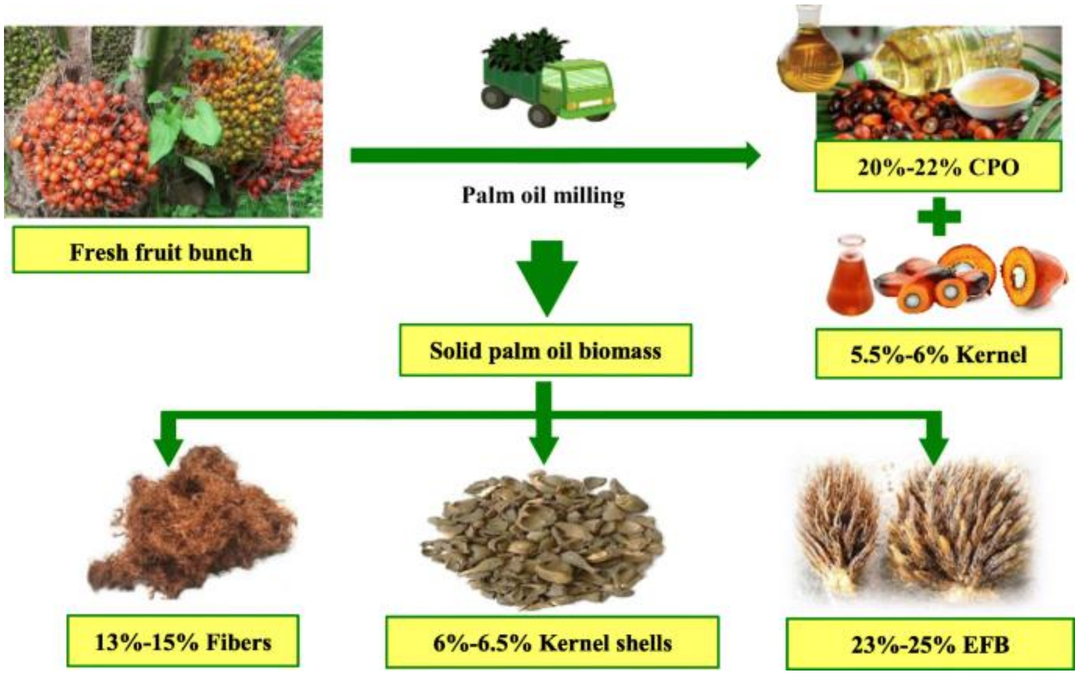


Figure 2.3: Types of waste and its percentage from fresh fruit bunch.

The biomass that is left over after the fresh oil palm fruits have been plucked from the fruit bunch is known as empty oil palm fruit bunches. Globally, palm oil farms generate enormous amounts of EFB, which are frequently disposed of as waste where they naturally decompose or burned to produce more carbon dioxide and methane emissions. One-third of the dry biomass produced by the manufacture of crude palm oil is made up of empty oil palm fruit bunches. Global production of EFB is close to 99 million metric tonnes per year (Geng, 2013). These massive piles of EFB might be absorbed into the soil, reducing emissions caused by its cremation or decomposition. Several studies have discussed the use of EFB that was applied to soils either raw (as organic mulch) or after being pyrolyzed or composted (Anyaoha et al., 2018). EFBs primarily improve soil water retention when used as mulch. It enhanced soil water and nutrient content when applied in the composted form or as biochar, presumably as a result of the C losses from anaerobic respiration during composting or the pyrolysis during biochar manufacture (**Figure 2.4**) (Adu et al., 2022).

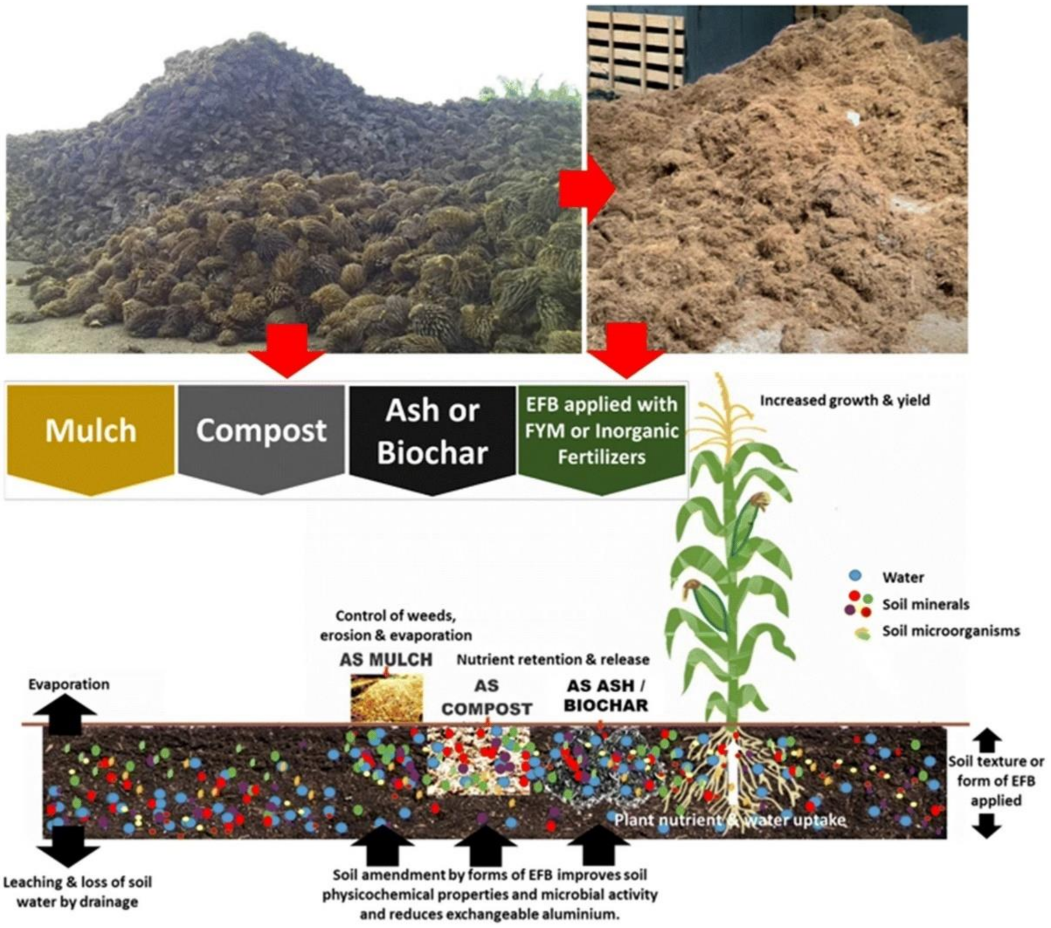


Figure 2.4: Usage of EFB ash.

EFB ash may be a low-cost organic fertilizer since they can improve soil organic carbon (SOC) and other soil chemical properties like pH and exchangeable K (Bakar et al. 2011).

2.9.2 Properties of Ash

Ash has been discovered to be very valuable in a variety of ways, but its increased usage in agriculture is of particular importance because of the high nutrient content for improving the soil and crops. Both gasification and combustion are significant heat conversion processes that produce ash as a byproduct. To create fuel gas, a partial oxidation process known as gasification is performed (Puig-Arnavat et al., 2010). Despite the fact that residue is an important component of the processes and can have an impact on the systems' overall performance, most studies on the gasification and combustion of FFB solid wastes concentrated on the fuel value with little attention paid to optimizing the yield of the residue for agricultural purposes (for example, soil improvement, which in turn affects crop growth and yield).

2.9.3 Fertilizer Value in Empty Fruit Bunch

According to the proximate analysis, the composition of EFB's ash contains 55.48% potassium oxide (K2O) which has a relatively high potassium content (Arfiana et al., 2021). Since potassium is one of the key ingredients in NPK fertilizer, which is also a macronutrient required by red onion plants, the high potassium content in EFB ash can be used to synthesize NPK fertilizer. Because the plants have shallow, sparsely branched roots, red onions require an intensive supply of nitrogen (N), phosphate (P), and potassium (K) to produce the greatest number of bulbs. In order to solve the issue of EFB solid waste in a palm oil mill and provide an alternative source of raw materials for the synthesis of NPK fertilizer, it may be possible to use EFB ash as a raw material for fertilizer.

From the experiment, the NPK fertilizer was created using a variety of ingredients, such as urea fertilizer to meet the nitrogen requirement, diammonium phosphate (DAP) to meet the phosphate requirement, potassium rich EFB ash, potassium chloride (KCl) to support the potassium requirement, zeolite as a matrix, micronutrient Mg from magnesium sulphate fertilizer and S from ZA fertilizer, as well as molasses as a binding agent (Arfiana et al., 2021). The yield of the red onion crop employing NPK ash fertilizer is then compared to the yield using commercial NPK, NPK char fertilizer, and no fertilizer treatments. **Figure 2.5** displays a comparison of agricultural yields.

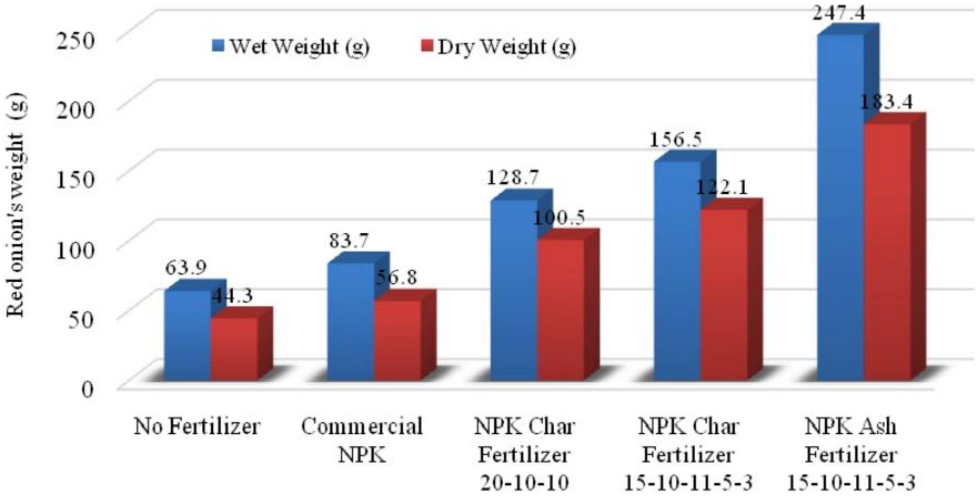


Figure 2.5: Yield of Red Onion comparison between char fertilizer and NPK ash fertilizer.

Compared to NPK char fertilizer, the usage of NPK ash fertilizer exhibited a more pronounced increase in red onion products to over 50%. In comparison to NPK char fertilizer without Mg and S micronutrient, it more than doubled, and in comparison, to no fertilizer treatment, it more than tripled (Arfiana et al., 2021). The release of nutrients occurs gradually with NPK ash fertilizer. Additionally, EFB ash increases soil quality by lessening soil acidity and enhancing the chemical characteristics of the soil, which in turn improves plant development.

CHAPTER 3

METHODOLOGY

3.1 Experiment Flow

A summary of the experiment flow is depicted in **Figure 3.1**.

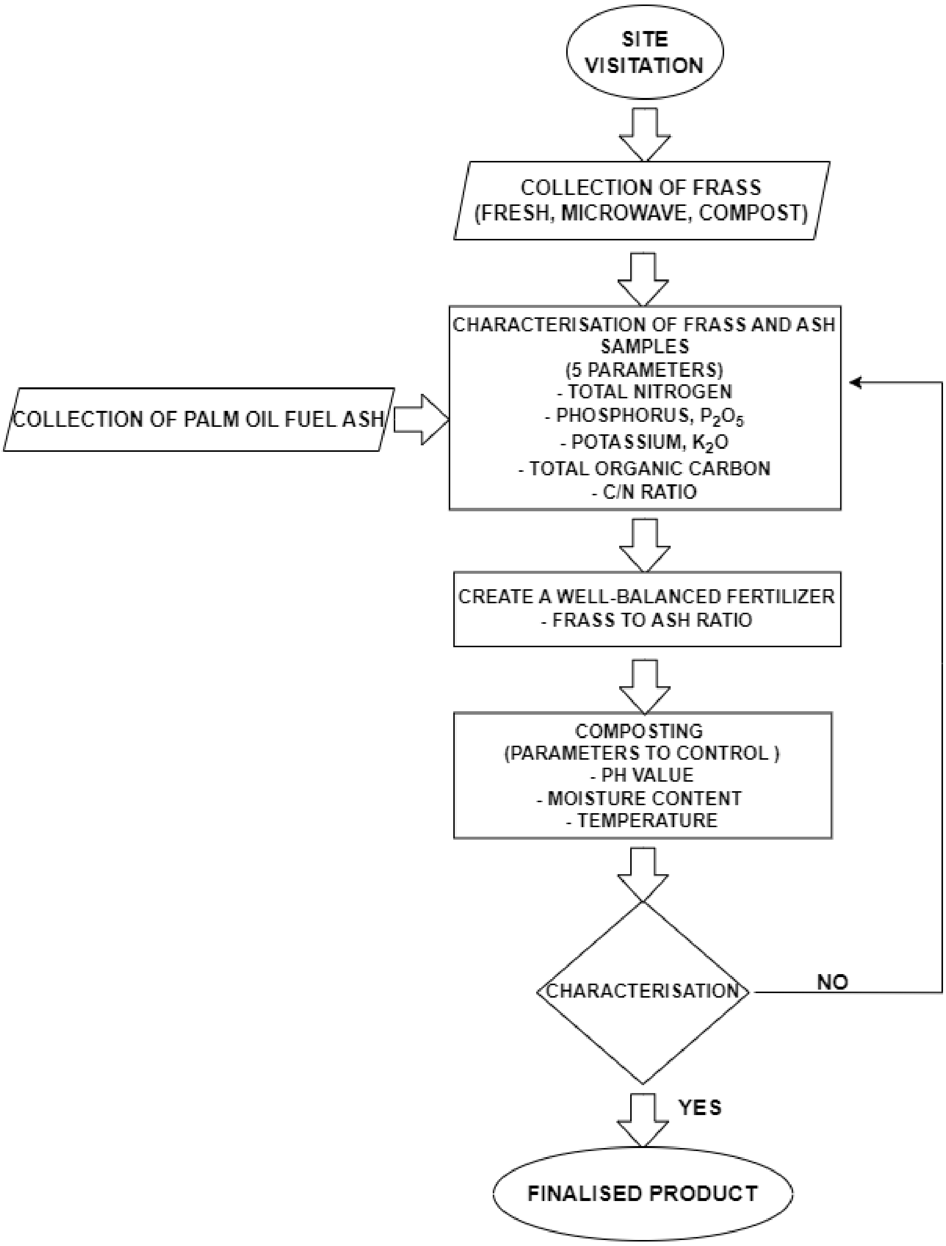


Figure 3.1: Experiment Procedures.

3.2 Company Description and Site Location

BioLoop - A Malaysia company, located at Lot 533, Bt 16 Jalan Bidor, Kg Air Hitam, 36030 Teluk Intan (**Figure 3.2**). The company's missions include revolutionizing organic waste management and advancing the field of biotechnology. They contend that the conventional approach to waste management needs to change fundamentally.

By applying biotechnology and Black Soldier Fly Larvae (BSFL), they can divert large amounts of organic waste where it just downgrades and emits carbon dioxide from landfills previously and upcycle the organic waste into value-adding products. Compared to conventional methods, this procedure is much faster, produces fewer greenhouse gas emissions, and is more sustainable. The second is feeding the world sustainably. The founders' shared goal is to successfully market high-quality, organic, and sustainable produce. For the pet food, aquaculture, and poultry industries, BSFL is a wholesome animal feed source. Besides, BSFL feces (frass) is nutrient-rich and doubles as compost, both of which improve the health of the soil.

Teluk Intan is a strategic location for the business's daily operation because it is surrounded by palm oil mills, from which the company can source a plentiful supply of palm oil waste, a key feedstock for BSF larvae. Our project involved the evaluation of three distinct types of frasses - fresh, compostable, and microwave-generated. We meticulously assessed these samples for various parameters, including pH, nitrogen, total phosphorus, total carbon, and potassium.

Figure 3.2: Location of BioLoop Sdn Bhd

3.3 Characterization Stage

3.3.1 Preparation of Air-dried sample

Prior to sending frass samples to the laboratory for analysis, it is essential to air-dry them. This crucial step halts ion exchange processes and soil microbial activity, ensuring the stability of the sample. Air-drying also facilitates a reduction in sample mass, optimizing the shipping process. The recommended procedure involves air-drying the sample within a temperature range of 25 to 35°C. This controlled drying environment helps maintain the integrity of the sample while ensuring its suitability for analysis. Furthermore, it is advisable to powder the frass before submission, enabling it to pass through a 2 mm filter post-drying (refer to **Table 3.1**). This additional preparation step enhances sample homogeneity and accuracy of results, thereby improving the overall analysis process. For all conventional analyses, a dried sample weighing 50 grammes is adequate (Osu.edu, 2023).

Table 3.1: Soil analyses and minimum sample weights for each parameter test.

Analysis	Minimum Sample Weight, (g)	Sieve Size, (mm)
Standard Analysis	15.0	2
Total N and C	5.0	2
Organic Matter	10.0	2
Soil extraction:		
• **Normal Ammonium Acetate**	2.0	2
• **Available Phosphorus**	2.0	2
• **Mehlich 3**	5.0	2

The frass samples, including fresh, composted, and microwave-dried frass, underwent a preliminary airing process to prevent any chemical alterations and ensure accurate analysis. This involved spreading the samples thinly within labeled plastic containers and placing them in a cool, dry environment to prevent cross-contamination. Over the course of several days, the samples were left uncovered to air-dry completely (**Figure 3.3**). This methodical approach allowed for the cessation of any ongoing chemical reactions, thereby preserving the integrity of the samples for subsequent analysis. Before shipment to the laboratory, the dried samples were securely covered to maintain their condition and prevent any potential contamination during transit. This meticulous handling protocol was crucial to ensure the reliability and accuracy of the analytical results. All the frass samples in the laboratory will be weighed using the AUX320 Analytical Balance (**Figure 3.4**) (Shimadzu, Japan).

Figure 3.3: Preparation of air-dried frass sample prior laboratory testing.

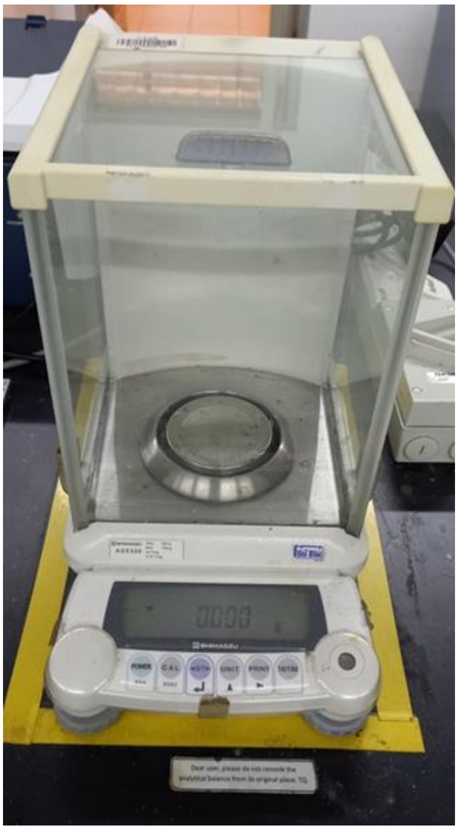


Figure 3.4: AUX320 Analytical Balance (Shimadzu, Japan)

3.3.2 pH Standard Liquid Preparation and Measurement

The pH value of the frass samples was tested by using the pH sensor in TYP series V13.0.1 (**Figure 3.5**). The measuring range is 1 to 14 with tolerance 0.1. Prior to sending frass samples.

Following the instructions in the Surechem Instruction's manual, a standard buffer with a pH of 4.00 is dissolved in a 50 mL beaker by adding distilled water. The resulting solution is then carefully transferred into a 250 mL volumetric flask and diluted with water until the volume reaches 250 mL. This process is repeated to prepare a standard solution with a pH of 9.18. These solutions are essential for calibrating pH meters and ensuring accurate measurements. The purpose of testing the acidity of the frass samples is to determine the appropriate reagent for analyzing their phosphorus content. Approximately 10g of each sample is placed into a 50 mL beaker and mixed with 25 mL of water. After stirring for 2 minutes, the mixture is allowed to settle for 30 minutes. This settling period allows any insoluble particles to separate, ensuring that subsequent tests are accurate and reliable.

Figure 3.5: pH sensor in TPY series V13.0.1 (Nanbeiinstrument, China).

3.3.3 Total Nitrogen

The three parameters, total nitrogen, total phosphorus, and potassium will be tested using NPK analyzer (TYP series V13.0.1) (**Figure 3.6**). For total nitrogen, three spoons of frass samples, each weighing approximately 4 grams, were collected and transferred into a labelled bottle marked as "Frass sample 1, 2, and 3". Using a syringe, 20 milliliters of water were injected into the bottle. About 1 gram of NO. 1 powder was then added using a spoon. The bottle was capped and vigorously shaken for a duration of 10 minutes. Once shaken, the mixture was filtered to obtain the N sample liquid.

For the blank liquid, two-thirds of water were carefully poured into a clean glass cuvette using a fresh pipette. As for the standard liquid, 900 microliters of water were added into a separate glass cuvette using a pipette. The "N standard liquid" was retrieved from the designated black reagent box and 100 microliters of it were added to the cuvette containing water, then thoroughly mixed. This standard liquid held a concentration of 20 mg/kg (20 ppm). Next, 1000 microliters of the previously obtained

N sample liquid were transferred into another glass cuvette. For both the standard and sample liquid cuvettes, 100 microliters of N1 reagent were separately introduced, shaken, followed by the addition, and shaking of 100 microliters of N2 reagent. These mixtures were allowed to rest for 10 minutes, and subsequently, 800 microliters of water were added to each cuvette. After a final shake, they were promptly placed into the testing channels of the instrument, ensuring this was done within 10 seconds. lines.

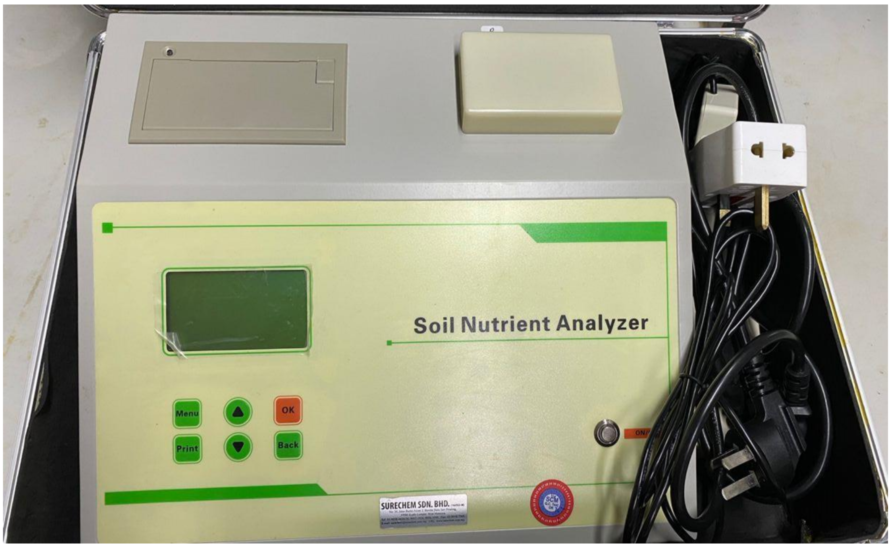


Figure 3.6: Soil nutrient analyzer TPY series V13.0.1 (Nanbei instrument, China).

3.3.4 Total Phosphorus – P_2O_5

For phosphorus the procedures are almost the same, three spoon of soil sample, each weighing 4 grams, were taken and placed into an empty white bottle within the reagent setup. Using an injector, 20 ml of water was added to the sample. Subsequently, 0.5 grams of NO.2 powder were added. The bottle was sealed, and the contents were vigorously shaken for 10 minutes. Note that for frass samples identified as acid oil, the addition of NO.2 powder was unnecessary; only 500 microliters of NO.3 powder needed to be added.

Following filtration, the P sample liquid was obtained. For the blank liquid, water was added to fill two-thirds of the first glass cuvette using a clean pipette. In the case of the standard solution, 900 microliters of water were introduced into the second glass cuvette with a clean pipette. The "P standard liquid" was obtained, and 100 microliters were added to the cuvette, subsequently mixed thoroughly. The concentration of the standard liquid was 20 mg per Kg (20 ppm). To create the sample liquid, a volume of P sample liquid along with 800 microliters of water were combined in the third glass cuvette. Moving on, 100 microliters of P1 reagent and 800 microliters of water were added separately to the second and third glass cuvettes containing the standard liquid and sample liquid. These mixtures were shaken well. An additional 50 microliters of P2 reagent were introduced in the same manner, followed by thorough shaking, after which they were immediately placed into the testing channel of the instrument within seconds.

3.3.5 Total Potassium – K_2O

Three spoon of soil sample, each weighing 4 grams, were obtained, and placed into an empty white bottle within the reagent box. An injector was used to add 20 ml of water to the soil sample. Next, 1 gram of NO.1 powder was added to the mixture. The bottle was sealed, and its contents were vigorously shaken for a period of 10 minutes. After shaking, the mixture was filtered to yield the K sample liquid.

For the blank liquid, water was added to fill two-thirds of the first glass cuvette using a clean pipette. In the case of the standard solution, 900 microliters of water were introduced into the second glass cuvette using a clean pipette. The "K standard liquid" was prepared by adding 100 microliters into the cuvette and then thoroughly mixing it. The concentration of the standard liquid was 100 mg/kg (100 ppm). To create the sample liquid, 1000 microliters of K sample liquid were pipetted into the third glass cuvette. Following this, 100 microliters of K1 reagent were respectively added to the second and third glass cuvettes containing the standard liquid and sample liquid. These mixtures were shaken vigorously. Subsequently, 100 microliters of K2 reagent were introduced to both cuvettes in the same manner. After shaking, the mixtures were left to rest for 5 minutes. Then, 800 microliters of water were added, the cuvettes were shaken again, and the contents were promptly placed into the testing channel of the instrument within 10 seconds. seconds.

3.3.6 Total Organic Carbon

The testing of the Total Organic Carbon (TOC) content of each frass and POFA sample was outsourced to a reputable laboratory outside for accuracy and precision. This meticulous approach ensures reliable results and allows for confident decisions regarding the blending of frass and POFA to achieve the desired composting outcomes.

To ensure the accuracy and reliability of all results, the NPK (nitrogen, phosphorus, and potassium) values of each sample were subjected to thorough testing once more by a designated laboratory. This meticulous verification process helps confirm the integrity of the data and provides a solid foundation for informed decision-making regarding compost blending and management strategies.

3.4 Post-treatment – Mixing and Composting Stage

3.4.1 Ideal Blended C/N Ratio Calculation for Mixing Compost

Organic matter comprises a significant amount of carbon (C) and a smaller proportion of nitrogen (N). This balance between carbon and nitrogen within an organism is referred to as the carbon-to-nitrogen ratio (C:N ratio). Maintaining the appropriate ratio is crucial for optimal composting, as it provides the necessary energy for composting microorganisms and facilitates protein production. Compost scientists have extensively studied this ratio and determined that the most efficient method for producing high-quality compost is to maintain a C:N ratio of approximately 25 to 30 parts carbon to 1 part nitrogen, denoted as 25-30:1. Deviating from this ratio can have adverse effects: a high C:N ratio, indicating excess carbon, leads to a slowdown in decomposition, while a low ratio, signaling excess nitrogen, results in malodorous compost (Planet Natural, 2023). Therefore, achieving and maintaining the ideal C:N ratio is essential for generating fertile, odor-free compost.

Numerous composting ingredients, including frass and palm oil fuel ash, inherently lack the optimal 25-30:1 carbon-to-nitrogen (C:N) ratio. Thus, creating an ideal compost mixture often involves blending various components to achieve what can be considered the "perfect compost recipe." The process of "recipe making" involves combining raw feedstock in appropriate amounts for composting.

When faced with high C:N ratios, characterized by excess carbon, remedies include incorporating materials such as grass clippings or manures, which are rich in nitrogen. Conversely, to address low C:N ratios, indicating an abundance of nitrogen, additions of paper, dry leaves, or wood chips can help balance the composition. Through thoughtful selection and blending of ingredients, composters can adjust the C:N ratio to create an environment conducive to efficient decomposition and the production of high-quality compost. Additionally, achieving the ideal carbon-to-nitrogen (C/N) ratio in compost can be accomplished by carefully distributing the appropriate proportion of each ingredient. This can be achieved through blended C/N ratio calculations, ensuring that the overall mixture aligns with the desired ratio for optimal composting.

In this experiment, only raw frass will be used for composting. Frass and ash are designated as two essential blending ingredients aimed at formulating an optimal compost recipe. Frass acts as the primary nitrogen source, complemented by ash, which serves as the carbon component. However, to attain the desired C/N ratio of the compost, precise calculations are required to determine the appropriate proportions of each sample for mixing. The calculations will be made using formula listed in **Eq 3.1** (Dougherty, 1999).

Assume 1kg dry matter carbon and nitrogen content for both frass and ash:

$$Desired\ C\ to\ N\ ratio = \frac{CF + CAX}{NF + NAX} \tag{3.1}$$

where,

C_F = Carbon mass in frass

C_A = Carbon mass in ash

N_F = Nitrogen mass in frass

N_A = Nitrogen mass in ash

X = Ash to frass proportion

3.4.2 Composting

The natural process of organic composting breaks down a pile of kitchen leftovers, yard garbage and other wastes. In the composting process, these substances are referred to as feedstocks. To start the decomposition process, the feedstocks are combined in a composting system, or pile. While there are several requirements, the process relies on one thing: the bacteria that carry out the breakdown process. Through their position of strength in numbers, these single-celled organisms convert your compostable trash into the rich humus that forms the basis of all excellent growing soils (Planet Natural, 2023).

Composting bacteria can be categorized into two main groups: anaerobes (**Eq 3.1**), which operate in environments devoid of oxygen, and aerobes (**Eq 3.2**), which thrive in oxygen-rich environments. The method of composting chosen will dictate the which type of bacteria to flourish. Sealed-container anaerobic composting favors anaerobic bacteria, while ventilated aerobic composting, such as in piles, heaps, and tumblers, promotes the growth of aerobic bacteria. efficiency (Planet Natural, 2023).

Anaerobic Composting:

$$Organic\ materials + water = Carbon\ dioxide + methane + hydrogen\ sulfide + energy \qquad (3.2)$$

Aerobic Composting:

$$Organic\ materials + oxygen + water = Carbon\ dioxide + water + energy \qquad (3.3)$$

3.4.3 Method of Composting

Selecting the appropriate composting method is crucial for the success of a composting program, as different methods have distinct requirements regarding labor, energy, land use, time, and volume of materials. By carefully considering these factors, composters can optimize their composting practices to align with their specific goals and resources, ultimately enhancing the efficiency and effectiveness of their composting efforts.

In this experiment, a static pile or bin with a passive aeration composting method was used. Passively aerated static piles, whether freestanding or contained in bins, offer a practical solution for processing smaller quantities of compostable materials, suitable for home, community, and on-site composting endeavors. These piles rely on natural air circulation rather than mechanical aeration to maintain ideal temperature and oxygen levels. Passive aeration occurs as warm air, generated by microbial activity, rises within the pile, while cooler air is drawn in through its sides and bottom, a phenomenon known as the "chimney effect." When blending organic materials within the pile, it's essential to ensure sufficient airspace throughout to facilitate aeration. Incorporating bulking agents like wood chips creates a less compact pile, enhancing oxygen flow. Additionally, periodically turning the pile promotes uniform decomposition.

3.4.4 Compost Pile Building and Parameters to Control

The passive aerated static pile which also known as controlled aerobic composting is a methodical process that transforms organic materials into a nutrient-rich soil amendment or mulch. This process relies on the presence of oxygen for efficient decomposition. Besides, composting necessitates a precise balance of carbon-to-nitrogen ratios in the added materials, alongside sufficient oxygen and moisture. These conditions foster thriving microorganisms responsible for digesting and decomposing organic matter. The composition of the compost pile itself is pivotal to the process, with factors such as particle size and pile density influencing oxygen and moisture levels. Throughout decomposition, the pile's temperature undergoes fluctuations, initially rising before gradually declining. This natural progression yields compost, a nutrient-rich, dark, crumbly substance with an earthy aroma.

During composting, five essential areas need to be managed for optimal results. Feedstock and nutritional balance are the first. A correct ratio of "green" and "brown" organic elements is necessary for composting, or controlled breakdown. Grass clippings, leftover food, and manure are examples of "green" organic materials that are high in nitrogen (US EPA, 2015). Dry leaves, wood chips, and branches are examples of "brown" organic materials because they are high in carbon but low in nitrogen. It takes time and experimentation to get the ideal nutrient combination. It is a component of the science and art of composting. In my case, the frass followed by a small number of citrus peels serves as the green materials while the palm oil fuel ash, shredded newspaper, and dried leaves will be the brown materials (**Figure 3.7**). The amount of frass and pofa is allocated according to the blending C/N ratio calculations. Additionally, the particle size of these materials plays a crucial role, as grinding, chipping, or shredding them increases the surface area for microorganisms to thrive. Smaller particles foster a more homogeneous compost mixture and enhance insulation, crucial for maintaining optimum temperatures. However, excessively fine particles may hinder airflow. So, the newspaper and dried leaves were shredded in a medium size which is larger than the pile’s particles but smaller than the original size.

Figure 3.7: Brown and green materials in the compost bin.

Adequate moisture content is essential since microorganisms within the compost rely on water for survival and to transport substances, which can come from organic materials or external sources like rainfall. The ideal moisture range for effective composting falls between 45% and 60%. Moisture levels exceeding 65% can lead to anaerobic conditions, where aerobic organisms perish due to oxygen depletion, allowing anaerobic organisms to dominate and emit a pungent ammonia odor. Conversely, moisture levels below 40% encourage fungal dominance, impeding full decomposition and hindering the production of quality compost. Once a pile becomes excessively dry, rehydration becomes challenging, potentially resulting in increased dust production. Should moisture levels plummet below 35%, biological activity ceases altogether, emphasizing the critical importance of maintaining appropriate moisture levels throughout the composting process (Lsuagcenter, 2022).

Besides, proper oxygen flow, facilitated by turning the pile or incorporating bulking agents, is vital for aeration, as it accelerates decomposition and prevents anaerobic conditions. Lastly, maintaining the right temperature range is critical, as it encourages rapid composting, pathogen and weed seed destruction, and microbial activity. In my case, the compost's moisture content, temperature, and pH value are monitored using a soil meter tester twice weekly to ensure the compost is in the right condition. (**Figure 3.8**).

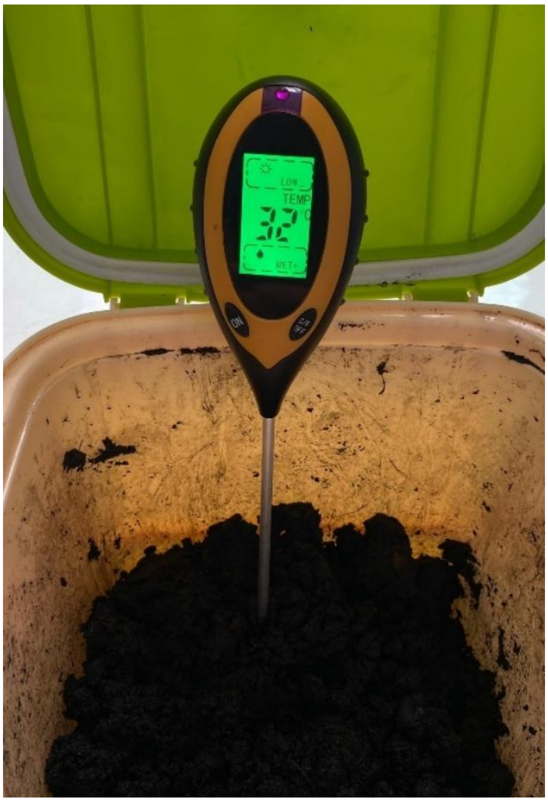


Figure 3.8: Measurement of temperature and moisture content of the compost.

For constructing the compost pile in this experiment, the traditional layer method was employed. Initially, a base layer of brown materials, including shredded newspaper and dried leaves, was evenly spread at the bottom of the compost bin. Following this, the measured quantity of frass was added on top, followed by a layer of palm oil fuel ash. Additionally, green materials, such as citrus peels, were incorporated onto the compost pile. This layering technique facilitates the creation of a balanced compost mixture, ensuring adequate carbon-to-nitrogen ratios and promoting efficient decomposition. Lastly, add some water to moisten the compost to encourage microbial activity.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 BioLoop- Field Observation

BioLoop, a leading biotechnology firm, is revolutionizing the management of organic waste, generating sustainable insect protein for animal consumption, and manufacturing natural fertilizer. The company is currently operating a Black Soldier Fly (BSF) facility in Perak, with a daily operational capacity of 20 metric tons of organic waste. To manage such a substantial workload efficiently, BioLoop has introduced the BioLoop intelligent feeding system (BIFS) (**Figure 4.1**). This innovative system integrates a robotic arm, an automated feeder, and conveyor belts to oversee four key functions: depalletizing, harvesting, feeding, and palletizing. Without the BIFS, the daily operations would involve manually shifting 20,000 kilograms of various organic waste materials, presenting significant physical challenges.

Figure 4.1: BioLoop Intelligent Feeding System (BIFS).

Moreover, BioLoop prides itself on its world-class breeding system, meticulously designed to prioritize the propagation of the BSF colony. This system ensures optimal fly size, mating frequency, and egg yield, which are critical for supporting downstream bioconversion processes. Equipped with advanced climate control systems, modular cages for seamless scalability, and specialized lighting emitting specific wavelengths to promote mating, BioLoop maintains direct control over its daily egg harvest to meet desired production targets.

4.1.1 BSFL Recovery products

4.1.2 BSF Larvae- An alternative protein source

In accordance with a methodical sequence of procedures encompassing harvesting, feeding, pelletizing, heating, and extraction, the company introduces four sustainable products to the market: whole dried Black Soldier Fly (BSF) larvae, defatted BSF larvae, BSF larvae oil, and frass. The whole dried BSF larvae exhibit an approximate composition of 40% crude protein, 25% crude fat, 16% total carbohydrate, and a gross energy value of 4400 kcal/kg. Conversely, the defatted BSF larvae meal boasts a crude protein content exceeding 50%, positioning it as an exceptional source of high-quality protein for animal feed. With a low crude fat content of less than 10%, it stands out as an ideal feed ingredient for animals necessitating a low-fat diet. The nutrient analyses are outlined in **Table 4.1**. Additionally, the defatted BSF larvae meal showcases less than 13% crude fiber, less than 2% moisture content, and a substantial crude energy content of 370 kcal/100g. As a high-protein, low-fat, and high-energy feed ingredient, defatted BSF larvae meal is the result of meticulous fat removal from BSF larvae, aiming to enhance protein content and reduce fat levels. However, the characteristics of substrates significantly influence the composition of BSF larvae.

Table 4.1: Nutritional content of BioLoop's BSF larvae.

Nutritional content	Whole Dried BSFL	Defatted BSFL
Crude protein (%)	40	>50
Crude fat (%)	25	<10
Total carbohydrate (%)	16	-
Gross energy vale (kcal/kg)	4400	3700

4.1.3 BSFL Frass- An alternative fertilizer

Beyond the recovery of protein in Black Soldier Fly (BSF) larvae products, the company is dedicated to nutrient reclamation through the production of frass. Frass, derived from the nutrient-rich excrement of BSFL, comprises approximately 70% organic matter, hosting beneficial microbes and bacteria crucial for optimal plant growth. Serving as a potent soil conditioner, BSFL frass enhances nutrient absorption, thereby promoting overall crop health, akin to traditional compost. BioLoop's frass products boast an impressive nutrient profile, containing over 3% nitrogen, more than 4% phosphorus, and approximately 0.5% potassium, making them invaluable resources for sustainable agricultural practices. Compared to a commercial organic fertilizer with a balanced NPK value of 2.3% (**Table 4.2**), the fertilizer value of frass surpasses it, suggesting its potential to replace conventional fertilizers.

Demonstrating their commitment to sustainable practices, BioLoop has effectively managed over 3500 metric tons of organic waste. Through this initiative, the company has successfully produced and sold over 1200 metric tons of frass, while also yielding 40 metric tons of insect proteins. These accomplishments underscore the company's dedication not only to waste management but also to the extraction of valuable resources, fostering a sustainable approach to resource recovery and utilization.

Table 4.2: Comparing frass nutrient profile over commercial fertilizer.

Fertilizer Content	**Analyst Result**	
	BioLoop's Frass (BioLoop, 2016)	**Commercial Organic Fertilizer** (Hamid et al., 2019)
Nitrogen	>3%	2.3%
Phosphorus	>4%	2.3%
Potassium	>0.5%	2.3%
Organic matter	>70%	-
pH Value	6-8	-
C:N ratio	12:1	-

4.2 Issues with the Raw Frass as Organic Fertilizer Replacement

A high-quality fertilizer typically originates from stable and mature compost. However, immediately after Black Soldier Fly Larvae (BSFL) rearing, frass exhibits high biological activity (Ivã Guidini Lopes, Jean Wh Yong, and Lalander, 2022). Biodynamic instability is a result of the rapid composting process, and potentially phytotoxic compounds present in BSF larvae frass (Nasir and Jean, 2021). In BioLoop's BSF facility, raw frass, sieved from the machine, is promptly piled for packaging without undergoing any post-treatment or secondary processing (**Figure**

4.2). Subsequently, the frass is dispatched to customers once a designated quantity has been accumulated. However, it's important to note that in this state, the raw frass remains unstable and immature, potentially impacting its effectiveness as a soil amendment.

Additionally, based on the macronutrient analysis result of the frass provided by the company (**Table 4.2**), the C/N ratio is 12:1, which is relatively low and may lead to nitrogen toxicity (Mortier, F. Velghe, and S. Verstichel, 2016). When an organic substrate has a C: N ratio between 1 and 15, rapid mineralization and release of nitrogen occur (Brust, 2019). Therefore, in BioLoop, before the frass was sent to packaging, it had not undergone any standard post-treatment like thermophilic composting. Besides, the issues with the low C/N ratio of the raw frass need to be addressed. It could be done by mixing it with another biowaste that contains high carbon content such as palm oil fuel ash to achieve an ideal blending C/N ratio of 25:1, enhance matrix stabilization and boost the efficacy of the combined fertilizer.

Figure 4.2: BioLoop’s BSFL frass.

4.3 Characterization of BSFL Frass and Palm Oil Fuel Ash

Frass samples (raw and microwave-treated) and ash samples were collected from the BSF facility at Teluk Intan and the palm oil factory at Air Kuning, respectively. These samples were characterized using the previously outlined methodology. Additionally, they were sent to BVAQ PERMULAB in Petaling Jaya for secondary verification. Discrepancies between our own results and those provided by the company were significant, prompting us to rely on the values obtained from BVAQ due to their higher accuracy. These results are presented in **Table 4.3**. For further details, refer to the certificates of analysis in **Appendix A**.

Table 4.3: Characterization of frass and ash samples.

Parameter	**Type of Sample**			
	Raw Frass (BioLoop)	**Raw Frass (BVAQ)**	**Microwave Frass**	**Palm Oil Fuel Ash**
Total Organic Carbon (%)	36.00	42.70	45.40	17.30
Total Nitrogen, N (%)	3.00	3.53	3.95	0.23
Phosphorus, P_2O_5 (%)	4.00	1.13	1.71	2.96
Total Potassium, K_2O (%)	0.5	3.03	3.96	3.11
Carbon: Nitrogen Ratio	12:1	**12:1**	**11:1**	75:1

The analysis results of the raw frass conducted by BVAQ revealed discrepancies in the NPK values compared to those provided by the company (**Figure 4.3**). This inconsistency suggests that the NPK values of the frass product vary due to the absence of a standardized post-treatment process on the frass. Given this finding, it is imperative to subject the frass to further treatment, such as composting, before packaging and sale to farmers. This additional step ensures the uniformity and reliability of the NPK values in the frass product, thereby enhancing its effectiveness as a soil amendment.

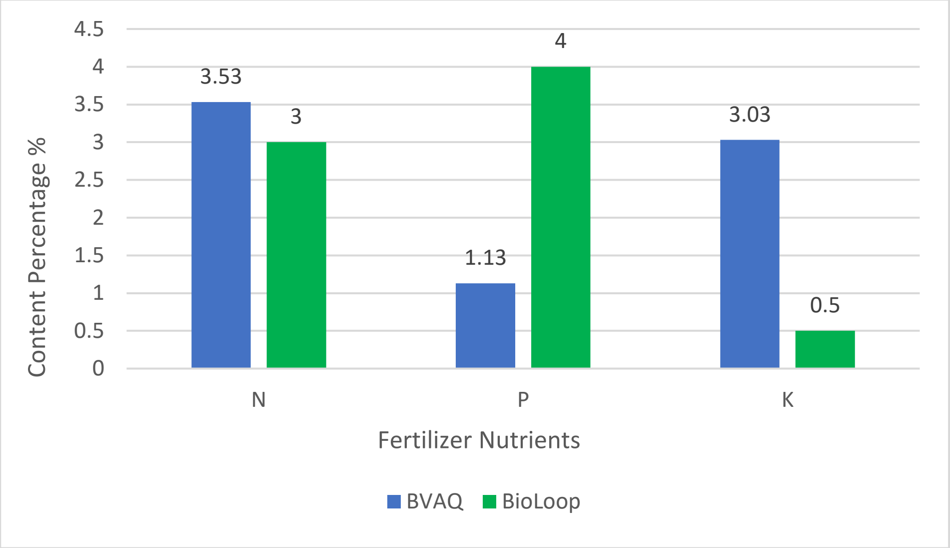


Figure 4.3: Comparing the nutrient analyses of BVAQ over BioLoop.

Furthermore, the analysis revealed that the NPK value of the microwave frass is slightly higher compared to the raw frass (**Figure 4.4**). This suggests that microwave frass exhibits a greater nutrient content and possesses a more favorable odor profile compared to its raw counterpart. Microwave frass undergoes a secondary treatment process involving heating and drying, which contributes to its improved smell and potentially enhanced nutrient availability. During the microwave treatment, volatile compounds responsible for unpleasant odors are often eliminated or reduced, resulting in a more pleasant aroma. This improvement in odor quality can enhance the acceptability and usability of microwave frass as a soil amendment. Despite these advantages, it is noteworthy that the C/N ratio of microwave frass remains relatively low, primarily due to its elevated nitrogen content. While this characteristic may facilitate rapid mineralization, caution should be exercised regarding its direct application on plants, as excessively high nitrogen levels can potentially lead to nutrient imbalances and affect plant health. Therefore, additional considerations and adjustments may be necessary to optimize the use of microwave frass as a soil amendment.

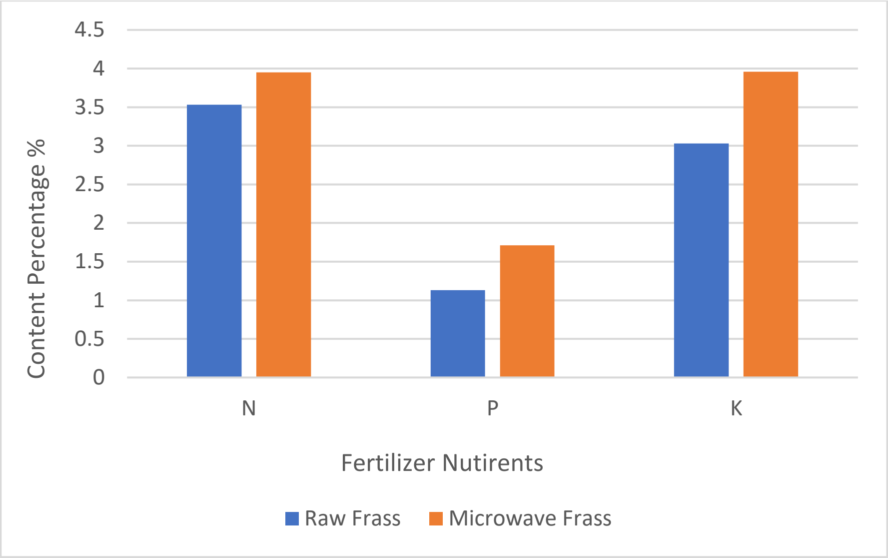


Figure 4.4: Comparing NPK value of Raw frass over Microwave frass.

Based on the observations above, it's evident that both raw and microwave frass have low C/N ratios. This suggests that further combinations are necessary to optimize the composting process and achieve the desired nutrient balance. To enhance the matrix stabilization and fertilizer nutrient content of the raw frass, it can be blended with palm oil fuel ash (POFA) and subjected to further composting, thereby increasing its overall fertilizer effectiveness. However, before proceeding, it is crucial to characterize the POFA to understand its nutrient profile accurately. Subsequently, a suitable proportion for each ingredient can be formulated based on the desired nutrient profile. As indicated in **Table 4.3**, the palm oil fuel ash exhibits the highest C/N ratio (75:1) among the samples analyzed. Palm oil fuel ash (POFA) has a high carbon content due to the organic residue present in the ash, which is a byproduct of burning oil palm shells at high temperatures (Syaizul, Abdul Karim Mirasa and Hidayati Asrah, 2015).

POFA, a by-product of the palm oil industry, is obtained through the incineration of various waste materials, including palm oil husks, fibers, and shells. During the incineration process, biomass is burned at temperatures ranging from 800 to over 1000°C, resulting in the production of POFA with a dark grey to black hue (**Figure 4.5**) (Alsubari, et al., 2016). This coloration is primarily attributed to significant levels of unburned carbon present in the ash (A.S.M. Abdul Awal and M.

Warid Hussin, 2011). Incorporating POFA into the compost mixture can contribute additional carbon content, aiding in the stabilization of the matrix and providing supplementary nutrients for plant growth.

Figure 4.5: Palm Oil fuel ash from Air Kuning palm oil factory.

However, on the flip side, the palm oil fuel ash (POFA) contains significant amounts of key fertilizer macronutrients such as phosphorus (P) and potassium (K), with impressive values of nearly 3% for each (**Table 4.3**). The high potassium content in POFA is primarily attributed to the presence of empty fruit bunches (EFBs), a prominent waste product of the oil palm industry. For every ton of fresh fruit bunch, approximately 23% of EFBs are generated, making it the largest contributor among all waste materials (Samadhi et al, 2020). Moreover, based on proximate analysis, the composition of EFB ash contains 55.48% potassium oxide (K2O), making it well-suited for fertilizer synthesis (Arfiana et al., 2021). This rich nutrient content highlights the potential of POFA as a valuable resource for enhancing soil fertility and promoting plant growth.

4.3.1 Mixing ratio of BSFL Frass and Ash for composting

In the process of creating composts, materials with high and low C:N ratios are combined to create an ideal C:N ratio of about 24:1 for soil microbes (SDSU Extension, 2024). In this experiment, the frass that was used for mixing and compost is the raw frass but not the microwave frass. The mixing proportion of frass and POFA components to achieve a desired blended C/N ratio for composting is determined using **Eq 3.1** (Dougherty, 1999). Firstly, the carbon and nitrogen content (dry weight) of each component are assessed. According to the analysis conducted by BVAQ (refer to Table 4.3), for 1 kg of frass, the carbon content is 0.427 grams, while the nitrogen content is 0.0353 grams. Similarly, for POFA, the carbon content is 0.173 grams, and the nitrogen content is 0.00231 grams. Subsequently, in the second step, an unknown variable "X" represents the proportion of ash required to be added to 1 kg of frass to achieve the desired C/N ratio (refer to **Appendix B**). Different value of desire C/N ratio are tabulated in **Table 4.4** for reference, and further analysis. It showed that the frass-to-ash ratio varies with the input of desired C/N ratio. This methodical approach allows for precise calculation and adjustment of component proportions, ensuring optimal nutrient balance in the compost mixture.

Table 4.4: Different mixing proportion given by different C/N ratio.

Desire C/N ratio	**Frass to Ash ratio**	
	Frass	**Ash**
20	1	2.20
25	1	3.95
30	1	6.09

4.4 Post-treatment performance

4.4.1 Final composting result

In this experiment, targeting a final compost C/N ratio of 25, **Table 4.4** suggests a mixing ratio of approximately 1:4 for frass to ash. This implies that 100 grams of frass will be blended with 400 grams of ash to achieve the desired ratio. Additionally, two additional sets of mixed compost were prepared using different frass-to-ash mixing ratios of 1:0.25 (frass dominated, rich in nitrogen) and 1:1 (balance in frass and ash ratio). This variation in mixing ratios allowed for the assessment of their impact on the fertilizer nutrient profile of the final compost. Following preparation, all mixed composts were placed in the compost bin and allowed to compost for a duration of 2 weeks. Subsequently, the final compost samples were sent to BVAQ PERMULAB for comprehensive testing. The analysis results are presented in detail in **Table 4.5**. This systematic approach enables a thorough evaluation of the effects of different mixing ratios on the nutrient composition such as NPK value and the C/N ratio of the compost.

Table 4.5: Nutrient composition of different ratio of Frass-Ash mixing compost.

Parameter	**Type of Compost**		
	Frass to Ash (1:0.25)	**Frass to Ash (1:1)**	**Frass to Ash (1:4)**
Total Organic Carbon (%)	34.30	27.80	17.40
Total Nitrogen, N (%)	3.23	1.86	0.81
Phosphorus, P_2O_5 (%)	1.65	2.14	2.25
Total Potassium, K_2O (%)	3.39	3.09	2.95
Carbon: Nitrogen Ratio	11:1	15:1	**22:1**
pH value	5-7	5-7	5-7

In this study, composting with different mixing ratios of ingredients resulted in changes in the nutrient composition, as indicated by the frass nutrient analysis presented in **Table 4.5**. The primary objective of this experiment was to achieve an ideal C/N ratio for the compost, guided by a desired target ratio of 25. Analysis of the final C/N ratios (**Figure 4.6**) revealed that the compost with a frass-to-ash mixing ratio of 1:4 (FA(1:4)), tailored according to previous calculations, exhibited the highest value of 22 compared to the frass-dominated compost (FA(1:0.25)), which had a ratio of 11, and the frass-ash balanced compost (FA(1:1)), with a ratio of 15. The elevated C/N ratio observed in the FA(1:4) compost is likely attributed to the higher initial composition of ash, leading to a greater proportion of carbon-rich material relative to nitrogen. Additionally, in compost mixtures with higher carbon content, microorganisms may initially prioritize carbon consumption, resulting in a slower rate of nitrogen mineralization and a higher C/N ratio during the early stages of composting. This phenomenon, known as N immobilization, occurs when microorganisms require a sufficient amount of nitrogen, typically in the form of ammonium or nitrate, to digest carbon-containing sources effectively (SDSU Extension, 2024). However, it's essential to recognize that factors such as moisture content and the stage of decomposition can also influence the C/N ratio. Variations in these factors throughout the composting process may contribute to fluctuations in nutrient availability and microbial activity, ultimately impacting the final C/N ratio of the compost.

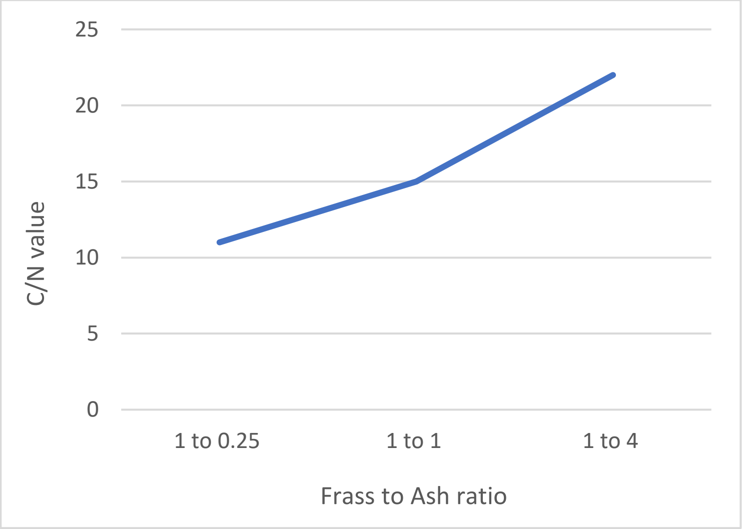


Figure 4.6: Comparing the C/N ratio of 3 different mixing ratio compost.

The initial carbon-to-nitrogen (C:N) ratio of the ash is 75:1. However, when it is blended with frass, which has a lower C:N ratio, and undergoes composting, this ratio significantly decreases to 22:1 (**Figure 4.7**). This reduction occurs because during microbial consumption of organic compounds, approximately two-thirds of the carbon is released as carbon dioxide, while the remaining one-third is incorporated, along with nitrogen, into microbial cells. Subsequently, when these cells die, the incorporated carbon and nitrogen are released back into the compost pile for further microbial utilization (Cornell.edu, 2024). Moreover, during the composting process, microorganisms primarily utilize carbon substrates as their main energy source. When the compost pile undergoes turning, the introduction of oxygen stimulates microbial growth, which leads to the breakdown of carbon substrates, releasing carbon dioxide (CO2). Assuming that nitrogen is preserved and not lost through ammonia gas emission or leaching, the carbon-to-nitrogen (C:N) ratio in an adequately moist and aerated compost pile decreases over time (Walter, 2020).

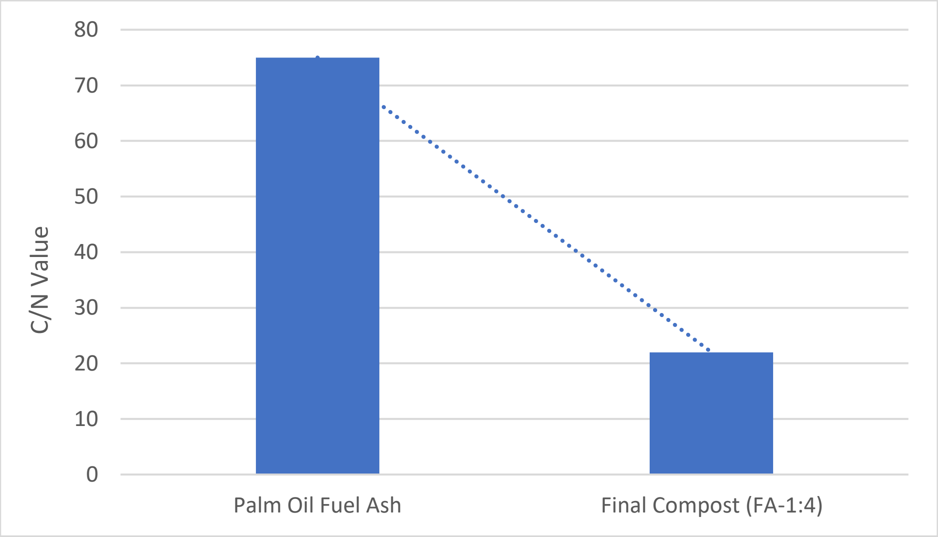


Figure 4.7: The difference in C/N ratio of POFA and final compost.

The analysis of the main fertilizer nutrients, nitrogen, phosphorus, and potassium (NPK), in the three final composts was visualized in a graph (**Figure 4.8**), with particular emphasis on the nitrogen values due to their substantial range of changes. While nitrogen exhibited notable variations across the compost blends, phosphorus and potassium content remained relatively stable with no significant changes observed. Analysis reveals a noticeable decline in nitrogen content within the final compost as the initial proportion of ash increases. In the frass-dominated compost (FA(1:0.25)), nitrogen content measures 3.23%, contrasting with 1.86% for the frass-ash balanced compost (FA(1:1)) and 0.81% for the (FA(1:4)) compost. This decline can be attributed to the inherent differences between frass and ash. Frass, being an organic material derived from insect larvae, typically contains a higher nitrogen content compared to ash, which is primarily composed of carbonaceous material. Therefore, when the proportion of ash increases in the compost mixture, there is a dilution effect on the overall nitrogen content due to the lower nitrogen content in ash. Furthermore, when provided with organic materials containing a high ratio of carbon to nitrogen, such as carbon-rich ash, microorganisms may prioritize the consumption of carbon substrates over nitrogen-containing compounds. This preference for carbon-rich materials can lead to a relatively slower rate of nitrogen mineralization compared to carbon decomposition. (Cornell.edu, 2024).

In compost mixtures with excess carbon relative to nitrogen, microorganisms may undergo a process known as nitrogen immobilization (Brust, 2019). During nitrogen immobilization, microorganisms absorb and incorporate available nitrogen into their biomass for growth and reproduction (University of California, 2016). As a result, nitrogen becomes temporarily "immobilized" in microbial cells, reducing its availability for other microbial processes and contributing to a decrease in the overall nitrogen content of the compost mixture. For instance, when there is an excess of carbon-rich materials, microbial activity is hindered as nitrogen becomes limited for optimal growth and metabolic functions. This limitation on nitrogen availability can slow down the breakdown of organic matter, leading to reduced nitrogen mineralization rates. Consequently, the microbial community may not efficiently convert nitrogen into forms that are readily available for plant uptake, resulting in greater losses of nitrogen through leaching or as gaseous ammonia (Brust, 2019).

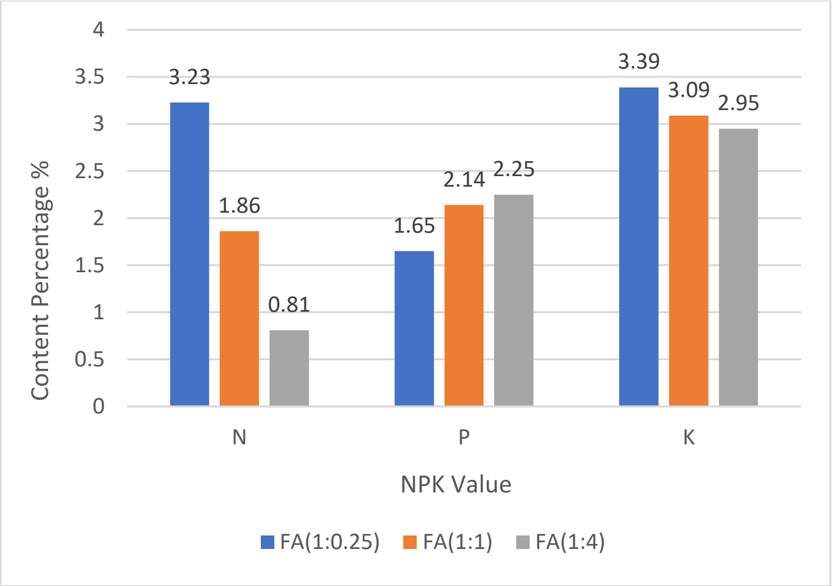


Figure 4.8: Comparison of NPK values among 3 different mixing ratio composts.

4.4.2 Economic Aspect - Cost Analysis

Incorporating Palm Oil Fuel Ash (POFA) into BioLoop's production of frass-based fertilizer presents a promising opportunity to significantly reduce the final cost of their fertilizer product. One of the primary cost advantages of using mixed compost is the potential savings on raw materials. POFA, often obtainable at a lower cost or even for free, especially if the company has access to nearby palm oil factories, provides a cost-effective alternative. BioLoop, situated in an area predominantly occupied by oil palm cultivation and palm oil production companies, stands to benefit from this proximity. Currently, BioLoop sells 25 kg of pure frass fertilizer for 35 ringgits. However, by incorporating POFA into the frass according to the calculated ratio, the amount of frass required is reduced. This reduction in frass usage not only lowers the final production cost but also increases the gross margin of the product. For example, a 25 kg bag of fertilizer produced from a mixing ratio of 1 to 4, based on previous calculations of frass and POFA, offers a higher nutrient value (but need to address the loss of nitrogen issues first) and lower material cost (due lower frass usage) compared to a 25 kg bag of full frass-based fertilizer. Although adopting POFA and frass for composting may require some initial investment in purchasing equipment to ensure consistency, the long-term benefits are significant. Increased production quantity, coupled with lower processing and handling costs, result in cost-effectiveness and a better return on investment (ROI) over time.

4.4.3 Aligning with Sustainable Development Goals

Blending Black Soldier Fly larvae (BSFL) frass with palm oil fuel ash (POFA) in an appropriate ratio and composting it as organic fertilizer presents a promising approach for nutrient cycling and establishing a circular economy model. By utilizing POFA, a byproduct of the palm oil industry, as a fertilizer additive, companies can contribute to reducing waste generated by palm oil mills, aligning with Sustainable Development Goal (SDG) 12 (Responsible Consumption and Production). This practice promotes sustainable consumption and production patterns by minimizing waste generation and promoting the efficient use of resources. Furthermore, incorporating POFA into frass-based fertilizer reduces the need for synthetic fertilizers, which are often manufactured through energy-intensive processes and may have negative environmental impacts, such as greenhouse gas emissions and water pollution. This supports SDG 13 (Climate Action) and SDG 14 (Life Below Water) by mitigating climate change and reducing pollution in aquatic ecosystems (Un.org, 2015).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

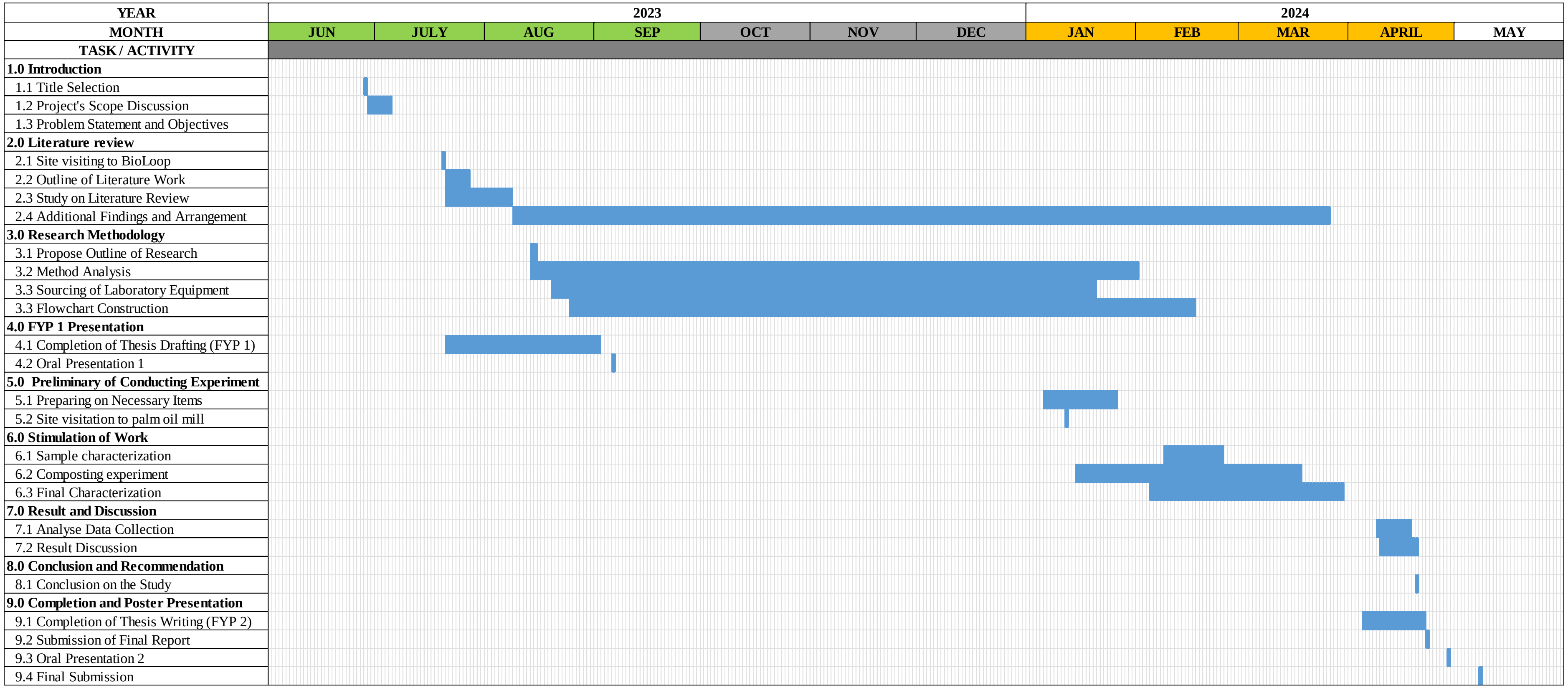
5.1 Conclusion

Aligned with objective (i), this study delved into characterizing BSF Larvae raw frass as an organic fertilizer, aiming to replace traditional fertilizers in sustainable agricultural practices. This review underscores the promising nature of BSFL frass resulting from composting wastes, calling for a comprehensive interdisciplinary investigation. Notably, the composition of frass exhibits notable variability, with phosphorus (P), potassium (K), and micronutrient concentrations being notably influenced by the feed substrate. Encouragingly, findings suggest that frass compost demonstrates a favorable nitrogen content ranging from 3 to 4%, phosphorus content from 1 to 2%, and potassium content about 3%, suggesting its potential as a substitute for conventional fertilizers. It is important to acknowledge, nevertheless, that different research have produced different results. While some have indicated that BSF larvae frass can increase crop output, others have pointed out adverse growth consequences that could be caused by phytotoxicity. Black Soldier Larvae frass is a product that lacks biological stability due to its fast-composting rate and the presence of chemicals that may be phytotoxic. This suggests that stabilizing post-treatments may be necessary to improve the product's usefulness as a fertilizing amendment. Moving to objective (ii), the study explored the blending of frass with palm oil fuel ash in various mixing ratios to boost fertilizer content and fulfill crop nutrient requirements. The mixing ratio determination was based on achieving the ideal C/N ratio (25:1) for microbial activity optimization. Subsequent composting of the mixed compost (FA(1:4)) for two weeks yielded a C/N ratio (22:1) which falls within acceptable range between 20 to 30. However, a notable change occurred in the major fertilizer nutrient, nitrogen content, within the mixed compost, decreasing significantly from approximately 3.5% to 0.9%. This points to significant nitrogen loss through processes like leaching and gaseous emissions during the composting process, underscoring the instability of the nitrogen content. Thus, lowering the ash proportion and extending the composting period can be done as amendments.

5.2 Recommendation

For future research, extending the composting period of the mixture could facilitate more thorough nitrogen mineralization. Lengthening the composting duration allows sufficient time for microbial activity to break down organic matter, releasing nitrogen in forms accessible to plants. By extending the composting period, researchers can better understand the kinetics of nitrogen transformation and optimize nutrient availability in the final compost product. Additionally, endeavors should focus on elucidating he effects of post-treatment procedures, such as heat-assisted composting on the production of humic compounds and the mitigation of phytotoxicity. This entails comprehensive investigations into the duration and temperatures of thermophilic, mesophilic, and maturation stages, alongside rigorous evaluation of emissions from these post-treatments to ensure environmental sustainability.

GANTT CHART



Project Timeline Schedule Gantt Chart.

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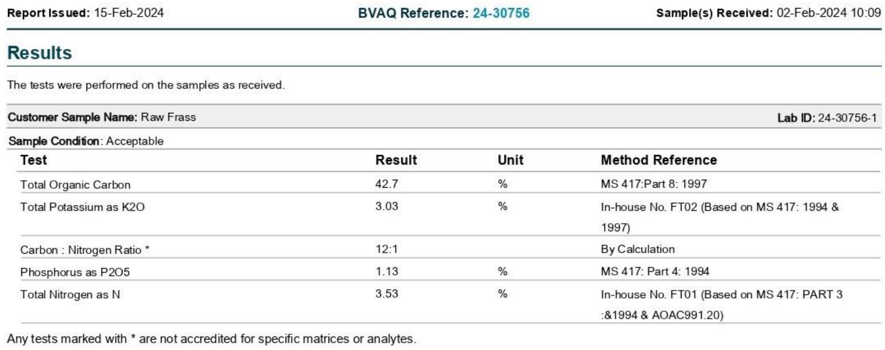
APPENDICES

APPENDIX A: Certificate of Analysis

The following are the certificates of analysis provided by BVAQ PERMULAB.



Report Issued: 15-Feb-2024 **BVAQ Reference:** 24-30756 **Sample(s) Received:** 02-Feb-2024 10:09

Results

The tests were performed on the samples as received.

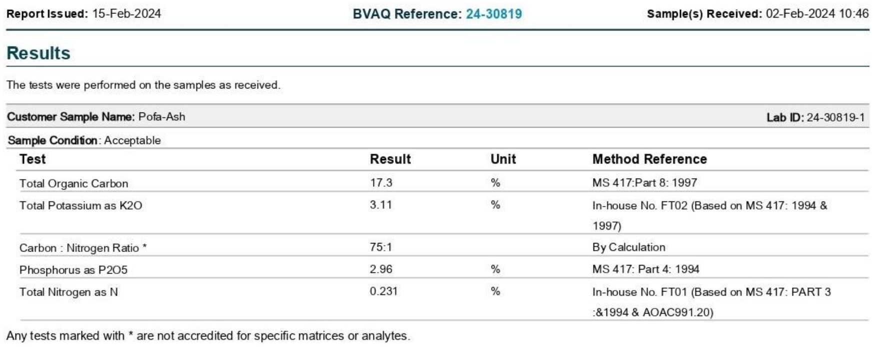
Customer Sample Name: Raw Frass **Lab ID:** 24-30756-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	42.7	%	MS 417:Part 8: 1997
Total Potassium as K2O	3.03	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	12:1		By Calculation
Phosphorus as P2O5	1.13	%	MS 417: Part 4: 1994
Total Nitrogen as N	3.53	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

Raw Frass Analysis



Report Issued: 15-Feb-2024 **BVAQ Reference:** 24-30819 **Sample(s) Received:** 02-Feb-2024 10:46

Results

The tests were performed on the samples as received.

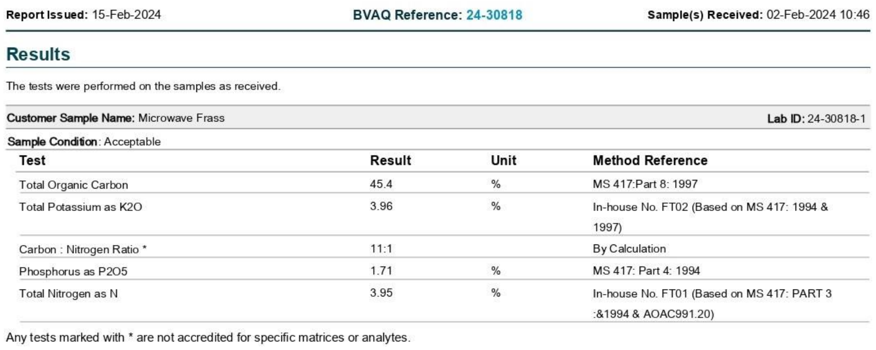
Customer Sample Name: Pofa-Ash **Lab ID:** 24-30819-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	17.3	%	MS 417:Part 8: 1997
Total Potassium as K2O	3.11	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	75:1		By Calculation
Phosphorus as P2O5	2.96	%	MS 417: Part 4: 1994
Total Nitrogen as N	0.231	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

POFA Analysis



Report Issued: 15-Feb-2024 **BVAQ Reference:** 24-30818 **Sample(s) Received:** 02-Feb-2024 10:46

Results

The tests were performed on the samples as received.

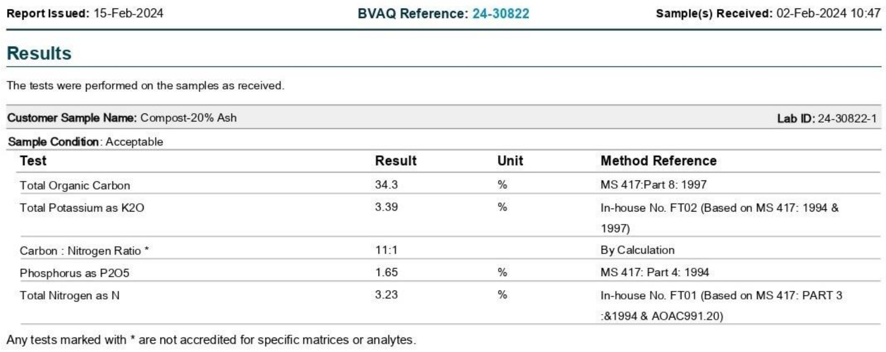
Customer Sample Name: Microwave Frass **Lab ID:** 24-30818-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	45.4	%	MS 417:Part 8: 1997
Total Potassium as K2O	3.96	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	11:1		By Calculation
Phosphorus as P2O5	1.71	%	MS 417: Part 4: 1994
Total Nitrogen as N	3.95	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

Microwave Frass Analysis



Report Issued: 15-Feb-2024 **BVAQ Reference:** 24-30822 **Sample(s) Received:** 02-Feb-2024 10:47

Results

The tests were performed on the samples as received.

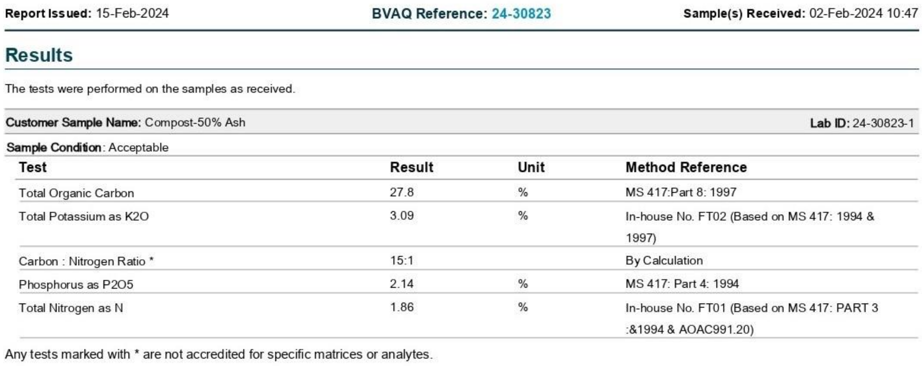
Customer Sample Name: Compost-20% Ash **Lab ID:** 24-30822-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	34.3	%	MS 417:Part 8: 1997
Total Potassium as K2O	3.39	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	11:1		By Calculation
Phosphorus as P2O5	1.65	%	MS 417: Part 4: 1994
Total Nitrogen as N	3.23	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

Frass to Ash Analysis (1:0.25)



Report Issued: 15-Feb-2024 **BVAQ Reference:** 24-30823 **Sample(s) Received:** 02-Feb-2024 10:47

Results

The tests were performed on the samples as received.

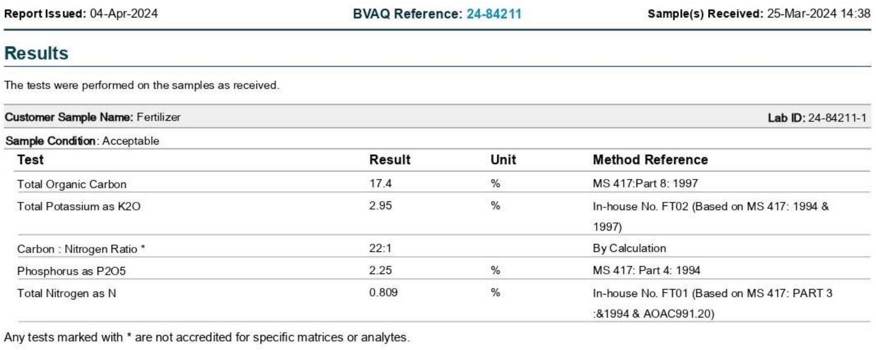
Customer Sample Name: Compost-50% Ash **Lab ID:** 24-30823-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	27.8	%	MS 417:Part 8: 1997
Total Potassium as K2O	3.09	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	15:1		By Calculation
Phosphorus as P2O5	2.14	%	MS 417: Part 4: 1994
Total Nitrogen as N	1.86	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

Frass to Ash Analysis (1:1)



Report Issued: 04-Apr-2024 **BVAQ Reference:** 24-84211 **Sample(s) Received:** 25-Mar-2024 14:38

Results

The tests were performed on the samples as received.

Customer Sample Name: Fertilizer **Lab ID:** 24-84211-1

Sample Condition: Acceptable

Test	Result	Unit	Method Reference
Total Organic Carbon	17.4	%	MS 417:Part 8: 1997
Total Potassium as K2O	2.95	%	In-house No. FT02 (Based on MS 417: 1994 & 1997)
Carbon : Nitrogen Ratio *	22:1		By Calculation
Phosphorus as P2O5	2.25	%	MS 417: Part 4: 1994
Total Nitrogen as N	0.809	%	In-house No. FT01 (Based on MS 417: PART 3 :&1994 & AOAC991.20)

Any tests marked with * are not accredited for specific matrices or analytes.

Frass to Ash Analysis (1:4)

APPENDIX B: Calculations for blending C/N ratio.

Desire blending C/N ratio = 20, 25, 30

Carbon mass in frass, $C_F = 0.427$

Carbon mass in ash, $C_A = 0.173$

Nitrogen mass in frass, $N_F = 0.0353$

Nitrogen mass in ash, $N_A = 0.00231$

Ash to frass proportion = X

$20 = (0.427 + 0.173 X) / (0.0353 + 0.00231 X)$

$X = 2.20 = 2.2$

$25 = (0.427 + 0.173 X) / (0.0353 + 0.00231 X)$

$X = 3.95 = 4$

$30 = (0.427 + 0.173 X) / (0.0353 + 0.00231 X)$

$X = 6.09 = 6$