



**DETERMINATION OF MICROPLASTICS IN
RICE BEETLE, *Sitophilus oryzae*
(COLEOPTERA: CURCULIONIDAE)**

By

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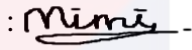
A report submitted in fulfillment of the requirements for the degree of
Bachelor of Applied Science (Natural Resources Science) with
Honours

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2024

DECLARATION

I declare that this thesis entitled “Determination of Microplastics in Rice Beetle, *Sitophilus oryzae* (Coleoptera: Curculionidae)” 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.

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Determination of Microplastics in Rice Beetle, *Sitophilus oryzae*
(Coleoptera: Curculionidae)

ABSTRACT

This study was done to identify the presence of microplastics in rice beetles and they are a major problem for the environment and human health in many different ecosystems. The purpose of this study is to clarify if rice beetles, a common bug linked to stored grains and a possible sign of food contamination, contain microplastics. A comprehensive process for collecting and processing samples is part of the study approach. Rice beetle samples were collected from local rice and stored in glass containers. During the dissection procedure, special attention was taken to avoid external contamination. Advanced analytical methods which is Fourier-transform infrared spectroscopy (FTIR) was used to detect and quantify microplastics. According to this study, rice beetles consume microplastics in their surroundings, such as contaminated grain surfaces or packaging materials. Microplastics enter the human food chain due to consumption such as plastic packaging, endangering consumers' health. The types of microplastics found in the beetles were identified by this study as polymethylpentene (PMP), ethylene-acrylic acid copolymer (EAA), styrene butadiene copolymer (SBC), and ethylene propylene diene monomer (EPDM). Given the widespread usage of these polymers in packing materials, the materials and storage methods utilised in grain storage facilities are directly related to the source of contamination. This study highlights the need for better storage procedures and strategies to lower contamination by detecting the presence of microplastics in grains that have been kept. Maintaining microplastic pollution-free storage facilities can improve food supply safety and quality while safeguarding consumer health.

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**Penentuan Mikroplastik dalam Kumbang Beras, *Sitophilus oryzae*
(Coleoptera: Curculionidae)**

ABSTRAK

Kajian ini dilakukan untuk mengenal pasti kehadiran mikroplastik dalam kumbang padi dan ia merupakan masalah utama bagi alam sekitar dan kesihatan manusia dalam banyak ekosistem yang berbeza. Tujuan kajian ini adalah untuk menjelaskan sama ada kumbang padi, pepijat biasa yang dikaitkan dengan bijirin yang disimpan dan kemungkinan tanda pencemaran makanan, mengandungi mikroplastik. Proses yang komprehensif untuk mengumpul dan memproses sampel adalah sebahagian daripada pendekatan kajian. Sampel kumbang beras dikumpul daripada beras tempatan dan disimpan dalam bekas kaca. Semasa prosedur pembedahan, perhatian khusus telah diambil untuk mengelakkan pencemaran luaran. Kaedah analisis lanjutan iaitu Fourier-transform infrared spectroscopy (FTIR) telah digunakan untuk mengesan dan mengukur mikroplastik. Menurut kajian ini, kumbang padi mengambil mikroplastik di persekitaran mereka, seperti permukaan bijirin yang tercemar atau bahan pembungkus. Mikroplastik memasuki rantai makanan manusia kerana penggunaan seperti pembungkusan plastik, membahayakan kesihatan pengguna. Jenis mikroplastik yang terdapat dalam kumbang telah dikenal pasti oleh kajian ini sebagai polymethylpentene (PMP), kopolimer asid etilena-akrilik (EAA), kopolimer stirena butadiena (SBC), dan etilena propilena diena monomer (EPDM). Memandangkan penggunaan meluas polimer ini dalam bahan pembungkusan, bahan dan kaedah penyimpanan yang digunakan dalam kemudahan penyimpanan bijirin adalah berkaitan secara langsung dengan sumber pencemaran. Kajian ini menyerlahkan keperluan untuk prosedur dan strategi penyimpanan yang lebih baik untuk mengurangkan pencemaran dengan mengesan kehadiran mikroplastik dalam bijirin yang telah disimpan. Mengekalkan kemudahan penyimpanan bebas pencemaran mikroplastik boleh meningkatkan keselamatan dan kualiti bekalan makanan sambil menjaga kesihatan pengguna.

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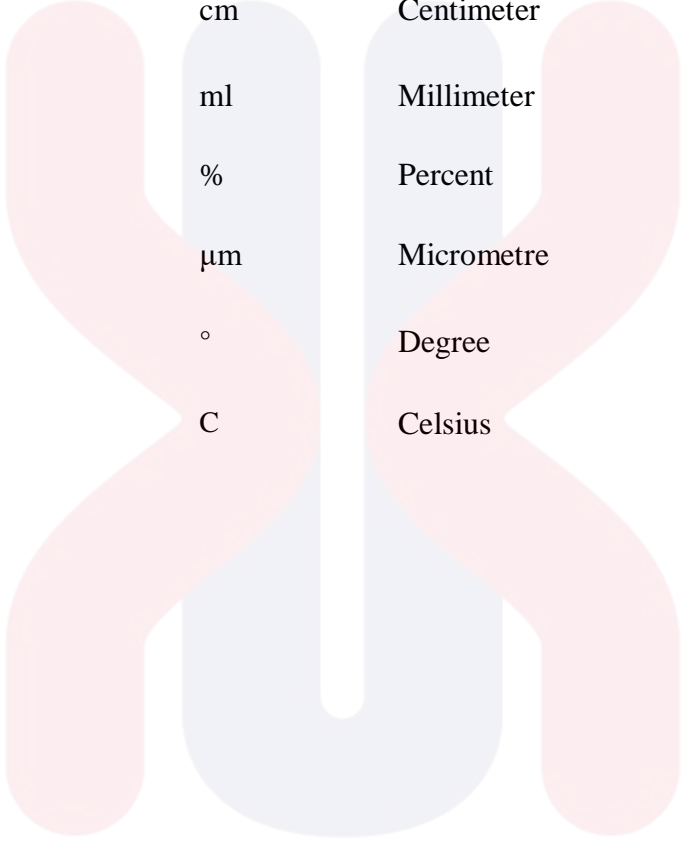
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LIST OF ABBREVIATIONS

FTIR	Fourier-transform infrared spectroscopy
MPs	Microplastics
EPDM	Ethylene Propylene Diene monomer
PMP	Polymethylpentene
SBC	Styrene Butadiene Copolymer
EAA	Ethylene-acrylic acid copolymer
HCl	Hydrochloric acid
NaCl	Sodium chloride
UV	Ultraviolet

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LIST OF SYMBOLS

cm	Centimeter
mm	Millimeter
%	Percent
μm	Micrometre
°	Degree
C	Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Microplastics are little plastic particles, often with a diameter of less than 5 millimeters. 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).

The relationship between rice beetles (*Sitophilus oryzae*) and a main food source makes them an interesting study subject. They are typically found in stored grains. Given their propensity to infest rice and other grains that have been stored, these insects might serve as a vector for the spread of pollutants up the food chain (Sousa et al., 203). In order to assess the possible consequences of consuming contaminated grains and address the wider ecological ramifications, it is imperative to determine the level of microplastic contamination in rice beetles.

This study also fits to provide useful information by examining the presence of microplastics in rice beetles that can be used for the purpose of reducing plastic pollution in food chain and environmental settings. To sum up, the study's motivation stems from the pressing need to comprehend and tackle the effects of microplastic pollution in a significant insect species linked to a staple food supply. In addition to advancing our knowledge of microplastics in terrestrial ecosystems, this research has applications in the fields of environmental preservation and food safety.

1.2 Problem Statement

The possible contamination of food chains and the threats that microplastics bring to the environment and human health are the issues that the study on the detection of microplastics in rice beetles attempts to address. Originating from the break-down of bigger plastic products, microplastics have spread across many ecosystems as contaminants. Microplastics have been well investigated in marine habitats, but less is known about how they arise in terrestrial ecosystems (Thushari et al., 2020), especially when they are present in the presence of agricultural species like rice beetles.

It is well known that rice beetles (*Sitophilus oryzae*) infest these primary food sources since they are frequently discovered in stored grains, particularly rice. There is a worry that rice beetles might act as carriers of microplastics into the human food chain due to their relationship with grains that humans eat. The main focus of the issue statement is the requirement to determine the level of microplastic contamination in rice beetles, identify the sources of these pollutants, and analyse the possible threats to ecosystems and public health.

Additionally, a gap in our knowledge of the ecological effects of plastic pollution is limited by the paucity of thorough data on microplastics in terrestrial creatures, particularly those implicated in the food chain. Developing practical methods to lessen the effects of microplastics on the environment and human health is complicated by this information gap.

1.3 Objectives

- i. To determine the occurrence of microplastics within the rice beetle.
- ii. To characterize the microplastics within the rice beetle.

1.4 Scope of Study

This study focused on rice beetle (*Sitophilus oryzae*). This investigation was carried out in the laboratory. The rice beetle was sampled from glass rice container. Microplastic characterisation was determined by using Fourier-transform infrared spectroscopy (FTIR). FTIR is a powerful analytical technique used to identify and analyze the chemical composition of substances based on their interaction with infrared light.

The determination of microplastics in rice beetles involves a comprehensive investigation aiming to understand the presence, sources, and potential impact of microplastics in these insects. The scope of this study strictly follows the proposed main objectives for this study, to ascertain if rice beetles contain microplastics and to characterize the microplastics that are present.

1.5 Significant of Study

This study is to provide an initial baseline data of microplastic types found in rice beetles. As a consequence, it will increase the knowledge of understanding the origins and movements of microplastics in terrestrial settings. For the purpose of creating focused initiatives to reduce plastic pollution, this knowledge is essential. Henceforth, risk evaluations related to microplastic exposure can benefit from research on microplastics in rice beetles. The results might direct the creation of mitigating measures to lessen the effects of microplastics on the environment and perhaps human health.

Environmental policy and management techniques can benefit from this study. The information may be used by governmental bodies and environmental groups to create plans for reducing microplastic contamination in agricultural 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.

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastics

Polymers, which are big molecules formed of repeating structural units, are the raw materials used to create synthetic materials like plastic. Because of its cost-effectiveness, durability, and adaptability, it has become an essential component of modern living. Plastics come in a variety of forms, each having special qualities and applications. Polystyrene, polypropylene, polyethylene, and polyvinyl chloride (PVC) are examples of common kinds. Polymerization is the process by which petrochemicals are usually converted into plastics. On the other hand, bio-based polymers manufactured from renewable resources like cornflour or sugarcane have been developed as a result of technological developments. Larger plastic items have the ability to degrade over time into tiny particles known as microplastics (Hassan, 2017). These microscopic particles have the potential to endanger human health and the environment. They may also find their way into the food chain.

Microplastics are tiny bits of garbage or detritus that are made of plastic and are less than 5 mm (0.2 inch) in size. Primary and secondary microplastics are the two different categories of microplastics. The classification of microplastics is different for primary and secondary types (Browne et al., 2015). Primary microplastics are directly produced within the micrometer measurement range, and they are likely to flow from residential or commercial drainage systems and into wastewater treatment channels. Laundry and washing machine discharges emit a large number of synthetic fibers into wastewater. Secondary microplastics are created because of continued UV

light exposure, the weathering process, and physical abrasion, as well as meso-plastics (> 5.0 mm) and macro-plastics (greater than a few centimetres) litter fragmentation (Gall et al., 2015). In fact, plastic is a UV-sensitive material, and exposure to UV light can produce oxidative processes that ultimately lead to deterioration, which shortens plastic's outdoor life. Another important reason for secondary microplastic is mechanical degradation, which happens when resistance forces that arise during movement through the various habitats of the environment tear resistant material into smaller particles (Li et al., 2018).

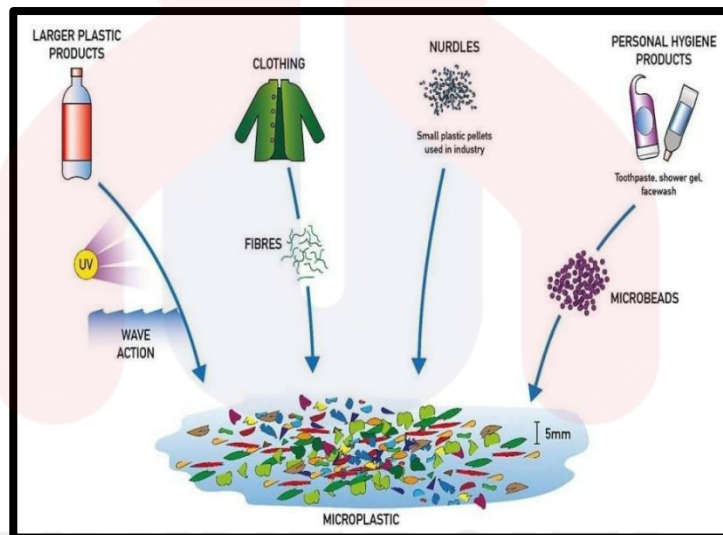


Figure 2.1: Occurrence of microplastics

2.2 Effects of microplastics to ecosystem and living biota

Microplastics may affect many levels of biological organisation, ranging from individual species to entire ecosystems, and can have a wide range of consequences on ecosystems (Alimba & Faggio, 2019). Fish, plankton, and invertebrates are among the aquatic creatures that frequently consume microplastics. When microplastics are ingested and migrate up the food chain, a process known as bioaccumulation may occur. As a result, predatory animals (including humans) may ingest more microplastics (Alfaro-Nunez et al., 2021). Microplastics that are ingested can physically hurt organisms by obstructing digestive systems, causing internal damage,

or preventing them from eating and absorbing nutrients (Ma et al., 2020). Microplastics have the ability to penetrate tissues and cells due to their tiny size, which may result in cellular damage, potential to carry infections as vectors and have the ability to attract pathogens, which might aid in their movement across ecosystems and perhaps aid in the transmission of illnesses among living things (Lu K. et al., 2018).

Microplastics' impact on live biota (organisms) can differ according on the kind of creature, the size and makeup of the microplastics, the length of exposure, and the particular environmental circumstances (Aragaw, 2021). Plankton and bivalves are examples of organisms that filter feed and can consume microplastics with their food. This consumption may result in impaired food absorption, decreased feeding efficiency, and bodily harm. By eating prey that has previously consumed microplastics, predatory creatures may unintentionally consume these particles. Higher trophic level creatures may accumulate larger amounts of microplastics as a result, a process known as bioaccumulation. Microplastics that are ingested have the potential to physically damage an organism's internal organs and digestive system. Microplastics can infiltrate cells due to their tiny size, which raises the possibility of oxidative stress, inflammation, and other physiological problems (De Sa et al., 2015). According to some research, hormonal systems in organisms may be upset by exposure to microplastics, which might have an impact on development and reproduction (Guvén et al., 2018). For animals with intricate life cycles and delicate reproductive systems, this is especially worrisome. In several species, exposure to microplastics has been connected to reproductive consequences. This involves decreased fertility, changed reproductive habits, and aberrant offspring development. Endocrine-disrupting chemicals and contaminants that adsorb into microplastics are possible. These chemicals have the ability to disrupt an organism's hormonal balance, which can impact its ability to reproduce (Mu et al., 2021).

2.3 Previous studies

Year	Author	Previous studies
2023	Jie Shen, Boying Liang, & Hui Jin	The impact of microplastics on insect physiology and the indication of hormesis.
2023	Jianqiang Zhu, Pengfei Wu, Nan Zhao, Shengtao Jiang, Huayue Zhu, & Hangbiao Jin	Microplastics in terrestrial insects, long-horned beetles (Coleoptera: Cerambycidae), from China.
2022	Witwisitpong Maneechag & Taeng On Prommi	Occurrence of microplastics in edible aquatic insect <i>Pantala</i> sp. (Odonata: Libellulidae) from rice fields.

Table 2.1: Previous studies on the microplastics in insect

Based on the previous study, there are three journal about effect of microplastics in different types of insect.

2.4 Description of the rice beetle

In general, the word "rice beetles" refers to the common pest *Sitophilus oryzae*, which is scientifically known to affect stored products. Small bugs known as rice beetles contaminate grains that have been kept, such as rice, wheat, barley, oats, and other cereal goods (Hunt et al., 2007). The length of an adult rice beetle is only 2 to 3 mm. Their body is oval-shaped and elongated and adults usually have a dark brown to black body colour. Their wing coverings may feature four different reddish-yellow patches. The long, thin snout of rice weevils is one of its distinguishing characteristics. The purpose of this snout is to drill into grains (Stork et al., 2015).

Known to infest a range of cereal goods, rice beetles are a significant pest of grains that are kept. They can get at the grains that are being kept by penetrating the packing materials. The egg, larva, pupa, and adult stages make up the rice beetle's life cycle. Grains are the larval host of female beetles, whose eggs hatch into kernels. In order to feed on the inside of grains, rice beetles harm the endosperm in both their adult and larval phases. Kitchens, food processing rooms, and storage facilities are vulnerable to infestations of rice weevils because they like warm, humid environments (McKenna et al., 2009).

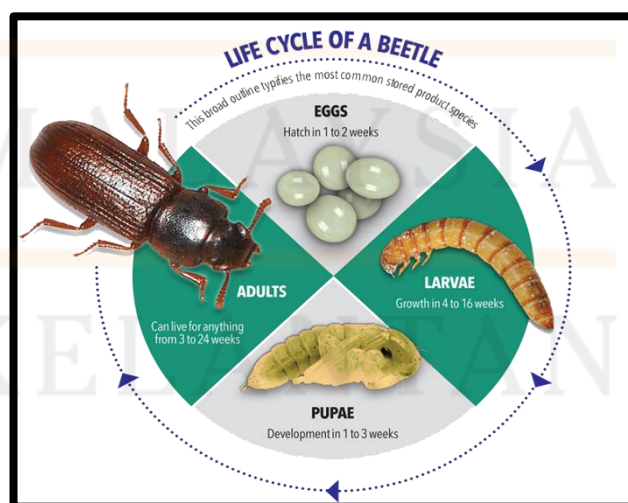


Figure 2.2: Life cycle of a rice beetle

2.5 Roles and feeding guilds of the rice beetle

Sitophilus oryzae, the rice beetle, has specialised functions in its ecological niche, however many of these functions are deemed harmful when it comes to stored grains. When it comes to stored grains, such as rice, wheat, barley, oats, and other cereal goods, rice beetles are mostly considered a nuisance. Food items that have been kept are contaminated and damaged by their infestation (Longstaff, 1981). The endosperm of grains is the primary food source for rice beetles, who harm stored harvests by eating them. Farmers and the food business may suffer financial losses as a result of this. Food items that are kept may become contaminated as a result of rice beetle infestations. As a result, neither humans nor animals should eat the impacted grains. Because of their ability to damage packing materials, rice beetles can enter stored items and multiply throughout them. This may make the infestation worse and more difficult to manage. Grain is where female rice beetle eggs are laid. As the larvae grow, they consume the inner part of the grain while developing inside the kernels. Although rice beetles are not usually thought of as useful in natural environments, they might aid in the breakdown of plant matter through their feeding activity in stored grains (Okram, 1963).

Sitophilus oryzae, often known as rice beetles, are granivores that feed mostly on grains and seeds. As specialised granivores, rice weevils mostly consume the endosperm of cereal grains, such as wheat, barley, oats, and rice, as well as other goods that have been kept. They have specialized mouthparts that allow them to pierce the grain and feed on the starchy interior. The rice beetle larvae grow inside the grain kernels. They eat the inside part of the grain, causing serious harm and degrading the quality of harvests that are kept. Rice beetles are seed predators that prey on plant reproductive organs, especially seeds. They can result in significant losses in grain products that are stored. Rice beetles may also be detritivores in their

native habitat, aiding in the breakdown of plant matter in the form of grains that have been stored (Subedi et al., 2009).

2.6 Types of method

Typically, a combination of sample preparation, extraction, and identification procedures are used in the investigation of microplastics in organisms. Gather rice beetles from the glass rice container first. Make sure to handle things properly to prevent infection when collecting. To visually detect and describe the microplastics in the sample, use microscopy methods like compound microscopy. Use methods such as Fourier-transform infrared spectroscopy (FTIR) to identify microplastics chemically. In some subsamples, microplastics may be manually counted under a microscope.

CHAPTER 3

MATERIAL AND METHODS

3.1 MATERIALS

In this study, to determine the microplastics in rice beetles using the following materials and equipment:

Table 3.1 Materials and apparatus

No.	Purpose	Items/ Apparatus
1.	Sampling rice beetles	<ul style="list-style-type: none">- Tools and glass container- Analytical balance- Forcep
2.	Identification of microplastics on external body part of rice beetles	<ul style="list-style-type: none">- Rice beetles sample- Microscope- Microscope slide- Petri dish
3.	Digestion of microplastics	<ul style="list-style-type: none">- Conical flask- Distilled water- Hydrochloride acid- Sodium chloride- Spatula- Graduated cylinder- Aluminium foil

4.	Filtering digestion	<ul style="list-style-type: none"> - Whatman cellulose acetate membrane filter - Buchner funnel - Vacuum pump - Tube - Filtering Flask - Drying oven - Dessicator
5.	Microplastics determination	<ul style="list-style-type: none"> - Fourier-transform infrared spectroscopy (FTIR) - Metal needle - Bunsen burner

3.2 METHODS

3.2.1 Adult rice beetle sampling

Samples of rice beetles were collected from glass rice container. The rice that was used to sampling rice beetles was the local brand, namely brand A, which is brand commonly consumed and among affordable. This brand A is one of the most popular varieties of rice, which is prone to rice beetle infestation. It is well known that these bugs deposit their eggs inside or on top of rice grains, causing the larvae to hatch and consume the insides of the grains. It can cause harm and lower the rice's quality.

The most effective way to rapidly locate many insect species is to search directly with the hand. To gather rice beetles from rice, the sampling technique that has been used was hand collecting because the rice beetles are small pest insects. The total number of rice beetle specimens collected was 3.3g and was separated into three

replicates. Rice beetles samples were collected enough to achieve statistical significance. Then, the gathered rice beetles were moved into tidy, glass containers. Plastic containers that potentially introduce more microplastics while collecting insects are kept away. After that, the samples were placed in a laboratory chiller (5°C- 35°C).



Figure 3.1 Rice beetles sample that has been collected.

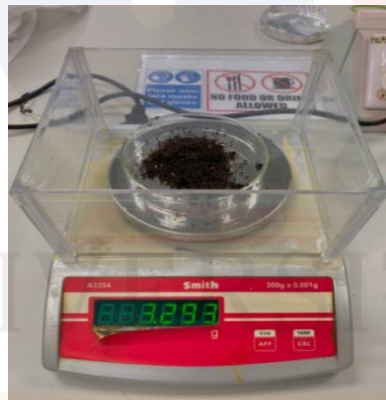


Figure 3.2 Rice beetles that has been collected was 3.3g.

3.2.2 Physical detection of microplastics on rice beetles

Physically inspecting the insects' surface to look for plastic particles is how microplastics on rice beetles are found. As an alternative, fundamental observations were made first with a high-quality magnifying lens. Following that, an examination under the microscope. The identification of the exterior microplastic particles as well as other particles within the rice beetle body was carried out under a microscope.

The microscope was utilised for a closer look. To observe minute particles, set the magnification setting appropriately. The individual rice beetle is picked up and put on the microscope slide and placed under the microscope.

The next step is to identify microplastics. Checks for microplastics is done on the body's surface, in the legs, and in other appendages. Search for particles that lack the usual forms, colours, and appearance of organic stuff. Microplastics can be translucent, coloured, and have unique forms and edges that set them apart from the beetle's natural characteristics (Kooi et al., 2019). It was discovered that the exterior body portions of rice beetles had microplastic particles adhered to them. For instance, based on their size and form, the kind of microplastics, such as fibers, were found. Microplastic that have been detected on the skin of rice beetle, it will be removed and kept in a petri dish for further examination, which is hot needle test. To improve vision and locate any microplastics that could be embedded or partially buried on the beetle's surface, use a light source. Samples are always handled properly to reduce the possibility of contamination throughout the inspection process. To eliminate exterior microplastic particles and other particles, rice beetles need to be separated from debris and rinse using distilled water. Following that, the material was allowed to dry.



Figure 3.3 Microplastic that have been found at rice beetle under the microscope.

3.2.3 Process of digestion & filtration

Digestion:

There are many processes involved in the extraction and identification of microplastics from rice beetles. Depending on the unique qualities of the sample, several extraction techniques may be used, and some modifications required. The rice beetles samples were placed into three replicates of the digestion vessels which is a conical flask. To separate microplastics and get rid of organic debris, use the right digesting technique. Digestion methods (enzymatic or chemical) need to be used to break down organic material while preserving microplastics. For chemical digestion, hydrochloride acid (HCl) is a helpful chemical. For instance, 5 ml (30%) of hydrochloric acid (HCl) and 30 ml (4M) of sodium chloride (NaCl) should be added to each sample. Organic substances are effectively broken down at this concentration without harming the majority of microplastic kinds. This is because HCl would effectively break down organic molecules at a concentration of 30%. Subsequently, the 4M NaCl would support the separation process by maintaining the solution's ionic strength stability. After that, the conical flasks need to be covered with aluminium foil. The samples were shaken for 120 hours or five days in the laboratory shaker and at 25°C to ensure thorough digestion of the organic material.



Figure 3.4 The samples are put in the laboratory shaker to shake for 5 days (25°C).

Filtration:

To expedite the filtering process, use a vacuum filtration system. To separate the microplastics from the digested samples, Whatman Cellulose Acetate membrane filter paper which has a pore size of $0.2\ \mu\text{m}$ was utilised. The filter paper is placed on the base and clamped to the funnel. Pour the digested sample onto the membrane filter and turn on the vacuum pump to create a pressure difference that will force the sample through the filter. For the purpose of getting rid of any impurities or left over digesting solution, rinse the filter with distilled water for each sample. After that, the filter was gently taken off of the base without damaging it and put into a petri dish. Following the filtering process, the filter paper was put in a labeled petri dish in the oven (47°C) for 2 hours. These filter papers were removed after two hours and allowed to cool in a desiccator.



Figure 3.5 Use a vacuum filtration system for the samples that have been shaken.



Figure 3.6 Filter papers in the petri dish and put it in the oven (47°C) for 2 hours.

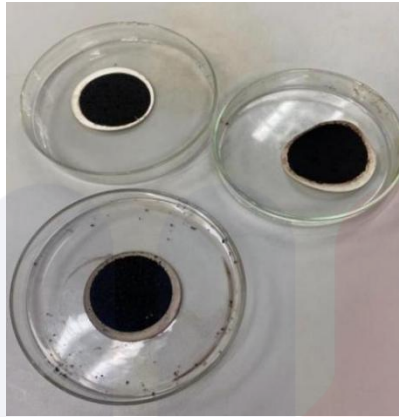


Figure 3.7 Filter papers were dried using the oven.



Figure 3.8 Filter papers were cooled in a desiccator.

3.2.4 Determination of microplastics

Hot needle test:

One easy way to find out if microplastics are present is to use the hot needle test. This test can assist in determining whether an insect, such as a rice beetles, has contained microplastics. Microplastics are defined as particles that melt or significantly alter in form when they come into contact with the hot needle (Zhang et al., 2020). Non-reacting particles are probably made of organic materials or other non-plastic materials. First, a metal needle is closed with microplastic particles after being heated to a suitable temperature. Verify that the needle is hot enough and close to the pieces. After then, examined how these microplastic fragments

responded to the heat. For instance, the fragments melt, which indicate the presence of microplastics in rice beetles. This is due to the fact that plastics have very low melting points and will exhibit distinct reactions to the heat applied by the needle.

Fourier-transform infrared spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) is a method used to evaluate the presence and composition of microplastics. The produced microplastic samples are then analysed using micro-FTIR. A tiny portion of the microplastic sample is exposed to infrared light when it is placed on the FTIR device. The obtained spectrum provided information regarding the chemical makeup of the microplastics.

In the laboratory, the model of Nicolet™ IZ10 FTIR Spectrometer was utilised. For instance, 32 co-added scan repetitions with a wavelength of 4000-500 cm^{-1} and a spectral resolution of 4 cm^{-1} be employed for the measurement of attenuated total reflectance (μATR) mode. After that, OMNIC software will be used to examine the graph.



Figure 3.9 Use FTIR to evaluate the presence and composition of microplastics.

3.3 RESEARCH FLOW CHART

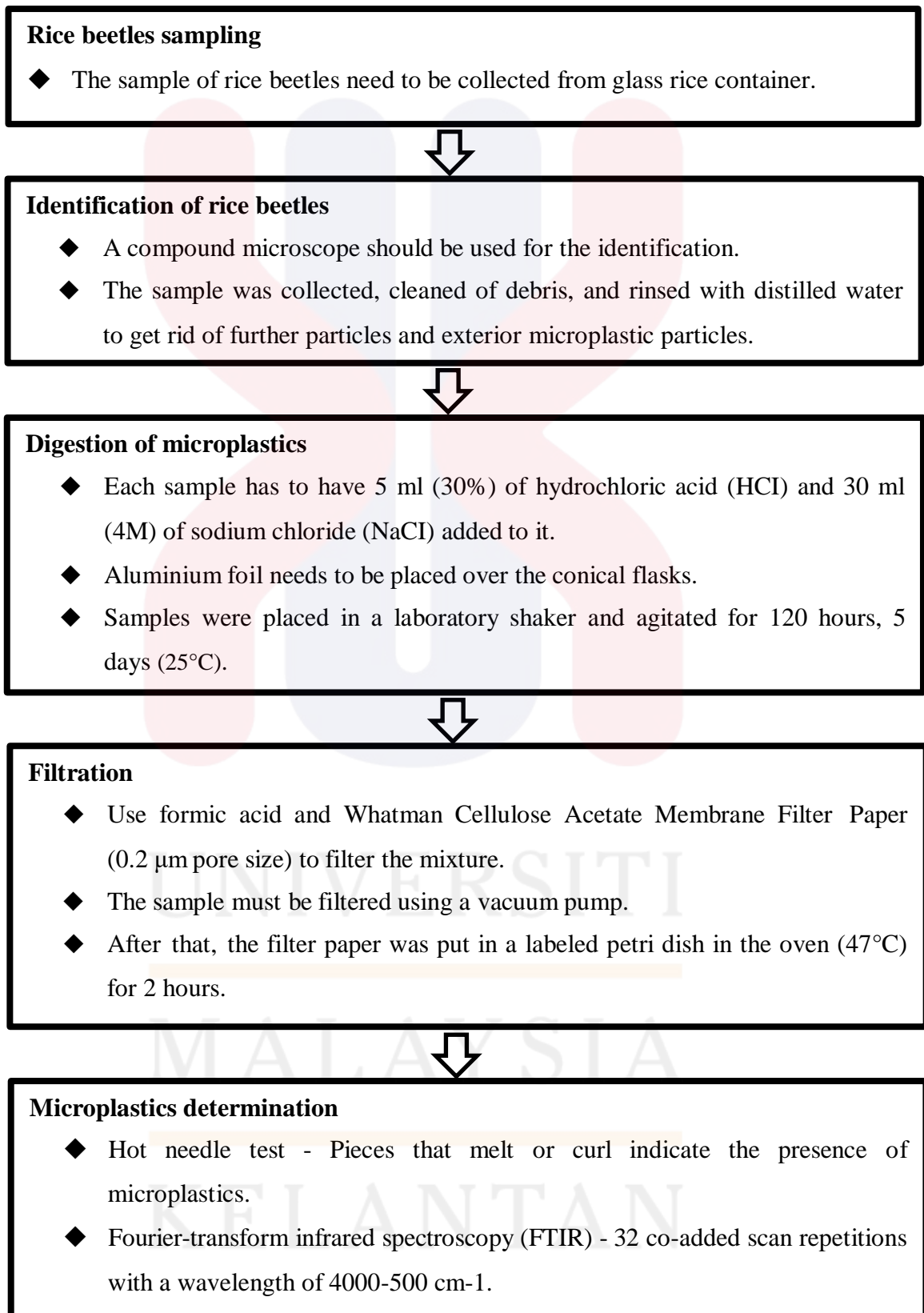


Figure 4.0 Research flow chart for determination of microplastics in rice beetles (*Sitophilus oryzae*).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result

4.1.1 Chemical composition of microplastics

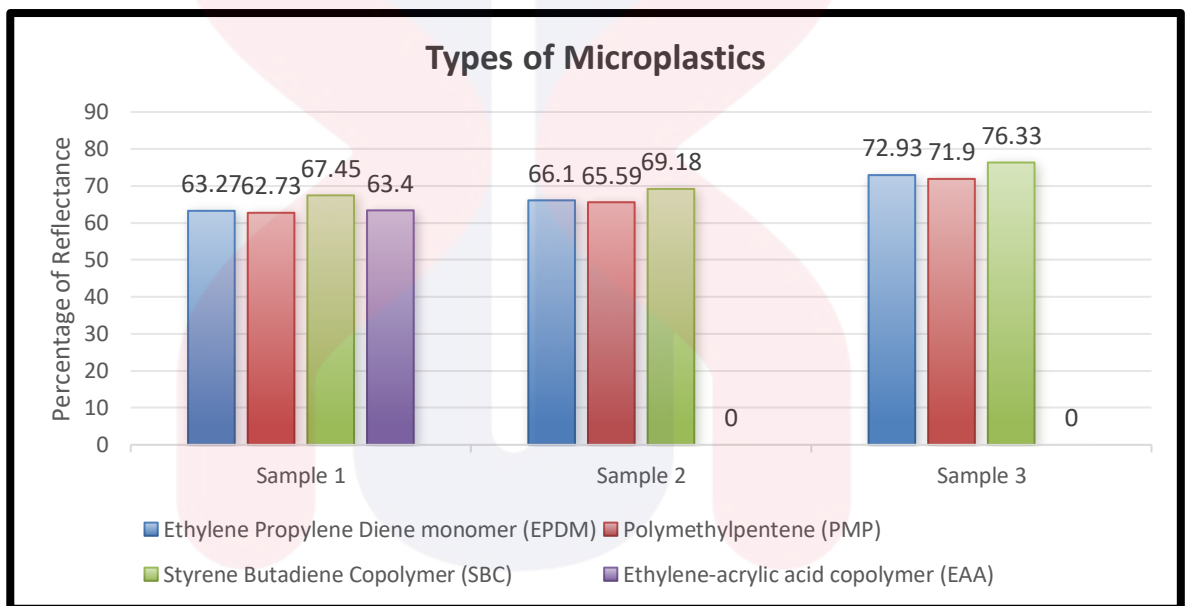


Figure 4.1 Percentage of Reflectance of chemical composition in microplastics.

Figure 4.1 indicates the percentage of reflectance from FTIR analysis for three samples of rice beetle. The most highest reflectance in Sample 1, Sample 2, and Sample 3 is Styrene Butadiene Copolymer (SBC), meanwhile the most lowest reflectance in each sample is Polymethylpentene (PMP). S1 shows the percentage of reflectance of Ethylene-acrylic acid copolymer (EAA), while S2 and S3 do not shows the percentage of reflectance of EAA.

4.1.2 Microplastic shape



Figure 4.2 Shape of microplastic were detected under the microscope.

Figure 4.2 indicates form of microplastic that was found on the rice beetle under the microscope is fiber. Most of the fibers are found at the part of the rice beetle's leg.

4.1.3 Microplastics occurrence

Types	Sample 1	Sample 2	Sample 3
Ethylene Propylene Diene monomer (EPDM)	+	+	+
Polymethylpentene (PMP)	+	+	+
Styrene Butadiene Copolymer (SBC)	+	+	+
Ethylene-acrylic acid copolymer (EAA)	+	-	-

Table 4.1 Microplastics recorded in rice beetles.

Table 4.1 indicates overall microplastics in three samples of rice beetle obtain from FTIR analysis.

4.1.4 Types of Microplastics

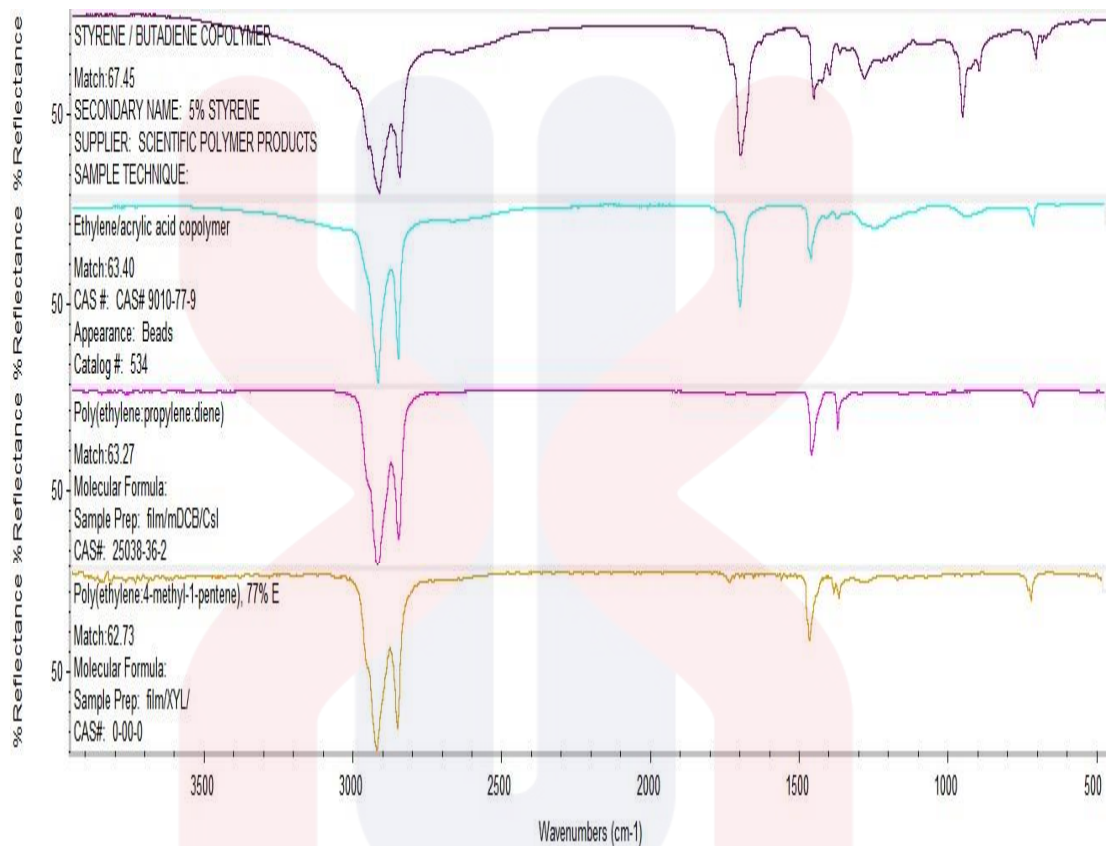


Figure 4.3 Types of Microplastics in Sample 1.

Figure 4.3 indicates four types of microplastics that been found in sample 1 which are Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP).

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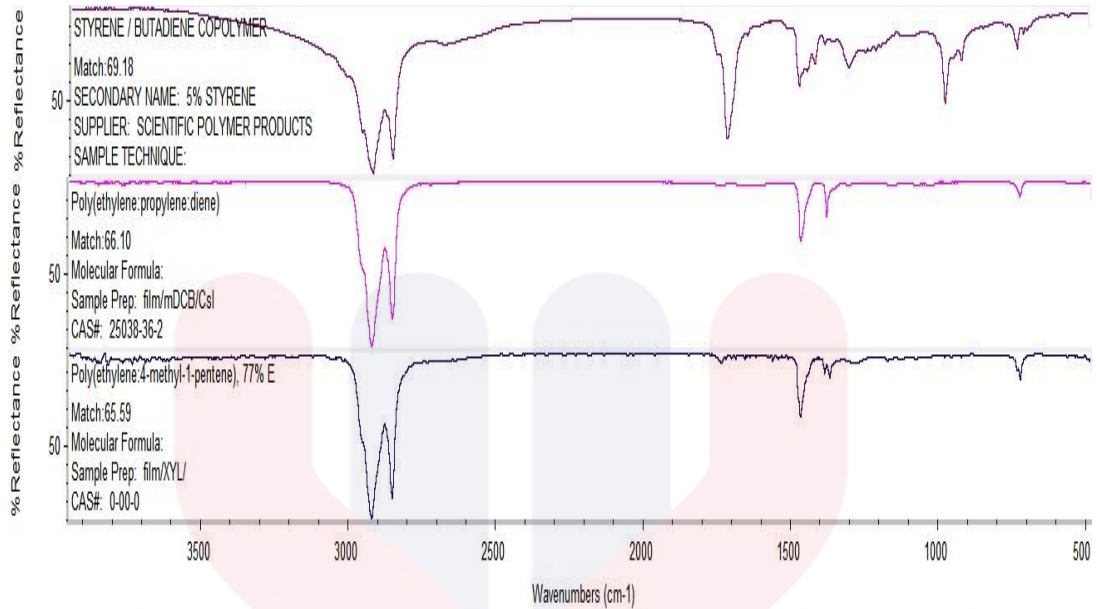


Figure 4.4 Types of Microplastics in Sample 2.

Figure 4.4 indicates three types of microplastics that been found in sample 2 which are Styrene Butadiene Copolymer (SBC), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP).

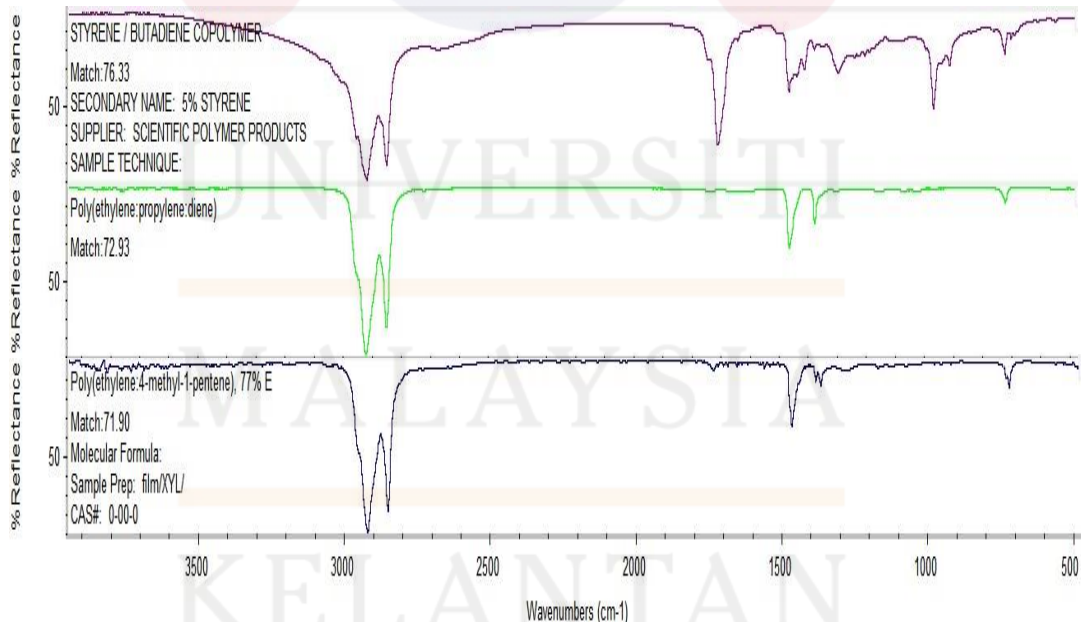


Figure 4.5 Types of Microplastics in Sample 3.

Figure 4.5 indicates three types of microplastics that been found in sample 3 which are Styrene Butadiene Copolymer (SBC), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP).

4.2 Discussion

Figure 4.1 shows the percentage of reflectance from FTIR analysis in the three samples of rice beetle which are S1, S2, and S3. Overall, the most abundance of Ethylene Propylene Diene monomer (EPDM) was in S3 which is 72.93%, followed by S2 is 66.10%. The lowest abundance of EPDM was in S1 which is 63.27%. The highest of Polymethylpentene (PMP) is S3 which is 71.90%, followed by S2 is 65.59%. The lowest PMP is in S1 which is 62.73%. On the other hand, the most abundance of Styrene Butadiene Copolymer (SBC) found in S3 which is 76.33%. The second most abundance is S2 which is 69.18%. The most least abundance is S1 which is 67.45%. Next, the most abundance of Ethylene-acrylic acid copolymer (EAA) was in S1 which is 63.40% because there are no EAA found in S2 and S3.

Overall, the most highest reflectance in S1, S2, and S3 is Styrene Butadiene Copolymer (SBC). SBC, having a minimum 70% styrene content, is an engineered thermoplastic material made from a copolymer of styrene and butadiene (McKeen, 2012). The second highest is Ethylene Propylene Diene monomer (EPDM). Copolymerization of ethylene/propylene and unsaturated diene results in EPDM, a saturated elastomeric thermoplastic material (Costa et al., 2024). Meanwhile the most lowest reflectance in each sample is Polymethylpentene (PMP). PMP is a thermoplastic polymer composed of monomers of methylpentene (Lopes et al., 2006). S1 shows the percentage of reflectance of Ethylene-acrylic acid copolymer (EAA), while S2 and S3 do not shows percentage of reflectance of EAA (Figure 4.1). Copolymers made of ethylene and acrylic acid are known as EAA (Leonard et al., 2024).

Fragments, fibers, films, foam, and beads are some of the typical form categories used to classify microplastics (Kooi et al., 2019). Under a microscope,

rice beetle microplastic shape is displayed in figure 4.2. Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP) are the four types of microplastics that are present in the samples have the same form which is fibers. Most fibers have been found in the external parts of the rice beetle's body such as legs, wings and heads of rice beetles. Microplastic fibers (MPF) usually have a diameter of 10 μ m-30 μ m and a cylindrical or angular form similar to a ribbon (Kooi et al., 2019) .

Table 4.1 display overall types of microplastics in three sample (S1, S2, S3) of rice beetle obtain from FTIR analysis. S1 was recorded to have the highest types of microplastics was found which is four types. The microplastics are Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP). Conversely, the minimal variety of microplastics was observed in S2 and S3, with only three distinct types present. The microplastics were Styrene Butadiene Copolymer (SBC), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP).

The existence of synthetic polymers in rice beetles, such as Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP), have serious detrimental effects. These effects are complex and have an influence on food safety, environmental health, and agricultural output. Each polymer has a particular effect on the beetles because of its own set of chemical characteristics. Multiple mechanisms can lead to SBC, EAA, EPDM, and PMP contamination in rice beetles. These insects are well-known for infesting grains that have been kept, and they frequently live in areas with polymer particles. SBC, EAA, EPDM, and PMP

particles can cling to the exoskeleton of the beetle or be consumed with food. These particles may affect the beetle's body chemically and physically once it is inside and at the same time effect the rice that contain this beetles.

Because of its flexibility, resilience to deterioration, and durability, Styrene Butadiene Copolymer (SBC) is a synthetic rubber that finds extensive application in the packaging, construction, and automotive sectors. Its widespread usage, however, causes environmental dispersion and microplastic contamination, which can seep into soil and water sources. Because SBC particles linger in the ecosystem, they are dangerous to many different kinds of creatures, including pests like rice beetles. Rice that has been kept become further polluted by SBC particles that seep into the grain as infected beetles decay. Consumer health may be directly at risk as a result of the leaching process's potential to release styrene and other hazardous degradation products into rice. Styrene exposure for an extended period of time is linked to a number of health concerns, such as skin irritation, respiratory disorders, and cancerous consequences (McKeen, 2012).

Excellent adhesive qualities, flexibility, and chemical resistance make Ethylene-acrylic acid copolymer (EAA) highly valued. These characteristics make it appropriate for use in industrial components, medical equipment, and food packaging. EAA is useful, but because of its environmental permanence, there are worries about how it may affect ecosystems. Since EAA is not easily broken down, it tends to accumulate in the environment and can come into touch with insects such as rice beetles. Physical contamination can occur when contaminated rice grains are directly contaminated by EAA particles introduced by contaminated rice beetles. Non-biodegradable polymer particles have the potential to lower rice quality, safety, and marketability. Because of worries about their health and safety, consumers are rejecting rice that has been tainted with synthetic polymers (Leonard

et al., 2024).

Synthetic rubber with high value that is resistant to environmental variables, flexible, and long-lasting is called Propylene Diene monomer (EPDM). Its capacity to tolerate extreme circumstances without deteriorating has led to its employment in a broad range of applications, including roofing materials and automobile seals. The fact that EPDM is stable under a variety of environmental circumstances also means that it is difficult to decompose, which causes it to accumulate in the environment and have an adverse effect on biological systems. Because EPDM is persistent in the environment, a variety of creatures may be exposed to it over time. Since birds and other insects hunt on rice beetles, the EPDM that beetles eat can enter the food chain and could have an impact on predators. Ecological equilibrium may be upset and predator population losses may result from this bioaccumulation (Costa et al., 2024).

High melting point, low density, and resilience to UV and chemicals are the characteristics of Polymethylpentene (PMP). Because of these qualities, it may be applied to a variety of situations where clarity and durability are crucial. PMP has the benefit of being stable and resistant to degradation, which allows it to stay in the environment for long periods of time. Because of its tenacity, PMP build up in environments where it interacts with a variety of creatures, including pests like rice beetles. Contamination of stored rice can be exacerbated by PMP particles found in infected beetles. Even though PMP is chemically inert, any additives or leftover chemicals employed in its manufacture may seep into the rice and endanger customers' health. Furthermore, PMP's harmful effects on predators that eat tainted beetles may cause wider ecological disruptions (Lopes et al., 2006).

The FTIR analysis results graph for S1,S2,S3 is shown in Figure 4.3. Sample 1 displays four different types of microplastics in the sample which are

Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP). Meanwhile, the FTIR analysis results graph for samples 2 and 3 can be seen in figures 4.4 and 4.5. The same three different forms of microplastics were discovered in both samples which are Styrene Butadiene Copolymer (SBC), Ethylene Propylene Diene monomer (EPDM), and Polymethylpentene (PMP).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

To conclude, microplastics in rice beetles (*Sitophilus oryzae*) collected from grain storage have been found in this study, and this information opens our eyes to the larger problem of ecosystems and food safety. The primary types of microplastics identified were Styrene Butadiene Copolymer (SBC), Ethylene-acrylic acid copolymer (EAA), Ethylene Propylene Diene Monomer (EPDM), and Polymethylpentene (PMP), which are commonly used in packaging materials.

Common pests in stored grains, rice beetles pose a serious threat to agriculture. These insects' microplastic presence emphasises how ubiquitous plastic contamination is. Through investigating the effects of microplastics on rice beetles' ecology, and food safety, we can learn important lessons about the widespread effects of synthetic polymer pollution.

Microplastic pollution has significant ecological ramifications. As a component of the food chain, beetles can pass microplastics to birds and other insect predators. Higher trophic levels are at danger from this bioaccumulation, which can also cause ecological imbalances. Because synthetic polymers like SBC, EAA, EPDM, and PMP are persistent in the environment, their effects can endure a long period and may eventually change ecosystems and biodiversity.

There are serious concerns to food safety when grains that have been kept are contaminated with microplastics. Microplastics have a greater chance of getting into the human food chain when rice beetles eat grains. Consuming food items

tainted with microplastic particles can have negative health effects, especially if the polymers release toxic chemicals into the food. To safeguard the public's health, food supplies must be guaranteed to be pure and safe.

Finding microplastics in rice beetles (*Sitophilus oryzae*) brings attention to a serious problem for the environment and public health. By implementing enhanced waste management strategies, substituting pesticides, and closely observing the situation, we can lessen the effects of polymers on farming and protect ecosystems and public health. The all-encompassing approaches described offer a way forward for a future that is more sustainable and for a cleaner, safer environment.

5.2 Recommendation

Future recommendation to ensure a representative analysis, samples of rice beetles should be collected from diverse kinds of rice. This approach will provide a comprehensive understanding of microplastic contamination in different types of rice as well as be able to compare which rice is of higher quality.

Next, it is essential to disseminate the results of the microplastic monitoring program to the general public and pertinent parties, such as farmers, storage facility managers, and policymakers, in order to increase awareness and driving collective action. Transparency in reporting will build trust and encourage best practices.

According to this study, all rice manufacturing should switch to fiberglass packaging instead of plastic. This is due to the fact that fiberglass can lessen microplastic pollution and can keep rice beetles from consuming microplastics contained in rice plastic bags.

REFERENCES

Zhihao Yuan, Rajat Nag, & Enda Cummins. (2022). *Human health concerns regarding microplastics in the aquatic environment - from Marine to Food Systems*. Science of The Total Environment.
<https://www.sciencedirect.com/science/article/pii/S0048969722008221>.

Sousa, I. G., Oliveira, J., Mexia, A., Barros, G., Almeida, C., Brazinha, C., Vega, A., & Brites, C. (2023). *Advances in environmentally friendly techniques and circular economy approaches for insect infestation management in stored rice grains*. National Center for Biotechnology Information.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9914097/>.

G.G.N Thushari, & J.D.M. Senevirathna. (2020). *Plastic pollution in the marine environment*. Heliyon.
<https://www.sciencedirect.com/science/article/pii/S2405844020315528>.

Hassan. (2017). *Polymers in our daily life*. BioImpacts : BI.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5524987/>.

Browne, M. A., Underwood, A. J., Chapman, M. G., Williams, R., Thompson, R. C., & Frecker, J. A. (2015). *Linking effects of anthropogenic debris to ecological impacts*. The Royal Society Publishing, 282.

Gall, S., & Thompson, R. (2015). *The impact of debris on marine life*. Marine Pollution Bulletin, 170-9.

Li, J., Liu, H., & Chen, J. (2018). *Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection*. Water Research, 137, 362- 374.

Alimba, C. G., and Faggio, C. (2019). *Microplastics in the marine Environment: Current Trends in Environmental Pollution and Mechanisms of Toxicological Profile*. Environ. Toxicol. Pharmacol. 68, 61–74.
 doi:10.1016/j.etap.2019.03.001.

Alfaro-Nunez, A., Astorga, D., Cáceres-Farías, L., Bastidas, L., Villegas, C. S., Macay, K., et al. (2021). *Microplastic Pollution in Seawater and marine Organisms across the Tropical Eastern Pacific and Galápagos*. Scientific Rep. 11 (1), 1–8.

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., and Xing, B. (2020). *MPs in Aquatic Environments: Toxicity to Trigger Ecological Consequences*. Environ. Pollut. 261, 114089. doi:10.1016/j.envpol.2020.114089.

Lu, K., Qiao, R., An, H., and Zhang, Y. (2018). *Influence of MPs on the Accumulation and Chronic Toxic Effects of Cadmium in Zebrafish (Danio rerio)*. *Chemosphere* 202, 514–520. doi:10.1016/j.chemosphere.2018.03.145.

Aragaw, T. A. (2021). *Microplastic Pollution in African Countries' Water Systems: a Review on Findings, Applied Methods, Characteristics, Impacts, and Managements*. *SN Appl. Sci.* 3 (6), 1–30. doi:10.1007/s42452-021-04619z.

De Sa, L. C., Luis, L. G., and Guilhermino, L. (2015). *Effects of MPs on Juveniles of the Common Goby (Pomatoschistus Microps): Confusion with Prey, Reduction of the Predatory Performance and Efficiency, and Possible Influence of Developmental Conditions*. *Environ. Pollut.* 196, 359–362.

Guyen, O., Bach, L., Munk, P., Dinh, K. V., Mariani, P., and Nielsen, T. G. (2018). *Microplastic Does Not Magnify the Acute Effect of PAH Pyrene on Predatory Performance of a Tropical Fish (Lates calcarifer)*. *Aquat. Toxicol.* 198, 287–293. doi:10.1016/j.aquatox.2018.03.011.

Mu, X., Qi, S., Liu, J., Yuan, L., Huang, Y., Xue, J., et al. (2021). *Toxicity and Behavioral Response of Zebrafish Exposed to Combined Microplastic and Bisphenol Analogues*. *Environ. Chem. Lett.*, 1–8. doi:10.1007/s10311-021-01320-w.

Jie Shen, Boying Liang, Hui Jin. (2023). *The impact of microplastics on insect physiology and the indication of hormesis*. *TrAC Trends in Analytical Chemistry*. <https://www.sciencedirect.com/science/article/abs/pii/S0165993623002170>.

Jianqiang Zhu, Pengfei Wu, Nan Zhao, Shengtao Jiang, Huayue Zhu, Hangbiao Jin. (2023). *Microplastics in terrestrial insects, long-horned beetles (Coleoptera: Cerambycidae), from China*. *Science of The Total Environment*. <https://www.sciencedirect.com/science/article/abs/pii/S0048969723028188>.

Witwisitpong Maneechan and Taeng On Prommi. (2022). *Occurrence of microplastics in edible aquatic insect pantala sp. (Odonata: Libellulidae) from Rice Fields*. *PeerJ*. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8833222/>.

Hunt T, Bergsten J, Levkanicova Z, Papadopoulou A, John OS, Wild R, et al. *A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation*. *Science*. 2007;318:1913–6. <https://doi.org/10.1126/science.1146954>.

Stork NE, McBroom J, Gely C, Hamilton AJ. *New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods*. *Proc Natl Acad Sci U S A*. 2015;112:7519–23. <https://doi.org/10.1073/pnas.1502408112>.

McKenna DD, Sequeira AS, Marvaldi AE, Farrell BD. *Temporal lags and overlap in the diversification of weevils and flowering plants*. Proc Natl Acad Sci U S A. 2009;106:7083–8. <https://doi.org/10.1073/pnas.0810618106>.

Longstaff B.C. *Biology of the grain pest species of the genus Sitophilus (Coleoptera: Curculionidae): A critical review*. Prot. Ecol. 1981;2:83– 130.

Okram S., Hath T.K. *Biology of Sitophilus oryzae (L.) (Coleoptera: Curculionidae) on Stored Rice Grains during Different Seasons in Terai Agro- Ecology of West Bengal*. Int. J. Curr. Microbiol. Appl. Sci. 2019;8:1963. doi: 10.20546/ijcmas.2019.804.229.

Subedi Y.D.G.C., Thapa R.B., Rijal J.P. *Rice weevil (Sitophilus oryzae L.) host preference of selected stored grains in Chitwan Nepal*. J. Inst. Agric. Anim. Sci. 2009;30:151–158.

Kooi, M., Koelmans, A. (2019). *Simplifying microplastic via continuous probability distributions for size, shape, and density* | environmental science & technology letters. <https://pubs.acs.org/doi/10.1021/acs.estlett.9b00379>.

Zhang, Y., Kang, S., Allen S.(2020). *Atmospheric microplastics: A review on current status and Perspectives*. Earth-Science Reviews. <https://www.sciencedirect.com/science/article/pii/S001282521930621X>.

McKeen, L. (2012). *Styrene-butadiene copolymer. Styrene-Butadiene Copolymer - an overview* | ScienceDirect Topics. <https://www.sciencedirect.com/topics/chemistry/styrene-butadiene-copolymer>.

Leonard, L., Liddle, R., Atherall, A. (2024). *Microplastics in human blood: Polymer types, concentrations and characterisation using μ ftir*. Environment International. <https://www.sciencedirect.com/science/article/pii/S0160412024003374>.

Costa, N. L., Hiranobe, C. T., Cardim, H. P., Dognani, G., Sanchez, J. C., Carvalho, J. A. J., Torres, G. B., Paim, L. L., Pinto, L. F., Cardim, G. P., Cabrera, F. C., dos Santos, R. J., & Silva, M. J. (2024). *A review of EPDM (ethylene propylene diene monomer) rubber-based nanocomposites: Properties and progress*. MDPI. <https://www.mdpi.com/2073-4360/16/12/1720>.

Lopez, C., Wilkes, L., Stricklen, M., White, S. (2006). *4-methyl-1-pentene*. National Center for Biotechnology Information. PubChem Compound Database. <https://pubchem.ncbi.nlm.nih.gov/compound/4-Methyl-1-pentene>.

APPENDIX A

Image of selected microplastics in each sample under the microscope.



APPENDIX B

Image of Fourier-Transform Infrared Spectroscopy (FTIR) raw data of S1,S2, S3.

