DETERMINATION OF MICROPLASTICS IN COMMERCIAL BLACK SOLDIER FLY LARVAE *Hermetia illucens*, (DIPTERA: STRATIOMYIDAE)

by

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DECLARATION

I declare that this thesis entitled "DETERMINATION OF MICROPLASTICS IN COMMERCIAL BLACK SOLDIER FLY LARVAE *Hermetia illucens*, (DIPTERA: STRATIOMYIDAE)" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : 

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Date : 12 August 2024

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ABSTRACT

The use of plastics has been widely used and is a major contributor to environmental and ecosystem pollution. There have been many studies that have been reported in the presence and effects of microplastics in many environments but no studies have been done in Malaysia on the commercial black soldier fly. Therefore, the purpose of this study is to determine the occurrence of microplastics within the commercial black soldier fly larvae (*Hermetia illucens*). The sample of commercial black soldier fly larvae was acquired from local supplier of A BSFL Farming in Pulau Pinang, B BSFL Farming in Batu Caves Selangor and C BSFL Farming in Hulu Langat Selangor. The sample was separated into nine replicates. There were five types of microplastics polymers that have been identified through fourier transform spectroscopy (FT-IR) namely Polyethylene (PE), Polypropylene (PP), Polydiene, Nylon and Polyethylene. This study shows that there were microplastics in BSFL.

Keyword: Commercial Black Soldier Fly Larvae, microplastics.

PENENTUAN MIKROPLASTIK DALAM KOMERSIAL LARVA LALAT ASKAR HITAM *Hermetia illucens*, (DIPTERA: STRATIOMYIDAE)

ABSTRAK

Penggunaan plastik telah digunakan secara meluas dan merupakan penyumbang utama kepada pencemaran alam sekitar dan ekosistem. Terdapat banyak kajian yang telah dilaporkan mengenai kehadiran dan kesan mikroplastik dalam banyak persekitaran tetapi tiada kajian dilakukan di Malaysia mengenai komersial lalat askar hitam. Oleh itu, tujuan kajian ini adalah untuk menentukan kejadian mikroplastik dalam komersial larva lalat askar hitam (Hermetia illucens). Sampel komersial larva lalat askar hitam diperoleh daripada pembekal tempatan A BSFL Farming di Pulau Pinang, B BSFL Farming di Batu Caves Selangor dan C BSFL Farming di Hulu Langat Selangor. Sampel dipisahkan kepada sembilan replika. Terdapat lima jenis polimer mikroplastik yang telah dikenal pasti melalui spektroskopi transformasi fourier (FT-IR) iaitu Polietilena (PE), Polipropilena (PP), Polidiena, Nylon dan Polietilena. Kajian ini menunjukkan terdapat mikroplastik dalam BSFL.

Kata kunci: Komersial Larva Lalat Askar Hitam, mikroplastik.

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LIST OF ABBREVIATIONS

BSFL	Black Soldier Fly Larvae
PE	Polyethylene
PS	Polystyrene
PP	Polypropylene
PVC	Polyvinyl chloride
PET	Polyethylene terephthalate
PU	Polyurethane
FTIR	Fourier Transform Infrared
SEM	Scanning Electron Microscopy
HCl	Hydrochloric acid
NaCl	Sodium Chloride
RPM	Revolutions per minutes
G	Gram
KG	Kilogram
Mm	Milimetre
µm	Micrometre
mL	Mililitre

LIST OF SYMBOLS

ºC	Degree Celcius
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Plastics are wide in the world. There are the common types of plastics that can be found as microplastics such as polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC) to produced the combs and button, polyethylene terephthalate (PET), polyurethane (PU), and other plastic polymers. On the bad side, the existence of plastic has a long-term negative effect due to the very long decay process. The decline in the quality of life of plastic elements also affects the health of terrestrial and aquatic ecosystems, attracting multiple hazards and damages. Reducing the amount of primary microplastics used in consumer goods and controlling plastic trash to stop the production of secondary microplastics are two ways to lessen the pollution caused by microplastics.

Microplastics are small plastic particles. They can be produced on purpose at this tiny size for particular uses, such in some cleaning or cosmetic goods, or they can be the consequence of bigger plastic things breaking down because of mechanical action, weathering, and sunshine (Zhihao et al., 2022). Microplastics can divided to two forms, primary and secondary microplastics. The harm of plastic and microplastic debris negatively affects in particular on the fauna and human health. The latest technology is insufficient in effectiveness to measure the existence of small microplastics that are invisible to the naked eye in ecosystems and their potential for marine and human biota (Barboza et al., 2018).

Black Soldier Fly originates from Southeastern United State. It is not consider as pest because it not disturb human habitat and food also does not carried disease according by (Tomberlin et al., 2002). When adult it only survives on the large fat body stored from the larval stages that cause it not consume any feed mentioned (Newton et al., 2005). Commonly, the Black Soldier Fly help decaying organic matter like cow dung, garbage and spoiled feed in livestock and agriculture farm. (Sheppard et al. 2002) have observed that Black Soldier prepupae consist of 40% protein and 30% fat depending on the feed that they consume.

Commercial Black Soldier Fly Larvae (BSFL) have familiar or popular relationship with a main food protein source to fishes, swine and chicken and makes them an interesting study subject to get new exploration about this insect. Other studies have worked on the nutrient and fiber content of organic substances, but this latest study has the opportunity to detect the existence of microplastics in the Commercial Black Soldier Fly Larvae (BSFL) to improve the quality of this insect so that it is not harmful to other organisms. This is because when microplastics found in Commercial Black Soldier Fly Larvae (BSFL) may give potential negative impact to consumers base on their food chains related.

1.2 Problem Statement

Commercial Black Soldier Fly Larvae (*Hermetia illucens*) can convert organic waste into best-quality nutrients for swine, fish and poultry feeds. Better commercial BSFL food formulations and necessary nutritional care need to be planned and systematic because commercial BSFL eat any type of organic waste such as starch or mashed potato, worm, rotting meat, decomposing fruit and vegetables include plastics.

Can change to fresh food and avoid feeding things that contain plastics to produce a nutrient ingredient from the insect's body. If this insect consumes the plastics or microplastics will effect bioaccumulation in food chain. They may be transferred and stored further down the food chain. Based on the latest survey and paper research, there show no study carry about microplastics in Commercial Black Soldier Fly Larvae.

1.3 Objectives

i. To determine the occurrence of microplastics within the commercial black soldier fly larvae (*Hermetia illucens*).

ii. To characterize the microplastics within the commercial black soldier fly larvae (*Hermetia illucens*).

1.4 Scope of Study

This study focuses on the microplastic in Commercial Black Soldier Fly Larvae (*Hermetia illucens*). As we know in Commercial Black Soldier Fly Larvae (*Hermetia illucens*) will be used in aquaculture or aquafarming to feedings. Commercial Black Soldier Fly Larvae food sources to terrestrial life such as predators are fish, swine and poultry. Commercial Black Soldier Fly Larvae (*Hermetia illucens*) are part of the food chain, it is possible that they will absorb or contains microplastics, which may then be passed on to trophic level related.

The Commercial Black Soldier Fly Larvae (*Hermetia illucens*) was studied thoroughly and ethically to detect microplastics in its body and to understand the existence, causes, and potential effects of microplastics in these insects. The results of this study can help explain the role of the Commercial Black Soldier Fly Larvae (*Hermetia illucens*) and the possible consequences of microplastics in fisheries and animal husbandry ecosystems, thereby broadening our understanding of microplastic pollution. Microplastics were determined by using Fourier Transform Infrared (FTIR) spectroscopy to analyse the chemical composition in insect.

1.5 Significant of Study

There were many studies that conducted in past year regarding using of Black Soldier Fly but very less or no research regarding microplastics in Commercial Black Soldier Fly Larvae (*Hermetia illucens*). The aim of this study to provide an initial baseline data of understanding the kinds of microplastic types found in Commercial Black Soldier Fly Larvae (*Hermetia illucens*). As a consequence, it will increase the new knowledge and advance understanding the presence of microplastics in the commercial insect. The data from the analysis may provide an opportunity for the supplier or seller to handle and manage these insects free from plastic pollution.

Environmental policy and management techniques can benefit from this study. The information may be use by governmental bodies and environmental groups to create plans for reducing microplastic contamination in aquaculture or aquafarming environments. In addition, the study raises people's awareness of how widespread microplastic contamination is. Publicizing the results can help raise awareness among the general public of the value of ethical waste management as well as the possible repercussions of plastic contamination.

Entomologists, environmental policy and management techniques can take advantage of this study. The new meeting in this study could be used by government and private bodies, entomology students and environmental groups to come up with plans to reduce microplastic pollution in aquaculture or aquafarming environments. Nowadays, new or strange-looking information is also more likely to go viral so it provides an opportunity to expand the current knowledge regarding the rate of microplastics pollution in the insect to the public's awareness of the importance of ethical waste management. This effort is relevant.

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastics

Today's studies give the impression that microplastics are small fragments that are separate from plastic and have a millimeter-sized size range according by (Hidalgo-Ruz et al., 2012). Koelmans et al., (2017) were mentioned, have stated in their study that it is estimated that the percentage of total plastic fragments is half that of the other content. The sustainability of biodiversity is disturbed, the quality of ecosystems is affected, and human health deteriorates. Hidalgo-Ruz et al., 2012). Microplastics come from a variety of causes.

Figure 2.1.1: Occurrence of microplastics (sources: Google Image, 2020)

2.1.1 Characteristic of Microplastics

Microplastics have differences in shape, size, color and origin that are not the same due to the diversity of sources. Marine organisms cannot distinguish their food from microplastic because there is a diversity of types, sizes, shapes and colors that can confuse them (Hidalgo-Ruz, Gutow, Thompson, & Thiel, 2012).

2.1.2 Types of Microplastics

Microplastics has been categorized into two main types on the basis of their source. Primary microplastics are formed from cosmetics, personal care such as body scrub and face cleanser, medical or surgeries products and clothing fibre according by (Zhang et al., 2021). Secondary microplastics are formed from the breakdown of larger plastic products. Plastic fragments from plastic bags, plastic nets and resin containers are secondary microplastics into the environment.

2.1.3 Colour of Microplastics

One of the categories of microplastics that need excellent research is colour according by (Abu-Hilal & Al-Najjar, 2009). Microplastics can be found in a variety of colors whether light, dark, or transparent. Some microplastics will be available in green, red, yellow, blue and others colour. Supported by (Shiber, 1987) mentioned, mostly the polypropylene (PP) has typically transparent and clear coloured while for white plastic pellets have been categories polyethylene (PE).

2.2 Life Cycle Black Soldier Fly

During the adult stages, Black Soldier Fly appearance likes bees and wasps. Black Soldier Fly comes in various color of the body according by (Diclaro et al., 2012) but for adult commonly black and blue in color. It also consists of two translucent "window" that located on the first abdominal segment. The length of adult Black Soldier Fly is around 15 to 20 mm based on opinion (Sheppard et al., 2002). Their legs have white in color at the end while the antenna elongate by three dividing segment when adult.

In naturally the female Black Soldier Fly will deposit the mass of about 500-900 eggs at the space between the waste materials such as garbage, manure and others. It takes about 4 days to hatch into larvae. The oval egg of Black Soldier Fly has faded yellow or creamy white in color with the length about 1 mm (NCIPMI, 2015). Next, during larvae stages it looks dull and white color the length of the larvae about 27mm with width 6mm (Diclaro et al., 2012). It required 14 days to complete development of six instars (Hall & Gerhardt, 2002).

It known as insatiable feeder during that stage because of it behavior that continuously feed for growing and fulfill six instars. During the prepupal stages it disperses from the feeding site to the shelter areas to undergo process pupation. During this stages the development of darkens exoskeleton become prominent. Pupation stages take about two week to become adult according by (Hall & Gerhardt, 2002).

The life circle of a Black Soldier Fly (*Hermetia illucens*). Commercial Black soldier Fly (*Hermetia illucens*) life cycle (44-45 days total). They develop in the growth media and spend two stages as eggs (4 days) and maggots stage (18 days).

Only when they are transforming from pre-pupa (14 days) to adults (9 days) do they move away from the media to find a dry spot to complete the metamorphosis.

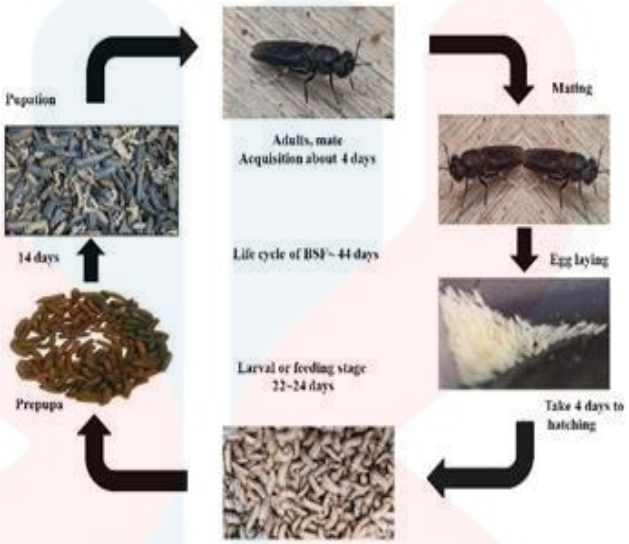

Figure 2.2.1: Life circle of Commercial Black Soldier Fly (*Hermetia illucens*), (sources: Google Image, 2021)

Figure 2.2.2: Life circle of a Commercial Black Soldier Fly (*Hermetia illucens*), (sources: Google Image, 2021)

2.3 Breeding of Black Soldier Fly Larvae

Tomberlin (2008) stated that temperature may affect the life spans and the growth rate of Black Soldier Fly. The larvae shows a better survivality at the

temperature around 27°C and 30°C while above than that temperature cause the less survivality rate (Prashant, 2009). Next, the suitable temperature for their mating purpose is around 28°C (Zhang et al., 2010). In our country, there is suitable in condition for the mating and breeding Black Soldier fly as we located at the tropical region.

In our country Malaysia, most of the farmers try to produce it larvae by using a fermentation of the coconut husk and the corn pulp. This fermentation will take about 1 week and above. Besides that, we also can be use the mixing of the kitchen scraps for attraction of the Black Soldier Fly to be laying egg as it may produce the strongest odor. From the previous study (Shepperd et al., 2002) noted that to begin a new colony of Black Soldier Fly Larvae by natural mating take about 2 weeks.

From the past study stated that during their research they found that the Black Soldier Fly can be found in coffee ground and the caffeine in the coffee may help in the development better metabolism for them (Shepperd et al., 2002). During the mating process male Black Soldier Fly will try to attract female to the strongest odor site. For the natural mating process may help to preparing the suitable medium for them to be mate.

2.4 Previous Studies

Year	Authors	Previous Studies
2023	Lu Huang, Shoufeng Zhang, Lingyun Li, Juying Siyu Zhang, Wang, Xiaoxing Liu, Weiwei Zhang	Research progress on microplastics pollution in polar oceans
2022	Shahida Anusha Siddiqui, Bridget Ristow, Rahayu, Nugroho Susetya Putra, Nasih Widya Yuwono, Khoirun Nisa', Bosco Mategeko, Sergiy Smetana, Morteza Saki, Asad Nawaz & Andrey Nagdalian	Black soldier fly larvae (BSFL) and their affinity for organic waste processing
2021	Wan Iffah Binti Wan Ismail	The effect of antibacterial activity of the black soldier fly larvae (*hermetia illucens*) extracted from the selected solvent against *Aeromonas hydrophila*

2021	Muhammad Salam, Fakhri Alam, Shi Dezhi, Ghulam Nabi, Amina Shahzadi, Shabi Ul Hassan, Muhammad Ali, Mian Abdal Saeed, Jamil Hassan, Nisar Ali & Muhammad Bilal	Exploring the role of Black Soldier Fly Larva technology for sustainable management of municipal solid waste in developing countries
2020	Mimi Lovianna Anak Bitlius	Determinations of microplastics in sediment in Kelantan and Pattani Bay

2.5 Effect of Microplastics to Ecosystem and Living Biota

Plastic is easily available in the waters of the oceans. Plastic waste floating above the bottom of the sea surface and many plastic polymer drifts in the sea until it passes the sea boundary. This is a critical challenge, without borders, these pollutants will affect the entire world's oceans according by (P. Strafella, M. Lopez Correa, I. Pyko, S. Teichert & A. Gomiero, 2020).

Microplastics are omnipresent and pose a global threat of problems to the environment due to their robustness, long-lasting resilience, and existence, which take a long time to become extinct. Most microplastics research has made observations about marine ecosystems. Existing scientific evidence suggests that exposure to microplastics causes a variety of toxic insults, from eating disorders to reproductive effectiveness physiological changes in water quality, the synergistic and and the

deterioration of producers to consumer trophics according by (Krishna, Shreya, & Sadasivam, 2020).

The deterioration of microplastics in wastewater involves various complex mechanisms that can go through the process of biological decomposition through decomposition agents such as bacteria or fungus. It takes thousands of years for microplastics to completely degrade. These process is impacted by a number of variables, including an appropriate pH balance and temperature. Wastewater can break down microplastics in a number of ways. The process of oxidation brought on by ultraviolet light is called photodegradation mentioned (Sarno et al., 2020).

The impact of microplastics on marine life and fresh water is particularly worrying due to the increasing level of pollution. Microscopic plastic particles are difficult to dissolve in aquatic environments. Because of their small size and look similar to food, many marine animals, including fish, corals, zooplankton, phytoplankton, algae, oysters, sea urchins, and lobsters, frequently pick up these particles. As a result, plastic particles are moved up the trophic chain to a higher level by aquatic life. The impact of microplastics on marine life is a worry since they can entangle and ingest marine organisms, potentially leading to the death of aquatic fauna.

Lakes, rivers and marine environments become centers for the collections of plastics. It is anticipated that when benthic organisms are connected to the seafloor, they may experience new negative impacts. These organisms include vertebrates and invertebrates with different feeding methods. Because microplastics in aquatic environments can impact an organism's development, poor nutritional status, gut bacteria, immune systems, and population and reproduction are stunted, they may constitute a hazard to aquatic life.

According by (Magni et al., 2018) have demonstrated that filter feeders like mussels, lalas, shellfish, and oysters may be vulnerable to microplastic intake. Based on his scientific research that this species has microplastic in line with plastic pollution in sea water. Microplastics have already been found in the mussel and crab species mentioned by (Farrell and Nelson, 2013), indicating that higher trophic levels are consuming microplastics through their prey or food chain according by (Wright et al., 2013).

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2.6 Roles and Feeding Guilds of the Black Soldier Fly

Known as "worm phoenix," Black Soldier Fly Larvae (BSFL) are utilised for fish or swan supplements, insect and animal management and organic waste bioconversion according by (Rehman et al., 2019, 2021; Sheppard, 1992). BSFL is alternative ingredient for animal feeds. Breeding these insect colonies is easy and fast so opens up human opportunities and interests to build mass breeding in the production of these species on commercialized interests and values. For instance, employing fats to replace less sustainable edible fats such in butter and producing biodiesel (Rehman et al., 2018) are examples of new proteins utilised in poultry, aquaculture, and cattle feed. According to studies conducted by Bruni et al. (2020), Jahan and Haque (2021),

and Melenchón et al. (2021) in aquaculture, BSFL meal improved on positive impact health conditions.

According to Rehman and Hollah (2020) and Veldkamp et al., (2021) commercial black soldier flies are a viable bioconversion agent for organic waste and a potentially nutrient-rich source of protein for aquaculture, swine, and poultry. Commercial Black Soldier Fly are high in nutrients, minerals, amino acids, and omega-3 fatty acids. Up to 43% of their body weight can be made up of protein. established by Zhu et al., (2019); Liu et al., 2017). This insect will be used as waste to produce protein, not a pest or danger to other organisms. It has efficient production, sustainable rearing, high nutrition value, and can be commercial in economic value.

Black soldier fly larvae as a feed ingredient. Recently, BSFL has been studied as a potential protein-rich feed element for other species such as pigs and poultry mentioned by (Awoniyi et al., 2003). According to (Sealey et al., 2011) Black Soldier Fly Larvae as a feed ingredient is potential animal feed components that have been extensively studied in fish but not as widely in monogastric and other animals according by (Sealey et al., 2011). However, nowadays BSFL is the preferred diet of choice for cattle farming or ruminants animal.

2.7 Types of Methods

Typically, a combination of sample preparation, extraction, and identification procedures are used in the investigation of microplastics in organisms. With Fourier-transform Infrared Spectroscopy (FTIR), peak variations are more visible and the overall composition of the sample's interior and surface may be monitored. Its broad coverage and good spectral resolution are advantages (Larkin, 2011). An effective and

non-destructive method for obtaining comprehensive details on an insect's shape, chemistry, and structure is scanning electron microscopy (SEM). Imaging micro-nano structures can be accomplished with great efficiency using scanning electron microscopy. To improve image quality, it has advanced significantly. Certain microplastics can be identified using fluorescence microscopy, especially if they have been tagged with fluorescent dyes. Microplastics may be manually counted under a microscope in some subsamples. For quantitative analysis, use analytical techniques like gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). To prove the microplastics conditions, it's has advanced use hot needle test because if the pieces melt its can show pieces contains microplastics (Witte et al., 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Material

In this study, the determination of microplastics in commercial black soldier fly larvae (*Hermetia illucens*) used the following materials and apparatus:

Table 3.1.1 Materials and apparatus

Laboratory material and apparatus	1) Chemical substance: ▪ Sodium chloride (NaCl) ▪ Hydrochloric acid 37% (HCl) ▪ Ethanol 2) Lab material: ▪ 250ml Beaker ▪ 250ml Conical flask ▪ 100ml Glass measuring cylinder ▪ Petri dish 3) Lab instruments: ▪ Chiller ▪ Laminar flow cabinet ▪ Rax Vision Microscope ▪ Fourier transform infrared spectrometer (FT-IR) ▪ Vacuum pump

	▪ Filtering flask ▪ Silicon tubing hose ▪ Buncher funnel ▪ Bunsen burner 4) Others equipment: ▪ Forceps ▪ Cotton glove ▪ Microscope slides ▪ Lab coat ▪ Filter paper ▪ Tissue (free MPs)

3.2 METHODS

3.2.1 Commercial Black Soldier Fly Larvae (*Hermentia illucens*) Sampling and Preparation

Commercial Black Soldier Fly Larvae were acquired from the purchase and obtained of A BSFL Farming in Pulau Pinang, B BSFL Farming in Batu Caves Selangor and C BSFL Farming in Hulu Langat Selangor. This insect is the main material necessary to carry out research to detect and identify the presence of microplastics in their body.

The method of recruitment is transparent and safe for the purpose of scientific studies. The procedure for larvae management is to wear glass beaker container elements for storage and covered by aluminium foil. Elements containing plastics are avoided to minimize pollution and preserve the purity of insects as well as any

microplastic particles that have the potential to determine the accuracy of the presence of microplastics in this insect. The sample was separately from external microplastics particles and put in the petri dish and capture the microplastics photo use Rax Vision Microscope.

The black soldier fly larvae were weighed using an analytical balance scale to obtain accurate measurements of the sample mass. The weight required for each nine replicate is 30 grams. Analytical balance scales are extremely sensitive laboratory tools that are often applied in physical and analytical chemistry. It's can be supported by (R. Schulthess, 2003) mentioned analytical balance scale read ability reacts sensitively to loaded weights.

Figure 3.1. 30g of BSFL weight in a beaker weighed using analytical balance scales

Figure 3.2. The beakers are neatly covered by aluminium foil and labelled. Petri dish are used to store microplastics found on the outer surface of BSFL

3.2.2 Physical Detection of Microplastics on Black Soldier Fly Larvae (*Hermentia illucens*)

Black Soldier Fly Larvae (*Hermentia illucens*) microplastic was identified by using a Rax Vision Microscope because this tool is easier to view the entire insect at once and do not require slide preparation. Researcher will put 10x to 45x magnification range of most standard microscope more than enough for most applications. If there was a microplastic detected on the skin of BSFL, it was removed and kept in a petri dish for further examination. Number of microplastics was done with manual counting and recorded separately the different source.

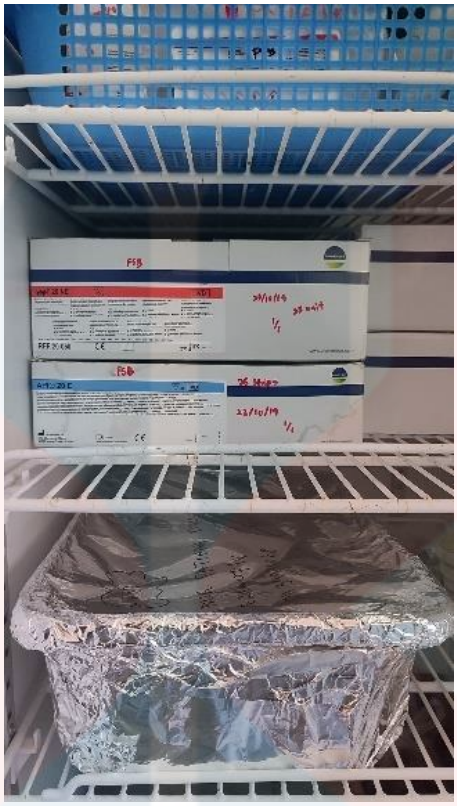


Figure 3.3. Store each BSFL sample into the chiller at the appropriate temperature level

3.2.3 Digestion of Black Soldier Fly Larvae

Sample preparation of BSFL will be preserve in an appropriate preservative to protect their conditions. Proper digestive techniques for break down the organic material in these insect samples, was used appropriate solvent sodium chloride (NaCl) solution to float microplastics from the digested samples. Sodium chloride can control plastics and could provide a safe because it's can maintain the right balance of fluid. Digestion methods enzymatic or chemical need to be used to break down organic material while preserving microplastics. Hydrochloric acid (HCl) is a useful chemical for BSFL digestion and destroyed because of its corrosive nature.

Before digestion began, the material were rinsed with ultra-pure distilled water to remove any possible laboratory contaminants and the sample were crushed for a more stable charge and transferred straight into 250mL conical flask. Each sample were added 100ml of hydrochloric acid (HCl) and 30g sodium chloride (NaCl) to each conical flask filled with sample in laminar flow cabinet. The conical flasks were to be covered with aluminium foil. The samples were be put in the laboratory shaker to be shaken for 72 hours or 120 hours equivalent to 5 days according by (Akindele et al., 2020). The shaker will configured at a temperature of 26.9 ºC and a speed of 100 RPM (Catarino, Thompson, Sinderson & Henry, 2016).

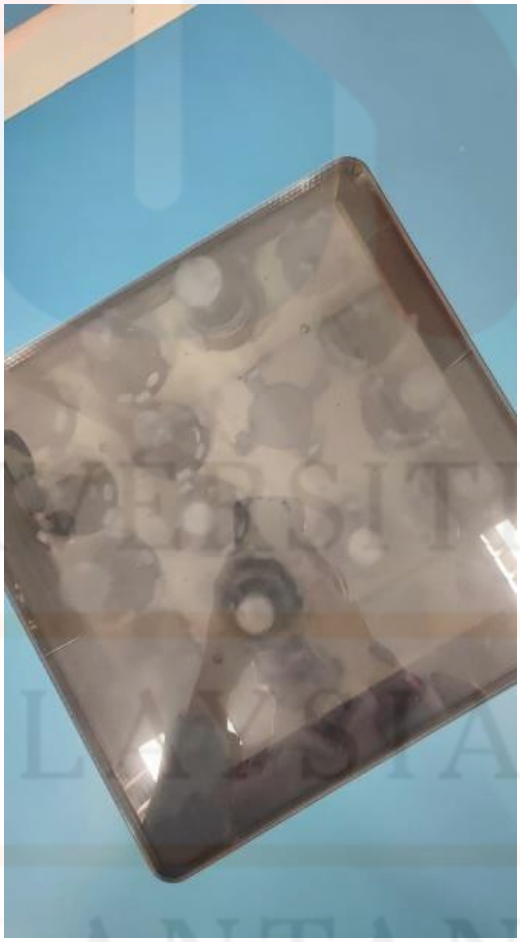


Figure 3.4. The digested samples were put in the shaker for 120 hours. Shaking with setting revolutions per minute **(**RPM SV 100) and temperature SV 26.9 ºC.

3.2.4 Filteration of Digestion

Each sample was be neutralized with distilled water and filter using Bioflow Cellulose Acetate Membrane filter paper 0.45 µm pore size or can be used Whatman Cellulose Acetate membrane filter paper (0.2 µm pore size) according by (Akindele et al., 2020). Vacuum pump was be used to filter the sample. After filtering, the filter paper was placed in a petri dish with the labelling and kept in the desiccator for 6 days at room temperature (25°C).

Figure 3.5. Bioflow Cellulose Acetate Membrane filter paper 0.45 µm pore size

3.2.5 Process of Extraction and Fourier-transform Infrared Spectroscopy

The fourier transform the most accessible method for determining the identity of polymers or microplastics is Fourier Transform Infrared Spectroscopy (FTIR). Method employed to eliminate microplastics. Utilise to describe and classify microplastics. Chloroform was be used to fully clean the capsule press mechanism before using organic tissue to dry them. Chloroform and organic tissue was be used to carefully clean and wipe the crystal. The sample was be placed on the crystal layer, and after scanning, the data will be shown. Adequate pressure will be applied, approximately 90 force, to obtain good spectra on the screen. Perform a library search to see the microplastics type search value.

Figure 3.6. Placing the filter paper on the FT-IR surface

3.2.6 Determination of Microplastics

3.2.6.1 Micro-FTIR (Micro-Fourier Transform Infrared Spectroscopy).

Micro-FTIR is an effective technique for identifying and classifying microplastics according to their chemical composition. Micro-FTIR is then used to analyse the microplastic samples that have been prepared. Infrared light is delivered through a small part of the microplastic sample that is placed on the FTIR apparatus. The resulting spectrum defined something about the microplastics' chemical composition. The model of Nicolet™ IZ10 FTIR Spectrometer will be used in the laboratory. For example, measurement of attenuated total reflectance (μATR) mode with 32 co- added scans replicates with the wavelength of 4000-500 cm-1 at a spectral resolution of 4cm-1 needs to be used. Then, the graph will be analysed using OMNIC software the microplastics.

3.2.6.2 Hot needle test.

Hot needle tests need to be used to determine microplastics in black soldier fly larvae. For example, we might see if the pieces melt or curl, meaning that pieces contain microplastics (Witte et al., 2012). Make sure the needle is hot enough and close to the pieces (Witte et al., 2012).

RESEARCH FLOW CHART

This research project took 6 months, where several key activities have been carried out.

The following activities below.

1. BSFL sampling
   - Black Soldier Fly Larvae will collect from the purchase at A BSFL Farming in Pulau Pinang, B BSFL Farming in Batu Caves Selangor and C BSFL Farming in Hulu Langat Selangor.
   - Random sampling from different three source local farming company.
   - 270 gram died and dry BSFL needed in glass container.

2. BSFL identification
   - The sample is separately from different source.
   - The identification will use stereomicroscope or microscope Rax Vision.
   - The sample is separately from external microplastics particles and put in the petri dish and capture the microplastics photo use microscope Rax Vision.
   - Use water and double cleaning the sample with distilled water.

3. Digestion of microplastics
   - 100ml hydrochloric acid (37%), 30g sodium chloride,
   - The conical flasks will cover with aluminium foil.
   - The sample were put in the laboratory shaker to be shaked for 72hour or 120 hours equivalent to 5 days according by (Akindele et al., 2020).

4. Filtering digestion

- Each sample neutralized with distilled water and filter using Bioflow Cellulose Acetate membrane filter paper (0.45µm pore size) (Akindele et al., 2020).
- Vacuum pump was used to filter the sample
- After filtering, the filter paper was placed in a petri dish with the labelling and kept in the oven or dessiccator for 6 days at room temperature (25°C).
- Three replicate

5. Microplastics determination

- Use micro-Fourier-transform infrared (µFTIR) spectroscope to determine microplastics in BSFL.
- Use hot needle test.
- If the pieces melt or curl, means that pieces contains microplastics (Witte et al., 2012).
- Make sure the needle is hot enough and close to the pieces (Witte et al., 2012).

6. Quantification

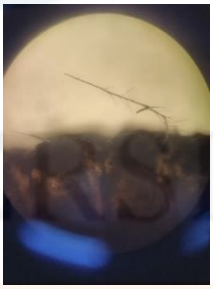
- The particle -per-gram and particle-per-organism units used in quantifying.

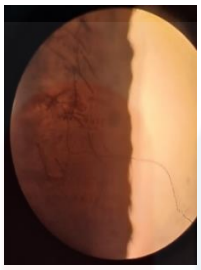
CHAPTER 4

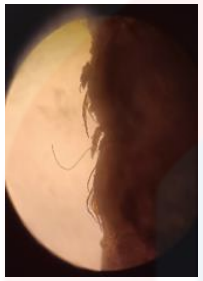
RESULT AND DISSCUSSION

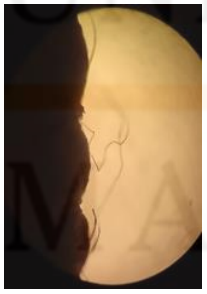
4.1 Shape and Colour of Microplastics

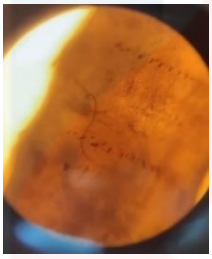
Results for the classification amounts of the microplastics are shown in Table 4.1 and Table 4.2. The microplastics that have been collected. In this research, two types of microplastics were identified which are filament and thread by using Rax Vision microscope. Filament is the most common type of microplastics that were found in B Farming compare to C Farming and A Farming. The shape of filament can be supported by scientific paper mentioned the results for filaments are in agreement with the elongated shape of this category of microplastics.

Microplastics		
Replicate 1 (B)	Replicate 2 (B)	Replicate 3 (B)
 		











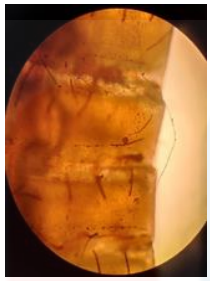
		

Table 4.1. Results of the microplastics capture on Rax Vision microscope B Farming. Replicate 1 (filament) founded. Replicate 2 (filament) founded. Replicate 3 (filament and thread) founded.

Based on Table 4.1, it showed that filament types of microplastics were found in the B Farming in three replicates. The amount of filament found were higher than the other types of microplastics which is 8 filaments. The abundance of the microplastics filament are high in replicate 1 (B) compare to replicate 2 (B) and replicate 3 (B). According by (Estahbanati & Fahrenfeld, 2016) mentioned primary microplastics consist of smooth edges or texture and symmetrical shape.

Based on table 4.2, its showed that the number of microplastics filament, thread and fibre were detected at the Black Soldier Fly Larvae surface from B Farming. Filament is the most popular types of microplastics at the Black Soldier Fly Larvae which has (14) followed by thread (1) and fibre (1). These research can be supported by (Hidalgo-Ruz et al., 2012), mentioned today's studies give the impression that microplastics are small fragments that are separate from plastic and have a millimetre-sized range.

Hot needle tests result shown the microplastics from manual counting is burn and melt when needle hot enough touched the surface. According by (Witte et al., 2012), mentioned if the pieces melt or curl, meaning that pieces contain microplastics and make sure the needle is hot enough and close to the pieces. The needle was to sterile with fire by bunsen burner to safety and contamination.

Polymer shape	Colour	Total
Filament	White transparent	14
Thread	Black	1
Fibre	White transparent	1

Table 4.2. Polymer shape, colour and total used in manual counts of physical detection of microplastics in Black Soldier Fly Larvae

There are two colours of microplastics that have been detected at the Black Soldier Fly Larvae. White transparent was the most common colour that found at the Black Soldier Fly Larvae from B Farming. The black colour of microplastics also been identified in the physical detection manual collected. According by (Abu-Hilal & Al-Najjar, 2009), he mentioned the colours are one of the classifications of microplastics and for the majority polypropylene has transparent and clear coloured.

4.2 Composition of Microplastics

There were 5 types of microplastics particles that have been collected in BSFL farming. There are diverse types of microplastics polymers that have been identified through fourier transform spectroscopy (FT-IR), including Polyethylene (PE),

Polypropylene (PP), Polydiene, Polyethylene Mix and Nylon 12. The results of fourier transform spectroscopy (FT-IR) are shown in Table 4.3.

Types	A BSFL Farming	B BSFL Farming	C BSFL Farming
Polyethylene (PE)	+	+	-
Polypropylene (PP)	+	+	-
Polydiene	+	+	-
Polyethylene Mix	-	+	-
Nylon 12	-	+	-

Table 4.3. The occurrence of microplastics from FT-IR analysis

Based on Table 4.3, there were 5 totals of plastics types according to the composition of the microplastics that were found in Black Soldier Fly Larvae. As the largest number of microplastics type found in this research, Polyethylene (PE) and followed by Polypropylene (PP) and Polydiene. According by (Lenz et al., 2015; Antunes et al., 2013; Gesamp, 2016), mentioned polymers of polyethylene (PE) and polypropylene (PP) that dominantly found in all environment section.

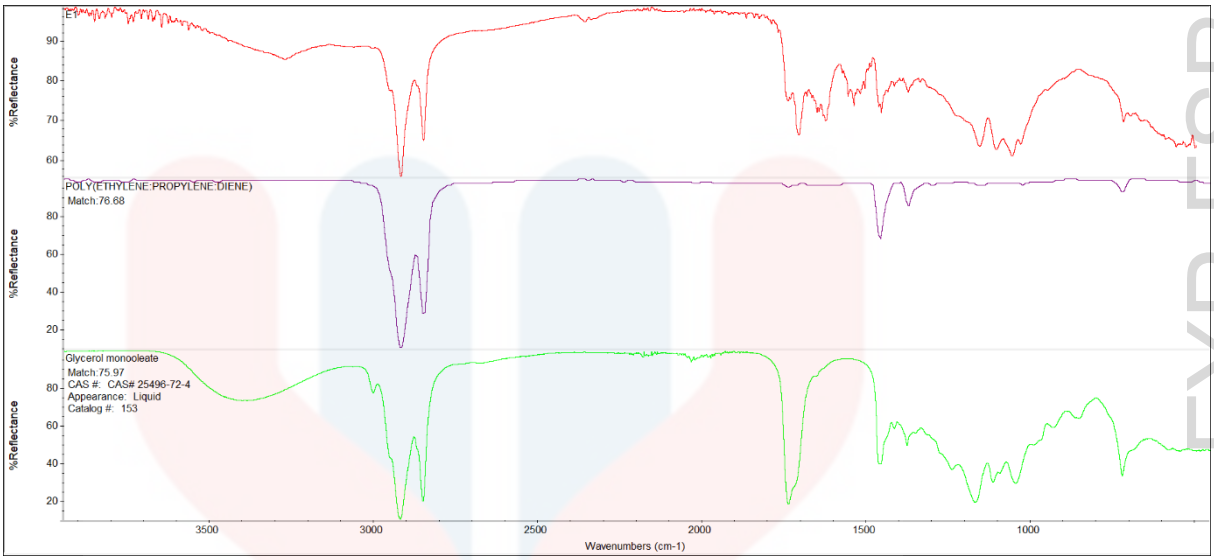


Figure 4.1. FT-IR spectrum showing the Polyethylene (PE), Polypropylene (PP), and Polydiene composition in BFSL (replicate 1) from A Farming

Polyethylene (PE) is the most economical and widely used which is utilised in every circumstance or product. PE can be processed easily and can be transformed into varieties of shapes and forms. PE has the ability to alter easily during processing, thus gives relatively longer chain length, density, and crystallinity, allowing PE products to have tailored properties for a variety of applications.

Polyethylene is a thermoplastic material, an extremely large range of application and industries also can become a thermoset plastic when modified according by (Plastics Europe, 2017). Thermoset a type of plastic which cannot be recycle due its composite chemical structure. In Figure 1, Figure 2 and Figure 3, the FT-IR spectrum showed the Polyethylene (PE), Polypropylene (PP), and Polydiene found in BFSL (replicate 1) from A Farming.

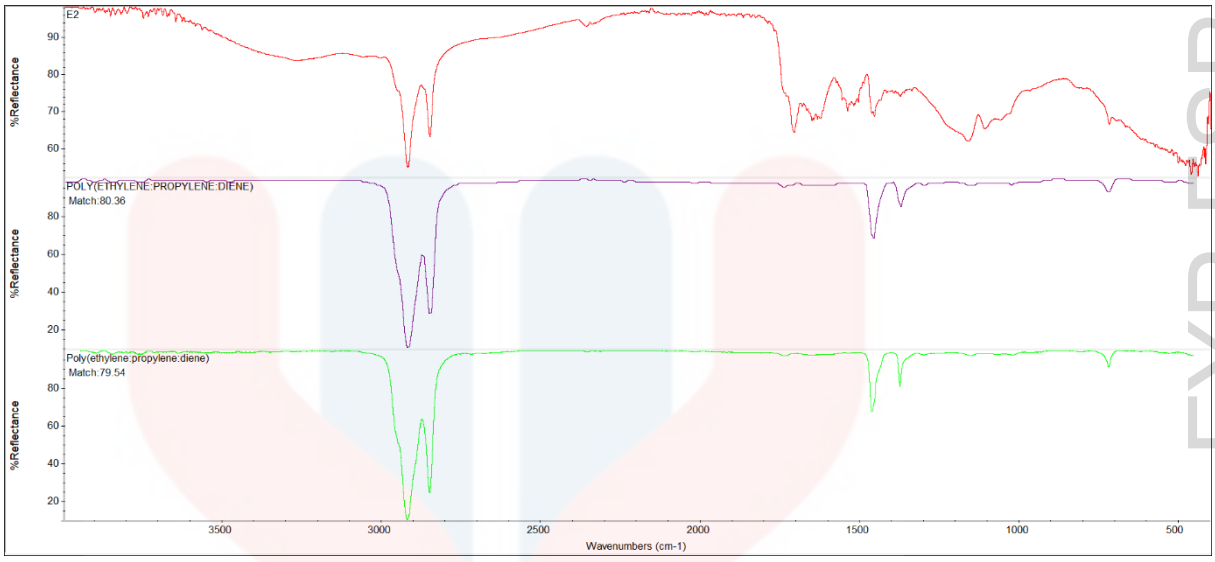


Figure 4.2. FT-IR spectrum showing the Polyethylene (PE), Polypropylene (PP), and Polydiene composition in BFSL (replicate 2) from A Farming

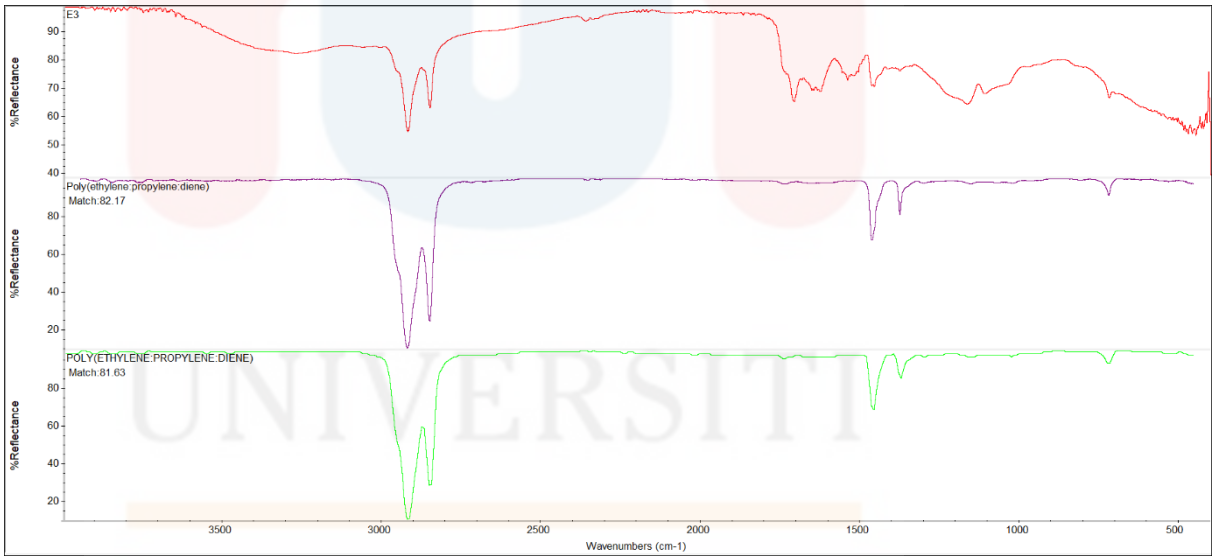


Figure 4.3. FT-IR spectrum showing the Polyethylene (PE), Polypropylene (PP), and Polydiene composition in BFSL (replicate 3) from A Farming

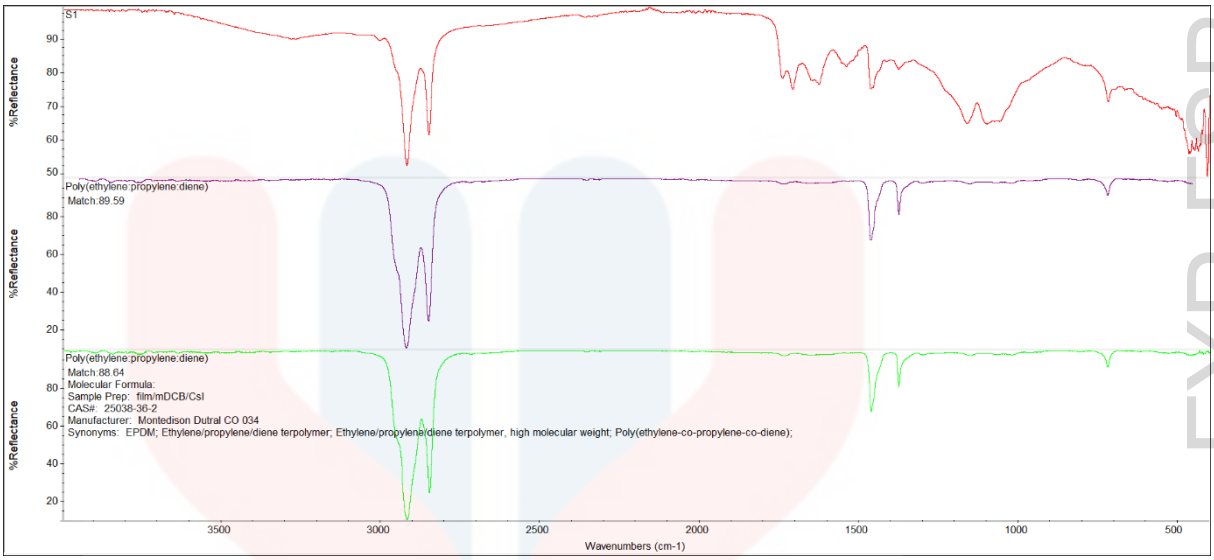


Figure 4.4. FT-IR spectrum showing the Polyethylene (PE), Polypropylene (PP), and Polydiene composition in BFSL (replicate 1) from B Farming

Polypropylene (PP) is a thermoplastic material used in numerous applications that includes household items, labeling, packaging and textiles. These can be supported by other research, mentioned PP is broadly manufactured polymers, particularly for the auto industry because of its low cost and high processability. In Figure 4, FT-IR spectrum showed the Polyethylene (PE), Polypropylene (PP), and Polydiene found in BFSL (replicate 1) from B Farming.

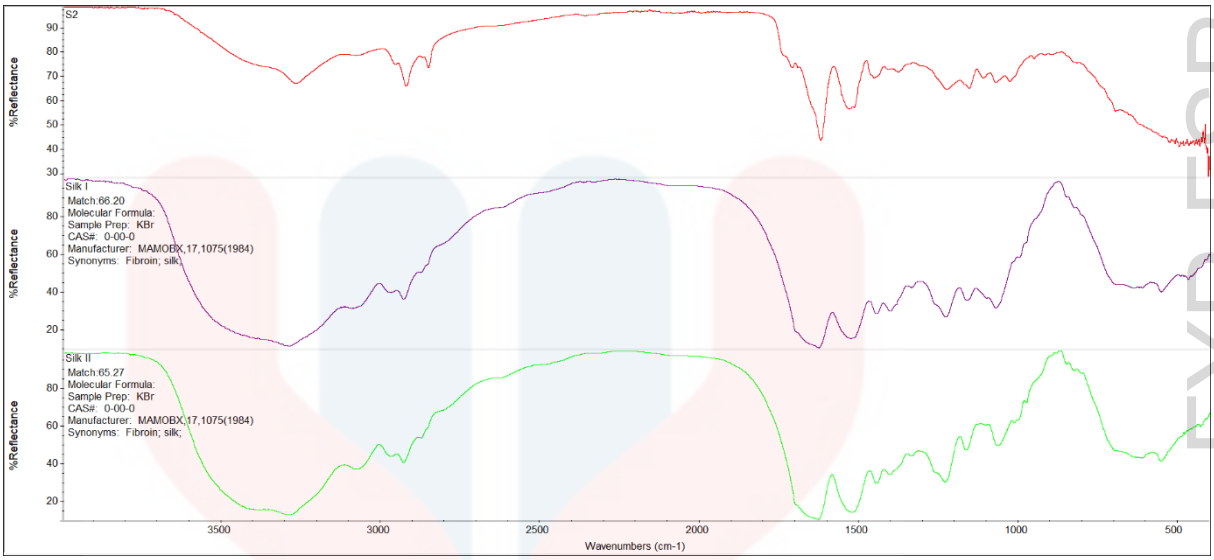


Figure 4.5. FT-IR spectrum showing no microplastics composition in BFSL (replicate 2) from B Farming

Silk generally comes from nature in fibre form from cocoons. Silks are generally recognized for their unique combination of mechanical properties, including remarkable strength and toughness. Silk is composed of natural protein fibre known as fibroin. Silk has played an significant part in the advancement of loom and weaving technology. Silks belong to a group of organic polymers with high molecular weights according by (Altman et al., 2003). In Figure 5, FT-IR spectrum showed the Silk I and Silk II found in BFSL (replicate 2) from B Farming.

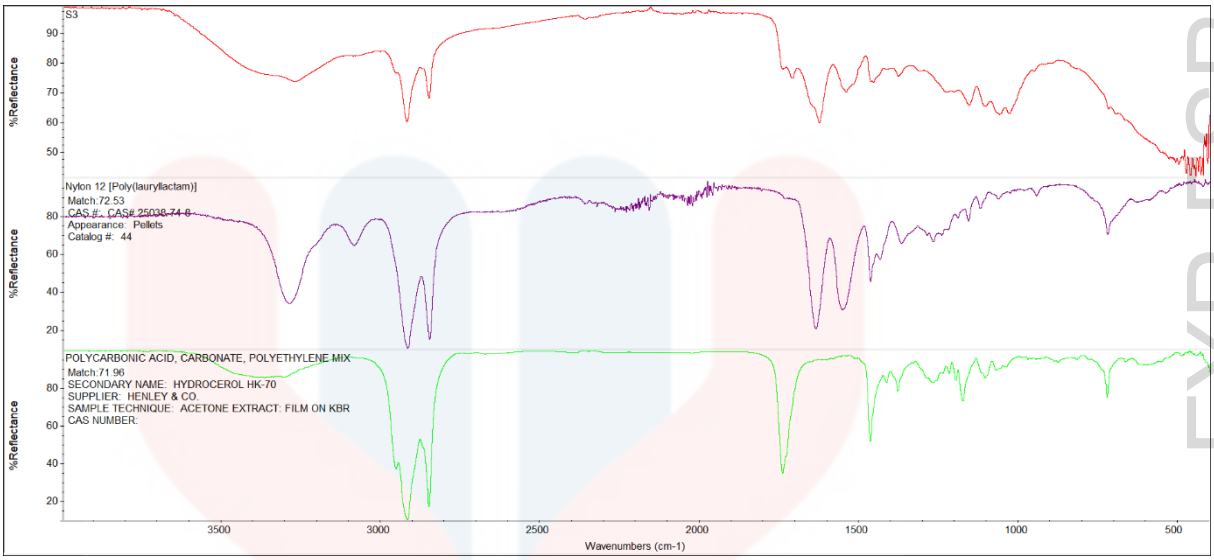


Figure 4.6. FT-IR spectrum showing the Nylon 12 and Polyethylene Mix composition in BFSL (replicate 3) from B Farming

Nylon is a versatile and thermoplastic polymer that can be converted to fibres, films, and different shapes through the melting, forming, and cooling processes. Nylon has excellent mechanical qualities because it has been widely used to produce clothes, manufactured plastics, and food packing films. Nylon is a semi crystalline synthetic polymer that belongs to the family of polyamides. Nylon 12 is utilised in various industrial areas regardless of having longer aliphatic chains, poorer mechanical strength, and melting point. Figure 6, FT-IR spectrum showed the Nylon 12 and Polyethylene Mix found in BFSL (replicate 3) from B Farming.

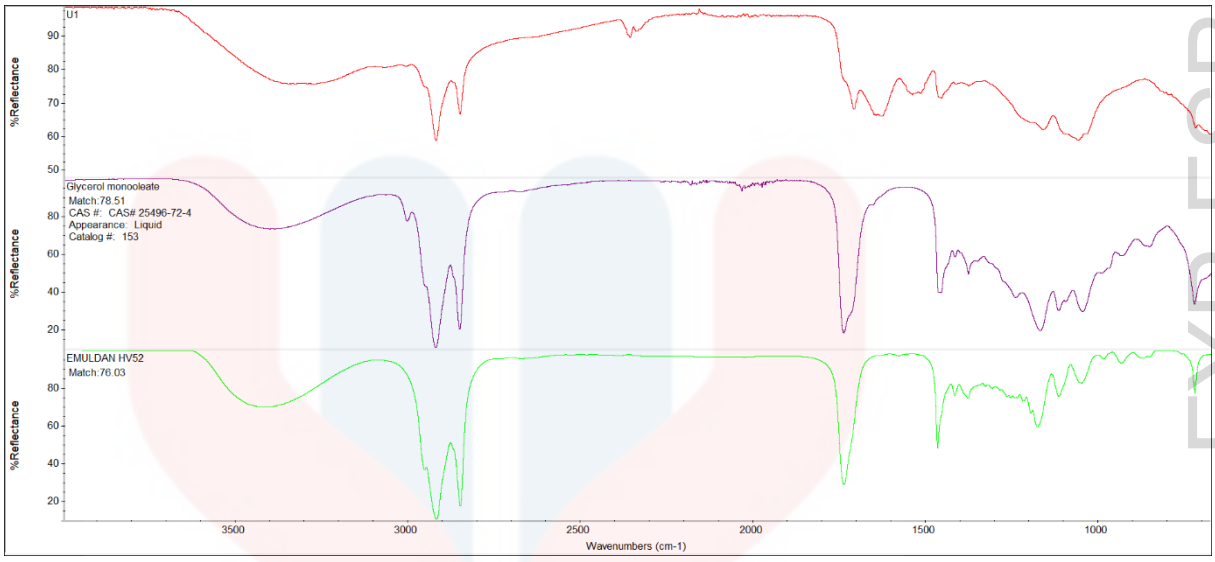


Figure 4.7. FT-IR spectrum showing no microplastics composition in BFSL (replicate 1) from C Farming

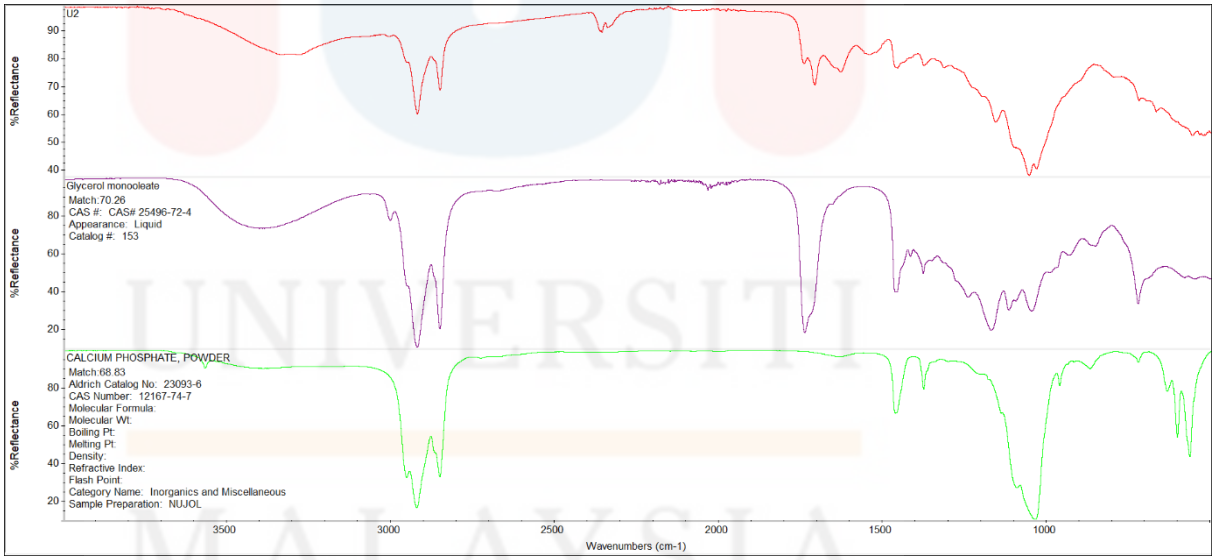


Figure 4.8. FT-IR spectrum showing no microplastics composition in BFSL (replicate 2) from C Farming

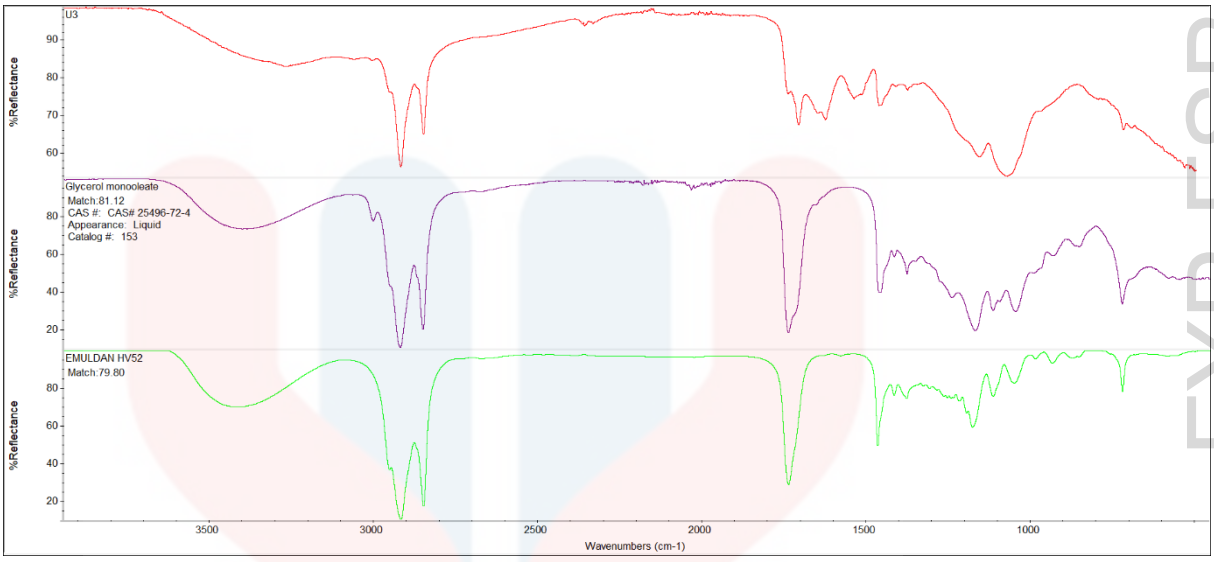


Figure 4.9. FT-IR spectrum showing no microplastics composition in BFSL (replicate 3) from C Farming

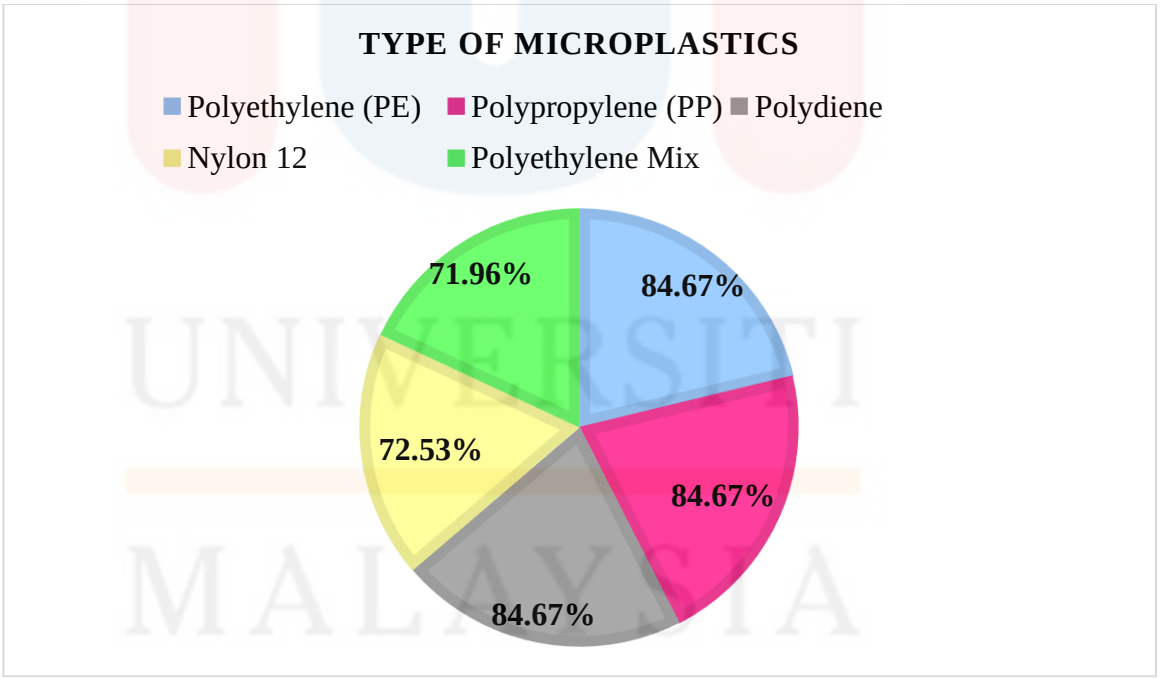


Figure 4.10. The percentage of reflectance for microplastics from FT-IR spectrum result for overall

Plastics have changed our life since their appearance in the middle of the last century according by (Thompson et al., 2009). They are massively used as they replaced traditional materials due to their multiple advantages. Plastic was designed to make life better and quality to human, but today it has become a serious threat to the environment and the safety of the planet according by (Millican J.M., 2021).

Factor influencing the presence of microplastics may come from plastic packaging. Contact between larvae and plastic packaging is almost always the cause of mutual transfers between container and contents. The quality of food or larvae products is influenced by the contamination resulting from the interaction with the substances in the composition of the packaging, sometimes the alteration of the nutritional qualities being doubled and affecting the safety of consumption.

Application of resources from nylon and silk during larvae management can be factor influencing the presence of microplastics. The quality of glove come from fibres can influenced by the contamination resulting from the interaction with the substances in the composition of the feeding procedure for larvae. The fibres attaches to the outer member of the larvae indirectly and involuntarily.

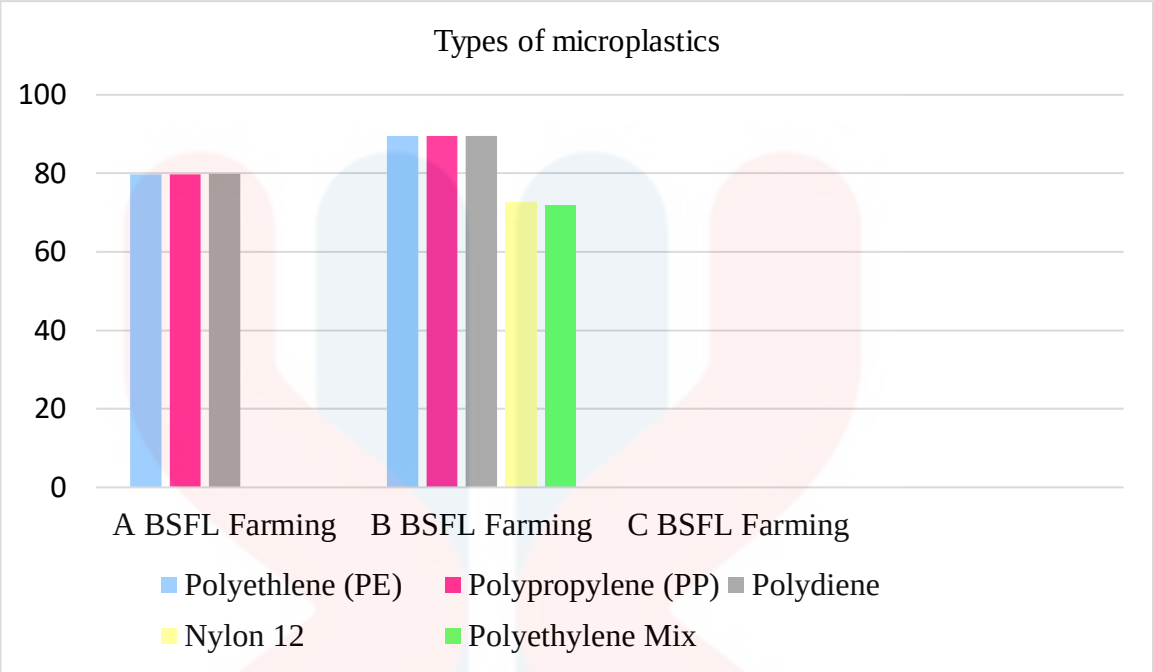


Figure 4.11. Types of microplastics found through FT-IR spectrum result

Based on Figure 11, there result shown A BSFL Farming is higher Polyethlene (PE), Polypropylene (PP), and Polydiene of microplastics 79.74%. B BSFL Farming is highest Polyethlene (PE), Polypropylene (PP), and Polydiene of microplastics 89.59%. B BSFL shown is more types of microplastics include Polyethlene (PE), Polypropylene (PP), Polydiene, Nylon 12 and Polyethylene Mix. C BSFL Farming shown is none microplastics.

4.3 Effect microplastics to consumers

The gradual accumulation of microplastics in the environment is becoming a significant concern for both the environment and human health. Microplastics can enter human bodies through ingestion, contact, and inhalation. Humans get their protein sources especially through fish, chicken, seafood and eggs. It's can be

supported by (Anderson A.G, 2016) mentioned the research shows that the most common exposure comes from ingestion, so the main mode of exposure is diet. Microplastics particles were found in human feces, chiefly polyethylene terephthalate and polypropylene according by (Schwabl et al., 2019), and single microplastics particles were found in human placenta mentioned by (Ragusa et al., 2021).

According to recent research, plastic materials, micro and nano particle-sized plastic particles, the compounds are linked to may be harmful to human health according by (Rist et al., 2018). The number of toxicological investigations on microplastics is rising quickly. According to experiments, exposure to microplastics may cause a range of negative consequences, such as oxidative stress, metabolic disorders, immunological responses, neurotoxicity, and developmental and reproductive toxicity mentioned by (Sarawut Sangkham, 2022).

Microplastics exist in our daily necessities like drinking water, seafood and protein sources according by (Praveena, 2021). Black Soldier Fly Larvae which contains microplastics also have been widely distributed in fish and chicken, especially in aquacultural systems. They especially with negative charge can get into the human body because human consume the protein source everyday. Once microplastics enter human it's will cause food pollution and raise the possibility of human exposure.

Fish contaminated with microplastics will experience a negative impact on their development. Microplastics were found in the dorsal muscles and gastrointestinal system of the specimens that were roughly inspected. According to (Barboza et al., 2019), exposure to microplastics in fish causes elevated levels of lipid peroxidation in

the brain, gills, and spinal muscles. It likewise boosts the activity of acetylcholinesterase in the brain.

Comprehensive review of the experimental research showing substantial harmful or infectious effects of microplastics in fish was carried out by (Yong, Valiyaveetill, and Tang, 2020). Toxic reactions generally arise from smaller particles less than five micrometre. Microplastics causes alterations in the gut microbiome, oxidative stress and neutrophil infiltration of the mucosa and structural damage.

Microplastics can disturb the feeding behavior and motor activity in adult fish and fry, also to negatively affect fertility. Microplastics slow down the growth of fry and reduce the expression of genes important in development which lowers the fry's post-hatching survival rate. This is can be supported by other researcher mentioned, there is evidence that mothers can pass on microplastics to their children has been documented (Pitt et al., 2018), along this addition to proof that early fry development is impacted by prenatal exposure to microplastics according by (Wang et al., 2019).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, microplastics were detected in the Commercial Black Soldier Fly Larvae. The abundance of microplastics in B Farming is higher which is linked with the factor influencing the presence of microplastics may come from plastic packaging and application of resources from nylon and silk during larvae management. The amount of filament found were higher than the other types of microplastics and white transparent is the most common colour of microplastics that were detected from Black Soldier Fly Larvae for one farming only. The most common type of microplastics are Polyethlene (PE), Polypropylene (PP), and Polydiene are the main polymer compositions found in the Black Soldier Fly Larvae that have been observed by FTIR. Based on this study, the microplastics pollution needs an concentration and good attention to commercial insect. The presence of microplastics in larvae poses a significant threat to the food chain. These tiny plastic particles can accumulate as they move up the food chain, potentially leading to harmful effects on both aquatic living entities and humans who consume these organisms. The ingestion of microplastics by larvae can introduce toxic substances and disrupt biological processes, making them unsafe for consumption and raising serious environmental and health concerns. This is particularly concerning because commercial BSFL are widely used as a food source in aquaculture. An extensive study of microplastics, particularly in BSFL in Malaysia, is necessary to gain more detailed information on microplastics contamination.

5.2 Recommendation

Through the progress in conducting this research study, there are several limitations have been encountered and opinions have been suggested in order to provide advantages and convenience for others researchers to acknowledge and develop modification.

The first limitation that has been discovered is the controlling speed chemicals during digestion. Hydrochloric acid should be poured slowly and gradually into the material lab or glass material containing the sample to avoid high reactions such as foam overflow. This is because hydrochloric acid is highly acidic and quick to react. This accident also can effected the sample amounts. The sample need to be extra in case the sample suffered damage due to chemical abuse.

The second limitation that has been discovered is the unsuitable chemicals used at the part of digestion examination. The density separation procedure is one of the extraction techniques that can impact the concentration of microplastics. Choose Zinc Chloride with a higher density than Sodium Chloride is essential for scenarios where the density of the salt may disrupt the density separation process. This is because high density solution is more suitable to use during digestion.

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APPENDIX

FT-IR analysis.
This is FT-IR spectrum result for nine replicates from A BSFL Farming, B BSFL Farming and C BSFL Farming.