COMPARATIVE STUDIES ON PLASTIC DEGRADATION CAPACITY AND ENZYMATIC ANALYSIS IN LARVAL INSTARS OF *GALLERIA MELLONELLA* AND *ACHROIA GRISELLA*

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Zoology

By

Nikhita Kapahi

Registration Number: 11919194

Supervised By

Dr. Lovleen (18863)

Professor

Department of Zoology

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**COMPARATIVE STUDIES ON PLASTIC DEGRADATION CAPACITY AND ENZYMATIC ANALYSIS IN LARVAL INSTARS OF *GALLERIA MELLONELLA* AND *ACHROIA GRISELLA***" in fulfillment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Lovleen, working as Professor, Department of Zoology, School of Bioengineering and Biosciences, of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Nikhita Kapahi

Research Scholar

Reg. No. 11919194

School of Bioengineering and Biosciences

Lovely Professional University

Punjab

India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"COMPARATIVE STUDIES ON PLASTIC DEGRADATION CAPACITY AND ENZYMATIC ANALYSIS IN LARVAL INSTARS OF *GALLERIA MELLONELLA* AND *ACHROIA GRISELLA*"** submitted in fulfillment of the requirement for the award of degree of Doctor of Philosophy (Ph.D.) in the Department of Zoology, School of Bioengineering and Biosciences, is a research work carried out by Ms. Nikhita Kapahi, 11919194, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Date:

Dr. Lovleen

Professor,

Department of Zoology,

School of Bioengineering and Biosciences,

Lovely Professional University,

Punjab

India

ABSTRACT

Polyethylene and polypropylene are two common types of non-biodegradable plastic that significantly contribute to environmental pollution. In the present study it is shown that *Galleria mellonella* (*G. mellonella*) and *Achroia grisella* (*A. grisella*) caterpillars can consume plastics at remarkable speeds. Although these larval insects are believed to play a crucial role in plastic waste biodegradation, the extent of their contribution to the process is still not well understood and is a matter of debate.

The current thesis centers on investigation of the plastic consumption and biodegradation capabilities of three commonly utilised plastics: Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), and Polypropylene (PP). These commonly employed polymers, polypropylene and polyethylene, play a substantial role in the global accumulation of plastic waste. The primary aim of the thesis is to investigate how well larval instars of wax moth can biodegrade these plastics. The objectives conducted to accomplish the present research work are: evaluation of the plastic degradation capacity of different larval instars of wax moth, examination of the plastic degradation ability of different types of the plastics by wax worms and analysis of the enzymatic activity of the wax moth instars after ingestion of the plastics.

For the first objective, the study investigated the biodegradation of long-chain hydrocarbons (LDPE, HDPE, and PP) by two species of wax worms, the greater wax moth (*G. mellonella*) and the lesser wax moth (*A. grisella*), without the involvement of gut bacteria. Initially, the plastics were exposed to soil for a year and then their biodegradation capacity was assessed. Subsequently, both soil-treated and naive plastics were exposed to all larval instars of the wax moth species for two days to evaluate their ability to biodegrade LDPE, HDPE, and PP.

Further, for second and third objective, the current research aimed to assess the ability of larvae to consume plastic by measuring the decrease in polymer mass and observing the survival rates of the larval instars following their exposure to the plastic materials, analysis of consumed plastic film, investigation of biodegraded remains of the plastics in the excretory waste of the larva and the enzyme analysis of the homogenate of

the larva. Moreover, to observe the plastic degradation ability of the larval instars of wax moth, the larvae were administered with the antibiotics. After exposure of antibiotics, the larvae were exposed to the plastics for two days. To determine the plastic consumption ability, the weight loss of plastics and survival rates of the larval instars after the experiment was recorded. Additionally, Scanning Electron Microscopy (SEM) images were used to compare the degradation of plastic films exposed to wax moth larvae with those of naive plastic films. Gas Chromatography and Mass Spectroscopy (GC-MS) analysis of the frass was conducted to assess plastic degradation, while enzyme assays of Alcohol Dehydrogenase (ADH) and Lactate Dehydrogenase enzymes (LDH) was analysed to track polymer degradation by the wax moths.

All larval stages of the *G. mellonella* and *A. grisella* were exposed to both untreated and soil-treated LDPE, HDPE, and PP films for 48 hours to assess their ability to consume plastic waste. The results showed that the seventh instar larva of the greater wax moth exhibited the highest plastic consumption rate of 6.64% when fed soil-treated LDPE film, while the fifth instar larva of the lesser wax moth consumed the maximum plastic of 7.51% when fed untreated LDPE film. When exposed to HDPE film, the seventh instar larva of *G. mellonella* showed the highest plastic consumption rate of 8.89% when fed to soil-treated films. Similarly, the fifth instar larva of *A. grisella* displayed a highest plastic consumption rate of 7.55% when fed soil-treated HDPE film. After exposure to PP films, the seventh instar larvae of *G. mellonella* consumed the maximum plastic at a rate of 8.44% when fed soil-treated films, while the fifth instar larvae of the lesser wax moth consumed 1.82% of plastic when fed soil-treated PP film.

The survival rates of wax worms fed on different types of plastic films were studied. The results showed that the maximum survival rate of the all larval instars of the greater wax moth that were fed on the naive and soil treated LDPE for two days was 94.666% in seventh larval instars fed with naive LDPE film whereas for lesser wax moth the highest survival rate was found to be 92% in fifth instar larvae exposed to naive LDPE film. For HDPE, the maximum survival rate was 94.66% when sixth instar larvae

of *G. mellonella* were fed on soil-treated HDPE film. Further, when all larval instars of *A. grisella* were exposed to HDPE film, the highest survival rate of 94.66% was exhibited by the fourth instar larvae exposed to pretreated HDPE film. Additionally, the highest survival rate for greater wax moth larvae was 96.66% for seventh larval instar stage when fed on soil-treated PP film for 48 hours. Similarly, the highest survival rate for lesser wax moth larvae was 96.66% for fifth instar stage when fed on pretreated PP film for 48 hours. These findings highlight the impact of different plastic films on the survival rates of wax moth larvae.

To investigate the inherent plastic degradation abilities of two wax moth species, initially, the gut microbiomes of the larval insects were killed by feeding them with antibiotics (ampicillin, polymyxin B, kanamycin, neomycin, and vancomycin) solution. This allowed analysing the gut-independent plastic consumption capacity of the larvae in the present study. So, the gut independent plastic consumption capacity was analysed by recording plastic consumption capacity, survival rate of the larva, SEM images of the remains of the plastic film left after the experiment, GC-MS of the frass of the larva collected after the experiment, maximal enzyme activity for ADH and LDH enzyme.

To assess the plastic ingestion ability of different larval stages of greater and lesser wax moths without gut microbiota, the larvae were treated with antibiotics for 24 hours. Following this treatment, the larvae were given LDPE, HDPE, and PP films for a period of 48 hours to evaluate their plastic consumption ability. The results showed that for greater wax moth larvae fed with naive LDPE film, fourth instar larvae without any antibiotic treatment exhibited the highest plastic consumption rate of 4.84%. Whereas, for lesser wax moth larvae, the fifth instar group without antibiotics demonstrated the highest plastic consumption rate of 7.51% when provided with LDPE film. When exposed to HDPE film, without antibiotics seventh instar greater wax moth larvae showed the maximum plastic consumption rate of 7.01%, while the fourth instar lesser wax moth larvae treated with antibiotics displayed the highest plastic consumption rate of 6.83%. Upon exposure to naive PP films, the seventh instar greater wax moth larvae without

antibiotics exhibited the highest plastic consumption rate of 8.4%. On the other hand, for lesser wax moth larvae consuming PP film, the fifth instar larvae administered with antibiotics showed the maximum plastic consumption rate of 3.66%.

In the present research work the survival rates of greater and lesser wax moth larvae when fed on different plastic materials for 48 hours was examined. The maximum survival rate of the greater wax moth larvae that were fed on the naive LDPE was 94.66% by seventh instar without antibiotic administered larvae whereas for lesser wax moth the highest survival rate was 92% for without antibiotic fifth instar larva. For HDPE, the maximum survival rate for *G. mellonella* when fed on the naive plastics was 98% observed in without antibiotic seventh larval instar group. Further, when all larval instars of *A. grisella* were exposed to HDPE film, the highest survival rate of 81.33% was observed for without antibiotic fifth instar group. As all larval instars of greater wax moth larva were fed on the PP for 48 hours, the maximum survival rate was 94.66% for without antibiotic seventh instar larvae while for lesser wax moth larvae the highest survival rate was 99.33% for antibiotic treated fifth instar group. The results demonstrate the varying tolerance levels of these two moth species' larvae when exposed to different plastic substrates over a 48-hour period. The survival rates provide insights into the potential of the insects to survive on the plastics as sole diet.

The SEM images of the residual films from LDPE, HDPE, and PP, after ingestion by the greater wax moth, displayed pits (5µm-50µm), cracks (5µm-20µm), depressions (10µm-20µm) and holes (0.5mm- 2.62mm) indicating the intake of these plastics by the larvae. Likewise, when studying the remnants of plastic films using SEM, the lesser wax moth revealed cracks, pits, and holes that indicate the larvae have consumed the plastic.

The GC-MS of excretory waste of the *G. mellonella* and *A. grisella* revealed the presence of the alkanes (Heptadecane, Heneicosane, Tetracosane), alcohols (2-Pentanol, 3-Hexanol), aldehydes (trans-5-Methyl-2-isopropyl-2-hexen-1-al), acids (Propanoic acid, 2-methyl-, 2-propenyl ester, Butanoic acid, tridec-2-ynyl ester) and other functional groups (Propanoic acid, 2-methyl-, 2-propenyl ester, Decyl pentyl ether) of low molecular

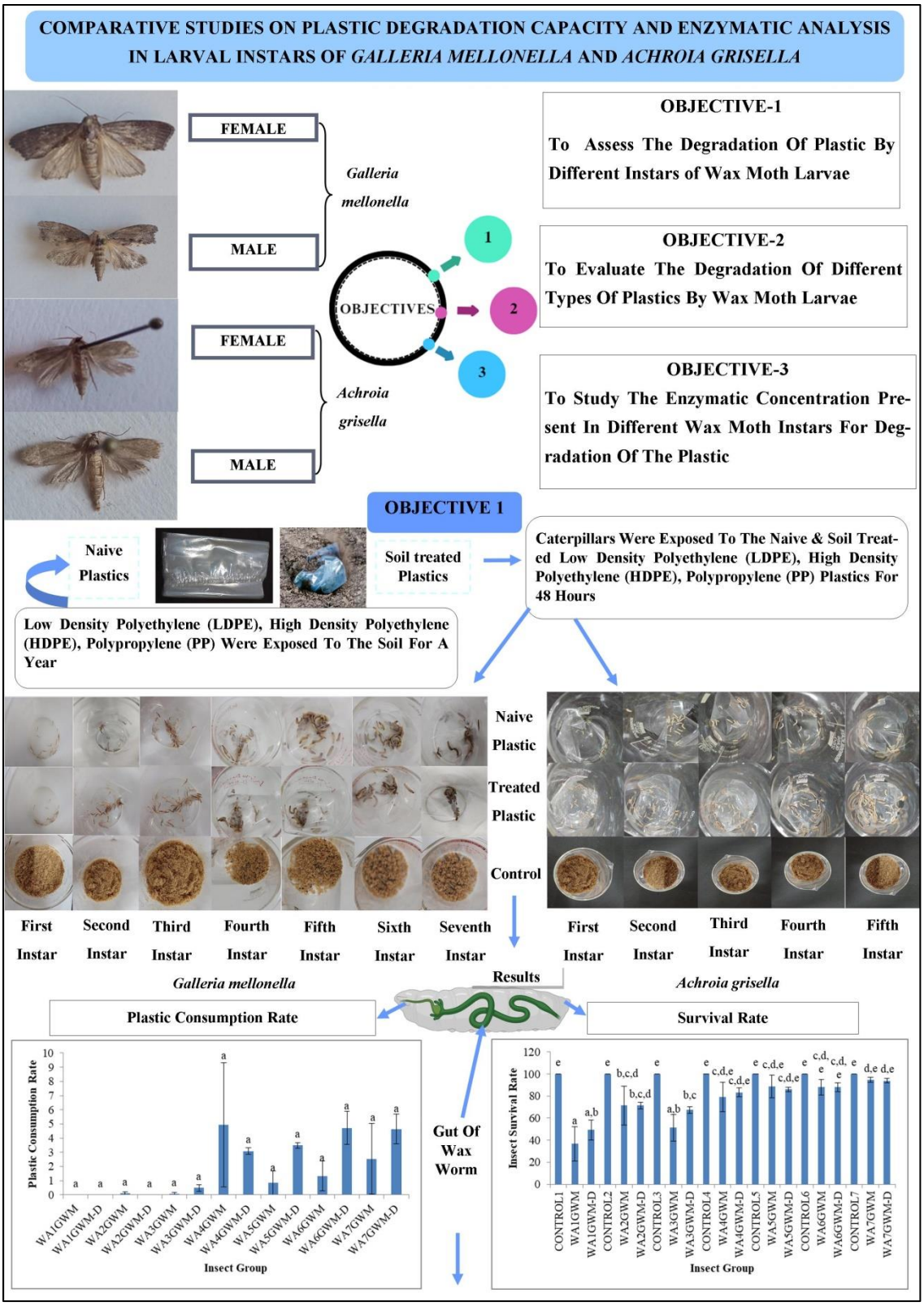
weight as compared to the molecular weight of the LDPE, HDPE and PP plastics.

The highest alcohol dehydrogenase enzyme activity of 3.32 mU/min was recorded for without antibiotic sixth instar larva of *G. mellonella* when fed on the LDPE film as sole diet. For *A. grisella* larva, the maximum enzymatic activity of ADH enzyme when fed on the LDPE as sole diet was 5.14 mU/min for without antibiotic fifth instar group. Moreover, for HDPE film when fed to *G. mellonella* larva, the highest enzymatic activity for ADH was 4.6 mU/min for antibiotic treated fifth instar larva while the highest enzymatic activity for ADH enzyme when fed on the HDPE for all larval instars of *A. grisella* larva was 5.35 mU/min for third instar without antibiotic group. For *G. mellonella* larvae fed PP film as their sole diet, the maximum ADH enzyme activity was 3.90 mU/min for the second instar group without antibiotics. In lesser wax moth larvae fed PP plastic as their only food source, the highest ADH enzyme activity was 5.62 mU/min for the fifth instar group with antibiotics.

The maximum enzymatic activity of lactate dehydrogenase for greater wax moth larva when fed on LDPE film was 2.94 mU/min for with antibiotic seventh instar larva. Similarly, for lesser wax moth larva, the highest enzymatic activity of LDH when fed on the LDPE plastics as sole diet was 0.89 mU/min for with antibiotic third instar larva. For HDPE plastics, the maximum enzymatic activity for LDH enzyme of various larval instar groups of greater wax moth was 1.28 mU/min for with antibiotic seventh instar larva. On the other hand, for HDPE plastics when fed to *A. grisella* larva, the maximum enzymatic activity for LDH enzyme was 0.80 mU/min for with antibiotic fifth instar larva. The maximum enzymatic activity of lactate dehydrogenase for greater wax moth larva when fed on PP film was 3.67 mU/min for without antibiotic seventh instar larva whereas the lesser wax moth larva when fed on PP plastics, the highest enzymatic activity for LDH enzyme was 5.78 mU/min for without antibiotic second instar larva.

The findings of this study indicate that greater and lesser wax moth larvae are capable of ingesting various polymers (PP, HDPE, and LDPE). Both species exhibit a significant consumption of plastic, suggesting that wax worms could play a role in

polymer breakdown. The higher survival rates of the later larval stages in both species suggest that these insects may have the ability to consume long linear hydrocarbons and potentially reduce plastic pollution. Examination of SEM images of the plastic remnants consumed by *G. mellonella* and *A. grisella* larvae revealed surface irregularities such as pits, holes, and depressions, confirming plastic consumption. GC-MS study of the frass (wax worms fed exclusively on plastics) revealed the presence of many functional groups, including ethers, alcohols, esters, and alkanes. These compounds detected in the GC-MS have a low molecular weight in contrast to the non-biodegraded plastics, this shows that the wax moths biodegrade the polymers into biodegradable compounds. While more complex functional systems may be required to utilize honey bee pests for plastic biodegradation, a thorough investigation into the genetic elements and potential pathways involved in plastic biodegradation by wax moths is essential.


COMPARATIVE STUDIES ON PLASTIC DEGRADATION CAPACITY AND ENZYMATIC ANALYSIS IN LARVAL INSTARS OF GALLERIA MELLONELLA AND ACHROIA GRISELLA
FEMALE
MALE
Galleria mellonella
FEMALE
MALE
Achroia grisella
OBJECTIVES
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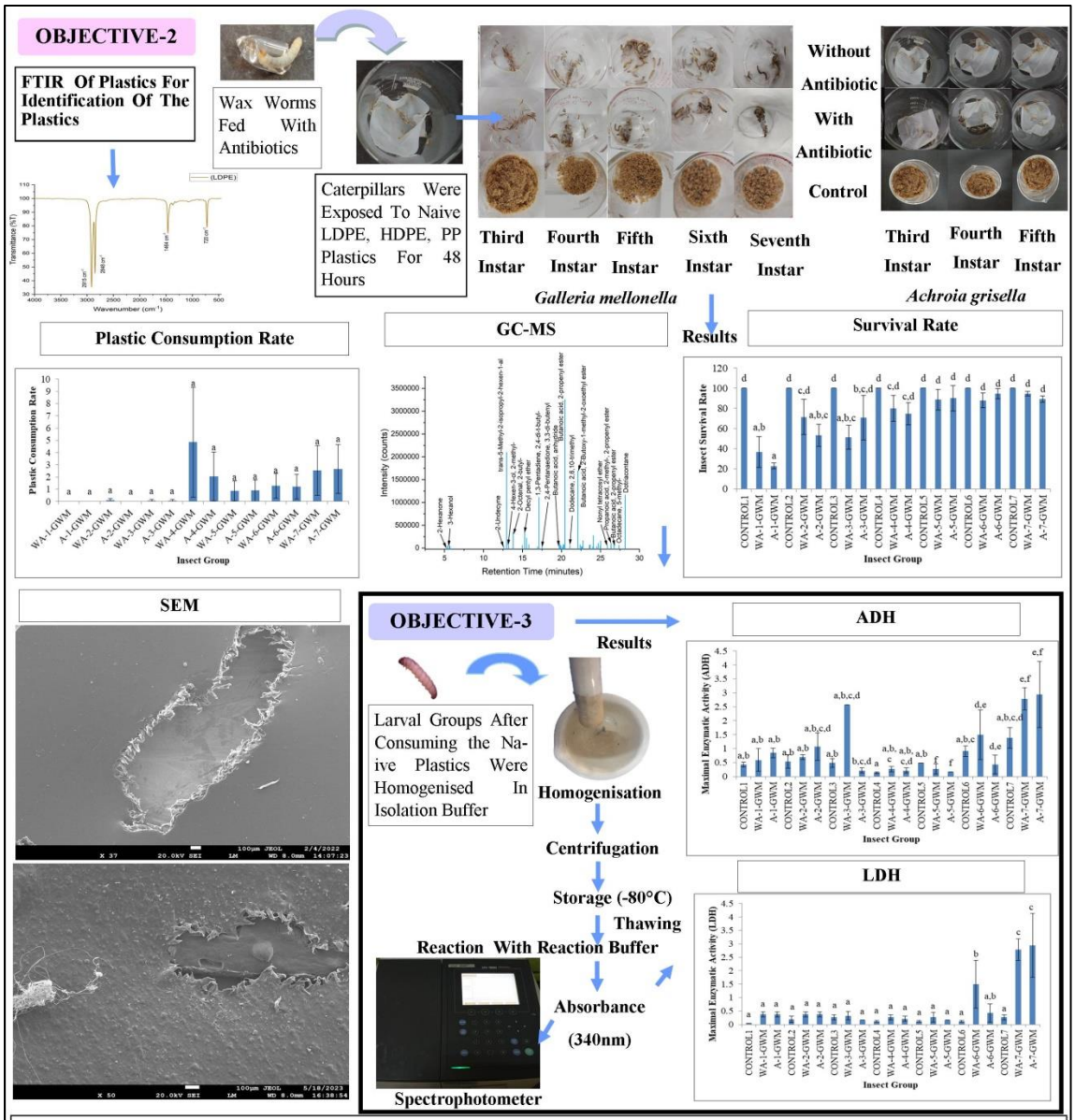
OBJECTIVE-1
To Assess The Degradation Of Plastic By Different Instars of Wax Moth Larvae
OBJECTIVE-2
To Evaluate The Degradation Of Different Types Of Plastics By Wax Moth Larvae
OBJECTIVE-3
To Study The Enzymatic Concentration Present In Different Wax Moth Instars For Degradation Of The Plastic
OBJECTIVE 1
Naive Plastics
Soil treated Plastics
Caterpillars Were Exposed To The Naive & Soil Treated Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Polypropylene (PP) Plastics For 48 Hours
Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Polypropylene (PP) Were Exposed To The Soil For A Year
Naive Plastic
Treated Plastic
Control
First Instar
Second Instar
Third Instar
Fourth Instar
Fifth Instar
Sixth Instar
Seventh Instar
Galleria mellonella
Achroia grisella
Results
Plastic Consumption Rate
Survival Rate
Gut Of Wax Worm
Plastic Consumption Rate
Insect Group
Insect Survival Rate
Insect Group



Conclusion

* The study found that the larvae of two species of wax moths, *Galleria mellonella* and *Achroia grisella*, are capable of consuming various types of plastic films, including Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), and Polypropylene (PP). Notably, this is the first report to claim that the larvae of the lesser wax moth, *Achroia grisella*, can consume polypropylene. In the present study, the maximum plastic consumption capacity was observed during the later larval instar stages, specifically the fifth to seventh instar stages for the greater wax moth and the fourth to fifth instar stages for the lesser wax moth. When the larvae of both species were fed a diet consisting solely of these plastic materials, they exhibited a high survival rate, exceeding 90%. This suggests that the wax moth larvae are able to thrive on a diet composed entirely of these plastic polymers.

* Scanning Electron Microscopy (SEM) images revealed surface irregularities on the remnants of the film, that confirmed plastic consumption by the larvae. While first instar larvae showed no significant weight loss for LDPE and PP films, but the SEM images revealed pits on the remnants, indicating some interaction. Moreover, the decrease in the molecular weight of plastic in the form of compounds such as 3-Hexanol, 1,3-Pentadiene, 2,4-di-t-butyl, Propanoic acid, 2-methyl-, 2-propenyl ester, Decyl pentyl ether, and Butanoic acid, tridec-2-ynyl ester found in the Gas Chromatography and Mass Spectroscopy (GC-MS) of the frass of wax worm indicated digestion of the plastics by the insect.

* The rise in maximum enzymatic activity of Alcohol Dehydrogenase (ADH) and Lactate Dehydrogenase (LDH) in the larvae fed with plastic, with or without antibiotic treatment, compared to the control group, demonstrated the breakdown of the plastics. These findings indicated that both *Galleria mellonella* and *Achroia grisella* were capable of consuming LDPE, HDPE, and PP plastics, suggesting that wax worms have the potential to serve as effective degraders of these plastic materials.

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Nikhita Kapahi

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Abbreviations

LDPE	Low- Density Polyethylene
HDPE	High-Density Polyethylene
PP	Polypropylene
PS	Polystyrene
PE	Polyethylene
G.	*Galleria*
A.	*Achroia*
%	Percentage
°C	Degree Celsius
UV	Ultraviolet
NP	North Pacific
NA	North Atlantic
mm	Millimeters
G	Grams
Km	Kilometers
nm	Nanometers
ml	Milliliters
mg	Milligrams
mU	Milliunits
µm	Micrometers
µl	Microliters
R+M	Radius+ Median wing vein
ZSI	Zoological Survey of India
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
GC-MS	Gas-Chromatography Mass Spectroscopy
WA1GWM	without antibiotic first instar greater wax moth

WA1GWM-D	without antibiotic first instar greater wax moth fed with pretreated plastic film
WA2GWM	without antibiotic second instar greater wax moth
WA2GWM-D	without antibiotic second instar greater wax moth fed with pretreated plastic film
WA3GWM	without antibiotic third instar greater wax moth
WA3GWM-D	without antibiotic third instar greater wax moth fed with pretreated plastic film
WA4GWM	without antibiotic fourth instar greater wax moth
WA4GWM-D	without antibiotic fourth instar greater wax moth fed with pretreated plastic film
WA5GWM	without antibiotic fifth instar greater wax moth
WA5GWM-D	without antibiotic fifth instar greater wax moth fed with pretreated plastic film
WA6GWM	without antibiotic sixth instar greater wax moth
WA6GWM-D	without antibiotic sixth instar greater wax moth fed with pretreated plastic film
WA7GWM	without antibiotic seventh instar greater wax moth
WA7GWM-D	without antibiotic seventh instar greater wax moth fed with pretreated plastic film
WA1LWM	without antibiotic first instar lesser wax moth
WA1LWM-D	without antibiotic first instar lesser wax moth fed with pretreated plastic film
WA2LWM	without antibiotic second instar lesser wax moth
WA2LWM-D	without antibiotic second instar lesser wax moth fed with pretreated plastic film
WA3LWM	without antibiotic third instar lesser wax moth
WA3LWM-D	without antibiotic third instar lesser wax moth fed with pretreated plastic film

WA4LWM	without antibiotic fourth instar lesser wax moth
WA4LWM-D	without antibiotic fourth instar lesser wax moth fed with pretreated plastic film
WA5LWM	without antibiotic fifth instar lesser wax moth
WA5LWM-D	without antibiotic fifth instar lesser wax moth fed with pretreated plastic film
WA-1-GWM	without antibiotic first instar greater wax moth
A-1-GWM	antibiotic administered first instar greater wax moth
WA-2-GWM	without antibiotic first instar greater wax moth
A-2-GWM	antibiotic administered second instar greater wax moth
WA-3-GWM	without antibiotic third instar greater wax moth
A-3-GWM	antibiotic administered third instar greater wax moth
WA-4-GWM	without antibiotic fourth instar greater wax moth
A-4-GWM	antibiotic administered fourth instar greater wax moth
WA-5-GWM	without antibiotic fifth instar greater wax moth
A-5-GWM	antibiotic administered fifth instar greater wax moth
WA-6-GWM	without antibiotic sixth instar greater wax moth
A-6-GWM	antibiotic administered sixth instar greater wax moth
WA-7-GWM	without antibiotic seventh instar greater wax moth
A-7-GWM	antibiotic administered seventh instar greater wax moth
WA-1-LWM	without antibiotic first instar lesser wax moth
A-1-LWM	antibiotic administered first instar lesser wax moth
WA-2-LWM	without antibiotic first instar lesser wax moth
A-2-LWM	antibiotic administered second instar lesser wax moth
WA-3-LWM	without antibiotic third instar lesser wax moth
A-3-LWM	antibiotic administered third instar lesser wax moth
WA-4-LWM	without antibiotic fourth instar lesser wax moth
A-4-LWM	antibiotic administered fourth instar lesser wax moth

WA-5-LWM	without antibiotic fifth instar lesser wax moth
A-5-LWM	antibiotic administered fifth instar lesser wax moth

CHAPTER 1

INTRODUCTION

Plastics are hydrocarbon-based petroleum products (Vuppaladadiyam et al., 2023) used in both residential and commercial sectors (Ruiz Barrionuevo et al., 2022). Chemically, plastic polymers are composed of a chain of molecules with links comprised of carbon, hydrogen, oxygen, and/or silicon (Kumar, 2018). Plastics play a significant role in the packaging, healthcare, building, and transportation industries because to their durability and affordability (Kumar, 2018). Global plastic production has significantly increased since the early 2000s, reaching nearly 400 million metric tons annually by 2021 (https://www.statista.com/topics/5401/global-plastic-waste/), which is further increased to 450-460 million metric tonnes by 2023 (https://ourworldindata.org/plastic-pollution; https://www.rts.com/blog/plastic-pollution-in-the-ocean-2023-facts-and-statistics/). The packaging sector currently accounts for 26% of all plastic consumption, and by 2050, that percentage is predicted to have multiplied four times (Geyer et al., 2017; Kumar, 2018). These packaged plastics are designed for single use and ends up in the water bodies, landfills and other natural ecosystems causing long term harm to the nature. The improper disposal of the packaged plastics also leads to formation of the microplastics. Small plastic fragments measuring below 5 millimeters, known as microplastics, have been discovered across various ecosystems, even in the remote regions of the Arctic (Bergmann et al., 2019), the deep sea (Woodall et al., 2014), and even in human food and drinking water (Cox et al., 2019). The existence of plastic waste in the environment has been shown to have negative impacts on wildlife, such as entanglement and ingestion (Bergmann et al., 2015), and the potential effects on human health are still being investigated (Barboza et al., 2018).

1.1. Plastics

Plastics are used in most aspects of day-to-day life as they are inexpensive, bear low production costs, and are durable. Production, together with the usage of plastic products, has been increasing logarithmically over the past ten years for a wide range of consumer goods used in daily life (Cai et al., 2018). Over the past few decades, the annual production of plastic materials has increased rapidly, with almost 370 million tonnes

produced in 2019 (Plastics Europe & Conversio Market & Strategy GmbH, 2019). According to a report published in 2022, the worldwide production of resin and fiber was estimated to be 2 million tonnes in 1950; it rose to 380 million tonnes in 2015; a certain decrease to 367 million tonnes was visible in 2020 as a result of COVID-19's effects on the sector (Hossain et al., 2022). The demand for plastics is strong and expected to continue, as evidenced by a 4% growth in global output to more than 390 million tonnes in 2021 (Plastic Europe: Plastics – The Facts, 2022). Polyethylene (PE) and polypropylene (PP) account for approximately ninety two percent of all synthetic plastics produced and are utilised to manufacture plastic bottles, bags, disposable containers, and other packaging materials (Ghatge et al., 2020). In 2018, 23% of the world's plastic manufacturing was composed of polypropylene, one of the most important plastic polymers for consumer products (Hossain et al., 2022).

Plastics are ubiquitous across residential, commercial, and non-commercial sectors, playing a crucial role in the economy, infrastructure, and several industries. They find application in agriculture, construction, consumer goods, telecommunications, healthcare, and medicine, contributing to the manufacturing of a wide range of items. From computers, office supplies, and plastic furniture to packaging for food, water, and personal care products, as well as components for vehicles, water storage tanks, and plumbing fittings, plastics are integral to the manufacturing processes of diverse sectors (Nithin & Goel, 2017). Apart from being the extremely useful material to mankind, the decomposition of plastic in the natural surroundings is utmost difficult.

In 2016, global plastic production reached approximately 280 million tonnes. The leading producer of thermoplastics and polyurethanes is China, responsible for nearly a third of the world's output at 29%. Europe and the North American Free Trade Agreement region are the second and third largest manufacturers, contributing 19% and 18% to the total production, respectively (Plastics Europe, 2017; Padgelwar et al., 2021). Although India and China have almost identical populations, India's need for polymers is just one-fifth to that of China, indicating that less plastic consumption in India. India finds considerable

regional differences in plastic usage, with northern India accounting for 23%, western India accounting for 47% and southern India accounting for 21%, making plastics a necessary evil. Further, Gujarat state is number one in the nation for having the most plastic production and processing facilities, with 5000 units. Reliance Industries Limited fulfills approximately 75–80% of the plastic demand, with the remaining 20% being provided by four government entities: Bharat Petroleum Corporation Ltd., Indian Oil Corporation Ltd., Haldia Petrochemicals, and Gas Authority of India Ltd (Padgelwar et al., 2021).

According to recent estimates, the global production of non-biodegradable plastic ranges from 350 million to 400 million metric tons per year. Out of this vast quantity, a significant portion, ranging from 5 million to 13 million metric tons, ends up as waste plastic in the world's oceans annually. This alarming amount of plastic pollution poses a serious threat to marine ecosystems and the overall health of the planet's oceans (Ghatge et al., 2020). Numerous types of plastics make up a sizeable fraction of the overall waste produced in the environment. Estimated levels of improperly handled plastic garbage range from 2% in the US to 89% in underdeveloped nations like Myanmar (Jambeck et al., 2015; Dhanshyam & Srivastava, 2021).

1.1.1. Plastic Pollution- The extensive usage, improperly man handling of plastic leads to accumulation of the polymers in the terrestrial and aquatic environment. Due to the poor recycle properties and single-use nature of the majority of consumed plastics, their rising production and use worldwide have resulted in historic levels of plastic waste generation and extensive plastic pollution (Borrelle et al., 2020; Lau et al., 2020; Walker & Fequet, 2023). According to global statistics, a mere fraction of plastic waste, amounting to less than one-tenth, has undergone the recycling process. An additional 12% has been disposed of through incineration, while the vast majority, nearly four-fifths, has accumulated over time, finding its way into various ecosystems across the planet (Geyer et al., 2017; Walker & Fequet, 2023). The amount of plastic garbage produced globally in 2016 that entered aquatic environments was estimated by Borrelle et

al., 2020 to be between 19 and 23 metric tonnes, but by 2030, that amount is expected to increase to up to 53 metric tonnes yearly (Walker & Fequet, 2023). Also, a study published in 2023, shows that the amount and dispersion of plastics in the ocean's surface layer have significantly increased since the turn of the century. Plastic concentrations varied from 1990 to 2005 during this trendless period, which was followed by a sharp upward trend starting in 2006. This demonstrates unequivocally that improper disposal methods and a lack of natural breakdown features result in the detrimental disposal of plastics in the ocean (Eriksen et al., 2023).

1.1.2. Existing Plastic Waste Management Techniques- Naturally degradation of plastics occur at a slow pace, which generates huge amounts of plastic waste that is accumulated on the planet (Webb et al., 2013a,b). Plastic waste treatment consists of 10% recycling of decay, 13% incineration, and 77% reclamation due to the lack of suitable degrading techniques (Verma et al., 2016). The release of harmful substances into the environment occurs during incineration, whereas groundwater and soil pollution occur during reclamation (Verma et al., 2016; Karn & Jenkinson, 2019). As a result, neither strategy is suitable for long-term use (Verma et al., 2016). Furthermore, according to a research report published in 2021 stated that every 20 residences, as often as possible, have a cluster of plastics burning on a daily basis in Dehradun, Uttrakhand, India. The act of setting fire to plastic materials releases a multitude of hazardous substances into the atmosphere, such as dioxins, polychlorinated biphenyls (PCBs), mercury, and furans. These noxious chemicals pose a significant threat to various life forms across the ecosystem. The toxic fumes generated by the incineration of plastics contaminate both the air and the surrounding environment, leading to detrimental consequences for the well-being of all living organisms in the affected areas (Karn, 2021). It was the goal of a study report that was published in 2021 to create a comprehensive analysis of the plastic waste problem in India and to determine the best possible combination of policies for trash reduction. According to the research, composite policy combinations offer a more potent policy mix than individual policy interventions for successful degradation of plastic waste. A good composite policy mix includes a disposal fee and a recycling

subsidy. However, kerbside recycling is a much more effective combination because it can offset and lessen the negative effects of both of these policies when they are implemented separately (Dhanshyam & Srivastava, 2021). But successful implementation of such techniques cannot completely minimize the plastic waste. So, the existing methods for the degradation and recycling of the plastics are harmful for the environment. There is immense need for the sustainable methods for degradation of the plastics.

1.1.3. Biological Degradation of Plastics- Degradation refers to the process by which a material's physical or chemical characteristics are modified. This change can occur through various mechanisms, including chemical reactions, exposure to light or heat, mechanical stress, or the action of biological processes. These processes can lead to significant alterations in the material's structure and properties. Polymer degradation primarily occurs through two fundamental mechanisms: hydrolysis, which involves the breaking of chemical bonds due to reaction with water, and oxidation, where the material reacts with oxygen. These processes can be driven by both chemical and biological factors, ultimately leading to the breakdown and decomposition of the polymer structure (Fotopoulou & Karapanagioti, 2017). Amongst all the degradation methods biodegradation is the most recent and unexplored method of disintegration of plastics. Biodegradation of plastics primarily occurs by microorganisms (Danso et al., 2019) and insects (Jang & Kikuchi, 2020). Studies on the biodegradation of plastics have been conducted using either complex microbial communities from a variety of pure cultures found in terrestrial (soil from waste sites, composting) or marine habitats. In recent times, interest has also been raised in the microbes that degrade plastic in insects' gut (Cassone et al., 2020; Ren et al., 2019; Zhang et al., 2020).

The natural breakdown of plastics could take hundreds of years, but insect-based biodegradation of plastics is becoming a more dependable way to deal with the plastic trash already in the environment. The selection of the current research topic- **“Comparative Studies on Plastic Degradation Capacity and Enzymatic Analysis in**

Larval Instars of *Galleria mellonella* and *Achroia grisella*" was influenced by the issue of accumulated plastic debris in many natural environments.

1.2. Wax Moths as Potential Bio-degraders of Plastics

Wax moth is a familiar term referred to moth species that infect and destroy the bee hives as well as bee keeping equipment (Ellis et al., 2013; Kwadha et al., 2017; Wojda et al., 2020; Kapahi & Marwaha, 2022). They are also recognised as bee miller, wax miller, wax worm, bee moth (Paddock, 1918; Ellis et al., 2013; Kwadha et al., 2017). The Greater wax moth (*Galleria mellonella* Linnaeus), Lesser wax moth (*Achroia grisella* Fabricius), Indian meal moth (*Plodia interpunctella* Hubner), Bumble bee wax moth (*Aphomia sociella* Linnaeus), and Mediterranean flour moth (*Anagasta kuchniella* Zeller) are the species of moth that are often referred to as wax moth (Williams, 1978; Kwadha et al., 2017; Marwaha, 2023b). The larval stage of wax moth species is recognized as the most destructive pest of honeybee colonies, renowned for both causing significant damage and their ability to biodegrade the plastics.

Plastics are composed of synthetic polymers extracted from fossil sources and are highly resistant to natural biodegradation. Apart from being resistant to biodegradation plastics have become most integral part of the modern society. Due to their numerous beneficial characteristics, such as strength, hardness, and affordability, plastics are the most often used polymer in day to day life (Danso et al., 2019). Plastic waste has grown into a severe global issue with negative effects on living things. One of the major concerns is the overuse of plastic in our ordinary routine. Excessive accumulation of plastics in the terrestrial and aquatic habits leads to the consumption of the plastics by the living organisms. Animals that consume plastic have excess polymers accumulated in their intestines (Barnes et al., 2009). Certain additives called plasticizers are also added in the plastics to increase the tensile strength of the polymers. Numerous endocrine organs experience dysfunction as a result of plasticizers, including bisphenol A, polybrominated diphenyl ethers, phthalates, and tetrabromobisphenol A. Additionally cancer-causing, plasticizers influence the peroxisome proliferator-activated receptor alpha, which results

in cancer in mammals (Talsness et al., 2009; Shahnawaz et al., 2019). Furthermore, plastics hinder plant growth; degrade soil, and lower chlorophyll levels (Bosker et al., 2019a).

In the current study, the biodegradation of polyethylene and polypropylene films, the two most commonly used plastics for industrial, residential, and packaging purposes will be investigated. Also, in the present study we will discuss about two wax worm species greater wax worms and lesser wax worms that are potential degraders of plastics (Bombelli et al., 2017; Chalup et al., 2018).

1.2. 1. *Galleria mellonella* (*G. mellonella*) – The *G. mellonella* (Lepidoptera: Pyralidae) known as greater wax moth are a constant threat to honey bee (*Apis mellifera*) colonies all over the world. Moth larvae are destructive to the stressed colonies and severely harm the stored beekeeping equipment, honey bee colonies and wax combs. Due to the considerable financial impact of the wax moth in apiculture, researchers have conducted numerous investigations into various aspects of this species, including its life cycle, interactions with the environment, genetics, molecular biology, physiology and methods for managing its population. The economic significance of wax moth has prompted several studies on the biology, ecology, study of biology at molecular level, physiology, and control of wax moth. Beyond their notorious reputation as pests in bee colonies, these insects are emerging as valuable model organisms for a wide range of research fields, including physiology, genetics, and proteomics. The growing interest in wax moths from their substantial impact on the beekeeping industry, as well as their unique ability to break down plastic materials through biological processes; these moths are now being recognized for their multifaceted potential in both agricultural and environmental contexts. The wax moth, on the other hand, goes through a complete metamorphosis and has the life stages including eggs, larvae, pupae, and adults (Figure 1.1).

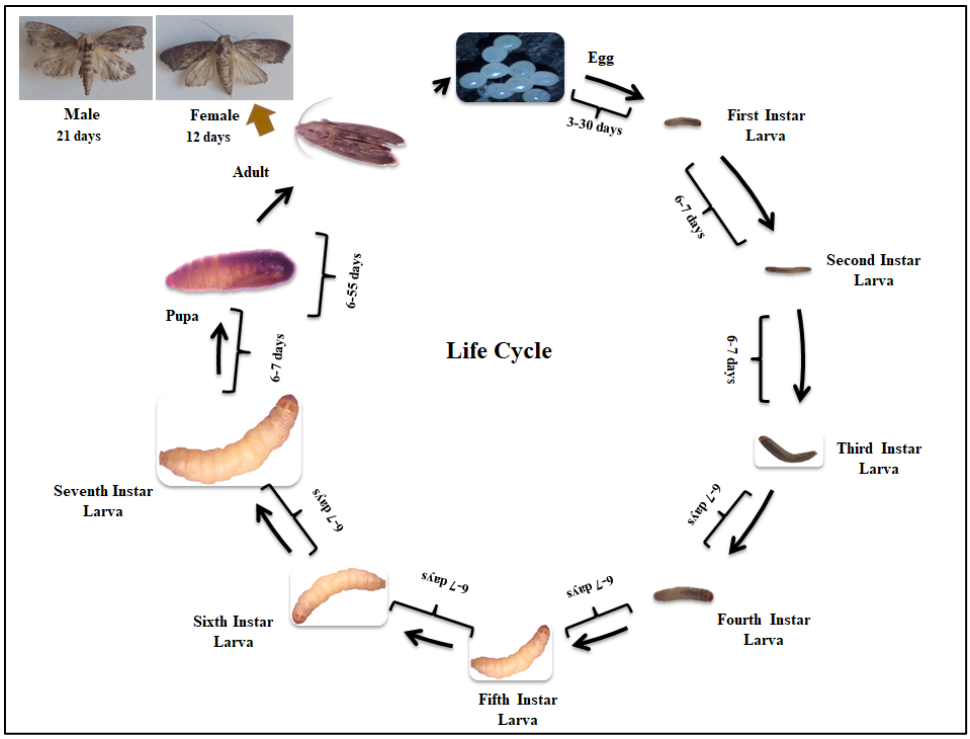


Figure 1.1 Illustrates the holometabolus life cycle of *Galleria mellonella* including life stages egg (Magnification- 80X), larva (first-last instar) (Magnification- 16X), pupa (Magnification- 16X) and adult (male-Magnification- 12X, female- Magnification-10X)**.** The Life span of greater wax moth can vary according to the environmental conditions

1.2. 2. *Achroia grisella* (*A. grisella*)- The *A. grisella* (Lepidoptera: Pyralidae) most commonly called as lesser wax moth are amongst the major pests of honey bee colonies (Kapahi & Marwaha, 2022, Marwaha 2023a,b). Moth larvae are pests of wax combs in honey bee colonies, especially in stressed colonies and damaging to stored beekeeping equipment. Studies on the biology, ecology, molecular biology, physiology, and management of wax moth have been driven by the insects' economic importance. The wax moth are holometabolous insects that show complete metamorphosis, have the life stages of eggs, larvae, pupae, and adults (Figure 1.2).

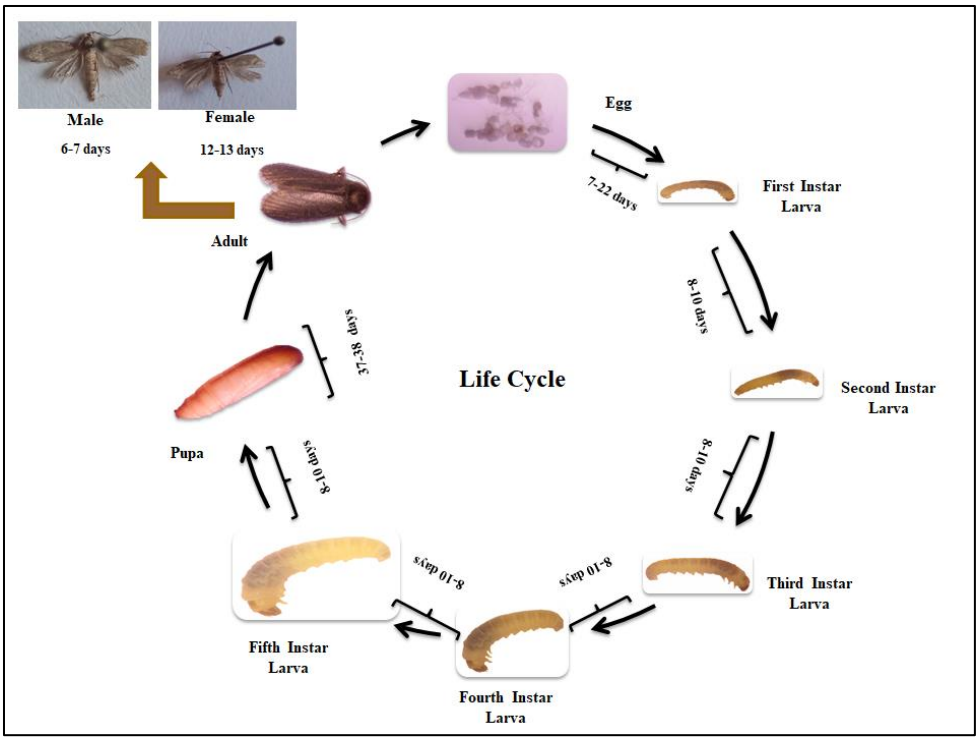


Figure 1.2 represents the complete metamorphosis in the *Achroia grisella* depicting life stages egg (Magnification- 20X), larva (first- last instar) (Magnification- 16X), pupa (Magnification- 20X), and adult (male-Magnification- 18X; female- Magnification- 16X). The Life span of lesser wax moth can vary according to the environmental conditions

1.3. Plastic Biodegradation by Wax Moths

Wax worms are the most commercially significant pest of honey wax comb because they reside as nest parasites in the honeybee colonies and ingest wax comb (Kwadha et al., 2017). Wax worms are extensively cultivated and utilized as a premium, profitable feed for various domestic animals, reptiles, amphibians, and birds, as well as being highly effective bait for fishing. These larvae typically consume wax combs, which contain a complex assortment of lipid molecules such as alkenes, alkanes, esters, and fatty acids (Kundungal et al., 2021). Moreover, the synthetic plastics and wax combs possess most prevalent hydrocarbon bond, which is identical chemical structure in the plastics and bee wax. This similarity in the chemical composition is the main reason behind the easy consumption of plastic polymers by wax worms.

Previous studies have explored the ability of various insect species, particularly the larvae of *G. mellonella* and *A. grisella*, to biodegrade different types of polyethylene. Bombelli et al. (2017) had initially reported the biodegradation of polyethylene by *G. mellonella*, while Chalup et al., (2018) observed *A. grisella* larvae ingesting silo bags made of UV filters and polyethylene layers.

The majority of studies in this field suggest that the degradation of plastics occurs due to the action of microorganisms residing in the digestive tract of wax worms (Zhang et al., 2020; Cassone et al., 2020; Montazer et al., 2021; Shikha et al., 2022). However, the current research thesis takes a different approach by investigating the breakdown of long linear chained plastics by the larval stage of the wax moth, without the involvement of the gut microbiome.

1.4. Present Study

The Polyethylene and polypropylene are amongst the most utilised plastics in the world. Additionally these are most accumulated plastics globally. Therefore, the present study explores the biodegradation of long-chain hydrocarbons (LDPE, HDPE and PP). In the current investigation the biodegradation capacity of the wax worms' *G. mellonella* and *A. grisella* is explored. The present research work concentrates on the biodegradation of wax worms independent of gut microbiota for plastics. There is expectation of biodegradation of plastics by enzymes present in the gut cells of the wax worms as well as the enzymes present in the gut microbes. Figure 1.3 depicts a speculative pathway that when insects are fed on the antibiotic solution there is expectation of biodegradation of the plastics by the enzymes present in the gut and fat body of the bee moth caterpillars. Administration of various antibiotics can lead into the disruption of intestinal microbiota into the insect that will lead to either accumulation of fragments of plastics in the excreta or digestion of plastic fragments. Alkane monooxygenase in the plasma membrane takes the oligomers of plastics and breaks down the polymers into alcohol. Alcohol dehydrogenase, a cytosolic enzyme, converts alcohol into aldehyde. Aldehyde is further transformed into a carboxylic acid by the mitochondrial enzyme aldehyde

dehydrogenase. The carboxylic acid formed is either stored in the body or consumed by β oxidation and tri-carboxylic acid cycle. Whereas the insects not fed with any antibiotic solution possess gut microbes as well the body cells of the gut of larval insect that metabolize the plastics collectively. It is presumed that the microbes as well as the cells of digestive cells and fat body cell collectively biodegrade the plastics. The microbial cell secretes the exoenzymes that act on fragments of long hydrocarbons chains and convert them into short hydrocarbon chains. The short hydrocarbon chains or oligomers are taken up by the microbial cells and endoenzymes present in the microbial cells act on the convert them into metabolic byproducts. Some of the short oligomers are degraded by the cells of the digestive tract and fat body cells.

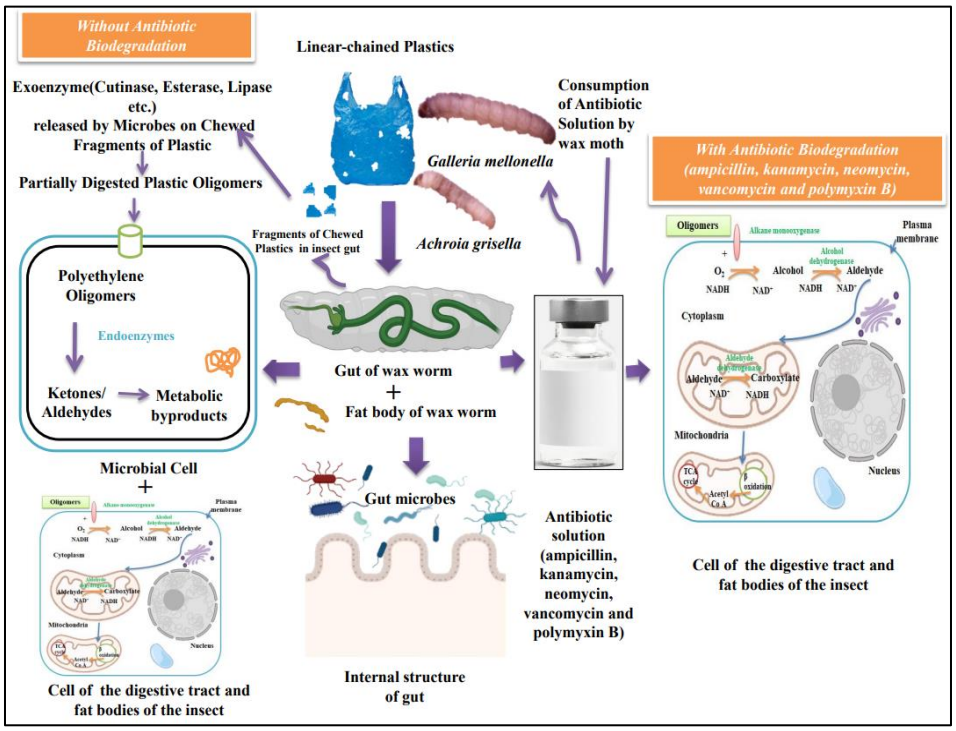


Figure 1.3 illustrates speculative mechanism for linear chained plastics with the dependence of gut microbiota and without any gut microbiota. The larvae of both *Galleria mellonella* and *Achroia grisella* are fed with antibiotic solution in order to observe the extent of biodegradation of plastics without intestinal microbiota (Copyright Protected)

Wax worms are prominent pests of honey bee colonies and beehives. So, open culturing of the wax worms can lead to increase in the population of the concerned pests and lead

to ecological imbalance in the nature. Consequently, there is need of a closed system that can be used as a tool for biological disintegration of the plastics.

CHAPTER 2

REVIEW

OF

LITERATURE

A clean and healthy environment is very essential for survivability of living organisms but to increase human comfort there is continuous use of certain synthetic polymers, synthetic chemicals, xenobiotic and others, which are making environment misfit for existence and establishment of life on this planet. The scientific community has witnessed a remarkable surge in the utilization of synthetic polymers, with plastics emerging as a material of paramount significance in contemporary society. The inherent stability and durability of plastics have rendered them as indispensable components of human comfort and convenience. The exponential growth in plastic consumption, from a mere 5 million metric tonnes in the 1950s to an astonishing 367 million metric tonnes in 2020, can be attributed to the material's versatile properties and wide-ranging applications across various industries. This unprecedented rise in plastic usage has been facilitated by the material's unique combination of strength, lightweight nature, and resistance to degradation, making it a preferred choice for infinite products and processes. However, the rapid expansion of plastic production and consumption has also raised concerns regarding its environmental impact and the need for sustainable management strategies (Napper & Thompson, 2023). Due to extensive use of these synthetic polymers it is expected that plastic manufacturing would quadruple over the next 20 years (Vuppaladadiyam et al., 2023).

Although plastics are useful, cost effective and durable but these polymers are non-biodegradable which eventually results in their accumulation in terrestrial ecosystem, such as landfills and aquatic environments like lakes, rivers, seas, oceans and beaches (Ali et al., 2021). This massive accumulation of plastic ultimately result in plastic pollution (Napper & Thompson, 2023).

The polymers accumulate as a result of the global growth in plastic production and manufacturing rates. A buildup of plastics due to piled plastic waste and a lack of appropriate disposal options have negative effects on the ecosystem. As there aren’t enough mass- producible landfills available for disposal of plastics so these polymers are burned which releases hazardous and toxic gases into the atmosphere, contributing to air

pollution. Due to lack of efficient degradation methods, plastic burning is most popular disposable method in many less developed and developing nations. Furthermore, there are a lot of inappropriate disposal techniques, such as open dumping, unregulated incineration, irrational composting, and improper landfilling which further enhance the plastic pollution. Usage of such techniques increase the unwanted environmental concerns (Nithin & Goel, 2017).

In addition to having an adverse effects on the environment, plastic trash can seriously harm the biotic as well as abiotic components of freshwater, marine, and terrestrial ecosystems. The accumulation of plastics not only contaminates the environment but also affects the growth of living organisms. Moreover, numerous marine (zooplanktons, jellyfishes, lobsters, seals, turtles, bald eagle, parakeet, baleen whales, goose barnacles, dolphins, etc.) and land animals (sheep, goats, cows and buffaloes) are killed by plastic debris after choking or becoming entangled (Otsyina et al., 2018; Kosior & Crescenzi, 2020; Law et al., 2020; Napper & Thompson, 2023).

2.1. Consequences of Plastics on Life Forms

Plastic pollution is primarily caused by inadequate waste management practices, irresponsible human actions, and accidental contamination. Properly managed landfills are designed to be self-contained systems, surrounded by barriers to prevent waste from being carried away by the wind and regularly covered with soil or other materials. Plastics, being non-biodegradable, can remain intact for many years until they are incinerated or recycled. The improper disposal of plastic waste, when not disposed in designated landfills, often results in its dispersion across terrestrial environments, transported by wind until it infiltrates aquatic systems which afterwards accumulate in oceanic habitats. This form of environmental contamination is a direct consequence of human negligence, manifested through actions such as the indiscriminate discarding of litter, the deposition of waste in areas not sanctioned for collection, or the deliberate introduction of plastics into marine ecosystems (Barnes et al., 2009; Hammer et al., 2012).

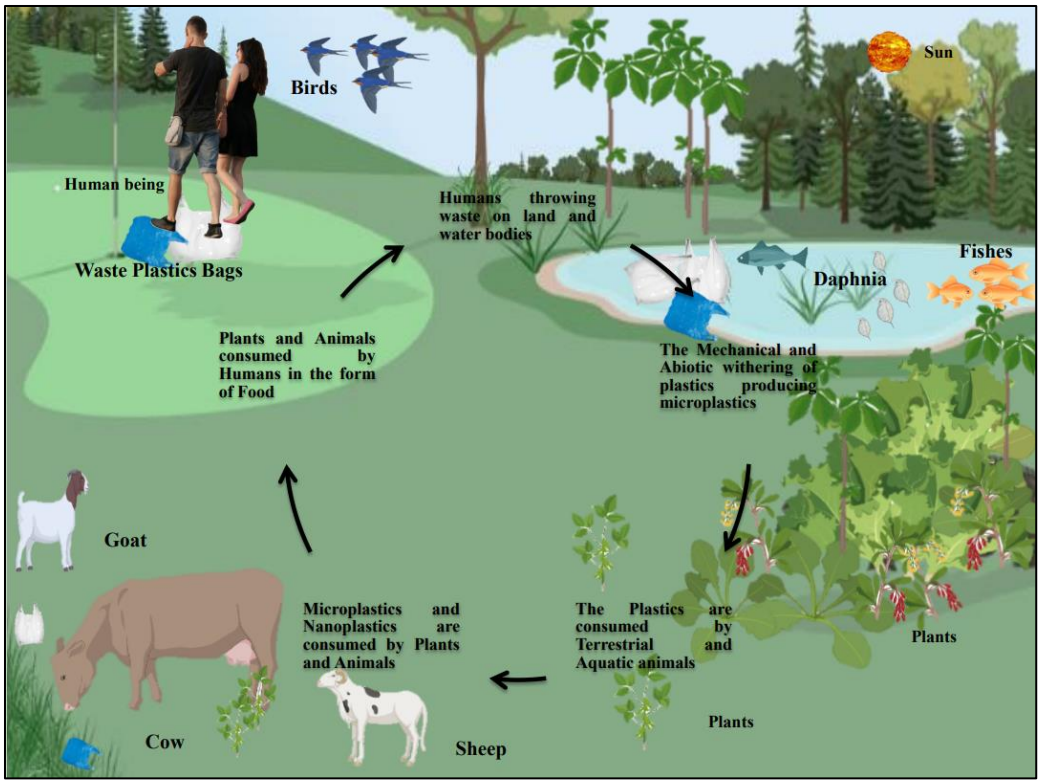


Figure 2.1 Effect of plastics on the life forms- The widespread use of single-use plastics has led to a significant environmental problem, as these materials are often carelessly discarded and end up in both land and water ecosystems. This plastic waste can have detrimental effects on various life forms inhabiting these environments. When organisms accidentally ingest plastic debris, it accumulates in their bodies over time. As a result, the consumed plastics can potentially transfer through the food chain, ultimately reaching humans who consume these affected flora and fauna. The ingestion of plastic-contaminated food by humans raises concerns about the development of various health issues and disorders

2.1. 1. Impact of Plastic waste on Fauna- The growing abundance of plastic pollution in natural ecosystems represents a major threat to animals, especially those living in the marine habitat. The accumulation of plastic debris in the oceans directly threatens the well-being of aquatic life. Marine animals frequently mistake floating plastic for food and consume it, or they become trapped in the debris, which can hinder their mobility and lead to suffocation. As the quantity of plastic waste entering the environment continues to grow, it will further impact both marine and freshwater ecosystems. In the study conducted by Browne et al. (2013), the lugworm species *Arenincola marina* was subjected to sand containing a mixture of 5% microplastics, various additives such as PBDE-47 and triclosan, and pollutants like phenanthrene and nonylphenol, highlighting

the potential harm caused by plastic contamination. Pollutants accumulated significantly in the intestinal tissues of lugworms after being transferred from their general tissues. Ingesting nonylphenol from the sand has led to a reduction of up to 60% in the ability of coelomycetes to eliminate harmful bacteria. Exposure to microplastics made of polyvinyl chloride has made lugworms more vulnerable to oxidative stress. Triclosan consumption has severely impaired the worms' ability to process sediments, leading to the mortality of insect species. The presence of nanoplastics in the environment of *Daphnia magna* has negatively impacted the zooplankton's development, causing physical deformities and reproductive issues (Besseling et al., 2014). Plastic pollution in aquatic environments can disrupt the delicate balance of predator-prey interactions by interfering with interspecies chemical communication. The presence of plastic waste can absorb kairomones, which are chemicals released by predators that prey species, such as *Daphnia longicephala*, rely on to detect and avoid danger. As a result, the ability of prey to effectively sense and respond to the presence of predators is compromised, potentially leading to altered behavior and increased vulnerability. This disruption in chemical signaling highlights the far-reaching consequences of plastic contamination on the intricate relationships within aquatic ecosystems (Trotter et al., 2019).

Plastic waste presents a serious danger to seals, especially to the younger and more inquisitive ones. They are drawn to the plastic, often swimming with it or sticking their heads through loops, which can lead to harmful entanglements. Due to the backward growth of their hair, seals find it difficult to remove plastic rings, loops, or lines from around their necks. As the seal matures, the plastic collar becomes increasingly constrictive, eventually strangling the animal or obstructing its blood flow. Additionally, many seals, especially those with poor vision in the North Sea, become trapped in underwater fishing nets while hunting for food. Unable to escape from the floating plastic debris, these animals tragically drown.

Certain species of whales are susceptible to entrapment in marine debris, which can lead to fatal consequences. Some whale species may find themselves unable to break free

from the entanglement, resulting in drowning. Conversely, larger whales frequently become intertwine in fishing gear, which they inadvertently carry with them. This latter form of entrapment can cause the whale to experience malnutrition and suffocation, as the entanglement prevents their ability to consume food in a normal manner (Hammer et al., 2012). According to a research report ingestion of micro and nanoplastics present in the shoreline and sea floor by marine organisms can lead to severe harm to their body (Eriksen et al., 2023).

Since plastics cannot be broken down by the digestive system, they accumulate within an organism's body rather than being excreted. This accumulation of plastic can lead to blockages in the digestive tract, suppressing the appetite and potentially deteriorating the stomach lining (Laist, 1987). The California condor (*Gymnogyps californianus*), a species facing the threat of extinction, has experienced population declines due to the presence of plastic waste in their land-based habitats, which the birds accidentally consume (Thompson et al., 2009). Otsyina et al. (2018) investigated the impact of discarded plastic on sheep and goats in grazing areas. The researchers found that many participants in the study used plastic bags because they were inexpensive. A significant percentage of responders highlighted animal deaths as the eventual result of ingesting trash plastic bags (Otsyina et al., 2018).

Plastic utilisation raises environmental problems due to the quantity of garbage produced as well as the toxins that leach from the plastic. Polymers include plasticizers to improve their tensile strength, but when these plasticizers come into contact with humans, they may seriously damage their body organs. Di(2-ethylhexyl)phthalate is a plasticizer that is used all over the world, therefore many individuals probably come into contact with it every day. Studies on animals revealed that this substance is a nongenotoxic carcinogen. Mono-and dicarboxylic acids, which are Di(2-ethylhexyl)phthalate metabolites, transactivate the peroxisome proliferator-activated receptor α, which has been linked to nongenotoxic carcinogenesis (Ito & Nakajima, 2008). Furthermore, Tetrabromobisphenol A, polybrominated diphenyl ethers, bisphenol A, and phthalates are examples of

components used in plastics that are emitted from plastic items. These substances are also referred to as endocrine disruptive chemicals due to their capacity to alter the endocrine system. Evidence from both human and animal studies suggests that endocrine disrupting chemicals may contribute to the occurrence of cancer, reduction in sperm counts, temporally rising rates of male reproductive tract developmental abnormalities, and the trend towards precocious puberty in females (Talsness et al., 2009).

2.1. 2. Repercussions of Plastic waste on Flora- Inhibiting plant development, lowering chlorophyll concentrations, and lowering soil quality seem to be just a handful of the effects plastic waste has on plants. But these impacts of plastics might have a significant negative influence on the ecosystem. The accumulation of waxy material possibly plastics on the pores' surface in *Glycine max* (soybean) seeds can seriously impair water intake (Calero et al., 1981). Moreover, the plastics not only affect the seeds of higher plants but they also affect the photosynthesis in the algal species. *Scenedesmus* and *Chlorella* did not undergo photosynthesis as there was blockage of light air flow physically by nanoparticles when they were exposed to polystyrene particles that were 20 nm in size, according to Bhattacharya et al. (2010). Furthermore, microplastics are taken up by the epidermis or by cells; these tiny polymers are more likely to be consumed by the plant (Bandmann et al., 2012). In contrast to algae (*Scenedesmus obliquus*), there was a decrease in chlorophyll content after 72 hours of exposure to polystyrene nanoplastics of 70 nm (Besseling et al., 2014). According to Kalčíková et al. (2017), *Lemma minor*, a species of aquatic duckweed, experienced root development inhibition when exposed to microplastics. It was estimated that, microplastics may physically impede plant roots, which might inhibit plant development (Kalčíková et al., 2017). According to Bosker et al. (2019), plastic particles accumulate in the testa of *Lepidium sativum* and significantly impair metabolic processes. The biophysical characteristics of soil can be significantly harmed by microplastics. Microplastics in the soil have a serious negative impact on the normal metabolic processes and development of the plants (De Souza Machado et al., 2019).

2.2. Accumulation of Plastics- One of the most frequently consumed everyday items is plastic, and plastic product usage has dramatically expanded globally. Less than 60 years ago, the first bulk production of plastics was observed; now, the bulk of items that people use practically everywhere on Earth are made of plastics (Barnes et al., 2009). Since 1950, almost 830 million tonnes of plastic have already been manufactured. The remaining 93% of garbage is either dumped in landfills or discharged into the surroundings, with just 7% of it being recycled (Boucher et al., 2019). Each day, 27,000 tonnes of abandoned plastics end up into the oceans. There are significant environmental contamination problems caused by the distributed plastic mounds of microplastics and plastic trash (Friot & Boucher, 2017). A third of all manufactured plastic is utilised for packaging and is then quickly discarded. In the ocean, landfills, and natural terrestrial environments, accumulation of plastics waste is reported (Thompson et al., 2009). Plastic garbage that has accumulated over years pollutes the environment and piles up in all ecosystems.

2.2. 1. Plastic Debris on Land- Plastics are semi-synthetic or synthetic materials manufactured through the polymerization process (Ghayebzadeh et al., 2020). The main issue is that, although being durable materials, synthetic plastics are widely utilized for transient applications like packing. The amount of plastic packaging that is collected for recycling is only 14%, and the plastics that are recycled are typically used in low-value applications that cannot typically be recycled once they have been used. Additionally, only 72% of plastic packaging is recycled, 32% escapes the collection system, indicating that either no plastic packaging is collected at all or that it is collected but subsequently improperly disposed of or dumped and 40% is disposed of in landfills (Kosior & Crescenzi, 2020)**.** Just a small portion of the plastic garbage that is dumped every day gets recycled or incinerated at dump sites. The majority of it ends up in landfills, where it decomposes over a period of up to 1000 years, during which time harmful compounds seep into the soil and water. Although plastic pollution was originally discovered and acknowledged in the aquatic environment, current investigations have revealed that plastic remnants are present in significant amounts in the soil. According to some reports,

soil contamination from plastics is worse and worsens quickly, ranging from 4 to 23 times worse than plastic pollution in water. The accumulation of microplastics in the soil is detrimental to the soil biota. As a result, once plastic material has accumulated in the soil, it is incorporated into the soil's organic matter and mineral replacements and survives for many hundred years (Tudor et al., 2019).

2.2. 2. Plastics in the Aquatic Water Bodies- Plastic are persistent and hence assemble in the environment. Dominance of plastic waste is observed in the marine ecosystem presently. Plastic pollution can be found even in the most remote regions of the world because the lightweight and durable nature of plastics allows them to travel more easily than other types of waste. Thus, proportion of plastic particles in marine garbage rises with increasing distance from the source of the debris. The majority of the times, plastic items are discovered floating in the water or washed up on shorelines. According to research conducted in the North Sea, of the plastic waste that is dumped into the ocean each year, 15% of it floats to the top, 15% washes up on beach, and the remaining 70% eventually sinks to the bottom of the ocean (Barnes et al., 2009).

2.2. 2. 1. Plastics Floating in Water Bodies- As plastics are fabricated of light polymeric materials or have forms that allow air to be trapped inside of them, many plastic objects (e.g., bottles and bags) float. Most plastic items remain afloat up till they accumulate enough biota on their surface to become heavy or become saturated with water and sink (Hammer et al., 2012). Marine convergence zones, confined seas, and ocean currents appear to be places where floating trash tends to amass most often. Debris is pushed towards a central region where winds and currents cease to exist by the North Pacific (NP) central gyre, a region of large air pressure and ocean current in clock wise direction. Plastic trash has been extensively studied and sampled in this area. Furthermore, the heart of the NP gyre is now referred to as the Great Pacific "Garbage Patch" or "Pacific Trash Vortex" because to the imperceptible buildup of plastic garbage, primarily meso-and micro-plastic particles (Hammer et al., 2012).

Moore et al. (2001) sampled 11 randomly selected locations in the eastern portion of the NP central gyre using a manta trawl. Unknown fragments, polystyrene polymers, plastic resin pellets, polypropylene polymers, and thin plastic film particles were the five groups into which the individual plastic bits were divided. In the examined region, the average quantity of plastic particles was 334,271 particles per square kilometer with a mass of 5,114 grams per square kilometer. Though, plankton was found to be 5 times more abundant than plastic, but plastic particles found in the region had a mass that was around 6 times that of plankton. These plastic particles in abundance in the water bodies are a serious matter of concern (Moore et al., 2001). Pairs of bongo mesh were utilised in 2002 to sample a different region in the eastern NP central gyre. The mean particle density in the samples taken at both depths was 0.017 particles/m^3, which is a factor of 100 lower than the concentrations discovered at the surface of the identical locations that were studied previously (Moore et al., 2005).

Further to measure the density of plastic particles in the water was carried out in the Kuroshio Current region, on the western edge of the NP gyre. 76 sites were sampled in this area between April 2000 and April 2001 using a manta trawl. Plastics were divided into the following categories: pellets of plastic resin, plastic goods, and pieces of plastic goods, rubber, fiber, styrofoam, thin plastic sheets, and sponge. The mass (0-153,000g/km^2) and abundance (0-3,520,000particles/km^2) varied between the regions. The number of plastic particles grew with increasing distance from the shore, and the Kuroshio Current's region had the highest abundance, suggesting that this current is involved in the movement and dispersion of plastics from Japan and Indonesia throughout the NP Ocean (Yamashita & Tanimura, 2007).

There are only five gyres on earth, including the one in the North Pacific (NP). Research organisations were functioning on mapping their data as they have also investigated into the North Atlantic (NA) gyre. Amid 1986 and 2008, the Sea Education Association maintained an eye out for plastics in the NA gyre. Various research vessels carried out for greater than 6,100 surface plankton mesh tows. Amongst them plastic was present in 62%

of all tows, and the enormous sample included 1,069 pieces, or 580,000 pieces per kilometre. It is notable that this study indicated an increment in the quantity of plastic waste up to the year 2000, but from the year 2000 to 2008 exhibited hardly any growth in plastic debris, even though plastic manufacturing climbed consistently after the year 2000 (Law, 2010).

Given the vast surface area of the seas, it is nearly hard to get knowledge about the magnitude of floating plastic waste. However, there is relatively limited available data about the prevalence of floating plastic garbage in the water. Nevertheless, the research has provided sufficient evidence to suggest that humanity should be wary of the scale of floating plastic pollution and recognize it as a significant waste production issue.

2.2. 2. 2. Plastic Debris at Beds of Water Bodies- The marine debris left behind by fishing boat trawls was investigated in a research carried out in the Patras and Echinadhes Gulfs in Western Greece. In these two Gulfs, the debris density was 89 and 240 pieces per km^2, respectively. Plastic pieces became the primary type of debris at the beds of water bodies (Stefatos et al., 1999). According to survey findings, the seafloor in the North Sea has an average of 110 pieces of trash per square kilometre. There would be 600,000 m^3 of marine debris on the bottom if this figure were expanded to the whole North Sea. When scanning France and Corsica, it was discovered that there were 300 million pieces of marine trash in the Mediterranean at a depth of 2,500 metres (Miljo, 2001). In various Greek coastal locations in the eastern Mediterranean in 2004, the quantities of marine benthic debris were examined. Plastics constituted the largest portion of the debris at 55.47%, with an average total density of marine debris estimated at 15 items per square kilometer, ranging from 0 to 251 items per square kilometer (Katsanevakis & Katsarou, 2004).

Plastic waste is accumulating in the terrestrial and aquatic ecosystems. The presence of plastic waste can lead to harmful effects on the organisms living in these environments. The piling of plastic waste can negatively impact the health, survival and overall well-being of both terrestrial and aquatic organisms.

2.3. Need of Biodegradation of Plastics- Plastic manufacturing and use have dramatically increased globally as a result of the fast urbanisation and economic expansion in many nations. Almost 100 million tonnes of plastic garbage are generated each year and are adding to the environment at shocking rate (Shahnawaz et al., 2019). 50–80% of the garbage that pollutes marine ecosystems globally is made up mostly of plastic, which is the major component (Fotopoulou & Karapanagioti, 2017). The problem of plastic pollution has developed into a hazard to the world's environment because of plastic's resistance to deterioration and its abundance in industry (Webb et al., 2013). The recovery rate of plastic waste remains quite low because of its limited recycling value and the absence of adequate specialized support systems (Kong et al., 2017).

Many dangerous and environmentally harmful impacts are spurred on by plastic contamination in the marine environment. Wildlife is directly endangered by plastic waste that has been proven to negatively affect a wide variety of animals. For the majority of species, ingesting and being entangled in plastic materials pose the greatest risks. Particularly juvenile animals frequently become entangled in plastic trash, which can cause major harm as the animal matures and restrict mobility, and even prevent mammals from breathing properly from properly eating. A broad range of marine organisms, along with seabirds, sea turtles, whales, fur seals, sharks, and filter feeders were significantly distressed by plastic pollution. These animals are particularly susceptible to ingesting plastic items and they mistake plastics for food are marine birds (Webb et al., 2013). The digestive tract of these animals can get clogged with plastic after ingesting it, which can reduce the amount of feeding stimulus, impede digestion, reduce digestive enzyme output, and lower levels of steroid hormones, which can interfere with reproduction (Laist, 1987; Thompson et al., 2009).

2.4. Biodegradation of Plastics- Degradation is the term for any alteration of a substance's physical or chemical characteristics and can result from chemical, physicochemical processes, as well as biological ones (Fotopoulou & Karapanagioti, 2017). The term "biodegradation" refers to the process by which materials are

decomposed into substances that are safe for the environment, such as biomass, carbon dioxide, and water, using naturally occurring organisms (Karak, 2016). For the initial time, a comparable plastic biodegradation test of lignin and paraffins due to bacterial action was investigated by growing bacteria on several types of alkenes as the only carbon source available. Researchers continued by claiming that bacteria can only degrade polymers with molecular weights up to 4800 (Fuhs, 1961; Shahnawaz et al., 2019). Eventually, studies of plastic breakdown by bacteria began to proliferate in the literature from all over the world. Moreover, the larvae of *Plodia interpunctella* were the first to demonstrate polyethylene biodegradation (Yang et al., 2014).

2.5. Biodegradation of Plastics by Insects- Plastic waste consumption by arthropods is a new approach; several plastic-eating worms have been reported that may digest plastic and transform it into a non-hazardous substance (Yang et al., 2014; Bombelli et al., 2017). Till recently, seven types of plastics have been damaged by insects: polyethylene, polystyrene polyvinyl chloride, polypropylene, polyphenylene sulphide, ethylene-vinyl acetate, and extruded polystyrene. The process of plastic decomposition in insects is still being studied; however it is assumed that enzymes and gut bacteria play a role in insects (Bilal et al., 2021).

2.5. 1. History of Biodegradation of Plastics by Insects- From 1950s, when commercial plastic production first began, there has been a remarkable growth in the industry. It is estimated that from 1950 to 2018, approximately 6.3 billion tonnes of plastic were generated worldwide (Alabi et al., 2019; Bilal et al., 2021). The inherent slow decomposition rate of plastic leads to an accumulation of plastic waste, which presents a significant threat to the ecosystem. The rate at which plastic degrades is influenced by various factors, including age, exposure to weather conditions, the specific type of polymer, temperature, pH levels, and exposure to radiation (Akbay & Özdemir, 2016).

The first recorded instance of polyethylene biodegradation was observed in the larvae of *Plodia interpunctella* (Yang et al., 2014)**.**

2.5.2. Insects' Biodegrading Plastics- Many invertebrates, including *Plodia interpunctella* (Yang et al., 2014), *Zophobas atratus* (Kim et al., 2020), *Galleria mellonella* (*G. mellonella*) (Bombelli et al., 2017), *Tenebrio molitor* (Brandon et al., 2018), *Achroia grisella* (*A. grisella*) (Chalup et al., 2018), *Achatina fulica* (Song et al., 2020), *Hermetia illucens* (Beale et al., 2022) and others, have demonstrated plastic biodegradation.

2.5.3. Biodegradation of Plastics by Wax Moth- According to their nest parasitic lifestyle and ability to consume honey wax comb, wax worms are the pest of honey wax comb that has the most economic impact on apiculture industry (Kwadha et al., 2017; Wojda et al., 2020). Waxworms are cultivated widely and used as great and profitable pet food, reptile, amphibian, and bird food, as well as good fishing bait. Wax comb, which contains a wide range of lipid compounds including alkenes, alkanes, esters, and fatty acids, is frequently consumed by waxworms (Kundungal et al., 2021). The fundamental cause of wax worms' ease in consuming polymers is the CH_2-CH_2 link, which is the most frequent hydrocarbon bond in wax combs and is also present in many plastics due to the latter's similarity to bee wax. Bombelli et al. (2017) were the first to report *G. mellonella's* polyethylene biodegradation. In Argentina, apiculturists cover their beehives with silo-bags, which are made of three layers of polyethylene and one UV filter. According to reports, *A. grisella* larvae ended up eating these silo bags (Chalup et al., 2018). Further, Kundungal et al., (2019) revealed that *A. grisella* was responsible for the biodegradation of High-Density Polyethylene (HDPE). Low-Density Polyethylene (LDPE) received a solar pretreatment that accelerated *G. mellonella's* biodegradation of the polymers (Kundungal et al., 2021). Researchers had isolated the *Aspergillus flavus* from the *G. mellonella's* intestines that had demonstrated the ability to destroy microplastics of HDPE (Zhang et al., 2020). Moreover, the activity of the enzymes lipase, fatty acid metabolism, and carboxylesterase was examined to determine how the gut microbiota affects the biodegradation of LDPE and beeswax (Kong et al., 2019). The effects of co-diet on the gut microbiota of *G. mellonella* as well as the caterpillar's ability to biodegrade polyethylene and polystyrene was explored by Lou et al. (2020). From the

digestive tract of the greater wax moth, Cassone et al. (2020) recovered *Acinetobacter*, which has the capacity to biodegrade the LDPE. Peydaei et al.(2020) has identified the impact of polyethylene ingestion on the *G. mellonella* salivary gland. The ability of the complete live larva and homogenate of the caterpillar, *G. mellonella*, to biodegrade was also investigated in this research article (Peydaei et al., 2020). In this research report, technological issues with the potential for biodegradation were also examined (Billen et al., 2020). Lemoine et al. (2020) employed biochemical and RNA sequencing techniques to evaluate the *G. mellonella's* capacity to degrade polyethylene. Intestinal transcript sequencing revealed that plastic-fed larvae maintained normal intestinal metabolism with increased metabolic activity for lipids (Lemoine et al., 2020). *Bacillus aryabhattai*, *Lysinibacillus fusiformis*, and *Microbacterium oxydans* were also isolated from the whole-body extracts of *G. mellonella*. These bacterial species have the ability to biodegrade LDPE, and their biodegradation capacity was investigated by weighing the polyethylene film, monitoring cell biomass production, and identifying the byproducts of the polymers' biodegradation (Montazer et al., 2021). Contrastingly, Peydaei et al. (2021) confirmed that greater wax moth larvae of could chew the polyethylene, polystyrene and polypropylene plastics. The chewing of plastics modified the gut microbiota diversity in the insect whereas the microbes were not affected by the plastics as diet. On the other hand, *G. mellonella's* biodegradation capability for the polymers used in electronic equipment was examined by Zhu et al. in 2021. Also, the feeding preferences of *G. mellonella* were also examined in contrast to used trash plastics and naive plastics (Zhu et al., 2022). Furthermore, examination of development time, duration of the larval stage, and survival rate revealed an impact of LDPE, Polystyrene (PS), and Polypropylene (PP) intake on the growth of *G. mellonella* (Ruiz Barrionuevo et al., 2022). Shikha et al. (2022) examined the development of gut isolates of *G. mellonella* larva on plastics and the identification of gut microbiota using a method that was culture dependent. Beale et al. (2022) had observed the biodegradation potential of wax worm, black soldier fly and meal worm. The metabolism of sole carbon diet, and the metabolites produced were also analysed. Moreover, the biodegradation of polystyrene and polyethylene as diet by

greater wax moth larva was observed. It was concluded that ingestion of polystyrene was more by the insect as compared to polyethylene, interaction of the plastic material with the insect lead to formation of new chemicals, that validated the biodegradation (Burd et al., 2023). Still the biodegradation capacity of *G. mellonella* and *A. grisella* independent of gut microbiota for long linear chained plastics is unexplored.

Polyethylene and polypropylene are two of the most widely used polymers in the entire globe. Also, they are the most accumulating plastics on a worldwide scale. As a result, the current study investigates the biodegradation of long-chain hydrocarbons (LDPE, HDPE and PP). The current study explores on the biodegradation potential of the wax worms *G. mellonella* and *A. grisella*. The present investigation focuses on the biodegradation of wax worms in the absence of gut microbial diversity for plastics. Plastics are expected to be biodegraded by enzymes found in the gut cells of wax worms as well as enzymes found in intestinal bacteria. Figure 2.2 displays a hypothetical process in which when insects are fed an antibiotic solution, the plastics are expected to be biodegraded by enzymes found in the stomach and fat body of bee moth larvae. Antibiotic administration can disturb the insect's intestinal microbiota, resulting in either the buildup of plastic polymers in the excreta or the digestion of plastic pieces. Alkane monooxygenase in the plasma membrane degrades polymers into alcohol by taking oligomers of plastics. A cytosolic enzyme called alcohol dehydrogenase transforms alcohol to aldehyde. The mitochondrial enzyme aldehyde dehydrogenase converts aldehyde into a carboxylic acid. The generated carboxylic acid is either retained in the body or consumed through β oxidation and the tri-carboxylic acid cycle.

In contrast, insects that have not been administered any antibiotic solution have gut microorganisms along with body cells. Plastics are thought to be biodegraded by microorganisms, digestive cells, and fat body cells working together. Exoenzymes are secreted by the microbial cell and act on pieces of long hydrocarbon chains to convert them to short hydrocarbon chains. The microbial cells take up the short hydrocarbon chains or oligomers, and endoenzymes contained in the microbial cells act on them to

transform them into metabolic byproducts. Some of the short oligomers are destroyed by digestive tract and fat body cells.

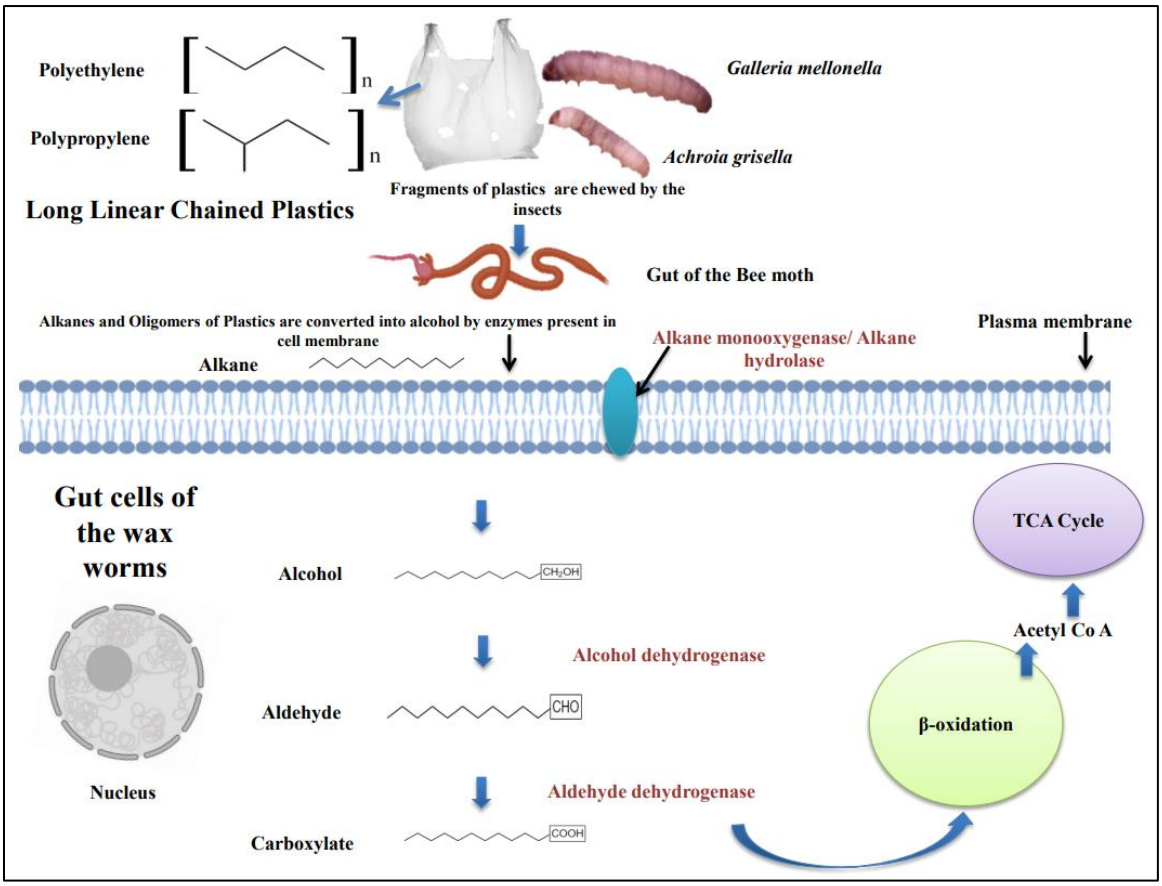


Figure 2.2 The cellular hypothetical mechanism suggesting biodegradation of linear plastics by gut and fat body cells of the wax worms (Copyright Protected)

CHAPTER 3

HYPOTHESIS

Synthetic plastics have become indispensable materials in modern society due to their remarkable properties such as affordability, stability, and durability. However, these same qualities make most plastics resistant to biodegradation, leading to their accumulation in landfills and ecosystems like oceans and beaches. This plastic pollution poses severe threats to both terrestrial and marine life, as animals often consume or become entangled in plastic debris, leading to starvation and mortality.

Moreover, the use of plastics raises environmental concerns due to the waste generated and the release of harmful chemicals like plasticizers and endocrine-disrupting compounds into the environment. These substances have been linked to various health issues, including cancer, reproductive disorders, and developmental abnormalities.

While technologies such as reclamation, recycling, and incineration are currently used to help plastics disintegrate, these methods can contaminate groundwater and soil, releasing toxic compounds into the environment. Therefore, there is an urgent need for effective biological methods to biodegrade plastics.

Interestingly, wax moths, which are common pests in the apiculture sector, have caterpillars capable of biodegrading plastics. The current study aims to investigate the biodegradation potential of all larval stages of *Galleria mellonella* (greater wax moth) and *Achroia grisella* (lesser wax moth) on the most widely used long linear chained polymers, namely Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), and Polypropylene (PP).

In this study, the polymers had been exposed to all larval instars of both wax moth species for two days to observe their biodegradation potential. To assess the extent of biodegradation, the weight loss of the polymers is recorded, which indicated the amount of plastic consumed by the larvae. Furthermore, the survival rate of the insects throughout the experiment is noted. The Scanning Electron Microscopy (SEM) has been utilised to visually examine the degree of degradation on the plastic film's surface. Additionally, the frass (insect excrement) using Gas Chromatography and Mass

Spectroscopy (GC-MS) was analysed to confirm the biodegradation of the plastics. The study also focuses on identifying potential of the enzymes responsible for plastic biodegradation in these wax moth larvae.

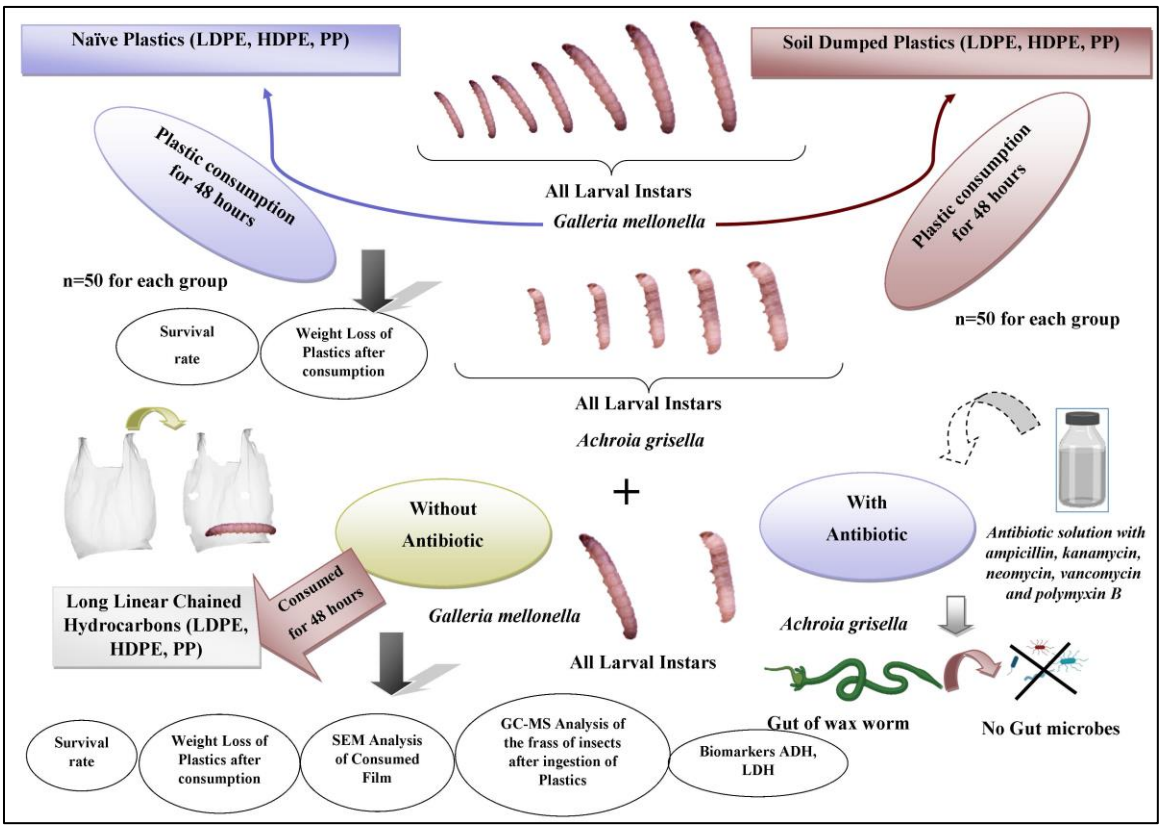


Figure 3.1 Schematic representation of the research work that will be undertaken in the present study. In the current research investigation fifty larval instars for *G. mellonella* and *A. grisella* will be fed with naive and soil dumped polyethylene for two days to study the biodegradation capacity on inactivated and activated plastics. Further, the most utilised long linear chained hydrocarbons (LDPE, HDPE, and PP) were exposed to all the larval instars of both the species. After biodegradation, the survival rate of larval instars, the weight loss of the consumed plastics, the SEM of the left over film after consumption and GC-MS of the larvae after plastic consumption was recorded

As wax moths are pest of apiculture industry, so there is need of a closed environment in which plastics could be degraded by these insects. It is expected that the current research investigation can provide a solution for the biodegradation of the most abundant long linear chained plastics accumulated in the ecosystem. In near future it is anticipated that the present research could be used for waste plastic treatment.

CHAPTER 4

AIM AND RESEARCH OBJECTIVES

Aim

The present research study deals with the plastic degradation capacity of all larval instars of *Galleria mellonella* and *Achroia grisella* independent of gut microbiota.

Research Objectives

The following are the short-term goals of the current research:

- To assess the degradation of plastic by different instars of wax moth larvae.
- To evaluate the degradation of different types of plastics by wax moth larvae.
- To study the enzymatic concentration present in different wax moth instars for degradation of the plastic.

CHAPTER 5

MATERIALS

AND

METHODS

Galleria mellonella (*G. mellonella*) and *Achroia grisella* (*A. grisella*) were chosen for the current study because they represent serious problems for the apiculture sector in the chosen study location. To assess the plastic biodegradation capacity of the wax worms collection of the insect, identification of the insect, identification of various types of plastics by FTIR, biodegradation experiments for various types of linear long chained hydrocarbons, various parameters including weight loss, survival rate, SEM of the biodegraded film, GC-MS of the frass of the larvae and enzymatic analysis for enzyme biodegrading the plastics.

5.1. Collection, Rearing and Identification of Wax Moths

5.1.1. Collection of Test Organism

Adults, pupae, larvae, and eggs of both species have been collected from the Big Bee Agro Farm, Tiwana Bee Farm, Kashmir Apiaries, Doraha (76° 2' 0.1896" E, 30° 48' 14.1084" N), Punjab, India (Figure 5.1). Visualisations and monographs from prior literature are used to initially identify *G. mellonella* (greater wax moth) and *A. grisella* (lesser wax moth) species (Williams, 1978; Solis, 2006; Ellis et al., 2013; Kwadha et al., 2017).

5.1.1.1. Test Organism- *G. mellonella* and *A. grisella* are holometabolous insects having four major developmental phases in their life cycle: egg, larva, pupa, and adult. *G. mellonella* and *A. grisella* of the order Lepidoptera's, family Pyralidae were used as experimental models.

(A)

(B)

Figure 5.1 (A) Depicts store of Tiwana bee farm with frames infested with wax moth. (B) A frame infested with wax moth

5.1.1.2. Rearing and Identification of the Test Organism

The test organisms have been collected from bee farms in Doraha, Punjab. Eggs, larvae, and pupae, were then reared in separate rearing boxes in insectaries under controlled conditions of temperature (27 ± 5°C), photoperiod (dark 24 hours), and humidity (60 to

75%) on an artificial diet of bran (420 g), honey (150 ml), glycerine (150 ml), tap water (30 ml), and ground brood comb (20 g) (Ellis et al., 2013). Pupae were transferred in the separate rearing boxes for adult emergence. Females after mating with males were housed in glass beakers with crumbled paper for the current study and were permitted to oviposit. The eggs of the wax moth were collected by allowing the gravid females to oviposit on crumbled paper holding 3 to 4 specimens at a time. The eggs collected in this manner were allowed to hatch within the BOD incubator under regulated conditions as mentioned above until they reached the desired stage (Ellis et al., 2013). Visualisations and descriptions, as well as morphological traits of collected eggs, larvae, pupae, and adults, were used to identify test models (Paddock, 1918; Smith, 1965; Williams, 1978; Ellis et al., 2013; Egelie et al., 2015; Kwadha et al., 2017). Additionally, adult insect samples were sent to the Zoological Survey of India (ZSI) in Solan for identification (No.F. 48-2-2015/tech193).

5.1.1.3. Characteristics of *Galleria mellonella* and *Achroia grisella*

5.1.1.3.1. Description of *G. mellonella*- The *Galleria mellonella* possess four developmental stages namely eggs, larvae, pupae and adults. The Larval instars of greater wax moth are honey bee pests and are cream-colored with dark grey to greyish patterns. The head has four stemmata on each side, and the spiracles have a yellowish peritreme of constant thickness (Gorham, 1991; Ellis et al., 2013). The larvae hatch with a diameter of 0.12-0.15 mm and a length of 1-3 mm (Paddock, 1918; Smith, 1965). Before pupation, late instar larvae are 25 to 30 mm long and 5-7 mm in diameter (Smith, 1965).

Morphological Characteristics and Life Stages-

Eggs- Wax moth eggs range in size, measuring 0.478 ± 0.04 mm in length and 0.394 ± 0.02 mm in width on average. The eggs have a rough texture due to its spheroidal shape and interspersed wavy lines (Paddock, 1918; Ellis et al., 2013; Kwadha et al., 2017). According to Smith (1965), the eggs are white coloured and spherical shaped. The hue of an egg's shell can vary, encompassing shades of pink, creamy white, and pure white.

However, the precise biological processes responsible for these colour differences remain largely unexplainable (Warren & Huddleston, 1962; Kwadha et al., 2017). Flexibility exists in the shell membrane. At the egg's cephalic pole, the micropile appears to be a single, tiny pore. The egg's surface does not have any projections (Smith, 1965). Eggs are typically laid in groups in depressions, crevasses, and fissures. Because they are flexible, they flatten on the sides that are next to each other or the surface they are placed against. When eggs are deposited, a cement-like layer covers them, causing them to adhere to the surface or to one another. The larva emerges from the egg by biting through the "shell" of the egg (Smith, 1965).

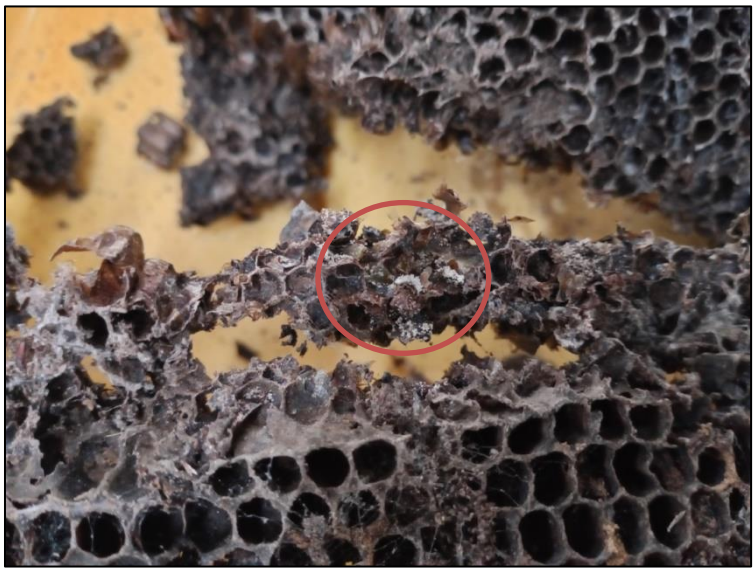


Figure 5.2 Photographic image of the egg cluster laid by female of greater wax moth larva

Larvae- *Galleria* larvae transform from the first larval instar through the dormant pupal stage into the imago, as is typical of the holometabolous insects. Only during the larval stage is food consumed (Smith, 1965; Ellis et al., 2013). Wax moth larvae hatch out at a size of 0.12-0.15 mm in diameter and 1-3 mm in length (Paddock, 1918; Smith, 1965). Last larval instar measure 25 to 30 mm in length and 5-7 mm in diameter before pupation (Figure 5.3) (Smith, 1965). The sclerotized structures, which take on different colours of light to dark brown depending on the amount of time that has passed since each ecdysis, are the only pigmented parts of the larvae. Throughout all of the larval instars, they

consume food while spinning the tunnels in the bee hive, although they spin more in the later instars. A silky sheen is left behind as the larva burrows through and over the bee combs, which are its food, and it also creeps over the walls of the culture bottles. Before the larva spins a white silken cocoon for itself in the final stage, there is no order or design to the spinning process. The final larval instar spins a cocoon around itself and converts itself into pupa. It takes tiny bite-sized pieces of the available material and fastens them to the exterior of its cocoon. This is how it protects itself. The larva constructs its cocoon such that the imago may easily emerge via a type of roughly hinged trap door at the end of the pupa case (Smith, 1965).

Due to the lack of sex-specific exterior physical characteristics, sex differentiation between male and female is not yet achievable in larval stages. The larva is polipod (eruciform), with three thoracic and eleven abdominal segments. The larvae possess several prolegs on the third to sixth abdominal segments and six legs on the thorax. Normal sclerotization occurs in the three pairs of the four-jointed thoracic legs. A single pronged hook or claw is present on each leg's terminal end. There are four pairs of prolegs in the third to sixth abdominal segments. A planta with a circle of around 35 uniserial biordinal sclerotized hooks or crochets is given for each proleg. Large suranal plate is positioned on tenth abdominal segment. The eleventh abdominal segment consists of two anal plates and two anal prolegs that are lateroventrally positioned and have a semicircle of 24 biordinal crochets. In-between the anal prolegs are the anal aperture. There are tactile hairs around the base of each body segment. Slightly oval shaped spiracle with evident sclerotised ring called peritreme is visible on the body segments. There are large lateral lobes on the body wall laterodorsal to each abdominal leg or proleg, as well as at equivalent positions on the legless segments. Sclerites are absent from the majority of body segments, although the cuticle is heavy while being flexible. In the ninth, tenth, and eleventh abdominal segments as well as the first thoracic terga, sclerites are visible. The head is highly pigmented and sclerotized (Smith, 1965). The sclerotized body parts of the larva are cream-colored, yet as it grows and moults successfully, its colour darkens (Smith, 1965; Kwadha et al., 2017).

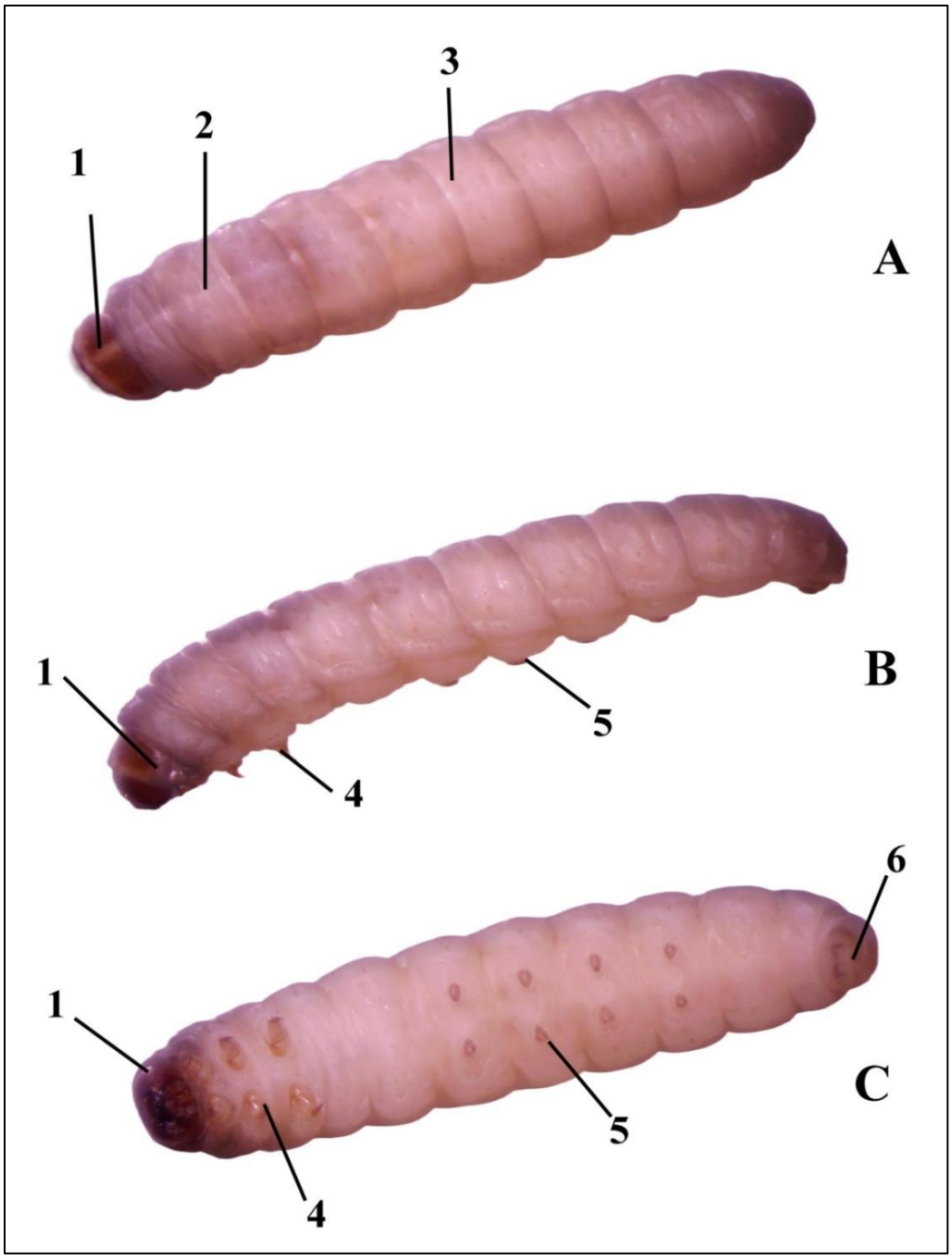


Figure 5.3 The microscopic image of larval stage of *Galleria mellonella* under the stereo microscope (A) Dorsal view (Magnification 16 X), (B) Lateral view (Magnification 14 X), (C) Ventral view under magnification of 16X: 1. Head with stemmata, 2. Thorax, 3. Abdomen, 4. Thoracic legs, 5. Prolegs, 6. Anal Prolegs

Microspines, also known as "asperities," (Figure 5.4) are exocuticular spines that cover the membraneous portions of the body wall (Hinton, 1943; Smith, 1965). These measure around 5 microns in diameter and 8 microns in length, and they are grouped in checkered rows. Following each ecdysis, the spines initially have very little pigmentation but over time, this pigmentation increases. As it gets closer to the next ecdysis, this results in the larval skin darkening (Smith, 1965).

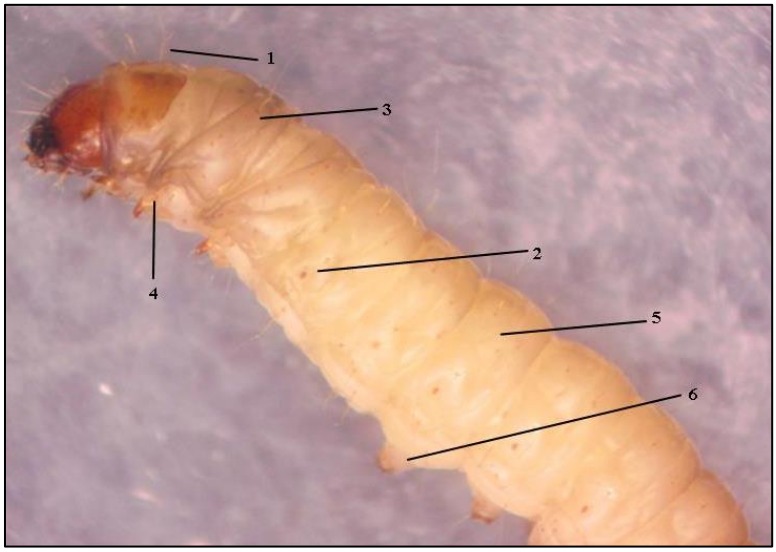


Figure 5.4 Stereo-microscopic image of a portion of the larval body of *Galleria mellonella* depicting: 1. microspines, 2. spiracles, 3. thorax, 4. thoracic legs, 5. a few abdominal segments and 6. prolegs (Magnification 40X)

The first eight abdominal and thoracic segments' initial tergums, as well as the lateral edges of each, are where the **spiracles** (Figure 5.3B, 5.4) are found in the pleura. Except for the prothoracic and eighth abdominal segments' spiracles, which are around three times bigger, all the spiracles are of the same size. Each spiracle is encircled by the peritreme- an oval brown cutin sclerite (Smith, 1965).

Galleria's head is a highly intricate structure. Presence of **stemmata** is observed in the head of the larva (Figure 5.9C) (Ellis et al., 2013). Between the lateral frontal arms and extending upward is a clypeus called the adfrontal. Four pairs of ocelli are located just behind the slender, three-jointed antennae. The mandibles are situated between the

antennae and are strongly sclerotized. Although they lack subapical teeth, they have three fully formed apical teeth. Each mandible has a single condyle and two mandibular setae on the lateromesial border. The labrum bears tormae at its dorsolateral corners and is bilobed or cleft on the medial aspect of its distal border. It appears to lack labral punctures, although it clearly has labral setae. The labrum overlaps mandibles dorsally. The mouth is present between hypopharynx and base of labrum. The spinneret protrudes forward from the hypopharynx and is situated on its anterior mesial border. On each side of the spinneret are the labial palpi. The prementum and postmentum are located ventro-posterior to each other on the hypopharynx's ventral side. The maxillary palpi, which extend ventrolaterally into the hypopharynx, and the maxillary lobes, which each have three tactile projections, are dorsal to them. The postmental sclerite is located in the midventral region of the postmentum. The stipes is located lateral to the postmental sclerites. The cardo is located posterior to the stipes, while the submental sclerites are located medially to each cardo.

Morphometric studies- Various larval instars of *Galleria* could be distinguished by morphometric studies. Morphometric studies are characterization of body size various stages of insects at entire life span of the insects. Using straightforward linear measurements, such as the head capsule width or body length, one may determine the size of an insect (Dyar, 1890). Even with significant limitations, counting and identifying the number of immature instars is one of the most often used applications with measurements of breadth of the head capsule (McClellan & Logan, 1994). However, when it is not feasible or desirable to weigh insects directly, body length measures have been used to assess the biomass of insects or, less frequently, to determine the growth rate of immatures (Costa & Gomes-Filho, 2002). Matsumoto & Yano (1995) monitored the frequency distribution of the head width of larvae raised at various temperatures. In the populations raised at 20 °C and 25 °C, there were eight instars, and at 30 °C and 35 °C, there were seven. In this study we are rearing culture at 30 °C, therefore we are considering seven instars for the study of plastic biodegradation.

Pupae- The larva reaches a quiescent stage at the conclusion of its last larval stage, quits feeding, spins a cocoon, and begins to change into a pupa. The pupal case is completely white at this stage, meaning it lacks colour. Within a day, a thick pigmentation, particularly on the dorsal side, occurs (Smith, 1965). The pupae of greater wax moth are 5-7 mm in diameter and 12-20 mm in length (Paddock, 1918; Smith, 1965; Ellis et al., 2013). Generally, female pupae are larger than male pupae. The pupal stage is obtect, with a fluid produced during ecdysis that secures all of its appendages to the body. The pupa initially exhibits a white to yellow coloration, but with aging and development, it progressively transforms to brown and ultimately dark brown (Figure 5.5). Similar to the adult stage, sexual dimorphism also exists in the pupal stage. In the male pupa, pair of small, rounded external knobs are present on the ventral aspect of the ninth abdominal segment, resembling phallomeres. In contrast, the female pupa features a bifurcated sternum on the eighth abdominal segment, which functions as an opening for the bursa copulatrix. *Galleria* pupae have a slight appearance of pilifers, which are widespread in several lepidoptera families. One of the characteristics that distinguish females from from males in the adult is their big labial palpi that are visible in female pupae. Except for the eleventh abdominal segment, the body is covered in setae, or tactile hairs (Smith, 1965). The pupa has two prominent eyes, and antennae are grafted onto the mesowing. Pupation often occurs within cocoons that have been spun and subsequently coated with feces and other debris, featuring an opening that enables the mature adult to emerge (Kwadha et al., 2017).

The dorsal surface of the pupal case is adorned with numerous protrusions resembling knobs. A series of larger spines or knobs forms a connection between the tenth abdominal segment and the head. This series is duplicated across the thoracic region. During the eclosion process, as the adult emerges from the pupal stage, the pupal case splits open along the double series of these dorsomesal spines, specifically through the tergal area of the thorax (Smith, 1965).

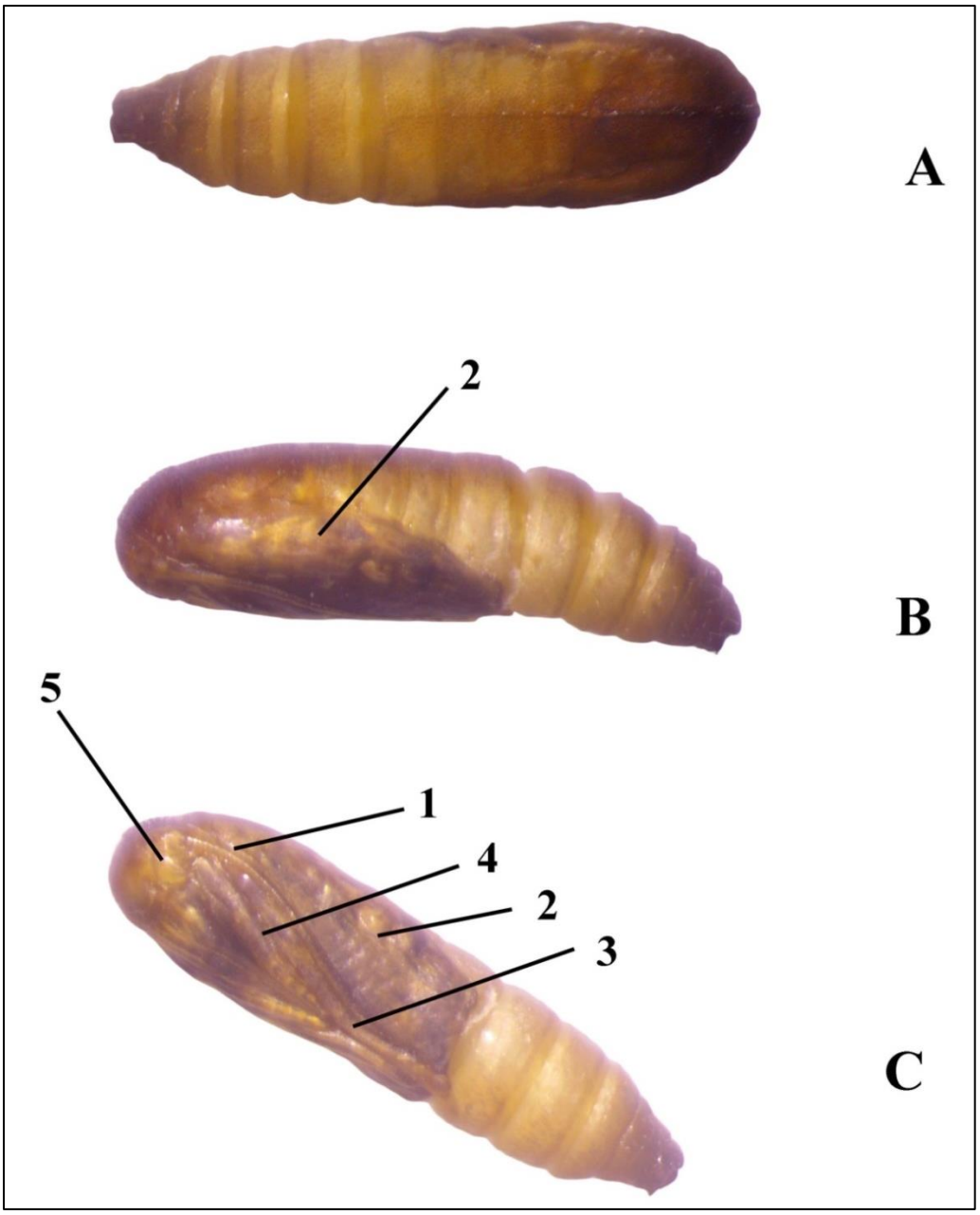


Figure 5.5 Microscopic image of obtect pupa of *Galleria mellonella* under the stereo- microscope (A) Dorsal view (18X- magnification), (B) Lateral view (18X- magnification), (C) Ventral view the magnification of 16X: 1. Antenna, 2. Mesothoracic wing, 3. Mesothoracic leg, 4. Prothoracic leg, 5. Compound eye

Adults- The greater wax moth adults clearly display sexual dimorphism. The female wax moth weighs 169 mg and has an average body length and wingspan with a width and length of 31 mm and 15-20 mm, respectively (Ellis et al., 2013; Kwadha et al., 2017;

Paddock, 1918; Williams, 1978). Comparatively to the female, the male is significantly smaller and has a lighter coat of colour (Ellis et al., 2013; Williams, 1978). The pigmentation on the forewings of both sexes varies in intensity. The anterior two-thirds are covered in scales, resulting in a consistent darker coloration, while the posterior one-third features a combination of stripes with varying shades of dark and light pigmentation. The lengths of development and larval diets have been found to affect adult body colour (Kwadha et al., 2017).

Scales- There is a wide range of forms in the scales of *Galleria*. There are thirteen shapes of scales detected in the greater wax moth. They encompass the legs, wings, and antennae in addition to the rest of the body (Smith, 1965).

Head- Except for a few distinct variants, the head is typically characteristic of Lepidoptera. About two-thirds of the head's overall width is occupied by the eyes. The compound eyes are massive in size and ellipsoidal. They have 4,500 to 4,650 ommatidia and have an average diameter of 0.65 mm. Each ommatidium is around 0.0125 mm in size. The typical eye appears shiny black in the dark. It appears light, icy grey after being exposed to strong natural or artificial light (Figure 5.6).

All of the head's sclerites and its appendages are typically covered in a thick coating of scales. The midventral region, right and left halves, and the fronto-clypeus are all divided by a line rather than by sutures. The vertex and fronto-clypeus are clearly separated by a transverse suture.

In the female, the three segmented labial palps protrude slightly upward and forward, whereas in the male, they curve abruptly upward and hook inward. A spine with a sharp bend resides at the distal end of the male palp segment. The male is therefore described to have a "snub nose," whereas the female has a "pointed nose" visually.

(A)

(B)

Figure 5.6 Photographic image of adult of *Galleria mellonella*. (A) Female (Magnification 20X), (B) Male (Magnification 20X)

The junction of the inner median and lateral grooves of the paired galeae forms the proboscis in Lepidoptera that feed during the adult stage. *Galleria* does not consume food during the adult stage; hence the proboscis is undeveloped and typically bifurcates at the distal end, separating the galeae distally.

Filiform in appearance, the antennae have an expanded, prolonged scape with a bulb at their distal end. At the intersection of the fronto-clypeus, vertex, and inner edge of the eye, the scape bulb articulates with the head. The pedicel lies in the size range between the proximal antennal segments and the scape. Male anatennal segments range from 40 to 50, whereas female anatennal segments range from 50 to 60. The antennae of the male are slightly shorter than those of the female because the segments in the male and the female are around the same length. The distal segments are gradually more slender and larger (0.20 mm) than the proximal segments, which are larger in diameter and shorter (0.10 mm). Each antennal segment's ventral side is slightly flattening and is covered with tiny tactile hairs called microtrichia (100-200 to each segment). Around the centre of each segment, there are three pairs of longer, thicker tactile hairs organised in a vertical row. Each segment has two crossbands of scales that cover its dorsal and lateral sides. The segmentation seems to be twice as common as it actually is due to the alternating bands of scales that overlap the junctions of the segments when viewed from the dorsal side. Therefore, only the bands of scales are visible from above, not the membranous articulations between neighbouring segments. Normal development includes the maxillary and labial palpi. The mandibles are only faintly represented by minor remnants (Smith, 1965).

Thorax- Typical lepidopteran thorax comprises of prothorax, mesothorax, metathorax. A pair of curving stalked plates, known as patagia, which are located immediately posterior to the cervix and hook rearward over the scutum of the mesothorax provide as visual evidence of the prothorax when seen from the dorsal side. Both parts of the pterothorax are smaller than the prothorax. Spiny scales of a particular form cover the patagia. Generally, they resemble a wide collar. The first pair of spiracles is located slightly anterior to the anepisternum of the mesothoracic pleuron, between the pro-and metathorax. The anterior articular sclerites of the wings are the broad with triangular tegulae of the total four axillary sclerites. The mesowings exhibit sex diversity. The distal border (outermost margin) is essentially straight in the female whereas it is notched in the male. Cell R+M in the female is also closed. This cell has a demarcation running down

the middle that reveals the presence of an ancestral median vein. The male has a similar scenario, but the r-m and m-cu veins are hardly visible, resulting in the appearance of an open R+M cell. Between the meso-and metathorax, right posterior to the subalare 2, the second pair of very large spiracles may be seen. It is expanded type with the sealable lips. The scutellum of the meso thorax substantially encloses the metathorax. Only the postnotum and the two lateral sclerites of the metascutum are visible from the dorsal view. In the male, the humeral lobe at the wing's humeral angle forms the frenulum, which is made up of a single, massive spine. It is around one-third the length of the wing's costal edge. The frenular hook, also known as the retinaculum, is a wing-coupling device that secures it to the forewing. The female's frenulum is made up of bundles of three, somewhat smaller bristles that emerge from the same location. The cubitas vein's proximal end is where a cluster of jugal bristles that emerge from the forewing's jugal lobe hold them in place. A comparable group of bristles, which appear to serve no purpose, is present in the male (Smith, 1965).

Legs- Lepidopteran legs normally have the characteristic attachment with the thorax. There is one notable difference, though: Each prothoracic leg has a substantial tibial epiphysis. There are no tibial spurs on the prothoracic legs. At the distal end of each tibia, the mesothoracic legs have a single pair of sizable tibial spurs. Two pairs of spurs—one pair distally and the other submesially-are present on the metathoracic tibia. The tibial spurs are long, pointed cones covered in many hair-like sensillae. Each leg has one distal pretarsus and five tarsal segments. The pretarsus is made up of a dorsal lobe called the unguifer, to which a pair of curved claws are laterally attached, the ungues, a median stalked terminal pad called the arolium, a pair of finger-like ventral extensions called the pulvillae, and three or more pairs of plumose hairs that emerge from the unguifer to the side of the pair of claws (Smith, 1965).

Abdomen- Seven pregenital abdominal segments are observed in both males and females. For attachment with the metathorax, the first abdominal segment has undergone significant modification. Near the centre of the first abdominal pleura, there is the first

pair of abdominal spiracles. A significant portion of the first abdominal sternum is covered by two chordotonal organs, as demonstrated by the size of the tympani. Segments 2 to7, respectively, are normal, with each segment's pleura containing two spiracles. The spiracles have incomplete peritremes and are of the biforans type. Both sexes' eighth segments are devoid of spiracles. The eighth sternum in males has been altered to become claspers or pseudovalves. The united terga, which houses the aedeagus, represents the modified segments 9-11 in the male. Segments 9-11 in the female are changed to produce the long, telescoping ovipositor. The terga and sterna's sclerotized regions progressively integrate into the pleura's membranous region (Smith, 1965).

5.1.1.3.2. Description of *A. grisella*- Lesser wax worms have four developmental stages namely eggs, larvae, pupae and adults (Williams, 1978; Ellis et al., 2013).

Morphology Characteristics and life stages -

Eggs- Eggs are often laid by adult females close to a food source in concealed crevices. The eggs are round and creamy white (Figure 5.7). The time it takes for eggs to hatch varies, and higher temperatures accelerate the development of all life stages. Hatching of eggs normally takes five to eight days (Egelie et al., 2015; Ellis et al., 2013; Kapahi & Marwaha, 2022).

Figure 5.7 The photographic image of the egg cluster of laid by female of *Achroia grisella*

Larvae- The larvae have pronotal shields and narrow, white bodies, with brown heads (Figure 5.8). Larval development typically takes six to seven weeks at 29 to 32°C and can last anywhere from one to five months. Larvae mature at around 20 mm in length and undergo most of their development in the last two instars (Egelie et al., 2015).

Morphometric Studies- As mentioned in the previous section for *Galleria mellonella* in the same way for *Achroia grisella* morphometric studies are used to distinguish the larval stages. The measures of the larval head capsule and head girth, according to Mahgoub et al. (2015), can be used to discriminate between the five instars of the *Achroia grisella* larval stage (Mahgoub et al., 2015). In the present study for biodegradation of plastics we will study the biodegradation of the plastics on five larval instars.

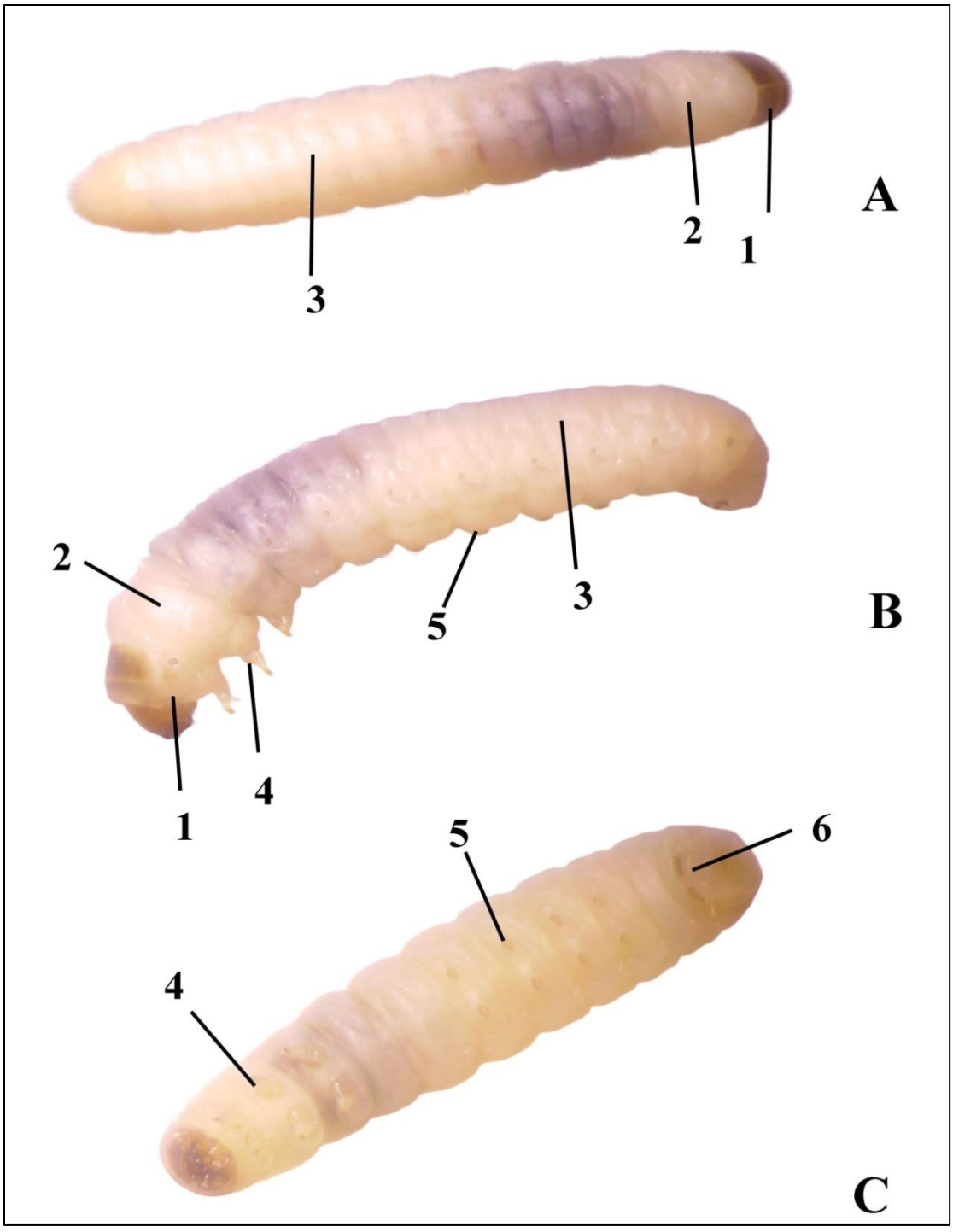


Figure 5.8 The microscopic image of fully grown larval stage of *Achroia grisella* under the stereo microscope (A) Dorsal view, (B) Lateral view, (C) Ventral view under magnification of 20X: 1. Head without stemmata, 2. Thorax, 3. Abdomen, 4. Thoracic legs, 5. Prolegs, 6. Anal Prolegs

Table 5.1 Differences in life phases of greater and lesser wax moths

Life Stage	Character	Greater Wax Moth	Lesser Wax Moth	References
Egg	Size	0.44 ± 0.04 mm length and 0.36 ± 0.02 mm width	0.41 ± 0.02 mm in length and 0.31 ± 0.01 mm in width	(Arbogast et al., 1980)
	Colour	They lay 50-150 cream-pinkish white coloured egg cluster (Figure 5.2)	Spherical shaped, creamy-whitish in coloured eggs (Figure 5.7)	(Williams, 1978)
	Life span	3 to 30 days, depending on the overall condition of the environment	7 to 22 days, depending on the overall condition of the environment	(Williams, 1978; Sharma et al., 2011)
	Characters	The entire surface has reticulation, with uniformly widened carinae encircling the primary cells.	Reticulation is restricted to the anterior end, and the carinae enclosing the primary cells are noticeably wider along the cell's outer edges.	(Arbogast et al., 1980)
Larva	Size	Fully grown larva is 12-20	Fully grown larva is 1-20 mm	(Williams, 1978; Sharma

		mm in length and 5-7mm in width	in length	et al., 2011)
	Colour	Slightly pointed reddish head, creamy whitish body with dark grey markings (Figure 5.9A)	Brown heads with pronotal shields and white coloured narrow bodies (Figure 5.9B)	(Williams, 1978)
	Life span	At 29-30°C temperature, a life span of 6-7 weeks is observed.	A life span of 6-7 weeks has been recovered at 29-30°C.	(Williams, 1978; Sharma et al., 2011)
	Characters	Four stemmata are present on the each side of the head(Figure 5.9C), spiracles with uniformly thick yellowish peritreme (Figure 5.9E)	Stemmata is absent on the head (Figure 5.9D)	(Ellis et al., 2013)
Pupa	Size	12-20 mm in length and 5-7 mm wide	Length: 11.3 ± 0.4 mm, width: 2.80 ± 1.89 mm	(Williams, 1978; Sharma et al., 2011)
	Characters	Parchment-thick cocoon that is off-white in	White coloured cocoon covered with debris and	(Williams, 1978)

		colour with dark reddish brown coloured pupa (Figure 5.5)	frass with tan-yellow coloured pupa (Figure 5.10)	
	Life span	6-55 days, depending on the overall condition of the environment	37.3 days (approximately)	(Williams, 1978; Sharma et al., 2011)
Adults	Size	15 mm long insect with 31mm long wingspan (Figure 5.6) (approximately)	Female=13 mm (length) Male= 10 mm (length) (Figure 5.11)	(Ellis et al., 2013)
	Colour	Reddish brown coloured, heavy bodied insect with spotted forewings and hind wings are lightly fringed cream coloured	Prominent yellow head with small silver bodied, forewings are oval shaped and hindwings are heavily fringed	(Williams, 1978)
	Life Span	Female- average life span is 12 days. Male- average life span is 21 days	Female- average life span is 6.90 days. Male- average life span is 12.90 days	(Williams, 1978; Sharma et al., 2011)

	Characters	Labial palps are protruding and long; breadth of forewing is 5 to 7 mm; termen of forewing is concave; Cu of hindwing is 4 branched	Labial palps are prominently short; pincerlike labial palps in males which are transversely incurved; breadth of forewing is less than 5mm; termen of forewing is convex (males possess concave termen in hindwing); hindwing of the insect possess the Cu which is 3-branched	(Ellis et al., 2013)

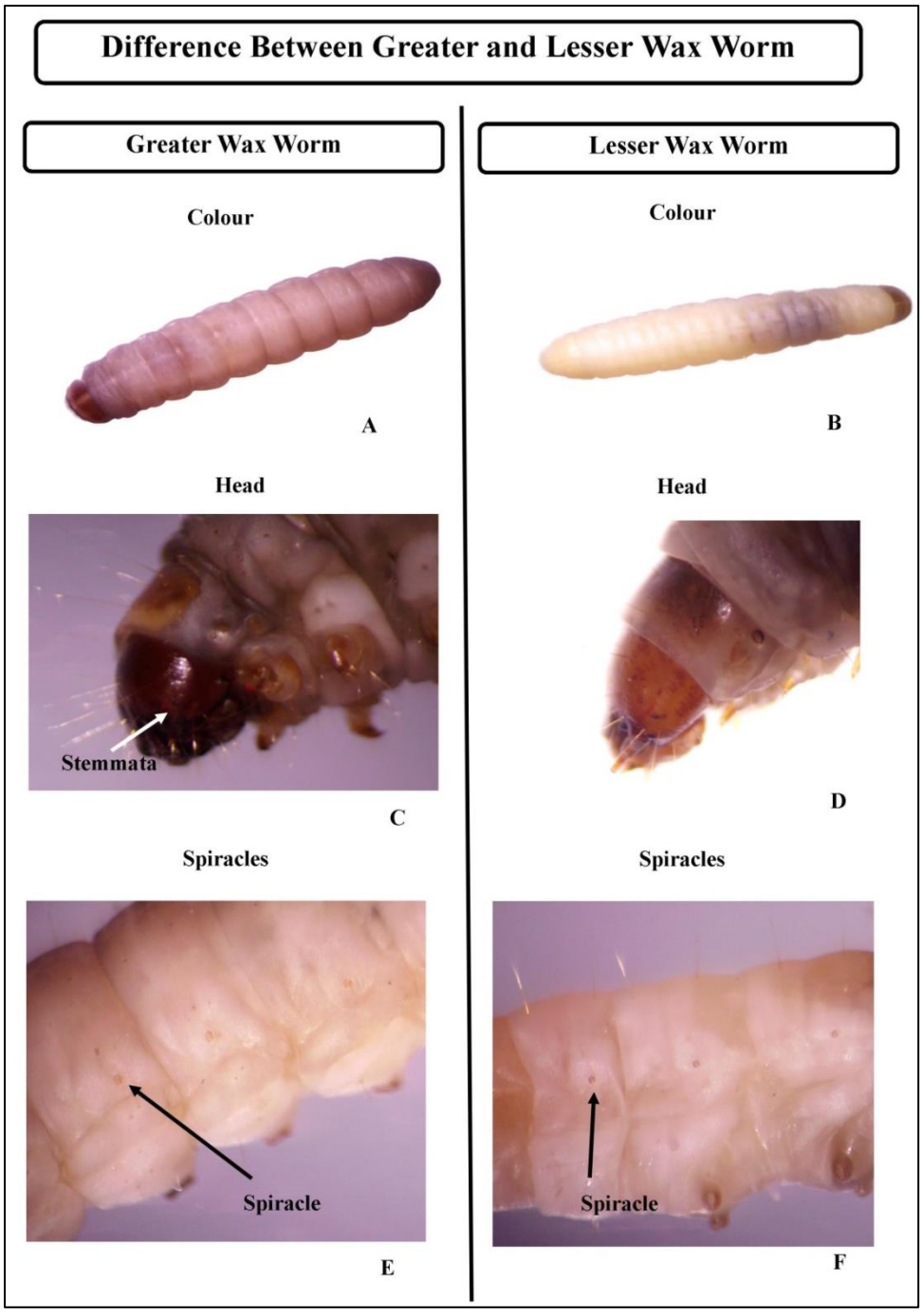


Figure 5.9 The Difference between larva of *G. mellonella* and *A. grisella* (A) Dorsal view of greater wax moth (magnification 18X), (B) Dorsal view of lesser wax moth under magnification of 20X, (C) Head of greater wax worm under magnification of 60X, (D) Head of lesser wax worm under magnification of 80X, (E) Spiracles of greater wax worm under magnification of 60X, (F) Spiracles of lesser wax worm under magnification of 60X

Pupae- Mature larvae within the honey bee hive will undergo pupation at any spot within the hive. Prior in doing so, they spin thick silk cocoons around themselves. Pupae are approximately 11 mm length and are tan-yellow in colour (Figure 5.10). Due of their frequent frass and other debris coverings, cocoons can be challenging to distinguish. Pupae can develop for up to two months, but on average, it takes around 37 days for an adult to emerge (Egelie et al., 2015).

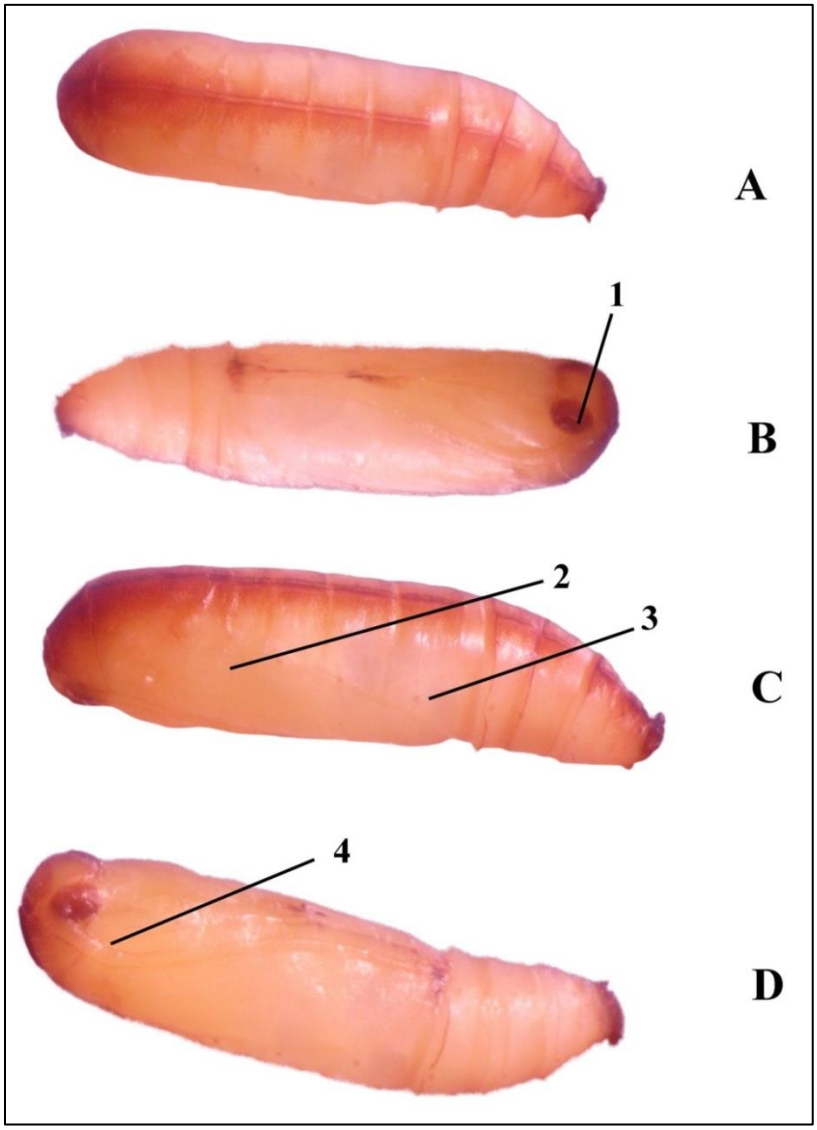


Figure 5.10 Microscopic image of *Achroia grisella* under the stereo microscope (A) Dorsal view under the magnification of 20X, (B) Ventral view (Magnification 24X), (C) and (D) Lateral view (Magnification 50X): 1. Compound eye, 2. Mesothoracic wing, 3. Spiracles, 4. Antenna

Adults- The adult of lesser wax moths have thin bodies and are around 1-20 mm long (Figure 5.11). They have wings that are approximately 1/2 inch wide. Males are often smaller than females. They have a conspicuous yellow head and a colouring that varies from silver-gray to beige. Adults have an average lifespan of around one week and are most active at night. Within honey bee colonies, males use ultrasonic signals to persuade females to their mating places. Additionally, females deposit their eggs overnight. Adults hide in trees and shrubs close to hives throughout the day (Egelie et al., 2015).

(A)

(B)

Figure 5.11 Photographic image of adult of *Achroia grisella* (A) Female (Magnification 10X), (B) Male (Magnification 12X)

The following parameters were adopted to conduct the current research project and are briefly detailed below-

1. **To assess the degradation of plastic by different instars of wax moth larvae-** All life stages (eggs, larvae, pupae and imago) were gathered from certain bee farms in doraha and housed in an insectary under temperature 30±1°C and humidity controls of 70% (Ellis et al., 2013). After rearing larvae/pupae, imagoes were recognised using the visual taxonomic keys of (Ellis et al., 2013; Kwadha et al., 2017; Paddock, 1918; Williams, 1978). All larval instars were separated by following morphometric study and incubated with naive and used polyethylene to assess the biodegradation capability of the larval instars (Matsumoto and Yano, 1995; Mahgoub et al., 2015). Sample sizes of fifty larvae of each instar were segregated and incubated with the naive as well as soil treated polyethylene film for two days. After biodegradation the weight loss of the film and survival rate of the instars was recorded.
2. **To evaluate the degradation of different types of plastics by wax moth larvae-** The larvae of both the species of wax moth was separated according to morphometrics of the insect. One group of fifty insects from each larval instar stage was administered with 10 microliter of antibiotic solution (with antibiotics- ampicillin, kanamycin, vancomycin, polymyxin B, and neomycin). All the larval instar stage with and without antibiotic along with control were fed with LDPE, HDPE, PP for 48 hours. After biodegradation the weight loss of the plastic film, SEM of the biodegraded film, survival rate of the insect and GC-MS of the frass of the insect was recorded (Bombelli et al., 2017; Kundungal et al., 2019; Peydaei et al., 2020; Billen et al., 2020).
3. **To study the enzymatic concentration present in different wax moth instars for degradation of the plastic**- Control (C), Without Antibiotics (WA), and With Antibiotics (A) larvae were homogenised in the isolation buffer and centrifuged at 15000g for 15 minutes at 40°C. At 340 nm, the enzymes test for Alcohol

dehydrogenase and Lactate dehydrogenase were performed, and spectrophotometrically. Findings were recorded for 3 minutes.

5.2. Chemicals for Experimentation

5.2.1. Plastics- Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), and Polypropylene (PP) were used for this investigation. LDPE, the most widely used thermoplastic, is also the most affordable packaging film due to its low cost. This polyethylene is soft, extremely flexible, and transparent, with good tear and moisture resistance but is poor gas barrier. It is utilised for a wide range of purposes. It is simple to process and may be combined with a range of ingredients to change its fundamental qualities, such as polyolefins, fillers, and colours. HDPE is a low-cost semi-translucent milky white thermoplastic. It is more stiff and stronger than LDPE, with high impact strength and greater puncture resistance. It has similar chemical resistance, release qualities, and vapour properties to LDPE; however it has weak gas barrier and weathering properties. PP is a low-cost thermoplastic with exceptional clarity, gloss, and tensile strength. It has a greater melting point than polyethylene, making it appropriate for high-temperature sterilisation applications. It also has less haze and a greater gloss (https://polymerdatabase.com/Films/PE%20Films.html).

Some of the more important physicochemical properties, as well as extra pertinent information, of all three plastics are listed here:

Table 5.2- Some Characteristic Properties of the Plastics

Plastics Properties	**Low-Density Polyethylene**	**High-Density Polyethylene**	**Polypropylene**
Molecular formula	$(C_2H_4)_n$	$(C_2H_4)_n$	$(C_3H_6)_n$
Manufacturer	Durga Polypack, Derabassi, Punjab, India.	Durga Polypack, Derabassi, Punjab, India.	Durga Polypack, Derabassi, Punjab, India.

Chemical structure	-CH_2-CH_2-CH- | CH_2 | CH_2-	-CH_2-CH_2-CH_2-	-CH-CH_2-CH-CH_2- | | CH_3 CH_3
Melting point	106-112° C	120-130° C	130-171° C
Recycling code			
Colour	Transparent	White coloured	Transparent
Uses	Packaging material, plastic bags, wash bottles, tubing dispenser bottles, laboratory equipments and parts of computer, etc.	Toys, chemical containers, shampoo bottles, pipe systems, milk jugs, recycling bins, cereal box liners, grocery bags	Fibers, textile, packaging materials, bags, parts of equipments and machinery

5.2.2. Antibiotics- Five antibiotics, ampicillin, kanamycin, polymyxin B, vancomycin, and neomycin, were chosen for the gut microbiota independent biodegradation of plastics by insects in the present investigation. In order to explore the involvement of the intestinal microbiota in plastic breakdown, intestinal microbiota were eliminated by providing antibiotics to all larval instars via food (Kong et al., 2019) as well as orally via Durham tube (Figure 5.12) (Kesti & Thimmappa, 2019). Ampicillin is used for eliminating gram-negative bacteria. Kanamycin is used to eradicate both gram-positive and gram-negative bacteria, while neomycin is used to destroy yeast; vancomycin is used to eliminate gram-positive bacteria, and polymyxin B is used to target gram-negative bacteria (Kong et al., 2019).

Figure 5.12 Oral feeding of antibiotic cocktail solution (ampicillin, kanamycin, vancomycin, neomycin, polymyxin B) to larvae of lesser wax moth by Durham tube

Some of the more essential physicochemical features, as well as additional relevant information of all five antibiotics is comparatively summarised below:

Table 5.3 Characteristic Properties of the Antibiotics

Antibiotics / Properties	Ampicillin	Kanamycin	Neomycin	Vancomycin	Polymyxin B
Molecular formula	$C_{16}H_{19}N_3O_4S$	$C_{18}H_{36}N_4O_{11}$	$C_{23}H_{46}N_6O_{13}$	$C_{66}H_{75}Cl_2N_9O_{24}$	$C_{56}H_{98}N_{16}O_{13}$
Molecular weight	349.4	484.5	614.6	1449.254	1385.60
Manufacturer	HiMedia Laboratories Pvt. Ltd.	HiMedia Laboratories Pvt. Ltd.	Sisco Research Laboratories Pvt. Ltd.	HiMedia Laboratories Pvt. Ltd.	Central Drug House (P) Ltd.
Melting point	208°C	172-192°C	334-336°C	>175°C	>203°C
Odour	Odourless or have a slight characteristic odour of	Odourless	Odourless	Odourless	Odourless

	penicillin.				
Colour	Crystalline white powder	Crystalline white powder	White amorphous powder	Brown or tan coloured powder	White coloured powder
Uses	Ampicillin is a drug used to control and cure specific bacterial infections.	Kanamycin is recommended for management of bacterial infections by pathogens: *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus species*, and *Acinetobacter species*.	Neomycin exhibits a wide range of antibacterial action. It works against most yeast, Gram-negative and certain Gram-positive bacteria, including *pneumococci*, *meningococci*, *staphylococci*, and *gonococci*.	Serious Gram-positive bacterial infections are treated with the drug vancomycin.	Serious infections with multidrug-resistant gram-negative bacteria, particularly those caused on by *Pseudomonas aeruginosa*, *Enterobacteriaceae*, and *Acinetobacter baumannii*, can be treated with polymyxin B.

Source- https://pubchem.ncbi.nlm.nih.gov

5.2.2.1. Antibiotic Dosage Selection for Gut Microbiota Destruction- Force-feeding for antibiotic treatments with 10 µl of autoclaved water supplemented with ampicillin (targeting Gram-negative bacteria; 1 mg ml^{-1}), vancomycin (Gram-positive bacteria; 0.5 mg ml^{-1}), polymyxin B (Gram-negative bacteria; 1 mg ml^{-1}), kanamycin (Gram-positive and Gram-negative bacteria; 1 mg ml^{-1}), and neomycin (yeast; 1 mg ml^{-1}) (Kong et al.,

2019). The durhaam tube was cut into half and filled with antibiotic solution for force feeding (Kesti & Thimmappa, 2019). To validate the absence of gut bacteria, the insects were administered antibiotic solution mixed with meal in 1:9 ratios to guarantee the insect group was free of gut microbes.

5.2.2.2. Validations of Gut Microbiota Destruction- Five larvae from each group fed with and without antibiotic were used to isolate guts. Surface sterilisation was performed on the larvae using 70% ethanol and sterile water. The intestines were extracted and placed in 1.5 ml tubes with 500 µl of sterile saline water (0.9%). The tubes were quickly vortexed, and the gut tissues were carefully removed with a sterile pipette, and the suspension was utilised as an inoculum. 200 µl of the suspension from both tubes was distributed on nutritional agar plates and incubated for 24 hours at 37 °C (Yang et al., 2014; Kesti & Thimmappa, 2019).

5.2.3. Other Experimental Chemicals- Phosphate buffered saline, Sucrose, EDTA, Tris HCl, Ethanol, NAD^+, HEPES, Imidazole, NADH, Na-pyruvate, Triton X-100.

5.3. Selection and Identification of the Plastics Used During Experiment

5.3.1. Selection of the Plastics- For the present research investigation, three plastics namely, Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE) and Polypropylene (PP) has been selected. Polyethylene and polypropylene are two of the world's most extensively used polymers (Bombelli et al., 2017). Moreover, plastics' environmental effect is becoming a global problem since treatment and disposal solutions are limited, while manufacturing and consumption rates are growing. The burning of plastic waste releases toxic and hazardous fumes into the atmosphere, contributing to air pollution. This practice is common in many developing and underdeveloped countries due to the lack of properly designed and managed landfill facilities for disposing of these wastes. Moreover, in countries like India, various improper disposal methods are frequently employed, including open dumping, unregulated burning, unscientific composting, and poorly managed landfilling (Kandakatla et al., 2013; Nithin & Goel,

2017). The improper, disposal practices and extensive usage leads to accumulation of these plastics in the atmosphere.

5.3.2. Identification of the Plastics- Before doing any experiment involving plastic ingestion, it is critical to validate the plastics utilised in the wax moth experiment. Fourier Transform Infrared Spectroscopy (FTIR) was carried out on the naive polymer sheets to identify the polymers. The transmittance wave number range was 4000-450 cm^{-1} (Lou et al., 2020).

5.3.3. Preparation of Plastic Sheets- In order to avoid any type of thermal and photo-oxidation the naive plastic films are kept in the vacuum desiccator in the dark conditions (Figure 5.13).

Figure 5.13 Storage of plastic films in the vacuum desiccator. The plastic films were stored in the controlled conditions to avoid any thermal or photo-oxidation

5.4. Pretreatment to Plastic Films

Since old or discarded LDPE, HDPE and PP films are frequently discovered in landfills, the naive films have been treated in soil to discover the biodegradation capacity of the larva for the used film. The naive LDPE, HDPE and PP films were exposed to soil in Ayushya Vatika in Lovely Professional University (75° 42' 20.7252" E**,** 31° 15' 19.4544" N) in 3 September 2021 to 3 September 2022. All the soil treated films are washed with distilled water and dried in the room temperature in the shaded area.

5.5. Protocol to Study the Consumption of Naive and Pretreated Plastic Films by all Larval Instars of Wax Moth

The larval instars are segregated according to the morphometric studies from the culture of greater and lesser wax moth. In the present study, fifty larvae of all the seven larval developmental stage of *G. mellonella* (Matsumoto and Yano, 1995) and of all five larval stage of *A. grisella* (Mahgoub et al., 2015) were exposed to 12×18 cm of naive and soil treated LDPE, HDPE and PP film for 48 hours at 30 °C in 250 ml glass beaker that was covered with muslin cloth. In order to limit the impact of the preceding diet, the larvae were starved for four hours before to the experiment. Then, 50 wax moth larvae were incubated in each beaker under regulated temperature and humidity conditions for consumption naive and soil treated LDPE, HDPE as well as PP film (Figure 5.14). Following the experiment, the larvae were transferred to a different container. The weight loss of the plastic consumed and survival rate of the insects was recorded.

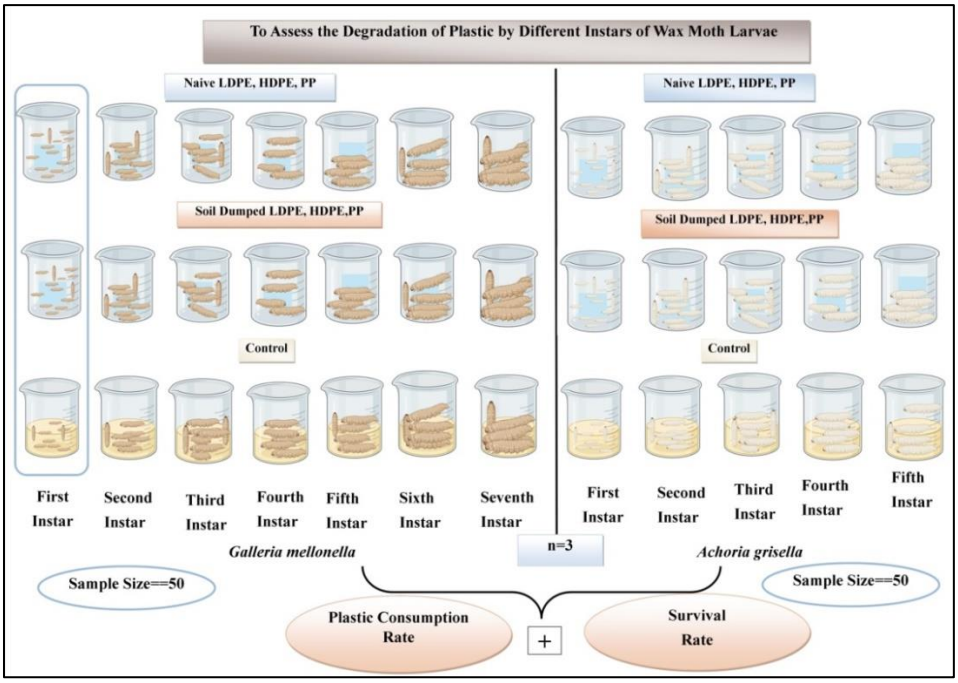


Figure 5.14 Schematic representation of experiment setup of the consumption of naive and pretreated plastic films by all larval instars of wax moth

5.6. Protocol to Study the Degradation of Different Types of Plastics by all Larval Instars of Wax Moth

The larval instars are divided based on morphometric studies of greater and lesser wax moth cultures. A sample of fifty larvae of all the larval developmental stages of *G. mellonella* and *A. grisella* were force fed with 10 µl antibiotic cocktail solutions (ampicillin, kanamycin, neomycin, polymyxin B, and vancomycin). Larval instars from all developmental stages fed with antibiotic solution and without any prior antibiotic treatment along with control group were starved for four hours before the experiment. All the larval development stage fed with the antibiotic and without any antibiotic treatment was exposed to naive LDPE, HDPE, PP films of 12×18 cm for 2 days in 250 ml glass beaker covered with muslin cloth. Moreover, the control group of *G. mellonella* and *A. grisella* were fed with artificial diet comprising bran (420 g), honey (150 ml), glycerine (150 ml), tap water (30 ml), and ground brood comb (20 g) for 48 hours. All the groups were incubated at 30 °C temperature, complete dark conditions, and 60 to 75% of relative humidity during the experiment. The plastic consumption by the larvae, survival rate during the experiment was recorded (Figure 5.15). The frass of wax worms from all the groups is collected and stored in -20 °C for GC-MS analysis. The remnants of the consumed films are stored in vacuum desiccator for SEM analysis.

5.7. Plastic Consumption Index

Prior to and during the larvae's biodegradation, the plastic film (Naive LDPE, HDPE, PP and Soil treated LDPE, HDPE, PP) was pre-weighed. The approach used by (Kundungal et al., 2019, 2021) was adopted in order to calculate the percentage of weight reduction.

$$\text{Weight Loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

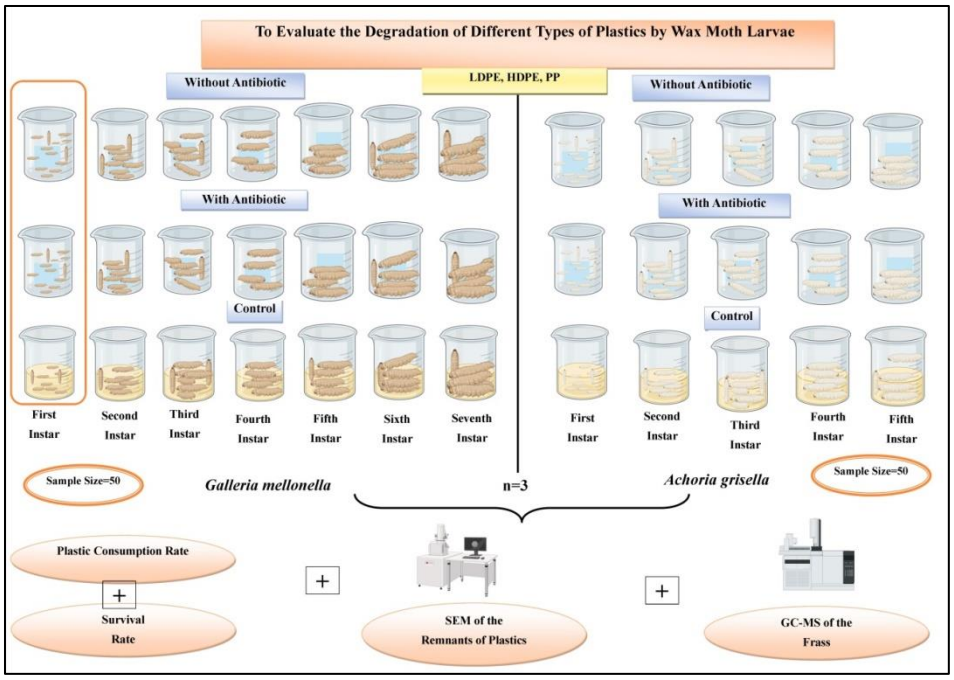


Figure 5.15 Schematic representation of the experimental set up of the degradation of different types of plastics by all larval instars of wax moth

5.8. Survival Rate of the Larvae

After the consumption of plastic by larvae, the larvae survived during the experiment were recorded and survival rate was calculated (Kundungal et. al., 2021).

$$\text{Survival Rate (\%)} = \frac{\text{No. larvae after experiment}}{\text{No. larvae before experiment}} \times 100$$

5.9. Sample Preparation for SEM

For SEM analysis, the ingested LDPE, HDPE and PP film remains were carefully packed in the sample bag. The plastic sheets were gold coated. The sample was sputter-coated with a thin layer of gold intended to increase the conductivity of the polymer and improve visibility. The SEM pictures have a magnification of 1-100 µm. To assess the physical changes in the LDPE, HDPE, PP following ingestion by greater and lesser wax

moth caterpillars, the SEM images of the films before and after biodegradation were compared (Peydaei et al., 2020; Kapahi & Marwaha, 2023).

5.10. Sample Preparation for GC-MS

After the LDPE, HDPE, PP film biodegradation in all groups, the GC-MS determined the intermediates and products for the excreta of insects. Tetrahydrofuran (5 ml) was used to extract 0.025 g of frass. Tetrahydrofuran was used to heat the frass samples for two hours at 40°C and they were immersed for 15 hours. After the evaporation of tetrahydrofuran, the residual polymers were dissolved in pure hexane for the analysis. The programming for the oven's temperature started at 40°C with a hold of 4 minutes, then ascended to 280°C at a rate of 10°C per minute with a hold of 5 minutes. The compounds detected were recognised by the NIST17 database (Lou et al., 2020; Kapahi & Marwaha, 2023).

5.11. Protocol for Protein Extraction

Fifty wax moth larvae of each group for greater and lesser wax moth were fed with LDPE, HDPE, and PP for 2 days under controlled conditions. The control group was fed with artificial diet for 48 hours. The caterpillars were anaesthetized by placing them in the ice for 5 minutes. The whole larva for all the groups along with control (100 mg) was placed in 2 ml Eppendorf tube with 1000 µl isolation buffer (0.24 M sucrose, 50 mM PBS, pH 7.4, 0.06%, Triton X-100, 0.5 mM EDTA) for Alcohol dehydrogenase (ADH) activity. For homogenisation of larvae for determination of enzyme activity of Lactate dehydrogenase (LDH) activity the isolation buffer is 20 mM HEPES, 1 mM EDTA, 0.1% Triton. Using a motor pestle, the tissue was homogenised, which was subsequently incubated for 15 minutes on ice. Prior to further analysis, the samples were vortexed and centrifuged at 15000g for 20 minutes at 4 °C, and the soluble supernatant was frozen at 80 °C (Lemoine et al., 2020).

5.12. Procedure for Enzyme Activity Assays

The maximal enzymatic activity of the alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) was detected to observe the plastic digestion capacity of the wax worms. The activity of enzymes has been detected spectrophotometrically. The absorbance was set at 340 nm, and the temperature was fixed at the animals' rearing temperature (30 °C). ADH enzyme assays were performed in reaction buffer (2 mM NAD^+, 50 mM Tris-HCl pH 8.6) with 200 mM ethanol as substrate. Meanwhile, LDH enzyme assays were carried out in a reaction solution containing 50 mM imidazole, 0.15 mM NADH, and 0.2 mM Na-pyruvate as substrate. Whole larva homogenates were thawed on ice and incubated for 5 minutes at 30 °C. In 3 ml of assay buffer 40 µl substrate is added. To begin the reaction 100 µl homogenate is added and incubated at 30 °C for 5 minutes to start the reaction. One negative control (no homogenate) was conducted and 2-3 technical replicates for each sample after the reaction for 3 minutes to calculate V_{max} (mU/min) (Lemoine et al., 2020) (Figure 5.16). After the experiment, the absorbance for all the homogenates was recorded and enzymatic activity was calculated by (Papaneophytou et al., 2021):

$$\text{Enzyme Activity (mU/min)} = \frac{\Delta A \times V_{total} \times 1000}{\varepsilon \times d \times t}$$

Here: ΔA is the change in the absorbance;

V_{total} is the total volume of the reaction mixture

ε is the molar absorptivity

d is the path length of the cuvette

t is the time in minutes

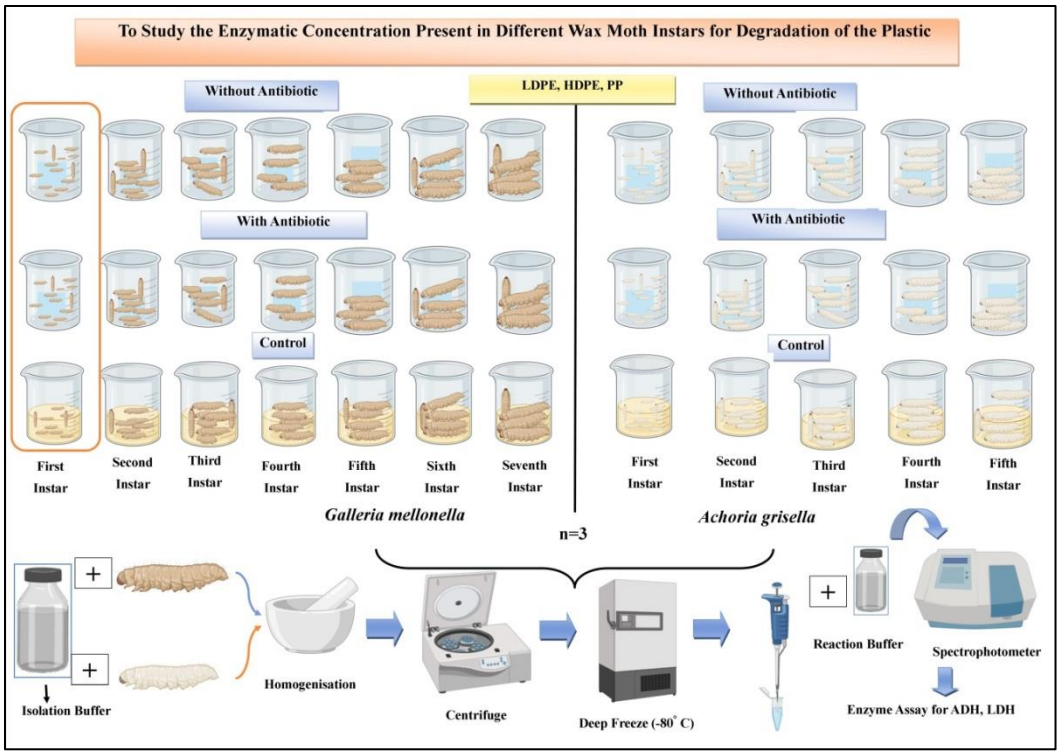


Figure 5.16 Schematic representation of procedure for maximal enzyme activity assays

5.13. Statistical Analysis

Every experiment was performed in triplicate and the results were provided as mean ± standard deviation (S.D.) for every result, with fifty insects in each group. The statistical analysis was carried out using the one-way analysis of variance (ANOVA) study, which was followed by the Tukey's honestly significant difference test using SPSS software (version 16). The results were considered to be statistically significant if the p-values were 0.05 or below.

CHAPTER 6

RESULTS

AND

DISSCUSSION

The greater (*Galleria mellonella*) and lesser (*Achroia grisella*) wax moths are most devastating pest in apiculture industry globally. They are known to destruct the bee colonies and bee keeping equipments. Both wax moth species are also known to biodegrade the plastics. For the present biodegradation analysis, larvae of *Galleria mellonella* (*G. mellonella*) and *Achroia grisella* (*A. grisella*) has been used for biodegradation of long linear chained plastics. In order to determine the biodegradation capacity of all larval instars of greater and lesser wax moth the larval instars were exposed to plastics and parameters including plastic consumption index, survival rate of insect, SEM of the leftover plastic film, GC-MS of the excreta of the larvae was analysed. In addition to the earlier described parameters, the estimation of maximal enzymatic activity of enzymes (ADH and LDH) was conducted to validate the digestion of the plastics by wax worms.

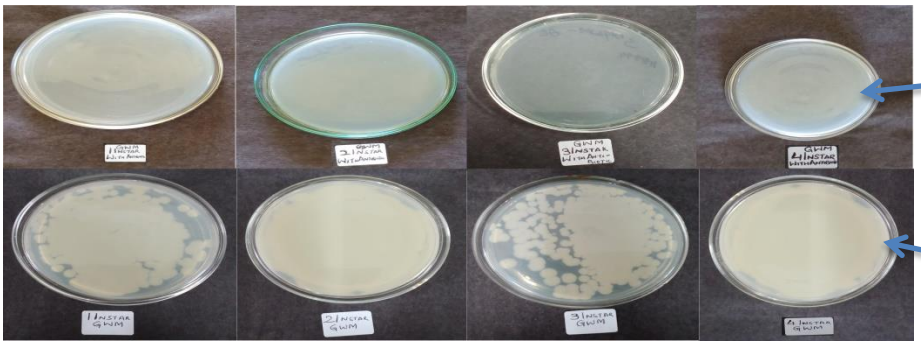
Initially, before conducting the research work a few preliminary tests like validation of gut microbiota destruction and identification of plastics by FTIR, have been followed.

6.1. Validation of Gut Microbiota Destruction

For validation of gut microbiota destruction the larvae of greater and lesser wax moth were fed with 10 µl of antibiotic solution (ampicillin, kanamycin, vancomycin, neomycin, polymyxin B) by Durham tube (Kesti & Thimmappa, 2019). The ampicillin was primarily used to eradicate gram negative bacteria, kanamycin for elimination of both gram negative and gram positive bacteria, neomycin for yeast removal and polymyxin B for gram-negative bacteria. The homogenate of all the larval instars was (with antibiotic as well as homogenate of without any antibiotic treatment caterpillars) smeared on the nutrient agar plate and efficacy of antibiotics in the homogenate was studied by culture dependent method for 24 hours (Kesti & Thimmappa, 2019).

The homogenate of the larvae that were orally force fed with antibiotic solution showed no growth on the nutrient agar plate whereas the homogenate of larvae that were not administered with any antibiotic solution showed growth of certain microbes on the

media plates (Figure 6.1). No growth of microbes on the antibiotic fed larvae illustrated validation of gut microbiota destruction in the antibiotic fed larvae.



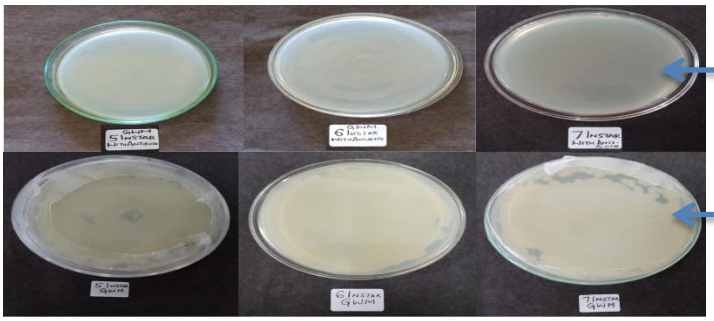
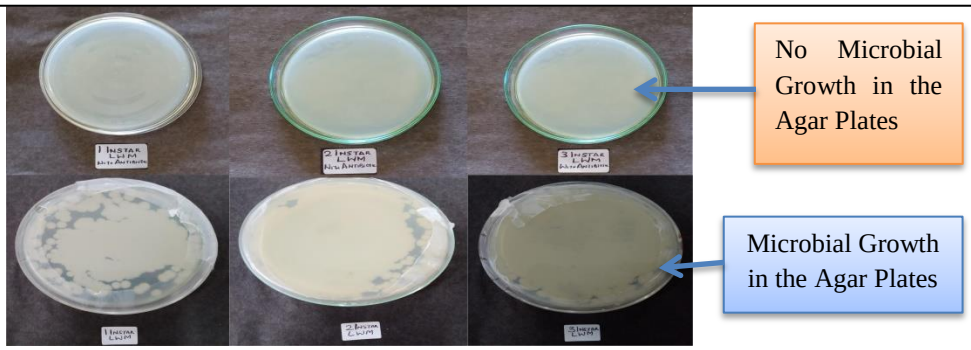


Figure 6.1(A)



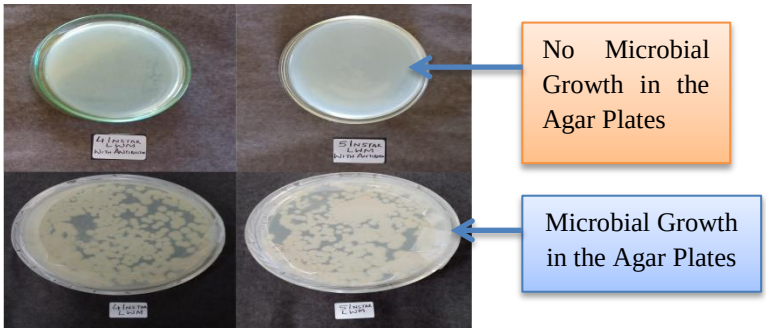


Figure 6.1(B)

Figure 6.1 Validation of gut-microbiota destruction by antibiotic treatment. (A) Culture dependent validation of the presence of gut microbiota for homogenate of all larval instars of *G. mellonella* fed with antibiotics and without any antibiotics. (B) Culture dependent validation of the presence of gut microbiota for homogenate of all larval instar stages of *A. grisella* fed with antibiotics and without any prior antibiotic treatment. The upper row of the images A&B depict antibiotic fed larval homogenate smeared nutrient agar plate and lower row represents without antibiotic fed larval homogenate smeared nutrient agar plated with the growth of the microbes

6.2. Identification of Various Types of Plastics

The composition of solids, liquids, and gases could possibly be determined using FTIR spectra. Identification of unknown materials and verification of manufacturing materials are the most frequent uses of FTIR. Most of the time, the information content is quite particular, allowing for precise differentiation between similar things. Thus, for the present research work various types of plastics (LDPE, HDPE, and PP) were identified by FTIR. The thin films of plastics are fixed in the ATR-FTIR (Attenuated Total Reflectance- Fourier Transform Infrared Spectroscopy) spectroscope and transmittance spectra of 450-4000 cm^{-1} was fixed on the instrument. The FTIR spectra of plastic films revealed a few transmittance peaks of various functional groups belonging to specific type of the plastic films.

FTIR of naive Low-Density Polyethylene (LDPE) film- The various types of long linear plastics were identified using FTIR. FTIR analysis of naive LDPE film revealed the transmittance at 720 cm^{-1} which is characteristic peak for CH_2 bond present in LDPE. Transmittance around is 1464 for CH_2 deformation, 2916, 2848 cm^{-1}, which are signature peaks for CH stretch in LDPE (Figure 6.2A).

FTIR of naive High-Density Polyethylene (HDPE) film- FTIR of Naive HDPE film possesses the characteristic peaks for CH_2 rocking peak at 719 cm^{-1}, C-C stretching peak at 873 cm^{-1}, CH_2 twisting peak at 1048 cm^{-1}, CH_2 scissoring at 1463 cm^{-1}, symmetric CH_2 stretching at 2360 cm^{-1}, asymmetric CH_2 stretching at 2848 cm^{-1}, symmetric CH stretching at 2915 cm^{-1}, which confirm the used plastic as HDPE (Figure 6.2B).

FTIR of naive Polypropylene (PP) film- The infrared spectra of naive PP films exhibits peaks at 795, 847, 968, 1000, 994, 1175, 1379, 1462, 2895-2899, 2922, 2958 cm-1 for C-

C stretch, C-H and CH_3 rocking, C-H stretch and CH_3 rocking, C-H wagging and CH_3 rocking, CH_3 symmetrical bending, CH_3 stretching, CH_2 asymmetrical stretch, CH_3 asymmetrical stretch, respectively (Figure 6.2C).

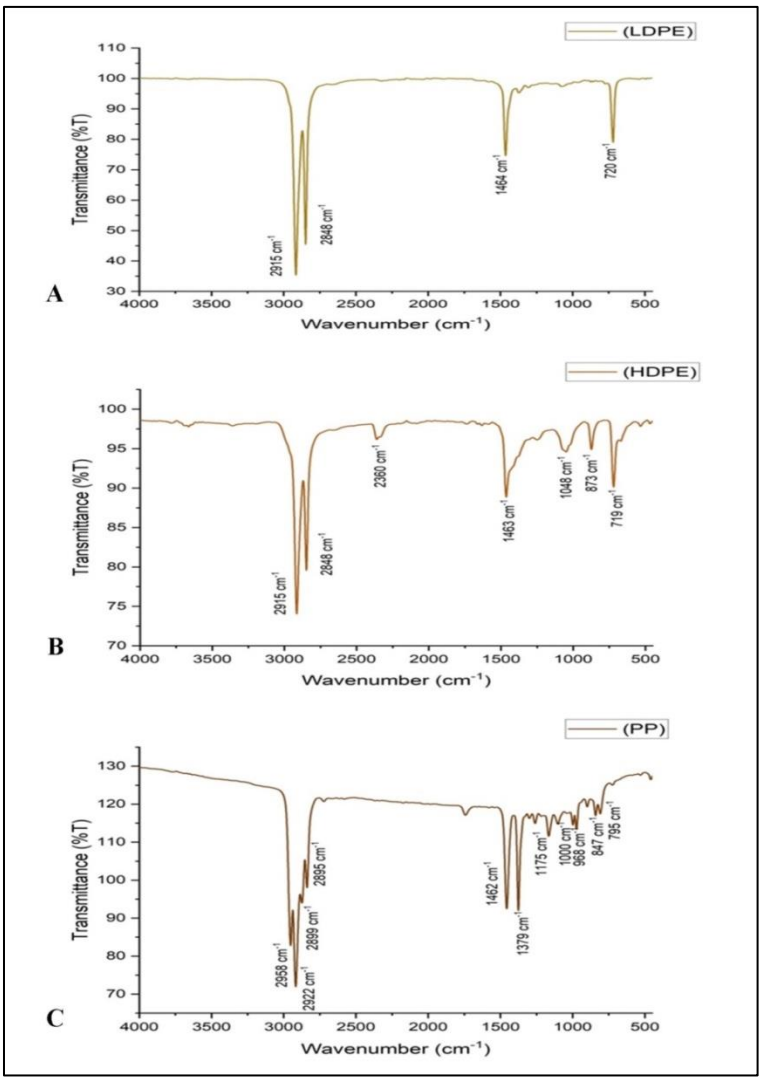


Figure 6.2 Fourier Transform Infrared Spectroscopy (FTIR) of naive plastic films; on the x-axis wavenumber of the transmission range is plotted and on the y-axis the transmittance observed after emergence of the infrared waves. Here, (A) is FTIR graph naive LDPE film; (B) represents FTIR graph of naive HDPE film; (C) illustrates FTIR plot for naive PP film

For present research work these parameters have been followed:

- **Study of the Consumption of Naive and Pretreated Plastic Films by all Larval Instars of Wax Moth**
- **Study of the Degradation of Different Types of Plastics by all Larval Instars of Wax Moth**
- **Enzyme Activity Assays**

The plastic waste is usually found dumped in the landfills and natural surroundings. In order to observe the consumption of waste plastics present in the form of waste on the terrestrial environment and in the landfills, the plastic films (LDPE, HDPE, and PP) were dumped in the soil for a year. These soil dumped plastic films along with naive plastic films were fed to all the larval instars of greater and lesser wax moth for 48 hours. Moreover, the wax worms were force fed with the antibiotics to explore the plastic ingestion, digestion and biodegradation capacity of the wax moths for the plastics. Additionally, the enzymatic tests for the enzymes lactate dehydrogenase and alcohol dehydrogenase demonstrated the validity of the plastics' digestion.

6.3. Study of the Consumption of Naive and Pretreated Plastic Films by All Larval Instars of Wax Moth

All larval instar stages in the group of fifty larvae in each group of greater and lesser wax moth were exposed to the naive and soil treated LDPE, HDPE, and PP film for two days. After experiment, the plastic consumption capacity and survival rate of the larva was recorded. For analysis of plastic consumption capacity of greater wax moth for naive and pretreated plastic films, the first instar group fed with naive plastic film (WA1GWM), first instar group fed with pretreated plastic film (WA1GWM-D), second instar group fed with naive plastic film (WA2GWM), second instar group fed with pretreated plastic film (WA2GWM-D), third instar group fed with naive plastic film (WA3GWM), third instar group fed with pretreated plastic film (WA3GWM-D), fourth instar group fed with naive plastic film (WA4GWM), fourth instar group fed with pretreated plastic film

(WA4GWM-D), fifth instar group fed with naive plastic film (WA-5-GWM), fifth instar group fed with pretreated plastic film (WA5GWM-D), sixth instar group fed with naive plastic film (WA6GWM), sixth instar group fed with pretreated plastic film (WA6GWM-D), seventh instar group fed with naive plastic film (WA-7-GWM), seventh instar group fed with pretreated plastic film (WA7GWM-D) and control for first instar (CONTROL1), control for second instar (CONTROL2), control for third instar (CONTROL3), control for fourth instar (CONTROL4), control for fifth instar (CONTROL5), is control for sixth instar (CONTROL6) and control for seventh instar (CONTROL7) (Table 6.1).

Similarly for *A. grisella*, first instar group fed with naive plastic film (WA1LWM), first instar group fed with pretreated plastic film (WA1LWM-D), second instar group fed with naive plastic film (WA2LWM), second instar group fed with pretreated plastic film (WA2LWM-D), third instar group fed with plastic film LDPE film (WA3LWM), third instar group fed with pretreated plastic film (WA3LWM-D), fourth instar group fed with naive plastic film (WA4LWM), fourth instar group fed with pretreated plastic film (WA4LWM-D), fifth instar group fed with naive plastic film (WA5LWM), fifth instar group fed with pretreated plastic film (WA5LWM-D) and control for first instar (CONTROL1), control for second instar (CONTROL2), control for third instar (CONTROL3), control for fourth instar (CONTROL4), control for fifth instar (CONTROL5) (Table 6.2).

Table 6.1 Insect groups of greater wax moth for study of the consumption of naive and pretreated plastic films by all larval instars of wax moth

For *Galleria mellonella* Larva	
CONTROL1-control of first instar of greater wax moth	

WA1GWM- without antibiotic first instar of greater wax moth	WA1GWM-D- without antibiotic first instar of greater wax moth fed with pretreated plastic film
CONTROL2-control of second instar of greater wax moth	
WA2GWM- without antibiotic second instar of greater wax moth	WA2GWM-D-without antibiotic second instar of greater wax moth fed with pretreated plastic film
CONTROL3-control of third instar of greater wax moth	
WA3GWM- without antibiotic third instar of greater wax moth	WA3GWM-D-without antibiotic third instar of greater wax moth fed with pretreated plastic film
CONTROL4-control of fourth instar of greater wax moth	
WA4GWM- without antibiotic fourth instar of greater wax moth	WA4GWM-D-without antibiotic fourth instar of greater wax moth fed with pretreated plastic film
CONTROL5-control of fifth instar of greater wax moth	
WA5GWM- without antibiotic fifth instar of	WA5GWM-D-without antibiotic fifth instar of greater wax moth fed with pretreated plastic

greater wax moth	film
CONTROL6-control of sixth instar of greater wax moth	
WA6GWM- without antibiotic sixth instar of greater wax moth	WA6GWM-D-without antibiotic sixth instar of greater wax moth fed with pretreated plastic film
CONTROL7-control of seventh instar of greater wax moth	
WA7GWM- without antibiotic seventh instar of greater wax moth	WA7GWM-D-without antibiotic seventh instar of greater wax moth fed with pretreated plastic film

Table 6.2 Insect groups of lesser wax moth for study of the consumption of naive and pretreated plastic films by all larval instars of wax moth

For *Achroia grisella* Larva	
CONTROL1-control of first instar of lesser wax moth	
WA1LWM- without antibiotic first instar of lesser wax moth	WA1LWM-D- without antibiotic first instar of lesser wax moth fed with pretreated plastic film

CONTROL2-control of second instar of lesser wax moth	
WA2LWM- without antibiotic second instar of lesser wax moth	WA2LWM-D-without antibiotic second instar of lesser wax moth fed with pretreated plastic film
CONTROL3-control of third instar of lesser wax moth	
WA3LWM- without antibiotic third instar of lesser wax moth	WA3LWM-D-without antibiotic third instar of lesser wax moth fed with pretreated plastic film
CONTROL4-control of fourth instar of lesser wax moth	
WA4LWM- without antibiotic fourth instar of lesser wax moth	WA4LWM-D-without antibiotic fourth instar of lesser wax moth fed with pretreated plastic film
CONTROL5-control of fifth instar of lesser wax moth	
WA5LWM- without antibiotic fifth instar of lesser wax moth	WA5LWM-D-without antibiotic fifth instar of lesser wax moth fed with pretreated plastic film

Plastic Consumption by *Galleria mellonella*

Low-Density Polyethylene (LDPE) - The naive Low Density Polyethylene (LDPE) and Soil Dumped Low Density Polyethylene (LDPE-D) were exposed to greater wax moth larvae of all larval instar stage. In different experiments, fifty larval instars in each group were kept along with the control population was exposed to LDPE. After the completion of the experiment the plastic consumption and survival rate of the larvae was recorded.

Plastic Consumption Rate- The plastic consumption rate of the *G. mellonella* fed on the naive and pretreated plastic film was recorded after the exposure of the insect larva to the LDPE film for two days. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Plastic consumption rate for naive and pretreated LDPE for *G. mellonella* is given by various groups- WA1GWM, WA1GWM-D, WA2GWM, WA2GWM-D, WA3GWM, WA3GWM-D, WA4GWM, WA4GWM-D, WA5GWM, WA5GWM-D, WA6GWM, WA6GWM-D, WA7GWM and WA7GWM-D is 0, 0, 0.12 ± 0.11,0, 0.06 ± 0.1, 0.48 ± 0.22, 4.94 ± 4.38, 3.08 ± 0.23, 0.86 ± 0.83, 3.48 ± 0.18, 1.34 ± 1.05, 4.72 ± 1.15, 2.53 ± 2.5, 4.64 ± 1.05 %, respectively (Figure 6.3).

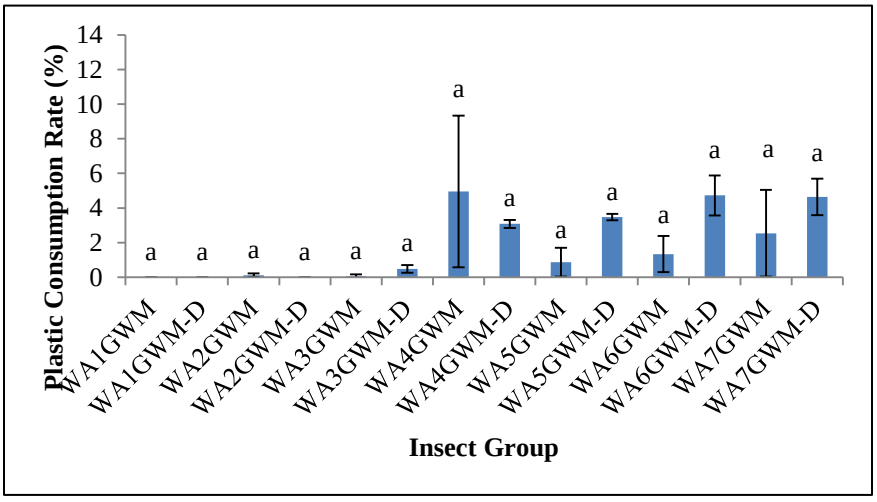


Figure 6.3 Plastic consumption rate of naive and pretreated LDPE film. The graph represents plastic consumption rate of LDPE by *G. mellonella*. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). In this context, without

antibiotic first instar group fed with naive LDPE film is WA1GWM, without antibiotic first instar group fed with pretreated LDPE film is represented as WA1GWM-D, without antibiotic second instar group fed with naive LDPE film is referred as WA2GWM, without antibiotic second instar group fed with pretreated LDPE film is depicted by WA2GWM-D, without antibiotic third instar group fed with naive LDPE film is referred as WA3GWM, without antibiotic third instar group fed with pretreated LDPE film is represented by WA3GWM-D, without antibiotic fourth instar group fed with naive LDPE film is WA4GWM, without antibiotic fourth instar group fed with pretreated LDPE film is referred as WA4GWM-D, without antibiotic fifth instar group fed with naive LDPE film is WA5GWM, without antibiotic fifth instar group fed with pretreated LDPE film is represented as WA5GWM-D, without antibiotic sixth instar group fed with naive LDPE film is depicted as WA6GWM, without antibiotic sixth instar group fed with pretreated LDPE film is referred as WA6GWM-D, without antibiotic seventh instar group fed with naive LDPE film is depicted as WA7GWM, without antibiotic seventh instar group fed with pretreated LDPE film is represented as WA7GWM-D

Insect Survival Rate- The insect survival rate of the *G. mellonella* fed on the naive and pretreated plastic film was recorded after the exposure of the wax worms to the LDPE film for 48 hours. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated LDPE for *G. mellonella* is given by various groups- Control1, WA1GWM, WA1GWM-D, Control2, WA2GWM, WA2GWM-D, Control3, WA3GWM, WA3GWM-D, Control4, WA4GWM, WA4GWM-D, Control5, WA5GWM, WA5GWM-D, Control6, WA6GWM, WA6GWM-D, Control7, WA7GWM and WA7GWM-D is 100 ± 0, 36.33 ± 15.53, 49.33 ± 9.01, 100 ± 0, 71.33 ± 17.47, 71.33 ± 3.05, 100 ± 0, 51.33 ± 12.05, 67.33 ± 3.05, 100 ± 0, 79.33 ± 13.31, 83.33 ± 4.16, 100 ± 0, 88.66 ± 10.26, 86 ± 2, 100 ± 0, 88 ± 7.21, 88 ± 4, 100 ± 0, 94.66 ± 2.30, 94 ± 2 % respectively (each test includes triplicates for each group) (Figure 6.4).

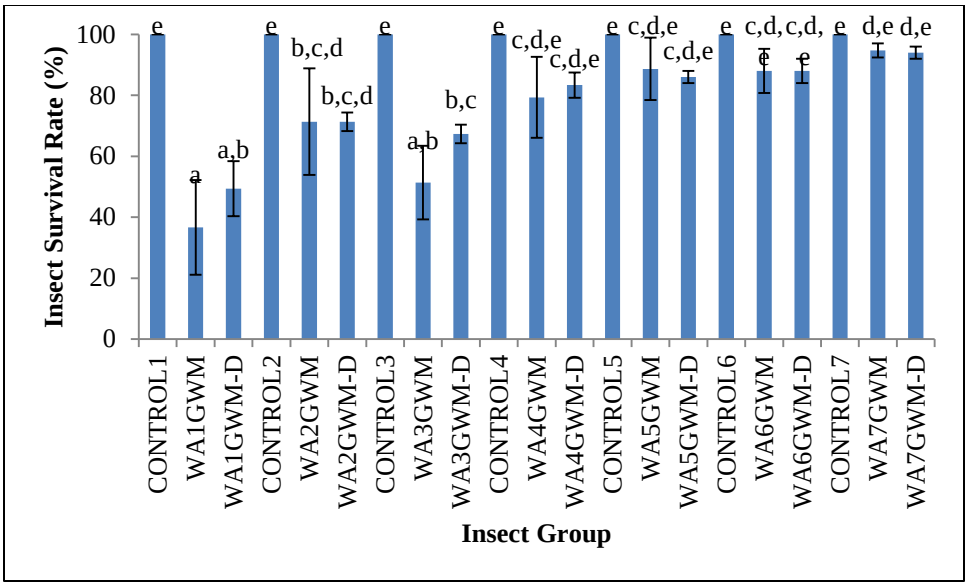


Figure 6.4 Insects survived when fed on naive and pretreated LDPE film. The graph represents *G. mellonella* insects survived when fed on naive and pretreated LDPE film. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-e). Note: CONTROL1= control first instar group, WA1GWM= without antibiotic first instar group fed with naive LDPE film, WA1GWM-D= without antibiotic first instar group fed with pretreated LDPE film, CONTROL2= control second instar group, WA2GWM= without antibiotic second instar group fed with naive LDPE film, WA2GWM-D= without antibiotic second instar group fed with pretreated LDPE film, CONTROL3= control third instar group, WA3GWM= without antibiotic third instar group fed with naive LDPE film, WA3GWM-D= without antibiotic third instar group fed with pretreated LDPE film, CONTROL4= control fourth instar group, WA4GWM= without antibiotic fourth instar group fed with naive LDPE film, WA4GWM-D= without antibiotic fourth instar group fed with pretreated LDPE film, CONTROL5= control fifth instar group, WA5GWM= without antibiotic fifth instar group fed with naive LDPE film, WA5GWM-D= without antibiotic fifth instar group fed with pretreated LDPE film, CONTROL6= control sixth instar group, WA6GWM= without antibiotic sixth instar group fed with naive LDPE film, WA6GWM-D= without antibiotic sixth instar group fed with pretreated LDPE film, CONTROL7= control seventh instar group, WA7GWM= without antibiotic seventh instar group fed with naive LDPE film, WA7GWM-D= without antibiotic seventh instar group fed with pretreated LDPE film

High-Density Polyethylene (HDPE) - For two days, the naive (HDPE) and soil-dumped (HDPE-D) groups were exposed to all stages of greater wax moth larvae, with a sample size of fifty larvae instars in each group, along with the control population. Following the experiment, the larvae's consumption of plastic and survival rate were noted.

Plastic Consumption Rate- Following a 48-hour exposure of the insect larvae to the HDPE film, the plastic consumption rate of *G. mellonella* was measured while feeding on the naive and pretreated plastic film. One-way ANOVA was used to determine the data's significance, and Tukey's significant difference test followed at a one-tailed significance

level of 0.05. The Plastic consumption rate for naive and pretreated HDPE for *G. mellonella* is given by various groups- WA1GWM, WA1GWM-D, WA2GWM, WA2GWM-D, WA3GWM, WA3GWM-D, WA4GWM, WA4GWM-D, WA5GWM, WA5GWM-D, WA6GWM, WA6GWM-D, WA7GWM and WA7GWM-D is 0.16 ± 0.13, 0.12 ± 0.21, 0.25 ± 0.25, 0.24 ± 0.21, 0.42 ± 0.12, 1.30 ± 0.44, 1.49 ± 0.93, 2.34 ± 1.13, 2.53 ± 0.06, 2.95 ± 1.35, 3.84 ± 2.18, 8.63 ± 1.55, 7.01 ± 4.93, 8.89 ± 0.99 %, respectively (Figure 6.5).

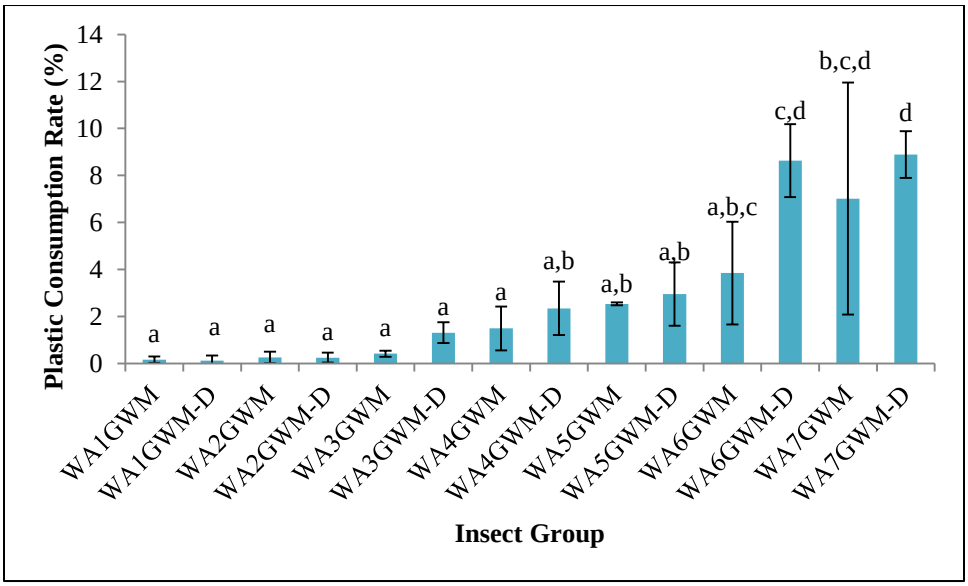


Figure 6.5 Plastic consumption rate of naive and pretreated HDPE film, in this graph, without antibiotic first instar group fed with naive HDPE film is referred as WA1GWM, without antibiotic first instar group fed with pretreated HDPE film is WA1GWM-D, without antibiotic second instar group fed with naive HDPE film is represented as WA2GWM, without antibiotic second instar group fed with pretreated HDPE film is referred WA2GWM-D, without antibiotic third instar group fed with naive HDPE film is represented as WA3GWM, without antibiotic third instar group fed with pretreated HDPE film is referred as WA3GWM-D, without antibiotic fourth instar group fed with naive HDPE film is depicted as WA4GWM, without antibiotic fourth instar group fed with pretreated HDPE film is WA4GWM-D, without antibiotic fifth instar group fed with naive HDPE film is referred as WA5GWM, without antibiotic fifth instar group fed with pretreated HDPE film is represented as WA5GWM-D, without antibiotic sixth instar group fed with naive HDPE film is represented as WA6GWM, without antibiotic sixth instar group fed with pretreated HDPE film is WA6GWM-D, without antibiotic seventh instar group fed with naive HDPE film is referred as WA7GWM, without antibiotic seventh instar group fed with pretreated HDPE film is WA7GWM-D. The graph represents plastic consumption rate of HDPE by *G. mellonella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-e)

Insect Survival Rate- The insect survival rate of the *G. mellonella* fed was recorded after the exposure of the wax worms to the HDPE film for two days. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated HDPE for *G. mellonella* is given by various groups- Control1, WA1GWM, WA1GWM-D, Control2, WA2GWM, WA2GWM-D, Control3, WA3GWM, WA3GWM-D, Control4, WA4GWM, WA4GWM-D, Control5, WA5GWM, WA5GWM-D, Control6, WA6GWM, WA6GWM-D, Control7, WA7GWM and WA7GWM-D is 100 ± 0, 36.66 ± 23.69, 54 ± 4, 100 ± 0, 47.33 ± 24.84, 67.33 ± 3.05, 100 ± 0, 83.33 ± 8.08, 81.33 ± 4.16, 100 ± 0, 92.66 ± 6.42, 76.66 ± 9.45, 100 ± 0, 98.66 ± 2.30, 94 ± 5.29, 100 ± 0, 91.33 ± 6.11, 92.66 ± 1.15, 100 ± 0, 98 ± 3.46, 96.66 ± 3.05 % respectively (each test includes triplicates for each group) (Figure 6.6).

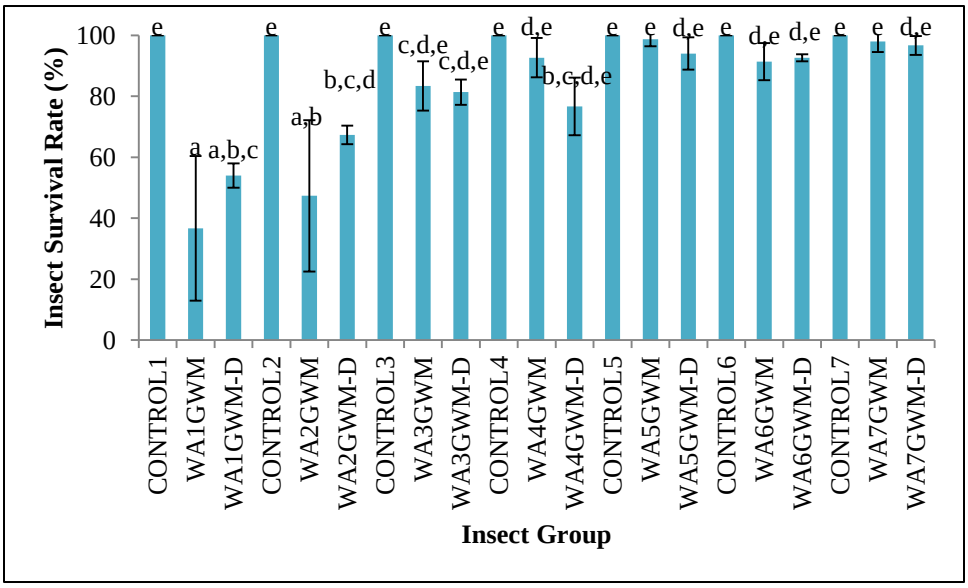


Figure 6.6 Insects survived when fed on naive and pretreated HDPE film. The graph represents *G. mellonella* insects survived when fed on naive and pretreated HDPE film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-e). Note: CONTROL1= control first instar group, WA1GWM= without antibiotic first instar group fed with naive HDPE film, WA1GWM-D= without antibiotic first instar group fed with pretreated HDPE film, CONTROL2= control second instar group, WA2GWM= without antibiotic second instar group fed with naive HDPE film, WA2GWM-D= without antibiotic second instar group fed with pretreated HDPE film, CONTROL3= control third instar group, WA3GWM= without antibiotic third instar group fed with naive HDPE film, WA3GWM-D= without antibiotic third instar group fed with pretreated HDPE film, CONTROL4= control fourth instar group, WA4GWM= without antibiotic fourth instar group fed with

naive HDPE film, WA4GWM-D= without antibiotic fourth instar group fed with pretreated HDPE film, CONTROL5= control fifth instar group, WA5GWM= without antibiotic fifth instar group fed with naive HDPE film, WA5GWM-D= without antibiotic fifth instar group fed with pretreated HDPE film, CONTROL6= control sixth instar group, WA6GWM= without antibiotic sixth instar group fed with naive HDPE film, WA6GWM-D= without antibiotic sixth instar group fed with pretreated HDPE film, CONTROL7= control seventh instar group, WA7GWM= without antibiotic seventh instar group fed with naive HDPE film, WA7GWM-D= without antibiotic seventh instar group fed with pretreated HDPE film

Polypropylene (PP) - The naive (PP) and soil dumped (PP-D) were exposed to greater wax moth larvae of all larval instar stage with a sample size of fifty larval instars in each group along with the control population for two days. After the completion of the experiment the plastic consumption and survival rate of the larvae was recorded.

Plastic Consumption Rate- The plastic consumption rate of the *G. mellonella* was recorded after the exposure of the insects to the PP film for 48 hours. One-way ANOVA was used to assess the data's significance, followed by Tukey's significant difference test at a one-tailed significance level of 0.05. The Plastic consumption rate for naive and pretreated PP for *G. mellonella* is given by various groups- WA1GWM, WA1GWM-D, WA2GWM, WA2GWM-D, WA3GWM, WA3GWM-D, WA4GWM, WA4GWM-D, WA5GWM, WA5GWM-D, WA6GWM, WA6GWM-D, WA7GWM and WA7GWM-D is 0, 0, 0, 0, 0.19 ± 0.19, 0.44 ± 0.28, 1.57 ± 1.29, 3.01 ± 2, 0.91 ± 0.76, 4.15 ± 3, 1.39 ± 1.33, 2.81 ± 1.28, 8.4 ± 7.6, 8.44 ± 0.42 % respectively.

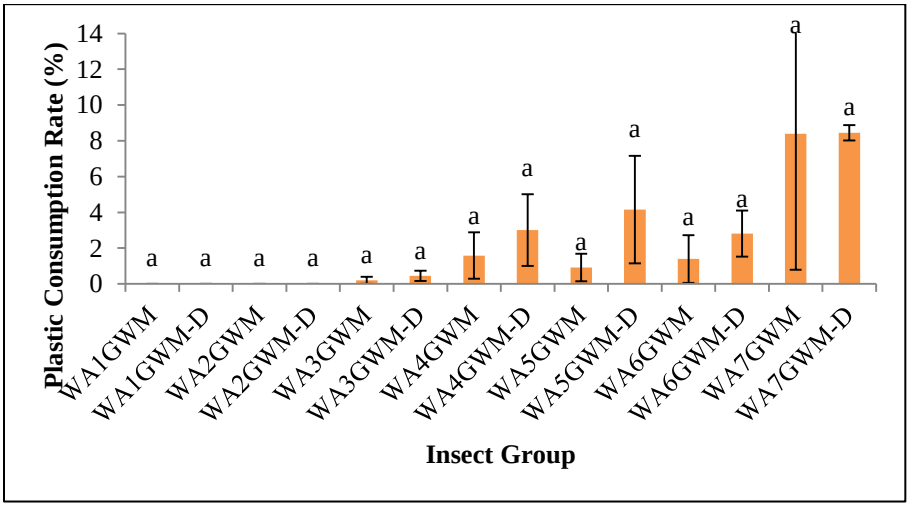


Figure 6.7 Plastic consumption rate of naive and pretreated PP film. The graph represents plastic consumption rate of PP by *G. mellonella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA

followed by Tukey's significant difference test which is represented by a). In this graph, without antibiotic first instar group fed with naive PP film is WA1GWM, without antibiotic first instar group fed with pretreated PP film is represented by WA1GWM-D, without antibiotic second instar group fed with naive PP film is referred to WA2GWM, without antibiotic second instar group fed with pretreated PP film is WA2GWM-D, without antibiotic third instar group fed with naive PP film is depicted by WA3GWM, without antibiotic third instar group fed with pretreated PP film is referred as WA3GWM-D, without antibiotic fourth instar group fed with naive PP film is WA4GWM, without antibiotic fourth instar group fed with pretreated PP film is represented by WA4GWM-D, without antibiotic fifth instar group fed with naive PP film is depicted by WA5GWM, without antibiotic fifth instar group fed with pretreated PP film is represented by WA5GWM-D, without antibiotic sixth instar group fed with naive PP film is WA6GWM, without antibiotic sixth instar group fed with pretreated PP film is referred as WA6GWM-D, without antibiotic seventh instar group fed with naive PP film is WA7GWM, without antibiotic seventh instar group fed with pretreated PP film is represented by WA7GWM-D

Table 6.3 Comparative data on plastic consumption (mg/day/insect) by *Galleria mellonella* for study of the consumption of naive and pretreated plastic films

Insect Group	LDPE	HDPE	PP
WA1GWM	0	0.06 ± 0.005	0
WA1GWM-D	0	0.003 ± 0.005	0
WA2GWM	0.006 ± 0.01	0.01 ± 0.01	0
WA2GWM-D	0	0.006 ± 0.005	0
WA3GWM	0.003 ± 0.005	0.016 ± 0.005	0.01 ± 0.01
WA3GWM-D	0.01 ± 0.005	0.03 ± 0.01	0.02 ± 0.01
WA4GWM	0.263 ± 0.37	0.06 ± 0.03	0.08 ± 0.11
WA4GWM-D	0.09 ± 0.01	0.07 ± 0.03	0.17 ± 0.16
WA5GWM	0.07 ± 0.03	0.1 ± 0.02	0.05 ± 0.04
WA5GWM-D	0.1 ± 0.05	0.09 ± 0.04	0.22 ± 0.23
WA6GWM	0.07 ± 0.1	0.15 ± 0.08	0.05 ± 0.1
WA6GWM-D	0.14 ± 0.04	0.21 ± 0.02	0.19 ± 0.06
WA7GWM	0.14 ± 0.14	0.25 ± 0.16	0.15 ± 0.13
WA7GWM-D	0.14 ± 0.03	0.23 ± 0.05	0.48 ± 0.02

Insect Survival Rate- Following the wax worms' 48-hour exposure to the PP film, the insect survival rate of *G. mellonella* was noted. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-

tailed significance of 0.05. The Insect survival rate for naive and pretreated PP for *G. mellonella* is given by various groups- Control1, WA1GWM, WA1GWM-D, Control2, WA2GWM, WA2GWM-D, Control3, WA3GWM, WA3GWM-D, Control4, WA4GWM, WA4GWM-D, Control5, WA5GWM, WA5GWM-D, Control6, WA6GWM, WA6GWM-D, Control7, WA7GWM and WA7GWM-D is 100 ± 0, 38 ± 15.62, 48.66 ± 13.31, 100 ± 0, 72 ± 21.66, 74 ± 10, 100 ± 0, 52 ± 14.42, 78 ± 7.21, 100 ± 0, 79.33 ± 15.01, 88 ± 2, 100 ± 0, 89.33 ± 9.45, 92 ± 5.29, 100 ± 0, 89.33 ± 5.03, 98 ± 2, 100 ± 0, 94.66 ± 2.30, 96.66 ± 3.05 % respectively (Figure 6.8).

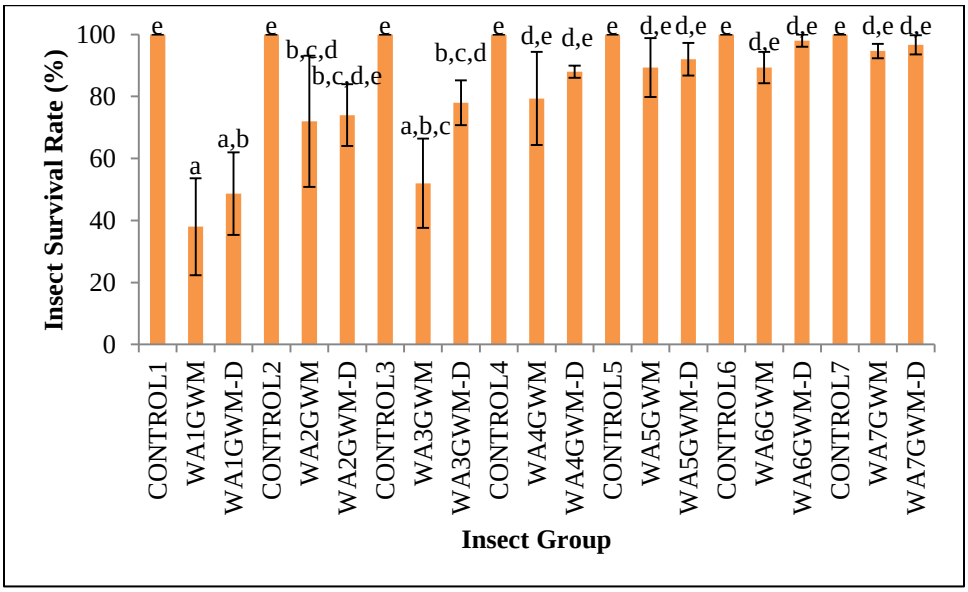


Figure 6.8 Insects survived when fed on naive and pretreated PP film. The graph represents *G. mellonella* insects survived when fed on naive and pretreated PP film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-e). Note: CONTROL1= control first instar group, WA1GWM= without antibiotic first instar group fed with naive PP film, WA1GWM-D= without antibiotic first instar group fed with pretreated PP film, CONTROL2= control second instar group, WA2GWM= without antibiotic second instar group fed with naive PP film, WA2GWM-D= without antibiotic second instar group fed with pretreated PP film, CONTROL3= control third instar group, WA3GWM= without antibiotic third instar group fed with naive PP film, WA3GWM-D= without antibiotic third instar group fed with pretreated PP film, CONTROL4= control fourth instar group, WA4GWM= without antibiotic fourth instar group fed with naive PP film, WA4GWM-D= without antibiotic fourth instar group fed with pretreated PP film, CONTROL5= control fifth instar group, WA5GWM= without antibiotic fifth instar group fed with naive PP film, WA5GWM-D= without antibiotic fifth instar group fed with pretreated PP film, CONTROL6= control sixth instar group, WA6GWM= without antibiotic sixth instar group fed with naive PP film, WA6GWM-D= without antibiotic sixth instar group fed with pretreated PP film, CONTROL7= control seventh instar group, WA7GWM= without antibiotic seventh instar group fed with naive PP film, WA7GWM-D= without antibiotic seventh instar group fed with pretreated PP film

Plastic Consumption by *Achroia grisella*

Low-Density Polyethylene (LDPE) – Lesser wax moth larvae in all larval instar stages were exposed to both the naive Low Density Polyethylene (LDPE) and the Soil Dumped Low Density Polyethylene (LDPE-D). For each larval group, fifty larval instars along with control were exposed to LDPE for 48 hours. Following the experiment, the larvae's consumption of plastic and survival rate were noted.

Plastic Consumption Rate- Following two days of exposure to the LDPE film, the plastic consumption rate for all larval stages of *A. grisella* was recorded. One-way ANOVA was used to determine the data's significance, followed by Tukey's significant difference test at a one-tailed significance level of 0.05. The Plastic consumption rate for naive and pretreated LDPE for *A. grisella* is given by various groups- WA1LWM, WA1LWM-D, WA2LWM, WA2LWM-D, WA3LWM, WA3LWM-D, WA4LWM, WA4LWM-D, WA5LWM, and WA5LWM-D, is 0, 0, 0, 0.43 ± 0.14, 0.18 ± 0.18, 0.46 ± 0.19, 0.91 ± 0.54, 0.33 ± 0.01, 7.51 ± 7.12, 2.64 ± 1.77 %, respectively (Figure 6.9).

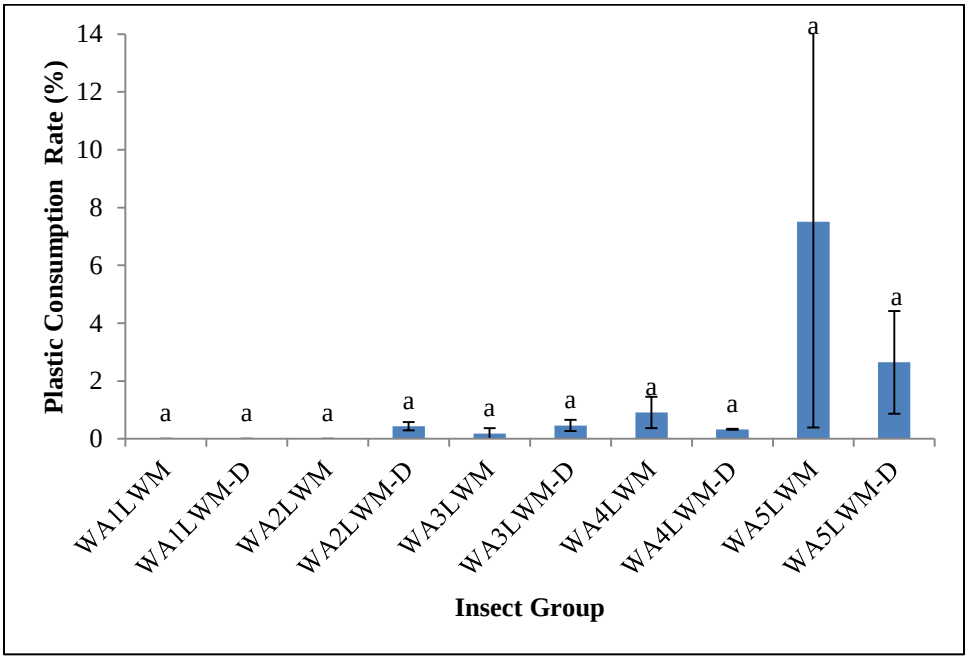


Figure 6.9 Plastic consumption rate of naive and pretreated LDPE film. The graph represents plastic consumption rate of LDPE by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA

followed by Tukey's significant difference test which is represented by a). In this graph, without antibiotic first instar group fed with naive LDPE film is WA1LWM, without antibiotic first instar group fed with pretreated LDPE film is represented by WA1LWM-D, without antibiotic second instar group fed with naive LDPE film is referred by WA2LWM, without antibiotic second instar group fed with pretreated LDPE film is depicted by WA2LWM-D, without antibiotic third instar group fed with naive LDPE film is WA3LWM, without antibiotic third instar group fed with pretreated LDPE film is represented by WA3LWM-D, without antibiotic fourth instar group fed with naive LDPE film is depicted by WA4LWM, without antibiotic fourth instar group fed with pretreated LDPE film is represented by WA4LWM-D, without antibiotic fifth instar group fed with naive LDPE film is referred as WA5LWM, without antibiotic fifth instar group fed with pretreated LDPE film is represented as WA5LWM-D

Insect Survival Rate- After the wax worms were exposed to the LDPE film for two days, the insect survival rate of *A. grisella* was recorded. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated LDPE for *A. grisella* is given by various groups- Control1, WA1LWM, WA1LWM-D, Control2, WA2LWM, WA2LWM-D, Control3, WA3LWM, WA3LWM-D, Control4, WA4LWM, WA4LWM-D, Control5, WA5LWM, and WA5LWM-D 100 ± 0, 35.33 ± 9.23, 64 ± 10.39, 100 ± 0, 53.33 ± 9.01, 84 ± 3.46, 100 ± 0, 76 ± 8.71, 93.33 ± 3.05, 100 ± 0, 82 ± 10.58, 86.66 ± 11.54, 100 ± 0, 92 ± 5.29, 93.33 ± 4.16 % respectively (each test includes triplicates for each group), respectively (Figure 6.10).

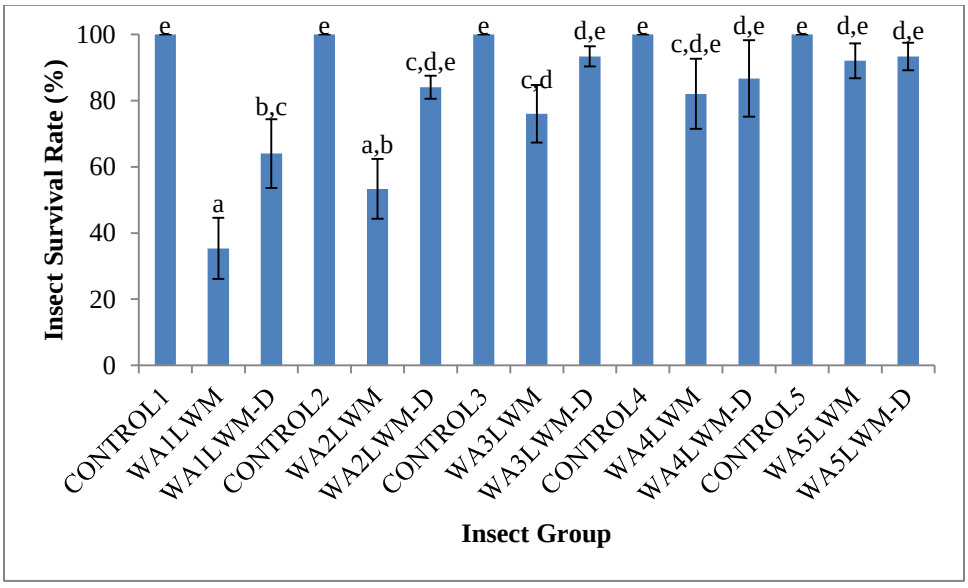


Figure 6.10 Insects survived when fed on naive and pretreated LDPE film. The graph represents *A. grisella* insects survived when fed on naive and pretreated LDPE film. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-e). Note:

CONTROL1= control first instar group, WA1LWM= without antibiotic first instar group fed with naive LDPE film, WA1LWM-D= without antibiotic first instar group fed with pretreated LDPE film, CONTROL2= control second instar group, WA2LWM= without antibiotic second instar group fed with naive LDPE film, WA2LWM-D= without antibiotic second instar group fed with pretreated LDPE film, CONTROL3= control third instar group, WA3LWM= without antibiotic third instar group fed with naive LDPE film, WA3LWM-D= without antibiotic third instar group fed with pretreated LDPE film, CONTROL4= control fourth instar group, WA4LWM= without antibiotic fourth instar group fed with naive LDPE film, WA4LWM-D= without antibiotic fourth instar group fed with pretreated LDPE film, CONTROL5= control fifth instar group, WA5LWM= without antibiotic fifth instar group fed with naive LDPE film, WA5LWM-D= without antibiotic fifth instar group fed with pretreated LDPE film

High-Density Polyethylene (HDPE) - The naive (HDPE) and soil dumped (HDPE-D) were exposed to all larval instar stages of lesser wax moth. For 48 hours, the control group and the sample size of fifty caterpillars each larval instar stage were exposed to plastics. After the completion of the experiment the plastic consumption and survival rate of the larvae was recorded.

Plastic Consumption Rate- The plastic consumption rate of the *A. grisella* was recorded after the exposure of the insects to the HDPE film for two days. One-way ANOVA was used to determine the data's significance, followed by Tukey's significant difference test at a one-tailed significance level of 0.05. The Plastic consumption rate for naive and pretreated HDPE for *A. grisella* is given by various groups- WA1LWM, WA1LWM-D, WA2LWM, WA2LWM-D, WA3LWM, WA3LWM-D, WA4LWM, WA4LWM-D, WA5LWM, and WA5LWM-D, is 0, 0, 0.79 ± 0.8, 2.44 ± 2.20, 1.38 ± 1.23, 4.89 ± 3.58, 3.05 ± 2.19, 4.95 ± 4.20, 4.41 ± 1.9, 7.55 ± 0.9 %, respectively (Figure 6.11).

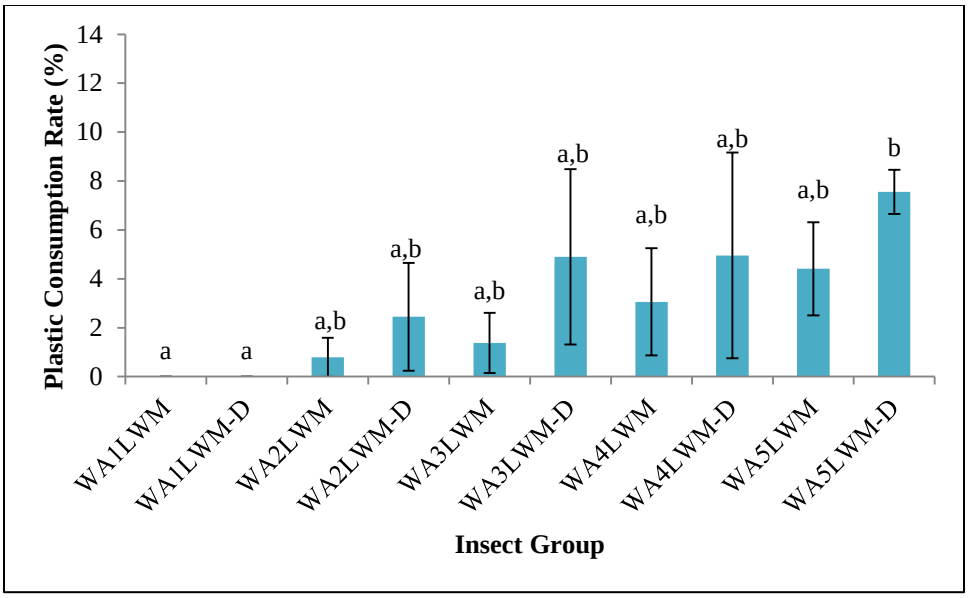


Figure 6.11 Plastic consumption rate of naive and pretreated HDPE film. The graph represents plastic consumption rate of HDPE by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). In this context, without antibiotic first instar group fed with naive HDPE film (WA1LWM), without antibiotic first instar group fed with pretreated HDPE film (WA1LWM-D), without antibiotic second instar group fed with naive HDPE film (WA2LWM), without antibiotic second instar group fed with pretreated HDPE film (WA2LWM-D), without antibiotic third instar group fed with naive HDPE film (WA3LWM), without antibiotic third instar group fed with pretreated HDPE film (WA3LWM-D), without antibiotic fourth instar group fed with naive HDPE film (WA4LWM), without antibiotic fourth instar group fed with pretreated HDPE film (WA4LWM-D), without antibiotic fifth instar group fed with naive HDPE film (WA5LWM), without antibiotic fifth instar group fed with pretreated HDPE film (WA5LWM-D)

Insect Survival Rate- Following the wax worms' 48-hour exposure to the HDPE film, the insect survival rate of *A. grisella* was noted. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The for naive and pretreated HDPE For *A. grisella* fed on HDPE, insect survival rate by various groups- Control1, WA1LWM, WA1LWM-D, Control2, WA2LWM, WA2LWM-D, Control3, WA3LWM, WA3LWM-D, Control4, WA4LWM, WA4LWM-D, Control5, WA5LWM, and WA5LWM-D is 100 ± 0, 32 ± 14.42, 55.33 ± 13.61, 100 ± 0, 60 ± 12, 86.66 ± 4.16, 100 ± 0, 71.33 ± 6.11, 84 ± 10.58, 100 ± 0, 80.66 ± 4.16, 94.66 ± 4.16, 100 ± 0, 81.33 ± 7.02, 94 ± 6 % respectively (each test includes triplicates for each group) (Figure 6.12).

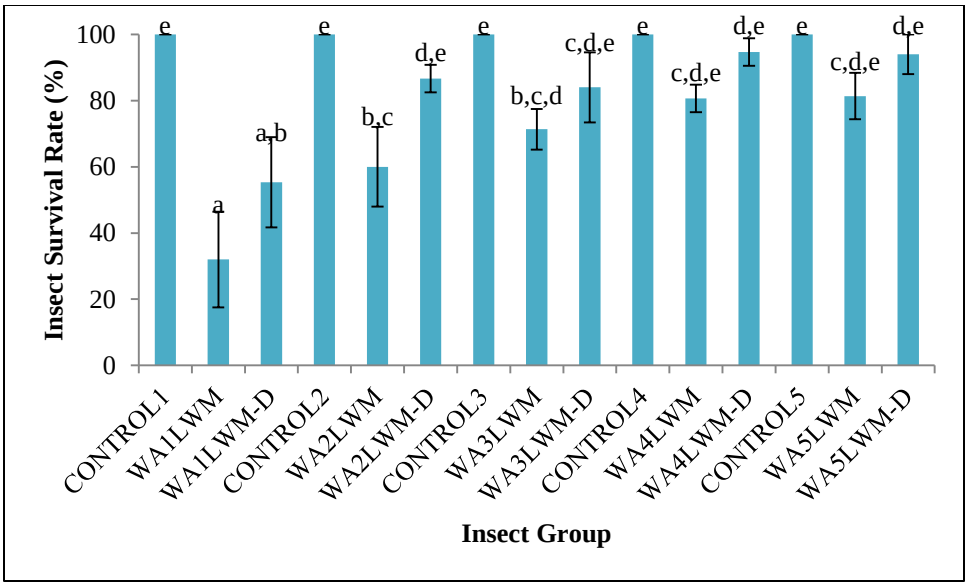


Figure 6.12 Insects survived when fed on naive and pretreated HDPE film. The graph represents *A. grisella* insects survived when fed on naive and pretreated HDPE film. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-e). Note: CONTROL1= control first instar group, WA1LWM= without antibiotic first instar group fed with naive HDPE film, WA1LWM-D= without antibiotic first instar group fed with pretreated HDPE film, CONTROL2= control second instar group, WA2LWM= without antibiotic second instar group fed with naive HDPE film, WA2LWM-D= without antibiotic second instar group fed with pretreated HDPE film, CONTROL3= control third instar group, WA3LWM= without antibiotic third instar group fed with naive HDPE film, WA3LWM-D= without antibiotic third instar group fed with pretreated HDPE film, CONTROL4= control fourth instar group, WA4LWM= without antibiotic fourth instar group fed with naive HDPE film, WA4LWM-D= without antibiotic fourth instar group fed with pretreated HDPE film, CONTROL5= control fifth instar group, WA5LWM= without antibiotic fifth instar group fed with naive HDPE film, WA5LWM-D= without antibiotic fifth instar group fed with pretreated HDPE film

Polypropylene (PP) - The naive (PP) and soil dumped (PP-D) were exposed to all larval instar stages lesser wax moth larvae. After the completion of the experiment the plastic consumption and survival rate of the larvae was recorded.

Plastic Consumption Rate- After the insects were exposed to the PP film for two days, the rate at which *A. grisella* consumed plastic was recorded. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Plastic consumption rate for naive and pretreated PP for *A. grisella* is given by various groups- WA1LWM, WA1LWM-D, WA2LWM, WA2LWM-D, WA3LWM, WA3LWM-D, WA4LWM, WA4LWM-D, WA5LWM, and

WA5LWM-D, is 0.24 ± 0.22, 0.26 ± 0.23, 0.24 ± 0.22, 0.63 ± 0.47, 1.05 ± 0.92, 0.53 ± 0.21, 1.6 ± 1.39, 0.5 ± 0.23, 0.9 ± 0.8, 1.82 ± 0.77 % respectively (Figure 6.13).

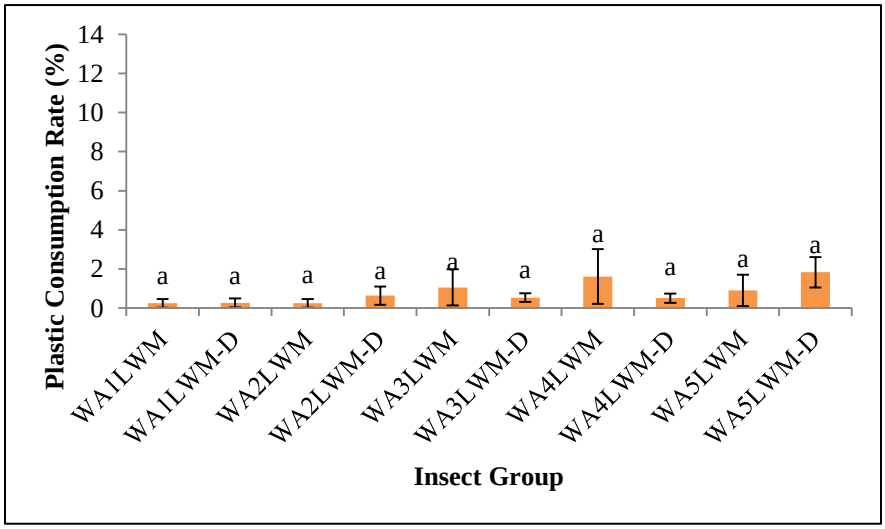


Figure 6.13 Plastic consumption rate of naive and pretreated PP film. The graph represents plastic consumption rate of PP by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). In this context, without antibiotic first instar group fed with naive PP film (WA1LWM), without antibiotic first instar group fed with pretreated PP film (WA1LWM-D), without antibiotic second instar group fed with naive PP film (WA2LWM), without antibiotic second instar group fed with pretreated PP film (WA2LWM-D), without antibiotic third instar group fed with naive PP film (WA3LWM), without antibiotic third instar group fed with pretreated PP film (WA3LWM-D), without antibiotic fourth instar group fed with naive PP film (WA4LWM), without antibiotic fourth instar group fed with pretreated PP film (WA4LWM-D), without antibiotic fifth instar group fed with naive PP film (WA5LWM), without antibiotic fifth instar group fed with pretreated PP film (WA5LWM-D) are the insect groups

Table 6.4 Comparative data on plastic consumption (mg/day/insect) by *Achroia grisella* for study of the consumption of naive and pretreated plastic films

Insect Group	LDPE	HDPE	PP
WA1LWM	0	0	0.003 ± 0.003
WA1LWM-D	0	0	0.006 ± 0.005
WA2LWM	0	0.03 ± 0.05	0.006 ± 0.01
WA2LWM-D	0	0.06 ± 0.07	0.01 ± 0.01
WA3LWM	0.01 ± 0.005	0.05 ± 0.04	0.02 ± 0.02
WA3LWM-D	0.01 ± 0.01	0.12 ± 0.09	0.01 ± 0.005

WA4LWM	0.05 ± 0.02	0.09 ± 0.09	0.03 ± 0.03
WA4LWM-D	0.01 ± 0	0.12 ± 0.1	0.01 ± 0.005
WA5LWM	0.42 ± 0.62	0.13 ± 0.06	0.02 ± 0.02
WA5LWM-D	0.07 ± 0.05	0.23 ± 0.02	0.04 ± 0.01

Insect Survival Rate- Following a 48-hour exposure of the wax worms to the PP film, the insect survival rate of the *A. grisella* fed on the naive and pretreated plastic film was recorded. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated PP for *A. grisella* is given by various groups- Control1, WA1LWM, WA1LWM-D, Control2, WA2LWM, WA2LWM-D, Control3, WA3LWM, WA3LWM-D, Control4, WA4LWM, WA4LWM-D, Control5, WA5LWM, and WA5LWM-D is 100 ± 0, 66.66 ± 12.85, 70 ± 12.16, 100 ± 0, 80.66 ± 17.009, 85.33 ± 3.05, 100 ± 0, 98 ± 3.46, 96 ± 5.29, 100 ± 0, 85.33 ± 18.9, 96 ± 2, 100 ± 0, 90 ± 5.29, 96.66 ± 3.05 % respectively (Figure 6.14).

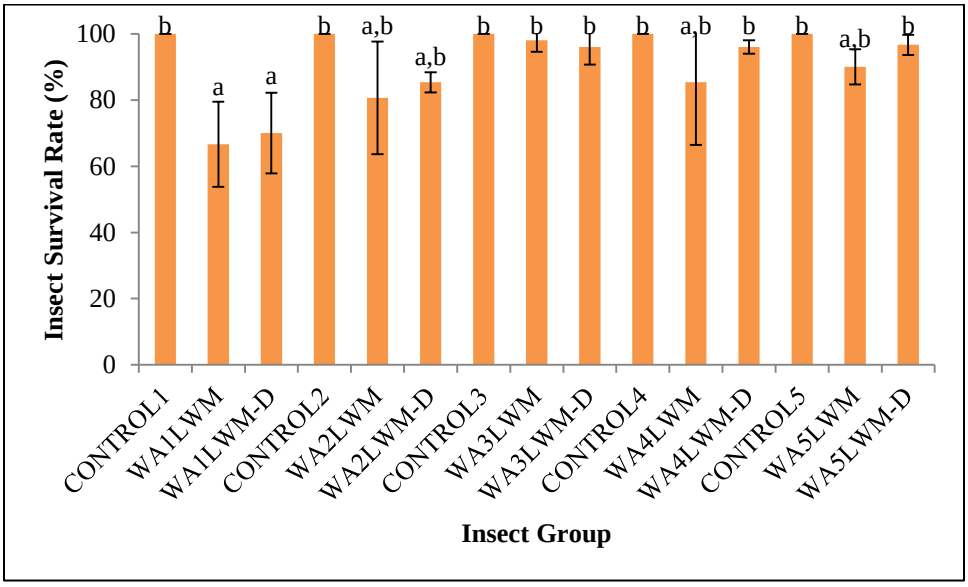


Figure 6.14 Insects survived when fed on naive and pretreated PP film. The graph represents *A. grisella* insects survived when fed on naive and pretreated PP film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). Note:

CONTROL1= control first instar group, WA1LWM= without antibiotic first instar group fed with naive PP film, WA1LWM-D= without antibiotic first instar group fed with pretreated PP film, CONTROL2= control second instar group, WA2LWM= without antibiotic second instar group fed with naive PP film, WA2LWM-D= without antibiotic second instar group fed with pretreated PP film, CONTROL3= control third instar group, WA3LWM= without antibiotic third instar group fed with naive PP film, WA3LWM-D= without antibiotic third instar group fed with pretreated PP film, CONTROL4= control fourth instar group, WA4LWM= without antibiotic fourth instar group fed with naive PP film, WA4LWM-D= without antibiotic fourth instar group fed with pretreated PP film, CONTROL5= control fifth instar group, WA5LWM= without antibiotic fifth instar group fed with naive PP film, WA5LWM-D= without antibiotic fifth instar group fed with pretreated PP film

6.4. Study the Degradation of Different Types of Plastics by All Larval Instars of Wax Moth

In order to observe the consumption and degradation capacity of plastics the naive polymer films (LDPE, HDPE, and PP) were fed to both wax moth species. The larvae were fed with antibiotics in order to observe the biodegradation capacity of the larvae. These naive plastic films were fed to all the larval instars of greater and lesser wax moth for 48 hours.

To study the plastic consumption capacity of greater wax moth the control group of first instar is CONTROL1, first instar group without antibiotic is WA-1-GWM, first instar group antibiotic fed is A-1-GWM, control group of second instar is CONTROL2, second instar group without antibiotic treatment is WA-2-GWM, second instar group administered to antibiotics is A-2-GWM, control group of third instar is CONTROL3, third instar group without any prior antibiotic treatment is WA-3-GWM, third instar group antibiotic fed is A-3-GWM, control group of fourth instar is CONTROL4, fourth instar group without antibiotics is WA-4-GWM, fourth instar group administered with antibiotics is A-4-GWM, control group of fifth instar is CONTROL5, fifth instar group without antibiotics is WA-5-GWM, fifth instar group antibiotic fed is A-5-GWM, control group of sixth instar is CONTROL6, sixth instar group without antibiotic administration is WA-6-GWM, sixth instar group fed with antibiotics is A-6-GWM, control group of

seventh instar is CONTROL7, seventh instar group without antibiotics is WA-7-GWM, seventh instar group fed antibiotic solution is A-7-GWM (Table 6.5).

Similarly, for lesser wax moth control group of first instar is CONTROL1, first instar group without antibiotic treatment is WA-1-LWM, first instar group administered with antibiotic solution is A-1-LWM, control group of second instar is CONTROL2, second instar group without antibiotic is WA-2-LWM, second instar group fed with antibiotics is A-2-LWM, control group of third instar is CONTROL3, third instar group without antibiotic treatment is WA-3-LWM, third instar group administered with antibiotics is A-3-LWM, control group of fourth instar is CONTROL4, fourth instar group without any prior antibiotic administration is WA-4-LWM, fourth instar group fed with antibiotics is A-4-LWM, control group of fifth instar is CONTROL5, fifth instar group without antibiotic treatment is WA-5-LWM, fifth instar group fed with antibiotics is A-5-LWM are the insect groups (Table 6.6).

Table 6.5 Insect groups of *Galleria mellonella* for study the degradation of different types of plastics by all larval instars of wax moth

For *Galleria mellonella* Larva	
CONTROL1-control of first instar of greater wax moth	
WA-1-GWM- without antibiotic first instar of greater wax moth	A-1-GWM- antibiotic fed first instar of greater wax moth fed with pretreated plastic film
CONTROL2-control of second instar of greater wax moth	

WA-2-GWM- without antibiotic second instar of greater wax moth	A-2-GWM- antibiotic fed second instar of greater wax moth fed with pretreated plastic film
CONTROL3-control of third instar of greater wax moth	
WA-3-GWM- without antibiotic third instar of greater wax moth	A-3-GWM- antibiotic fed third instar of greater wax moth fed with pretreated plastic film
CONTROL4-control of fourth instar of greater wax moth	
WA-4-GWM- without antibiotic fourth instar of greater wax moth	A-4-GWM- antibiotic fed fourth instar of greater wax moth
CONTROL5-control of fifth instar of greater wax moth	
WA-5-GWM- without antibiotic fifth instar of greater wax moth	A-5-GWM- antibiotic fed fifth instar of greater wax moth
CONTROL6-control of sixth instar of greater wax moth	
WA-6-GWM- without antibiotic sixth instar of greater wax moth	A-6-GWM- antibiotic fed sixth instar of greater wax moth

CONTROL7-control of seventh instar of greater wax moth	
WA-7-GWM- without antibiotic seventh instar of greater wax moth	A-7-GWM- antibiotic fed seventh instar of greater wax moth

Table 6.6 Insect groups of *Achroia grisella* for study the degradation of different types of plastics by all larval instars of wax moth

For *Achroia grisella* Larva	
CONTROL1-control of first instar of lesser wax moth	
WA-1-LWM- without antibiotic first instar of lesser wax moth	A-1-LWM- antibiotic fed first instar of lesser wax moth fed with pretreated plastic film
CONTROL2-control of second instar of lesser wax moth	
WA-2-LWM- without antibiotic second instar of lesser wax moth	A-2-LWM- antibiotic fed second instar of lesser wax moth fed with pretreated plastic film
CONTROL3-control of third instar of lesser wax moth	
WA-3-LWM- without antibiotic third instar of	A-3-LWM- antibiotic fed third instar of lesser

lesser wax moth	wax moth fed with pretreated plastic film
CONTROL4-control of fourth instar of lesser wax moth	
WA-4-LWM- without antibiotic fourth instar of lesser wax moth	A-4-LWM- antibiotic fed fourth instar of lesser wax moth
CONTROL5-control of fifth instar of lesser wax moth	
WA-5-LWM- without antibiotic fifth instar of lesser wax moth	A-5-LWM- antibiotic fed fifth instar of lesser wax moth

Plastic Consumption by *Galleria mellonella*

Low-Density Polyethylene (LDPE) - The naive LDPE films were exposed to all larval instar stages of greater wax moth with a sample size of fifty larvae in each group along with the control population for two days. After the completion of the experiment the plastic consumption and survival rate of the larvae, the SEM of the exposed LDPE film and GC-MS of the frass of the greater wax was recorded.

Plastic Consumption Rate- The plastic consumption rate of the *G. mellonella* fed on the naive and plastic film was recorded after the exposure of the insects to the LDPE film for two days to the larvae administered without and with antibiotics. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The plastic consumption rate for LDPE for *G. mellonella* by various groups- WA-1-GWM, A-1-GWM, WA-2-GWM, A-2-GWM, WA-3-GWM, A-3-GWM, WA-4-GWM, A-4-GWM, WA-5-GWM, A-5-GWM, WA-6-GWM, A-6-GWM, WA-7-GWM and A-7-GWM is 0, 0, 0.12 ± 0.1, 0, 0.06 ± 0.09, 0.06

± 0.09, 4.84 ± 4.48, 2.03 ± 2, 0.86 ± 0.83, 0.89 ± 0.87, 1.28 ± 1, 1.2 ± 1.02, 2.53 ± 2.02, 2.65 ± 2.01 %, respectively (Figure 6.15).

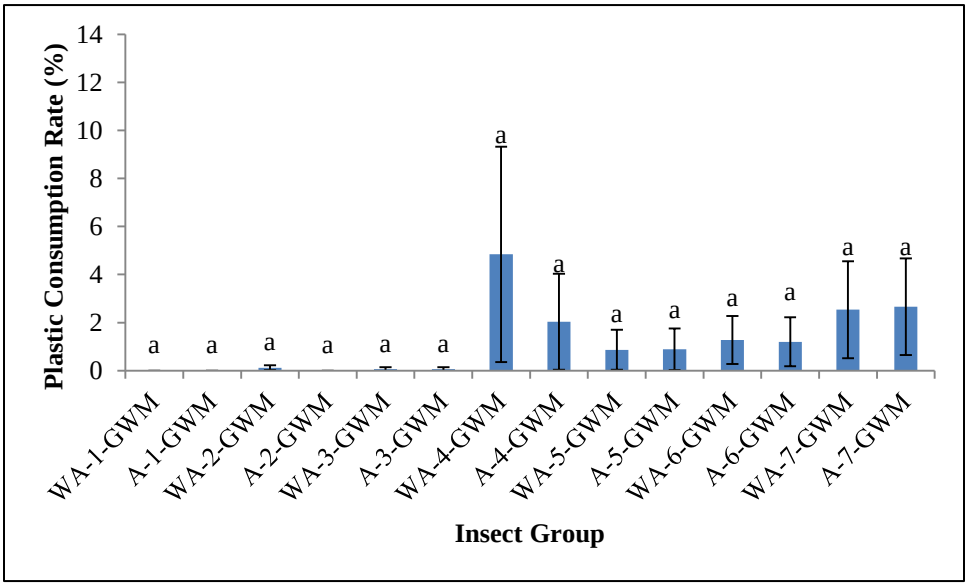


Figure 6.15 Plastic consumption rate of gut microbiota dependent and independent *G. mellonella* larvae. The graph represents plastic consumption rate of LDPE by *G. mellonella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). Note: WA-1-GWM= without antibiotic first instar group fed with naive LDPE film, A-1-GWM= antibiotic administered first instar group fed with naive LDPE film, WA-2-GWM= without antibiotic second instar group fed with naive LDPE film, A-2-GWM= antibiotic administered second instar group fed with naive LDPE film, WA-3-GWM= without antibiotic third instar group fed with naive LDPE film, A-3-GWM= antibiotic administered third instar group fed with naive LDPE film, WA-4-GWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive LDPE film, WA-5-GWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive LDPE film, WA-6-GWM= without antibiotic sixth instar group fed with naive LDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive LDPE film, WA-7-GWM= without antibiotic seventh instar group fed with naive LDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive LDPE film

Insects Survival Rate- The insect survival rate of the *G. mellonella* fed on the antibiotics and without antibiotics was recorded after the exposure of the wax worms to the LDPE film for two days. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate LDPE for *G. mellonella* is given by various groups- Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM,

Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 100 ± 0, 36.66 ± 15.53, 22.66 ± 3.05, 100 ± 0, 71.33 ± 17.47, 53.33 ± 11.01, 100 ± 0, 51.33 ± 12.05, 70.66 ± 22.03, 100 ± 0, 80 ± 13.11, 74.66 ± 11.01, 100 ± 0, 88.66 ± 10.26, 90 ± 12.49, 100 ± 0, 88 ± 7.21, 94.66 ± 5.03, 100 ± 0, 94.66 ± 2.3, 89.33 ± 3.05 %, respectively (Figure 6.16).

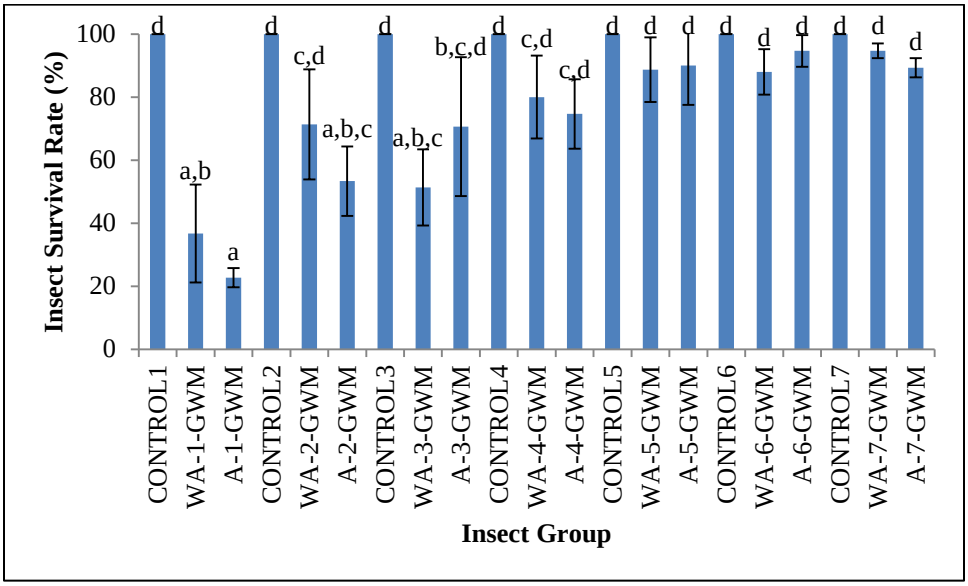


Figure 6.16 Insect survival rate when fed LDPE film. The graph represents with and without gut microbiota *G. mellonella* insects survived when fed on naive LDPE film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-d). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive LDPE film, A-1-GWM= antibiotic administered first instar group fed with naive LDPE film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic second instar group fed with naive LDPE film, A-2-GWM= antibiotic administered second instar group fed with naive LDPE film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive LDPE film, A-3-GWM= antibiotic administered third instar group fed with naive LDPE film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive LDPE film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive LDPE film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive LDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive LDPE film, CONTROL7= control group of seventh instar,WA-7-GWM= without antibiotic seventh instar group fed with naive LDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive LDPE film

SEM- Scanning electron microscopy of the leftover consumed LDPE film was performed for the larvae of greater wax moth. The SEM naive LDPE depicted clear surface without any structural modifications on the topography of the film (Figure 6.17). The minute

structural modifications and pits were observed in without antibiotic and with antibiotic first instar stage (Figure 6.18(i) A and B). A large pit was observed in LDPE film for without antibiotic second instar group (WA-2-GWM) (Figure 6.18(ii) C). As compared to WA-2-GWM group, the antibiotic fed second instar group depicted minute pits and roughness on the surface (Figure 6.18(ii) D). Some pits are present on the surface of the LDPE film insect consumed film by without antibiotic third instar group (WA-3-GWM) (Figure 6.18(ii) E). Some surface roughness is visible in the SEM images of with antibiotic group of third instar (A-3-GWM) (Figure 6.18(ii) F). Holes and pits are visible on the surface of the LDPE film consumed by without antibiotic fourth instar (WA-4-GWM) on the edges of the polymer film (Figure 6.18(ii) G). Large holes, pits are visible on the center and edges of the LDPE film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-GWM) (Figure 6.18(ii) H). Large hole is visible in SEM image for without antibiotic fifth instar (WA-5-GWM) (Figure 6.18(iii) I). In the antibiotic fed fifth instar group (A-5-GWM) disintegration of plastics is visible at the edges of the film in the SEM images (Figure 6.18(iii) J). For the without antibiotic sixth instar larval group (WA-6-GWM) the disintegration of polymers are visible on the edges (Figure 6.18(iii) K). The SEM images of the antibiotic administered sixth instar larval group (A-6-GWM) revealed disintegration of plastics on the edges of the film by the larval instars (Figure 6.18(iii) L). In the SEM images for seventh instar without antibiotic group (WA-7-GWM) the disintegration of plastics is visible on the edges (Figure 6.18(iii) M). The SEM images for antibiotic administered seventh instar (A-7-GWM) revealed consumed remains of plastics on the edges of the film (Figure 6.18(iii) N).

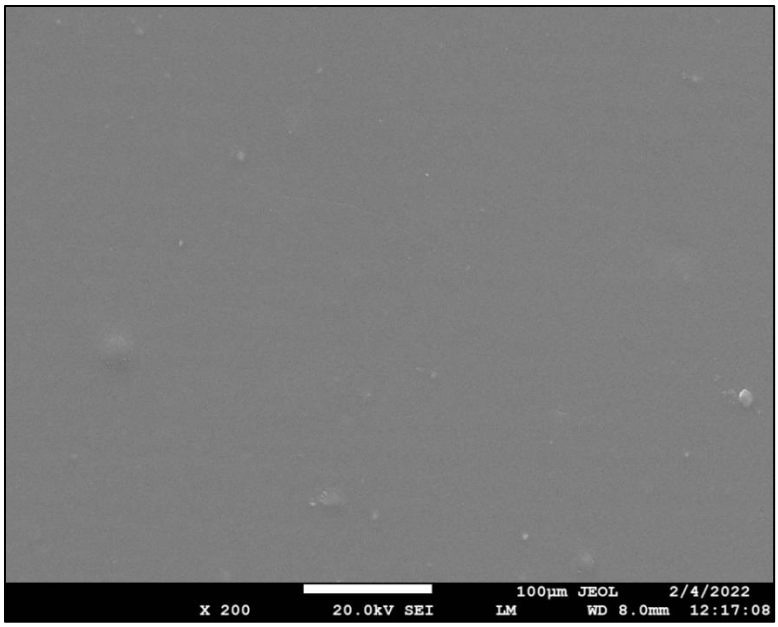


Figure 6.17 Scanning electron microscopic image of naive LDPE film

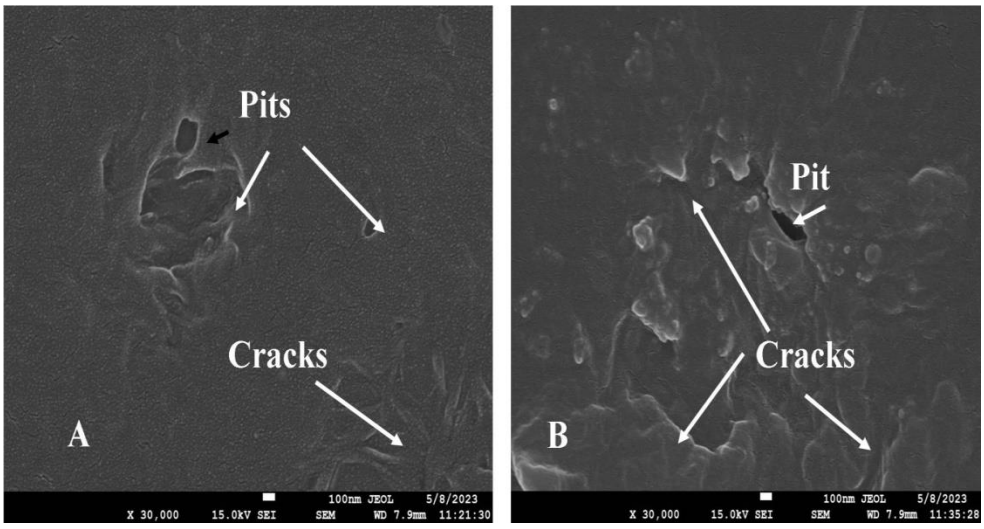


Figure 6.18 (i)

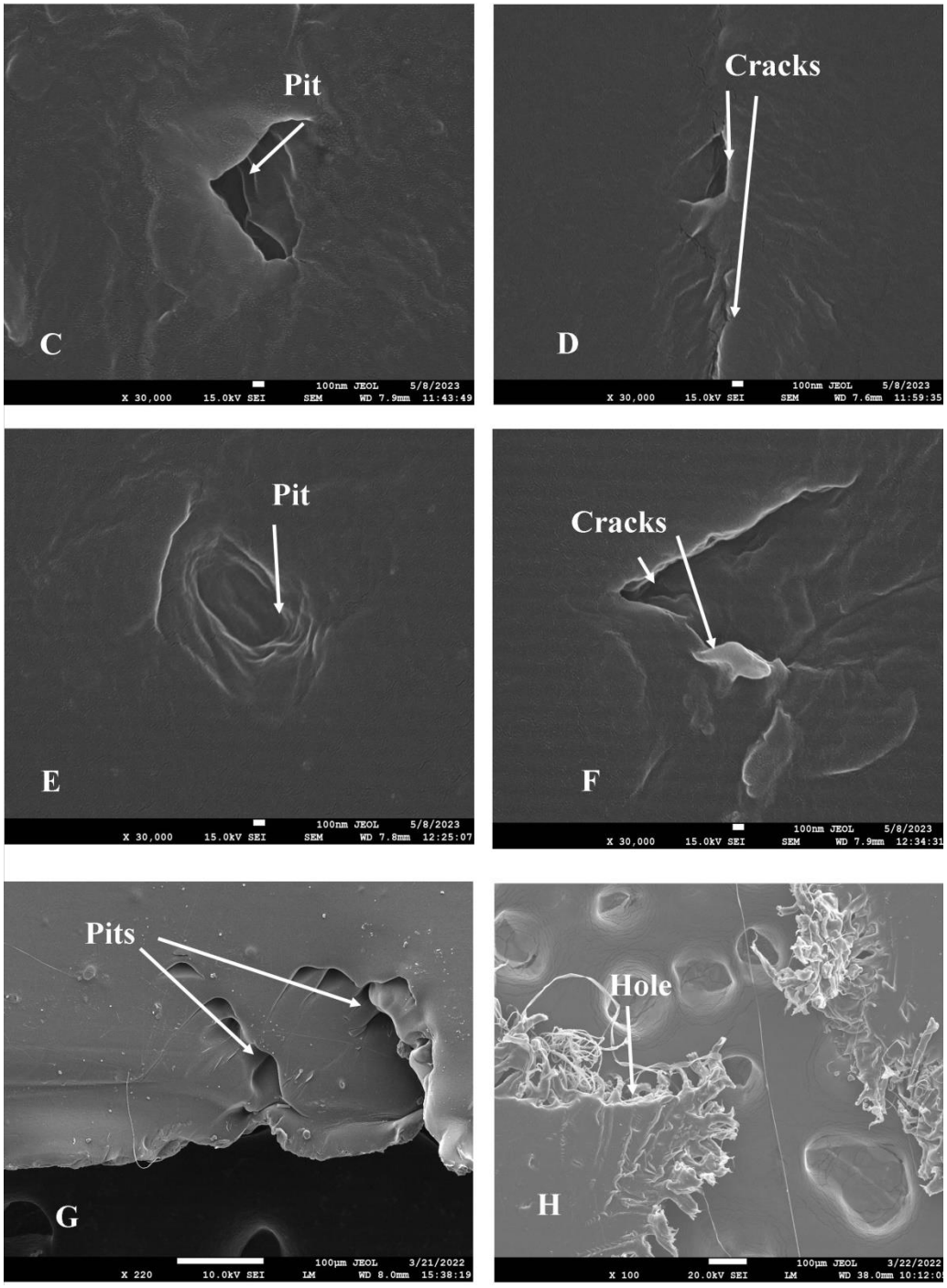


Figure 6.18 (ii)

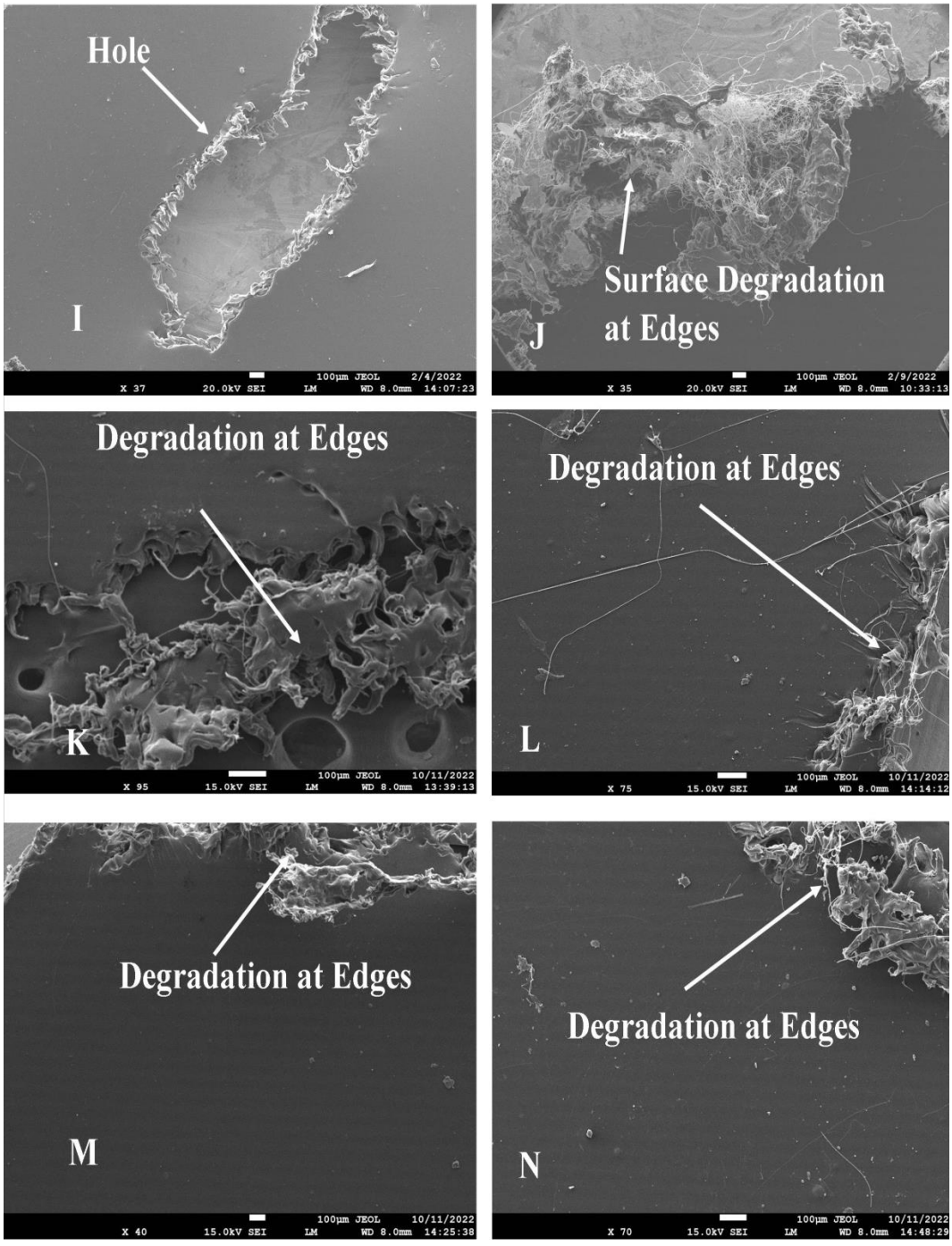


Figure 6.18 (iii)

Figure 6.18 Scanning electron microscopic image of leftover remains LDPE film consumed by *G. mellonella.* Figure (i) (A) LDPE film consumed by without antibiotic group of first instar (WA-1-GWM); (B) LDPE film consumed by antibiotic fed group of first instar (A-1-GWM); Figure (ii) (C) remains of

LDPE film consumed by without antibiotic group of second instar (WA-2-GWM); (D) LDPE film consumed by antibiotic administered group of second instar (A-2-GWM); (E) remains of LDPE film consumed by without antibiotic group of third instar (WA-3-GWM); (F) LDPE film consumed by antibiotic fed group of third instar (A-3-GWM); (G) LDPE film consumed by without antibiotic group of fourth instar (WA-4-GWM); (H) remains of LDPE film consumed by with antibiotic group of fourth instar (A-4-GWM); Figure (iii) (I) LDPE film consumed by without antibiotic group of fifth instar (WA-5-GWM); (J) remains of LDPE film consumed by with antibiotic group of fifth instar (A-5-GWM); (K) LDPE film remains of polymers fed by sixth instar of without antibiotic group (WA-6-GWM); (L) LDPE film consumed by antibiotic administered group of sixth instar (A-6-GWM); (M) LDPE film consumed by without antibiotic group of seventh instar (WA-7-GWM); (N) LDPE film remains of LDPE film consumed by antibiotic fed insects of seventh instar (A-7-GWM)

GC-MS- The Gas chromatography and mass spectroscopy was performed for the frass sample of the wax moth larvae in order to ensure the plastics ingested by the caterpillar are digested or biodegraded by the larvae into small degradable compounds.

In Control-LDPE, showed compounds Octane (5.546 minutes- retention time), Octane, 4-methyl- (7.094), Octane, 2-methyl- (7.136), Octane, 3-methyl- (7.284), Nonane (7.936), 2,2'-Bifuran, octahydro- (12.037), Heptadecane (18.825), Heneicosane (21.087), Hexadecanamide (24.850), Eicosane (25.881), Tetrapentacontane (26.495), Heneicosane (28.322), 6,6-Diethylhoctadecane (28.952) (Figure 6.19(i) A).

For WA-1-GWM group, 3-Hexanone (5.254), 2-Hexanone (5.375), 3-Hexanol (5.606), 2-Hexanol (5.685), Butanoic acid, 4-hydroxy (8.175), Spiro[2.4]heptane, 1,2,4,5-tetramethyl-6-mete- (17.999), Nonadecane (18.827), Hexadecane (21.088), Sulfurous acid, 2-pentyl tetradecyl ester (21.155), Heneicosane (23.137), 7-Hexadecenal, (Z)- (24.098), Heneicosane (25.010), Dotriacontane (29.200) (Figure 6.19(ii) B).

Figure 6.19(ii) C shows the compounds 3-Hexanol (5.565), 2-Pentanol, 4-methyl- (5.672), 3-Hexanol, 3-ethyl- (5.755), Butanoic acid, 4-hydroxy (8.194), 2-Butyl-2,7-octadien-1-ol (13.797), 1,3-Pentadiene, 2,4-di-t-butyl (17.113), 2,4-Pentanedione, 3,3-di-2-butenyl (17.643), 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate (18.309), Hexadecane (18.830), Butanoic acid, 2-propenyl ester (20.449), Heneicosane (21.088), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.117), Octadecane (23.150), Octane, 2,6,6-trimethyl- (25.012) for A-1-GWM group.

For WA-2-GWM group depicted compounds- 3-Hexanone (5.265), 2-Hexanone (5.355), 3-Hexanol (5.599), 2-Pentanol, 4-methyl- (5.698), Sulfurous acid, butyl undecyl ester (16.680), 5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)- (16.843), Acetate, 4-(1,1-dimethylethyl)-1-methyl-4-penten-2-ynyl ester (16.990), 2,4-Pentanedione, 3,3-di-2-butenyl (17.509), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.998), Nonadecane (18.826), Hexadecane (21.088), Propanoic acid, 2-methyl-, 2-propenyl ester (22.112), Heptadecane (25.007), Dotriacontane (29.199) (Figure 6.19(ii) D).

In A-2-GWM group revealed 3-Hexanone (5.250), 2-Hexanone (5.374), 3-Hexanol (5.575), 2-Hexanol (5.680), 1,6-Heptadien-4-ol (11.838), 2-Undecyne (12.606), 5-Methyl-2-isopropyl-2-hexen-1-al (12.967), 4-Hexen-3-ol, 2-methyl (13.085), 4-Hexen-3-ol, 2-methyl (13.200), Decenyl tiglate, 4E- (13.613), 2-Octenal, 2-butyl (13.793), 6-Methyl-hept-2-en-4-ol (15.799), 2,2,7-Trimethyloctane-3,5-dione (17.047), 1,3-Pentadiene, 2,4-di-t-butyl (17.113), 5-Methyl-Z-5-docosene (19.915), Heptadecane (19.992), 2-Oxo-n-valeric acid (20.450), Heneicosane (21.085), Sulfurous acid, pentyl tetradecyl ester (21.155), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.120), Sulfurous acid, 2-propyl tridecyl ester (22.545), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.772), 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl (22.873), Hexadecane (23.155), 13-Methylpentadec-14-ene-1,13-diol (23.420), Succinic acid, tridec-2-yn-1-yl 3-methylbut-2-yl ester (23.590), Butyric acid, 2,2-dimethyl-, vinyl ester (25.016), Butanoic acid, 2-propenyl ester (25.108), Methyl hexadec-9-enoate (28.005) compounds for GC-MS of frass (Figure 6.19(ii) E).

For WA-3-GWM group chemical peaks for 3-Hexanone (5.270), 2-Hexanone (5.367), 3-Hexanol (5.594), 2-Hexanol (5.693), Butanoic acid, 4-hydroxy (8.191), Propanoic acid, 2-methyl-, anhydride (12.906), 5-Methyl-2-isopropyl-2-hexen-1-al (12.963), 4-Hexen-3-ol, 2-methyl (13.071), 2-Octenal, 2-butyl (13.788), 6-Methyl-3,5-heptadiene-2-one (14.967), Decyl pentyl ether (15.275), 6-Methyl-hept-2-en-4-ol (15.791), 1,3-Pentadiene, 2,4-di-t-butyl (17.112), 9-Octadecene, 1,1-dimethoxy-, (Z)- (19.750), Oleyl alcohol, trifluoroacetate (19.909), Propanoic acid, 2-methyl-, 2-propenyl ester (20.231), 2,6,10-

Trimethyltridecane (21.080), Octane, 2,6,6-trimethyl (21.152), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.750), Butanoic acid, tridec-2-ynyl ester (22.877), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (25.107), Tetrapentacontane (26.049), 1,5-Heptadiene-3,4-diol (26.346), Butyric acid, 2,2-dimethyl-, vinyl ester (26.745), Dotriacontane (28.163) were present in the frass of *G. mellonella* larvae (Figure 6.19(ii) F).

In A-3-GWM group GC-MS revealed chemical peaks for 3-Hexanone (5.267), 2-Hexanone (5.377), 2-Hexanol (5.700), Butanoic acid, 4-hydroxy- (8.185), 1,6-Heptadien-4-ol (11.836), 2-Hepten-3-ol, 4,5-dimethyl (11.984), Butyric acid, neopentyl ester (12.054), 1,6-Heptadien-4-ol (12.125), 2-Undecyne (12.604), Propanoic acid, 2-methyl-, anhydride (12.907), 5-Methyl-2-isopropyl-2-hexen-1-al (12.965), 4-Hexen-3-ol, 2-methyl (13.199), Decenyl tiglate, 4E- (13.611), 2-Octenal, 2-butyl (13.790), 6-Methyl-3,5-heptadiene-2-one (14.973), 6-Methyl-hept-2-en-4-ol (15.795), 2,5-Heptanedione, 3,3,6-trimethyl- (17.045), Butanoic acid, anhydride (19.709), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.756), Heptacos-1-ene (19.910), Octacosyl pentyl ether (20.014), 2-Oxo-n-valeric acid (20.450), Heneicosane (21.085), Sulfurous acid, pentyl tridecyl ester (21.154), Hexadecanal (21.293), Propanoic acid, 2-methyl-, 2-propenyl ester (22.119), Butanoic acid, tridec-2-ynyl ester (22.751), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.410), Butanoic acid, tridec-2-ynyl ester (23.583), 1-Decanol, 2-hexyl (24.098), Butyric acid, 2,2-dimethyl-, vinyl ester (25.013) that were present in the excreta of the waxworms (Figure 6.19(ii)G).

The GC-MS graph for WA-4-GWM group showed peaks for 3-Hexanol (5.593), 2-Hexanol (5.700), Hydroperoxide, 1-ethylbutyl (8.948), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.966), 2-Octenal, 2-butyl (13.792), 2,4,4-Trimethyl-1-hexene (15.277), 1,3-Pentadiene, 2,4-di-t-butyl- (17.112), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.754), Ethylene glycol di-n-butyrate (19.872), Octadecane (19.985), Propanoic acid, 2-methyl-, 2-propenyl ester (20.232), Isophytol (21.150), Butanoic acid, 2-propenyl ester (22.119), Butanoic acid, tridec-2-ynyl ester (22.642), Butanoic acid, 2,6-

dimethylnon-1-en-3-yn-5-yl ester (22.754), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl (23.415), 17-Pentatriacontene (24.102), 11-Tetradecyn-1-ol (26.010), Butyric acid, 2,2-dimethyl-, vinyl ester (26.346), Propanoic acid, 2-methyl-, 2-propenyl ester (26.824), Dotriacontane (28.163), Tetrapentacontane (29.202) (Figure 6.19(iii)H).

For A-4-GWM group chemical peaks are- 3-Hexanol (5.598), 2-Hexanol (5.705), Butanoic acid, 4-hydroxy- (8.159), Acetic acid, 4-t-butyl-4-hydroxy-1,5-dimethyl-hex-2-enyl ester (13.954), 1,3-Pentadiene, 2,4-di-t-butyl (17.115), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.509), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.999), Nonadecane (18.827), Heneicosane (21.090), Isophytol (21.155), Heptadecane (23.140) that were detected in the GC-MS for frass of greater wax worm larvae (Figure 6.19(iii)I).

The GC-MS graph for WA-5-GWM group revealed peaks 3-Hexanol (5.597), 2-Hexanol (5.689), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.965), 4-Hexen-3-ol, 2-methyl- (13.072), Decyl pentyl ether (15.284), Decyl pentyl ether (15.530), 3,7-Dimethyl-4,6-nonandione (16.936), 1,3-Pentadiene, 2,4-di-t-butyl- (17.113),Decane, 2,3,4-trimethyl- (18.882), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.756), Butanoic acid, anhydride (19.871), Butyric acid, 2,2-dimethyl-, vinyl ester (20.232), 2-Oxo-n-valeric acid (20.450), Tridecanol, 2-ethyl-2-methyl (21.085), Octane, 2,6,6-trimethyl (21.148), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.118), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.554), Butanoic acid, tridec-2-ynyl ester (22.642), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.750), 1,6-Heptadien-4-ol, 4-propyl (23.670), 15-Hexenoic acid, 14-hydroxy-14-methyl (23.853), 6-Octadecenoic acid, methyl ester, (Z)- (24.099), Propanoic acid, 2-methyl-, 2-propenyl ester (26.346), Fumaric acid, hexadecyl octyl ester (26.436), Propanoic acid, 2-methyl-, 2-propenyl ester (26.744), Propanoic acid, 2-methyl-, 2-propenyl ester (26.820), Dotriacontane (28.161), Oxalic acid, hexyl pentadecyl ester (28.322), Tetrapentacontane (29.199) compounds in the excreta of larvae of *G. mellonella* (Figure 6.19(iii) J).

In A-5-GWM the GC-MS graph revealed chemical peaks for 3-Hexanone (5.287), Methyl Isobutyl Ketone (5.378), 3-Hexanol (5.594), 2-Hexanol (5.685), 3,5-Octadiene,

2,2,4,5,7,7-hexamethyl-, (E,Z)- (16.992), 1,3-Pentadiene, 2,4-di-t-butyl- (17.116), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.510), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.999), Heptadecane (18.827), Hexadecane (21.089), Isophytol (21.155), Propanoic acid, 2-methyl-, 2-propenyl ester (22.114), Heneicosane (23.139) compounds in the frass of the larvae (Figure 6.19(iii) K).

For WA-6-GWM, the GC-MS data obtained revealed the peaks for compounds named as Heptane (5.287), 2-Hexanone (5.352), 3-Hexanol (5.599), 2-Pentanol, 4-methyl- (5.679), Hydroperoxide, 1-ethylbutyl (8.947), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.964), 4-Hexen-3-ol, 2-methyl- (13.196), 2-Octenal, 2-butyl- (13.789), 6-Methyl-3,5-heptadiene-2-one (14.974), Decyl pentyl ether (15.272), 1,3-Pentadiene, 2,4-di-t-butyl- (17.111), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.753), Heptacos-1-ene (19.908), Phytol (20.012), Butyric acid, 2,2-dimethyl-, vinyl ester (20.232), 2-Oxo-n-valeric acid (20.448), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.118), Hexadecanoic acid, methyl ester (22.384), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.750), Butanoic acid, tridec-2-ynyl ester (22.877), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl (23.065), Butanoic acid, 2-methyloct-5-yn-4-yl ester (23.416), Butanoic acid, tridec-2-ynyl ester (23.587), 11,14-Octadecadienoic acid, methyl ester (24.041), 9-Octadecenoic acid, methyl ester, (E)- (24.097), 2-Nonen-4-one (24.496), Ethyl Oleate (24.708), 11-Eicosenoic acid, methyl ester (25.879), 1,5-Heptadiene-3,4-diol (26.354), Propanoic acid, 2-methyl-, 2-propenyl ester (26.746), Tetracontane (27.380), 2-Ethylbutyric acid, eicosyl ester (27.460), 11-Methyltricosane (27.538), Dotriacontane (28.163), Nonacosane (29.199) (Figure 6.19(iii) L).

In A-6-GWM, the GC-MS graph shows peaks for chemicals- 3-Hexanone (5.277), 2-Hexanone (5.375), 3-Hexanol (5.580), 2-Hexanol (5.677), Pentanoic acid, 2-propenyl ester (8.955), 2-Octenal, 2-butyl- (13.795), 2,4,4-Trimethyl-1-hexene (15.306), 6-Methyl-hept-2-en-4-ol (17.046), 2,5-Heptanedione, 3,3,6-trimethyl (17.113), 1,3-Pentadiene, 2,4-di-t-butyl (19.757), Ethylene glycol di-n-butyrate (19.870), 1-Dodecanol (19.915), Heneicosane (19.991), 2-Dodecyloxyethanol acetate (ester) (20.310), Butanoic acid, 2-

propenyl ester (20.451), Dodecane, 4-methyl- (21.092), 14-Octadecenal (21.481), Propanoic acid, 2-methyl-, 2-propenyl ester (22.119), Hexadecanoic acid, methyl ester (22.390), Butanoic acid, tridec-2-ynyl ester (22.554), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.642), Phytol (23.515), Butanoic acid, tridec-2-ynyl ester (23.585), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (24.042), 9-Octadecenoic acid, methyl ester, (E)- (24.100), Butyric acid, 2,2-dimethyl-, vinyl ester (25.014), Nonyl tetradecyl ether (25.881), 2,6-Octadiene-4,5-diol (26.662), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (26.746), Dotriacontane (28.166) compounds were detected in the frass of wax moth (Figure 6.19(iii) M).

For WA-7-GWM, the GC-MS data reveals 3-Hexanone (5.266), 2-Hexanone (5.383), 3-Hexanol (5.595), 2-Hexanol (5.688), Hydroperoxide, 1-ethylbutyl (8.949), Hydroperoxide, 1-methylpenty (9.142), Acetate, 4-(1,1-dimethylethyl)-1-methyl-4-penten-2-ynyl ester (16.985), 1,3-Pentadiene, 2,4-di-t-butyl (17.114), Butanol, 1-[2,2,3,3-tetramethyl-1-(3-methyl-1-penynyl)cyclopropyl]- (17.508), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.998), Heptadecane (18.826), Heneicosane (21.089), Propanoic acid, 2-methyl-, anhydride (21.155), Hexadecanoic acid, methyl ester (22.113), Heneicosane (22.387), 9-Octadecenoic acid, methyl ester, (E)- (23.138), 2-Nonen-4-one (24.497), Ethyl Oleate (24.710), Tetrapentacontane (27.382), 2-Methyl-Z,Z-3,13-octadecadienol (27.998), Dotriacontane (28.164) (Figure 6.19(iii)N).

In A-7-GWM, the GC-MS graph reveals 2-Hexanone (5.365), 3-Hexanol (5.591), 2-Pentanol, 4-methyl- (5.692), 2-Undecyne (12.608), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.965), 4-Hexen-3-ol, 2-methyl- (13.200), 2-Octenal, 2-butyl- (13.790), Hepta-2,4-dienoic acid, methyl ester (14.972), Decyl pentyl ether (15.278), Decyl pentyl ether (15.522), 6-Methyl-hept-2-en-4-ol (15.794), 2,5-Heptanedione, 3,3,6-trimethyl- (16.943), 1,3-Pentadiene, 2,4-di-t-butyl- (17.112), 2,4-Pentanedione, 3,3-di-2-butenyl (17.503), Butanoic acid, anhydride (19.709), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.753), Propanoic acid, 2-methyl-, anhydride (19.870), n-Pentadecanol (19.909), Butanoic acid, anhydride (20.098), Propanoic acid, 2-methyl-, 2-propenyl ester (20.233),

Sulfurous acid, decyl pentyl ester (20.315), Butanoic acid, 2-propenyl ester (20.450), Dodecane, 2,6,10-trimethyl (21.085), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.119), Hexadecanoic acid, methyl ester (22.386), Butanoic acid, tridec-2-ynyl ester (22.552), Butanoic acid, tridec-2-ynyl ester (22.641), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.749), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (23.585), Butanoic acid, tridec-2-ynyl ester (23.669), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (24.030), 9-Octadecenoic acid, methyl ester, (E)- (24.097), 2-Nonen-4-one (24.499), Ethyl Oleate (24.708), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- (24.964), [Butyric acid, 2,2-dimethyl-, vinyl ester (25.012), Nonyl tetracosyl ether (25.881), Propanoic acid, 2-methyl-, 2-propenyl ester (26.345), Butanoic acid, 2-propenyl ester (26.744), Octadecane, 5-methyl- (27.378), Dotriacontane (28.162) compounds were detected in the frass of the *G. mellonella* (Figure 6.19(iii)O).

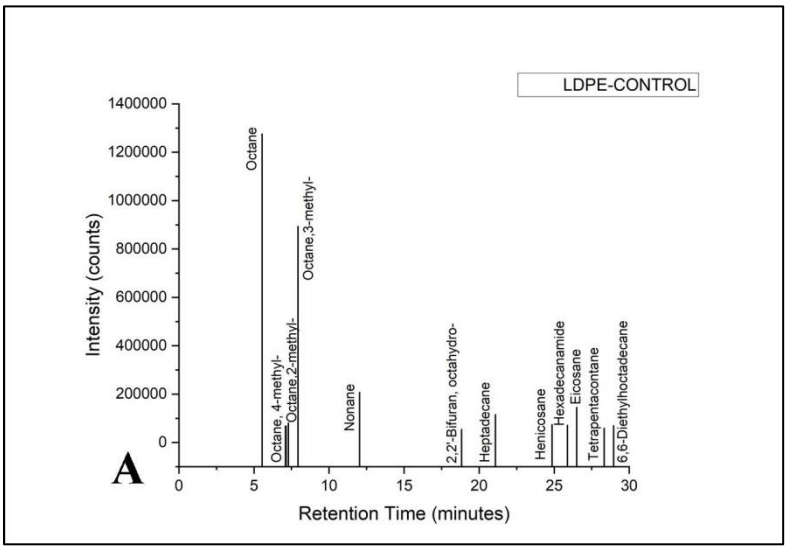


Figure 6.19(i)

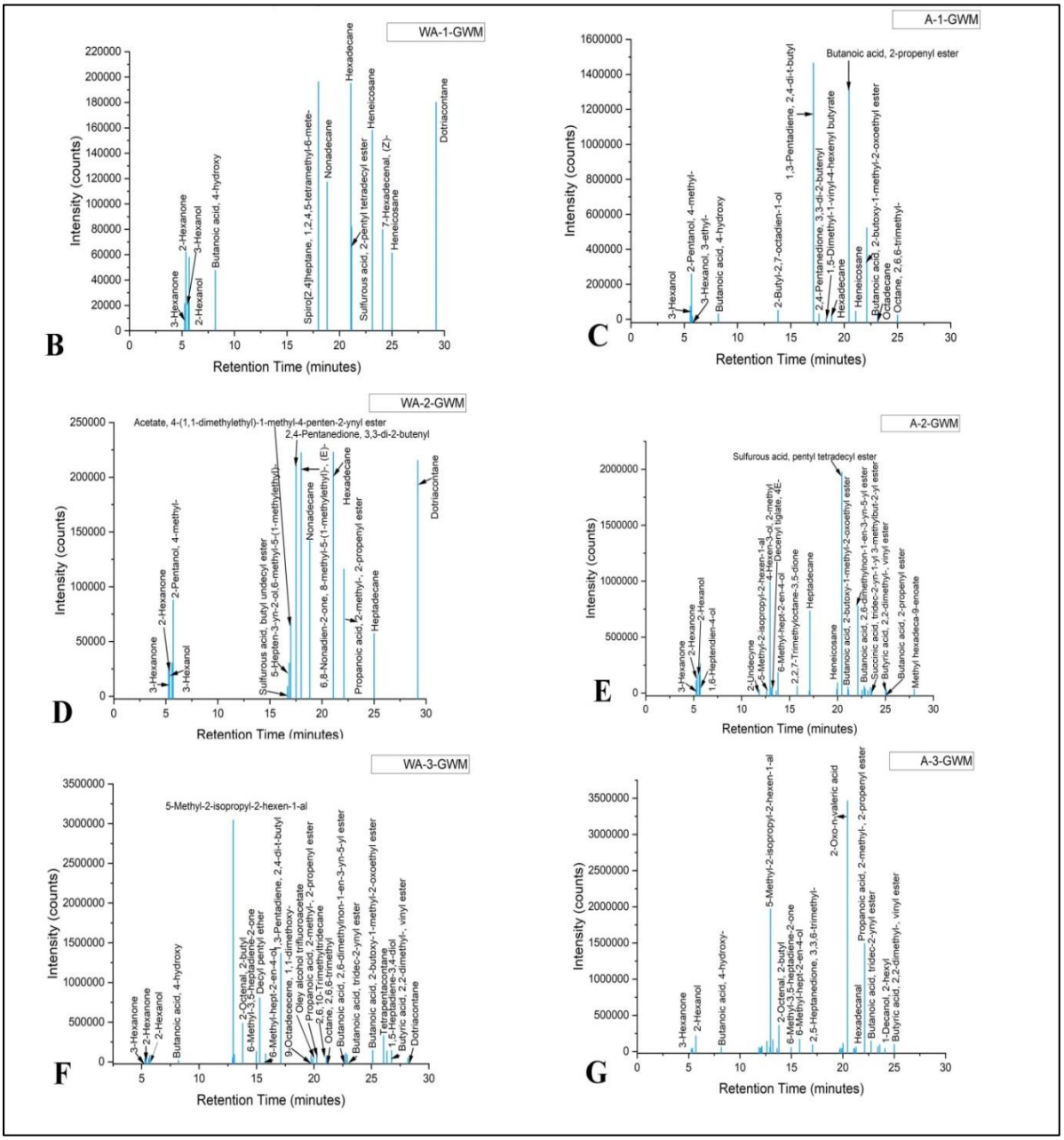


Figure 6.19(ii)

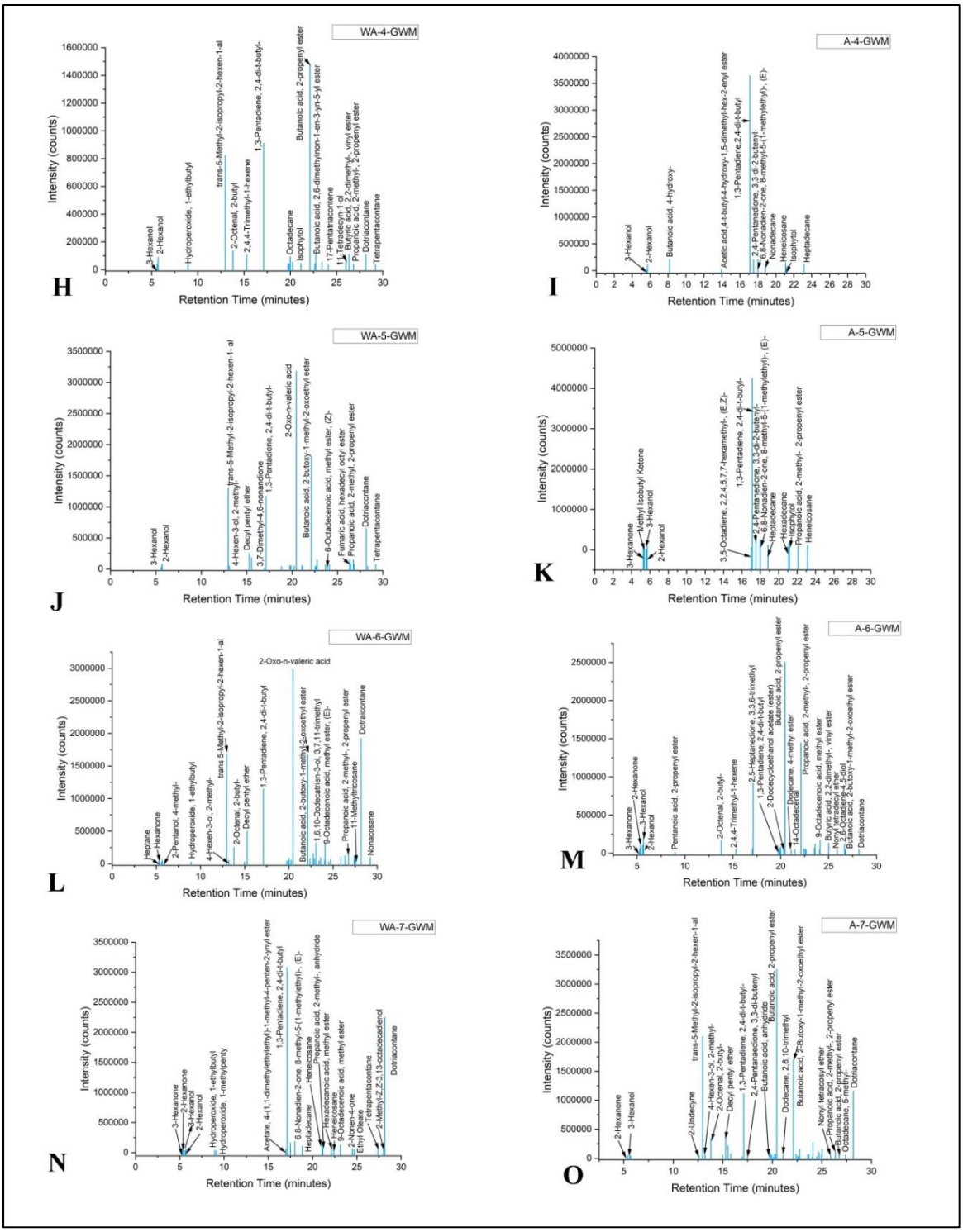


Figure 6.19(iii)

Figure 6.19 Gas chromatography and mass spectroscopy analysis for frass of *G. mellonella* fed with LDPE. Figure (i) (A) GC-MS graph for retention time and intensity for LDPE film (Control-LDPE); Figure (ii) (B) GC-MS graph for frass of without antibiotic group for first instar fed with LDPE (WA-1-GWM); (C)The graph for frass of antibiotic fed first instar group (A-1-GWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-GWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-GWM) ; (F) The GC-MS graph of frass for third instar without antibiotic group fed with LDPE (WA-3-GWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-GWM). Figure (iii) (H) GC-MS graph of without antibiotic fourth instar group (WA-4-GWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-GWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-GWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-GWM); (L) The GC-MS graph of retention time and intensity for frass of without antibiotic sixth instar group (WA-6-GWM); (M) GC-MS graph for frass of antibiotic administered sixth instar group (A-6-GWM); (N) The GC-MS graph for frass without antibiotic seventh instar group (WA-7-GWM); (O) The GC-MS graph of retention time and intensity for frass of antibiotic fed of seventh instar group (A-7-GWM)

High-Density Polyethylene (HDPE) - For duration of two days, the naive HDPE films were exposed to a sample of fifty larval instars from each group of greater wax moth larvae, as well as the control population. After the completion of the experiment the plastic consumption and survival rate of the larvae, the SEM of the exposed HDPE film and GC-MS of the frass of the greater wax moth was recorded.

Plastic Consumption Rate- After the insects were exposed to HDPE film for two days, the rate of plastic ingestion by *G. mellonella* larvae (both with and without antibiotics) fed on naive plastic film was recorded. One-way ANOVA followed by Tukey's significant difference test was used to determine the data's significance at a one-tailed significance level of 0.05. The plastic consumption rate for HDPE for *G. mellonella* by various groups- WA-1-GWM, A-1-GWM, WA-2-GWM, A-2-GWM, WA-3-GWM, A-3-GWM, WA-4-GWM, A-4-GWM, WA-5-GWM, A-5-GWM, WA-6-GWM, A-6-GWM, WA-7-GWM and A-7-GWM is 0.16 ± 0.13, 0.09 ± 0.17, 0.25 ± 0.25, 0.08 ± 0.14, 0.04 ± 0.12, 0.43 ± 0.26, 1.49 ± 0.93, 1.18 ± 0.53, 1.78 ± 1.32, 0.58 ± 0.33, 3.86 ± 2.16, 3.75 ± 2.07, 7.01 ± 4.93, 5.08 ± 1.3 %, respectively (Figure 6.20).

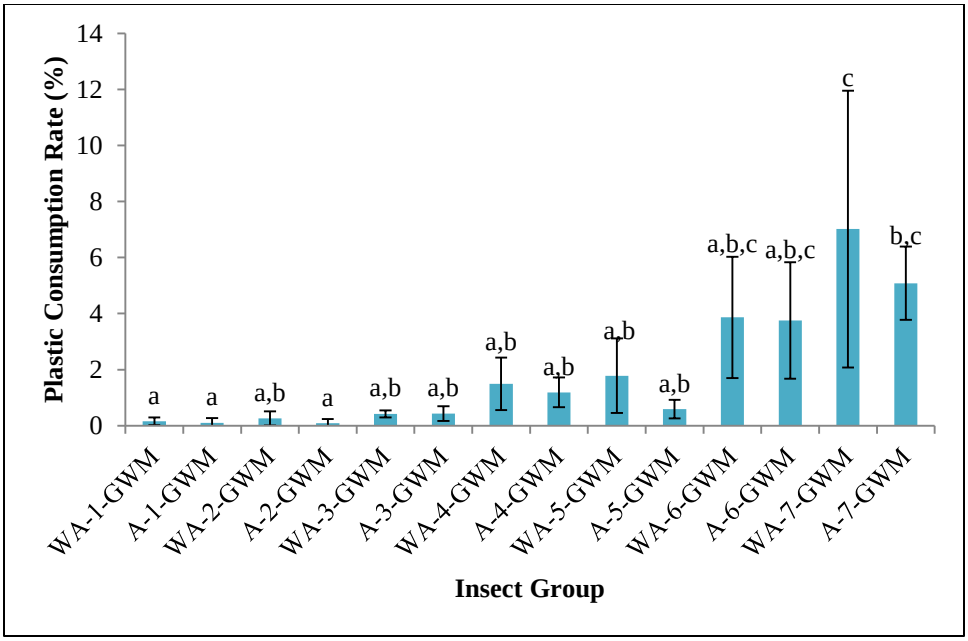


Figure 6.20 Plastic consumption rate of gut microbiota dependent and independent *G. mellonella* larvae. The graph represents plastic consumption rate of HDPE by *G. mellonella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-c). Note: WA-1-GWM= without antibiotic first instar group fed with naive HDPE film, A-1-GWM= antibiotic administered first instar group fed with naive HDPE film, WA-2-GWM= without antibiotic second instar group fed with naive HDPE film, A-2-GWM= antibiotic administered second instar group fed with naive HDPE film, WA-3-GWM= without antibiotic third instar group fed with naive HDPE film, A-3-GWM= antibiotic administered third instar group fed with naive HDPE film, WA-4-GWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive HDPE film, WA-5-GWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive HDPE film, WA-6-GWM= without antibiotic sixth instar group fed with naive HDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive HDPE film, WA-7-GWM= without antibiotic seventh instar group fed with naive HDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive HDPE film

Insects Survival Rate- The *G. mellonella* larva insect survival rate was measured 48 hours after the wax worms were exposed to the HDPE film. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive HDPE for *G. mellonella* is given by various groups- Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM,

A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 100 ± 0, 36.66 ± 23.69, 26 ± 22.53, 100 ± 0, 47.33 ± 24.84, 49.33 ± 17.009, 100 ± 0, 83.33 ± 8.08, 68 ± 8.71, 100 ± 0, 92.66 ± 6.42, 92 ± 2, 100 ± 0, 94 ± 7.21, 84 ± 10.58, 100 ± 0, 91.33 ± 6.11, 92.66 ± 8.08, 100 ± 0, 98 ± 3.46, 91.33 ± 6.11 %, respectively (Figure 6.21).

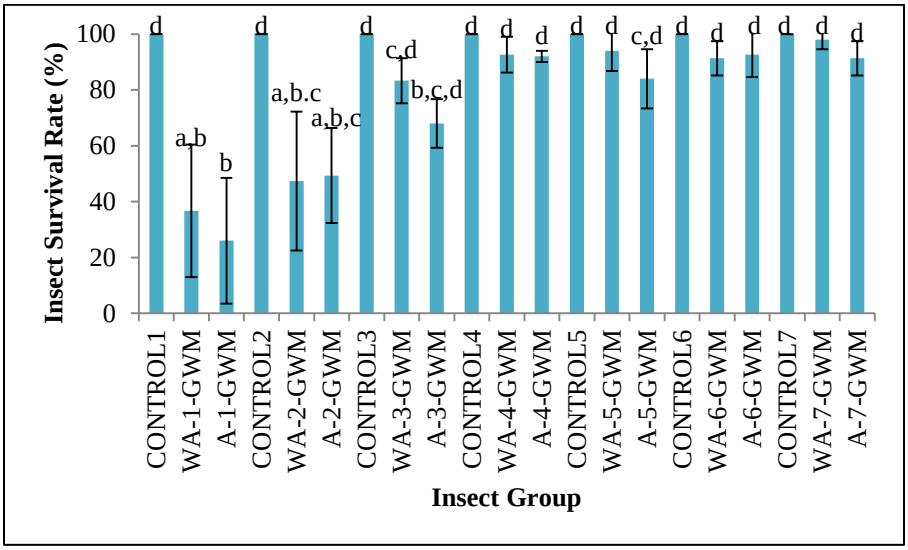


Figure 6.21 Insect survival rate when fed HDPE film. The graph represents with and without gut microbiota *G. mellonella* insects survived when fed on naive HDPE film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-d). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive HDPE film, A-1-GWM= antibiotic administered first instar group fed with naive HDPE film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic second instar group fed with naive HDPE film, A-2-GWM= antibiotic administered second instar group fed with naive HDPE film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive HDPE film, A-3-GWM= antibiotic administered third instar group fed with naive HDPE film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive HDPE film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive HDPE film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive HDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive HDPE film, CONTROL7= control group of seventh instar,WA-7-GWM= without antibiotic seventh instar group fed with naive HDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive HDPE film

SEM- Scanning electron microscopy of naive and consumed HDPE film was performed to visualise and compare the extent of biodegradation of the plastics by greater wax

worms. The SEM naive HDPE depicted clear surface without any structural modifications on the topography of the film (Figure 6.22). The minute structural modifications, ridges and pits were observed in without antibiotic first instar stage (WA-1-GWM) (Figure 6.23(i) A). On the surface topography of with antibiotic first instar stage (A-1-GWM) a few ridges are visible in the SEM images (Figure 6.23(i) B). A pit was observed in HDPE film for without antibiotic second instar group (WA-2-GWM) (Figure 6.23(ii) C). As compared to WA-2-GWM group, the antibiotic fed second instar group depicted minute roughness on the surface (Figure 6.23(ii) D). Some large holes are present on the surface of the HDPE film insect consumed film by without antibiotic third instar group (WA-3-GWM) (Figure 6.23(ii) E). Some large holes are visible in the SEM images of with antibiotic group of third instar (A-3-GWM) (Figure 6.23(ii) F). Holes and pits are visible on the surface of the HDPE film consumed by without antibiotic fourth instar (WA-4-GWM) on the edges of the polymer film (Figure 6.23(ii) G). Large holes are visible on the center and edges of the HDPE film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-GWM) (Figure 6.23(ii) H). Large hole is visible on the edges of HDPE film in SEM image for without antibiotic fifth instar (WA-5-GWM) (Figure 6.23(iii) I). In the antibiotic fed fifth instar group (A-5-GWM) disintegration of plastics is visible at the edges of the film in the SEM images (Figure 6.23(iii) J). For the without antibiotic sixth instar larval group (WA-6-GWM) the disintegration of polymers are visible in the form of holes in the HDPE film (Figure 6.23(iii) K). The SEM images of the antibiotic administered sixth instar larval group (A-6-GWM) revealed disintegration of plastics on the in the film as well as on the edges of the film by the larval instars (Figure 6.19(iii) L). In the SEM images for seventh instar without antibiotic group (WA-7-GWM) the disintegration of plastics is visible on the edges of the HDPE film (Figure 6.23(iii) M). The SEM images for antibiotic administered seventh instar (A-7-GWM) revealed consumed remains of plastics on the edges of the plastic film (Figure 6.23(iii) N).

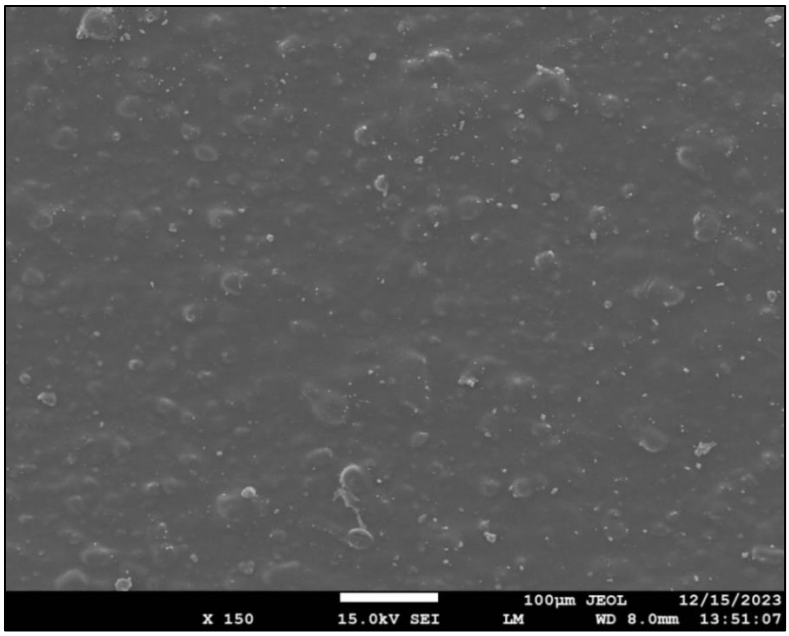


Figure 6.22 Scanning electron microscopic image of naive HDPE film

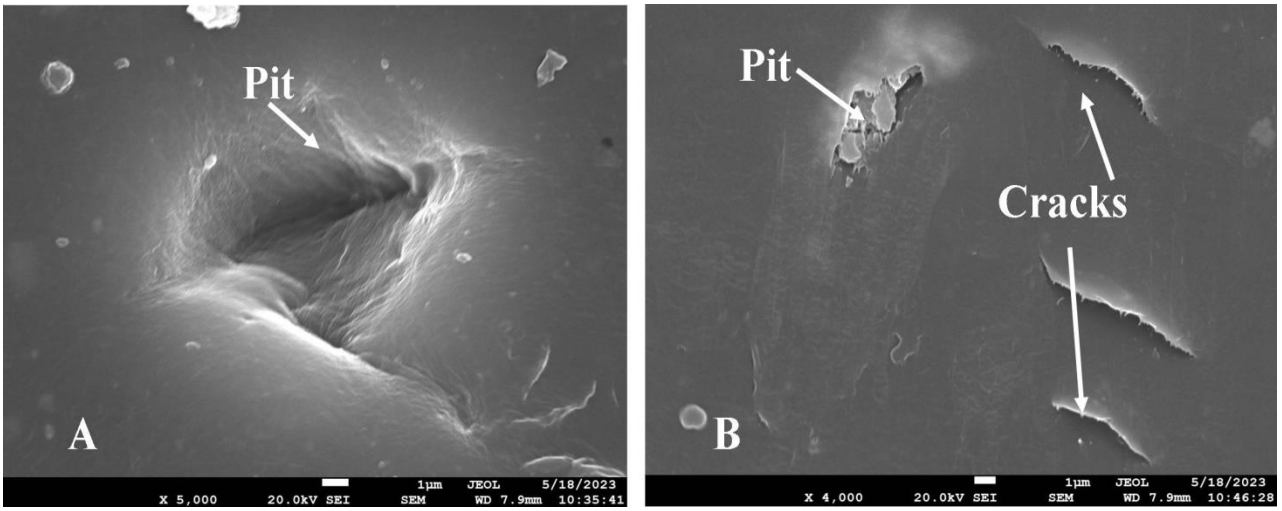


Figure 6.23(i)

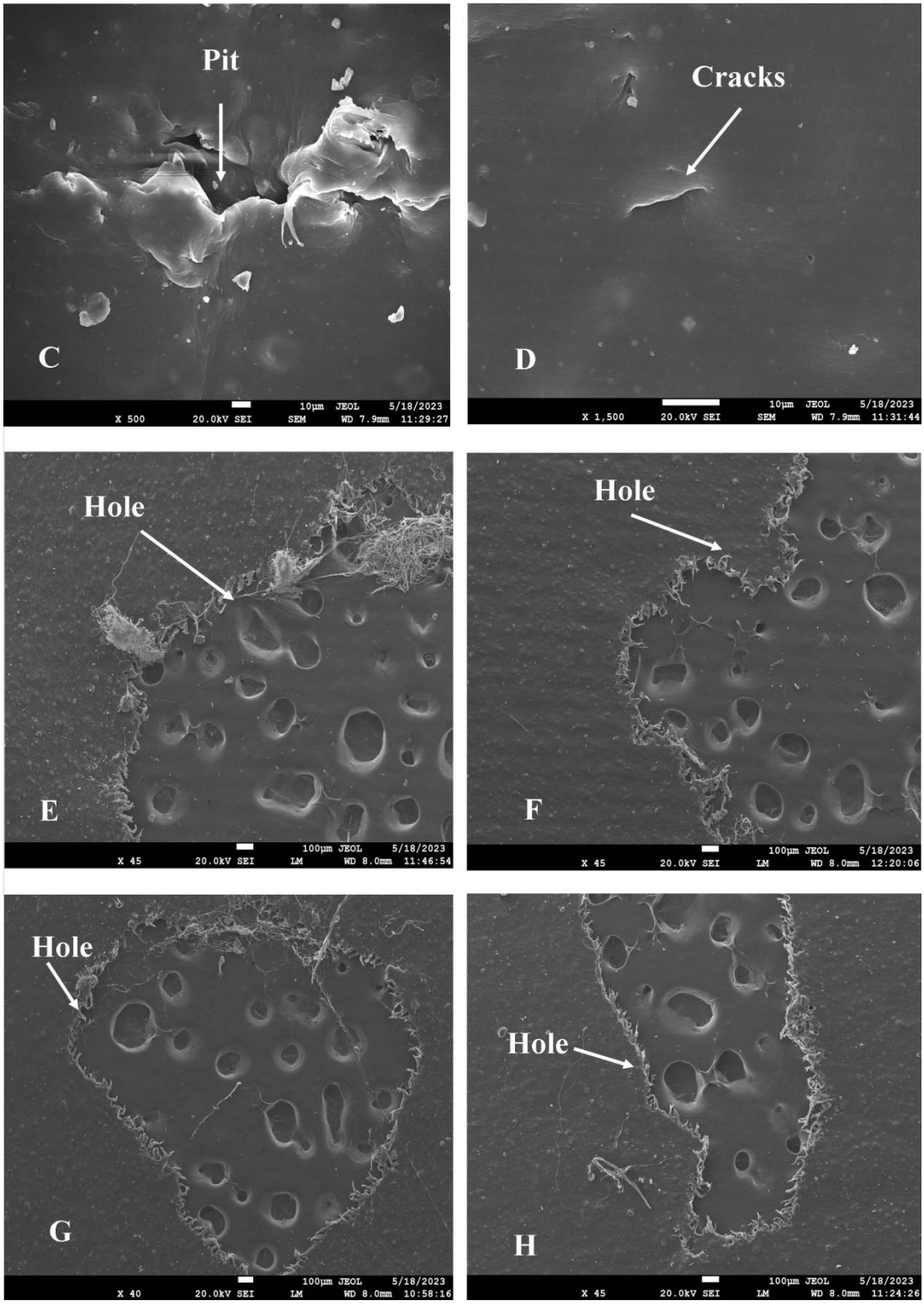


Figure 6.23(ii)

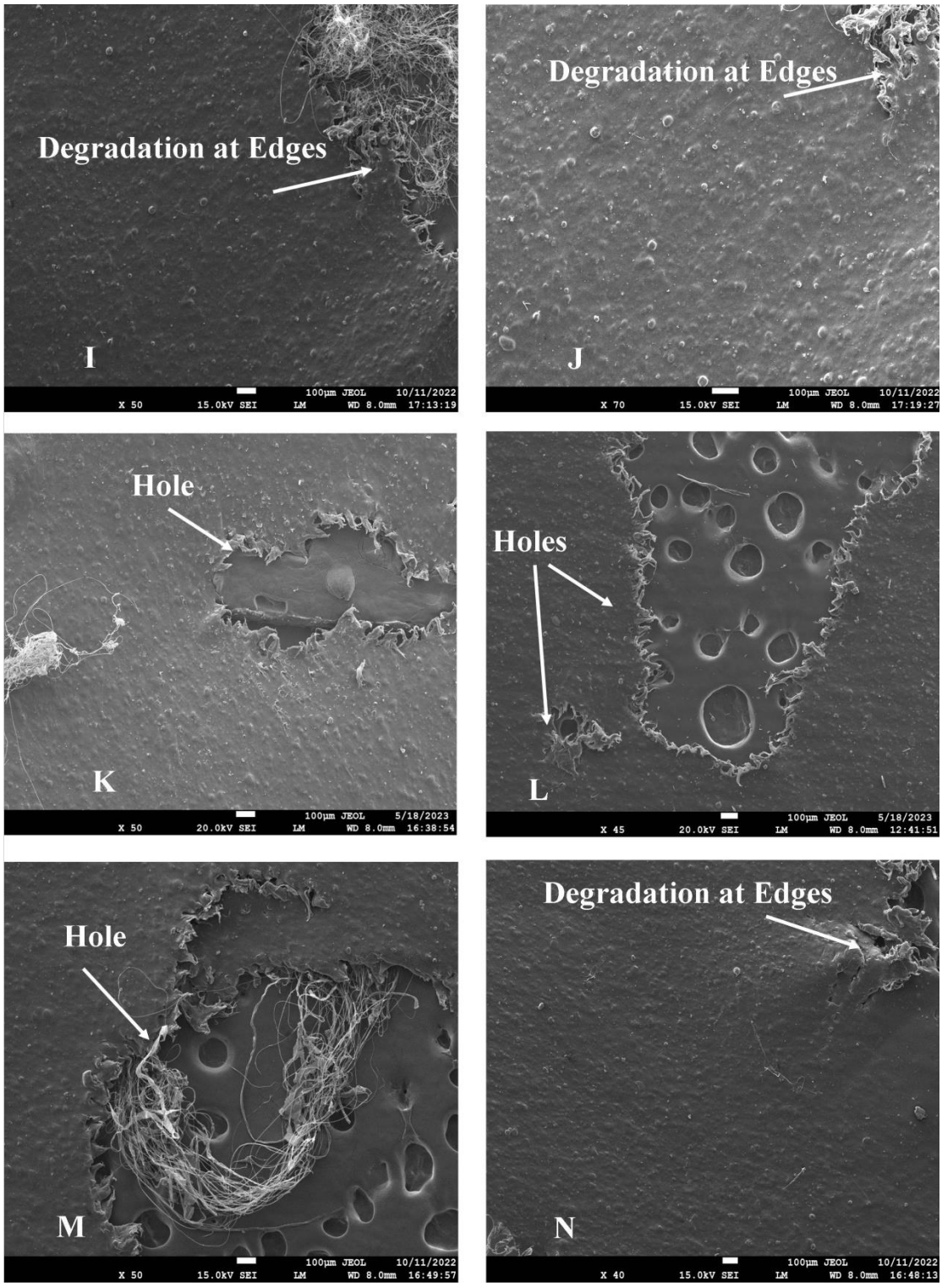


Figure 6.23(iii)

Figure 6.23 Scanning electron microscopic image of leftover remains HDPE film consumed by *G. mellonella.* Figure (i) (A) HDPE film consumed by without antibiotic group of first instar (WA-1-GWM); (B) HDPE film consumed by antibiotic fed group of first instar (A-1-GWM); Figure (ii) (C) remains of HDPE film consumed by without antibiotic group of second instar (WA-2-GWM); (D) HDPE film consumed by antibiotic administered group of second instar (A-2-GWM); (E) remains of HDPE film consumed by without antibiotic group of third instar (WA-3-GWM); (F) HDPE film consumed by antibiotic fed group of third instar (A-3-GWM); Figure (iii) (G) HDPE film consumed by without antibiotic group of fourth instar (WA-4-GWM); (H) remains of HDPE film consumed by with antibiotic group of fourth instar (A-4-GWM); (I) HDPE film consumed by without antibiotic group of fifth instar (WA-5-GWM); (J) remains of HDPE film consumed by with antibiotic group of fifth instar (A-5-GWM); (K) HDPE film remains of polymers fed by sixth instar of without antibiotic group (WA-6-GWM); (L) HDPE film consumed by antibiotic administered group of sixth instar (A-6-GWM); (M) HDPE film consumed by without antibiotic group of seventh instar (WA-7-GWM); (N) HDPE film remains of film consumed by antibiotic fed insects of seventh instar (A-7-GWM)

GC-MS- Gas chromatography and mass spectroscopy was conducted for the frass sample of wax moth larvae to verify that the plastics consumed by the caterpillar are broken down or otherwise biodegraded into minute degradable chemicals by the larvae.

In Control-HDPE compounds detected are Nonane (7.937), 2,2'-Bifuran, octahydro- (12.035), 2,2'-Bifuran, octahydro- (12.263), 2,3'-Bifuran, octahydro- (13.066), Furan, 2-butyltetrahydro- (13.185) , Furan, 2-butyltetrahydro- (22.114), Heneicosane (23.134), Hexadecanamide (24.829), Eicosane (25.882), Dotriacontane (26.725), Tetrapentacontane (27.234), Bis(2-ethylhexyl) phthalate (27.812), 2-Methylpentacosane (27.980), 3-Methylpentacosane (28.106), 3-Methylhexacosane (28.947), 5-Methylnonacosane (29.667), 2-Methylheptacosan (29.747) (Figure 6.24(i)A).

For WA-1-GWM group, revealed chemical peaks named as 2-Pentanol (5.703), Butanoic acid, 4-hydroxy (8.200), Acetic acid, 4-t-butyl-4-hydroxy-1,5-dimethyl-hex-2-enyl ester (13.964), Phosphonoacetic Acid, 3TMS derivative (14.888), 7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione (16.846), 1,3-Pentadiene, 2,4-di-t-butyl (17.125), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.009), Heptadecane (18.836), Heneicosane (21.099), Tetrapentacontane (22.262), Hexadecane (23.148), Heneicosane (25.017) for compounds that were detected in the frass of *G. mellonella* larvae (Figure 6.24(ii)B).

The GC-MS graph for frass of A-1-GWM detected compounds 2-Hexanol (5.743), Butanoic acid, 4-hydroxy- (8.228), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.000), 1,3-Pentadiene, 2,4-di-t-butyl- (17.125), 6,8-Nonadien-2-one, 8-methyl-5-(1-

methylethyl)-, (E)- (18.008), Heptadecane (18.834), Hexadecane (21.097), Heneicosane (23.144) (Figure 6.24(ii)C).

For WA-2-GWM group revealed the chemical peaks named as 2-Pentanol (5.731), Butanoic acid, 4-hydroxy- (8.200), Phosphonoacetic Acid, 3TMS derivative (14.888), 5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)- (16.853), 1,3-Pentadiene, 2,4-di-t-butyl- (17.126), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.010), Heptadecane (18.838), Heneicosane (21.102), 3-Buten-2-ol, 1-bromo-2-methyl (22.123), Heptadecane (23.150) in the frass of the greater wax moth larvae (Figure 6.24(ii)D).

In A-2-GWM group compounds detected are 2-Pentanol (5.729), Butanoic acid, 4-hydroxy- (8.194), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (16.999), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.008), 8-Hydroxy-2,2,8-trimethyldeca-5,9-dien-3-one (18.172), Heptadecane (18.834), Heneicosane (21.097), Tetracosane (23.146), Eicosane (25.019) in the frass of *G. mellonella* larvae (Figure 6.24(ii)E).

The GC-MS graph for WA-3-GWM group revealed compounds named as- 2-Pentanol, 4-methyl- (5.709), Butanoic acid, 4-hydroxy- (8.191), 1,3-Pentadiene, 2,4-di-t-butyl- (17.127), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.523), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.012), Nonadecane (18.837), Heneicosane (21.100), Propanoic acid, 2-methyl-, 2-propenyl ester (22.123), Heptadecane (23.150), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, tetradecyl ester (24.421) in the excreta of the *G. mellonella* larvae (Figure 6.24(ii)F).

For A-3-GWM group the chemical peaks for compound detected are 1-Propene, 1-methoxy- (4.051), 2-Pentanol, 4-methyl- (5.773), 1,3-Propanediol (7.614), Butanoic acid, 4-hydroxy- (8.186), Heptadecane, 2,6,10,15-tetramethyl- (16.313), 1,3-Pentadiene, 2,4-di-t-butyl- (17.122), Hexadecane (17.461), 1,18-Nonadecadien-7,10-dione (17.650), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.004), Nonadecane (18.834), Eicosane (19.996), 2,6,10-Trimethyltridecane (21.098), 3-Ethyl-2-nonanone (21.489),

Tetracosane (23.147), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexyl ester (24.416) in the excreta of greater wax moth larvae (Figure 6.24(ii)G).

In WA-4-GWM group the peaks revealed compounds namely 2-Hexanol (5.718), Butanoic acid, 4-hydroxy- (8.180), 1,3-Pentadiene, 2,4-di-t-butyl- (17.126), 17-Pentatriacontene (17.817), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.008), Heptadecane (18.837), Eicosane (19.997), Heneicosane (21.100), Dotriacontane (22.262), Heptadecane (23.149), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, isohexyl ester (24.420), Tetracosane (25.018), Tetracontane (26.738), Hexatriacontane (27.547) in the frass of *G. mellonella* larvae (Figure 6.24(iii)H).

For A-4-GWM group the GC-MS graph peaks revealed compounds namely 2-Pentanol, 4-methyl (5.784), 1,3-Propanediol (7.609), Butanoic acid, 4-hydroxy- (8.185), 1,3-Pentadiene, 2,4-di-t-butyl- (14.885), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.122), Heptadecane (18.006), 3-Ethyl-2-pentadecanone (18.832), Octadecanoic acid, ethenyl ester (21.488), 1-Pentanamine, N-pentyl- (21.928), Heneicosane (22.044), 2,2',2''-Nitrilotriethanol, triethyl ether (23.146), 2-Methylhexacosane (28.332) in the excreta of *G. mellonella* larvae (Figure 6.24(iii)I).

Figure 6.24(iii)J shows the compounds for WA-5-GWM group named as- 2-Hexanol (5.714), Butanoic acid, 4-hydroxy- (8.196), 5-t-Butyl-hexa-3,5-dien-2-one (17.002), 1,3-Pentadiene, 2,4-di-t-butyl- (17.127), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.010), Hexadecane (18.838), Octadecane (21.100), Propanoic acid, 2-methyl-, anhydride (22.128), Heneicosane (23.149), Heptadecane, 8-methyl- (25.020), Tetracontane (26.740), Dotriacontane (28.175) in the frass of greater wax moth larvae.

In A-5-GWM group the GC-MS graph peaks revealed compounds named as- 2-Pentanol (5.709), Butanoic acid, 4-hydroxy- (8.186), 5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)- (14.883), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (16.848), 1,3-Pentadiene, 2,4-di-t-butyl- (16.990), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.123), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (17.517), Nonadecane (18.006),

Heneicosane (18.832), Hexadecane (19.994), Heptadecane (21.095), Hexadecane (23.144), 3,5-Decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl- (23.488), Hexacosane (24.102), Tetracosane (25.017), Triacontane (25.895), Nonacosane (26.736), Dotriacontane (27.545), Tetracontane (28.336), Dotriacontane (29.211) in the excreta of *G. mellonella* larvae (Figure 6.24(iii)K).

For WA-6-GWM group the GC-MS graph peaks revealed compounds namely 2-Hexanol (5.706), Butanoic acid, 4-hydroxy- (8.211), Phosphonoacetic Acid, 3TMS derivative (14.888), 1,3-Pentadiene, 2,4-di-t-butyl- (17.126), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.521), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.009), Heptadecane (18.835), Heneicosane (21.100), Propanoic acid, 2-methyl-, anhydride (22.127), Heptadecane (23.148), 3,5-Decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl (23.490), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexyl ester (24.421), Eicosane (25.020), Dotriacontane (26.740), Tetracosane (27.547), Tetrapentacontane (28.338) that are present in the excreta of greater wax worms (Figure 6.24(iii)L).

In A-6-GWM group the GC-MS graph peaks revealed compounds- 2-Hexanol (5.696), Butanoic acid, 4-hydroxy- (8.183), Phosphonoacetic Acid, 3TMS derivative (14.884), Tetradecane (16.310), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (16.998), 1,3-Pentadiene, 2,4-di-t-butyl- (17.123), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.521), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.008), Carbonic acid, eicosyl vinyl ester (18.171), Hexadecane (18.833), Eicosane (19.996), Heneicosane (21.096), Isophytol (21.165), Propanoic acid, 2-methyl-, anhydride (22.120), Heptadecane (23.145), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexyl ester (24.419), Octacosane (25.016), Nonacosane (26.737), Dotriacontane (27.547), Bis(2-ethylhexyl) phthalate (27.827), Tetrapentacontane (28.336), Tetracontane (29.211) that are present in the excreta of *G. mellonella* larvae (Figure 6.24(iii)M).

For WA-7-GWM group the GC-MS data obtained revealed the peaks for compounds named as 2-Hexanol (5.712), Butanoic acid, 4-hydroxy- (8.216), Phosphonoacetic Acid, 3TMS derivative (14.886), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.003), 1,3-

Pentadiene, 2,4-di-t-butyl- (17.128), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.523), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.011), Tetradecane (18.837), Heneicosane (21.099), Sulfurous acid, octyl 2-pentyl ester (21.160), Hexadecanoic acid, methyl ester (22.396), Hexadecanoic acid, ethyl ester (23.075), Heptadecane (23.147), 9,12-Octadecadienoic acid, methyl ester (24.050), 9-Octadecenoic acid, methyl ester, (E)- (24.109), Ethyl Oleate (24.720), Octacosane (25.019), Tetracosane (26.740), Dotriacontane (27.389), Tetrapentacontane (28.172) present in the frass of the greater wax worm larvae (Figure 6.24(iii)N).

For A-7-GWM group the GC-MS data obtained revealed the peaks for compounds named as 2-Pentanol, 4-methyl- (5.713), Butanoic acid, 4-hydroxy- (8.187), Phosphonoacetic Acid, 3TMS derivative (14.885), Tetradecane (16.311), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (16.999), 1,3-Pentadiene, 2,4-di-t-butyl- (17.123), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.519), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.007), Heptadecane (18.832), Heneicosane (21.096), Butane, 1-bromo-2-methyl-, (S)- (21.365), Heneicosane (23.146), Tetracosane (25.017), Dotriacontane (25.894), Nonacosane (26.738), Tetracontane (27.546), Hexatriacontane (28.336), Eicosane (29.210) present in the excreta of *G. mellonella* larvae (Figure 6.24(iii)O).

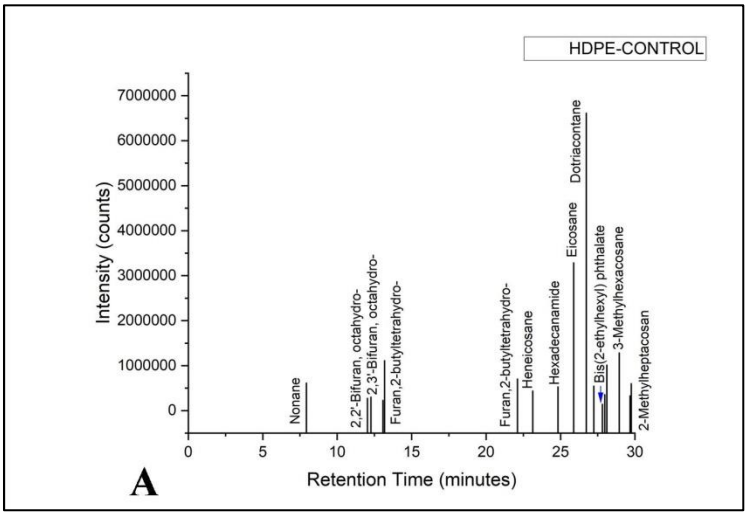


Figure 6.24(i)

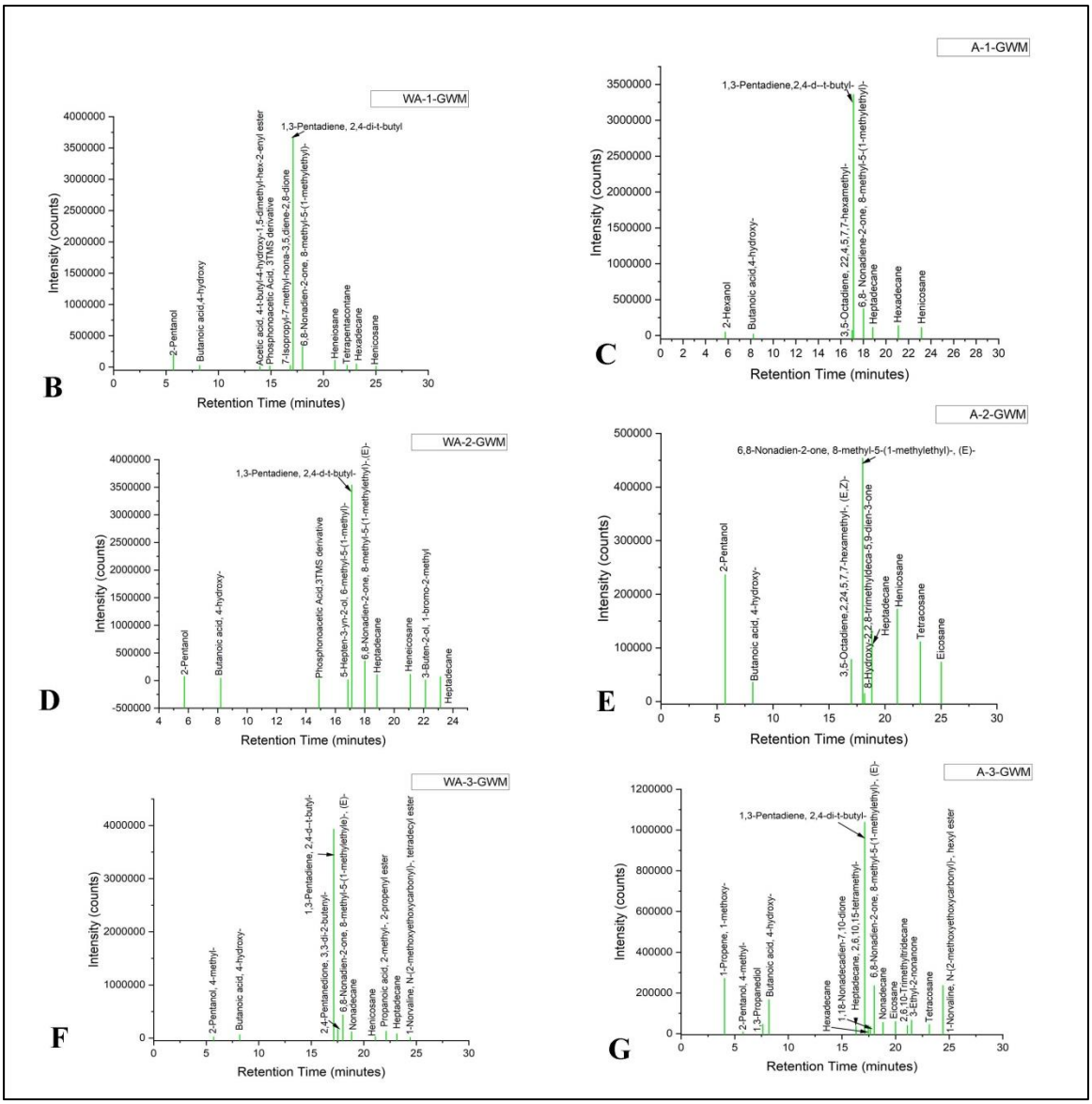


Figure 6.24(ii)

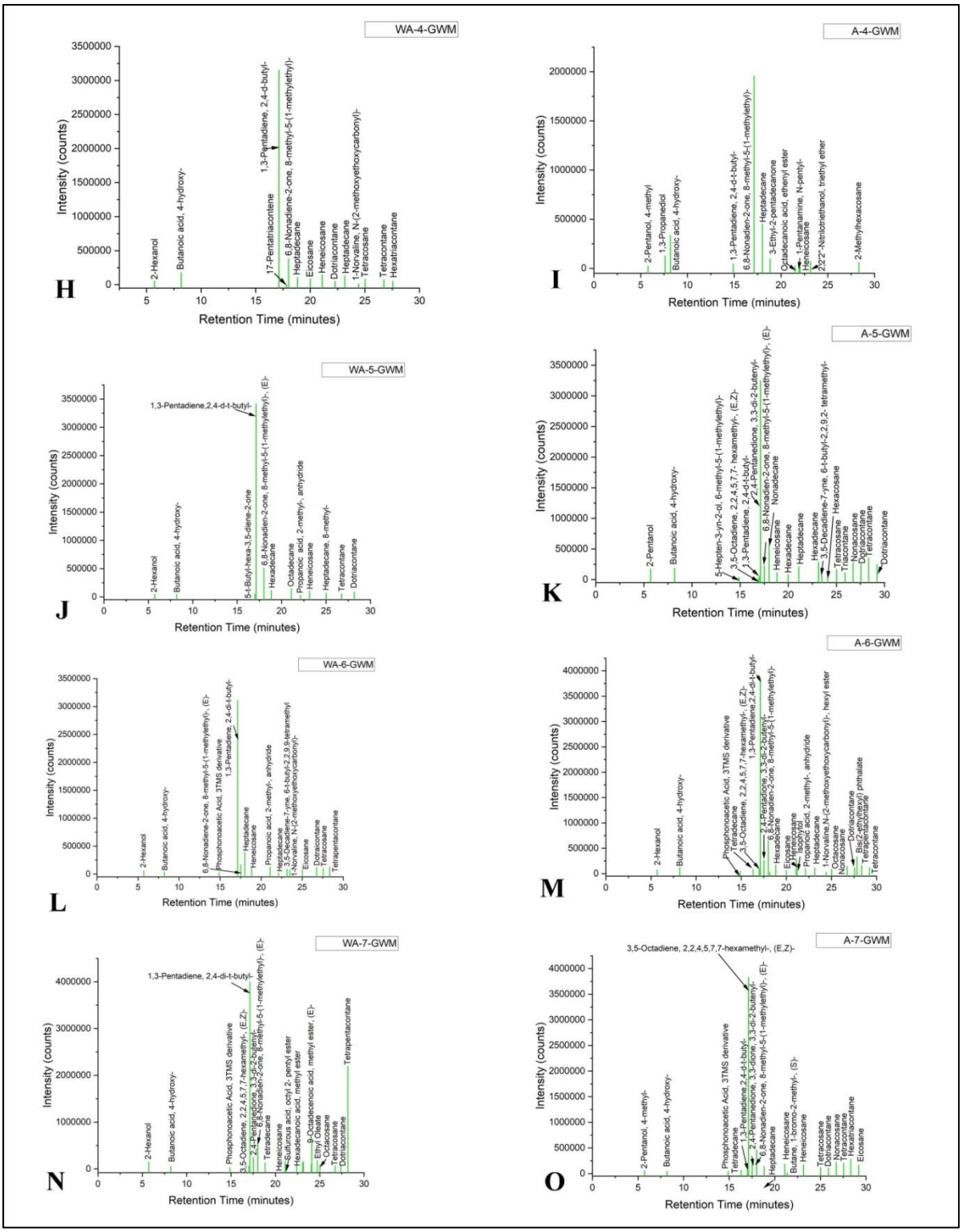


Figure 6.24(iii)

Figure 6.24 Gas chromatography and mass spectroscopy analysis for frass of *G. mellonella* fed with HDPE. Figure (i) (A) GC-MS graph for retention time and intensity for HDPE film (Control-HDPE); Figure (ii) (B) GC-MS graph for frass of without antibiotic group for first instar fed with HDPE (WA-1-GWM); (C)The graph for frass of antibiotic fed first instar group (A-1-GWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-GWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-GWM) ; (F) The GC-MS graph of frass for third instar without antibiotic group fed with HDPE (WA-3-GWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-GWM). Figure (iii) (H) GC-MS graph of without antibiotic fourth instar group (WA-4-GWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-GWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-GWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-GWM); (L) The GC-MS graph of retention time and intensity for frass of without antibiotic sixth instar group (WA-6-GWM); (M) GC-MS graph for frass of antibiotic administered sixth instar group (A-6-GWM); (N) The GC-MS graph for frass without antibiotic seventh instar group (WA-7-GWM); (O) The GC-MS graph of retention time and intensity for frass of antibiotic fed of seventh instar group (A-7-GWM)

Polypropylene (PP) - Greater wax moth larvae of all larval instar stages were exposed to the naive PP films for two days, with a sample size of fifty larval instars in each group and the control population. Following the end of the experiment, data were collected on the larvae's consumption of plastic, survival rate, and the SEM of the exposed PP film as well as the GC-MS of the greater wax worm's frass.

Plastic Consumption Rate- After the insects were exposed to PP film for two days with both antibiotic- and non-antibiotic-administered larvae, the plastic ingestion rate of *G. mellonella* fed on the naive plastic film was noted. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The plastic consumption rate for PP for *G. mellonella* by various groups- Control, WA-1-GWM, A-1-GWM, WA-2-GWM, A-2-GWM, WA-3-GWM, A-3-GWM, WA-4-GWM, A-4-GWM, WA-5-GWM, A-5-GWM, WA-6-GWM, A-6-GWM, WA-7-GWM and A-7-GWM is 0,0,0,0, 0.193 ± 0.193, 0.122 ± 0.106, 0.310 ± 0.127, 0.912 ± 0.910, 0.919 ± 0.768, 0.891 ± 0.871, 1.393 ± 1.231, 0.787 ± 0.805, 8.400 ± 7.609, 2 ± 2.017 %, respectively (Figure 6.25).

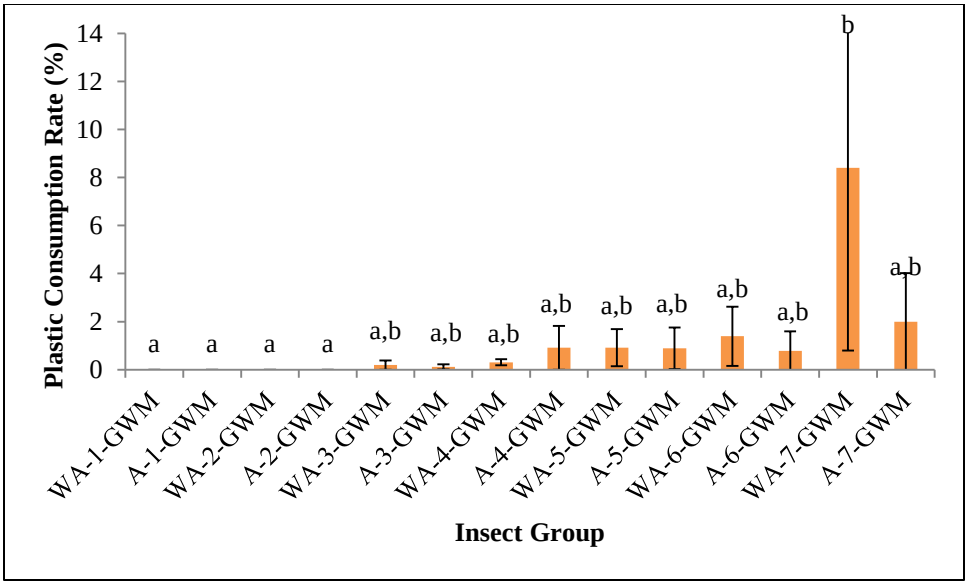


Figure 6.25 Plastic consumption rate of gut microbiota dependent and independent *G. mellonella* larvae. The graph represents plastic consumption rate of PP by *G. mellonella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-b). Note: WA-1-GWM= without antibiotic first instar group fed with naive PP film, A-1-GWM= antibiotic administered first instar group fed with naive PP film, WA-2-GWM= without antibiotic second instar group fed with naive PP film, A-2-GWM= antibiotic administered second instar group fed with naive PP film, WA-3-GWM= without antibiotic third instar group fed with naive PP film, A-3-GWM= antibiotic administered third instar group fed with naive PP film, WA-4-GWM= without antibiotic fourth instar group fed with naive PP film, A-4-GWM= antibiotic administered fourth instar group fed with naive PP film, WA-5-GWM= without antibiotic fifth instar group fed with naive PP film, A-5-GWM= antibiotic administered fifth instar group fed with naive PP film, WA-6-GWM= without antibiotic sixth instar group fed with naive PP film, A-6-GWM= antibiotic administered sixth instar group fed with naive PP film, WA-7-GWM= without antibiotic seventh instar group fed with naive PP film, A-7-GWM= antibiotic administered seventh instar group fed with naive PP film

Table 6.7 Comparative data on plastic consumption (mg/day/insect) by *Galleria mellonella* for study the degradation of different types of plastic films

Insect Group	LDPE	HDPE	PP
WA-1-GWM	0	0.006 ± 0.005	0
A-1-GWM	0	0.003 ± 0.005	0
WA-2-GWM	0.006 ± 0.01	0.01 ± 0.01	0
A-2-GWM	0	0.003 ± 0.005	0
WA-3-GWM	0.003 ± 0.005	0.01 ± 0.005	0.01 ± 0.01
A-3-GWM	0.003 ± 0.005	0.01 ± 0.01	0.006 ± 0.005

WA-4-GWM	0.25 ± 0.38	0.06 ± 0.03	0.08 ± 0.11
A-4-GWM	0.1 ± 0.1	0.04 ± 0.02	0.05 ± 0.04
WA-5-GWM	0.05 ± 0.04	0.07 ± 0.05	0.05 ± 0.04
A-5-GWM	0.05 ± 0.04	0.02 ± 0.01	0.05 ± 0.04
WA-6-GWM	0.07 ± 0.1	0.15 ± 0.08	0.08 ± 0.1
A-6-GWM	0.07 ± 0.1	0.15 ± 0.08	0.05 ± 0.05
WA-7-GWM	0.14 ± 0.14	0.25 ± 0.16	0.15 ± 0.13
A-7-GWM	0.15 ± 0.11	0.2 ± 0.06	0.12 ± 0.08

Insects Survival Rate- Following the wax worms' 48-hour exposure to the PP film, the insect survival rate of the *G. mellonella* fed on the film was observed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05.The Insect survival rate for naive and pretreated PP for *G. mellonella* is given by various groups- Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 100 ± 0, 38 ± 15.62, 24 ± 2, 100 ± 0, 72 ± 21.166, 52.666 ± 9.865, 100 ± 0, 52 ± 14.422, 73.333 ± 23.007, 100 ± 0, 79.333 ± 15.011, 74 ± 3.114, 100 ± 0, 89.333 ± 9.451, 89.333 ± 13.613, 100 ± 0, 89.333 ± 5.033, 94 ± 6, 100 ± 0, 94.666 ± 2.309, 89.333 ± 1.154 %, respectively (Figure 6.26).

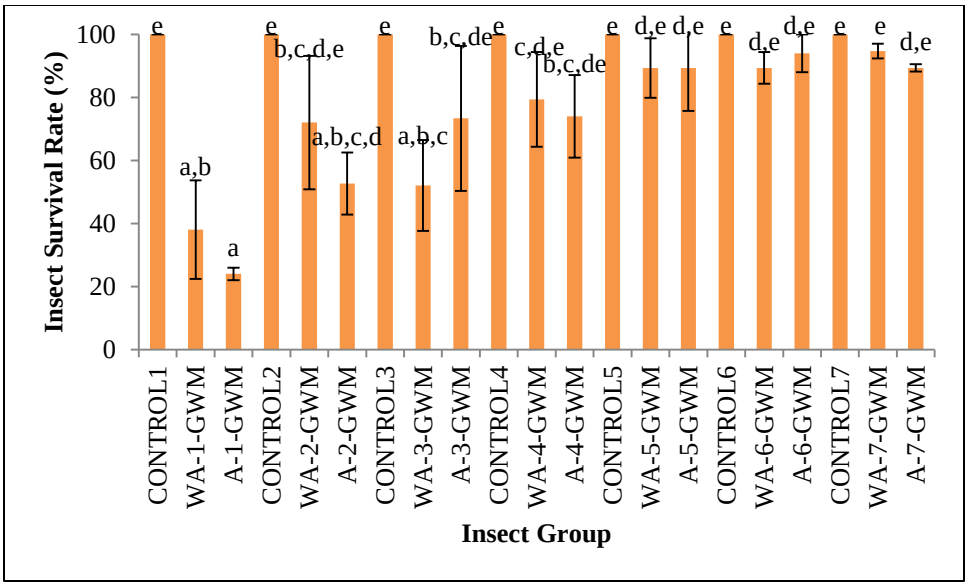


Figure 6.26 Insects survived when fed PP naive film. The graph represents with and without gut microbiota *G. mellonella* insects survived when fed on naive PP film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-e). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive PP film, A-1-GWM= antibiotic administered first instar group fed with naive PP film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic second instar group fed with naive PP film, A-2-GWM= antibiotic administered second instar group fed with naive PP film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive PP film, A-3-GWM= antibiotic administered third instar group fed with naive PP film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive PP film, A-4-GWM= antibiotic administered fourth instar group fed with naive PP film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive PP film, A-5-GWM= antibiotic administered fifth instar group fed with naive PP film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive PP film, A-6-GWM= antibiotic administered sixth instar group fed with naive PP film, CONTROL7= control group of seventh instar, WA-7-GWM= without antibiotic seventh instar group fed with naive PP film, A-7-GWM= antibiotic administered seventh instar group fed with naive PP film

SEM- Scanning electron microscopy of naive and consumed PP film was performed to visualise and compare the extent of biodegradation of the plastics by greater wax worms. The SEM naive PP depicted clear surface without any structural modifications on the topography of the film (Figure 6.27). There were no structural modifications observed in without antibiotic first instar stage (WA-1-GWM) as compared to naive PP film (Figure 6.28(i) A). On the surface topography of with antibiotic first instar stage (A-1-GWM) no structural modifications were visible in the SEM images (Figure 6.28(i) B). A pit was observed in PP film for without antibiotic second instar group (WA-2-GWM) (Figure

6.28(ii) C). The antibiotic fed second instar group (A-2-GWM) depicted negligible changes on the surface of plastic film as compared to naive PP film (Figure 6.28(ii) D). Some holes are present on the edges of the PP film insect consumed film by without antibiotic third instar group (WA-3-GWM) (Figure 6.28(ii) E). Some disintegrated polymers are visible in the SEM images on the edges of the PP film consumed by with antibiotic group of third instar (A-3-GWM) (Figure 6.28(ii) F). A large disintegrated area is visible on the surface of the PP film consumed by without antibiotic fourth instar (WA-4-GWM) on the edges of the polymer film (Figure 6.28(ii) G). The SEM image of the film revealed large disintegrated area is present on the edges of the PP film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-GWM) (Figure 6.28(ii) H). A large disintegrated area is visible on the edges of PP film in SEM image for without antibiotic fifth instar (WA-5-GWM) (Figure 6.28(iii) I). In the antibiotic fed fifth instar group (A-5-GWM) disintegration of plastics is visible at the edges of the film in the SEM images (Figure 6.28(iii) J). For the without antibiotic sixth instar larval group (WA-6-GWM) the disintegration of polymers are visible as disintegrated area on the edges in the PP film (Figure 6.28(iii) K). The SEM images of the antibiotic administered sixth instar larval group (A-6-GWM) revealed disintegration of plastics on the in the film as well as on the edges of the film by the larval instars (Figure 6.28(iii) L). In the SEM images for seventh instar without antibiotic group (WA-7-GWM) the disintegration of plastics is visible on the edges of the PP film (Figure 6.28(iii) M). The SEM images for antibiotic administered seventh instar (A-7-GWM) revealed consumed remains of plastics on the edges of the plastic film (Figure 6.28(iii) N).

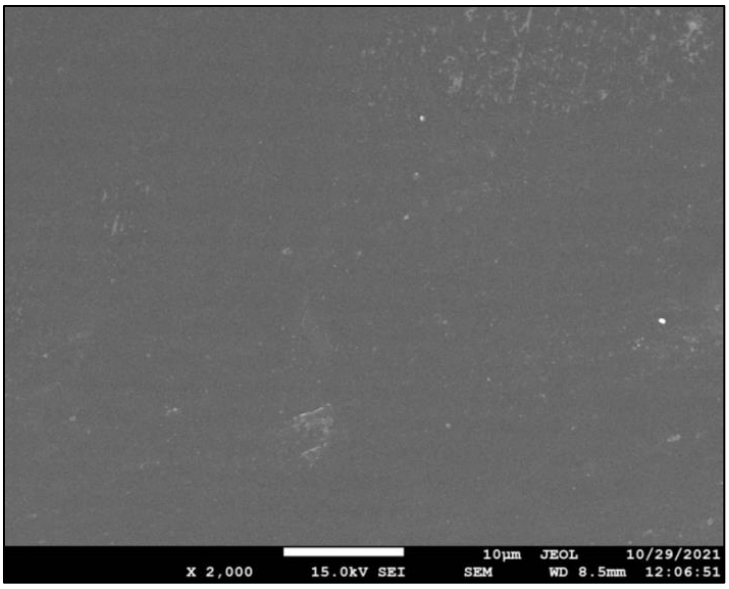


Figure 6.27 Scanning electron microscopic image of naive PP film

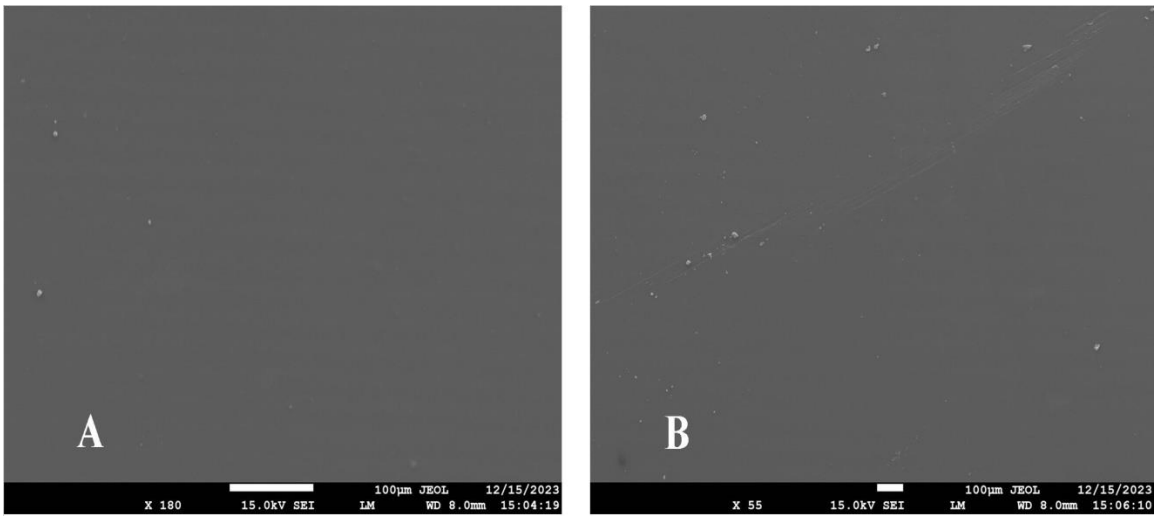


Figure 6.28(i)

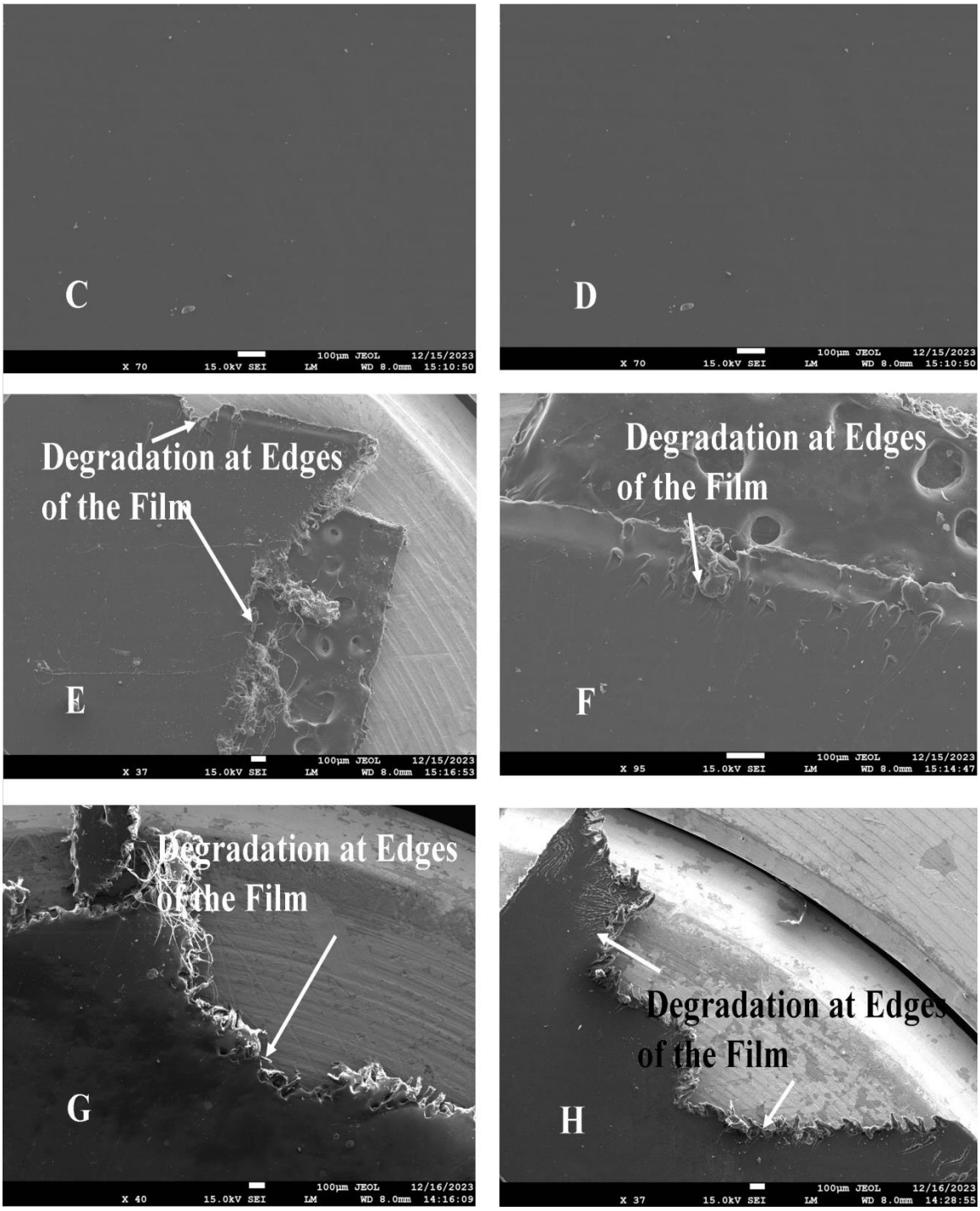


Figure 6.28(ii)

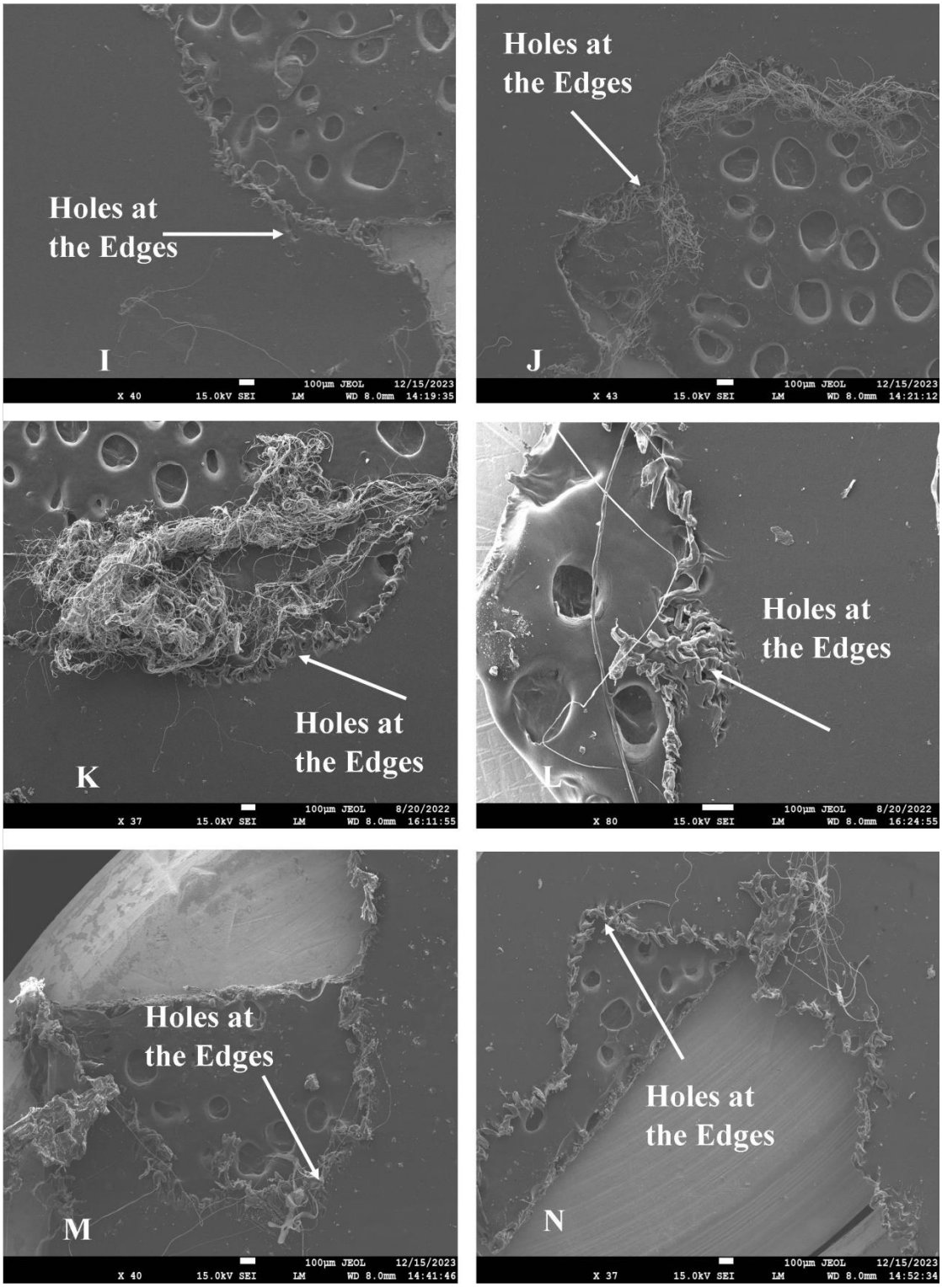


Figure 6.28(iii)

Figure 6.28 Scanning electron microscopic image of leftover remains PP film consumed by *G. mellonella.* Figure (i) (A) PP film consumed by without antibiotic group of first instar (WA-1-GWM); (B) PP film consumed by antibiotic fed group of first instar (A-1-GWM); Figure (ii) (C) remains of PP film consumed by without antibiotic group of second instar (WA-2-GWM); (D) PP film consumed by antibiotic administered group of second instar (A-2-GWM); (E) remains of PP film consumed by without antibiotic group of third instar (WA-3-GWM); (F) PP film consumed by antibiotic fed group of third instar (A-3-GWM); (G) PP film consumed by without antibiotic group of fourth instar (WA-4-GWM); (H) remains of PP film consumed by with antibiotic group of fourth instar (A-4-GWM). Figure (iii) (I) PP film consumed by without antibiotic group of fifth instar (WA-5-GWM); (J) remains of PP film consumed by with antibiotic group of fifth instar (A-5-GWM); (K) PP film remains of polymers fed by sixth instar of without antibiotic group (WA-6-GWM); (L) PP film consumed by antibiotic administered group of sixth instar (A-6-GWM); (M) PP film consumed by without antibiotic group of seventh instar (WA-7-GWM); (N) PP film remains of film consumed by antibiotic fed insects of seventh instar (A-7-GWM)

GC-MS- In order to confirm that the plastics consumed by the caterpillar are fragmented or otherwise biodegraded into tiny degradable compounds by the larvae, gas chromatography and mass spectroscopy were carried out to the frass sample of wax moth larvae.

In Control-PP, the GC-MS graph revealed compounds- 1,3-Propanediol (6.397), Butane, 1-chloro-2-methyl- (7.269), Nonane (7.926), Butanoic acid, 4-hydroxy- (8.168), 1,2,6-Hexanetriol (9.128), 2,2'-Bifuran, octahydro- (12.033), 2,2'-Bifuran, octahydro- (12.261), Propanoic acid, 2-methyl-, anhydride (12.897), 2,3'-Bifuran, octahydro- (13.063) (Figure 6.29(i)A).

WA-1-GWM group the compounds detected were 5,8,11,14-Eicosatetraynoic acid, methyl ester (4.360), Heptane, 2,4-dimethyl- (5.503), Octane, 2-methyl- (7.048), Octane, 3-methyl- (7.201), Nonane (7.846), Propanoic acid, 2-methyl-, anhydride (12.773), 5-Methyl-2-isopropyl-2-hexen-1-al (12.834), Propanoic acid, 2-methyl-, anhydride (13.060), Decyl pentyl ether (15.136), Decyl pentyl ether (15.375), 3-Buten-2-ol, 1-bromo-2-methyl- (20.093), 2-Oxo-n-valeric acid (20.309), Sulfurous acid, octyl 2-pentyl ester (21.013), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.981), Butanoic acid, tridec-2-ynyl ester (22.419), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.614), 2-Methylbutyl 8-methylnon-6-enoate (22.743), Octadecanoic acid, ethenyl ester (23.375), 1,6-Heptadien-4-ol, 4-propyl- (23.536), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.518), Butyric acid, 2,2-dimethyl-, vinyl ester (26.609),

Propanoic acid, 2-methyl-, 2-propenyl ester (26.683), Tetracosane (29.046) (Figure 6.29(i)B).

For A-1-GWM group the compounds detected are Heptane, 2,4-dimethyl- (5.524), Octane, 2-methyl- (7.055), Octane, 3-methyl- (7.214), Nonane (7.856), Butanoic acid, 4-hydroxy- (8.039), Borinic acid, diethyl-, methyl ester (11.916), 1,6-Heptadien-4-ol (11.988), 2-Undecyne (12.466), Propanoic acid, 2-methyl-, anhydride (12.771), 5-Methyl-2-isopropyl-2-hexen-1-al (12.831), 4-Hexen-3-ol, 2-methyl- (12.936), Decyl pentyl ether (15.141), Decyl pentyl ether (15.381), Butanoic acid, tridec-2-ynyl ester (21.574), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.978), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.608), Succinic acid, tridec-2-yn-1-yl 3-methylbut-2-yl ester (23.276), 2,6,6,10-Tetramethyl-undeca-8,10-diene-3,7-dione (23.370), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.442), Propanoic acid, 2-methyl-, anhydride (24.966), Tetrapentacontane (25.235), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.513), Propanoic acid, 2-methyl-, 2-propenyl ester (26.605), 1,5-Heptadiene-3,4-diol (26.678), Octacosane, 2-methyl- (28.521) (Figure 6.29(i)C).

WA-2-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.521), Octane, 3-methyl- (7.205), Nonane (7.856), Butanoic acid, 4-hydroxy- (8.037), Butyric acid, neopentyl ester (11.919), 1,6-Heptadien-4-ol (11.989), 2-Undecyne (12.467), Propanoic acid, 2-methyl-, anhydride (12.770), 4-Hexen-3-ol, 2-methyl- (13.058), Decyl pentyl ether (15.141), Decyl pentyl ether (15.383), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.610), Butane, 2-iodo-3-methyl- (19.728), 1,6-Heptadien-4-ol (19.801), Undecane, 5,7-dimethyl- (19.845), Propanoic acid, 2-methyl-, anhydride (19.953), 4-Hexen-3-ol, 2-methyl- (20.027), Butanoic acid, tridec-2-ynyl ester (21.575), 3-Methyl-hepta-1,6-dien-3-ol (21.632), Propanoic acid, 2-methyl-, 2-propenyl ester (21.979), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (22.607), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.739), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.276), Butanoic acid, tridec-2-ynyl ester (23.530), 2-

Oxo-n-valeric acid (23.599), Butanoic acid, 2-propenyl ester (24.873), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.516), 2,6-Octadiene-4,5-diol (26.603), Dotriacontane (29.040) (Figure 6.29(i)D).

For A-2-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.527), Octane, 2-methyl- (7.063), Octane, 3-methyl- (7.210), Nonane (7.857), Butanoic acid, 4-hydroxy- (8.004), Butyric acid, neopentyl ester (11.911), 1,6-Heptadien-4-ol (11.986), Borinic acid, diethyl-, methyl ester (12.139), 2-Undecyne (12.368), Propanoic acid, 2-methyl-, anhydride (12.769), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.830), 4-Hexen-3-ol, 2-methyl (12.934), Propanoic acid, 2-methyl-, anhydride (13.052), Butanoic acid, anhydride (13.398), Propanoic acid, 2-methyl-, 2-propenyl ester (13.714), Decyl pentyl ether (15.381), Butanoic acid, anhydride (19.565), 4-Iodobutanal (19.728), Heptadecane (19.850), Butanoic acid, anhydride (19.954), 4-Hexen-3-ol, 2-methyl- (20.028), Heneicosane (20.951), Butanoic acid, tridec-2-ynyl ester (21.574), 3-Methyl-hepta-1,6-dien-3-ol (21.630), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.979), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.607), 2,6,6,10-Tetramethyl-undeca-8,10-diene-3,7-dione (22.738), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (23.277), Butanoic acid, 2-methyloct-5-yn-4-yl ester (23.444), 1,7-Octadiene, 3-methoxy- (23.597), 2-Methyltetracosane (23.961), Butyric acid, 2,2-dimethyl-, vinyl ester (24.875), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (24.966), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.517), Butyric acid, 2,2-dimethyl-, vinyl ester (26.604), Dotriacontane (27.409), Tetrapentacontane (29.043) (Figure 6.29(i)E).

In WA-3-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.516), Octane, 2-methyl- (7.059), Octane, 3-methyl- (7.206), Nonane (7.856), Butyric acid, neopentyl ester (12.143), 2-Undecyne (12.370), Propanoic acid, 2-methyl-, anhydride (12.770), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.831), 4-Hexen-3-ol, 2-methyl- (12.936), 2-Butyl-2,7-octadien-1-ol (13.649), Decyl pentyl ether (15.141), 1,7-Octadien-3-ol, 2,6-dimethyl- (15.383), 4-Iodobutanal (19.565), Succinic

acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.611), Butanoic acid, anhydride (19.729), Propanoic acid, 2-methyl-, anhydride (19.953), Butanoic acid, tridec-2-ynyl ester (21.575), Pentane, 3-bromo- (21.631), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.979), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.609), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (22.741), Butanoic acid, 2-methyloct-5-yn-4-yl ester (23.375), Butanoic acid, 2-methyloct-5-yn-4-yl ester (23.447), 2-Hepten-3-ol, 4,5-dimethyl- (23.602), 6-Octadecenoic acid, methyl ester, (Z)- (23.963), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.516), Propanoic acid, 2-methyl-, 2-propenyl ester (26.606), Tetrapentacontane (28.032), Dotriacontane (29.042) (Figure 6.29(i)F).

For A-3-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.517), Octane, 2-methyl- (7.061), Octane, 3-methyl- (7.223), Nonane (7.856), Butanoic acid, 4-hydroxy- (8.064), Butanoic acid, 2-methylbutyl ester (11.920), 2-Undecyne (12.467), Propanoic acid, 2-methyl-, anhydride (12.770), 4-Hexen-3-ol, 2-methyl- (12.937), Propanoic acid, 2-methyl-, anhydride (13.053), Butanoic acid, anhydride (13.398), Decyl pentyl ether (15.132), 1,7-Octadien-3-ol, 2,6-dimethyl- (15.378), 2,5-Heptanedione, 3,3,6-trimethyl- (16.796), 2-Oxo-n-valeric acid (20.307), Butanoic acid, tridec-2-ynyl ester (21.629), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.978), Butanoic acid, tridec-2-ynyl ester (22.608), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.738), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (23.276), 1,6-Heptadien-4-ol, 4-propyl- (23.370), (+-)-3-Methyl-1-penten-3-ol (23.595), 1,5-Hexadien-3-ol, bromomethyldimethylsilyl ether (23.710), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.515), Propanoic acid, 2-methyl-, 2-propenyl ester (26.605), 1,5-Heptadiene-3,4-diol (26.677), Dotriacontane (29.038) (Figure 6.29(i)G).

For WA-4-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.520), Octane, 2-methyl- (7.065), Octane, 3-methyl- (7.216), Nonane (7.856), Butanoic acid, 4-hydroxy- (8.003), Butyric acid, neopentyl ester (11.910), 1,6-Heptadien-4-ol (11.985), Borinic acid, diethyl-, methyl ester (12.137), 2-Undecyne (12.667), Propanoic acid, 2-methyl-, 2-propenyl ester (12.769), Propanoic

acid, 2-methyl-, anhydride (12.829), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.934), 4-Hexen-3-ol, 2-methyl- (13.647), 2-Octenal, 2-butyl- (15.141), Decyl pentyl ether (15.383), 1,7-Octadien-3-ol, 2,6-dimethyl- (16.795), 2,5-Heptanedione, 3,3,6-trimethyl- (16.904), 2,5-Heptanedione, 3,3,6-trimethyl- (19.730), Butane, 2-iodo-3-methyl- (20.028), 4-Hexen-3-ol, 2-methyl- (21.575), Butanoic acid, tridec-2-ynyl ester (21.979), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (23.276), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.610), 4-Hexen-3-ol, 2-methyl- (23.961), 9-Octadecenoic acid, methyl ester, (E)- (24.874), Butyric acid, 2,2-dimethyl-, vinyl ester (24.965), Pentane, 3-bromo- (26.513), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.603), Propanoic acid, 2-methyl-, 2-propenyl ester (26.677), 1,5-Heptadiene-3,4-diol (28.028), Dotriacontane (29.040) (Figure 6.29(ii)H).

In A-4-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 2,4-dimethyl- (5.519), Octane, 2-methyl- (7.060), Octane, 3-methyl- (7.207), Nonane (7.854), Butanoic acid, 4-hydroxy- (8.011), Borinic acid, diethyl-, methyl ester (11.910), 1,6-Heptadien-4-ol (11.985), Butyric acid, neopentyl ester (12.137), 2-Undecyne (12.367), Propanoic acid, 2-methyl-, 2-propenyl ester (12.664), Propanoic acid, 2-methyl-, anhydride (12.769), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.828), 4-Hexen-3-ol, 2-methyl- (12.932), 2-Myristynoic acid (13.647), Decyl pentyl ether (15.139), 1,7-Octadien-3-ol, 2,6-dimethyl- (15.382), 2,5-Heptanedione, 3,3,6-trimethyl- (16.794), Propanoic acid, 2-methyl-, anhydride (19.564), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (19.608), Butyric acid, thio-, S-decyl ester (19.685), 1,6-Heptadien-4-ol (19.798), 4-Hexen-3-ol, 2-methyl- (20.027), 3-Methyl-hepta-1,6-dien-3-ol (21.573), Butanoic acid, tridec-2-ynyl ester (21.630), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (21.976), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.608), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.529), 4-Hexen-3-ol, 2-methyl- (23.597), 6-Octadecenoic acid, methyl ester, (Z)- (23.960), Hexanedioic acid, bis(2-ethylhexyl) ester (26.510), 1,5-Heptadiene-3,4-diol (26.604), 2,6-Octadiene-4,5-diol (26.678), Chloro(trihexyl)silane (26.730), Tetrapentacontane (29.039) (Figure 6.29(ii)I).

For WA-5-GWM group the chemical peaks for compounds present in the frass are named as 1-Propene, 1-methoxy (4.057), 2-Butene-1,4-diol (5.249), Octane (5.546), 1,2-propanediol butyrate (6.097), Hexanoic acid, 3-oxo-, ethyl ester (7.089), 3-Isopropoxypropylamine (7.510), Nonane (7.936), Butanoic acid, 4-hydroxy- (8.145), Propanoic acid, 2-methyl-, 2-propenyl ester (8.362), Butyric acid, neopentyl ester (12.033), 1,6-Heptadien-4-ol (12.108), Borinic acid, diethyl-, methyl ester (12.261), 2-Undecyne (12.494), Propanoic acid, 2-methyl-, anhydride (12.898), 4-Hexen-3-ol, 2-methyl- (13.064), Butanoic acid, anhydride (13.528), 1,7-Octadien-3-ol, 2,6-dimethyl (15.270), Decyl pentyl ether (15.535), 3,5-Heptanedione, 2,2,6,6-tetramethyl- (16.935), 1,3-Pentadiene, 2,4-di-t-butyl- (17.106), Butyric acid, 2,2-dimethyl-, vinyl ester (25.009), Pentane, 3-bromo- (25.100), Tetrapentacontane (25.976), Propanoic acid, 2-methyl-, 2-propenyl ester (26.347), Butanoic acid, 2-propenyl ester (26.655), Propanoic acid, 2-methyl-, 2-propenyl ester (26.814), 1-Chloroeicosane (27.464), Eicosyl isopropyl ether (27.812), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (28.095), Pentatriacontane (28.325), Triacontane (29.202), Hexacontane (29.838) (Figure 6.29(ii)J).

In A-5-GWM group the chemical peaks for compounds present in the frass are named as Heptane, 3-methyl- (4.870), 2-Butene-1,4-diol (5.114), Heptane, 2,4-dimethyl (5.547), 2-Butanol, 3-(2,2-dimethylpropoxy)- (6.107), 1,3-Dimethylbutyl butyrate (7.092), Octane, 2-methyl- (7.135), Heptane, 2,5-dimethyl- (7.286), Nonane (7.937), Butanoic acid, 4-hydroxy- (8.129), 3-Tetradecyn-1-ol (11.749), Borinic acid, diethyl-, methyl ester (12.036), 1,6-Heptadien-4-ol (12.110), 2-Undecyne (12.591), Propanoic acid, 2-methyl-, anhydride (12.899), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.958), 4-Hexen-3-ol, 2-methyl- (13.066), Sulfurous acid, bis(2-pentyl) ester (13.465), Butanoic acid, anhydride (13.532), 2-Octenal, 2-butyl- (13.785), Propanoic acid, 2-methyl-, 2-propenyl ester (13.852), 6-Methyl-3,5-heptadiene-2-one (14.982), 1,7-Octadien-3-ol, 2,6-dimethyl (15.273), Decyl pentyl ether (15.514), 6-Methyl-hept-2-en-4-ol (15.787), 2,5-Heptanedione, 3,3,6-trimethyl (16.935), 1,3-Pentadiene, 2,4-di-t-butyl- (17.107), Nona-2,3-dienoic acid, ethyl ester (17.912), Butanoic acid, tridec-2-ynyl ester (18.005), Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester (18.085), 1,5-Heptadiene-3,4-diol

(20.227), Butanoic acid, tridec-2-ynyl ester (22.740), Pentane, 1,1'-sulfonylbis- (22.877), 9-Octadecenoic acid, methyl ester, (E)- (24.094), 2-Oxo-n-valeric acid (25.009), Butanoic acid, 2-propenyl ester (25.101), Propanoic acid, 2-methyl-, 2-propenyl ester (26.342), 4-Methyl-3-heptanol, 2-methylpropionate (isomer 2) (26.654), Propanoic acid, 2-methyl-, 2-propenyl ester (26.740), Dotriacontane (28.158) (Figure 6.29(ii)K).

For WA-6-GWM group the chemical peaks for compounds present in the frass are named as Propane, 2,2-dimethoxy- (2.136), Boric acid, trimethyl ester (2.238), Hexane-1,3,4-triol, 3,5-dimethyl- (2.688), 3-Butynoic acid (2.932), Acetic acid (3.300), Decane (7.919), Undecane (9.593), 3-Hexadecene, (Z)- (13.899), Tetradecane (14.007), Pentacos-1-ene (15.660), 1-Decanol, 2-hexyl- (16.182), 1-Pentadecene (16.445), Heptadecane (16.534), 2-Methyl-Z-4-tetradecene (17.946), 8-Octadecanone (18.508), 1-Nonadecene (18.732), 9-Heptadecanone (19.583), Hexadecanoic acid, methyl ester (20.101), 2-Undecene, 3-methyl-, (Z)- (20.742), 1-Nonadecene (20.804), 9,12-Octadecadienoic acid, methyl ester (21.763), 9-Octadecenoic acid, methyl ester, (E)- (21.824), Oleic Acid (22.162), 2-Nonen-4-one (22.218), cis-9-Octadecenoic acid, propyl ester (23.310) (Figure 6.29(ii)L).

In A-6-GWM group the chemical peaks for compounds present in the frass are named as 3-Hexanol (5.342), Heptane, 2,4-dimethyl- (5.888), Heptane, 2,2,3,3,5,6,6-heptamethyl- (9.413), Decane (9.927), Decane, 5-methyl- (10.180), Decane, 4-methyl- (10.358), Dodecane, 2,6,10-trimethyl- (10.986), Undecane, 4-methyl- (11.089), Decane, 3,7-dimethyl- (11.811), Nonane, 5-(2-methylpropyl)- (12.924), Undecane, 3-methyl- (13.031), Undecane (13.089), Dodecane (13.525), Undecane, 2,4-dimethyl- (13.671), Undecane, 4,6-dimethyl- (13.726), Dodecane, 4-methyl- (13.866), Tetradecane (14.168), Pentadecane (14.257), 2,4-Dimethyldodecane (14.500), Dodecane, 2,6,11-trimethyl- (14.700), Dodecane, 4,6-dimethyl- (14.831), Dodecane, 4,6-dimethyl- (14.961), Hexadecane (15.089), Dodecane, 2,6,11-trimethyl- (15.402), Heptadecane (16.026), Eicosane, 10-methyl- (16.126), Tetradecane (16.549), Tetradecane, 5-methyl- (16.650), Octadecane (16.699), Heptadecane (16.764), Tetradecane (16.835), Heptadecane (17.235), Octadecane (17.317), 2,6,10-Trimethyltridecane (17.375), Undecane, 2,4-

dimethyl- (17.414), Eicosane (17.695), Heneicosane (17.755), Heptadecane (17.849), 2,6,10-Trimethyltridecane (17.896), Dodecane, 2,6,11-trimethyl- (18.355), Pentadecane, 3-methyl- (18.834), Hexadecane (19.208), Heptadecane (19.292), Hexadecane, 4-methyl- (19.460), Hexacosane, 1-iodo (19.539), Heneicosane (19.580), Hexadecane (19.788), Eicosane (19.914), Eicosane, 2,4-dimethyl- (19.982), Triacontane (20.021), Hexadecane (20.084), Heptadecane (20.182), Octadecane, 5-methyl- (20.235), 2,6,10-Trimethyltridecane (20.293), Dotriacontane (20.366), Hexadecane (20.429), Eicosane (20.479), Hexadecane, 7,9-dimethyl- (20.540), Heneicosane (20.595), Heptadecane, 8-methyl- (20.645), Tetrapentacontane (20.765), Eicosane (20.890), Heneicosane (20.944), Decyl isopropyl ether (21.200), Heptadecane, 3-methyl- (21.257), Heneicosane (21.591), Tridecanal (21.815), Eicosane (22.002), Pentacosane (22.040), 11-Methylpentacosane (22.232), Tetrapentacontane (22.428), Heneicosane (22.473), Octadecane, 5-methyl- (22.523), 2,6,10-Trimethyltridecane (22.575), Nonacosane (22.637), Heptadecane, 3-methyl- (22.730), 5,5-Diethylpentadecane (22.817), Eicosane (22.952), Tetrapentacontane (23.118), Decane, 1-iodo- (23.195), Tetrapentacontane (23.267), Eicosane (23.322), Octadecane, 1-iodo- (23.454), Octacosane (23.748), Dotriacontane (24.219), Tetracosane (24.609), Hexatriacontane (24.645), Tetrapentacontane (24.711), 2-Methylpentacosane (24.759), Dotriacontane (24.865), Tetrapentacontane (24.967), Hexadecyl nonyl ether (25.280), Tetrapentacontane (25.374), Heneicosane (25.716), Tetrapentacontane (26.237), Carbonic acid, eicosyl vinyl ester (26.618), Tetrapentacontane (26.725), 2-Methylheptacosane (26.853), Octacosane (26.929), Hexatriacontane (27.291), Heptadecane, 8-methyl- (27.523), Nonacosane (28.081), Heptadecane, 3-methyl- (28.385), Octadecane, 2,6,10,14-tetramethyl- (28.440), Hexacontane (28.751), Tetrapentacontane (29.154) (Figure 6.29(ii)M).

For WA-7-GWM group the chemical peaks for compounds present in the frass are named as 3-Butene-1,2-diol (5.093), Heptane, 2,4-dimethyl- (5.545), Nonane (7.935), Butanoic acid, 4-hydroxy-(8.124), 3-Tetradecyn-1-ol (11.750), Borinic acid, diethyl-, methyl ester (12.035), 1,6-Heptadien-4-ol (12.109), 8-Hexadecyne (12.590), Propanoic acid, 2-methyl-, anhydride (12.898), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.957), 4-

Hexen-3-ol, 2-methyl- (13.064), Butanoic acid, anhydride (13.531), 2-Myristynoic acid (13.785), 6-Methyl-3,5-heptadiene-2-one (14.975), Decyl pentyl ether (15.277), 1,7-Octadien-3-ol, 2,6-dimethyl (15.519), 2,5-Heptanedione, 3,3,6-trimethyl- (16.832), 2,5-Heptanedione, 3,3,6-trimethyl- (17.043), 1,3-Pentadiene, 2,4-di-t-butyl- (17.108), 2-Isopropyl-4-methylhex-2-enal (17.634), 3-Decyn-2-ol (17.909), Butanoic acid, tridec-2-ynyl ester (18.085), Isobutyryl bromide (19.745), Butanoic acid, tridec-2-ynyl ester (19.867), Propanoic acid, 2-methyl-, anhydride (20.167), 4-Hexen-3-ol, 2-methyl- (20.981), Butanoic acid, tridec-2-ynyl ester (21.716), 2-Hepten-3-ol, 4,5-dimethyl- (21.774), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (22.113), Hexadecanoic acid, methyl ester (22.381), 9-Octadecenoic acid, methyl ester, (E)- (24.093), Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester (25.101), Tetrapentacontane (25.970), Propanoic acid, 2-methyl-, 2-propenyl ester (26.341), Butanoic acid, anhydride (26.740), 2-Methyltetracosane (27.490), 5-Butyl-5-ethylheptadecane (28.910) (Figure 6.29(ii)N).

In A-7-GWM group the chemical peaks for compounds present in the frass are named as 1-Propene, 1-methoxy- (4.058), 2-Butene-1,4-diol (5.175), Heptane, 2,4-dimethyl- (5.544), 2-Butanol, 3-(2,2-dimethylpropoxy)- (6.097), Hexanoic acid, 3-oxo-, ethyl ester (7.086), Octane, 3-methyl- (7.283), 3-Isopropoxypropylamine (7.513), Nonane (7.934), Butanoic acid, 4-hydroxy- (8.131), 3-Tetradecyn-1-ol (11.748), Borinic acid, diethyl-, methyl ester (12.033), 1,6-Heptadien-4-ol (12.108), 2-Undecyne (12.589), Propanoic acid, 2-methyl-, anhydride (12.898), trans-5-Methyl-2-isopropyl-2-hexen-1-al (12.960), 4-Hexen-3-ol, 2-methyl- (13.064), Butanoic acid, anhydride (13.529), Butanoic acid, 6-ethyl-3-octyl ester (15.270), 1,7-Octadien-3-ol, 2,6-dimethyl- (15.513), 2,5-Heptanedione, 3,3,6-trimethyl- (17.039), 1,3-Pentadiene, 2,4-di-t-butyl- (17.106), Propanoic acid, 2-methyl-, 2-propenyl ester (22.113), Oxalic acid, neopentyl propyl ester (22.330), Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (22.745), Butanoic acid, tridec-2-ynyl ester (22.874), Pentane, 3-bromo- (25.100), Propanoic acid, 2-methyl-, 2-propenyl ester (26.340), 2,6-Octadiene-4,5-diol (26.737), 1-Chloroeicosane (27.490) (Figure 6.29(ii)O).

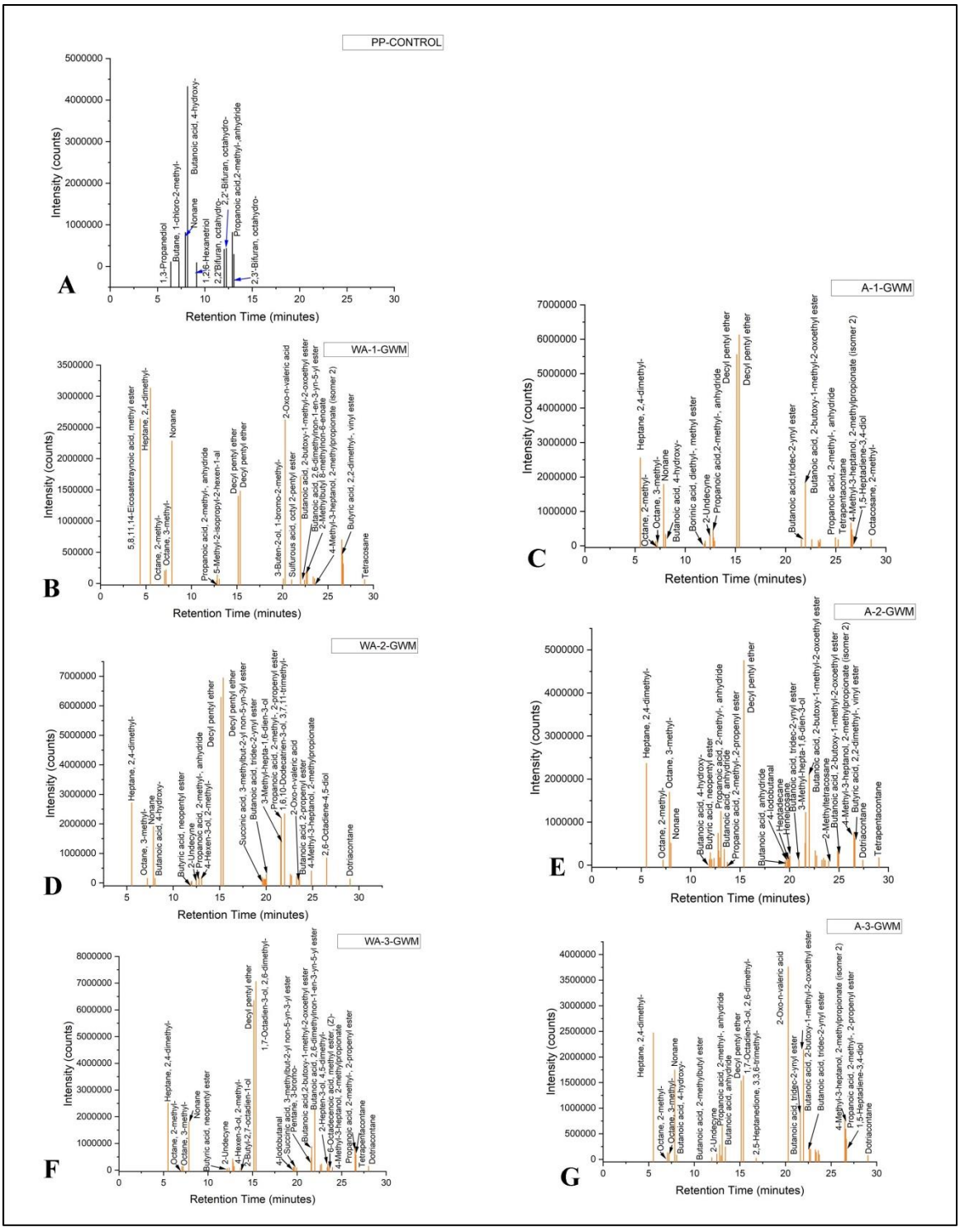

PP-CONTROL
A
Intensity (counts)
Retention Time (minutes)
1,3-Propanediol
Butane, 1-chloro-2-methyl-
Nonane
Butanoic acid, 4-hydroxy-
1,2,6-Hexanetriol
2,2'-Bifuran, octahydro-
Propanoic acid,2-methyl-,anhydride
2,3'-Bifuran, octahydro-
WA-1-GWM
B
5,8,11,14-Eicosatetraynoic acid, methyl ester
Heptane, 2,4-dimethyl-
Octane, 2-methyl-
Octane, 3-methyl-
Nonane
Propanoic acid, 2-methyl-, anhydride
5-Methyl-2-isopropyl-2-hexen-1-al
Decyl pentyl ether
3-Buten-2-ol, 1-bromo-2-methyl-
2-Oxo-n-valeric acid
Sulfurous acid, octyl 2-pentyl ester
Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester
2-Methylbutyl 8-methylnon-6-enoate
Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester
4-Methyl-3-heptanol, 2-methylpropionate (isomer 2)
Butyric acid, 2,2-dimethyl-, vinyl ester
Tetracosane
A-1-GWM
C
Heptane, 2,4-dimethyl-
Octane, 2-methyl-
Octane, 3-methyl-
Nonane
Butanoic acid, 4-hydroxy-
Boronic acid, diethyl-, methyl ester
2-Undecyne
Propanoic acid,2-methyl-, anhydride
Decyl pentyl ether
Butanoic acid,tridec-2-ynyl ester
Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester
Propanoic acid, 2-methyl-, anhydride
Tetrapentacontane
4-Methyl-3-heptanol, 2-methylpropionate (isomer 2)
1,5-Heptadiene-3,4-diol
Octacosane, 2-methyl-
WA-2-GWM
D
Heptane, 2,4-dimethyl-
Octane, 3-methyl-
Nonane
Butanoic acid, 4-hydroxy-
Butyric acid, neopentyl ester
2-Undecyne
Propanoic acid, 2-methyl-, anhydride
4-Hexen-3-ol, 2-methyl-
Decyl pentyl ether
Succinic acid, 3-methylbut-2-yl non-5-yn-3yl ester
Butanoic acid, tridec-2-ynyl ester
3-Methyl-hepta-1,6-dien-3-ol
Propanoic acid, 2-methyl-, 2-propenyl ester
1,7-Octadien-3-ol, 3,7,11-trimethyl-
2-Oxo-n-valeric acid
Butanoic acid, 2-propenyl ester
4-Methyl-3-heptanol, 2-methylpropionate
2,6-Octadiene-4,5-diol
Dotriacontane
A-2-GWM
E
Heptane, 2,4-dimethyl-
Octane, 2-methyl-
Octane, 3-methyl-
Nonane
Butanoic acid, 4-hydroxy-
Butyric acid, neopentyl ester
Propanoic acid, 2-methyl-, anhydride
Decyl pentyl ether
Butanoic acid, anhydride
4-Iodobutanal
Heptacosane
Heneicosane
Butanoic acid, tridec-2-ynyl ester
3-Methyl-hepta-1,6-dien-3-ol
Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester
2-Methyltetracosane
4-Methyl-3-heptanol, 2-methylpropionate (isomer 2)
Butyric acid, 2,2-dimethyl-, vinyl ester
Dotriacontane
Tetrapentacontane
WA-3-GWM
F
Heptane, 2,4-dimethyl-
Octane, 2-methyl-
Octane, 3-methyl-
Nonane
Butyric acid, neopentyl ester
2-Undecyne
4-Hexen-3-ol, 2-methyl-
2-Butyl-2,7-octadien-1-ol
Decyl pentyl ether
1,7-Octadien-3-ol, 2,6-dimethyl-
4-Iodobutanal
Succinic acid, 3-methylbut-2-yl non-5-yn-3-yl ester
2-Oxo-n-valeric acid
Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester
Butanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester
2-Heptan-3-ol, 4,5-dimethyl-
5-Octadecenoic acid, methyl ester, (Z)-
4-Methyl-3-heptanol, 2-methylpropionate
Propanoic acid, 2-methyl-, 2-propenyl ester
Tetrapentacontane
Dotriacontane
A-3-GWM
G
Heptane, 2,4-dimethyl-
Octane, 2-methyl-
Octane, 3-methyl-
Nonane
Butanoic acid, 4-hydroxy-
Butanoic acid, 2-methylbutyl ester
2-Undecyne
Propanoic acid, 2-methyl-, anhydride
Butanoic acid, anhydride
Decyl pentyl ether
1,7-Octadien-3-ol, 2,6-dimethyl-
2,5-Heptanedione, 3,3,6-trimethyl-
2-Oxo-n-valeric acid
Butanoic acid, tridec-2-ynyl ester
Butanoic acid, 2-butoxy-1-methyl-2-oxoethyl ester
Butanoic acid, tridec-2-ynyl ester
4-Methyl-3-heptanol, 2-methylpropionate (isomer 2)
Propanoic acid, 2-methyl-, 2-propenyl ester
1,5-Heptadiene-3,4-diol
Dotriacontane

Figure 6.29(i)

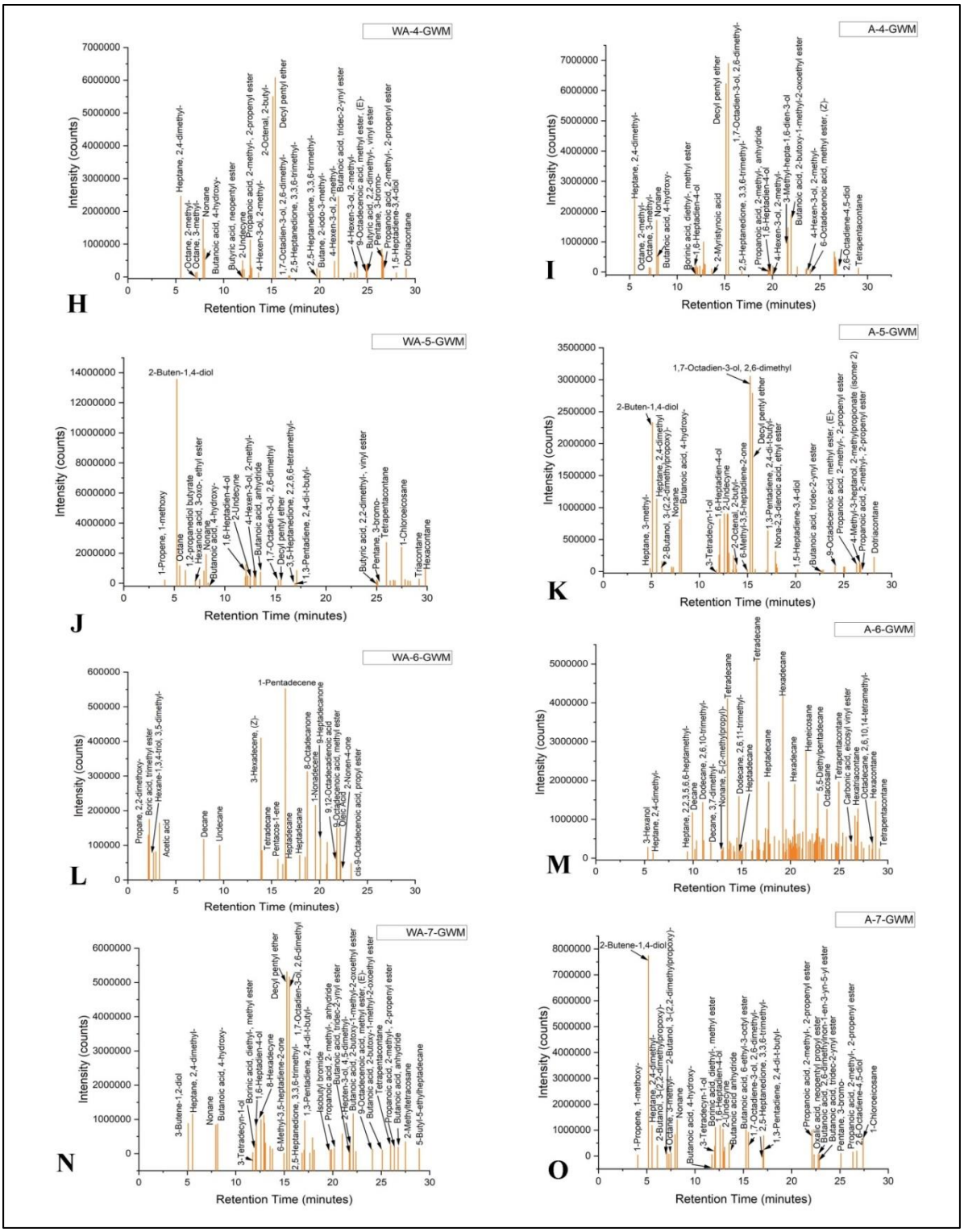


Figure 6.29(ii)

Figure 6.29 Gas chromatography and mass spectroscopy analysis for frass of *G. mellonella* fed with PP. Figure (i) (A) GC-MS graph for retention time and intensity for PP film (Control-PP); (B) GC-MS graph for frass of without antibiotic group for first instar fed with PP (WA-1-GWM); (C)The graph for frass of antibiotic fed first instar group (A-1-GWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-GWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-GWM) ; (F) The GC-MS graph of frass for third instar without antibiotic group fed with PP (WA-3-GWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-GWM). Figure (ii) (H) GC-MS graph of without antibiotic fourth instar group (WA-4-GWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-GWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-GWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-GWM); (L) The GC-MS graph of retention time and intensity for frass of without antibiotic sixth instar group (WA-6-GWM); (M) GC-MS graph for frass of antibiotic administered sixth instar group (A-6-GWM); (N) The GC-MS graph for frass without antibiotic seventh instar group (WA-7-GWM); (O) The GC-MS graph of retention time and intensity for frass of antibiotic fed of seventh instar group (A-7-GWM)

Plastic Consumption by *Achroia grisella*

LDPE- For a period of two days, the naive LDPE films were exposed to a sample of fifty larval instars from each group of lesser wax moth larvae, as well as the control population. The amount of plastic consumed by the larvae, their survival rate, the SEM of the exposed PP film, and the GC-MS of the greater wax worms' frass were all noted after the experiment was completed.

Plastic Consumption Rate- The plastic consumption rate of the *A. grisella* fed on the naive and plastic film was recorded after the exposure of the insects to the LDPE film for two days to the larvae administered without and with antibiotics. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The plastic consumption rate for LDPE for *A. grisella* by various groups- WA-1-LWM, A-1-LWM, WA-2-LWM, A-2-LWM, WA-3-LWM, A-3-LWM, WA-4-LWM, A-4-LWM, WA-5-LWM and A-5-LWM is 0, 0, 0, 0.05 ± 0.09, 0.18 ± 0.18, 0.05 ± 0.09, 0.91 ± 0.54, 0.32 ± 0.12, 7.51 ± 7.02, 2.77 ± 2.8 %, respectively (Figure 6.30).

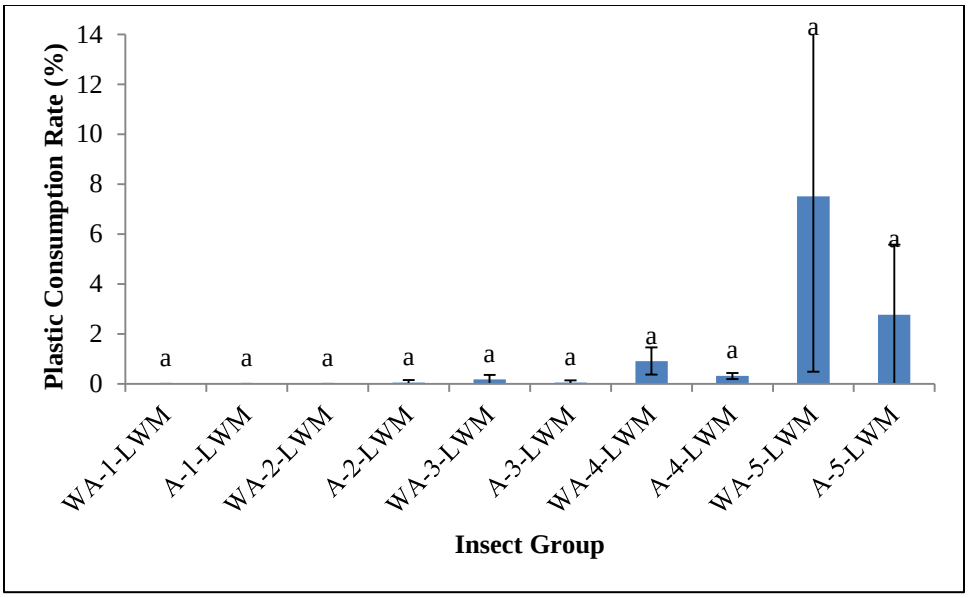


Figure 6.30 Plastic consumption rate of gut microbiota dependent and independent *A. grisella* larvae. The graph represents plastic consumption rate of LDPE by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). Note: WA-1-LWM= without antibiotic first instar group fed with naive LDPE film, A-1-LWM= antibiotic administered first instar group fed with naive LDPE film, WA-2-LWM= without antibiotic second instar group fed with naive LDPE film, A-2-LWM= antibiotic administered second instar group fed with naive LDPE film, WA-3-LWM= without antibiotic third instar group fed with naive LDPE film, A-3-LWM= antibiotic administered third instar group fed with naive LDPE film, WA-4-LWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive LDPE film, WA-5-LWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive LDPE film

Insect Survival Rate- The insect survival rate of the *A. grisella* fed on the naive plastic film was recorded after the exposure of the wax worms to the LDPE film for two days. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated LDPE for *A. grisella* is given by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM and A-5-LWM is 100 ± 0, 35.33 ± 9.23, 27.33 ± 11.71, 100 ± 0, 53.33 ± 9.01, 53.33 ± 15.14, 100 ± 0, 76 ± 8.71, 82.66 ± 9.45, 100 ± 0, 82 ± 10.58, 88 ± 2, 100 ± 0, 92 ± 5.29, 78.66 ± 16.28 %, respectively (Figure 6.31).

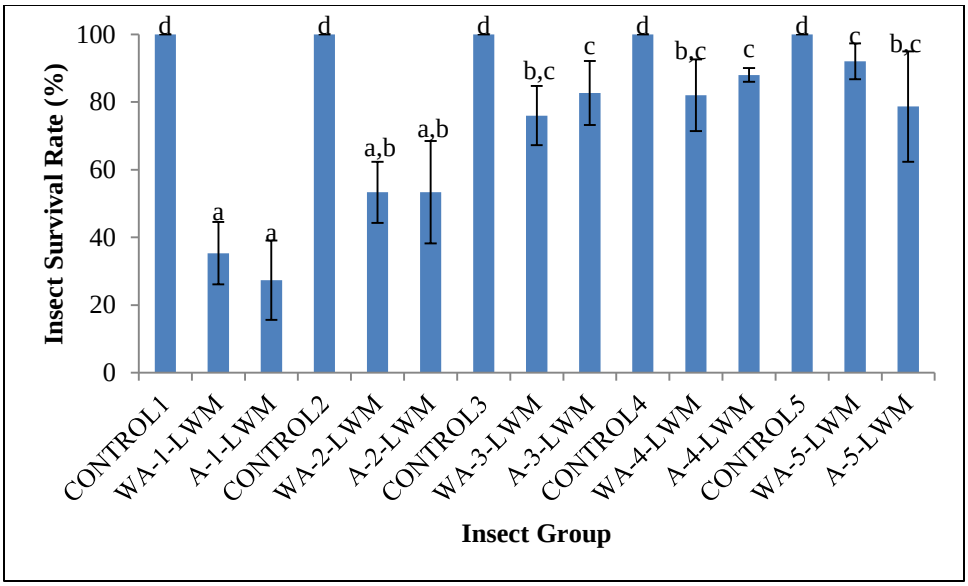


Figure 6.31 Insect survival rate when fed LDPE film. The graph represents with and without gut microbiota *A. grisella* insects survived when fed on naive LDPE film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-d). Note: Control1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive LDPE film, A-1-LWM= antibiotic administered first instar group fed with naive LDPE film, Control2= control group of second instar, WA-2-LWM= without antibiotic second instar group fed with naive LDPE film, A-2-LWM= antibiotic administered second instar group fed with naive LDPE film, Control3= control group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive LDPE film, A-3-LWM= antibiotic administered third instar group fed with naive LDPE film, Control4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive LDPE film, Control5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive LDPE film

SEM- Scanning electron microscopy of the leftover consumed LDPE film was performed for the larvae of lesser wax moth. The SEM naive LDPE depicted clear surface without any structural modifications on the topography of the film (Figure 6.17). No minute structural modifications were observed in without antibiotic and with antibiotic first instar stage (Figure 6.32(i) A and B). A large pit and ridges was observed in LDPE film for without antibiotic second instar group (WA-2-LWM) (Figure 6.32(i) C). As compared to WA-2-LWM group, the antibiotic fed second instar group depicted roughness on the surface (Figure 6.32(i) D). Some roughness on the surface is present on the LDPE film consumed by without antibiotic third instar group (WA-3-LWM) (Figure 6.32(ii) E). Some surface roughness is visible in the SEM images of with antibiotic group of third

instar (A-3-LWM) (Figure 6.32(ii) F). For without antibiotic fourth instar (WA-4-LWM) some degradation on the edges of the polymer film is visible (Figure 6.32(ii) G). Some surface roughness is visible on the center and edges of the LDPE film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-LWM) (Figure 6.32(ii) H). Large hole is visible in SEM image for without antibiotic fifth instar (WA-5-LWM) (Figure 6.32(ii) I). In the antibiotic fed fifth instar group (A-5-LWM) disintegration of plastics and some surface roughness is visible on the LDPE film in the SEM images (Figure 6.32(ii) J).

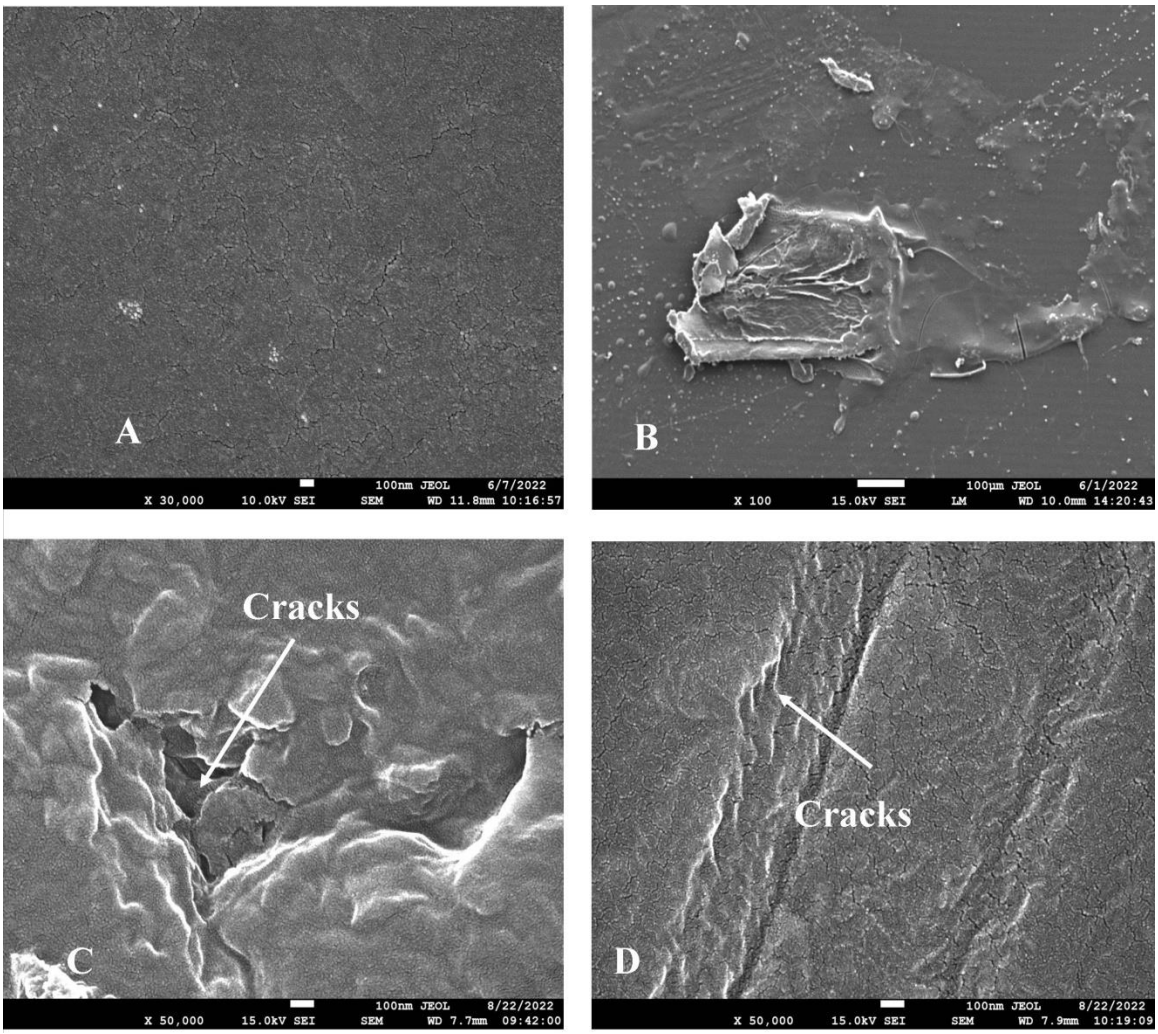


Figure 6.32(i)

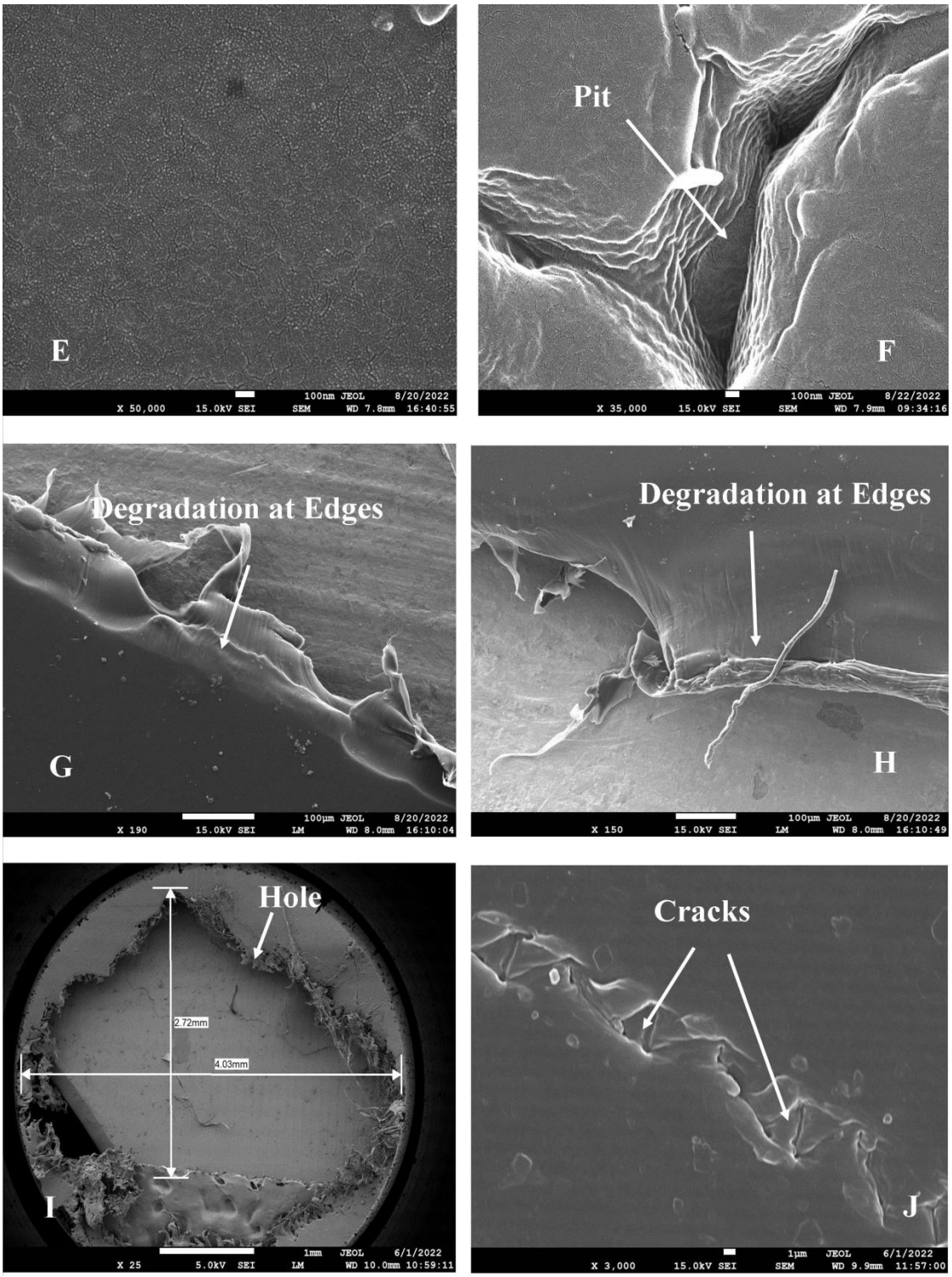


Figure 6.32(ii)

Figure 6.32 Scanning electron microscopic image of leftover remains LDPE film consumed by *A. grisella.* Figure (i) (A) LDPE film consumed by without antibiotic group of first instar (WA-1-LWM); (B) LDPE film consumed by antibiotic fed group of first instar (A-1-LWM); (C) remains of LDPE film consumed by without antibiotic group of second instar (WA-2-LWM); (D) LDPE film consumed by antibiotic administered group of second instar (A-2-LWM); Figure (ii) (E) remains of LDPE film consumed by without antibiotic group of third instar (WA-3-LWM); (F) LDPE film consumed by antibiotic fed group of third instar (A-3-LWM); (G) LDPE film consumed by without antibiotic group of fourth instar (WA-4-LWM); (H) remains of LDPE film consumed by with antibiotic group of fourth instar (A-4-LWM); (I) LDPE film consumed by without antibiotic group of fifth instar (WA-5-LWM); (J) remains of LDPE film consumed by with antibiotic group of fifth instar (A-5-LWM)

GC-MS- The wax moth larvae's frass sample was exposed to gas chromatography and mass spectroscopy to confirm that the plastics the caterpillar had ingested were fragmented down or otherwise biodegraded into small, biodegradable compounds by the larvae.

In Control-LDPE, showed compounds Octane (5.546 minutes- retention time), Octane, 4-methyl- (7.094), Octane, 2-methyl- (7.136), Octane, 3-methyl- (7.284), Nonane (7.936), 2,2'-Bifuran, octahydro- (12.037), Heptadecane (18.825), Heneicosane (21.087), Hexadecanamide (24.850), Eicosane (25.881), Tetrapentacontane (26.495), Heneicosane (28.322), 6,6-Diethylhoctadecane (28.952) (Figure 6.33(i)A).

For WA-1-LWM group the chemical peaks for compounds present in the frass are named as 2-Pentanol, 4-methyl (5.729), 5-t-Butyl-hexa-3,5-dien-2-one (17.006), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.527),6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.016), Hexadecane (18.843), Docosane, 1,22-dibromo (19.681), Heneicosane (21.104), Isophytol (21.175), 3-Buten-2-ol, 1-bromo-2-methyl- (21.373), Pentane, 3-bromo- (22.130), Isopentyl n-hexyl disulfide (23.085), Eicosane (23.155) (Figure 6.33(ii)B).

In A-1-LWM group the chemical peaks for compounds present in the frass are named as Succinic acid, hept-2-yl pent-4-en-1-yl ester (4.081), Nonanoyl chloride (4.180), 3-Hexanone (5.270), 2-Hexanone, 5-methyl- (5.360), Carbonic acid, octyl prop-1-en-2-yl ester (5.399), Valeric acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (5.485), 3-Hexanol (5.616), 2-Hexanol (5.707), Di-n-decylsulfone (9.844), Dodecane, 2,6,11-trimethyl- (14.569), Hexadecane (17.467), Dodecane, 2,6,11-trimethyl- (18.036), Methoxyacetic

acid, 4-hexadecyl ester (18.165), Eicosane, 1-iodo (20.003), Hexadecane (21.105), Heptadecane, 4-methyl- (21.168), Valeric acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester (23.585), Hexacontane (24.310), 11-Methyl-13-tetradecen-1-ol acetate (27.910), (Figure 6.33(ii)C).

For WA-2-LWM group the chemical peaks for compounds present in the frass are named as 3-Hexanone (5.290), 2-Hexanone (5.391), 3-Hexanol (5.625), 2-Hexanol (5.719), Sulfurous acid, pentyl undecyl ester (17.466), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.522), 2-Isopropyl-4-methylhex-2-enal (17.657), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (18.160), Octadecane, 5-methyl- (20.003), Phosphonoacetic Acid, 3TMS derivative (20.095), Dodecane, 4-methyl- (21.102), Sulfurous acid, octyl 2-pentyl ester (22.140), Tetracosane (22.265), Dodecane, 4-methyl- (23.159), 1,2-Propanediol, 3-(octadecyloxy)-, diacetate (27.350), Octadecanoic acid, 16-oxo-, methyl ester (28.344) (Figure 6.33(ii)D).

In A-2-LWM group the chemical peaks for compounds present in the frass are named as 3-Hexanone (5.290), Methyl Isobutyl Ketone (5.419), 3-Hexanol (5.610), 2-Pentanol, 4-methyl- (5.733), Dodecane, 4,6-dimethyl- (17.467), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.657), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.011), Heneicosane (20.003), 3-Ethyl-2,6,10-trimethylundecane (20.490), Hexadecane (21.103), 3-Buten-2-ol, 1-bromo-2-methyl- (21.165), Propanoic acid, 2-methyl-, anhydride (22.124), Tetracosane (22.265), Isopentyl n-hexyl disulfide (23.084), Tetracontane (23.154) (Figure 6.33(ii)E).

For WA-3-LWM group the chemical peaks for compounds present in the frass are named as 2-Hexanol (5.720), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.523), 2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl- (18.013), Hexadecane (18.839), Heneicosane (20.001), Heptadecane (21.101), Sulfurous acid, 2-pentyl tetradecyl ester (21.167), Heneicosane (22.149), Tetracosane (22.265), Hexadecanoic acid, ethyl ester (23.078), Heneicosane (23.151), Eicosane (25.023), Heptadecane (25.899), Tetrapentacontane (26.744) (Figure 6.33(iii)F).

In A-3-LWM group the chemical peaks for compounds present in the frass are named as 2,4-Pentanedione, 3,3-di-2-butenyl- (17.524), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.015), Hexadecane (18.842), Heptadecane (21.104), Butane, 1-bromo-2-methyl- (21.170), Propanoic acid, 2-methyl-, anhydride (22.127), Pentacosane (23.156) (Figure 6.33(iii)G).

For WA-4-LWM group the chemical peaks for compounds present in the frass are named as 3-Hexanol (5.620), 2-Hexanol (5.713), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.005), Eicosane (17.466), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.520), 2-Isopropyl-4-methylhex-2-enal (17.658), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.009), Hexadecane (18.840), Heneicosane (20.003), Sulfurous acid, octyl 2-pentyl ester (21.165), 3-Buten-2-ol, 1-bromo-2-methyl- (21.370), Butane, 2-bromo-2-methyl- (22.127), Tetracosane (22.265), Hexadecanoic acid, methyl ester (22.401), Carbonic acid, decyl octadecyl ester (22.877), Hexadecanoic acid, ethyl ester (23.082), 11-Methyltricosane (24.113), Dotriacontane (25.027), Tetracontane (26.746), Docosane, 1,22-dibromo- (28.343), Triacontane, 1-iodo- (29.222) (Figure 6.33(iii)H).

For A-4-LWM group the chemical peaks for compounds present in the frass are named as 3,5-Heptanedione, 2,2,4,6-tetramethyl- (5.280), Methyl Isobutyl Ketone (5.416), 3-Ethoxypropyl acetate (15.475), Carbamic acid, 2-(dimethylamino)ethyl ester (5.514), 3-Hexanol (5.622), 2-Pentanol, 4-methyl- (5.712), Acetic acid, 2,3-dibromo-4-methoxymethoxy-1-methyl-pent-2-enyl ester (5.830), Dodecane, 3-methyl- (9.860), Decane, 5-ethyl-5-methyl- (17.466), Hexadecane (18.846), Carbonic acid, eicosyl vinyl ester (18.927), Heptadecane (20.004), Heneicosane (21.106), Triacontane, 1-bromo- (22.262), Tetracosane (23.160), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (24.699), 11,13-Dimethyl-12-tetradecen-1-ol acetate (25.460) (Figure 6.33(iii)I).

In WA-5-LWM group the chemical peaks for compounds present in the frass are named as 2-Hexanone (5.082), 3-Hexanol (5.345), 2-Pentanol, 4-methyl- (5.451), Heptane, 2,4-dimethyl- (5.894), Octane, 4-methyl- (6.922), Nonane, 2-methyl- (9.203), Nonane, 2,5-dimethyl- (9.420), Decane (9.933), Decane, 5-methyl- (10.186), Nonane, 2,6-dimethyl-

(10.363), Dodecane, 2,6,10-trimethyl- (10.992), Dodecane, 4,6-dimethyl- (11.094), Decane, 3,7-dimethyl- (11.817), Heptadecane (12.929), Undecane, 3-methyl- (13.038), Tridecane (13.094), Dodecane (13.531), Undecane, 2,5-dimethyl- (13.731), Dodecane, 4-methyl- (13.871), Tetradecane (14.173), Pentadecane (14.263), 2,4-Dimethyldodecane (14.506), Dodecane, 2,6,11-trimethyl- (14.707), Dodecane, 4,6-dimethyl- (14.966), Tridecane (15.095), Dodecane, 4-methyl- (15.408), Heptadecane (16.031), Eicosane, 10-methyl- (16.130), Tetradecane (16.554), Tetradecane, 5-methyl- (16.655), Hexadecane (16.704), Heptadecane (16.769), Tetradecane, 4-methyl- (16.840), Heptadecane (17.239), Pentadecane (17.322), 2,6,10-Trimethyltridecane (17.379), Tetradecane, 5-methyl- (17.416), Pentadecane, 2,6,10,14-tetramethyl- (17.505), Eicosane (17.699), Hexadecane (17.760), Heptadecane (17.854), Heneicosane (18.360), Pentadecane, 3-methyl- (18.838), Hexadecane (19.214), Heneicosane (19.298), Heptadecane (19.545), Eicosane (19.585),Hexadecane (19.794), Pentadecane, 8-hexyl- (19.919), Eicosane, 2,4-dimethyl- (19.986), Heneicosane (20.027), Eicosane (20.090), Hexadecane (20.185), Octadecane, 5-methyl- (20.240), 2,6,10-Trimethyltridecane (20.298), Eicosane (20.371), Dotriacontane (20.434), Eicosane (20.485), Heneicosane (20.599), Tetrapentacontane (20.767), 2,4-Dimethyldodecane (20.900), Heptadecane, 8-methyl- (20.949), Tetradecanoic acid (21.190), Heptadecane, 3-methyl- (21.262), Heneicosane (21.596), Tetradecanal (21.819), Dotriacontane (22.008), Eicosane (22.045), 13-Methylheptacosane (22.226), Dotriacontane (22.433), Heneicosane (22.477), 5,5-Diethylpentadecane (22.526), 2,6,10-Trimethyltridecane (22.582), Tetrapentacontane (22.642), Heptadecane, 3-methyl- (22.736), Dotriacontane (22.823), Tetracosane (22.960), Tetrapentacontane (23.272), Eicosane (23.330), Triacontane, 1-iodo- (23.458), Eicosane (23.753), Dotriacontane (24.226), Tetracosane, 2,6,10,15,19,23-hexamethyl- (24.614), Tetrapentacontane (24.651), Tetracosane (24.713), Heptadecane, 3-methyl- (24.870), Tetrapentacontane (24.972), 6-Tetradecanesulfonic acid, butyl ester (25.275), Heneicosane (25.720), Dotriacontyl isopropyl ether (26.620), Tetracosane (26.857), Tetrapentacontane (26.930), Dotriacontane (27.294), Tetracosane (27.525),

Dotriacontane (28.080), Tetracosane (28.385), Tetracosane (28.437), Tetrapentacontane (28.650), Tetracosane (28.742), Tetrapentacontane (29.332) (Figure 6.33(iii)J).

For A-5-LWM group the chemical peaks for compounds present in the frass are named as 2-Hexanone (5.083), 3-Hexanol (5.346), 2-Pentanol, 4-methyl- (5.453), Heptane, 2,4-dimethyl- (5.895), Octane, 4-methyl- (6.922), Nonane, 2-methyl- (9.201), Nonane, 3-methyl- (9.339), Nonane, 2,5-dimethyl- (9.418), Decane (9.932), Decane, 5-methyl- (10.184), Nonane, 2,5-dimethyl- (10.290), Decane, 4-methyl- (10.362), Nonane, 5-(2-methylpropyl)- (10.854), Dodecane, 2,6,10-trimethyl- (10.990), Dodecane, 4,6-dimethyl- (11.093), Decane, 3,7-dimethyl- (11.815), Dodecane, 2,6,10-trimethyl- (11.915), Heptadecane (12.927), Undecane, 3-methyl- (13.036), Tridecane (13.091), Dodecane (13.529), Undecane, 4,6-dimethyl- (13.729), Dodecane, 4-methyl- (13.869), Tetradecane (14.171), Pentadecane (14.261), 2,4-Dimethyldodecane (14.504), Dodecane, 2,6,11-trimethyl- (14.704), Dodecane, 4,6-dimethyl- (14.964), Nonadecane (15.092), 2,6,10-Trimethyltridecane (15.186), Hexadecane (15.405), Heptadecane (16.029), Eicosane, 10-methyl- (16.128), Tetradecane (16.551),Tetradecane, 5-methyl- (16.650), Octadecane (16.702), Heptadecane (16.766), Tetradecane, 4-methyl- (16.838), Heptadecane (17.038), Heneicosane (17.237), Heptadecane (17.320), 2,6,10-Trimethyltridecane (17.376), Undecane, 2,4-dimethyl- (17.414), Dodecane, 2,6,11-trimethyl- (17.503), Hexadecane (17.696), Heneicosane (17.757), Heptadecane (17.851), Pentadecane (17.915), Hexadecane (18.357), Heneicosane (18.745), Pentadecane, 3-methyl- (18.835), 1-Heptadecene (19.117), Heptadecane (19.210), Heptadecane (19.299), Heneicosane (19.541), Eicosane (19.580), Hexadecane (19.791), Pentadecane, 8-hexyl- (19.916), 3-Ethyl-3-methylheptadecane (19.984), Heptadecane (20.024), Heneicosane (20.086), Pentadecane, 8-hexyl- (20.186), Octadecane, 5-methyl- (20.235), Eicosane (20.295), Heneicosane (20.370), Dotriacontane (20.431), Eicosane (20.482), Octacosane (20.598), Eicosane (20.763), 2,4-Dimethyldodecane (20.895), Dotriacontane (20.946), Heptadecane, 3-methyl-(21.260), Heneicosane (21.594), Eicosane (22.005), Heneicosane (22.045), Dotriacontane (22.211), Eicosane (22.431), Heptadecane (22.475), Dotriacontane (22.522), 2,6,10-Trimethyltridecane (22.579), Tetracosane (22.639),

Dotriacontane (22.734), Tetrapentacontane (22.820), Tetracosane (22.963), Dotriacontane (23.269), Octadecane, 1-iodo- (23.455), Heneicosane (23.750), Dotriacontane (24.223), Tetracosane (24.611), Eicosane (24.648), Tetrapentacontane (24.712), Dotriacontane (24.867), Tetrapentacontane (24.969), Eicosane (25.717), 2-Methylhexacosane (26.620), Tetrapentacontane (26.815), Dotriacontane (26.847), Tetrapentacontane (26.928), 2-Methyltetracosane (27.293), Heneicosane (27.524), Dotriacontane (28.078), Tetrapentacontane (28.385), Dotriacontane (28.438), Tetrapentacontane (28.753), 2-Methyltriacontane (29.151) (Figure 6.33(iii)K).

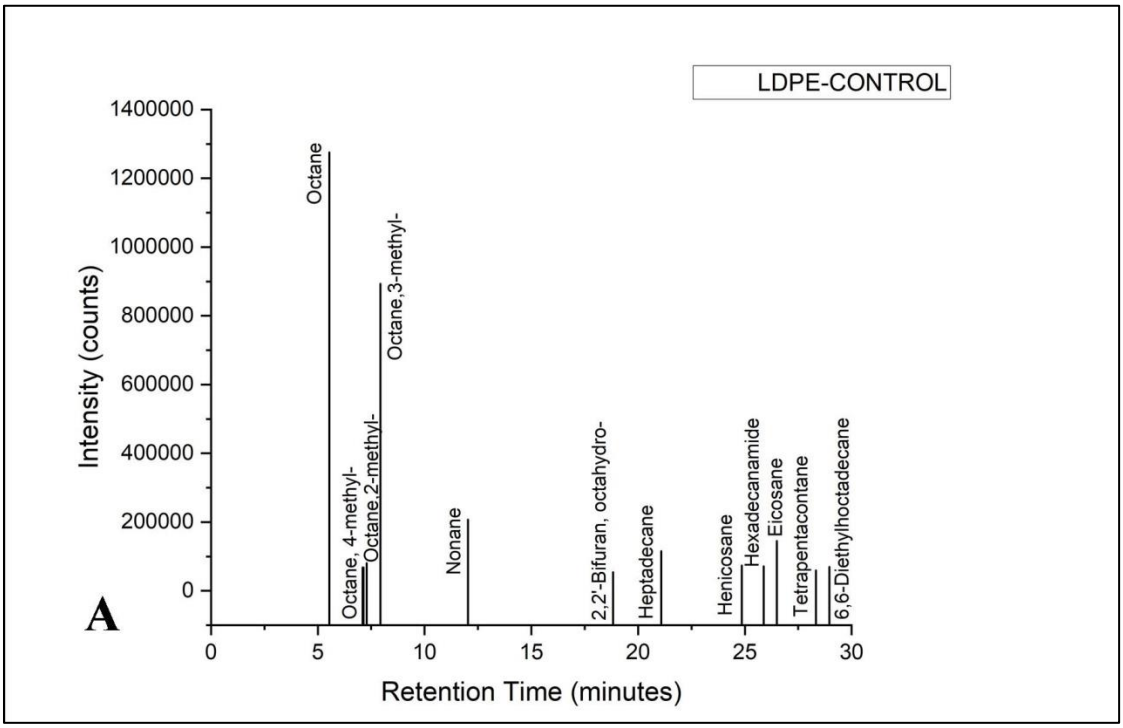


Figure 6.33(i)

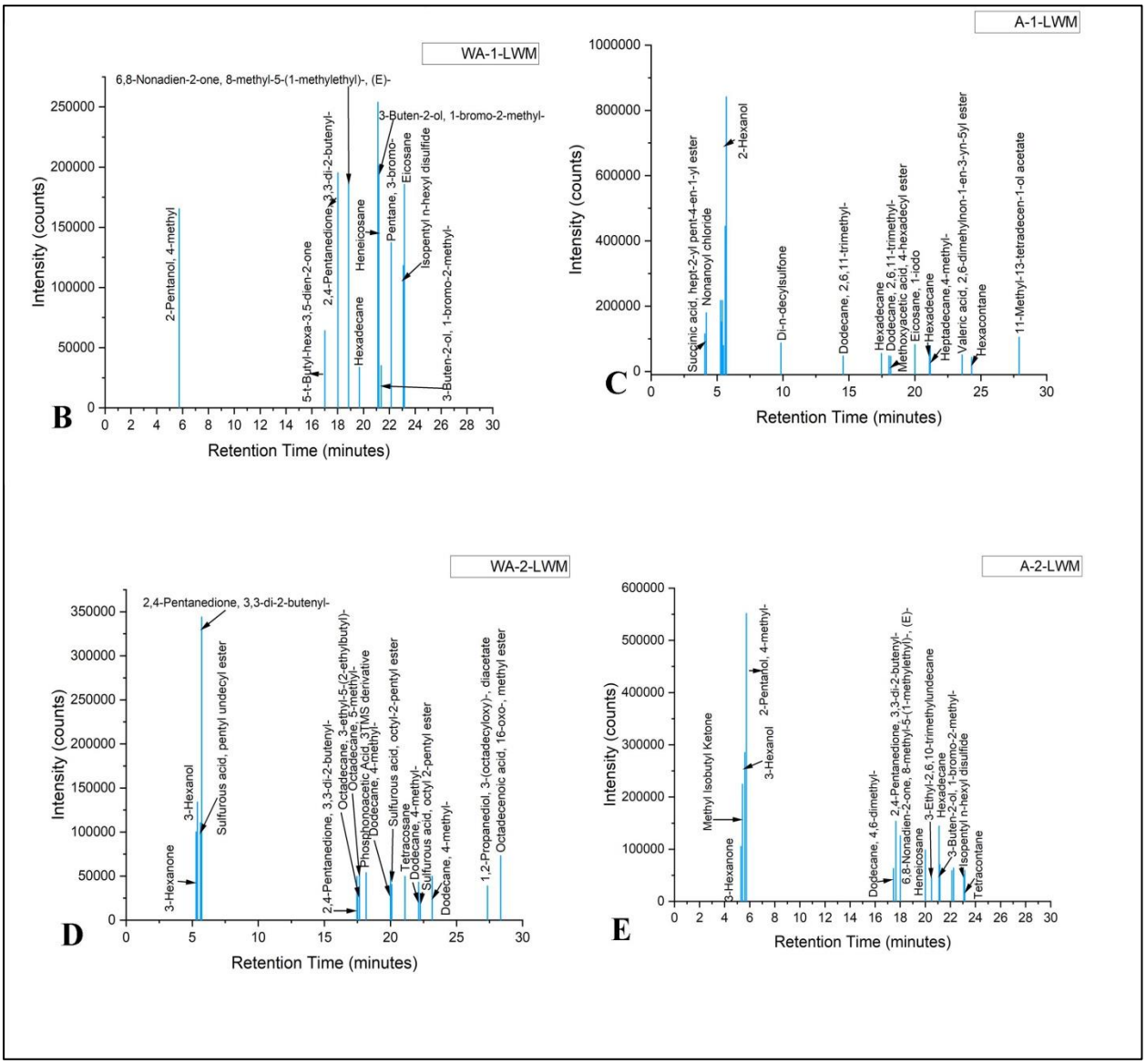


Figure 6.33(ii)

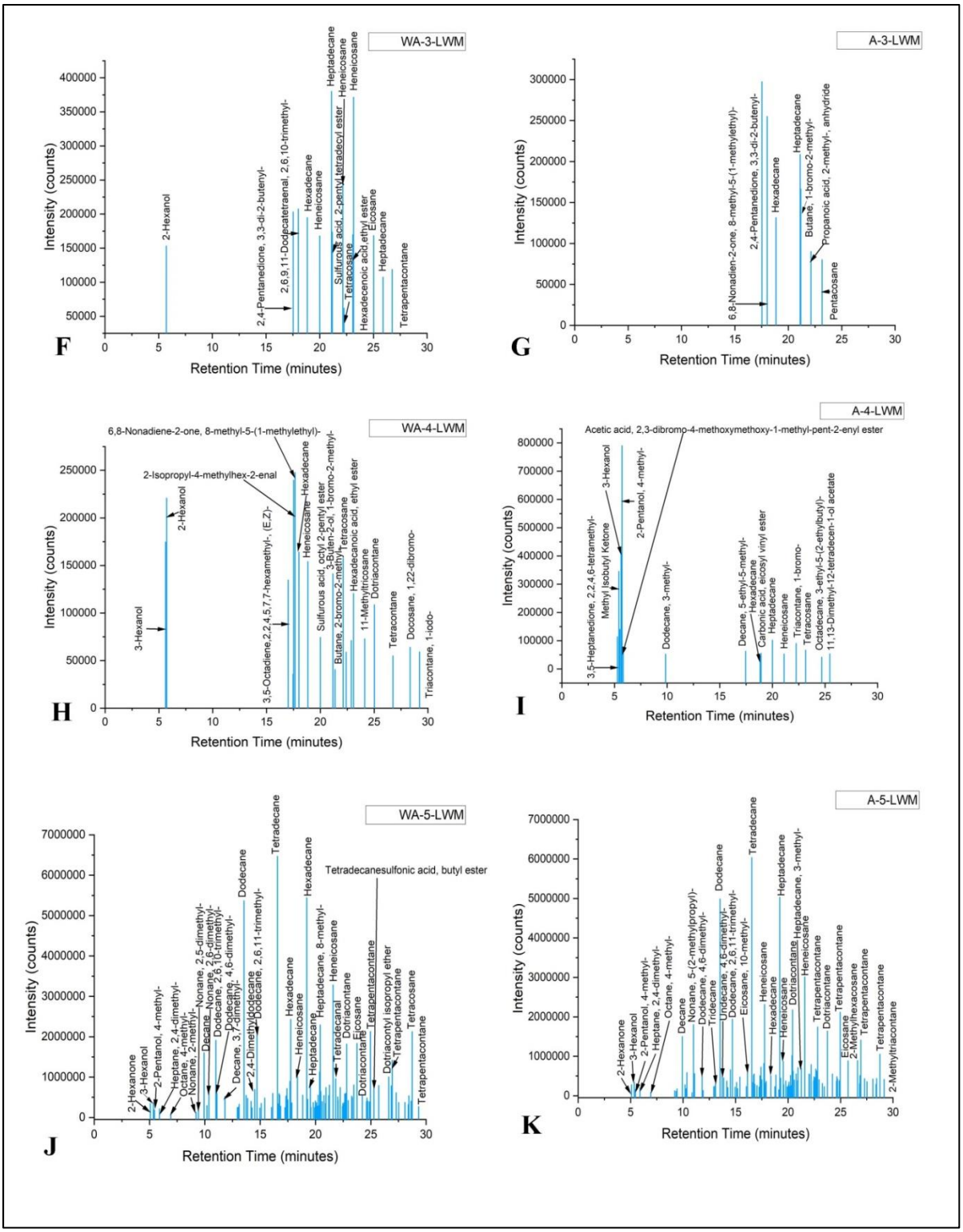


Figure 6.33(iii)

Figure 6.33 Gas chromatography and mass spectroscopy analysis for frass of *A. grisella* fed with LDPE. Figure (i) (A) GC-MS graph for retention time and intensity for LDPE film (Control-LDPE); Figure (ii) (B) GC-MS graph for frass of without antibiotic group for first instar fed with LDPE (WA-1-LWM); (C)The graph for frass of antibiotic fed first instar group (A-1-LWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-LWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-LWM). Figure (iii) (F) The GC-MS graph of frass for third instar without antibiotic group fed with LDPE (WA-3-LWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-LWM). (H) GC-MS graph of without antibiotic fourth instar group (WA-4-LWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-LWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-LWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-LWM)

High-Density Polyethylene (HDPE) – During a two-day period, fifty larval instars from each batch of lesser wax moth larvae, along with the control population, were exposed to the naive HDPE films. After the experiment's conclusion, data was collected on the larvae's intake of plastic, survival rate, and the SEM of the exposed PP film as well as the GC-MS of the greater wax worms' frass.

Plastic Consumption Rate- After the insects were exposed to the HDPE film for two days, the plastic consumption rate of *A. grisella* was recorded when they were fed on the naive plastic film. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The plastic consumption rate for HDPE for *A. grisella* by various groups- WA-1-LWM, A-1-LWM, WA-2-LWM, A-2-LWM, WA-3-LWM, A-3-LWM, WA-4-LWM, A-4-LWM, WA-5-LWM and A-5-LWM is 0, 0, 0, 0.79 ± 0.9, 0, 1.38 ± 1.23, 0.08 ± 0.15, 3.05 ± 2.19, 6.83 ± 6.54, 4.41 ± 1.9, 4.39 ± 1.93 %, respectively (Figure 6.34).

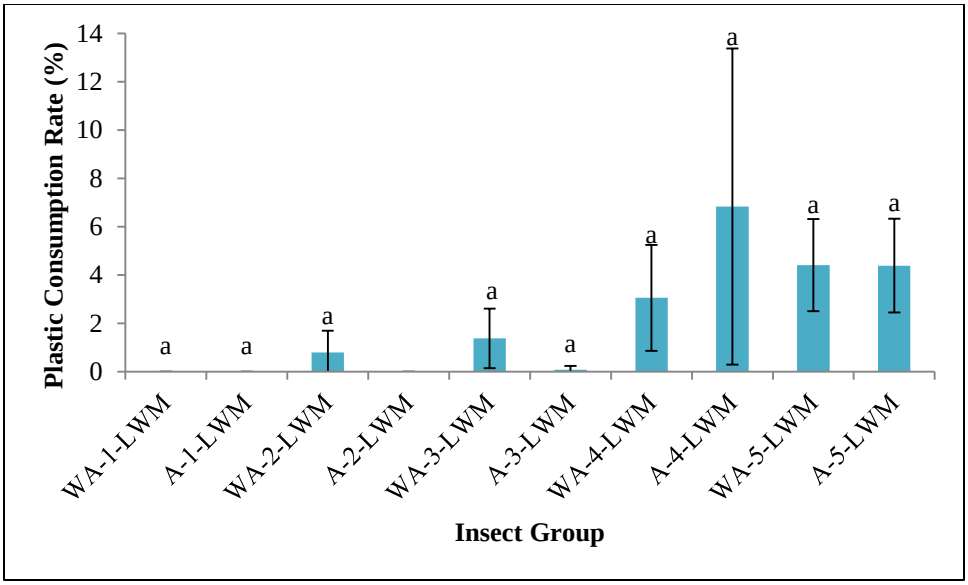


Figure 6.34 Plastic consumption rate of gut microbiota dependent and independent *A. grisella* larvae. The graph represents plastic consumption rate of HDPE by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). Note: WA-1-LWM= without antibiotic first instar group fed with naive HDPE film, A-1-LWM= antibiotic administered first instar group fed with naive HDPE film, WA-2-LWM= without antibiotic second instar group fed with naive HDPE film, A-2-LWM= antibiotic administered second instar group fed with naive HDPE film, WA-3-LWM= without antibiotic third instar group fed with naive HDPE film, A-3-LWM= antibiotic administered third instar group fed with naive HDPE film, WA-4-LWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive HDPE film, WA-5-LWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive HDPE film

Insects Survival Rate- The insect survival rate of the *A. grisella* was recorded after the exposure of the wax worms to the HDPE film for 48 hours. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive and pretreated HDPE for *A. grisella* is given by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM and A-5-LWM is 100 ± 0, 32 ± 14.42, 39.33 ± 23.007, 100 ± 0, 60 ± 12, 68.66 ± 11.01, 100 ± 0, 71.33 ± 6.11, 75.33 ± 11.71, 100 ± 0, 80.66 ± 4.16, 78.66 ± 4.16, 100 ± 0, 81.33 ± 7.02, 79.33 ± 13.01 %, respectively (Figure 6.35).

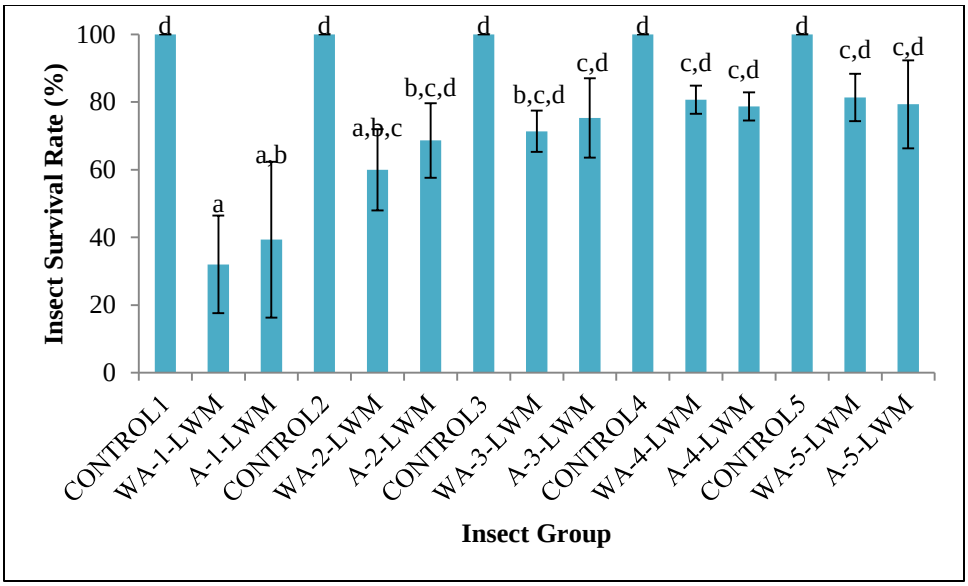


Figure 6.35 Insect survival rate when fed on HDPE film. The graph represents with and without gut microbiota *A. grisella* insects survived when fed on naive HDPE film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-d). Note: CONTROL1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive HDPE film, A-1-LWM= antibiotic administered first instar group fed with naive HDPE film, CONTROL2= control group of second instar, WA-2-LWM= without antibiotic second instar group fed with naive HDPE film, A-2-LWM= antibiotic administered second instar group fed with naive HDPE film, CONTROL3= control group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive HDPE film, A-3-LWM= antibiotic administered third instar group fed with naive HDPE film, CONTROL4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive HDPE film, CONTROL5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive HDPE film

SEM- Scanning electron microscopy of naive and consumed HDPE film was performed to visualise and compare the extent of biodegradation of the plastics by lesser wax worms. The SEM naive HDPE depicted clear surface without any structural modifications on the topography of the film (Figure 6.22). The minute structural modifications, ridges and pits were observed in without antibiotic first instar stage (WA-1-LWM) (Figure 6.36(i) A). On the surface topography of with antibiotic first instar stage (A-1-LWM) no structural modifications are visible in the SEM images (Figure 6.36(i) B). A hole was observed in HDPE film for without antibiotic second instar group (WA-2-LWM) (Figure 6.36(ii) C). As compared to WA-2-LWM group, the antibiotic fed second instar group depicted small hole on the surface (Figure 6.36(ii) D). Some holes are

present on the surface of the HDPE film insect consumed film by without antibiotic third instar group (WA-3-LWM) (Figure 6.36 (ii) E). Some large holes are visible in the SEM images of with antibiotic group of third instar (A-3-LWM) (Figure 6.36(ii) F). Holes are visible on the surface of the HDPE film consumed by without antibiotic fourth instar (WA-4-LWM) on the edges of the polymer film (Figure 6.36(iii) G). Large holes are visible on the center and edges of the HDPE film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-LWM) (Figure 6.36(iii) H). Large hole is visible on the edges of HDPE film in SEM image for without antibiotic fifth instar (WA-5-LWM) (Figure 6.36(iii) I). In the antibiotic fed fifth instar group (A-5-LWM) disintegration of plastics is visible at the edges of the film in the SEM images (Figure 6.36(iii) J).

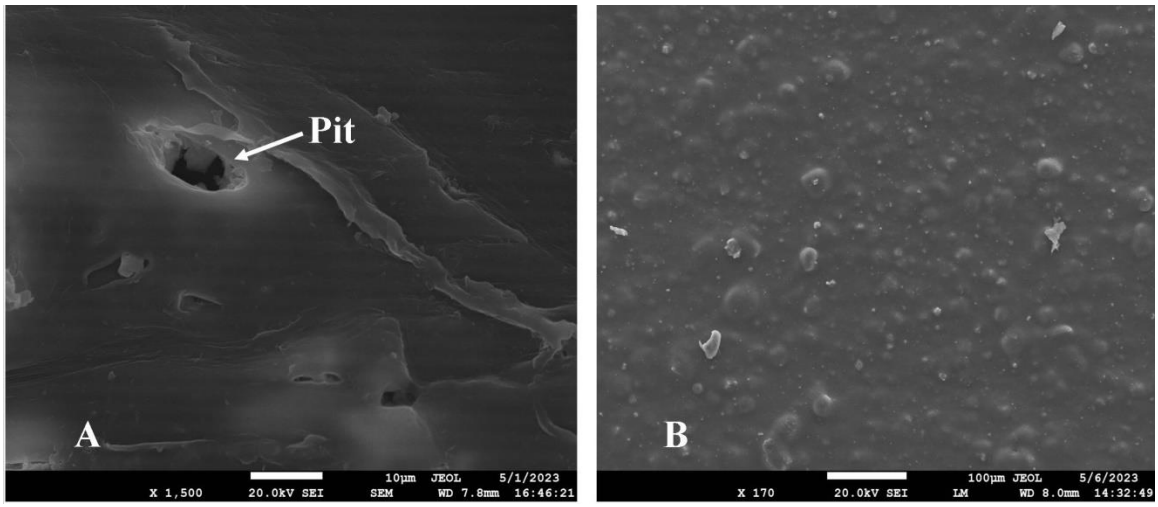


Figure 6.36(i)

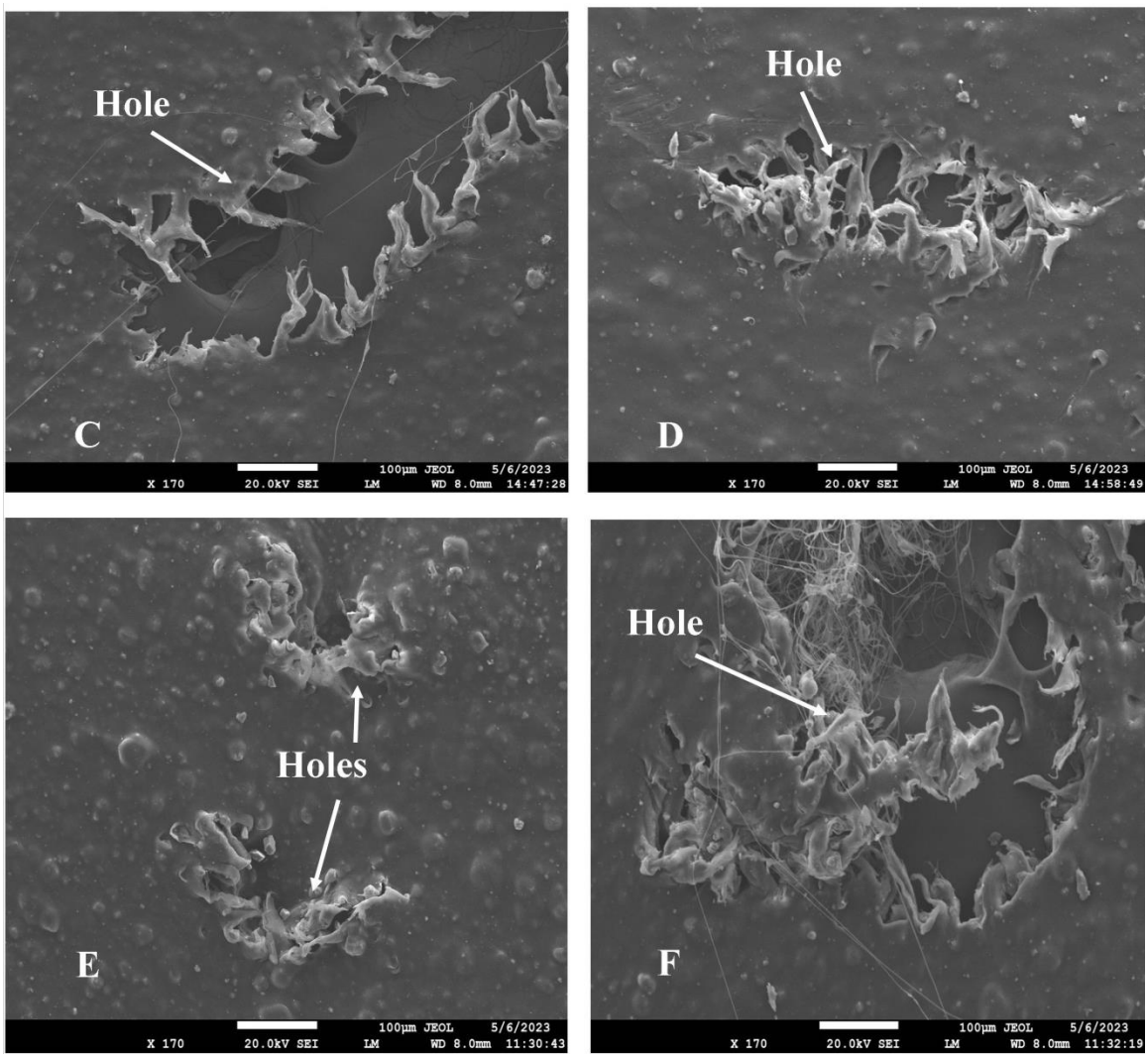


Figure 6.36(ii)

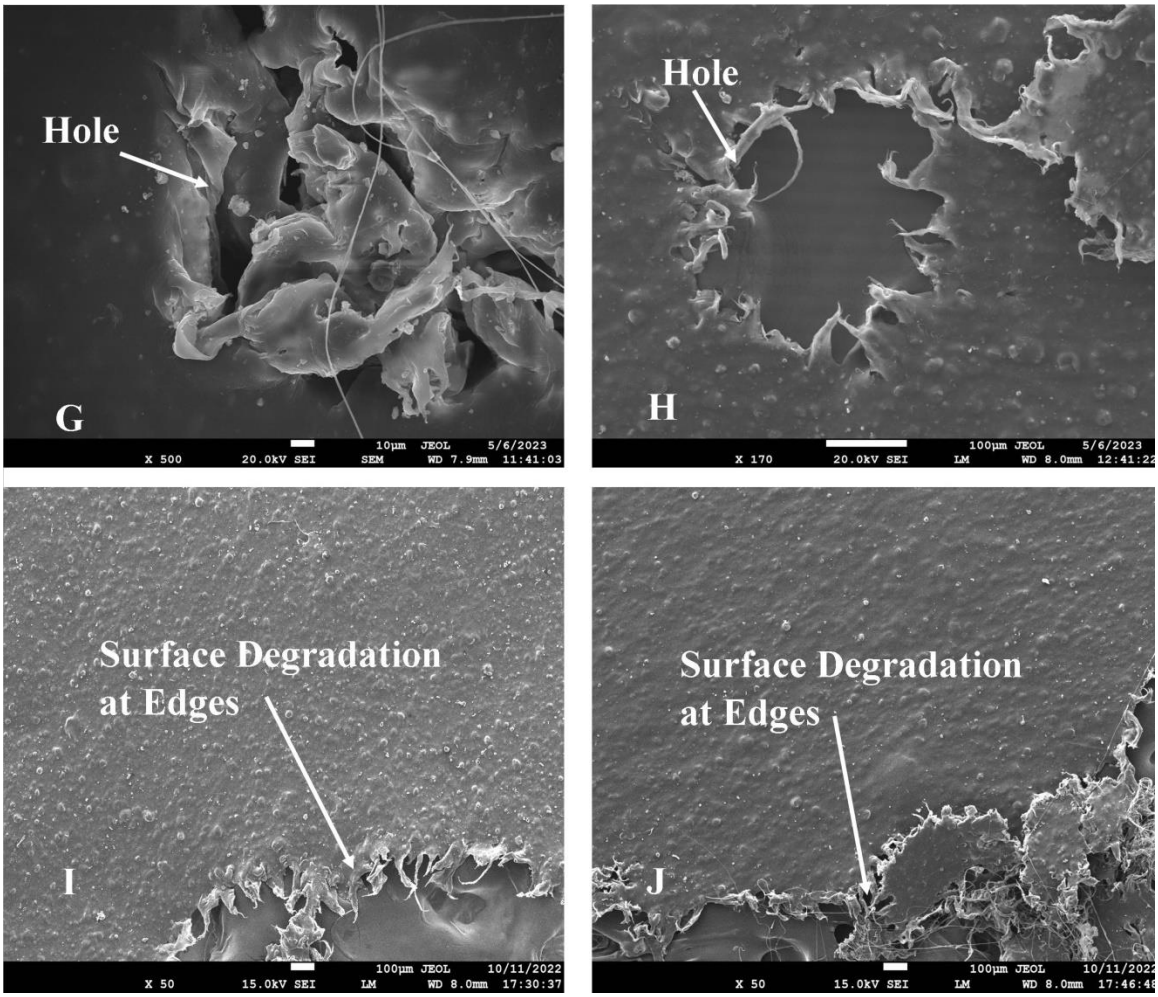


Figure 6.36(iii)

Figure 6.36 Scanning electron microscopic image of leftover remains HDPE film consumed by *A. grisella*. Figure (i) (A) HDPE film consumed by without antibiotic group of first instar (WA-1-LWM); (B) HDPE film consumed by antibiotic fed group of first instar (A-1-LWM); Figure (ii) (C) remains of HDPE film consumed by without antibiotic group of second instar (WA-2-LWM); (D) HDPE film consumed by antibiotic administered group of second instar (A-2-LWM); (E) remains of HDPE film consumed by without antibiotic group of third instar (WA-3-LWM); (F) HDPE film consumed by antibiotic fed group of third instar (A-3-LWM). Figure (iii) (G) HDPE film consumed by without antibiotic group of fourth instar (WA-4-LWM); (H) remains of HDPE film consumed by with antibiotic group of fourth instar (A-4-LWM); (I) HDPE film consumed by without antibiotic group of fifth instar (WA-5-LWM); (J) remains of HDPE film consumed by with antibiotic group of fifth instar (A-5-LWM)

GC-MS- Gas chromatography and mass spectroscopy were used to analyse the frass sample of wax moth larvae in order to verify that the plastics the caterpillar consumed

were disintegrated or otherwise biodegraded into tiny, biodegradable chemicals by the larvae.

In Control-HDPE compounds detected are Nonane (7.937), 2,2'-Bifuran, octahydro- (12.035), 2,2'-Bifuran, octahydro- (12.263), 2,3'-Bifuran, octahydro- (13.066), Furan, 2-butyltetrahydro- (13.185) , Furan, 2-butyltetrahydro- (22.114), Heneicosane (23.134), Hexadecanamide (24.829), Eicosane (25.882), Dotriacontane (26.725), Tetrapentacontane (27.234), Bis(2-ethylhexyl) phthalate (27.812), 2-Methylpentacosane (27.980), 3-Methylpentacosane (28.106), 3-Methylhexacosane (28.947), 5-Methylnonacosane (29.667), 2-Methylheptacosan (29.747) (Figure 6.37(i)A).

In WA-1-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.209), Phosphonoacetic Acid, 3TMS derivative (14.902), Tetradecane (16.320), 1,3-Pentadiene, 2,4-di-t-butyl- (17.136), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.531), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.019), Heptadecane (18.843), Heneicosane (21.105), 3-Buten-2-ol, 1-bromo-2-methyl- (21.180), Heptadecane (23.155) (Figure 6.37(i)B).

For A-1-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.187), Phosphonoacetic Acid, 3TMS derivative (14.895), Tetradecane (16.322), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.005), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.018), Heptadecane (18.843), Eicosane (20.004), Heneicosane (21.105), Isophytol (21.169), Propanoic acid, 2-methyl-, anhydride (22.131), Docosane, 1,22-dibromo- (22.273), Eicosane (23.154), 5-Butyl-5-ethylheptadecane (26.745) (Figure 6.37(i)C).

In WA-2-LWM group the chemical peaks for compounds present in the frass are named as Phosphonoacetic Acid, 3TMS derivative (14.896), 1,3-Pentadiene, 2,4-di-t-butyl- (17.135), 2,4-Pentanedione, 3,3-di-2-butenyl (17.529), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.018), Hexadecane (18.844), Heneicosane (20.006),

Hexadecane (21.106), Isophytol (21.175), Tetradecanal (21.308), Heneicosane (23.157), Nonacosane (25.027) (Figure 6.37(i)D).

For A-2-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.197), Phosphonoacetic Acid, 3TMS derivative (14.894), Tetradecane (16.320), 7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione (16.856), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.009), 1,3-Pentadiene, 2,4-di-t-butyl- (17.134), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.018), Isobutyl 8-methylnon-6-enoate (18.653), Hexadecane (18.844), Heneicosane (21.105), Butane, 1-bromo-2-methyl-, (S)- (21.169), Hexadecanal (21.306), Butane, 1-bromo-2-methyl-, (S)- (21.380), 3-Buten-2-ol, 1-bromo-2-methyl- (22.131), Eicosane (23.153), Heneicosane (25.026), Tetracontane (26.747), Dotriacontane, 1-iodo (28.344), Dotriacontane (29.221), (Figure 6.37(i)E).

For WA-3-LWM group the chemical peaks for compounds present in the frass are named as Phosphonoacetic Acid, 3TMS derivative (14.894), Tetradecane (16.322), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.013), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.531), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.020), Heptadecane (18.845), Heneicosane (21.107), 3-Buten-2-ol, 1-bromo-2-methyl (21.171), Hexadecanal (21.306), Butanoic acid, 2-propenyl ester (22.132), 9-Hexadecenoic acid, methyl ester, (Z)- (22.192), Ethyl 9-hexadecenoate (22.876), Isopentyl n-hexyl disulfide (23.084), 2-Methylhexacosane (23.157), 9-Octadecenoic acid (Z)-, methyl ester (24.114), Eicosane (25.026), 1,3-Propanediol, eicosyl ethyl ether (26.748), Dotriacontane (28.180), (Figure 6.37(ii)F).

In A-3-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.190), Phosphonoacetic Acid, 3TMS derivative (14.893), Tetradecane (16.320), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.009), 1,3-Pentadiene, 2,4-di-t-butyl- (17.133), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.017), Octadecane (18.841), Heneicosane (21.103), 3-Buten-2-ol, 1-bromo-2-methyl- (21.167), Hexadecanal (21.303), 3-Buten-2-ol, 1-bromo-2-methyl- (21.380),

Pentane, 3-bromo- (22.129), Octacosane, 1-iodo (22.270), Heneicosane (23.153), Dotriacontane (25.024), Eicosyl isopropyl ether (26.149), Tetracosane (26.745), Nonacosane (28.343), Tetrapentacontane (29.221) (Figure 6.37(ii)G).

For WA-4-LWM group the chemical peaks for compounds present in the frass are named as Phosphonoacetic Acid, 3TMS derivative (14.894), Tetradecane (16.321), Acetate, 4-(1,1-dimethylethyl)-1-methyl-4-penten-2-ynyl ester (17.011), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.532), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.019), Heptadecane (18.844), Hexadecane (20.006), Heneicosane (21.105), Isophytol (21.169), Hexadecanal (21.305), Propanoic acid, 2-methyl-, anhydride (22.130), 9-Hexadecenoic acid, methyl ester, (Z)- (22.193), Hexadecanoic acid, methyl ester (22.402), Ethyl 9-hexadecenoate (22.873), Dimethylmalonic acid, 3-methylbutyl nonyl ester (23.082), 1-Decanol, 2-hexyl (23.157), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (24.055), 9-Octadecenoic acid, methyl ester, (E)- (24.113), Methyl 9-cis,11-trans-octadecadienoate (24.665), Ethyl Oleate (24.723), 9-Octadecenoic acid (24.981), Heneicosane (25.025), Pentyl palmitoleate (25.244), 1,3-Propanediol, eicosyl ethyl ether (26.747), 9-Octadecenoic acid (Z)-, pentyl ester (26.906), Octyl tetradecyl ether (27.404), Nonacosane (27.553), Tetracontane (28.344), Dotriacontane (29.221), (Figure 6.37(ii)H).

In A-4-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.175), Phosphonoacetic Acid, 3TMS derivative (14.892), Tetradecane (16.319), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.008), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.017), 8-Hydroxy-2,2,8-trimethyldeca-5,9-dien-3-one (18.181), Nonadecane (18.842), Eicosane (20.004), Heneicosane (21.104), Butane, 1-bromo-2-methyl- (21.167), Hexadecanal (21.303), Butane, 1-bromo-2-methyl-, (S)- (21.371), Borane, diethyl[1-ethyl-2-(methoxymethyl)-1-butenyl]-, (Z)- (22.130), 9-Hexadecenoic acid, methyl ester, (Z)- (22.192), 2-Bromotetradecane (22.269), Ethyl 9-hexadecenoate (22.871), Heneicosane (23.152), 9-Octadecenoic acid, methyl ester, (E)- (24.111), 1-Norvaline, N-(2-methoxyethoxycarbonyl)-, tetradecyl ester (24.424), 5-Butyl-5-ethylheptadecane

(26.744), Tetrapentacontane (27.551), Nonacosane (28.340), Eicosane (29.218) (Figure 6.37(ii)I).

For WA-5-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.192), Phosphonoacetic Acid, 3TMS derivative (14.895), Tetradecane (16.322), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.010), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.534), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.019), Pentadecane (18.844), Heptadecane (20.005), Heneicosane (21.106), 3-Buten-2-ol, 1-bromo-2-methyl- (21.169), Hexadecanal (21.306), Butane, 1-bromo-2-methyl-, (S)- (21.380), Oxalic acid, neopentyl propyl ester (22.360), Isopentyl n-hexyl disulfide (23.088), Eicosane (23.156), 2-Methylhexacosane (24.115), Heneicosane (25.030), Nonacosane (26.749), Tetratetracontane (27.556), Nonacosane (28.345), Dotriacontane (29.223) (Figure 6.37(ii)J).

In A-5-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy- (8.189), Phosphonoacetic Acid, 3TMS derivative (14.890), Tetradecane (16.317), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.006), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.016), Hexadecane (18.841), Heptadecane (20.002), Heneicosane (21.102), 3-Buten-2-ol, 1-bromo-2-methyl- (21.166), Hexadecanal (21.303), 3-Buten-2-ol, 1-bromo-2-methyl (21.371), Pentane, 3-bromo- (22.129), Eicosane (23.152), 2-Methylhexacosane (24.110), Heneicosane (25.025), Dotriacontane (26.746), Eicosane (27.552), Dotriacontane (28.343), Eicosane (29.219) (Figure 6.37(ii)K).

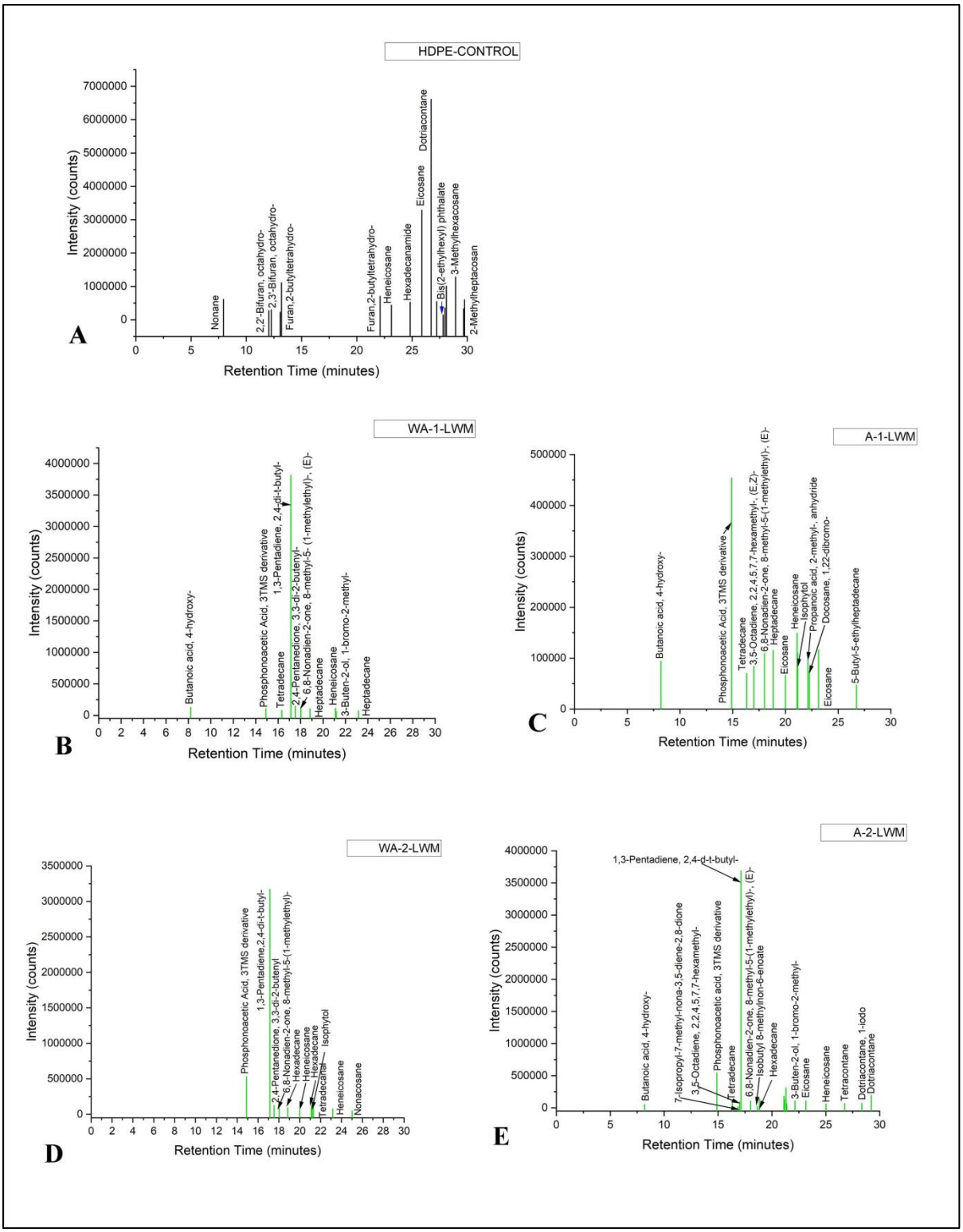

HDPE-CONTROL
Intensity (counts)
Retention Time (minutes)
Nonane
2,2'-Bifuran, octahydro-
2,3-Bifuran, octahydro-
Furan,2-butyltetrahydro-
Furan,2-butyltetrahydro-
Heneicosane
Hexadecanamide
Eicosane
Dotriacontane
Bis(2-ethylhexyl) phthalate
3-Methylhexacosane
2-Methylheptacosan
A
WA-1-LWM
Butanoic acid, 4-hydroxy-
Phosphonoacetic Acid, 3TMS derivative
1,3-Pentadiene, 2,4-di-t-butyl-
Tetradecane
2,4-Pentanedione, 3,3-di-2-butenyl-
6,8-Nonadien-2-one, 8-methyl-5- (1-methylethyl)-, (E)-
Heptadecane
Heneicosane
3-Buten-2-ol, 1-bromo-2-methyl-
Heptadecane
B
A-1-LWM
Butanoic acid, 4-hydroxy-
Phosphonoacetic Acid, 3TMS derivative
Tetradecane
3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-
6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-
Heptadecane
Eicosane
Heneicosane
Isophytol
Propanoic acid, 2-methyl-, anhydride
Docosane, 1,22-dibromo-
Eicosane
5-Butyl-5-ethylheptadecane
C
WA-2-LWM
Phosphonoacetic Acid, 3TMS derivative
1,3-Pentadiene,2,4-di-t-butyl-
2,4-Pentanedione, 3,3-di-2-butenyl
6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-
Hexadecane
Heneicosane
Hexadecane
Tetradecane
Isophytol
Heneicosane
Nonacosane
D
A-2-LWM
1,3-Pentadiene, 2,4-d-t-butyl-
Butanoic acid, 4-hydroxy-
7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione
3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-
Phosphonoacetic acid, 3TMS derivative
Tetradecane
6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-
Isobutyl 8-methylnon-6-enoate
Hexadecane
3-Buten-2-ol, 1-bromo-2-methyl-
Eicosane
Heneicosane
Tetracontane
Dotriacontane, 1-iodo
Dotriacontane
E

Figure 6.37(i)

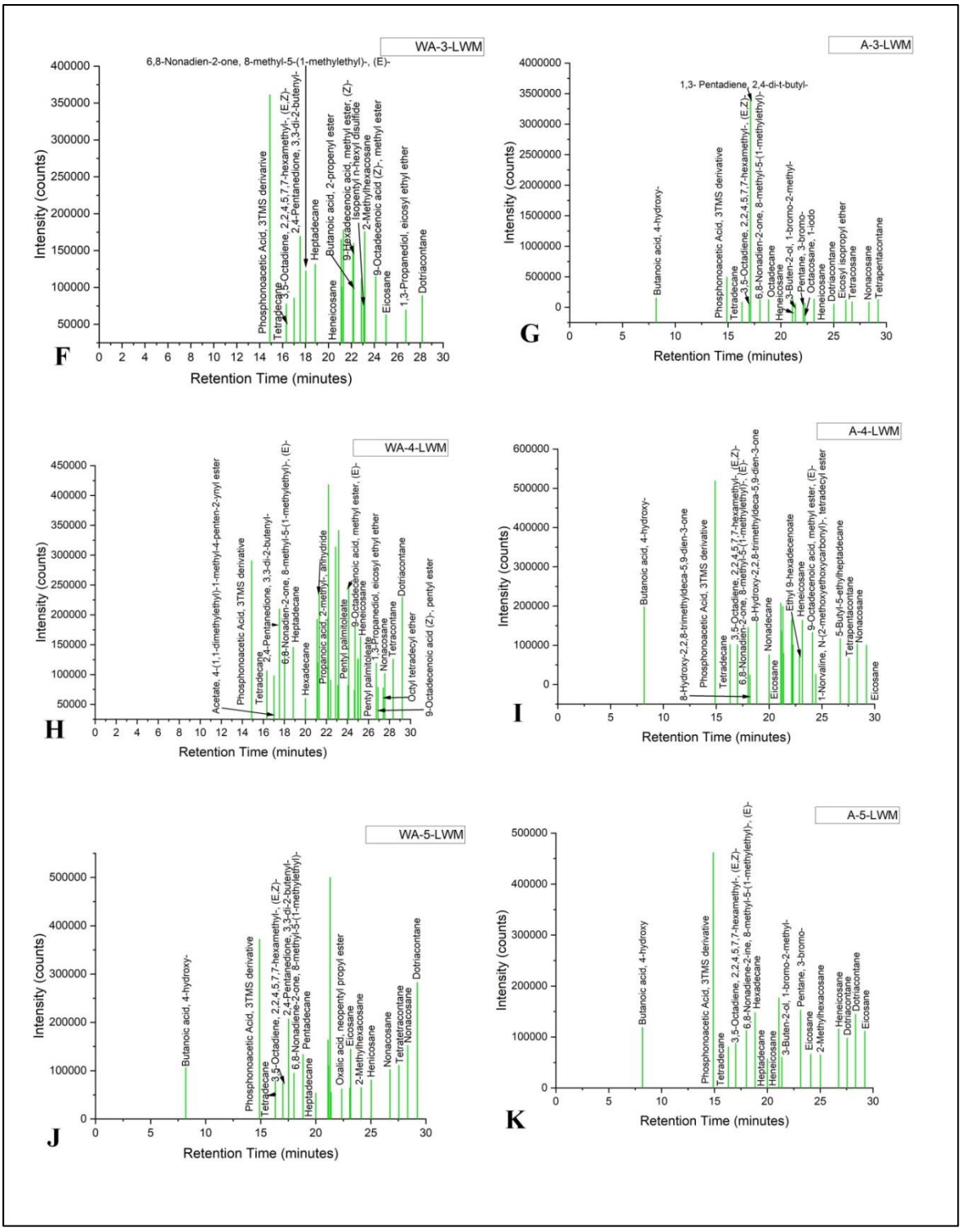


Figure 6.37(ii)

Figure 6.37 Gas chromatography and mass spectroscopy analysis for frass of *A. grisella* fed with HDPE. Figure (i) (A) GC-MS graph for retention time and intensity for HDPE film (Control-HDPE); (B) GC-MS graph for frass of without antibiotic group for first instar fed with HDPE (WA-1-LWM); (C)The graph for frass of antibiotic fed first instar group (A-1-LWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-LWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-LWM). Figure (ii) (F) The GC-MS graph of frass for third instar without antibiotic group fed with HDPE (WA-3-LWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-LWM). (H) GC-MS graph of without antibiotic fourth instar group (WA-4-LWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-LWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-LWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-LWM)

Polypropylene (PP) - For two days, the naive PP films were exposed to all larval instar stages of the lesser wax moth, with a sample size of fifty larval instars in each group. After the completion of the experiment the plastic consumption and survival rate of the larvae, the SEM of the exposed PP film and GC-MS of the frass of the greater wax was recorded.

Plastic Consumption Rate- The plastic consumption rate of the *A. grisella* was recorded after the exposure of the larval insects to the PP film for 48 hours to the larvae administered without and with antibiotics. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The plastic consumption rate for PP for *A. grisella* by various groups- WA-1-LWM, A-1-LWM, WA-2-LWM, A-2-LWM, WA-3-LWM, A-3-LWM, WA-4-LWM, A-4-LWM, WA-5-LWM and A-5-LWM is 0.24 ± 0.22, 0.16 ± 0.18, 0.24 ± 0.22, 0, 3.59 ± 3.44, 0.44 ± 0.45, 1.6 ± 1.39, 1.65 ± 0.56, 0.9 ± 0.9, 3.66 ± 3.08 %, respectively (Figure 6.38).

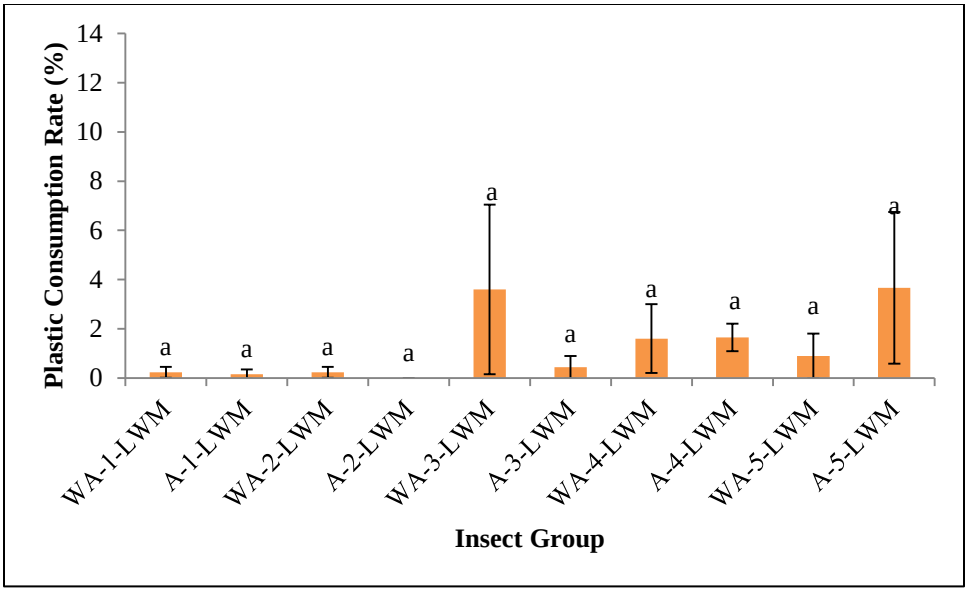


Figure 6.38 Plastic consumption rate of gut microbiota dependent and independent *A. grisella* larvae. The graph represents plastic consumption rate of PP by *A. grisella*. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a). Note: WA-1-LWM= without antibiotic first instar group fed with naive PP film, A-1-LWM= antibiotic administered first instar group, WA-2-LWM= without antibiotic second instar group fed with naive PP film, A-2-LWM= antibiotic administered second instar group fed with naive PP film, WA-3-LWM= without antibiotic third instar group fed with naive PP film, A-3-LWM= antibiotic administered third instar group fed with naive PP film, WA-4-LWM= without antibiotic fourth instar group fed with naive PP film, A-4-LWM= antibiotic administered fourth instar group fed with naive PP film, WA-5-LWM= without antibiotic fifth instar group fed with naive PP film, A-5-LWM= antibiotic administered fifth instar group fed with naive PP film

Table 6.8 Comparative data on plastic consumption (mg/day/insect) by *Achroia grisella* for study the degradation of different types of plastic films

Insect Group	LDPE	HDPE	PP
WA-1-LWM	0	0	0.003 ± 0.005
A-1-LWM	0	0	0.003 ± 0.005
WA-2-LWM	0	0.03 ± 0.03	0.006 ± 0.01
A-2-LWM	0.003 ± 0.005	0	0
WA-3-LWM	0.01 ± 0.01	0.05 ± 0.05	0.08 ± 0.12
A-3-LWM	0.003 ± 0.005	0.003 ± 0.003	0.01 ± 0.01
WA-4-LWM	0.05 ± 0.02	0.11 ± 0.07	0.03 ± 0.03
A-4-LWM	0.03 ± 0.01	0.28 ± 0.36	0.03 ± 0.01

WA-5-LWM	0.42 ± 0.62	0.2 ± 0.1	0.02 ± 0.02
A-5-LWM	0.15 ± 0.21	0.2 ± 0.1	0.08 ± 0.13

Insects Survival Rate- After a 48-hour exposure of the wax worms to the PP film, the insect survival rate of the *A. grisella* fed plastic film was noted. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The Insect survival rate for naive PP for *A. grisella* is given by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM and A-5-LWM is 100 ± 0, 66.66 ± 12.85, 50.66 ± 19.00, 100 ± 0, 80.66 ± 17.009, 88.66 ± 7.57, 100 ± 0, 98 ± 3.46, 96.66 ± 3.05, 100 ± 0, 78.66 ± 18.9, 93.33 ± 5.03, 100 ± 0, 80 ± 14.42, 99.33 ± 1.15 %, respectively (Figure 6.39).

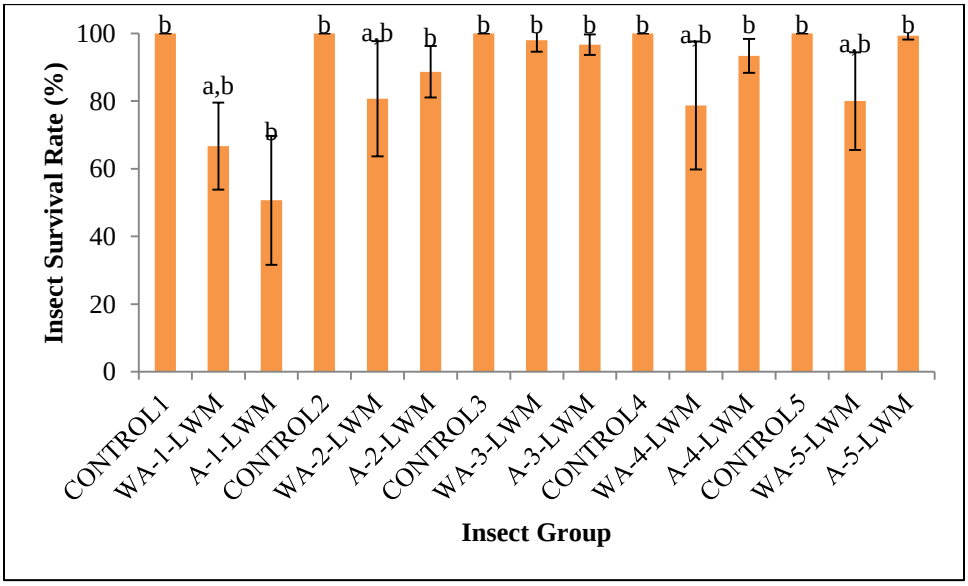


Figure 6.39 Insect survival rate when fed PP film. The graph represents with and without gut microbiota *A. grisella* insects survived when fed on naive PP film. Data represents mean ± S.D. (n = 3); $p<0.05$ (One - way ANOVA followed by Tukey's significant difference test which is represented from a-b). Note: CONTROL1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive PP film, A-1-LWM= antibiotic administered first instar group, CONTROL2= control group of second instar, WA-2-LWM= without antibiotic second instar group fed with naive PP film, A-2-LWM= antibiotic administered second instar group fed with naive PP film, CONTROL3= control group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive PP film, A-3-LWM= antibiotic administered third instar group fed with naive PP film, CONTROL4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive PP film, A-4-LWM= antibiotic administered

fourth instar group fed with naive PP film, CONTROL5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive PP film, A-5-LWM= antibiotic administered fifth instar group fed with naive PP film

SEM- The Scanning electron microscopy of naive and consumed PP film was performed to visualise and compare the extent of biodegradation of the plastics by lesser wax worms. The SEM naive PP depicted clear surface without any structural modifications on the topography of the film (Figure 6.27). There were no structural modifications observed in without antibiotic first instar stage (WA-1-LWM) as compared to naive PP film (Figure 6.40(i) A). On the surface topography of with antibiotic first instar stage (A-1-LWM) no structural modifications were visible in the SEM images (Figure 6.40(i) B). The pits and holes were observed in PP film for without antibiotic second instar group (WA-2-LWM) (Figure 6.40(i) C). The antibiotic fed second instar group (A-2-LWM) depicted holes and pits on the surface of plastic film as compared to naive PP film (Figure 6.40(i) D). Some holes and pits are present on the PP film insect consumed film by without antibiotic third instar group (WA-3-LWM) (Figure 6.40(i) E). Some pits are visible in the SEM images on the PP film consumed by with antibiotic group of third instar (A-3-LWM) (Figure 6.40(i) F). A large disintegrated area is visible on the surface of the PP film consumed by without antibiotic fourth instar (WA-4-LWM) on the edges of the plastic film (Figure 6.40(ii) G). The SEM image of the film revealed the presence of large disintegrated area on the edges of the PP film remains consumed by antibiotic administered fourth instar of greater wax moth (A-4-LWM) (Figure 6.40(ii) H). A large disintegrated area is visible on the edges of PP film in SEM image for without antibiotic fifth instar (WA-5-LWM) (Figure 6.40(ii) I). In the antibiotic fed fifth instar group (A-5-LWM) disintegration of plastics is visible at the edges of the film in the SEM images (Figure 6.40(ii) J).

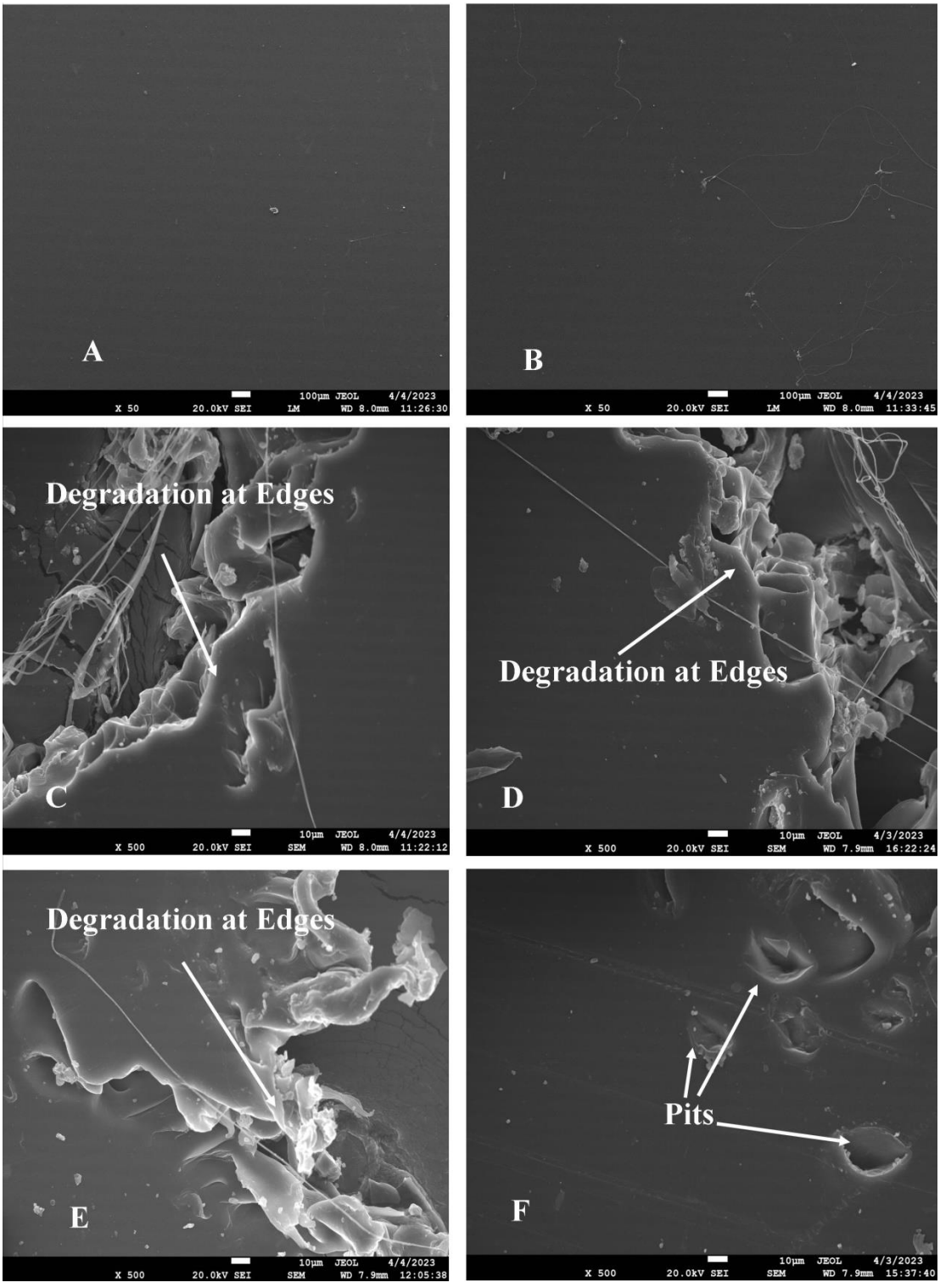

A
X 50 20.0kV SEI LM 100µm JEOL WD 8.0mm 4/4/2023 11:26:30
B
X 50 20.0kV SEI LM 100µm JEOL WD 8.0mm 4/4/2023 11:33:45
Degradation at Edges
C
X 500 20.0kV SEI SEM 10µm JEOL WD 8.0mm 4/4/2023 11:22:12
Degradation at Edges
D
X 500 20.0kV SEI SEM 10µm JEOL WD 7.9mm 4/3/2023 16:22:24
Degradation at Edges
E
X 500 20.0kV SEI SEM 10µm JEOL WD 7.9mm 4/4/2023 12:05:38
Pits
F
X 500 20.0kV SEI SEM 10µm JEOL WD 7.9mm 4/3/2023 15:37:40

Figure 6.40(i)

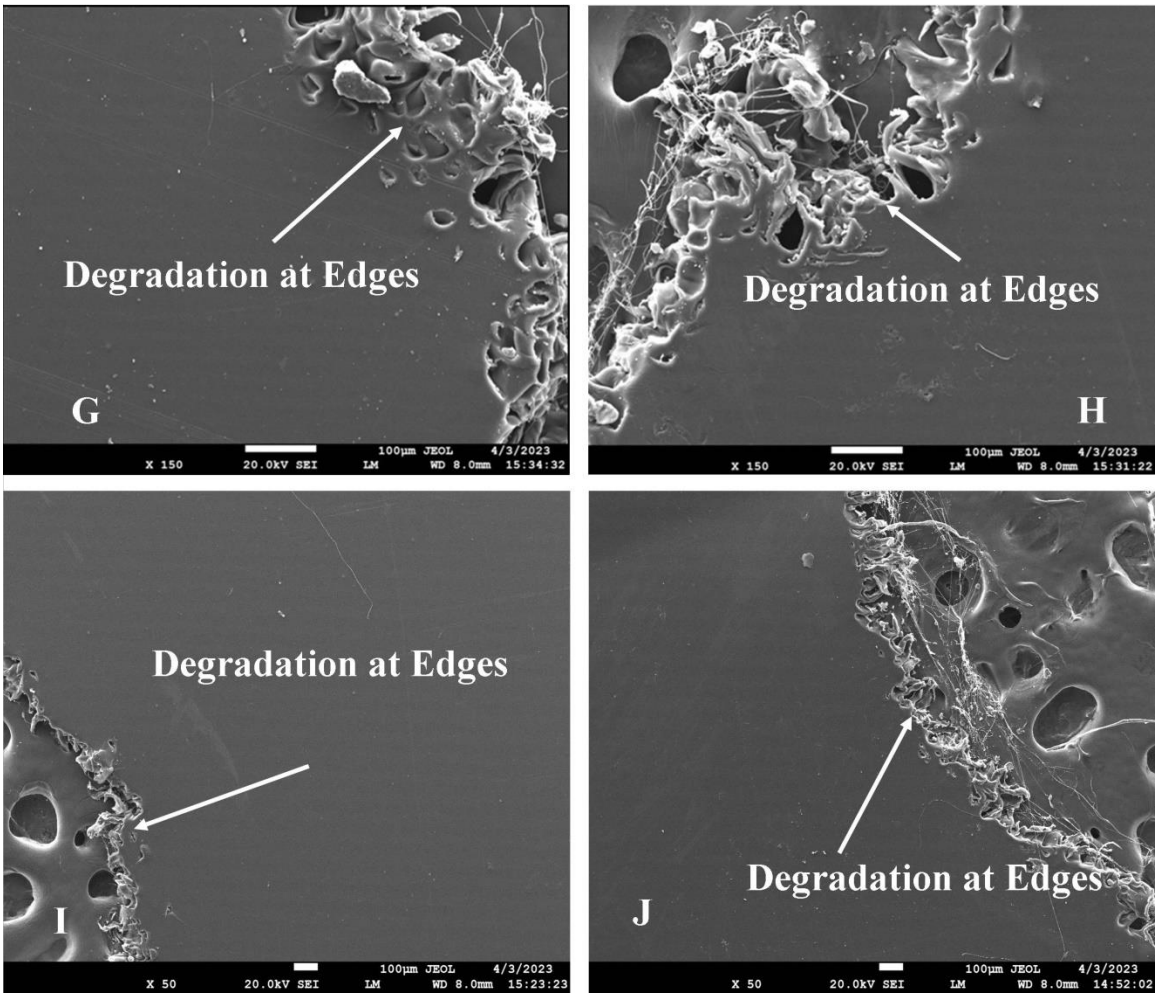


Figure 6.40(ii)

Figure 6.40 Scanning electron microscopic image of leftover remains PP film consumed by *A. grisella*. Figure (i) (A) PP film consumed by without antibiotic group of first instar (WA-1-LWM); (B) PP film consumed by antibiotic fed group of first instar (A-1-LWM); (C) remains of PP film consumed by without antibiotic group of second instar (WA-2-LWM); (D) PP film consumed by antibiotic administered group of second instar (A-2-LWM); (E) remains of PP film consumed by without antibiotic group of third instar (WA-3-LWM); (F) PP film consumed by antibiotic fed group of third instar (A-3-LWM); Figure (ii) (G) PP film consumed by without antibiotic group of fourth instar (WA-4-LWM); (H) remains of PP film consumed by with antibiotic group of fourth instar (A-4-LWM); (I) PP film consumed by without antibiotic group of fifth instar (WA-5-LWM); (J) remains of PP film consumed by with antibiotic group of fifth instar (A-5-LWM)

GC-MS- The frass sample of wax moth larvae was analysed using gas chromatography and mass spectroscopy to confirm that the caterpillar's consumption of plastics resulted in their disintegration or biodegradation into small, biodegradable compounds by the larvae.

In Control-PP, the GC-MS graph revealed compounds- 1,3-Propanediol (6.397), Butane, 1-chloro-2-methyl- (7.269), Nonane (7.926), Butanoic acid, 4-hydroxy- (8.168), 1,2,6-Hexanetriol (9.128), 2,2'-Bifuran, octahydro- (12.033), 2,2'-Bifuran, octahydro- (12.261), Propanoic acid, 2-methyl-, anhydride (12.897), 2,3'-Bifuran, octahydro- (13.063), Furan, 2-butyltetrahydro- (13.183), Butanoic acid, 2-propenyl ester (13.843), Hexanedioic acid, bis(2-ethylhexyl) ester (26.641), Propanoic acid, 2-methyl-, 2-propenyl ester (26.738) (Figure 6.41(i)A).

For WA-1-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy (8.177), Dodecane (13.450), Phosphonoacetic Acid, 3TMS derivative (14.889), Tetradecane (16.315), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.007), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.022), Hexadecane (18.839), Heptadecane (20.001), Heneicosane (21.101), Butane, 1-bromo-2-methyl- (21.166), Hexadecanal (21.301), 3-Buten-2-ol, 1-bromo-2-methyl- (21.372), Pentane, 3-bromo- (22.128), Methyl hexadec-9-enoate (22.195), 2-Methylhexacosane (22.871), Heneicosane (23.150), Eicosane (25.022) (Figure 6.41(ii)B).

In A-1-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 4-hydroxy (8.177), Dodecane (13.451), Phosphonoacetic Acid, 3TMS derivative (14.890), Tetradecane (16.316), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.008), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.018), Hexadecane (18.839), Heneicosane (21.101), Isophytol (21.166), Butane, 1-bromo-2-methyl (21.372), Propanoic acid, 2-methyl-, 2-propenyl ester (22.129), Heneicosane (23.150), 3,5-Decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl (23.496), Heneicosane (25.022) (Figure 6.41(ii)C).

For WA-2-LWM group the chemical peaks for compounds present in the frass are named as Dodecane (13.452), Dodecane, 2,6,11-trimethyl- (14.568), Phosphonoacetic Acid, 3TMS derivative (14.891), Tetradecane (16.316), Butylphosphonic acid, isobutyl undecyl ester (16.367), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.007), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.020), Hexadecane (18.839), Heneicosane (20.000), Heptadecane (21.100), Isophytol (21.165), 3-Buten-2-ol, 1-bromo-2-methyl- (21.370), Butanoic acid, 2-propenyl ester (22.129), Octadecanoic acid, ethenyl ester (22.195), Hexacosane, 1-iodo (22.269), Sulfurous acid, octadecyl 2-propyl ester (22.872), Tetracosane (24.109), Eicosyl isopropyl ether (24.328), Heneicosane (25.023), Tetrapentacontane (28.343) (Figure 6.41(ii)D).

In A-2-LWM group the chemical peaks for compounds present in the frass are named as Butanoic acid, 2-methyl- (7.615), Butanoic acid, 4-hydroxy- (8.186), Hydroperoxide, 1-methylhexyl (9.192), Dodecane (13.451), Phosphonoacetic Acid, 3TMS derivative (14.890), Tetradecane (16.316), Butyl triacontyl ether (16.450), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.008), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.023), Hexadecane (18.840), 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (19.337), Hexadecane (21.103), 3-Buten-2-ol, 1-bromo-2-methyl- (21.167), Propanoic acid, 2-methyl-, 2-propenyl ester (22.131), Oxalic acid, neopentyl propyl ester (22.356), 2-Methylhexacosane (22.875), Nonacosane (28.342) (Figure 6.41(ii)E).

In WA-3-LWM group the chemical peaks for compounds detected in the frass are named as Butanoic acid, 4-hydroxy (8.182), Methyl 2-methoxypropenoate (9.188), Nonane, 5-butyl- (11.839), Dodecane (13.453), Phosphonoacetic Acid, 3TMS derivative (14.892), Tetradecane (16.318), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.009), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.021), Hexadecane (18.841), Heneicosane (21.104), Butane, 1-bromo-2-methyl- (21.374), Propanoic acid, 2-methyl-, 2-propenyl ester (22.131), 2-Butanol, 3-(2,2-dimethylpropoxy)- (22.357), Ethyl 9-hexadecenoate (22.874), Eicosane (26.745) (Figure 6.41(ii)F).

For A-3-LWM group the chemical peaks for compounds present in the frass are named as Methyl 2-butoxyacetate (7.642), Butanoic acid, 2-methyl- (8.202), Pentanoic acid, 2-propenyl ester (9.012), Dodecane (13.456), Phosphonoacetic Acid, 3TMS derivative (14.893), Tetradecane (16.320), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.012), 2,4-Pentanedione, 3,3-di-2-butenyl- (17.540), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.024), Hexadecane (18.844), 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (19.341), Heneicosane (21.107), 3-Buten-2-ol, 1-bromo-2-methyl- (21.171), Butane, 1-bromo-2-methyl-, (S)- (21.376), 3-Ethyl-2-pentadecanone (21.499), Propanoic acid, 2-methyl-, 2-propenyl ester (22.135), Butanoic acid, anhydride (22.360), 1-Decanol, 2-hexyl- (22.878), Heneicosane (25.030), Tetrapentacontane (26.749) (Figure 6.41(ii)G).

In WA-4-LWM group the chemical peaks for compounds detected in the frass are named as Butanoic acid, 4-hydroxy- (8.177), Dodecane (13.454), Phosphonoacetic Acid, 3TMS derivative (14.891), Tetradecane (16.318), 9-Undecene-4,6-dione, 3,5,7,10-tetramethyl- (16.451), 5-t-Butyl-hexa-3,5-dien-2-one (17.011), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.024), Hexadecane (18.843), 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (19.337), Heneicosane (21.105), 3-Buten-2-ol, 1-bromo-2-methyl- (21.170), Hexadecanal (21.305), 3-Ethyl-2-pentadecanone (21.496), Propanoic acid, 2-methyl-, 2-propenyl ester (22.133), 9-Hexadecenoic acid, methyl ester, (Z)- (22.194), Borane, diethyl[1-ethyl-2-(methoxymethyl)-1-butenyl]-, (Z)- (22.359), Hexadecanoic acid, methyl ester (22.403), Ethyl 9-hexadecenoate (22.875), Hexadecanoic acid, ethyl ester (23.082), Pentadecane, 8-hexyl (23.155), 3,5-Decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl- (23.500), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (24.058), 9-Octadecenoic acid, methyl ester, (E)- (24.116), Linoleic acid ethyl ester (24.674), Ethyl Oleate (24.728), Methyl 5-eicosenoate (25.900), (Z)-Ethyl heptadec-9-enoate (26.453), Dotriacontane, 1-iodo (28.348) (Figure 6.41(iii)H).

For A-4-LWM group the chemical peaks for compounds present in the frass are named as 3-Isopropoxypropylamine (7.605), Butanoic acid, 2-methyl- (8.180), Propanoic acid, 2-methyl-, 2-propenyl ester (8.416), Pentanoic acid, 2-propenyl ester (8.997), Methyl 2-

methoxypropenoate (9.188), Dodecane (13.455), Phosphonoacetic Acid, 3TMS derivative (14.893), Tetradecane (16.319), 9,9-Dibutoxynonanoic acid, butyl ester (16.453), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (17.012), 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)- (18.027), Hexadecane (18.844), 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (19.340), Heneicosane (21.106), 3-Buten-2-ol, 1-bromo-2-methyl (21.171), Hexadecanal (21.306), Butane, 1-bromo-2-methyl-, (S)- (21.376), Pentane, 3-bromo- (22.133), 9-Hexadecenoic acid, methyl ester, (Z)- (22.195), Butanoic acid, anhydride (22.358), Hexadecanoic acid, methyl ester (22.402), Ethyl 9-hexadecenoate (22.876), Isopentyl n-hexyl disulfide (23.085), 11-Octadecenoic acid, methyl ester (24.115), (E)-9-Octadecenoic acid ethyl ester (24.728), Dotriacontane (26.747), Tetrapentacontane (28.346) (Figure 6.41(iii)I).

In WA-5-LWM group the chemical peaks for compounds present in the frass are named as 3-Hexanol (5.353), Dimethyl Sulfoxide (6.081), Decane (9.928), Decane, 5-methyl- (10.181), Decane, 4-methyl- (10.358), Dodecane, 2,6,10-trimethyl- (10.987), Decane, 4-methyl- (11.090), Decane, 3,7-dimethyl- (11.812), Dodecane (13.525), Undecane, 4,6-dimethyl- (13.726), Dodecane, 4-methyl- (13.866), Tetradecane (14.167), 2,4-Dimethyldodecane (14.501), Dodecane, 2,6,11-trimethyl- (14.701), Dodecane, 2,6,11-trimethyl- (15.402), Eicosane, 10-methyl- (16.125), Tetradecane (16.548), Tetradecane, 5-methyl- (16.650), Octadecane (16.698), Heptadecane (16.764), Tetradecane, 4-methyl- (16.834), Heptadecane (17.316), 2,6,10-Trimethyltridecane (17.375), Eicosane (17.695), Heptadecane (17.755), Heneicosane (17.851), Hexadecane (18.355), Pentadecane, 3-methyl- (18.834), Hexadecane (19.207), Heptadecane (19.293), Hexadecane, 4-methyl- (19.461), Heptadecane (19.539), Heneicosane (19.580), Octadecane (19.790), Heneicosane (20.021), Heptadecane (20.084), Heptadecane, 4-methyl- (20.235), Eicosane (20.292), Dotriacontane (20.366), Eicosane (20.429), Dotriacontane (20.479), Dodecane, 2,6,11-trimethyl- (20.596), Dotriacontane (20.944), Heptadecane, 3-methyl- (21.258), Heneicosane (21.590), Tridecanal (21.815), Eicosane (22.003), Heneicosane (22.040), 11-Methylpentacosane (22.224), Eicosane (22.429), Heneicosane (22.472), 2,6,10-Trimethyltridecane (22.575), Dotriacontane (22.637), Eicosane (22.731), Dotriacontane

(22.817), Tetracosane (22.961), 2-Methylhentriacontane (23.268), Octadecane, 1-iodo- (23.452), Heneicosane (23.748), Tetracosane (24.218), Eicosane (24.608), Dotriacontane (24.644), Tetrapentacontane (24.710), Heptadecane, 3-methyl- (24.866), Tetrapentacontane (24.968), Pentatriacontane (26.621), Tetracosane (26.848), Tetrapentacontane (26.925), 2-Methyltetracosane (27.294), Eicosane (27.523), Nonacosane (28.437), Tetracosane (28.748), Tetracontane (29.329) (Figure 6.41(iii)J).

For A-5-LWM group the chemical peaks for compounds present in the frass are named as Hexadecane (14.566), Phosphonoacetic Acid, 3TMS derivative (14.897), Sulfurous acid, 2-ethylhexyl isohexyl ester (16.457), Undecane, 1-bromo- (17.129), Heptadecane (17.404), Dodecane, 4,6-dimethyl- (17.464), 3-Ethyl-3-methylheptane (17.545), Hexadecane (20.000) (Figure 6.41(iii)K).

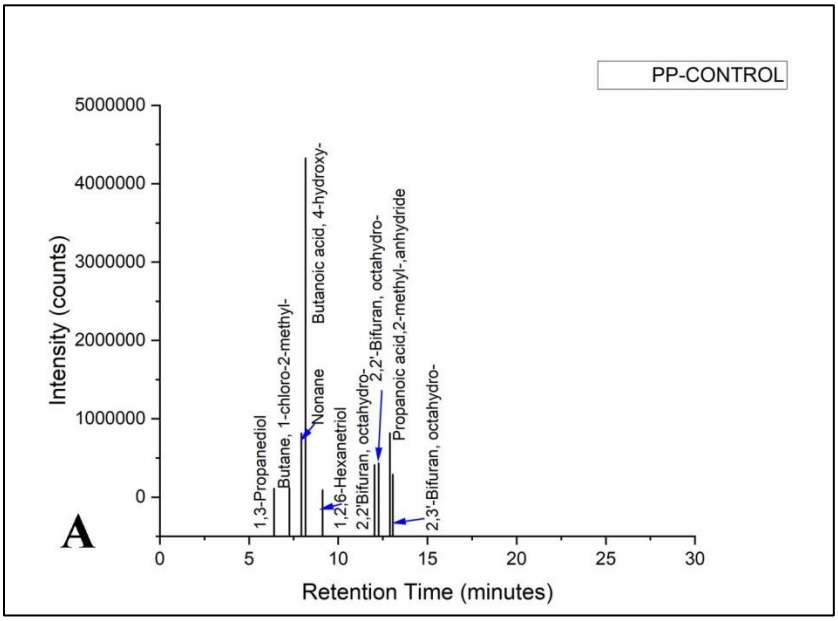


Figure 6.41(i)

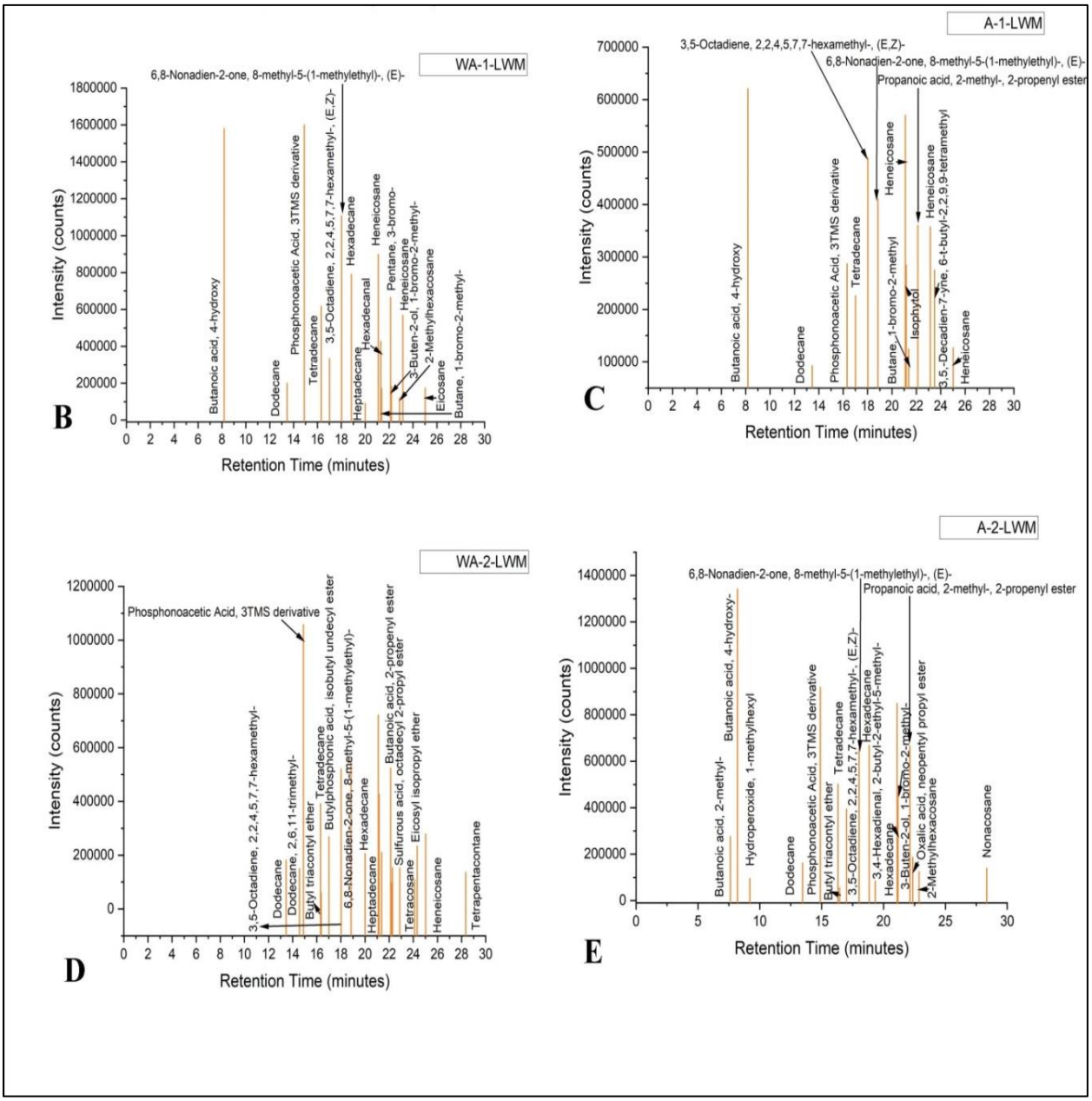


Figure 6.41(ii)

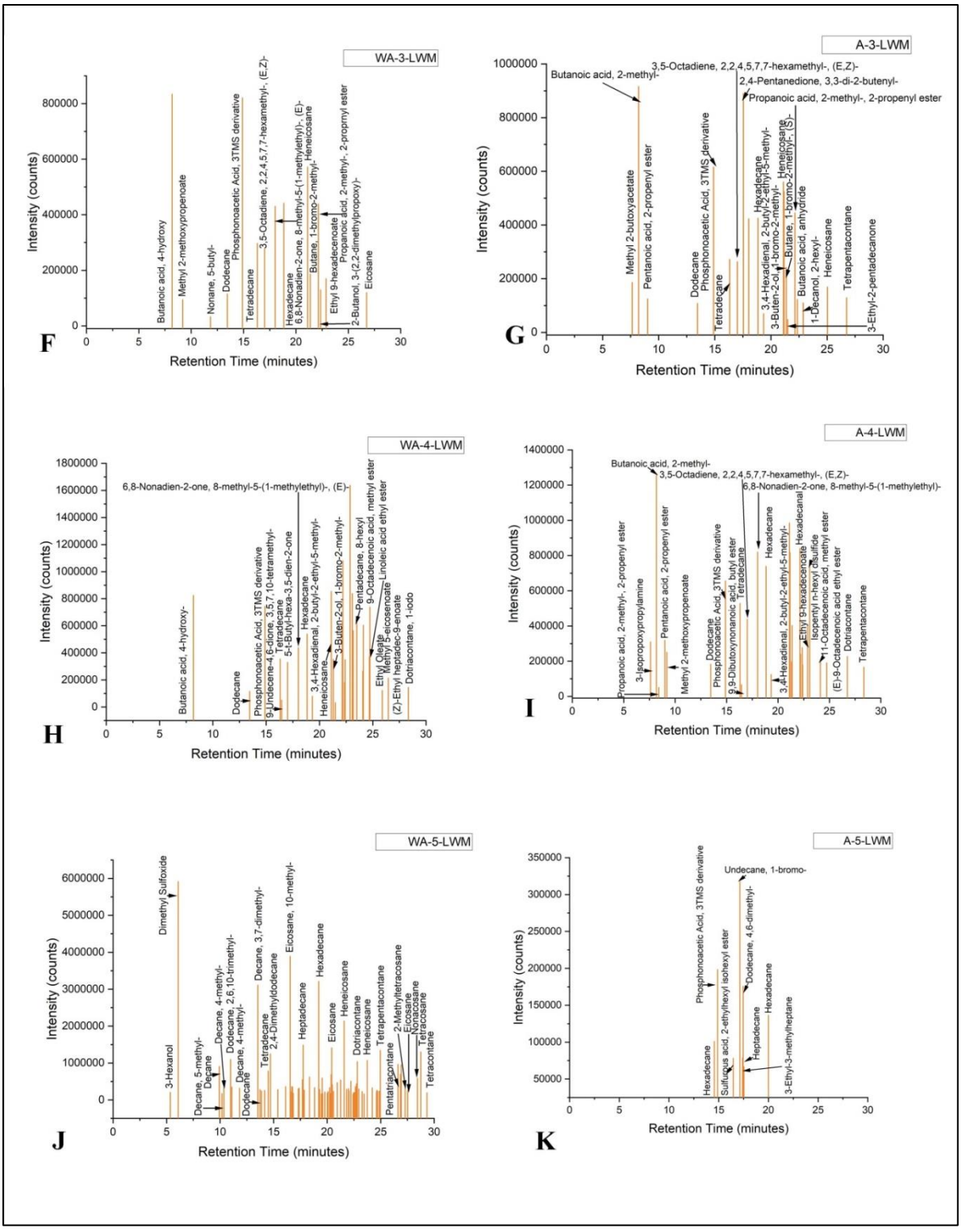


Figure 6.41(iii)

Figure 6.41 Gas chromatography and mass spectroscopy analysis for frass of *A. grisella* fed with PP. Figure (i) (A) GC-MS graph for retention time and intensity for PP film (Control-PP); Figure (ii) (B) GC-MS graph for frass of without antibiotic group for first instar fed with PP (WA-1-LWM); (C)The graph for frass of antibiotic fed first instar group (A-1-LWM); (D) Graph for frass of without antibiotic group of second instar (WA-2-LWM); (E) GC-MS graph of antibiotic fed second instar group (A-2-LWM). Figure (iii) (F) The GC-MS graph of frass for third instar without antibiotic group fed with PP (WA-3-LWM); (G) The GC-MS graph of antibiotic administered third instar group (A-3-LWM). (H) GC-MS graph of without antibiotic fourth instar group (WA-4-LWM); (I) The GC-MS graph of antibiotic fed fourth instar group (A-4-LWM); (J)The graph for frass of without antibiotic group of fifth instar (WA-5-LWM); (K) The GC-MS graph of antibiotic fed fifth instar group (A-5-LWM)

6.5. Enzyme Activity Assays

The larvae after consuming the long linear chained hydrocarbons (LDPE, HDPE, and PP) for 48 hours, the larvae were homogenised to analyse the extent of digestion ingestion of plastics and increment in the enzymes after consumption of complex high molecular weight polymers. In order to observe the plastic biodegradation capacity of the wax moth larval species the enzyme assays of alcohol dehydrogenase and lactate dehydrogenase was performed. In the present section, control group of first instar is CONTROL1, without antibiotic fed first instar group is WA-1-GWM, antibiotic fed first instar group is A-1-GWM, control group of second instar is CONTROL2, without antibiotic fed second instar group is WA-2-GWM, antibiotic fed second instar group is A-2-GWM, control group of third instar is CONTROL3, without antibiotic fed third instar group is WA-3-GWM, antibiotic fed third instar group is A-3-GWM, control group of fourth instar is CONTROL4, without antibiotic fed fourth instar group is WA-4-GWM, antibiotic fed fourth instar group is A-4-GWM, control group of fifth instar is CONTROL5, without antibiotic fed fifth instar group is WA-5-GWM, antibiotic fed fifth instar group is A-5-GWM, control group of sixth instar is CONTROL6, without antibiotic fed sixth instar group is WA-6-GWM, antibiotic fed sixth instar group is A-6-GWM, control group of seventh instar is CONTROL7, without antibiotic fed seventh instar group is WA-7-GWM, antibiotic fed seventh instar group is A-7-GWM are groups for greater wax moth (Table 6.5) whereas control group of first instar is CONTROL1, without antibiotic fed first instar group is WA-1-LWM, antibiotic fed first instar group is A-1-LWM, control group of second instar is CONTROL2, without antibiotic fed second instar group is WA-2-LWM, antibiotic fed second instar group is A-2-LWM, control group of third instar is

CONTROL3, without antibiotic fed third instar group is WA-3-LWM, antibiotic fed third instar group is A-3-LWM, control group of fourth instar is CONTROL4, without antibiotic fed fourth instar group is WA-4-LWM, antibiotic fed fourth instar group is A-4-LWM, control group of fifth instar is CONTROL5, without antibiotic fed fifth instar group is WA-5-LWM, antibiotic fed fifth instar group is A-5-LWM are groups for lesser wax moth (Table 6.6).

Maximal Enzymatic Activity of Alcohol Dehydrogenase- In the present section, the analysis of maximal enzymatic activity of alcohol dehydrogenase enzyme by greater and lesser wax moth is explored. As the conversion of food remains into aldehyde is facilitated by the enzyme alcohol dehydrogenase in the insects. During the consumption of plastics the small fragments of plastic are converted in to alcohol which is further converted into aldehyde by alcohol dehydrogenase (Lemoine et al., 2020). In addition to this, the plastics are large compounds with high molecular weight. So, eventually for the digestion of plastic large consumption of alcohol dehydrogenase is required. Thus, to detect the digestion of plastics the maximal enzyme assay of alcohol dehydrogenase is performed.

Galleria mellonella

Low-Density Polyethylene (LDPE) - After homogenisation, the maximal enzymatic activity of all larval instars of greater wax moth fed with LDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for LDPE for *G. mellonella* by various groups- Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.42 ± 0.09, 0.58 ± 0.4, 0.84 ± 0.17, 0.53 ± 0.24, 0.69 ± 0.09, 1.07 ± 0.49, 0.48 ± 0.16, 2.57 ± 0, 0.21 ± 0.09, 0.13 ± 0.03, 2.78 ± 0.37, 1.92 ± 0.8, 0.48 ± 0, 3.16 ± 0.76, 2.41 ± 0.32, 0.91 ± 0.18, 3.32 ±

0.56, 2.51 ± 1.04, 1.39 ± 0.37, 1.6 ± 0.42, 1.87 ± 0.4 mU/min, respectively (Figure 6.42).

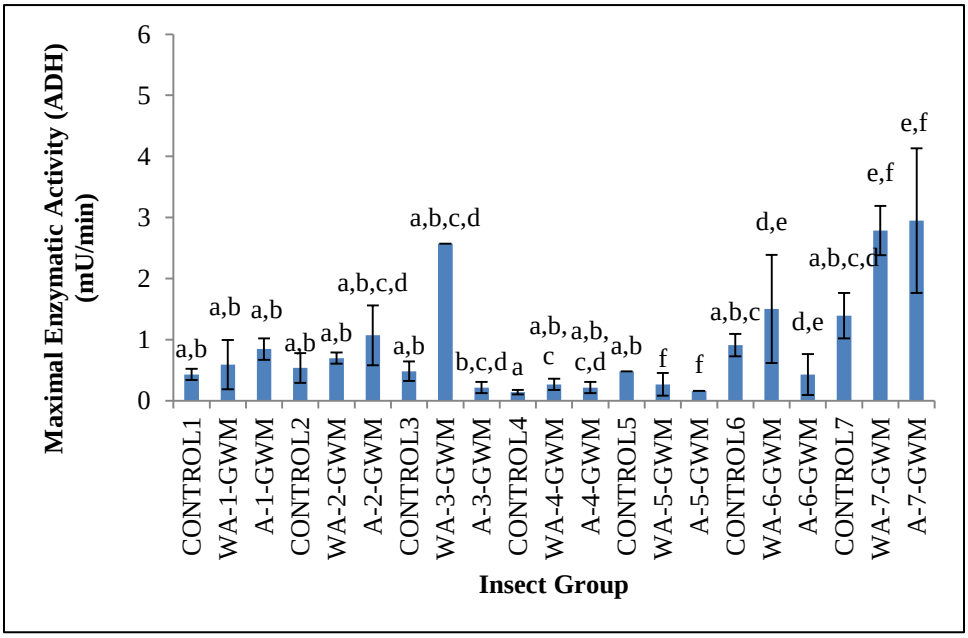


Figure 6.42 Effect of LDPE diet on the metabolism of *Galleria mellonella.* The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-f). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive LDPE film (WA-1-GWM), antibiotic administered first instar group fed with naive LDPE film (A-1-GWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive LDPE film (WA-2-GWM), antibiotic administered second instar group fed with naive LDPE film (A-2-GWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive LDPE film (WA-3-GWM), antibiotic administered third instar group fed with naive LDPE film (A-3-GWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive LDPE film (WA-4-GWM), antibiotic administered fourth instar group fed with naive LDPE film (A-4-GWM), control of fifth instar group (CONTROL5), without antibiotic fifth instar group fed with naive LDPE film (WA-5-GWM), antibiotic administered fifth instar group fed with naive LDPE film (A-5-GWM), control of sixth instar group (CONTROL6), without antibiotic sixth instar group fed with naive LDPE film (WA-6-GWM), antibiotic administered sixth instar group fed with naive LDPE film (A-6-GWM), control of seventh instar group (CONTROL7), without antibiotic seventh instar group fed with naive LDPE film (WA-7-GWM), antibiotic administered seventh instar group fed with naive LDPE film (A-7-GWM), are the insect groups

High-Density Polyethylene (HDPE) - After homogenisation, the maximal enzymatic activity of all larval instars of greater wax moth fed with HDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. For HDPE the maximal enzymatic activity of *G. mellonella* by various groups is Control1, WA-1-GWM, A-1-

GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.42 ± 0.09, 0.53 ± 0.18, 0.64 ± 0.27, 0.53 ± 0.24, 0.42 ± 0.18, 1.005 ± 0.1, 0.48 ± 0.16, 1.44 ± 0.27, 1.71 ± 0.24, 0.13 ± 0.03, 0.91 ± 0.18, 1.33 ± 0.09, 0.48 ± 0, 3.96 ± 1.61, 4.6 ± 0.49, 0.91 ± 0.18, 2.41 ± 0.48, 2.14 ± 0.18, 1.39 ± 0.37, 3.21 ± 0.8, 2.41 ± 0.27 mU/min, respectively (Figure 6.43).

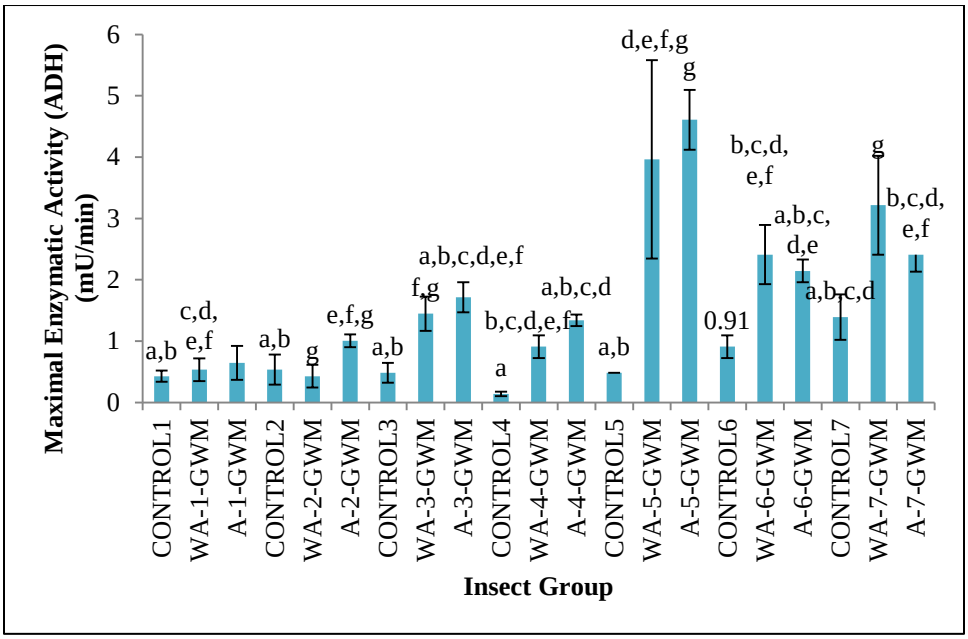


Figure 6.43 Effect of HDPE diet on the metabolism of *G. mellonella*. The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented from a-g). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive HDPE film (WA-1-GWM), antibiotic administered first instar group fed with naive HDPE film (A-1-GWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive HDPE film (WA-2-GWM), antibiotic administered second instar group fed with naive HDPE film (A-2-GWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive HDPE film (WA-3-GWM), antibiotic administered third instar group fed with naive HDPE film (A-3-GWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive HDPE film (WA-4-GWM), antibiotic administered fourth instar group fed with naive HDPE film (A-4-GWM), control of fifth instar group (CONTROL5), without antibiotic fifth instar group fed with naive HDPE film (WA-5-GWM), antibiotic administered fifth instar group fed with naive HDPE film (A-5-GWM), control of sixth instar group (CONTROL6), without antibiotic sixth instar group fed with naive HDPE film (WA-6-GWM), antibiotic administered sixth instar group fed with naive HDPE film (A-6-GWM), control of seventh instar group (CONTROL7), without antibiotic seventh instar group fed with naive HDPE film (WA-7-GWM), antibiotic administered seventh instar group fed with naive HDPE film (A-7-GWM), are the insect groups

Polypropylene (PP) - After homogenisation, the maximal enzymatic activity of all larval instars of greater wax moth fed with PP was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for PP for *G. mellonella* by various groups is Control1, WA-1-GWM, A-1-GWM, Control2,WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.42 ± 0.09, 2.03 ± 0.37, 2.08 ± 0.27, 0.53 ± 0.24, 3.9 ± 0.2, 2.9 ± 0.09, 0.48 ± 0.16, 3.0003 ± 1.52, 1.55 ± 0.09, 0.13 ± 0.03, 1.71 ± 0.09, 1.33 ± 0.92, 0.48 ± 0, 2.73 ± 0.16, 3.64 ± 0.56, 0.91 ± 0.18, 1.82 ± 0.24, 1.5 ± 0.24, 1.39 ± 0.37, 3.75 ± 0.66, 1.87 ± 0.09 mU/min, respectively (Figure 6.44).

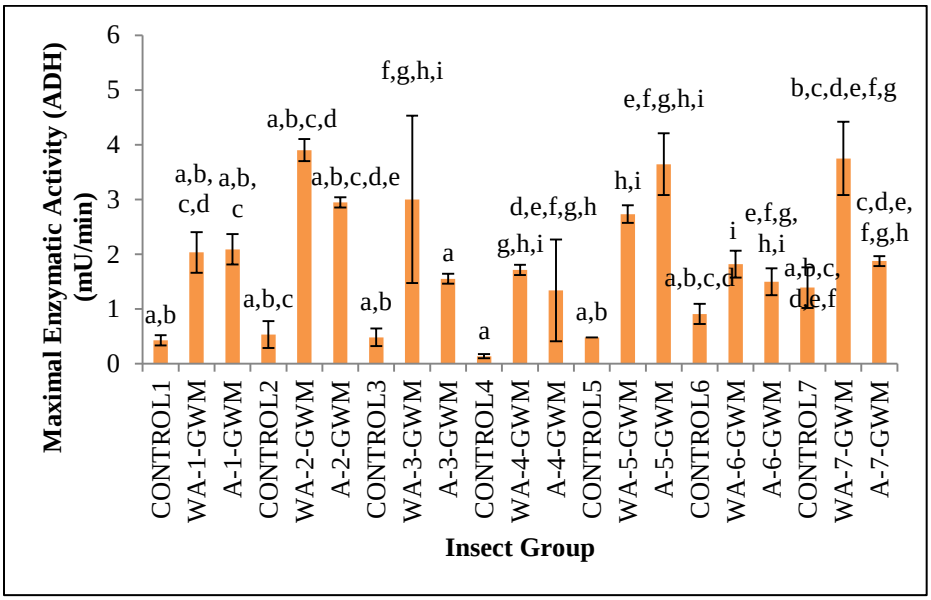


Figure 6.44 Effect of PP diet on the metabolism of *G. mellonella*. The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-i). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive PP film (WA-1-GWM), antibiotic administered first instar group fed with naive PP film (A-1-GWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive PP film (WA-2-GWM), antibiotic administered second instar group fed with naive PP film (A-2-GWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive PP film (WA-3-GWM), antibiotic administered third instar group fed with naive PP film (A-3-GWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive PP film (WA-4-GWM), antibiotic administered fourth instar group fed with naive PP film (A-4-GWM), control of fifth instar group

(CONTROL5), without antibiotic fifth instar group fed with naive PP film (WA-5-GWM), antibiotic administered fifth instar group fed with naive PP film (A-5-GWM), control of sixth instar group (CONTROL6), without antibiotic sixth instar group fed with naive PP film (WA-6-GWM), antibiotic administered sixth instar group fed with naive PP film (A-6-GWM), control of seventh instar group (CONTROL7), without antibiotic seventh instar group fed with naive PP film (WA-7-GWM), antibiotic administered seventh instar group fed with naive PP film (A-7-GWM), are the insect groups

Achroia grisella

Low-Density Polyethylene (LDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with LDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for LDPE for *A. grisella* by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is 0.26 ± 0.09, 1.92 ± 0.48, 3.69 ±1.004, 0.53 ± 0.09, 0.73 ± 0.73, 2.56 ± 0.73, 0.26 ± 0.09, 3.2 ± 1.11, 3.04 ± 1.004, 0.13 ± 0.03, 2.72 ± 0.73, 4.01 ± 0.28, 1.92 ± 0.48, 5.14 ± 1.004, 3.69 ± 1.004 mU/min, respectively (Figure 6.45).

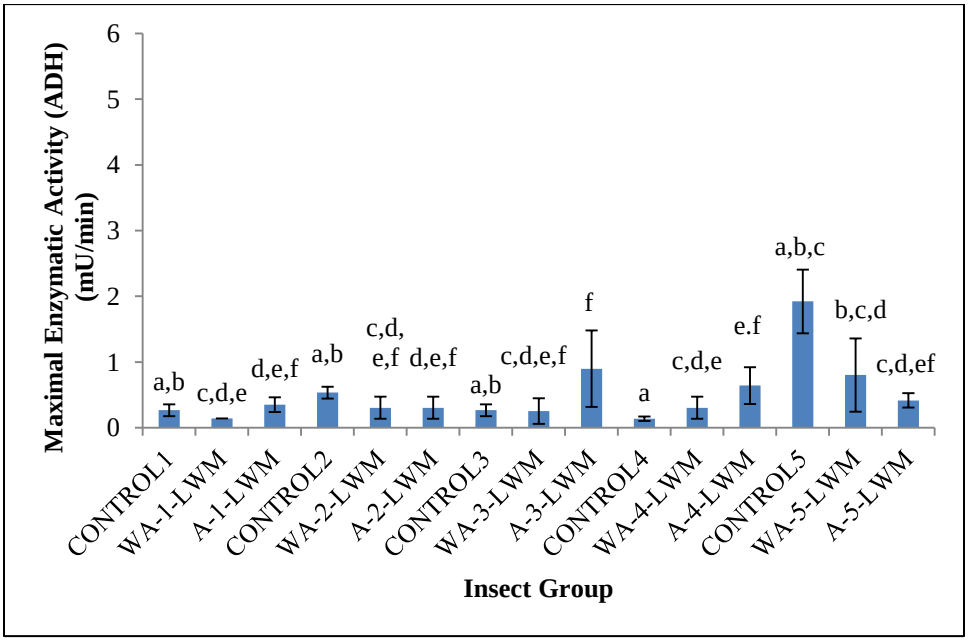


Figure 6.45 Effect of LDPE diet on the metabolism of *A. grisella.* The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n =3); $p<0.05$ (One -way

ANOVA followed by Tukey's significant difference test which is represented by a-f). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive LDPE film (WA-1-LWM), antibiotic administered first instar group fed with naive LDPE film (A-1-LWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive LDPE film (WA-2-LWM), antibiotic administered second instar group fed with naive LDPE film (A-2-LWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive LDPE film (WA-3-LWM), antibiotic administered third instar group fed with naive LDPE film (A-3-LWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive LDPE film (WA-4-LWM), antibiotic administered fourth instar group fed with naive LDPE film (A-4-LWM), control of fifth instar group (CONTROL5), without antibiotic fifth instar group fed with naive LDPE film (WA-5-LWM), antibiotic administered fifth instar group fed with naive LDPE film (A-5-LWM) are the insect groups

High-Density Polyethylene (HDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with HDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for HDPE for *A. grisella* by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is, 0.26 ± 0.09, 3.91 ± 1.03, 3.21 ± 0.7, 0.53 ± 0.09, 2.62 ± 0.09, 2.94 ± 0.09, 0.26 ± 0.09, 5.35 ± 0.56, 3.16 ± 1.68, 0.13 ± 0.03, 3.05 ± 0.42, 2.83 ± 0.24, 1.92 ± 0.48, 1.87 ± 0.88, 3.37 ± 0.73 mU/min, respectively (Figure 6.46).

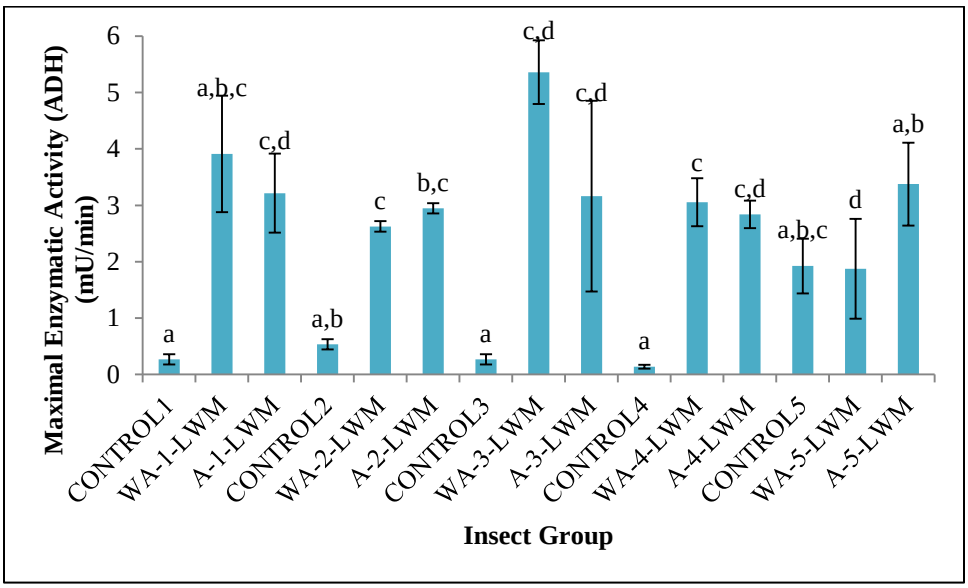


Figure 6.46 Effect of HDPE diet on the metabolism of *A. grisella.* The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n =3); $p<0.05$ (One -way

ANOVA followed by Tukey's significant difference test which is represented by a-c). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive HDPE film (WA-1-LWM), antibiotic administered first instar group fed with naive HDPE film (A-1-LWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive HDPE film (WA-2-LWM), antibiotic administered second instar group fed with naive HDPE film (A-2-LWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive HDPE film (WA-3-LWM), antibiotic administered third instar group fed with naive HDPE film (A-3-LWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive HDPE film (WA-4-LWM), antibiotic administered fourth instar group fed with naive HDPE film (A-4-LWM), control of fifth instar group (CONTROL5), without antibiotic fifth instar group fed with naive HDPE film (WA-5-LWM), antibiotic administered fifth instar group fed with naive HDPE film (A-5-LWM) are the insect groups

Polypropylene (PP) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with PP was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. For PP the maximal enzymatic activity of *A. grisella* by various groups- Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is, 0.26 ± 0.92, 3.37 ± 0.27, 4.34 ± 0.55, 0.53 ± 0.09, 3.75 ± 0.72, 4.23 ± 0.88, 0.26 ± 0.09, 3.69 ± 0.7, 5.62 ± 0.48, 0.13 ± 0.03, 3.16 ± 1.61, 5.14 ± 0.27, 1.92 ± 0.48, 2.25 ± 0.96, 3.59 ± 1.03 mU/min, respectively (Figure 6.47).

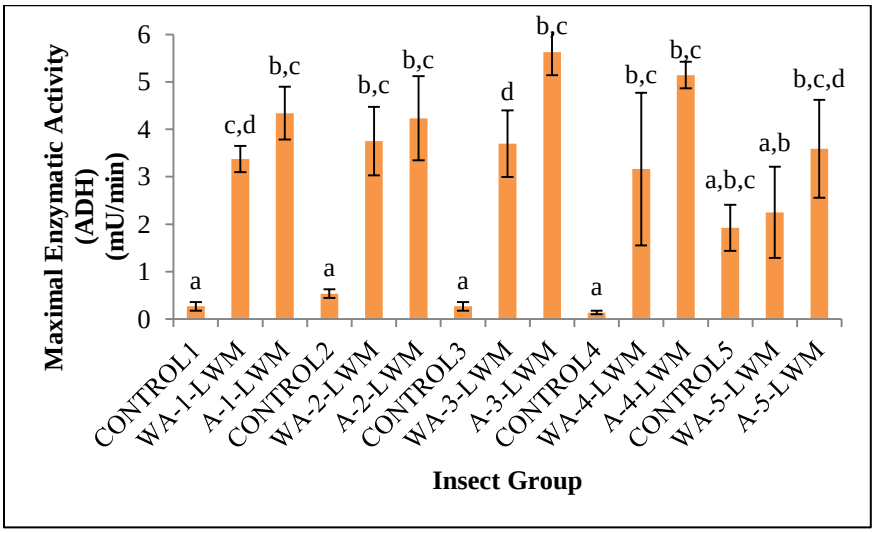


Figure 6.47 Effect of PP diet on the metabolism of *A. grisella*. The graph represents maximal enzyme activity of Alcohol dehydrogenase (ADH). Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way

ANOVA followed by Tukey's significant difference test which is represented by a-d). In this context, control group of first instar (CONTROL1), without antibiotic first instar group fed with naive PP film (WA-1-LWM), antibiotic administered first instar group fed with naive PP film (A-1-LWM), control group of second instar (CONTROL2), without antibiotic second instar group fed with naive PP film (WA-2-LWM), antibiotic administered second instar group fed with naive PP film (A-2-LWM), control group of third instar (CONTROL3), without antibiotic third instar group fed with naive PP film (WA-3-LWM), antibiotic administered third instar group fed with naive PP film (A-3-LWM), control of fourth instar group (CONTROL4), without antibiotic fourth instar group fed with naive PP film (WA-4-LWM), antibiotic administered fourth instar group fed with naive PP film (A-4-LWM), control of fifth instar group (CONTROL5), without antibiotic fifth instar group fed with naive PP film (WA-5-LWM), antibiotic administered fifth instar group fed with naive PP film (A-5-LWM) are the insect groups

Maximal Enzymatic Activity of Lactate Dehydrogenase- In this section, the maximal enzymatic activity of lactate dehydrogenase (LDH) in two lepidopteran species, *Galleria mellonella* (greater wax moth) and *Achroia grisella* (lesser wax moth), is investigated. Given that alcohol dehydrogenase (ADH) generates a surplus of reduced nicotinamide adenine dinucleotide (NADH), it was hypothesised that metabolic pathways may compensate by utilizing LDH to convert pyruvate to lactate, thereby regenerating oxidized nicotinamide adenine dinucleotide (NAD^+) and maintaining redox homeostasis within the cellular environment (Lemoine et al., 2020).

Galleria mellonella

Low-Density Polyethylene (LDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of greater wax moth fed with LDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for LDPE for *G. mellonella* by various groups is Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.04 ± 0, 0.37 ± 0.09, 0.37 ± 0.09, 0.19 ± 0.11, 0.37 ± 0.09, 0.37 ± 0.09, 0.26 ± 0.09, 0.32 ± 0.16, 0.16 ± 0, 0.11 ± 0.03, 0.26 ± 0.09, 0.21 ± 0.09, 0.11 ± 0.03, 0.26 ± 0.18, 0.16 ± 0, 0.11 ± 0.03, 1.5 ±

0.88, 0.42 ± 0.33, 0.26 ± 0.09, 2.78 ± 0.4, 2.94 ± 1.18 mU/min, respectively (Figure 6.48).

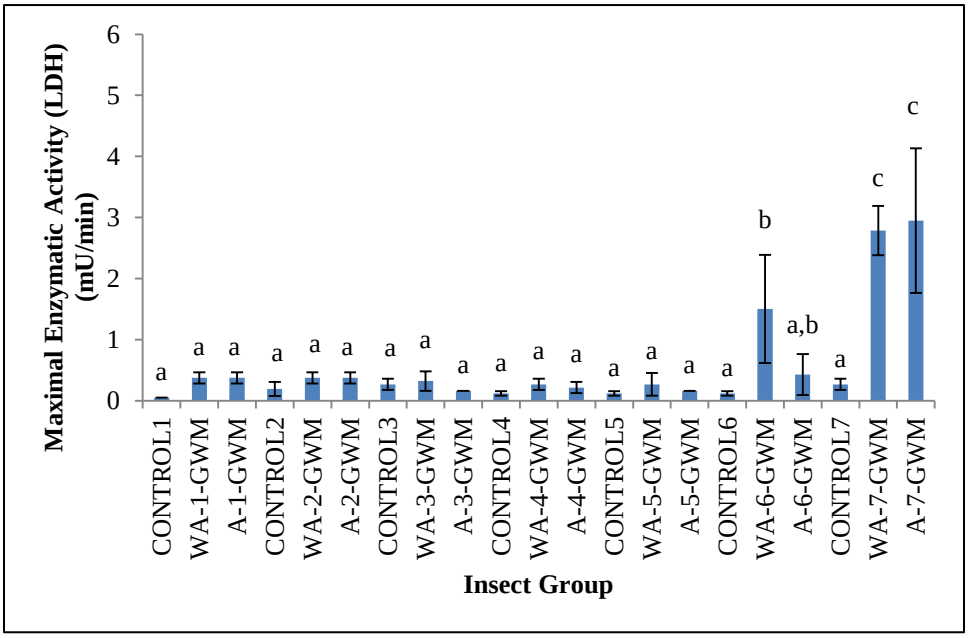


Figure 6.48 The enzymatic activity of LDH after consumption of LDPE as sole diet for *Galleria mellonella*. The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-c). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive LDPE film, A-1-GWM= antibiotic administered first instar group fed with naive LDPE film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic first instar group fed with naive LDPE film, A-2-GWM= antibiotic administered second instar group fed with naive LDPE film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive LDPE film, A-3-GWM= antibiotic administered third instar group fed with naive LDPE film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive LDPE film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive LDPE film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive LDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive LDPE film, CONTROL7= control group of seventh instar,WA-7-GWM= without antibiotic seventh instar group fed with naive LDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive LDPE film

High-Density Polyethylene (HDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of greater wax moth fed with HDPE was analysed. The maximal enzyme activity for HDPE for *G. mellonella* by various groups is Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM,

Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.04 ± 0, 0.16 ± 0, 0.16 ± 0, 0.19 ± 0.11, 0.21 ± 0.09, 0.021 ± 0.09, 0.26 ± 0.09, 0.02 ± 0.09, 0.21 ± 0.09, 0.11 ± 0.03, 0.26 ± 0.09, 0.58 ± 0.09, 0.11 ± 0.03, 0.16 ± 0, 0.16 ± 0, 0.11 ± 0.03, 1.07 ± 0.88, 0.64 ± 0.42, 0.26 ± 0.09, 0.21 ± 0.09, 1.28 ± 0.7 mU/min, respectively (Figure 6.49).

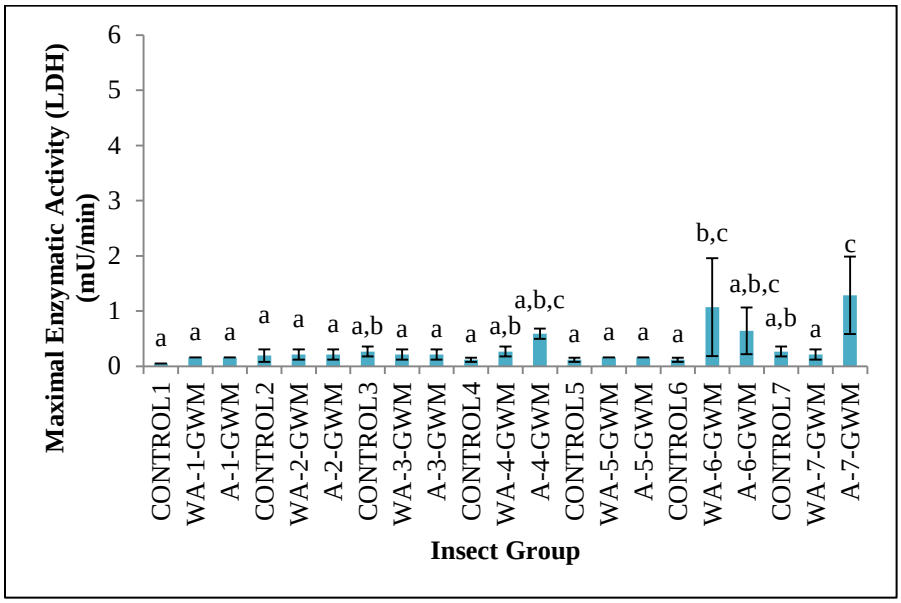


Figure 6.49 The enzymatic activity of LDH after consumption of HDPE as sole diet for *Galleria mellonella*. The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-c). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive HDPE film, A-1-GWM= antibiotic administered first instar group fed with naive HDPE film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic first instar group fed with naive HDPE film, A-2-GWM= antibiotic administered second instar group fed with naive HDPE film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive HDPE film, A-3-GWM= antibiotic administered third instar group fed with naive HDPE film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-GWM= antibiotic administered fourth instar group fed with naive HDPE film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-GWM= antibiotic administered fifth instar group fed with naive HDPE film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive HDPE film, A-6-GWM= antibiotic administered sixth instar group fed with naive HDPE film, CONTROL7= control group of seventh instar,WA-7-GWM= without antibiotic seventh instar group fed with naive HDPE film, A-7-GWM= antibiotic administered seventh instar group fed with naive HDPE film

Polypropylene (PP) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of greater wax moth fed with PP was analysed. Maximal enzyme

activity for PP for *G. mellonella* by various groups is Control1, WA-1-GWM, A-1-GWM, Control2, WA-2-GWM, A-2-GWM, Control3, WA-3-GWM, A-3-GWM, Control4, WA-4-GWM, A-4-GWM, Control5, WA-5-GWM, A-5-GWM, Control6, WA-6-GWM, A-6-GWM, Control7, WA-7-GWM and A-7-GWM is 0.04 ± 0, 0.48 ± 0.27, 0.48 ± 0.16, 0.19 ± 0.11, 0.27 ± 0.22, 1.07 ± 0.18, 0.26 ± 0.09, 0.85 ± 0.09, 1.07 ± 0.18, 0.11 ± 0.03, 0.21 ± 0.09, 1.33 ± 0.8, 0.11 ± 0.03, 1.17 ± 1.01, 0.21 ± 0.09, 0.11 ± 0.03, 1.23 ± 0.37, 0.32 ± 0.27, 0.26 ± 0.09, 3.69 ± 0.16, 2.78 ± 1.07 mU/min, respectively (Figure 6.50).

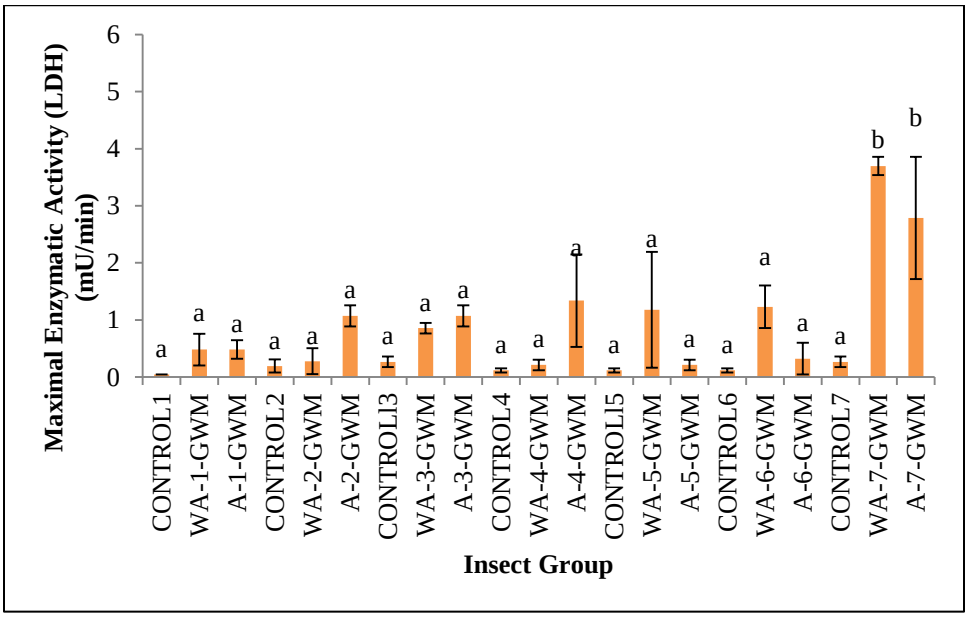


Figure 6.50 The enzymatic activity of LDH after consumption of PP as sole diet for *Galleria mellonella*. The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). Note: CONTROL1= control group of first instar, WA-1-GWM= without antibiotic first instar group fed with naive PP film, A-1-GWM= antibiotic administered first instar group fed with naive PP film, CONTROL2= control group of second instar, WA-2-GWM= without antibiotic first instar group fed with naive PP film, A-2-GWM= antibiotic administered second instar group fed with naive PP film, CONTROL3= control group of third instar, WA-3-GWM= without antibiotic third instar group fed with naive PP film, A-3-GWM= antibiotic administered third instar group fed with naive PP film, CONTROL4= control group of fourth instar, WA-4-GWM= without antibiotic fourth instar group fed with naive PP film, A-4-GWM= antibiotic administered fourth instar group fed with naive PP film, CONTROL5= control group of fifth instar, WA-5-GWM= without antibiotic fifth instar group fed with naive PP film, A-5-GWM= antibiotic administered fifth instar group fed with naive PP film, CONTROL6= control group of sixth instar, WA-6-GWM= without antibiotic sixth instar group fed with naive PP film, A-6-GWM= antibiotic administered sixth instar group fed with naive PP film, CONTROL7= control group of seventh instar, WA-7-GWM=

without antibiotic seventh instar group fed with naive PP film, A-7-GWM= antibiotic administered seventh instar group fed with naive PP film

Achroia grisella

Low-Density Polyethylene (LDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with LDPE was analysed. The significance of the data was calculated by one-way ANOVA followed by Tukey's significant difference test at a one-tailed significance of 0.05. The maximal enzyme activity for LDPE for *A. grisella* by various groups is Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is 0.08 ± 0.02, 0.14 ± 0, 0.35 ± 0.11, 0.11 ± 0.03, 0.3 ± 0.16, 0.3 ± 0.16, 0.11 ± 0.03, 0.25 ± 0.19, 0.89 ± 0.58, 0.1 ± 0.05, 0.3 ± 0.16, 0.64 ± 0.27, 0.8 ± 0.27, 0.8 ± 0.55, 0.41 ± 0.11 mU/min, respectively (Figure 6.51).

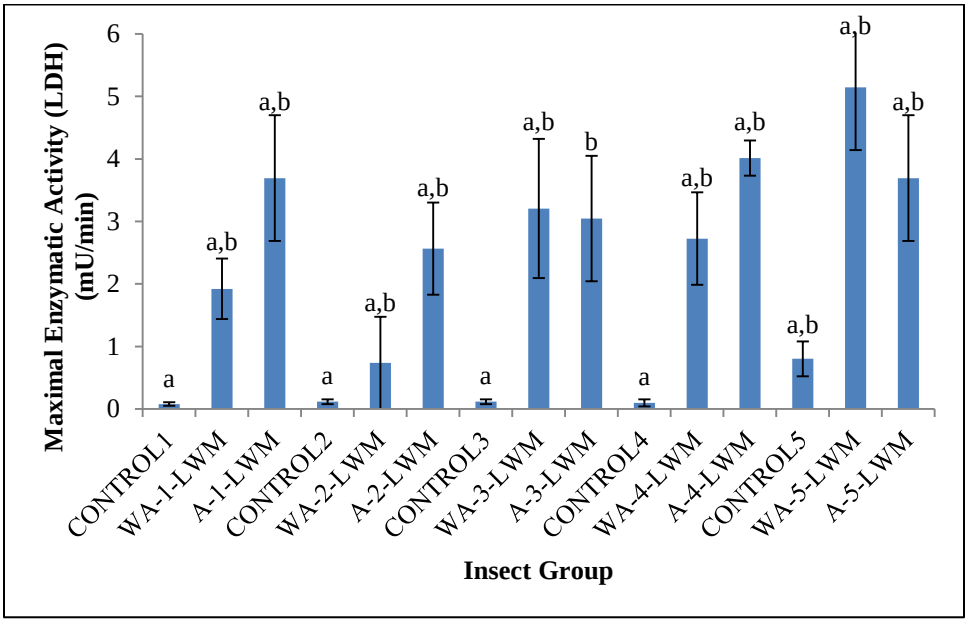


Figure 6.51 The enzymatic activity of LDH after consumption of LDPE as sole diet for *Achroia grisella.* The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). Note: Control1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive LDPE film, A-1-LWM= antibiotic administered first instar group fed with naive LDPE film, Control2= control group of second instar, WA-2-LWM= without antibiotic first instar group fed with naive LDPE film, A-2-LWM= antibiotic administered second instar group fed with naive LDPE film, Control3= control

group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive LDPE film, A-3-LWM= antibiotic administered third instar group fed with naive LDPE film, Control4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive LDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive LDPE film, Control5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive LDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive LDPE film

High-Density Polyethylene (HDPE) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with HDPE was analysed. For HDPE the maximal enzymatic activity (LDH) of *A. grisella* by various groups is Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is, 0.08 ± 0.02, 0.42 ± 0.24, 0.58 ± 0.09, 0.11 ± 0.03, 0.58 ± 0.09, 0.16 ± 0, 0.11 ± 0.03, 0.21 ± 0.09, 0.37 ± 0.37, 0.1 ± 0.05, 0.74 ± 0.24, 0.37 ± 0.18, 0.8 ± 0, 0.53 ± 0.24, 0.80 ± 0.27 mU/min, respectively (Figure 6.52).

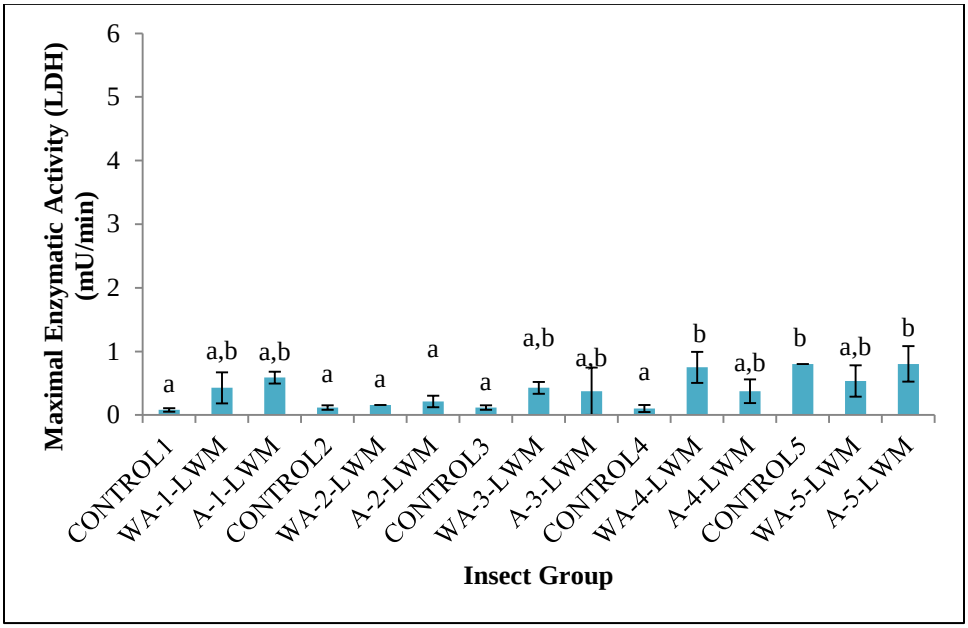


Figure 6.52 The enzymatic activity of LDH after consumption of HDPE as sole diet for *Achroia grisella.* The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n = 3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). Note: CONTROL1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive HDPE film, A-1-LWM= antibiotic administered first instar group fed with naive HDPE film, CONTROL2= control group of second instar, WA-2-LWM= without antibiotic first instar group fed with naive HDPE film, A-2-LWM= antibiotic administered second instar group fed with naive HDPE film, CONTROL3= control group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive HDPE film, A-3-LWM= antibiotic administered third instar group fed with naive HDPE film,

CONTROL4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive HDPE film, A-4-LWM= antibiotic administered fourth instar group fed with naive HDPE film, CONTROL5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive HDPE film, A-5-LWM= antibiotic administered fifth instar group fed with naive HDPE film

Polypropylene (PP) - After homogenisation of the larva, the maximal enzymatic activity of all larval instars of lesser wax moth fed with PP was analysed. The maximal enzyme activity for PP for *A. grisella* by various groups is Control1, WA-1-LWM, A-1-LWM, Control2, WA-2-LWM, A-2-LWM, Control3, WA-3-LWM, A-3-LWM, Control4, WA-4-LWM, A-4-LWM, Control5, WA-5-LWM, and A-5-LWM is, 0.08 ± 0.02, 1.28 ± 0.7, 3.85 ± 1.81, 0.11 ± 0.03, 5.78 ± 0.32, 4.98 ± 1.85, 0.10 ± 0.05, 0.74 ± 0.58, 0.64 ± 0.16, 0.8 ± 0.27, 0.53 ± 0.24, 0.53 ± 0.18, 0.8 ± 0.27, 0.53 ± 0.37, 0.32 ± 0.16 mU/min, respectively (Figure 6.53).

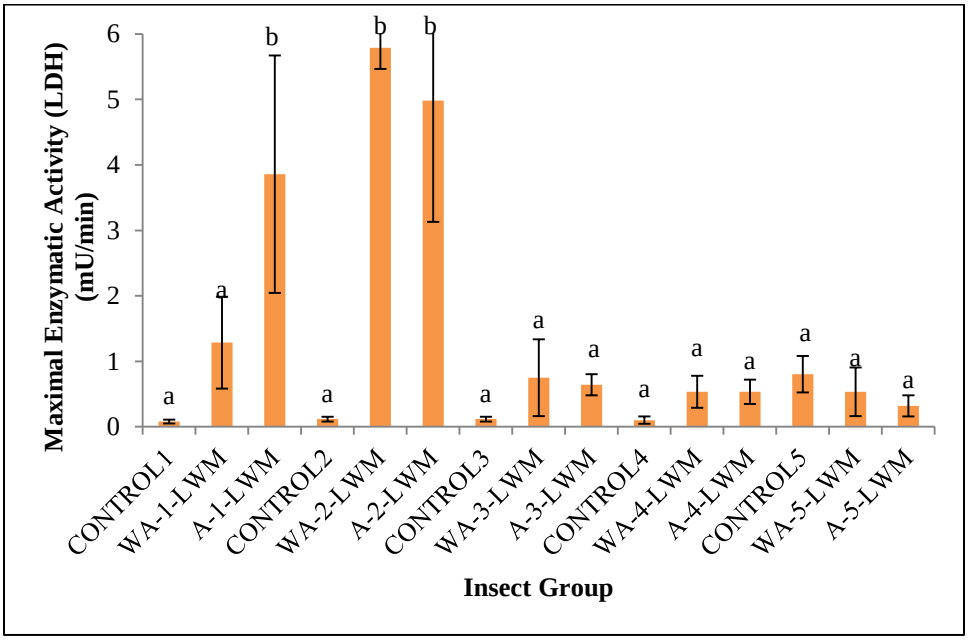


Figure 6.53 The enzymatic activity of LDH after consumption of PP as sole diet for *Achroia grisella.* The graph represents maximal enzyme activity of LDH. Data represents mean ± S.D. (n =3); $p<0.05$ (One -way ANOVA followed by Tukey's significant difference test which is represented by a-b). Note: CONTROL1= control group of first instar, WA-1-LWM= without antibiotic first instar group fed with naive PP film, A-1-LWM= antibiotic administered first instar group, CONTROL2= control group of second instar, WA-2-LWM= without antibiotic first instar group fed with naive PP film, A-2-LWM= antibiotic administered second instar group fed with naive PP film, CONTROL3= control group of third instar, WA-3-LWM= without antibiotic third instar group fed with naive PP film, A-3-LWM= antibiotic administered third instar group fed with naive PP film, CONTROL4= control group of fourth instar, WA-4-LWM= without antibiotic fourth instar group fed with naive PP film, A-4-LWM= antibiotic administered fourth instar

group fed with naive PP film, CONTROL5= control group of fifth instar, WA-5-LWM= without antibiotic fifth instar group fed with naive PP film, A-5-LWM= antibiotic administered fifth instar group fed with naive PP film

6.6. Discussion

Plastics are regarded as the most important compound in modern life. Plastics are becoming into materials that are essential to human comfort because of their stability and endurance. Because of its many uses, plastic material has seen a massive boom in consumption, the global consumption of plastics has witnessed an exponential surge, with annual production escalating from a modest 5 million metric tons in the 1950s to an astonishing 367 million metric tons as of 2020, underscoring the pervasive influence and ubiquitous presence of these materials in our daily (Napper & Thompson, 2023; Vuppaladadiyam et al., 2023). Moreover, recent research has shown that after the climate change accumulation of plastic debris in the environment has emerged as an ecological concern of utmost importance on a global scale (Bilal et al., 2021), and it affects all living forms, and natural environments (Dube & Okuthe, 2023; Napper & Thompson, 2023).

During the present research work, a few preliminary tests- validation of gut microbiota destruction when fed on the antibiotics and FTIR for the identification of the plastics were performed.

For the present research work the methodology by Yang et al., 2014 for *Plodia interpunctella* larva was followed to administer antibiotics in the wax moth. The homogenate of larval groups fed with antibiotics and without antibiotics was smeared on the nutrient agar plates. After incubation of twenty four hours, the growth of the microbes was observed in the nutrient agar plates of the homogenate of without antibiotic fed larvae whereas the homogenate of antibiotic fed larvae exhibited no growth on the culture plates (Figure 6.1). Thereafter, they supported the destruction of gut microbes in the homogenate of the insects administered with antibiotics. Similarly, Kong et al., 2019 ; Kesti & Thimmappa, 2019 for *G. mellonella* and *Corcyra cephalonica* observed the identical results when administered with antibiotics. Thus, in the present research work, the larvae were administered with antibiotic treatment for a day and homogenate of larvae was smeared on the nutrient agar plates along with homogenate of the larvae not

fed with any antibiotics. No growth of microbiota was observed in the former group whereas in the latter group microbiota growth was speculated in the 24 hours.

As a method for identifying polymers, FTIR is currently being used by the researchers (Camacho & Karlsson, 2001; Arnold et al., 2010; Barbeş et al., 2014; Cafiero et al., 2015; Jung et al., 2018) thus, various plastics used in the present research were identified by this technique. In the current research work, the research methodology of the Fang et al., 2012; Kundungal, et al., 2019; Peydaei et al., 2020 was followed for the identification of the plastic films used in the present study. FTIR analysis of LDPE film was recorded on 400-4000 cm^{-1} infrared spectra on transmission mode in order to characterise the LDPE film before biodegradation. The FTIR results before beginning of the experiments showed the characteristic peaks at 720, 1464, 2916, 2848 cm^{-1} confirming the plastic film used in the experiments is LDPE as depicted in Figure 6.2A. Further, FTIR analysis of HDPE film was recorded on 400-4000 cm^{-1} infrared spectra on transmission mode in order to characterise the HDPE film before biodegradation. The FTIR results before commencement of the experiments showed the characteristic peaks at 719, 873, 1048, 1463, 2360, 2848, 2915 cm^{-1} confirming the use of HDPE in the experiments as shown in Figure 6.2B. Similarly, FTIR analysis of PP film was recorded on 450-4000 cm^{-1} infrared spectra on transmission mode in order to characterise the PP film before biodegradation. The infrared spectra of naive PP films exhibits characteristic peaks at 795, 847, 968, 1000, 994, 1175, 1379, 1462, 2895-2899, 2922, 2958 cm^{-1} validating the use of PP in the experiments as shown in Figure 6.2C.

In the present research work, a batch of fifty *G. mellonella* larvae of each larval instar stage were fed with naive and soil treated LDPE, HDPE, and PP film. Further for the LDPE film, the plastic consumption capacity for the soil treated LDPE film was higher in all larval instars as compared to naive film. The maximum plastic consumption capacity of 6.64% was shown by seventh instar larva for soil treated film followed by 4.72% by sixth instar larvae fed with soil treated film in two days (Figure 6.3). But first and second instar when fed with naive and soil treated film revealed no plastic

consumption although third to fifth instar had shown significant plastic weight loss when exposed for 48 hours to the LDPE film. The results in the present research are in accordance with Kundungal et al. (2021), where 55.8% of plastic consumption was observed for solar exposed LDPE as compared to 18.57% consumption for naive LDPE film for fifteen larvae of fourth instar stage. Furthermore, 92 mg mass loss was observed in commercial polyethylene shopping bag in twelve hours by hundred greater wax moth larvae (Bombelli et al., 2017). Additionally, a batch of 100 caterpillars of *G. mellonella* had consumed 325µg of LDPE in one day and 768µg of LDPE in three days (Lemoine et al., 2020). When *G. mellonella* larvae were exposed to LDPE film for 12 hours, a large holes of 6.3 mm and pits of 14µm were observed (Peydaei et al., 2020). Similarly, a batch of 150 larvae consumed 1.95g of polyethylene in twenty one days of study (Lou et al., 2020). Moreover, a group of fifty larvae were able to consume 386.1 mg of polyethylene in eight days of exposure to the naive plastics (Peydaei et al., 2021).

Besides, LDPE consumption rate for fifty larvae of *G. mellonella* till they reach pupal stage (40 days) was 9.7% (Ruiz Barrionuevo et al., 2022). In the previous literature, mass loss of 0.015g of polyethylene/ greater wax moth larva was observed by Zhu et al., (2022). Further, naive polyethylene was exposed to greater wax moth larvae for five days and a mass loss of less than 5 mg per of plastic was observed per surviving larvae (Beale et al., 2022). According to Burd et al. (2023), twenty five larvae of greater wax moth exposed to naive LDPE film ingested 5.11% of the plastic in tenure of 7.25 days. In addition to this, shopping bags, trash, and freezer plastic were used as diet for the greater wax moth larvae. In the initial cohort, the consumption of polyethylene (PE) film was observed to be 71.6%, 89.5%, and 29.8% for freezer bags, trash bags, and shopping carrier bags, respectively. The subsequent generation of larvae, which were exclusively fed a plastic-based diet, exhibited digestion rates of 60%, 80%, and 20% for PE sourced from freezer bags, trash bags, and shopping carrier bags, respectively (Riabi et al., 2023).

Furthermore, no mortality was observed for the control groups from first to last larval instars fed with artificial diet when compared to other larval instars that were fed with

naive and soil treated LDPE film. For all larval instars fed with soil treated LDPE film revealed maximum survival rate as compared to naive LDPE film. But maximum survival rate was depicted as 94.66% in seventh larval instars fed with naive LDPE film whereas larvae exposed to soil treated LDPE film had 94% of survival rate (Figure 6.4). The first larval instars for both the naive and soil treated LDPE film fed groups had least survival rate amongst all the other larval instar groups. Though in previous studies the survival rate for solar radiations exposed LDPE film was 100% whereas the survival rate for naive LDPE film was 73.4% (Kundungal et.al., 2021). Moreover, the survival rate of polyethylene fed larvae after tenure of twenty one days was 35.3% (Lou et al., 2020). Similarly, the mortality rate of LDPE fed larvae was 28% in duration of forty days (Ruiz Barrionuevo et al., 2022). Additionally, survival rate of polyethylene fed larvae was 47.8% in an experimental period of ten days (Cassone et al., 2022). Likewise, survival rate of 93.75%-97.5% was observed by *G. mellonella* larvae fed on the plastics (Zhu et al., 2022). Besides, Beale et al. (2022), had reported increased survival rate in wax moth larvae when supplement with molasses as a supplement with the plastic diet. Further, survival rate of 40% was observed for greater wax moth larvae exposed to LDPE plastics (Burd et al., 2023). The percentage of larvae that survived on various feeding regimes was 98% for plastic bags frozen in freezers, 97% for plastic bags in the garbage, and 96.5% for plastic bags used for shopping (Riabi et al., 2023).

In the present section, plastic consumption capacity of the naive and soil treated HDPE films for *G. mellonella* is explored. From third instar larval stage to last instar stage, a significant increase in the plastic consumption capacity was observed for the soil treated plastic films. The highest plastic consumption rate as 8.89% for seventh instar fed with soil pretreated films followed by sixth larval instar exposed to pretreated HDPE films with the plastic consumption capacity of 8.63% (Figure 6.5). Besides, the plastic consumption capacity was least for first larval instar when fed on naive and soil treated films. Present exploration is the first research report that explores plastic consumption capacity of soil treated HDPE film. Additionally, 92 mg of mass loss was noted by 100 greater wax moth larvae in a twelve-hour period in a commercial polyethylene plastic

shopping bag (Bombelli et al., 2017). Also, over twenty-one days of investigation, a batch of 150 larvae of *G. mellonella* ingested 1.95g of polyethylene (Lou et al., 2020). Furthermore, after eight days of exposure to the naive polymers, a group of fifty larvae of greater wax moth managed to ingest 386.1 mg of polyethylene (Peydaei et al., 2021). In addition to this, Zhu et al. (2022) reported mass loss of 0.006 g of polyethylene/greater wax moth larva. On the other hand, after five days of exposure to *G. mellonella* larvae, naive polyethylene showed a mass loss of less than 5 mg of plastic for each caterpillar that survived (Beale et al., 2022).

Besides, in comparison to other larval instars fed with naive and soil-treated HDPE film, the control groups of all larval instars fed with artificial diet for first to seventh instar control, demonstrated 100% survival rate. When compared to naive HDPE film, a significantly higher survival rate was observed for all larval instars fed with soil-treated HDPE film. However, the greatest survival rate was shown to be 94.666% in sixth instar larvae fed with soil-treated HDPE film, and 94% in seventh instar larvae exposed to soil-treated HDPE film (Figure 6.6). Furthermore, in the previous literature, after twenty-one days, 35.3% survival rate was observed for the greater wax moth larvae exposed to polyethylene (Lou et al., 2020). On the contrary Cassone et al. (2022), throughout the course of 10 days of experimentation observed 47.8% of the survival percentage of larvae when fed with polyethylene. Further, larvae of *G. mellonella* that were fed with plastics showed a survival rate of 93.75%-97.5% (Zhu et al., 2022). In addition, Beale et al.(2022) found that adding molasses to the plastic diet enhanced the survival rate of wax moth larvae.

In the present section, plastic consumption capacity of the naive and soil treated PP films for *G. mellonella* is explored. The ability of the soil-treated plastic sheets to consume plastic increased significantly from the third to the last larval instar stage. The highest plastic consumption rate of 8.44% was noted for seventh-instar *G. mellonella* larvae fed with soil-pretreated films (Figure 6.7). This was followed by seventh-instar larvae given naive PP films, which had a plastic consumption capacity of 8.4% (Figure

6.7). Furthermore, the first and second larval instars have no potential to consume PP. This investigation is the first study report that examines *G. mellonella*'s ability to consume plastic for PP film treated with soil. Plastic consumption capacity for a group of fifty greater wax moth larvae was 159.3 mg in a period of eight days (Peydaei et al., 2021). Additionally, the percentage of PP used by fifty *G. mellonella* larvae up to the pupal stage (40 days) was 1.7% (Ruiz Barrionuevo et al., 2022). Zhu et al.(2022) also noted a mass loss of 0.005 g of polypropylene/ greater wax moth larva (approximately). Additionally, after five days of exposure to greater wax moth larvae on naive polypropylene, a mass loss of less than 1 mg of plastic was noted for each larva that survived (Beale et al., 2022).

The control groups of all larval instars provided artificial food, for control group of first instar larva to seventh instar larval stage showed 100% survival rate in contrast to other larval instars fed with naive and soil-treated PP film. Moreover, the highest survival rate was shown to be 96.66% in seventh instar larvae fed with soil-treated PP film, and 96% in sixth instar larvae exposed to soil-treated PP film (Figure 6.8). Similarly, throughout the course of forty days, the mortality rate of larvae fed PP was 18% (Ruiz Barrionuevo et al., 2022). Additionally, the survival percentage of *G. mellonella* larvae fed plastics ranged from 93.75% to 97.5% (Zhu et al., 2022). Furthermore, Beale et al. (2022) found that adding molasses to the plastic diet enhanced the survival rate of greater wax moth larvae.

This section examines plastic consumption ability of the naive and soil treated LDPE, HDPE, and PP films by larvae of *A. grisella*. For LDPE film, from the third to the last instar larval stage, the soil-treated plastic films' capacity to ingest plastic increased noticeably. For LDPE films, the maximum plastic consumption capacity was 7.51% exhibited by fifth larval instar fed on naive film followed by 2.64% by fifth instar fed on soil treated LDPE film (Figure 6.9). No plastic consumption capacity was detected in the first instar larva. Moreover, the lesser wax moth larvae were fed with the silo-bags for 12 days exhibited 90% of plastic consumption capacity (Chalup et al., 2018).

Compared to other larval instars fed with naive and soil-treated LDPE film, the control groups of all larval instars that were given artificial diet- identified as control1, control2, control3, control4, control5 showed a 100% survival rate. In general, all larval instars fed with soil-treated LDPE film showed a considerably greater survival rate as compared to naive LDPE film. However, the highest survival rate was found to be 92% in fifth instar larvae exposed to naive LDPE film followed by 93.333% in larvae fed with soil-treated LDPE film (Figure 6.10).

This section evaluates the *A. grisella* larvae's capacity to consume plastic from naive and soil-treated HDPE films. The larvae's ability to consume the soil-treated HDPE films increased substantially from the second to the last instar stage. The fifth larval instar fed on soil-treated HDPE film demonstrated a highest plastic consumption capacity of 7.55%, whereas the fourth instar fed on soil-treated HDPE film reported the capacity of 4.95% (Figure 6.11). Similarly, 43.3% consumption of HDPE films was reported for *A. grisella* in the previous literature (Kundungal et al., 2019).

The control groups of first to fifth larval instar stage that were fed artificial diet had a 100% survival rate in contrast to other larval instars fed with naive and soil-treated HDPE film. When compared to naive HDPE film, all larval instars fed with soil-treated HDPE film exhibited a significantly higher survival rate. However, the fourth instar larvae exposed to pretreated HDPE film had the highest survival rate (94.666%), whereas the fifth instar larvae fed with soil-treated HDPE film had the second highest survival rate of 94% (Figure 6.12). Moreover, the survival rate of *A. grisella* larvae when fed on the naive HDPE film is 74.6% (Kundungal et al., 2019).

The present report is the first report on polypropylene consumption by *A. grisella*. The larvae's ability to consume the soil-treated PP films increased substantially from the first to the last instar stage. The maximum plastic consumption capacity was 1.82% for the fifth larval instar fed on soil-treated PP film, whereas 1.6% was found for the fourth instar fed on naive PP film (Figure 6.13). No reports are available on the polypropylene plastic consumption by the lesser wax moth larva.

The control groups from first to last larval instar that were fed on the artificial diet had a 100% survival rate in contrast to other larval instars fed with naive and soil-treated PP film. When compared to naive PP film, all larval instars fed with soil-treated PP film exhibited a significantly higher survival rate. Further, the fifth instar larvae exposed to pretreated PP film had the highest survival rate (96.66%), whereas the fourth instar larvae fed with soil-treated PP film had the second highest survival rate of 96% (Figure 6.14). No reports are available on the survival rate of lesser wax moth larva after feeding on polypropylene plastic film.

In the present research work, all the larval instar were fed with the antibiotic solution and were exposed to the long linear chained plastics (LDPE, HDPE, PP) along with larval instars not fed with any antibiotic solution. In addition to this, the control groups were fed on the artificial diet for all larval instars. The exposure to plastics was for 48 hours to a group of fifty larvae of each larval instar stage. In the present exploration of the research report, the plastic consumption capacity of all larval instars, survival rate of the larvae, SEM of the remnants consumed film and GC-MS of the excretory waste produced by antibiotic fed and without antibiotic administered the larvae was examined.

In present section the plastic consumption capacity of naive LDPE film by *G. mellonella* is discussed. For naive LDPE film, the plastic consumption capacity of *G. mellonella* was maximum 4.84% for without antibiotic fourth instar larva followed by 2.65% for seventh instar larva fed with the antibiotics (Figure 6.15). For first instar no plastic consumption capacity was observed. Similarly in the previous literature the *G. mellonella* larvae fed with or without antibiotics could possibly consume the bee wax (Kong et al., 2019). Additionally, 92 mg of mass loss was noted by 100 greater wax moth larvae in twelve hours for a commercial plastic shopping bag (Bombelli et al., 2017). Kundungal et.al. (2021) had reported 18.57% weight loss of naive LDPE film when fed by greater wax moth larvae. Furthermore, 100 *G. mellonella* caterpillars consumed 325µg of LDPE in a single day and 768µg of polyethylene in a three-day period (Lemoine et al.,

2020). After being exposed to LDPE film for 12 hours, *G. mellonella* larvae showed the presence of large holes measuring 6.3 mm and pits of 14µm (Peydaei et al., 2020). Also, over twenty-one days of experiment, a batch of 150 larvae ingested 1.95g of polyethylene (Lou et al., 2020). Furthermore, after eight days of exposure to the naive polymers, a group of fifty larvae managed to ingest 386.1 mg of polyethylene (Peydaei et al., 2021). Additionally, fifty *G. mellonella* larvae consumed 9.7% of LDPE until they reached the pupal stage (Ruiz Barrionuevo et al., 2022). Zhu et al. (2022) also noted a mass loss of 0.015g of polyethylene/greater wax moth larva. Furthermore, after five days of exposure to greater wax moth larvae, naive polyethylene showed a mass loss of less than 5 mg of plastic for each caterpillar that survived (Beale et al., 2022). Furthermore, during the course of 7.25 days, 25 greater wax moth larvae exposed to naive LDPE film consumed 5.11% of the plastic (Burd et al., 2023).

In comparison to other larval instars that were fed without antibiotics and with antibiotic treatment, all larval instars in the control groups, feeding an artificial diet resulted in zero deaths. The maximum survival rate was exhibited as 94.66% by seventh instar without antibiotic administered larvae and with antibiotic sixth instar of greater wax moth exposed to naive LDPE film for 48 hours (Figure 6.16). Out of all the larval instar groups, the initial larval instars in both the antibiotic-containing and antibiotic-free groups had the lowest survival rate when exposed to LDPE films. While greater wax moth exposed to naive LDPE film had a survival rate of 73.4% in previous studies (Kundungal et.al., 2021). After twenty-one days, 35.3% of the larvae given polyethylene survived (Lou et al., 2020). Similarly, after forty days, 28% of the larval mortality was observed when fed on LDPE (Ruiz Barrionuevo et al., 2022). Additionally, throughout the course of 10 days of experimentation, the survival percentage of greater wax moth larvae fed polyethylene was 47.8% (Cassone et al., 2022). Besides, larvae of *G. mellonella* that were fed on plastics showed a survival rate of 93.75%-97.5% (Zhu et al., 2022). Furthermore, Beale et al. (2022), observed that adding molasses to the plastic diet enhanced the survival rate of greater wax moth larvae. Also, *G. mellonella* larvae exposed to LDPE plastics showed a 40% survival rate (Burd et al., 2023).

The SEM images of the remnants of the LDPE films revealed pits, cracks, holes, surface roughness and depressions on the surface of all the films consumed by all the larval instars of *G. mellonella* as compared to non-consumed LDPE film (Figure 6.17; 6.18). Although, no plastic consumption capacity was noted for first instar larva but some pits and surface roughness was detected on the film that indicated some extent of plastic consumption by the larvae (Figure 6.18 A,B). Moreover, the homogenate of the wax moth was smeared in the surface of the polyethylene film and there was significant differences in the surface morphology analysed by the AFM (Atomic Force Microscopy) (Bombelli et al., 2017). According to Ren et al. (2019), *Enterobacter* sp. D1 isolated from the guts of the greater wax moth larvae when exposed to LDPE film for fourteen days could significantly destruct the polyethylene film. Additionally, presence of cracks, roughness, and depressions were detected by SEM and AFM after incubation of the plastics with the bacterial species for a period of thirty one days. Similarly, AFM micrographs depicted surface roughness, pits and cavities on the LDPE films (Kundungal et.al., 2021). Moreover, increase in the pitting and surface deformations was observed in the SEM images of polyethylene film after exposure to the *G. mellonella* larvae (Peydaei et al., 2020). Likewise, the SEM images of polyethylene films revealed presence of filamentous structures with signs of cuts on the edges of the surface of the plastic film when ingested by greater wax moth larvae (Peydaei et al., 2021). In addition to this SEM images reported by Burd et al. (2023), also validated the presence of surface irregularities after consumption by the greater wax moth larvae. The SEM and AFM microscopy confirmed the biodegradation of the polyethylene by the *Enterobacter* sp. isolated from the gut of the greater wax moth larvae (Riabi et al., 2023).

The GC-MS results obtained in the present study revealed presence of hydrocarbons, alcohols, acids, esters, ethers and other functional groups in the frass of all the instars of greater wax moth fed with or without antibiotics that were exposed to the LDPE film for forty eight hours. As compared to the GC-MS of naive LDPE film that exhibited the presence of high molecular weight compounds whereas the chemical peaks in the GC-MS of the frass of greater wax moth larva fed with LDPE film, revealed the presence of low

molecular weight acids, esters, alkanes and other hydrocarbons which shows the signs of biodegradation of the polymers by the insect larva (Figure 6.19). Irrespective of the administration of antibiotics to the larva or non-antibiotic group of greater wax moth larva, presence of alkanes, alcohols, acids, esters and ethers in the excretory waste of larval instars points out towards biodegration of LDPE films by the larval instars of *G. mellonella* (Figure 6.19). The results in this section are in accordance with the previous literature (Kong et al., 2019; Kesti & Thimmappa, 2019). Moreover, when the solar pretreated LDPE film and naive LDPE film were fed to greater wax moth larvae, the GC-MS of the excreta of the wax worms revealed the presence of alkanes, acids, ester and ethers, indicating biodegradation of the polyethylene by the larva (Kundungal et.al., 2021). In addition to this, in polyethylene fed larvae decrease the molecular weight of the complex carboxylic acids and formation of long chained fatty acids proclaim the biodegradation of polyethylene by the greater wax worms (Lou et al., 2020).

The plastic consumption capacity of *G. mellonella* for HDPE film is discussed in the present section. When exposed to naive HDPE film, the plastic consumption capacity of *G. mellonella* was maximum 7.01% for seventh instar larva without any prior administration of antibiotics followed by 5.08% by seventh instar larvae fed with antibiotics (Figure 6.20). The least plastic consumption rate was observed in the first instar larvae (Figure 6.20). In the previous literature, 100 greater wax moth larvae ingested 92 mg of mass loss for a commercial polyethylene plastic shopping bag in twelve hours (Bombelli et al., 2017). Further, a fungal strain named *Aspergillus flavus* isolated from the gut of greater wax moth could degrade 3.9025% of HDPE microplastics in tenure of 28 days (Zhang et al., 2020). Additionally, during the course of the experiment, 1.95g of polyethylene was consumed by a batch of 150 larvae (Lou et al., 2020). According to Peydaei et al. (2021) , fifty larvae were able to consume 386.1 mg of polyethylene following eight days of exposure to the naive polymers. Zhu et al. (2022) similarly reported 0.015g of polyethylene/larva mass loss. Moreover, naive polyethylene demonstrated a mass loss of less than 5 mg of plastic for each caterpillar that survived five days of exposure to greater wax moth larvae (Beale et al., 2022).

The control groups of all larval instars fed on the artificial diet, revealed 100% survival rate in comparison to other larval instars that were without antibiotics and with antibiotic treatment. The maximum survival rate was 98% observed in without antibiotic seventh larval instar group followed by 94% in without antibiotic fourth instar group fed with HDPE film (Figure 6.21). Moreover, 35.3% of the larvae exposed to polyethylene survived after twenty-one days (Lou et al., 2020). Along with it, 47.8% survival rate was observed for larvae fed on polyethylene over the course of ten days (Cassone et al., 2022). Furthermore, the survival percentage of *G. mellonella* larvae fed on plastics was 93.75%-97.5% (Zhu et al., 2022). Additionally, Beale et al. (2022) found that increasing the amount of molasses in the plastic diet increased the survival rate of the wax moth larvae.

When comparing to the surface of all the films ingested by all the larval instars of *G. mellonella* to naive HDPE film (Figure 6.22), the SEM pictures of the consumed HDPE film remnants showed pits, fractures, holes, surface roughness, and depressions (Figure 6.23) whereas no such surface roughness was observed in the SEM images of the naive film. Although no weight loss of HDPE film was observed by the first instar larvae but some pits and surface roughness were visible on ingested film, suggesting that the larvae had consumed some plastic. In the previous literature, the wax moth homogenate was smeared on the polyethylene film's surface, and the AFM analysis revealed notable variations in the surface morphology by the homogenate of the insect, indicating biodegradation of the plastics (Bombelli et al., 2017). On the other hand, after being exposed to *G. mellonella*, a polyethylene film showed an increase in pitting and surface deformations (Peydaei et al., 2020). Similarly, filamentous structures with evidence of holes on the plastic film's surface have been observed in the SEM pictures of polyethylene films (Peydaei et al., 2021). The polyethylene's biodegradation by *Enterobacter* sp. was verified by SEM and AFM microscopy (Riabi et al., 2023).

The present study's GC-MS results showed the presence of hydrocarbons, alcohols, acids, esters, ethers, and other functional groups in the frass of all the greater wax moth instars

whether fed with antibiotics or without any prior antibiotic administration that were exposed to the HDPE film for 48 hours (Figure 6.24). In contrast to the GC-MS of naive HDPE film with chemical peaks for alkanes and other long linear chained hydrocarbons, the chemical peaks of the greater wax moth's frass revealed the presence low molecular weight hydrocarbons as compared to peaks observed for the naive film (Figure 6.24A). Regardless of whether the larvae of the greater wax moth were administered with antibiotics or not, the presence of alkanes, alcohols, acids, esters, and ethers in the excretory waste of larval instars indicates that *G. mellonella* larvae are biodegrading HDPE films (Figure 6.24). According to the previous literature, when greater wax worms are fed with polyethylene, after ingestion of the plastic, the GC-MS of the frass exhibited low molecular weight of the complex carboxylic acids and long-chained fatty acids are formed, indicating the biodegradation of the polyethylene (Lou et al., 2020).

In the present section, the plastic consumption capacity of naive PP film is discussed. For PP films, the maximum plastic consumption capacity was 8.4% observed in the without antibiotic treatment seventh instar larva followed by 2% for antibiotic fed seventh instar larval group (Figure 6.25). No plastic consumption capacity was observed for first and second instar larva. According to Peydaei et al. (2021), when greater wax moth larvae were exposed to polypropylene film for eight days, 159.6 mg mass loss of polypropylene was observed. Moreover, 1.7% of the plastic consumption capacity was observed for naive polypropylene plastics consumed by greater wax moth larvae (Ruiz Barrionuevo et al., 2022).

In contrast to other larval instars that were treated with antibiotics and those that were not, the control groups from first to seventh larval instars given artificial diet exhibited a 100% survival rate. The maximum insect survival rate was 94.66% for without antibiotic seventh instar larvae followed by 94% for with antibiotic sixth instar larvae (Figure 6.26). Furthermore, in the previous literature, the larval mortality rate was 18% for greater wax moth larvae when they consume the naive polypropylene plastics (Ruiz Barrionuevo et al., 2022).

The surface of every film consumed by all *G. mellonella* larval instar was compared to non-consumed PP film using a scanning electron microscopy. The results revealed pits, cracks, holes, rough surfaces, and depressions on the surface of SEM images for remnants of the plastic film (Figure 6.28) whereas no surface irregularities are visible on the SEM images of the naive film (Figure 6.27). But SEM images for first instar revealed no traces of plastic consumption by the larvae. In the previous literature, a hole as well as few scratches were visible on the PP film ingested by greater wax moth larvae (Peydaei et al., 2021).

The GC-MS results of the current investigation demonstrated the presence of several functional groups, including ethers, esters, alcohols, acids, and hydrocarbons, in the frass of all the greater wax moth instars that were exposed to the PP film for 48 hours that were either administered with antibiotics or no prior antibiotic treatment (Figure 6.29). The chemical peaks of the greater wax moth's frass indicated the existence of alkanes, alcohols, acids, esters, ethers and additional functional groups, in contrast to the naive PP film's GC-MS, which showed chemical peaks for alkanes and other long linear chained hydrocarbons (Figure 6.29A). The presence of alkanes, alcohols, acids, esters, and ethers in the excretory waste of larval instars of the greater wax moth suggests that *G. mellonella* larvae are biodegrading polypropylene films, regardless of whether the larvae were given antibiotics or not, i.e. gut microbiota is not solely responsible for biodegration of the plastics.

In the current section the plastic consumption capacity of naive LDPE film for *A. grisella* is discussed. In the present exploration maximum plastic consumption capacity was 7.51% observed in the without antibiotic fifth instar group larvae followed by 2.77% in the with antibiotic fifth instar group (Figure 6.30). No plastic consumption capacity was observed in first instar larva. On the contrary, 90% of the consumption of plastic was recorded in the previous literature for the lesser wax moth larvae that were fed silo-bags for a duration of 12 days (Chalup et al., 2018).

When provided an artificial diet, the control groups consisting of larvae at all developmental stages showed 100% survival in contrast to other larval instars that were treated with antibiotics and without antibiotics group. Additionally, the maximum survival rate was 92% for without antibiotic fifth instar larva followed by fourth instar larva fed with antibiotics followed by 88% for without antibiotic fourth instar larva fed with LDPE film (Figure 6.31).

The SEM images of the LDPE film remnants revealed pits, fractures, holes, surface roughness, and depressions (Figure 6.32) when the surfaces of all the films consumed by all *Achroia grisella* larval instars were compared to naive LDPE film.

In the present investigation, the GC-MS data showed that the frass of all *A. grisella* larvae that were exposed to the LDPE film for 48 hours exhibited a variety of functional groups, including ethers, esters, alcohols, acids, and hydrocarbons that indicate the biodegradation of the polymers by the wax moth (Figure 6.33). It is likely that *A. grisella* larvae biodegrade polyethylene films whether or not they were administered antibiotics since the excretory waste of lesser wax moth of all larval instars contains alkanes, alcohols, acids, esters, and ethers that confirm digestion of plastic film by the larva.

In the current section, the plastic consumption capacity of HDPE film for *A. grisella* larva is discussed. The maximum HDPE film consumption capacity was 6.83% for with antibiotic fourth instar followed by 4.41% for without antibiotic fifth instar group (Figure 6.34). No plastic consumption capacity was observed in the first instar larva. Likewise, 43.3% of HDPE film consumption for *A. grisella* was documented in previous investigations (Kundungal et al., 2019).

Irrespective of the larval developmental stage, control group demonstrated perfect survivability when consuming the provided artificial diet. The maximum insect survival rate was 81.333% observed for without antibiotic fifth instar stage followed by 80.666% for without antibiotic fourth instar group when fed on HDPE film (Figure 6.35). The minimum survival rate was observed for first instar stage. Furthermore, 74.6% of *A. grisella* larvae survived when fed on naive HDPE film (Kundungal et al., 2019).

The surface of remnants of all the films consumed by all *A. grisella* larval instar was compared to non-consumed HDPE film using a scanning electron microscope. The HDPE film remains displayed pits, cracks, holes, surface roughness, and depressions (Figure 6.36) whereas there were no such surface irregularities on the SEM of the naive film (Figure 6.22).

The current study's GC-MS data showed that the frass of all lesser wax moth instar that was exposed to the HDPE film for 48 hours and received antibiotics or no antibiotic treatment exhibited a variety of functional groups, such as ethers, esters, alcohols, acids, and hydrocarbons (Figure 6.37). Unlike the GC-MS analysis of the naive HDPE film, which revealed chemical peaks for alkanes and other long linear chained hydrocarbons, the chemical peaks of the frass of the lesser wax moth suggested the presence of additional functional groups. Despite the fact that the larvae of *A. grisella* were administered antibiotics, the excretory waste of antibiotic-fed larval instars of the lesser wax moth contains alkanes, alcohols, acids, esters, and ethers, indicating that the larvae are biodegrading polyethylene films. So, irrespective of presence or absence of the gut microbiota the larvae could degrade the plastics.

In the current section plastic consumption and biodegradation capacity of lesser wax moth larvae for polypropylene plastics is explored. **The present report is the first report that claims the consumption of polypropylene film by the *A. grisella* larvae.** The maximum plastic consumption rate was 3.66% for with antibiotic fifth instar larva followed by 1.65% for with antibiotic fourth instar group (Figure 6.38). No reports are available on the polypropylene plastic consumption by the lesser wax moth larva.

The maximum survival rate was 99.33% for with antibiotic fifth instar group followed by 96.66% for with antibiotic third instar group (Figure 6.39). The minimum survival rate was observed for the first instar larva. No reports are available on the survival rate of lesser wax moth larva when fed with PP film.

The surface of every film consumed by all *A. grisella* larval instar was compared to non-consumed PP film using a scanning electron microscope. The results revealed pits, cracks, holes, rough surfaces, and depressions (Figure 6.40) on the remnants of the wax worm ingested films as compared to the naive PP film.

The current study's GC-MS data showed that the frass of all lesser wax moth instar that was exposed to the PP film for 48 hours contained a variety of functional groups, such as ethers, esters, alcohols, acids, and hydrocarbons (Figure 6.41). In contrast to the naive PP film's GC-MS, which revealed chemical peaks for alkanes and other long linear chained hydrocarbons, the chemical peaks of the lesser wax moth's frass suggested the presence of additional functional groups with low molecular weight. Regardless of whether the larvae were administered antibiotics or not, the excretory waste of lesser wax moth larval instars contains alkanes, alcohols, acids, esters, and ethers, indicating that *A. grisella* larvae are biodegrading polypropylene films.

In addition to this, Ren et al. (2019) had isolated the *Enterobacter* sp. D1 from the gut of the greater wax moth larvae that could degrade the LDPE waste potentially. Moreover, *Aspergillus flavus* isolated from the guts of the greater wax moth could biodegrade the HDPE microplastics (Zhang et al., 2020). Saikia et al. (2022) had isolated a few gram-positive, gram-negative and a few micro algal species that were isolated from the guts of the greater wax moth larvae that could potentially degrade the plastics. Further, a bacterial species from genus *Acinetobacter* could grow on the LDPE plastics as the sole carbon source and degrade the plastics (Cassone et.al., 2020). Moreover, bacterial species isolated from the whole body extracts of *Microbacterium oxydans*, *Bacillus aryabhattai*, and *Lysinibacillus fusiformis* and there microbial consortia could biodegrade the LDPE efficiently (Montazer et al., 2021). Furthermore, a detailed analysis of gut microbiota of *G. mellonella* after consuming polyethylene and polystyrene was reported with strong emphasis on the bacterial and fungal diversity in wax moth larvae (Ruiz Barrionuevo et al., 2022b). A few microbial species were isolated from the gut of the greater wax moth named as *Bacillus* sp., *Enterococcus* sp., *Acinetobacter* sp., *Staphylococcus* sp.,

Aspergillus flavus had the potential to biodegrade the polyethylene plastics (Riabi et al., 2023). Although these microbial species could degrade the plastics but the plastic degradation capacity is quiet low as compared to the whole larvae comparatively. So, the wax moth could efficiently disintegrate the plastics.

The process of biodegradation in the wax moth is a complicated process. In the present thesis, the wax worms were firstly fed with the plastics, then their digestion and plastic biodegradation process was tracked by the analysis of the excretory waste of the larva and finally the rate of digestion could be inferred by enzyme assay. So, we can say that the process of biodegradation in the wax moth is divided into three steps- First step is fragmentation or ingestion of plastics by wax moths, second is digestion and third is energy production (Figure 6.54). The mouthparts of larva fragment the plastics, gut enzymes digest the plastics and the energy is produced by lactate dehydrogenase enzyme.

Further, to explore the biodegration capacity of the larvae for LDPE, HDPE and PP as sole diet, the enzymatic activity of enzymes alcohol dehydrogenase and lactate dehydrogenase was studied. The enzyme alcohol dehydrogenase plays important role in oxidation of fragments of diet into aldehyde. Moreover, it was hypothesised that since ADH enzyme is recorded to produce a surplus of the reduced form of nicotinamide adenine dinucleotide (NADH) as a byproduct, metabolic pathways may react by using lactate dehydrogenase (LDH) to convert pyruvate to lactic acid, which could regenerate NAD^+ (Figure 6.54, 6.55, 6.56) (Lemoine et al., 2020).

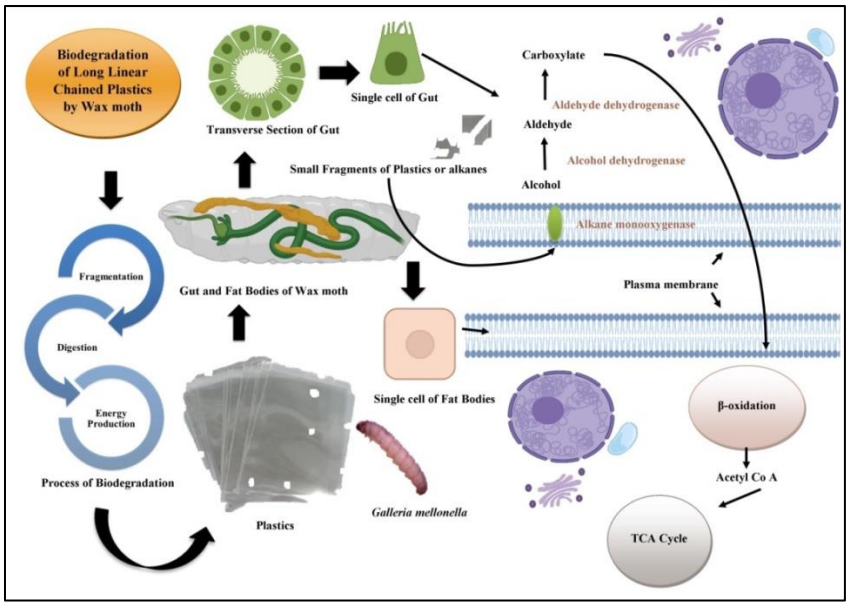


Figure 6.54 Process of the biodegradation in the wax moth. The image depicts the hypothetical mechanism of the process of the biological degradation of plastics in the gut of the wax worms (Lemoine et al., 2020) (Copyright Protected)

In the present section, the maximal enzymatic activity for enzyme alcohol dehydrogenase for *G. mellonella* when fed on LDPE, HDPE, PP as sole carbon diet is explored. For the LDPE plastics, the maximum enzymatic activity was 3.32 mU/min for without antibiotic sixth instar larva followed by 2.57 mU/min for without antibiotic third instar group (Figure 6.42). The least enzymatic activity was observed for first instar larva. Moreover, for the HDPE, the maximal enzymatic activity was 4.6 mU/min for with antibiotic fifth instar larva followed by 1.28 mU/min for with antibiotic seventh instar group (Figure 6.43). The least enzymatic activity was observed for first instar larva. Further, the maximum enzymatic activity for PP was 3.90 mU/min for without antibiotic second instar group followed by 3.7 mU/min for without antibiotic seventh instar group (Figure 6.44). Remarkably, researchers in the previous literature, noticed that in caterpillars given polyethylene, the peak activity of ADH enzyme was doubled (Lemoine et al., 2020).

In the present section, the maximal enzymatic activity for enzyme alcohol dehydrogenase for *A. grisella* when fed on LDPE, HDPE, PP as sole carbon diet is

explored. The maximum enzymatic activity for LDPE as the sole diet was 5.14 mU/min for without antibiotic fifth instar group followed by 4.01 mU/min for with antibiotic fourth instar group (Figure 6.45). Furthermore, the maximum enzymatic activity for HDPE polymer as sole diet was 5.35 mU/min for third instar without antibiotic group followed by 3.911 mU/min for without antibiotic first instar group and 3.375 mU/min for fifth instar with antibiotic group (Figure 6.46). Also, for the PP plastics as sole diet, the maximum enzymatic activity of 5.626 mU/min for with antibiotic fifth larval instar group followed by 5.144mU/min for with antibiotic fifth larval instar group (Figure 6.47). No research reports are available on ADH activity of lesser wax moth larvae when fed on the plastics.

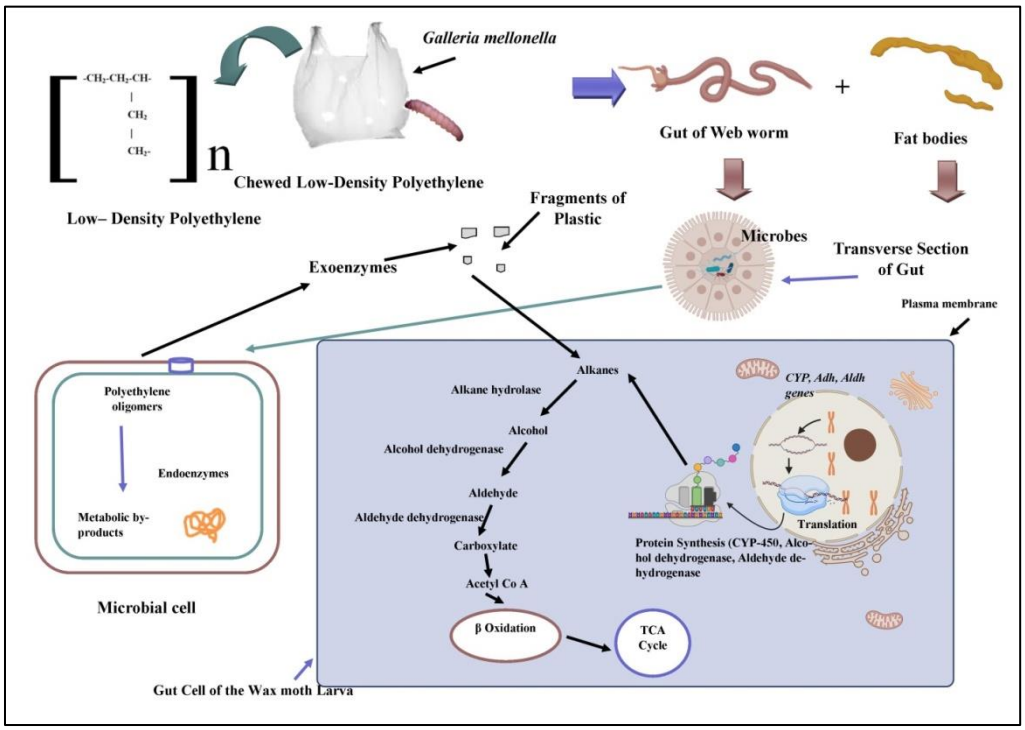


Figure 6.55 (A)

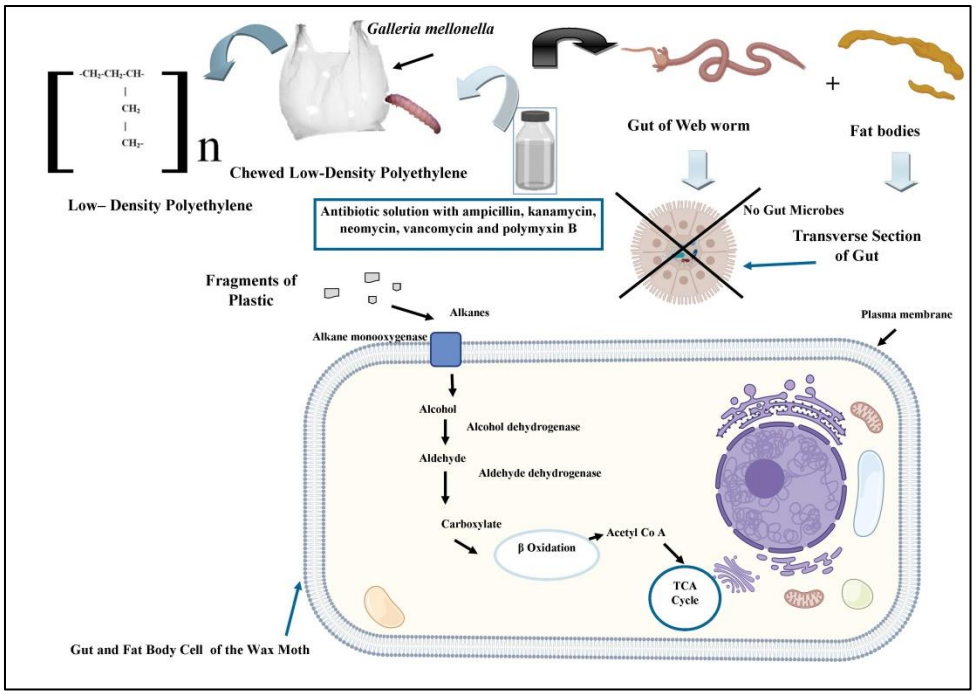


Figure 6.55 (B)

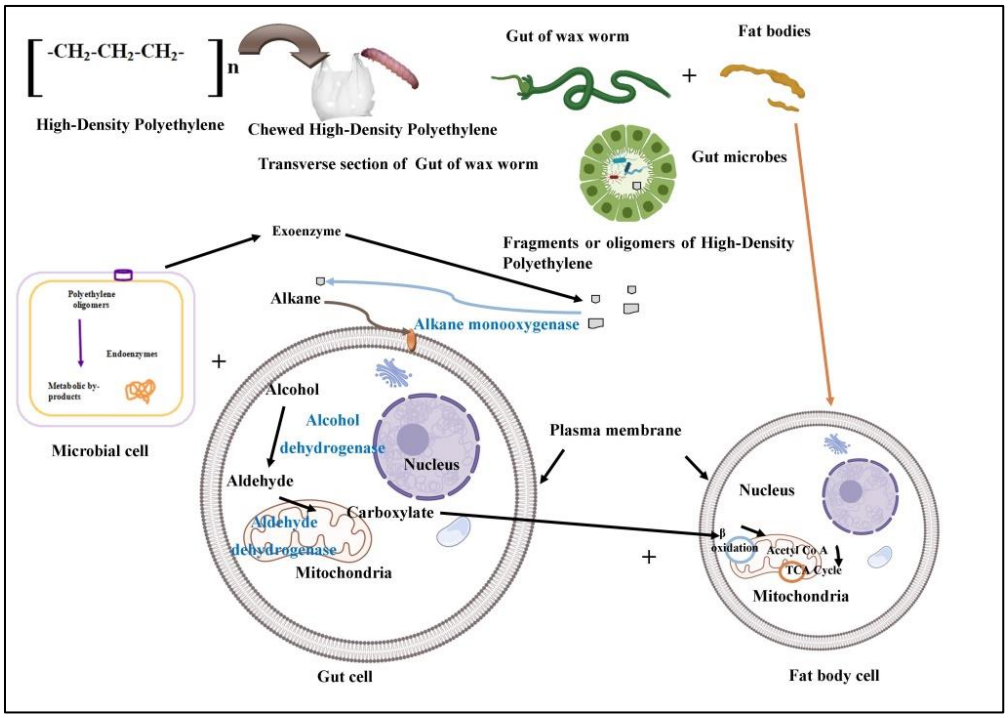
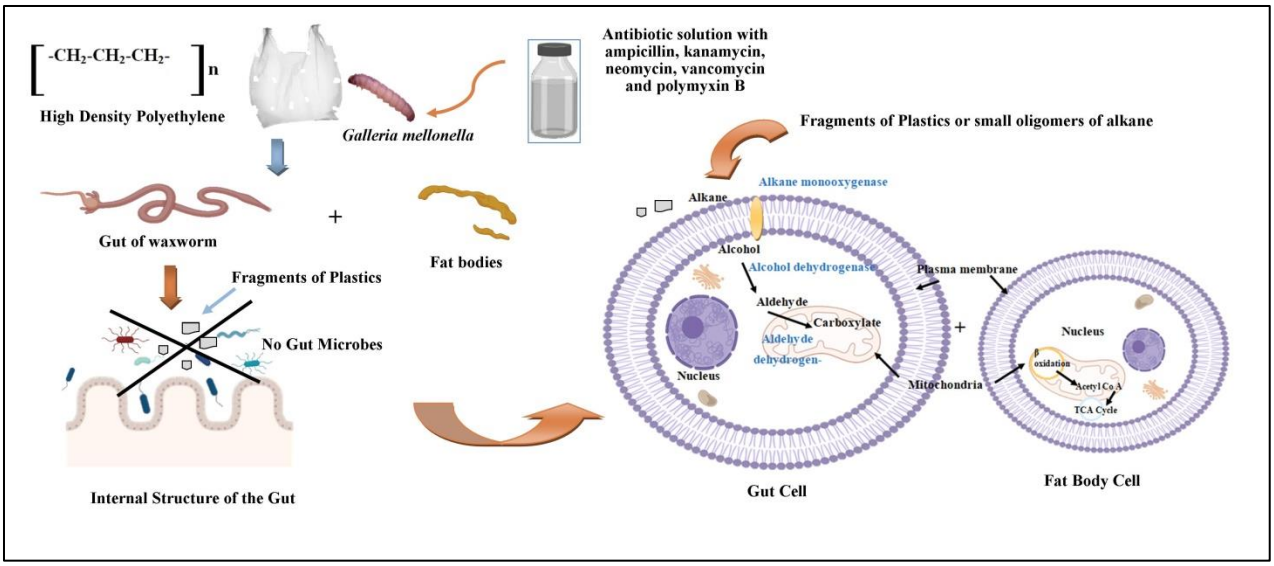


Figure 6.55 (C)



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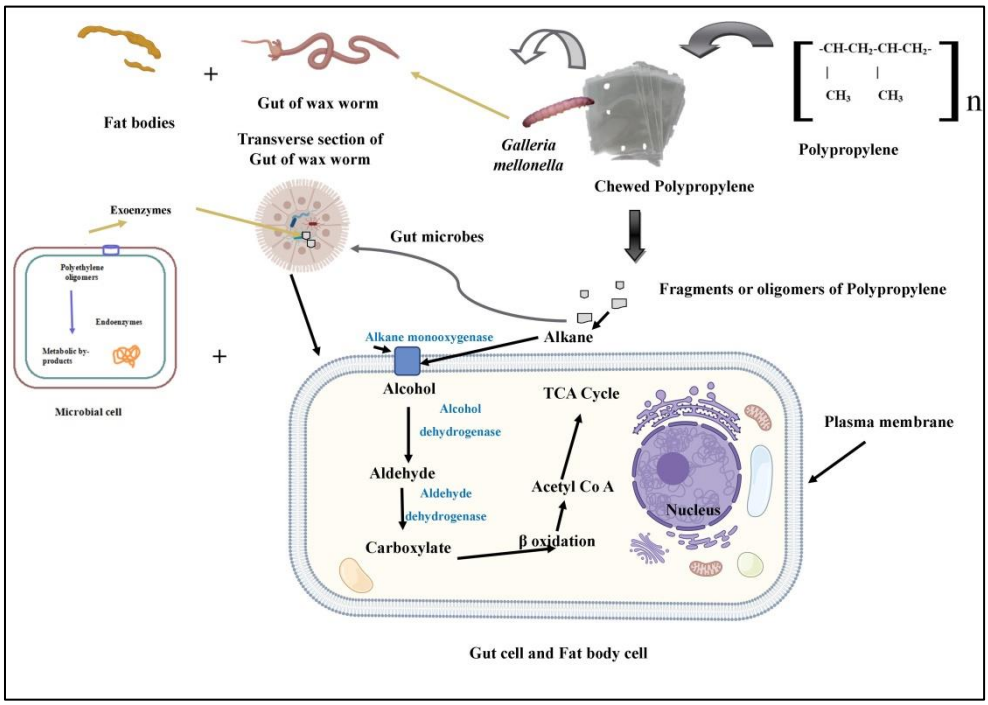

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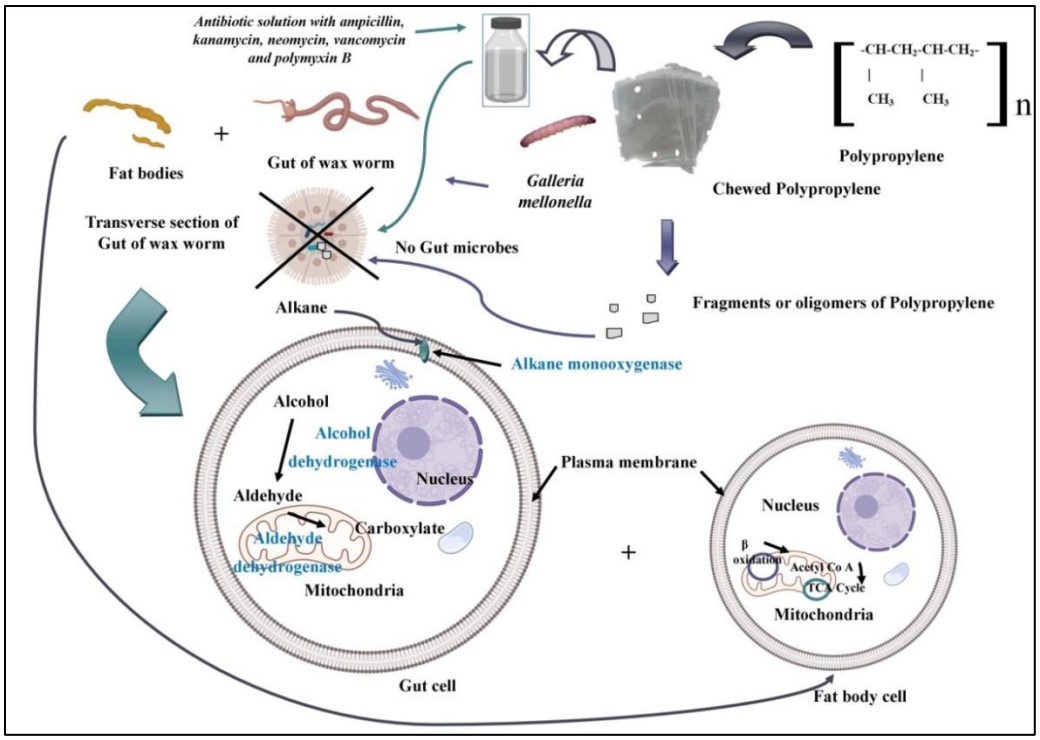
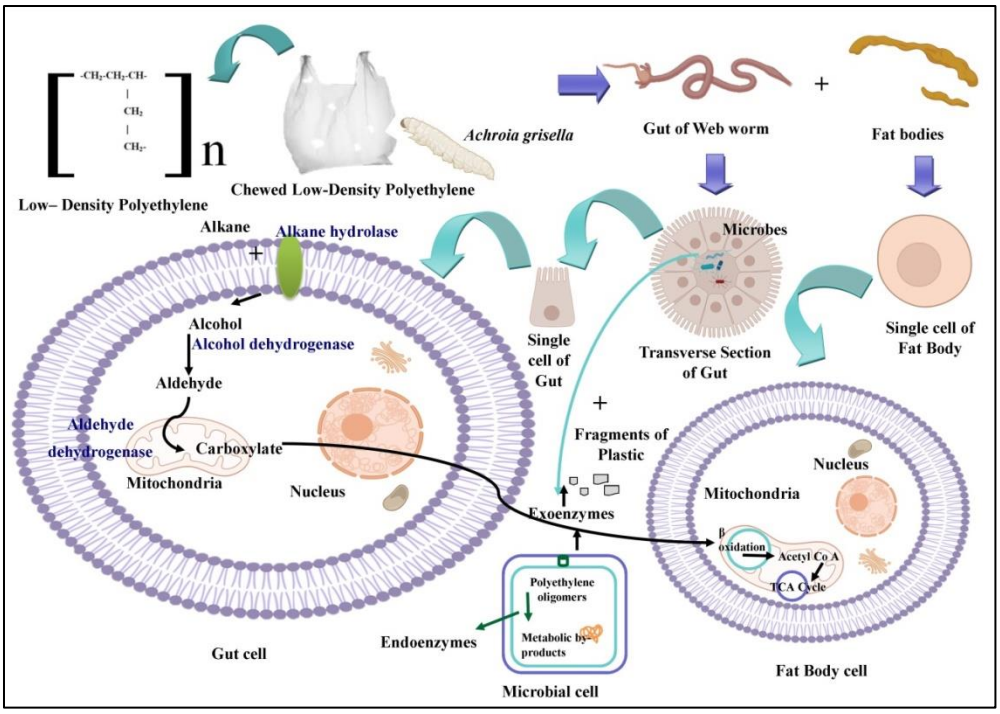


Figure 6.55 (F)

Figure 6.55 Hypothetical mechanisms of biodegradation of different types of plastics by *G. mellonella* (Copyright Protected). (A) Hypothetical mechanism of without antibiotic fed biodegradation in the larvae of greater wax moth for LDPE (Ghatge et al., 2020; Lemoine et al., 2020); (B) Hypothetical mechanism of antibiotic fed biodegradation in the larvae of greater wax moth for LDPE (Kong et.al., 2019; Lemoine et al., 2020); (C) Hypothetical mechanism of without antibiotic fed biodegradation in the larvae of greater wax moth for HDPE (Ghatge et al., 2020; Lemoine et al., 2020); (D) Hypothetical mechanism of antibiotic fed biodegradation in the larvae of greater wax moth for HDPE (Kong et.al., 2019; Lemoine et al., 2020); (E) Hypothetical mechanism of without antibiotic biodegradation in the larvae of greater wax moth for PP (Ghatge et al., 2020; Lemoine et al., 2020); (F) Hypothetical mechanism of antibiotic fed biodegradation in the larvae of greater wax moth for PP (Kong et.al., 2019; Lemoine et al., 2020)



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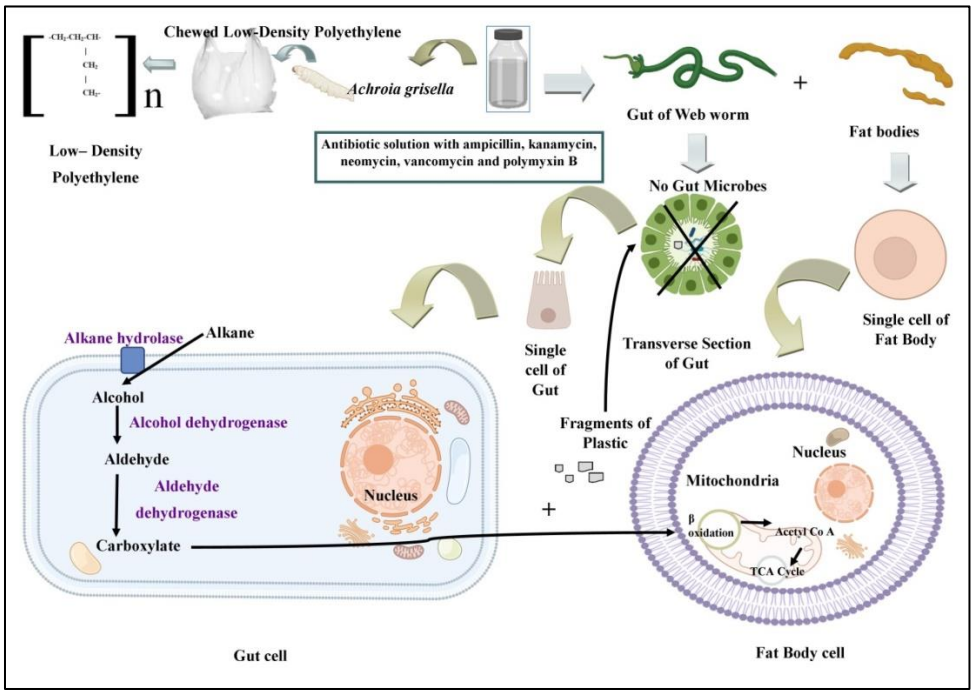

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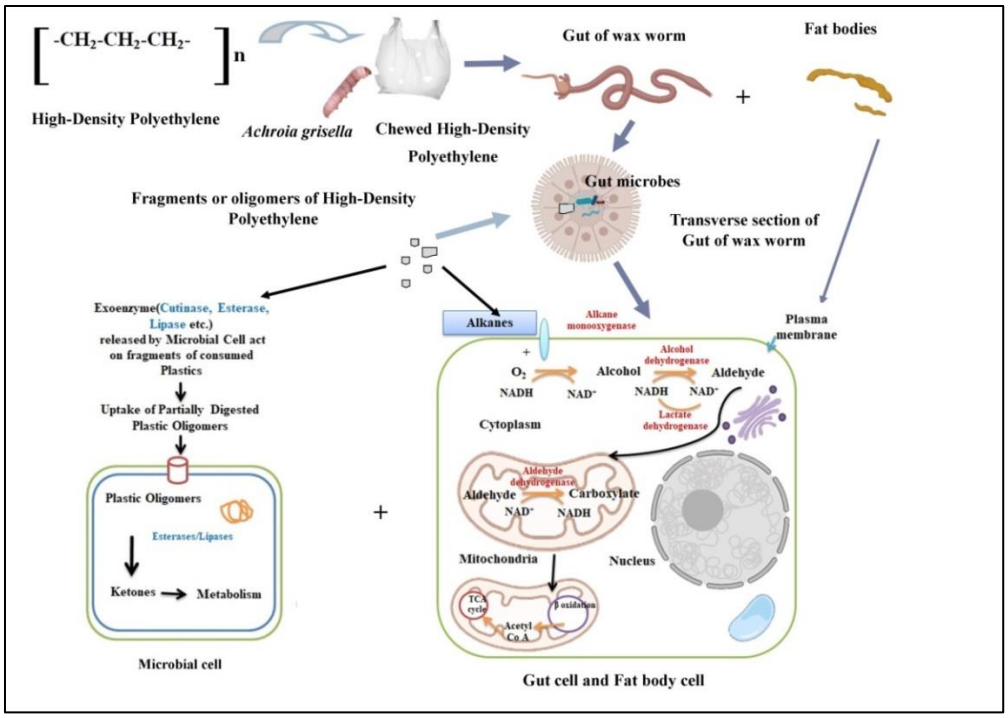

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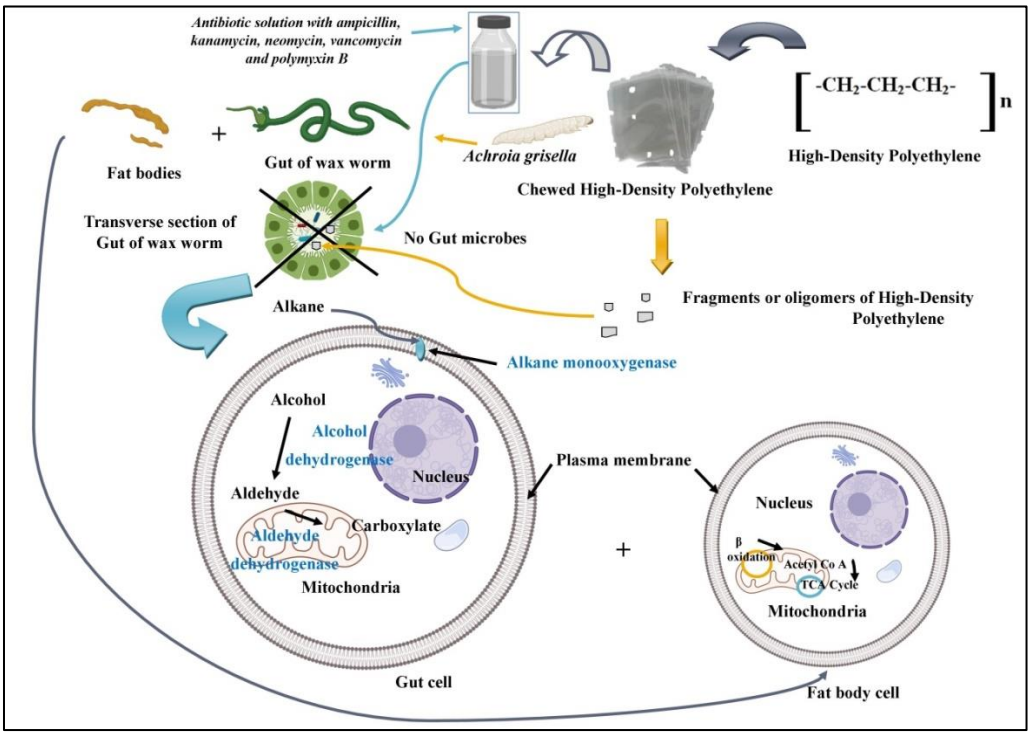

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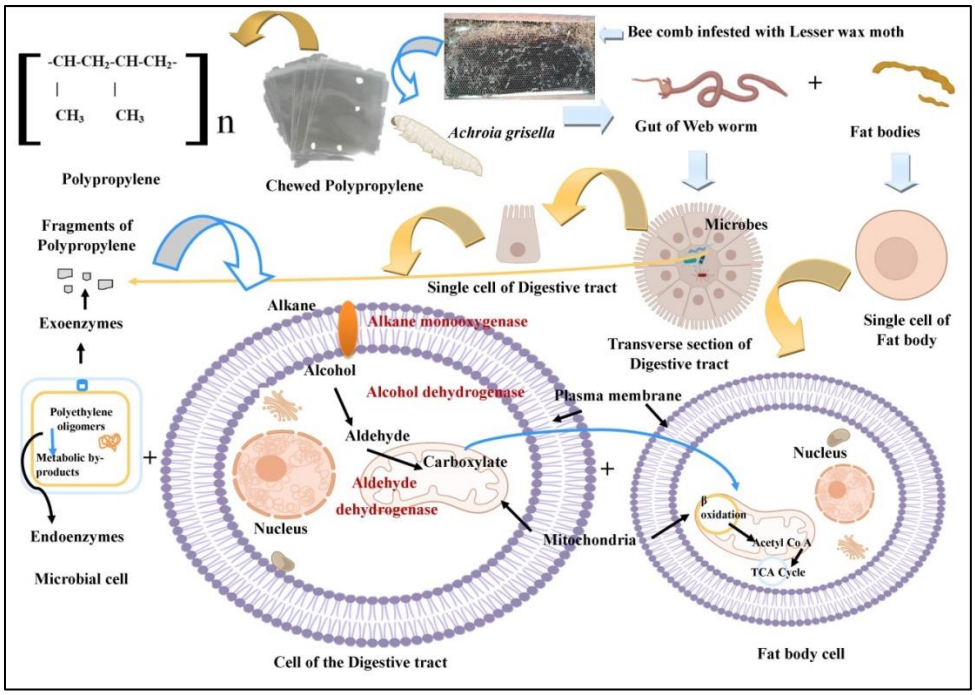

Figure 6.56 (E)

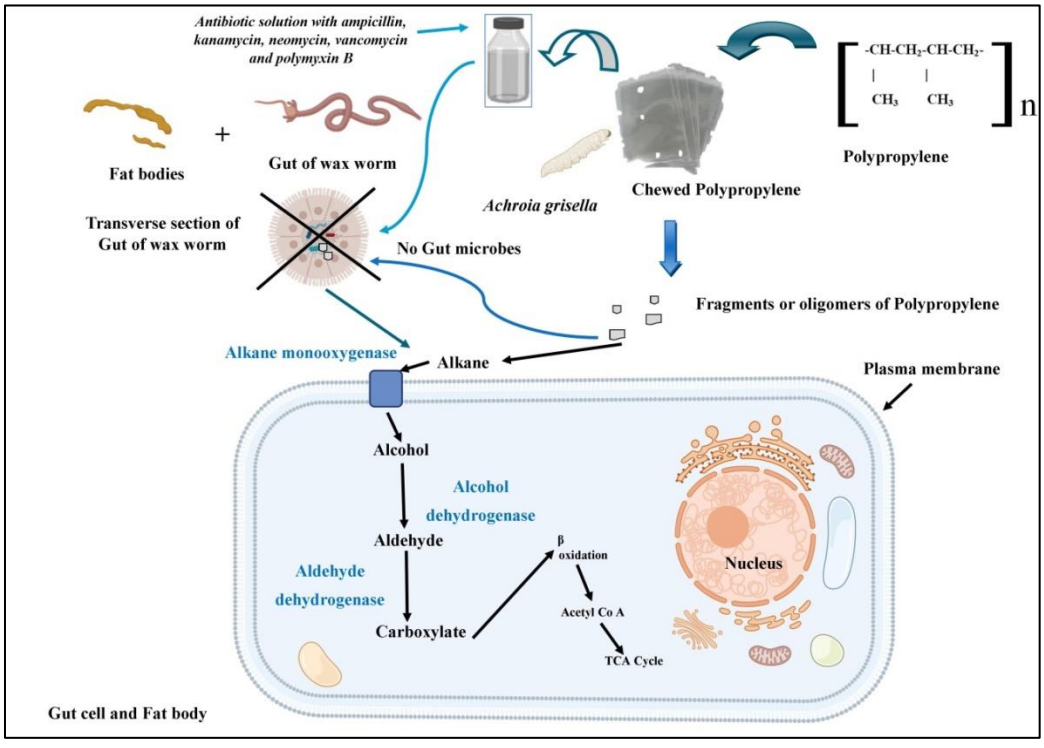

Figure 6.56 (F)

Figure 6.56 Speculative mechanisms for biodegration of different types of long linear chained hydrocarbons by *A. grisella* larva (Copyright protected). (A) Hypothetical mechanism of without antibiotic fed biodegradation in the lesser wax moth by for LDPE (Ghatge et al., 2020; Lemoine et al., 2020); (B) Hypothetical mechanism of antibiotic fed biodegradation in the lesser wax moth by for LDPE (Kong et.al., 2019; Lemoine et al., 2020); (C) Hypothetical mechanism of without antibiotic fed biodegradation in the lesser wax moth by for HDPE (Ghatge et al., 2020; Lemoine et al., 2020); (D) Hypothetical mechanism of antibiotic fed biodegradation in the lesser wax moth by for HDPE (Kong et.al., 2019; Lemoine et al., 2020); (E) Hypothetical mechanism of without antibiotic fed biodegradation in the lesser wax moth by for PP (Ghatge et al., 2020; Lemoine et al., 2020); (F) Hypothetical mechanism of antibiotic fed biodegradation in the lesser wax moth by for PP (Kong et.al., 2019; Lemoine et al., 2020)

In the present section, the maximal enzymatic activity for enzyme lactate dehydrogenase for *G. mellonella* when fed on LDPE, HDPE, PP as sole carbon diet is explored. For the LDPE plastics as the sole diet, the maximum enzyme activity (LDH) was 2.947 mU/min for with antibiotic seventh instar larva followed by 2.786 mU/min for without antibiotic seventh instar larva (Figure 6.48). Also, for the HDPE plastics as the sole diet, the maximum enzyme activity (LDH) was 1.285 mU/min for with antibiotic seventh instar larva followed by 1.071 mU/min for without antibiotic sixth instar larva (Figure 6.49). Moreover, for the PP plastics as the sole diet, the maximum enzyme activity was 3.676 mU/min for without antibiotic seventh instar larva followed by 2.786 mU/min for with antibiotic seventh instar larva (Figure 6.50). Previous research indicates that when greater wax moth larvae were given polyethylene, their LDH activity doubled, indicating a rise in lipid oxidation (Lemoine et al., 2020).

In the present section, the maximal enzymatic activity for enzyme lactate dehydrogenase for *A. grisella* when fed on LDPE, HDPE, PP as sole carbon diet is explored. For the LDPE plastics as the sole diet, the maximum enzyme activity was 0.899mU/min for with antibiotic third instar larva followed by 0.803 mU/min for without antibiotic fifth instar larva (Figure 6.51). Additionally, for the HDPE plastics as the sole diet, the maximum enzymatic activity was 0.803 mU/min for with antibiotic fifth instar larva followed by 0.749 mU/min for without antibiotic fourth instar larva (Figure 6.52). Moreover, for the PP plastics as the sole diet, the maximum enzyme activity was 5.787 mU/min for without antibiotic second instar larva followed by 4.983 mU/min for with antibiotic second instar larva (Figure 6.53). No research reports are available on LDH activity of lesser wax moth larvae when fed on the plastics.

The current research investigation focuses on the practical approach of the plastic consumption capacity and its digestion by the wax moth species. The results in the present research exploration provide positive observations on the plastic biodegradation capacity by the caterpillars. Moreover, the observations from the plastic consumption capacity prove that larval stages of both greater and lesser wax moth species could feed on the plastics (LDPE, HDPE, and PP) as sole diet. As the wax moths are pest to apiculture industry, so, for biodegradation of the plastics there is a need of a closed system for plastic biodegradation and appropriate artificial diet that can provide balanced diet for rapid growth of the insects. In the present research, a diet for growth of the insect was patent (Patent application number-202211073979 A) as well as closed system was designed and a patent was published on the same (Patent application number-202211020620 A). The present device focuses on completing the biodegradation of plastic garbage. Despite being a waste product from the apiculture business, wax worms have the ability to break down plastic. Consequently, the equipment in question is capable of degrading plastic and managing the trash generated by the beekeeping sector. The goal of the presented proposal is to produce biogas and use wax moth frass as fuel. Further, the different gases might be produced and compressed at different temperatures that could have a monetary worth.

The results in the present research proclaim that greater and lesser wax moth could ingest the plastics (LDPE, HDPE and PP). The significant plastic consumption rate by the larvae of the both the wax moth species reveal that wax worms could be used as the potential degraders of the polymers. The higher survival rate of later larval instar stages of both species signifies that the discussed insects could feed on the long linear chained hydrocarbons and help to reduce the plastic pollution. Further, the SEM images of remnants of the ingested plastic films revealed presence of holes, cracks, depressions and pits. These surface irregularities confirm the plastic consumption by *G. mellonella* and *A. grisella*. Moreover, the GC-MS results of the frass (wax worms when fed on the plastics as sole diet) revealed presence of alkanes, alcohols, aldehydes, esters, ethers, and other functional groups. These functional groups had low molecular weight as compared to the

naive plastics. This decrease in molecular weight of compounds found in the GC-MS of the frass of the wax worms indicates the biodegradation of the plastics into biodegradable compounds. But there is immense need for detailed study on the genetic elements involved in plastic biodegradation by wax moths and potential pathways responsible for biodegradation of the plastics. Also, there is need of more advanced functional system that can inhabit the honey bee pest and biodegrade the plastics. Furthermore, there is still extensive need on the enzymes and genetic elements present in the wax moth that are involved in the biodegradation of the plastics.

CHAPTER 7

CONCLUSION

Plastics are amongst the synthetic polymers that are considered as materials of biggest importance in the contemporary life. They are increasingly vital for human comfort due to their durability and resilience, leading to their widespread presence as plastic waste on Earth. Amongst all types of plastics, polyethylene and polypropylene are two of the most widely used polymers worldwide. These are also the most accumulated plastics worldwide. Consequently, the biodegradation of long-chain hydrocarbons (Low Density Polyethylene- LDPE, High Density Polyethylene- HDPE, and Polypropylene- PP) was investigated in the current work.

On the other hand wax worms are the most damage causing honey bee past. They are known to feed on the honey comb and beeswax naturally. The polyethylene and polypropylene are also long chained hydrocarbons similar to beeswax. Thus, in the current research work, the ability of *G. mellonella* (greater wax moth) and *A. grisella* (lesser wax moth), two species of wax worms, to biodegrade the plastics was investigated. Initially, the plastics (LDPE, HDPE and PP) had been left in the soil for a year in order to examine their capability for biodegradation after being accumulated in the environment. The wax moth's larval instars were then exposed to both these soil-treated plastics and naive plastics for 48 hours. Further, the plastic consumption capacity and survival rate of the larval insect on the naive and soil treated plastics was observed.

The focus of the present research is on wax worms' ability to biodegrade plastics without any involvement of gut microbes. In order to observe the biodegradation potential of all larval instars of greater and lesser wax moths, the larval insects were exposed to long linear chained hydrocarbons namely LDPE, HDPE and PP for two days. To observe the plastic consumption capacity of the larva, the weight loss of the polymers was recorded with the survival rate of the insects. Further, the extent of degradation of the plastic film was analysed by Scanning Electron Microscopic (SEM) images and compared with the SEM images of the naive plastic film. For determination of digestion and biodegradation of the plastics the Gas Chromatography and Mass Spectroscopy (GC-MS) of the frass was performed. The enzyme assay of the enzyme alcohol dehydrogenase

and lactate dehydrogenase as biomarker was performed to trace the digestion of the plastics by the wax moth.

When exposed to soil treated and naive LDPE, the maximum plastic consumption capacity was 6.64 % for seventh instar larva of *G. mellonella* fed with pretreated LDPE film whereas for HDPE film 8.89 % of plastic consumption capacity was observed by seventh instar larva fed soil treated film. Further, for PP film the maximum plastic consumption capacity was 8.44 % observed for seventh instar larva of greater wax moth. Whereas, for lesser wax moth, when exposed to LDPE film, the maximum plastic consumption capacity was 7.51 % observed for fifth instar larva fed with soil treated film. Likewise, for HDPE film, the maximum plastic consumption capacity was 7.55% observed for fifth instar larva fed with soil treated film. But for PP film the maximum plastic consumption capacity was 1.82 % for fifth instar larva fed with soil treated film.

The study investigated the survival rates of greater wax moth and lesser wax moth larvae when fed on various types of plastic films, including LDPE, HDPE, and PP. The maximum survival rate for greater wax moth larvae was 94.66% in the seventh instar stage when fed on naive and soil-treated LDPE films for two days. For HDPE films, the maximum survival rate for greater wax moth larvae was 94.66% in the sixth instar stage when fed on soil-treated film. The maximum survival rate for greater wax moth larvae was 96.66% in the seventh instar stage when fed on soil-treated PP film for 48 hours. Further, the highest survival rate for lesser wax moth larvae was 92% in the fifth instar stage when exposed to naive LDPE film. The highest survival rate for lesser wax moth larvae was 94.66% in the fourth instar stage when exposed to pretreated HDPE film. Moreover, the highest survival rate for lesser wax moth larvae was 96.66% in the fifth instar stage when exposed to pretreated PP film for 48 hours. The findings emphasize the survival capacity of the greater and lesser wax moth when consuming soil-treated and nave plastic films exclusively as their diet.

The study aimed to assess the naïve plastic ingestion ability of different larval stages *G. mellonella* and *A. grisella* in the absence of gut microbiota. To achieve this, the larvae

were treated with antibiotics for 24 hours to eliminate their gut microbiomes. Following the antibiotic treatment, the larvae were provided with LDPE, HDPE, and PP films for 48 hours to evaluate their plastic consumption ability. The fourth instar greater wax moth larvae without antibiotics exhibited the highest plastic consumption rate of 4.84% when fed on the naive LDPE film whereas, for HDPE films, the seventh instar greater wax moth larvae without antibiotics demonstrated the maximum plastic consumption rate of 7.01%. Further, after exposure to naive PP film, the seventh instar greater wax moth larvae without antibiotics exhibited the highest plastic consumption rate of 8.4%. Moreover, for *A. grisella*, fifth instar lesser wax moth larvae without antibiotics showed the maximum plastic consumption rate of 7.51% after exposure to the LDPE film while, the fourth instar lesser wax moth larvae treated with antibiotics displayed the highest plastic consumption rate of 6.83% when exposed to HDPE film. Further, for lesser wax moth fed with PP films, the maximum consumption rate was 3.66% for fifth instar larvae administered with antibiotics. The research provides insights into the potential role of wax moth larvae in plastic degradation and their ability to adapt to plastic-rich environments.

The current study investigated the survival rates of greater and lesser wax moth larvae when exposed to various plastic materials for duration of 48 hours. The highest survival rate of greater wax moth larvae (94.66%) was observed in the without antibiotics seventh instar larvae exposed to LDPE film. Moreover, for HDPE film, greater wax moth larvae had a maximum survival rate of 98% in the seventh instar larvae without antibiotics. For the PP film, greater wax moth larvae had a maximum survival rate of 94.66% in the seventh instar larvae without antibiotics. Further, lesser wax moth larvae showed a maximum survival rate of 92% in the without antibiotics fifth instar larvae fed with LDPE film while, larvae exhibited a highest survival rate of 81.33% in the without antibiotics fifth instar larval stage when exposed to HDPE film. The larvae fed with PP film showed the highest survival rate of 99.33% in the with antibiotics fifth instar larval stage. The survival rates illustrate the ability of these insects to survive when plastic is their sole diet, indicating their potential to survive on the plastics.

The SEM images of the remnants of plastic film ingested by greater wax moth revealed holes and pits on the surface of the polymers indicating the consumption of plastics by all larval instars of the discussed larval insect. Similarly, the SEM images of remnants of the plastic film consumed by lesser wax worms also revealed presence of holes, cracks and pits indicating plastic consumption by larval instars of the wax moth species.

The GC-MS of the frass sample collected after the plastic consumption by *G. mellonella* and *A. grisella* indicated the presence of alkanes, alcohols, aldehydes, acids and esters with less molecular weight as that of the naive plastics which indicated degradation of the polymer by the larval instars.

In the present research to confirm the digestion and biodegradation of long linear chained hydrocarbons (LDPE, HDPE and PP) the maximal enzymatic activity of alcohol dehydrogenase enzyme was analysed. For *G. mellonella* larvae the maximum enzymatic activity was observed as 3.90 mU/min for second instar larva exposed to PP film followed by 3.32 mU/min for without antibiotic sixth instar larva fed on LDPE as sole diet. But for greater wax moth larvae fed on the HDPE film the maximum enzymatic activity for alcohol dehydrogenase was 4.6 mU/min for with antibiotic fifth instar larva. Moreover, for *Achroia grisella* amongst all the plastic diet highest enzymatic activity was observed for PP film as 5.62 mU/min for with antibiotic fifth instar larva followed by 5.35 mU /min for HDPE for without antibiotic third instar larva. For LDPE as sole diet highest enzymatic activity was 5.35 mU /min for without antibiotic fifth instar larva of lesser wax moth.

Also, plastics have high molecular weight and during enzymatic reaction of ADH excess amount of reduced NADH, is produced which can be metabolized by the lactate dehydrogenase enzyme by converting pyruvate to lactate dehydrogenase which leads to production of NAD^+ (Lemoine et al., 2020). In the current research exploration for *G. mellonella* when fed on the plastic diet, amongst all the plastics maximum enzymatic activity was observed for PP by without antibiotic seventh larval instar. This was followed by 2.94 mU/min for without antibiotic seventh instar larva fed on the LDPE

film. The least enzymatic activity amongst all plastics was 1.28 mU/min observed for HDPE plastic film. Furthermore, for *A. grisella* amongst all the plastics fed as sole diet the maximum enzymatic activity was 5.78 mU/min for without antibiotic second instar larva for PP film followed by 0.89 mU/min for with antibiotic third instar larva fed with LDPE film. The least LDH enzymatic activity was 0.74 mU/min by without antibiotic fourth instar larva fed on HDPE plastics.

The current study's findings indicate that both greater and lesser wax moths may consume the polymers (LDPE, HDPE, and PP). The larvae of both species of wax moths consume a significant amount of plastic, suggesting that wax worms may be useful as polymer degraders. The fact that both species' later larval instar stages have a greater survival rate suggests that the insects under discussion may be able to consume long linear chained hydrocarbons and lessen the pollution caused by the plastics. Moreover, holes, fractures, depressions, and pits were seen in the SEM pictures of the remaining pieces of the consumed plastic films. The ingestion of plastic by *G. mellonella* and *A. grisella* is confirmed by these surface abnormalities. Further analysis of the frass (wax worms fed only on plastics) using GC-MS indicated the presence of several functional groups such as alcohols, esters, ethers, and alkanes. These functional groups detected in the GC-MS have a low molecular weight that indicates the plastic biodegradation. However, a thorough investigation of possible plastic biodegradation pathways in the larval insects is desperately needed. Also in future, research on the expression of the genetic elements responsible for degradation of plastics by the insect could be undertaken. As wax worms are honeybee pest, thus, they could be released in the environment. Therefore, there is requirement of more sophisticated functional systems are required in order to inhabit these honey bee pests and biodegrade plastics. Further, in near future plastic waste treatment plants can be established in the public places for the successful treatment of constantly accumulating plastic waste in the environment.

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APPENDICES

List of Publications

1. Kapahi, N., & Marwaha, L. (2022). Polyethylene degradation by larvae of wax moth. *Journal of Entomological Research*, *46*(3), 620-625.
2. Kapahi, N., Marwaha, L., Kaur, M., & Bawa, G. (2023). Comparison study on low-density polyethylene film biodegradation potential of *Achroia grisella* and *Galleria mellonella* larvae. *Journal of Environmental Biology*, *44*(5), 682-690.

List of Patents

1. Method of preparing nutritional diet for growth of *Galleria mellonella*
2. A novel device for plastic biodegradation

List of Copyrights

1. Process of Biodegradation of Long Linear Chained Plastics by *Galleria mellonella.*
2. Biological Control of Polyurethane foam by Larvae of *Galleria mellonella.*
3. Biological Control of Polypropylene Film by Larvae of *Galleria mellonella*.
4. Biological Control of Polypropylene Film by Larvae of *Achroia grisella.*
5. Biological Control of Polyethylene terephthalate microplastics by Larvae of *Galleria mellonella*.
6. Biodegradation of Polyethylene by *Achroia grisella*.
7. Biodegradation Capacity of *Achroia grisella* for High-Density Polyethylene Independent of Gut Microbiota.
8. Biological Control of High Density Polyethylene Film by Larvae of *Galleria mellonella.*
9. Mechanism for biodegradation of Plastic gut microbiota dependent and independent.
10. Generalized mechanism for Biodegradation of long linear chained plastics.
11. Mechanism for biodegradation of most extensively used Plastics by wax worms.

List of Conferences

1. Presented Oral Presentation on the topic entitled- A Research Account on Effect of Low-Density Polyethylene (LDPE) as diet on the larvae of *Galleria mellonella* and *Achroia grisella* in the International Conference on Bioengineering and Biosciences (ICBB-2022) held on 18-19 November 2022 organized by the Department of Biotechnology, School of Bio-engineering and Biosciences in association with the Society of Bioinformatics for Experimenting Scientists (Bioclues) & organized at Lovely Professional University, Punjab.
2. Presented Oral Presentation on the topic entitled- A Research Account on *Galleria mellonella*'s Biodegradation Capacity for Low-Density Polyethylene (LDPE) and Polypropylene (PP) Plastic Films in the International Conference on Science for Survival: To Explore The Unexplored Dimensions held on 10-11 February, 2023 organized at Govt. College For Women Udhampur & organised by Internal Quality Assurance Cell (IQAC) in association with Department of Botany, Govt. College for Women Udhampur, J&K (UT) India.

List of Workshops

1. Successfully Completed Training on "IPR & Technology Transfer" conducted as part of IIC Innovation Ambassador Training Series Organized by Institution's Innovation Council of MHRD's Innovation Cell, AICTE held at Lovely Professional University, Jalandhar, Punjab on 16-17 January 2020.
2. Participated in the program of international workshop on Combatting Microplastics Challenge in the Global Contex, including Microplastics an emerging contaminant: Challenges to analysis and mitigation Microplastics in fresh waters: impact on the ecosystem and ways to reduce it Analysis of microplastics in the integral water cycle: wastewater, sludge and drinking water by Micro-FTIR infrared spectroscopy on April 14, 2023.
3. Participated in IP Awareness/Training program under National Intellectual Property Awareness Mission on August 28, 2023.